The Nonhuman Primate in Nonclinical Drug Development and Safety Assessment

Edited by
Joerg Bluemel, Sven Korte,
Emanuel Schenck, Gerhard F. Weinbauer

VetBooks.ir

THE NONHUMAN PRIMATE IN NONCLINICAL DRUG DEVELOPMENT AND SAFETY ASSESSMENT

VetBooks.ir

THE NONHUMAN PRIMATE IN NONCLINICAL DRUG DEVELOPMENT AND SAFETY ASSESSMENT

Edited by

JOERG BLUEMEL
Medimmune, Gaithersburg, MD, USA

SVEN KORTE
Covance Laboratories GmbH, Münster, Germany

EMANUEL SCHENCK
Medimmune, Gaithersburg, MD, USA

GERHARD F. WEINBAUER
Covance Laboratories GmbH, Münster, Germany

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

VetBooks.ir

Academic Press is an imprint of Elsevier
125 London Wall, London, EC2Y 5AS, UK
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK

Copyright © 2015 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangement with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

For information on all Academic Press publications
visit our website at http://store.elsevier.com/

Printed and bound in the United States
15 16 17 18 10 9 8 7 6 5 4 3 2 1

ISBN: 978-0-12-417144-2

VetBooks.ir

Contents

VetBooks.ir

Contributors xiii
Preface xvii

I
GENETICS OF NONHUMAN PRIMATE SPECIES COMMONLY USED IN DRUG DEVELOPMENT

1. Diversity and Evolutionary History of Macaques with Special Focus on *Macaca mulatta* and *Macaca fascicularis*

CHRISTIAN ROOS AND DIETMAR ZINNER

Macaques 3
The Phylogenetic Position of Macaques Among Primates 5
Evolutionary History of Macaques 6
Taxonomic and Genetic Diversity of Rhesus and Long-Tailed Macaques 11
Conclusion 13
Acknowledgements 13
References 13

2. Evolutionary History and Genetic Variation of *Macaca mulatta* and *Macaca fascicularis*

BETSY FERGUSON AND DAVID GLENN SMITH

Origin and Dispersal of Cynomolgus Macaques 18
Origin and Dispersal of Rhesus Macaques 20
Genetic Diversity in Rhesus and Cynomolgus Macaques 22
Implications of Ancestral Divergence and Disease Susceptibility 23
NHP Genomic Studies 24
Genomic Sequence Analysis and Evolutionary Implications 25
Comparing Linkage Disequilibrium between Taxa 25
Introgression During Admixture Between Two Taxa 26
Genomic Discovery of Candidate Risk Alleles 27
Functional Allele Comparison in Rhesus and Cynomolgus Macaques 28
G Protein-Coupled Receptor Gene Variation in Rhesus and Cynomolgus Macaques 28
Genetic Variation and Drug Safety Testing 29
Gene Expression Variation 29
Solute Carriers of Organic Anions Sequence Variation in Cynomolgus Macaques 29
The Macaque Cytochrome P450 Gene Family 30
Immune Response Variation as a Variable in Drug Safety Testing 32
Conclusion 32
References 33

3. Genetic Variation of the Major Histocompatibility Complex in *Macaca mulatta* and *Macaca fascicularis*

LUTZ WALTER

General Aspects of the Major Histocompatibility Complex 37
The MHC of M. *mulatta* and M. *fascicularis* 40
MHC Disease Associations 45
Conclusion 46
Acknowledgments 46
References 46

4. Genetic Variations in the *Macaca fascicularis* Genome Related to Biomedical Research

TOBIAS HECKEL, ANJALI SINGH, ANDREAS GSCHWIND, ALEXANDRE REYMOND, AND ULRICH CERTA

Introduction: Animal Models and Drug Testing 53
Copy Number Variations in the Genome of M. *fascicularis* 55
Conclusion 61
References 62

II

COMPARATIVE PHYSIOLOGY OF NONHUMAN PRIMATE SPECIES USED IN NONCLINICAL SAFETY ASSESSMENT AND DRUG DEVELOPMENT

5. Basic Physiology of *Macaca fascicularis*

IRIS D. BOLTON

History and Introduction 67
Appearance and Morphology 69
Hematology 70
Serum Chemistry 73
Reproduction and Development 75
Cardiovascular 77
Hepatic and Urinary 78
Other Organ Systems 80
Endemic Diseases and Susceptibility 81
Behavior 82
References 83

6. Basic Physiology of *Macaca mulatta*

ANNE D. LEWIS AND KAMM PRONGAY

General Biology 88
Gastrointestinal Tract and Nutrition 89
Cardiovascular System 91
Respiratory System 96
Neurologic System and Special Senses 98
Development and Reproduction 100
Urinary System 105
Lymphatic System 107
Endocrine System 108
References 108

7. Basic Physiology of *Callithrix jacchus*

KERSTIN MÄTZ-RENSING AND SVEN KORTE

General Biology 115
Gastrointestinal Tract and Nutrition 117
Complete Blood Count 119
Cardiovascular System 122
Respiratory System 123
Development and Reproduction 123
Conclusion 129
References 129

8. Comparative Physiology, Growth, and Development

WAYNE R. BUCK

Introduction 136
Comparative Genetics 136
Comparative Growth and Development 139
Comparative Cardiovascular Physiology 151
Comparative Renal Physiology 155
Comparative Respiratory Physiology 156
Neurobiology 159
Visual System 161
References 162

9. Implications and Limitations of Reference Values and Historical Data for the Interpretation of Nonhuman Primate Toxicology Studies

HARRY YANG, LAURA RICHMAN, AND ROBERT HALL

Introduction 173
Utility of Reference Intervals for Nonhuman Primate Safety Studies 174
Limitations of Reference Intervals for Safety Assessment 178
Conclusion 182
References 182

III

PATHOPHYSIOLOGY OF NONHUMAN PRIMATE SPECIES USED IN NONCLINICAL SAFETY ASSESSMENT AND DRUG DEVELOPMENT

10. Nonhuman Primate Diseases of Relevance in Drug Development and their Impact on the Interpretation of Study Findings

CHANDRASSEGAR SARAVANAN, VITO G. SASSEVILLE, AND KEITH G. MANSFIELD

Introduction 188
Viral Infections 190

Bacterial Infections 199
Parasitic Infections 200
Fungal Infections 203
Conclusion 205
References 205

11. Consequences and Indicators of Test Article-Related Immune Suppression in Nonhuman Primates
JACINTHA M. SHENTON, KRISTIN L. HENSON, AND MARLON C. REBELATTO

Introduction 216
Selection of the NHP as the Nonclinical Safety Species for Immunomodulatory Drugs 218
Immunologically Relevant End Points in Standard Toxicology Studies of NHPs 219
Specialized Immunologic End Points in NHPs 222
Differential Diagnosis of On-Target, Off-Target, and Stress-Related Effects on the Immune System 224
Clinically Apparent Manifestations of Immune Suppression in NHPs Observed in Nonclinical Safety Studies: Case Examples 228
Identification of a Risk for Clinically Adverse Immune Suppression: Impact on Further Development 231
Conclusion 232
References 232

12. Toxicologic Pathology and Background Lesions of Nonhuman Primates
DANIEL J. PATRICK AND MARLON C. REBELATTO

Introduction 236
The Art and Practice of Toxicologic Pathology 236
Common Background Microscopic Changes in NHPs 244
Conclusion 254
References 255

13. Nonhuman Primate Models of Human Disease
JENNIFER A. CANN

Introduction 257
Type 2 Diabetes Mellitus, Cardiovascular Disease, and Metabolic Syndrome 259
Diseases of Aging 262
Female Reproductive Conditions 263
Immunosuppressive Conditions 266
Malaria 268
References 269

IV
THE 3 Rs AND ANIMAL WELFARE

14. Opportunities for Implementing the 3 Rs in Drug Development and Safety Assessment Studies Using Nonhuman Primates
KATHRYN CHAPMAN, KATHRYN BAYNE, JESSICA COUCH, THIERRY DECELLE, JOHN FINCH, LOLKE DE HAAN, TINA KOBAN, LARS FRIS MIKKELSEN, WOLFGANG MÜLLER, HELEN PALMER, AND MARK PRESCOTT

Introduction 282
Study Design Considerations to Minimize Nonhuman Primate Use 283
Refinement of Husbandry and Procedures to Improve Animal Welfare and Quality of Science 293
References 299

15. Training and Selection of (Mature) Nonhuman Primates for European Housing
WOLFGANG MÜLLER AND SUSAN HENWOOD

Introduction 303
Training of (Mature) Monkeys for European Housing 304
Selection of (Mature) Monkeys for European Housing 307
References 312

VetBooks.ir

VetBooks.ir

V

THE ROLE OF NONHUMAN PRIMATES IN SAFETY ASSESSMENT/REGULATORY TOXICOLOGY

16. Nonclinical Toxicology Testing Strategies and Applicable International Regulatory Guidelines for Using Nonhuman Primates in the Development of Biotherapeutics

MARQUE TODD AND MICHAEL W. LEACH

Introduction 316
Species of NHPs Used in Nonclinical Toxicity Studies 317
Overview of Nonclinical Toxicity Testing Strategy Using NHPs and Applicable International Regulatory Guidelines 317
Species Selection 319
In Vivo Pharmacology/Toxicology Studies 323
Considerations for Safety Pharmacology Assessment 326
Considerations for Developmental and Reproductive Toxicity Testing 327
Other Considerations for Using NHPs in Nonclinical Testing Programs 328
Conclusion 333
References 334

17. Alternatives to the Use of Nonhuman Primates in Regulatory Toxicology

GEORG SCHMITT, PAUL BARROW, AND MARKUS STEPHAN-GUELDNER

Introduction 337
Conventional Nonrodent Species as Alternatives to NHPs 339
Conventional Rodent Species as Alternatives to NHPs 349
Genetically Modified Rodent or Nonrodent Species as Alternatives to NHPs 350
Specific Animal Models for Targets Only Present in Humans/Human Disease 351
Alternative Lower-Order Species 352
Omission of Regulatory Toxicity Animal Studies If No Suitable Models Exist (Alternatives to In Vivo Testing) 352
References 352

VI

TRANSLATIONAL ASPECTS FOR THE USE OF NONHUMAN PRIMATES IN DRUG DEVELOPMENT

18. Pharmacokinetics of Human Therapeutics Characterized in Nonhuman Primates

CHIH-MING L. TSENG, RAFFAELLA FAGGIONI, AND LORIN K. ROSKOS

Nonhuman Primate Pharmacokinetic Study Designs 360
NHP PK Study Results 360
Determinants for PK in NHPs 367
Other Special PK Studies Using NHPs 370
PK Analysis of Data Obtained from NHPs 370
Species Difference and Prediction for Human PK 371
References 372

VII

NONHUMAN PRIMATE-SPECIFIC ASPECTS OF STANDARD TOXICOLOGY STUDIES

19. Advantages and Limitations of Commonly Used Nonhuman Primate Species in Research and Development of Biopharmaceuticals

ANNICK J. CAUVIN, CHRISTOPHER PETERS, AND FRANK BRENNAN

Introduction 379
Use of NHPs in Research and Development 382
Evaluation of Reproductive and Developmental Toxicity 385

Evaluation of Immune Functions 389
Conclusion 392
References 393

20. Nonhuman Primate Health Screening During Acclimation/Prestudy Conditioning

WILLIAM OLIVER IVERSON

Introduction 398
Animal Sourcing 398
Qualification of Animals at the Source 399
Quarantine of Animals in the Source Country 400
Transportation to the Test Facility 400
Quarantine in the Receiving Country 401
Acclimation 401
Study Selection 402
Socialization 402
Summary of Animal Sourcing and Acclimation 402
References 404

21. Study Design Considerations, Technical Procedural Aspects, Scheduling, and Dosing Sampling Volumes

WERNER FRINGS, MARC NIEHOFF, AND SVEN KORTE

Introduction 406
Animal Welfare, Care, and Use 406
Applicable Guidelines and Regulations 406
Justification of the Test System 407
References 415

22. Functional Assessments in Nonhuman Primate Toxicology Studies to Support Drug Development

JEAN-PIERRE VALENTIN, ANNIE DELAUNOIS, PIERRE LAINÉE, MATTHEW SKINNER, AND HUGO M. VARGAS

Introduction 419
Application to Specific Organ Functions 422
Conclusion 431
Acknowledgements 431
References 432

23. Considering the Use of *Callithrix jacchus*

C. MARC LUETJENS, JOACHIM KASPAREIT, SVEN KORTE, AND JOACHIM WISTUBA

Introduction 438
Evolutionary Origin of the Common Marmoset 439
Consequences of Phylogenetic Distance 439
Use of Marmosets in Reproductive and Regenerative Research 442
Practical Considerations 445
Use of Marmosets in Toxicological and Pharmacological Studies 447
References 453

24. Considerations on Immunogenicity

THOMAS GELZLEICHTER AND ERIC WAKSHULL

Introduction 457
Effect on PK and PD 458
Impact on Safety 459
Strategies to Minimize Effect 462
Methods to Measure Antibodies Against Protein Therapeutics 462
Conclusion 465
References 466

VIII

SPECIAL TOXICITY TESTING WITH NONHUMAN PRIMATES

25. Developmental and Reproductive Toxicity Testing

GERHARD F. WEINBAUER, CHRISTOPHER J. BOWMAN, WENDY G. HALPERN, AND GARY J. CHELLMAN

Introduction 472
Which NHP Model? 473
Fertility and Early Embryonic Development Study 474
Embryo-Fetal Development 479
Prenatal and Postnatal Development 482
Group Size and "3 R" Considerations 486
Relevance of ADAs 491
Placenta: Target Organ and Transport 491
Data Interpretation Challenges Associated with Background Changes in Pregnant NHPs 493

Conclusion 493
References 496

26. Juvenile Animal Studies and Experience
PAULINE L. MARTIN AND TIMOTHY P. COOGAN

Introduction and Regulatory Considerations 502
The Use of Nonhuman Primates for Juvenile Toxicity Testing 502
Organ Systems that Are Well Developed in Macaques at Birth or by 6 Months of Age 504
Organ Systems that Show Significant Development Beyond 6 Months of Age 506
Rationale for Not Conducting a Nonclinical Study Using NHPs 511
Rationale for Conducting a Nonclinical Study in NHPs 511
Conclusion 512
References 512

27. Immunotoxicology Testing of Monoclonal Antibodies in *Macaca fascicularis*: From Manufacturing to Preclinical Studies
MARIE-SOLEIL PICHÉ, BARBARA MOUNHO-ZAMORA, LYNNE LESAUTEUR, AND LEIGH ANN BURNS-NAAS

Immunotoxicology Regulatory Considerations 520
Product- and Process-Related Factors and Immunogenicity 523
State-of-the-Art Immunotoxicology Testing Preclinical Study Designs 525
Developmental Immunotoxicology Testing 533
References 540

28. Ophthalmological Investigations in Toxicity Studies
BIRGIT NIGGEMANN

Introduction 543
General Considerations 544
References 550

29. The Use of Nonhuman Primates in Cardiovascular Safety Assessment
JONATHAN R. HEYEN AND HUGO M. VARGAS

Introduction 552
Basic Cardiovascular Physiology 553
Cardiovascular Evaluation Approaches and Methods 555
Conclusion 571
Acknowledgements 571
References 572

30. Respiratory Function Measurements in Nonhuman Primates
DENNIS J. MURPHY

Introduction 579
Strategy for Respiratory Function Assessment 580
Methodologies for Assessing Respiratory Functions 582
References 588

31. Central Nervous System Assessment in Nonhuman Primates: Behavioral Assessment, Neurological (Electroencephalography, Nerve Conduction), Abuse Liability, and Cognitive Assessment
MARY JEANNE KALLMAN

Observation/General Behavior 592
Electrophysiological Assessment 593
Cognitive Assessment 599
Abuse Liability Potential 601
Conclusion 602
References 603

32. Skeletal Assessments in the Nonhuman Primate
JOHN L. VAHLE, YANFEI L. MA, AND DAVID B. BURR

Introduction to Skeletal Assessments in the Nonhuman Primate NHPs 606
NHP Bone Morphology and Physiology 607
Bone Densitometry 610
Bone Biomechanics 612
The Role of Biomarkers in Skeletal Assessment 615
Bone Histology and Histomorphometry 617
Implementing Skeletal Assessments in Drug Development Programs 620
References 623

VetBooks.ir

VetBooks.ir

33. Considerations for Special Administration Techniques
HELEN PALMER AND VIRGINIA FISHER

Introduction 628
Major Considerations 628
Considerations in Practice 632
Conclusion 645
References 645

IX
FUTURE TRENDS IN PRIMATE TOXICOLOGY

34. Considerations Regarding the Future Use of Nonhuman Primates to Support the Clinical Development of Biopharmaceuticals
JOY A. CAVAGNARO AND BEATRIZ SILVA LIMA

Introduction 650
Background 650
Regulatory Guidance 651
Improving Predictive Value 653
Species Selection 655
Study Design: General Toxicity 656
Reproductive/Developmental Toxicity 658
Immunogenicity 660
Product Class-Specific Considerations 661
Disease-Specific Considerations 662
Potential Impact of Scientific Innovation on the Use of NHPs 664
Conclusion 665
References 666

35. Gaps and Future Challenges
JOERG BLUEMEL, SVEN KORTE, EMANUEL SCHENCK, AND GERHARD F. WEINBAUER

References 671
Index 673

VetBooks.ir

VetBooks.ir

Contributors

Paul Barrow F. Hoffmann-La Roche Ltd., Basel, Switzerland

Kathryn Bayne AAALAC International, Frederick, MD, USA

Joerg Bluemel MedImmune, Gaithersburg, MD, USA

Frank Brennan UCB Pharma, New Medicines, Non-Clinical Development, Slough, UK

Iris D. Bolton University of Texas Medical Branch, Galveston, TX, USA

Christopher J. Bowman Pfizer Inc., Groton, CT, USA

Wayne R. Buck AbbVie, Inc., North Chicago, IL, USA

Leigh Ann Burns-Naas Gilead Sciences, Inc., Foster City, CA, USA

David B. Burr Lilly Research Laboratories, Indianapolis, IN, USA

Jennifer A. Cann MedImmune, Gaithersburg, MD, USA

Annick J. Cauvin UCB Biopharma, New Medicine, Non-Clinical Development, Braine L'Alleud, Belgium

Joy A. Cavagnaro Access BIO, Boyce, VA, USA

Ulrich Certa F. Hoffmann–La Roche Ltd, Pharma Research & Early Development, Basel, Switzerland

Kathryn Chapman National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, United Kingdom

Gary J. Chellman Charles River Laboratories, Reno, NV, USA

Timothy P. Coogan Janssen Research and Development, LLC, Spring House, PA, USA

Jessica Couch Genentech, South San Francisco, CA, USA

Lolke de Haan Medimmune, Cambridge, United Kingdom

Thierry Decelle Sanofi Pasteur, Marcy L'Etoile, France

Annie Delaunois Non-Clinical Development, UCB Biopharma SPRL, Braine-l'Alleud, Belgium

Raffaella Faggioni Clinical Pharmacology & DMPK, MedImmune LLC, Mountain View, CA, USA

Betsy Ferguson Oregon Health & Sciences University and Oregon National Primate Research Center, Beaverton, OR, USA

John Finch Charles River Laboratories, Edinburgh, United Kingdom

Virginia Fisher Huntingdon Life Sciences Ltd, Huntingdon, United Kingdom

Werner Frings Chugai Pharmaceutical Co., Ltd., Gotemba, Japan

Thomas Gelzleichter Genentech, South San Francisco, CA, USA

Andreas Gschwind Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

Robert Hall Covance Laboratories Inc., Madison, WI, USA

Wendy G. Halpern Genentech, South San Francisco, CA, USA

Tobias Heckel F. Hoffmann–La Roche Ltd, Pharma Research & Early Development, Basel, Switzerland

Kristin L. Henson Novartis Institutes for Biomedical Research, East Hanover, NJ, USA

Susan Henwood Covance, Madison, WI, USA

Jonathan R. Heyen Pfizer La Jolla, San Diego, CA, USA

VetBooks.ir

William Oliver Iverson MedImmune, Gaithersburg, MD, USA

Mary Jeanne Kallman Covance Laboratories, Greenfield, IN, USA

Joachim Kaspareit Covance Laboratories, Münster, Germany

Tina Koban Huntingdon Life Sciences, Huntingdon, United Kingdom

Sven Korte Covance Laboratories GmbH, Münster, Germany

Pierre Lainée Animal Welfare and Research, Sanofi, Montpellier, France

Michael W. Leach Pfizer, Inc., Andover, MA, USA

Lynne LeSauteur Human Health Therapeutics, National Research Council, Montreal, QC, Canada

Anne D. Lewis Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Beatriz Silva Lima iMED.ULisboa, Universidade de Lisboa, Faculty of Pharmacy, Lisboa, Portugal

Yanfei L. Ma Lilly Research Laboratories, Indianapolis, IN, USA

Keith G. Mansfield Novartis Institutes for Biomedical Research, Inc., Cambridge, MA, USA

C. Marc Luetjens Covance Laboratories GmbH, Münster, Germany

Pauline L. Martin Janssen Research and Development, LLC, Spring House, PA, USA

Kerstin Mätz-Rensing German Primate Center, Göttingen, Germany

Lars Fris Mikkelsen Independent

Barbara Mounho-Zamora ToxStrategies, Inc., Bend, OR, USA

Wolfgang Müller Covance, Münster, Germany

Dennis J. Murphy GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, USA

Marc Niehoff Covance Münster, Germany

Birgit Niggemann Covance Laboratories GmbH, Münster, Germany

Helen Palmer Huntingdon Life Sciences Ltd, Huntingdon, United Kingdom

Daniel J. Patrick MPI Research, Mattawan, MI, USA

Christopher Peters UCB Biopharma, New Medicine, Non-Clinical Development, Braine L'Alleud, Belgium

Marie-Soleil Piché Charles River Laboratories, Senneville, QC, Canada

Mark Prescott National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, United Kingdom

Kamm Prongay Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Marlon C. Rebelatto MedImmune, Biologics Safety Assessment, Gaithersburg, MD, USA

Alexandre Reymond Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

Laura Richman Translational Sciences, MedImmune, Gaithersburg, MD, USA

Christian Roos Gene Bank of Primates and Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

Lorin K. Roskos Clinical Pharmacology & DMPK, MedImmune LLC, Mountain View, CA, USA

Chandrassegar Saravanan Novartis Institutes for Biomedical Research, Inc., Cambridge, MA, USA

Vito G. Sasseville Novartis Institutes for Biomedical Research, Inc., Cambridge, MA, USA

Emanuel Schenck MedImmune, Gaithersburg, MD, USA

Georg Schmitt F. Hoffmann-La Roche Ltd., Basel, Switzerland

Jacintha M. Shenton Novartis Institutes for Biomedical Research, Cambridge, MA, USA

Anjali Singh F. Hoffmann–La Roche Ltd, Pharma Research & Early Development, Basel, Switzerland

Matthew Skinner AstraZeneca R&D Alderley Park, Macclesfield, Cheshire, England

VetBooks.ir

David Glenn Smith Department of Anthropology and California National Primate Research Center, University of California, Davis, CA, USA

Markus Stephan-Gueldner F. Hoffmann-La Roche Ltd., Basel, Switzerland

Marque Todd Pfizer, Inc., San Diego, CA, USA

Chih-Ming L. Tseng Clinical Pharmacology & DMPK, MedImmune LLC, Mountain View, CA, USA

John L. Vahle Lilly Research Laboratories, Indianapolis, IN, USA

Jean-Pierre Valentin UCB Biopharma SPRL, Braine-l'Alleud, Belgium

Hugo M. Vargas Department of Toxicology Sciences, Amgen Inc., Thousand Oaks, CA, USA

Eric Wakshull Genentech, South San Francisco, CA, USA

Lutz Walter Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

Gerhard F. Weinbauer Covance Laboratories GmbH, Münster, Germany

Joachim Wistuba Institute of Reproductive and Regenerative Biology, Centre of Reproductive Medicine and Andrology, University of Münster, Münster, Germany

Harry Yang Translational Sciences, MedImmune, Gaithersburg, MD, USA

Dietmar Zinner Cognitive Ethology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

VetBooks.ir

VetBooks.ir

Preface

Thalidomide, a small molecule that was tested preclinically in rats, mice, guinea pigs, rabbits, cats, and dogs without showing any severe toxicological effects became an over-the-counter drug in Germany in 1957. This drug alone resulted in about 10,000 cases of phocomelia in infants worldwide, and in addition to well-known limb deficiencies, thalidomide induced deformities of eyes, hearts, and the alimentary and urinary tracts, as well as blindness and deafness. These tragic events led to the development of much more stringent drug regulations and a new era of governmental control over drug development and use.

In addition to triggering a fundamentally different drug approval process, the thalidomide tragedy was also the starting point for the use of nonhuman primates (NHPs) in regulatory preclinical assessment of safety. Nowadays, especially with increasing numbers of large molecules, NHPs are explicitly required as the only relevant model of choice for a large percentage of all drug development projects. In addition to these general necessities, there are also many special relevant aspects of the NHP model that regularly result in requirements for use of the species in safety testing. For example, regarding the predictiveness of reproductive effects, the similarities in the menstrual cycle and anatomy and the physiology of the mammary gland are viewed in some cases as crucial for the assessment of toxicity in female genital organs. The ocular system also has some unique features (e.g., both NHPs and humans have a macula lutea/fovea) not found in other mammals. Therefore NHPs represent a more relevant model to test for specific ocular effects during the discovery and development of new pharmaceuticals.

Because NHPs have played a vital role in late-stage toxicology assessment for so many years—now with many of relevant peer-reviewed journal articles published—we perceived the need to make this tremendous knowledge base available to more individuals working in various different disciplines related to drug development and approval. The overall number of chapters of this book alone exemplifies the breadth of knowledge that has been generated. Among all these relevant topics we tried to highlight the importance of scientific integrity and ethical considerations throughout. We also dedicated a separate chapter to animal welfare and the "3 Rs" (replace, reduce, refine), as well as NHP accommodation and training, to emphasize all the relevant points. This book also was designed to give guidance on good study conduct in routine and regulatory studies, as well as to provide insight into specialty toxicology testing and translational aspects of the use of NHPs in drug development. We were convinced that these aims can be accomplished only if a comprehensively updated summary of the knowledge base in genetics, genomics, evolutionary history, comparative physiology, and the relevant aspects of primate pathology is provided. We also considered the three predominant NHP species (*Macaca fascicularis*, *Macaca mulatta*, and *Calithrix jacchus*) used in nonclinical safety assessment as much as is possible within the scope of a textbook.

VetBooks.ir

As editors, we are thankful to be able to assemble an outstanding team of globally renowned experts in the field, and we hope that this textbook is successful in summarizing the current state of knowledge in a manner that provides value for colleagues in academia, contract and pharmaceutical research, and regulatory environments alike.

Joerg Bluemel
Sven Korte
Emanuel Schenck
Gerhard F. Weinbauer

VetBooks.ir

PART I

GENETICS OF NONHUMAN PRIMATE SPECIES COMMONLY USED IN DRUG DEVELOPMENT

VetBooks.ir

VetBooks.ir

CHAPTER

1

Diversity and Evolutionary History of Macaques with Special Focus on *Macaca mulatta* and *Macaca fascicularis*

Christian Roos[*], *Dietmar Zinner*[†]

[*]Gene Bank of Primates and Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

[†]Cognitive Ethology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

OUTLINE

Macaques 3
The Phylogenetic Position of Macaques Among Primates 5
Evolutionary History of Macaques 6
Taxonomic and Genetic Diversity of Rhesus and Long-Tailed Macaques 11
Conclusion 13
Acknowledgements 13
References 13

MACAQUES

Macaques are a species-rich genus of Old World monkeys. They are the most widely distributed primate taxon besides humans. Barbary macaques (*Macaca sylvanus*) live in northwestern Africa (Morocco and Algeria) and on Gibraltar (most likely introduced there by humans), whereas the other 21 species range over large parts of south, east, and southeast Asia. They have even crossed the Wallace line and colonized Sulawesi and Timor [1–4]. Fossil

http://dx.doi.org/10.1016/B978-0-12-417144-2.00001-9
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

evidence further suggests that macaques historically had an even wider distribution during the Plio-Pleistocene era, encompassing large parts of Eurasia [5,6].

The name *macaque* derives from the Portuguese *macaco*(*a*), which itself derives from the west African Fiot word (*ma*)*kaku*, which simply means "monkey." Macaques are medium-sized primates. Females weigh 3-11 kg and males, 5-18 kg, with prominent differences in body mass among species. Sexual dimorphism in macaques is, however, less pronounced than in the related baboons or mandrills. Macaques are predominantly tropical primates, but they cover a considerable latitudinal gradient, from Timor in the south to the northern tip of Honshu Island in Japan. Well known are the "snow monkeys" (Japanese macaques, *M. fuscata*) in the Japanese Alps, which warm themselves in winter by bathing in thermal pools. The majority of the species, however, are associated with various tropical forests and other, more open tropical habitats on the Indian subcontinent, in southeast Asia, and in Sundaland. They are primarily arboreal, but several species have become semiterrestrial, with an array of anatomic adaptations for living on the ground. In contrast to arboreal species, which have longer hindlimbs than forelimbs and a long tail, making balancing on thin branches easier, macaques have forelimbs and hindlimbs of similar length, enabling more energy-efficient movement on a terrestrial substrate. Macaques also have shorter tails than, for example, the largely sympatric langurs (*Presbytis*, *Trachypithecus*, or *Semnopithecus*). Macaques feed predominantly on fruits, but depending on the ecological situation, their diet also includes other plant parts such as leaves, flowers, seeds, bark, buds, and lichens. They also eat invertebrates, for example, insects and crustaceans, and small vertebrates, such as birds, reptiles, and small mammals, as well as eggs [7–9].

In some of the macaque species, females produce an estrogen-dependent perineal swelling around the time of ovulation or they show intensive reddening of the sexual skin, both indicating ovulation [10–13]. Macaques are gregarious and live in medium to large social groups comprising several adult males and females and their offspring. Females are predominantly philopatric, whereas males leave their natal group when they reach adulthood. Within groups, females live in a kin-related social network, and both sexes exhibit dominance hierarchies that often influence access to resources and reproduction partners [14–16].

Macaques occupy diverse ecological niches; some species show remarkable ecological plasticity. It is therefore not surprising that some species live close to humans and take advantage of the resources voluntarily or involuntarily provided by humans [17,18]. At many temples in Asia, macaques are tolerated by people and even fed. But interactions of people with macaques are not restricted to temple sites. They also occur at tourist locations that benefit from the presence of macaques and in urban areas, where some species live commensally with humans. Macaques also raid crops, and in some regions they are regarded as pests. Although some species seem to be abundant in certain areas, the prospects of survival are bad for the majority of species. According to the International Union for Conservation of Nature Red List of Threatened Species, 2 of the 22 macaques species are critically endangered, 5 are endangered, 8 are vulnerable, 2 are near threatened, and 5 are assessed as least concern [19]. Among the species listed as least concern are the two most often used in biomedical research, rhesus (*M. mulatta*) and long-tailed macaques (*M. fascicularis*). These are also the two species with the widest distribution; however, genetic studies clearly show that intraspecific variation in these two species is high [20–31] and that both species may comprise several (sub)species. This makes new assessments of the conservation status of the various (sub)species necessary. Furthermore, the presence of deeply diverged

VetBooks.ir

genetic lineages within rhesus and long-tailed macaques likely also affect their use in biomedical research, and the problem of taxonomic, ecological, physiological, and genetic difference should no longer be ignored. Acknowledging patterns of genetic diversity among and within macaque species is relevant for biomedical studies. A macaque is not simply a macaque; likewise, a rhesus macaque is not simply a rhesus macaque and a long-tailed macaque is not simply a long-tailed macaque.

This chapter provides a brief overview of the diversity of macaques and their evolutionary history. We focus on the two species most commonly used as nonhuman primate models in biomedical research—rhesus and long-tailed macaques—and address problems of taxonomic, ecological, and physiological ignorance of "the macaque" or "the monkey" in biomedical research.

THE PHYLOGENETIC POSITION OF MACAQUES AMONG PRIMATES

Macaques (genus *Macaca*) represent one of the major lineages of the family Cercopithecidae (Old World monkeys). This family is the only extant family in the superfamily Cercopithecoidea, which, combined with the superfamily Hominoidea (human and apes), constitutes the infraorder Catarrhini (Figure 1.1) [33,34]. Accordingly, besides great apes (chimpanzees, gorillas, orangutans) and small apes (gibbons), Old World monkeys are our closest living relatives. According to fossil remains and genetic data, both superfamilies diverged ca. 32 million years ago (Ma) [32,35–37]. In contrast, marmosets, squirrel monkeys, and owl monkeys—species that are also widely used in biomedical research—are representatives of the infraorder Platyrrhini (New World monkeys) and diverged from Catarrhini ca. 46 Ma [32,35–37]. Old World monkeys are not only phylogenetically more closely related to humans than New World monkeys, they are also more similar to human in physiology, anatomy, genetics, immune response, and behavior [34,38]. Thus, Old World monkeys are the preferred model in biomedical research and drug safety assessment [39–41].

The Old World monkey family comprises 159 extant species in 23 genera and is by far the most speciose primate family [9]. Because of anatomic, morphological, and physiological differences, which are related to adaptations to a predominantly folivorous or frugivorous diet, the family is divided into two subfamilies: leaf-eating monkeys (Colobinae), for which the diet consists predominantly of leaves, and the cheek-pouched monkeys (Cercopithecinae), which are mainly frugivorous [9,33,34,42]. According to genetic data that support this classification, both subfamilies diverged ca. 22 Ma [32,35–37]. Cheek-pouched monkeys are mainly distributed in Africa, and only macaques occur in Asia [9]; based on differences in anatomy, morphology, behavior, and genetics, cheek-pouched monkeys are further split into the two tribes Papionini and Cercopithecini. Both diverged ca. 14 Ma [32,35–37]. In general, Papionini, comprising macaques, baboons, mangabeys, and related species, are more terrestrial and more robustly built than Cercopithecini, which are generally smaller and more slenderly built [9,34]. Cercopithecini are mainly arboreal, with only a few terrestrial species, and comprise guenons and related species, among them African green monkeys (*Chlorocebus* spp.), which are widely used in biomedical research [43]. Among papionins, both macaques and baboons are important models in biomedical research, for example, simian immunodeficiency virus research, drug safety research and risk assessment, or xenotransplantation [39,44–46].

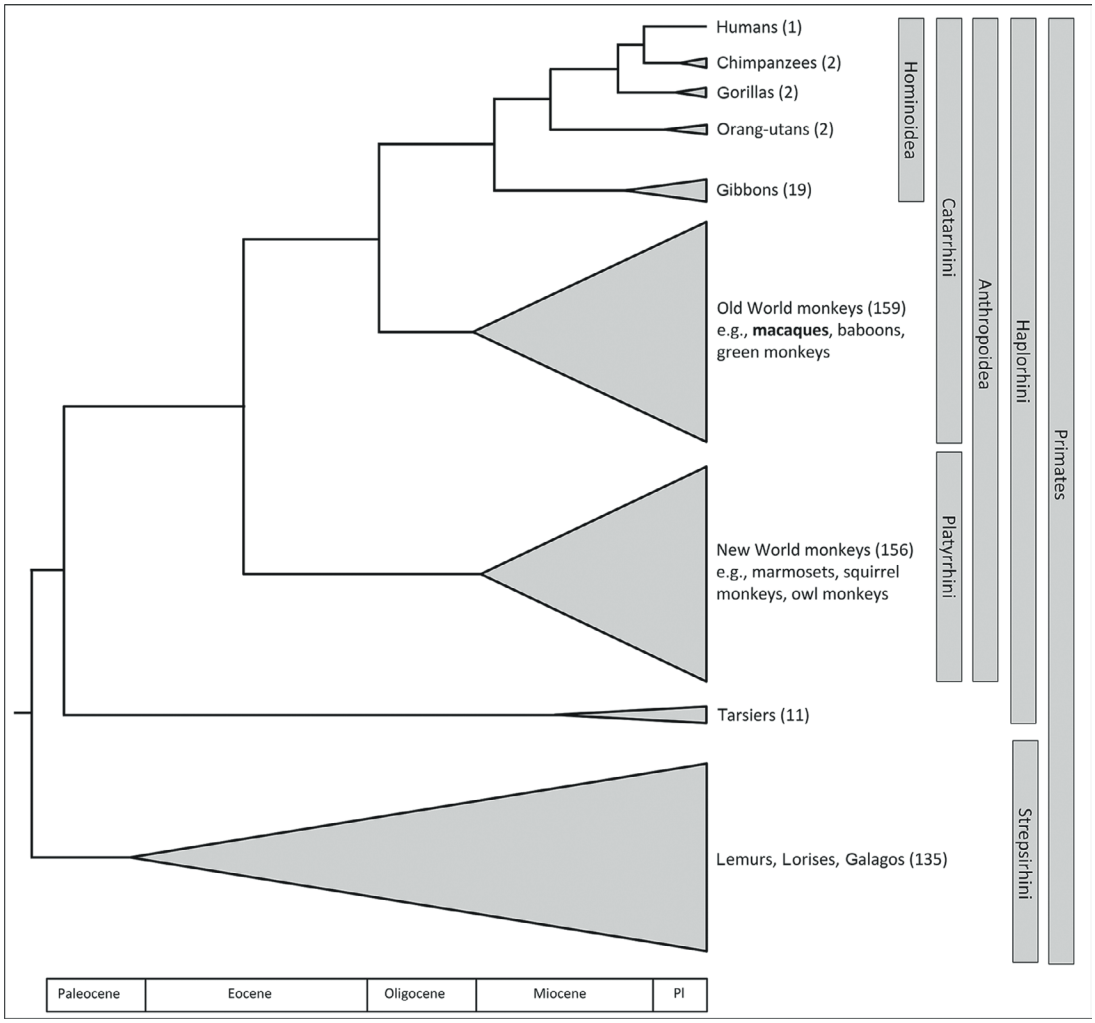


FIGURE 1.1 Phylogenetic relationships among major primate lineages. The phylogenetic position of macaques among Old World monkeys is highlighted in bold letters. Numbers in parentheses indicate the number of species in each group. Divergence ages are taken from Finstermeier et al. [32]. PI = Plio-Pleistocene.

EVOLUTIONARY HISTORY OF MACAQUES

The genus *Macaca* consists of 22 species and 37 taxa and is one of the most diverse Old World monkey genera (Table 1.1) [8]. Macaques are classified into species groups, but the number and composition of these groups has been a matter of debate in the last 50 years [1,2,5,33]. Division into seven species groups, as recently proposed [9], might best reflect the evolutionary history of macaques. Accordingly, there are three monotypic species groups with only one species each (*M. sylvanus* group, *M. arctoides* group, and *M. fascicularis* group)

VetBooks.ir

TABLE 1.1 Species Groups, Species and Subspecies of the Genus *Macaca* According to Anandam et al. [8] and Zinner et al. [9]

Species group	Species	Subspecies
M. sylvanus group	*M. sylvanus*	
M. silenus group	*M. silenus*	
	M. nemestrina	
	M. leonina	
	M. siberu	
	M. pagensis	
Sulawesi macaques group	*M. nigra*	
	M. nigrescens	
	M. hecki	
	M. tonkeana	
	M. maura	
	M. ochreata	*M. o. ochreata*
		M. o. brunnescens
M. sinica group	*M. sinica*	*M. s. sinica*
		M. s. aurifrons
		M. s. opisthomelas
	M. radiata	*M. r. radiata*
		M. r. diluta
	M. munzala	
	M. thibetana	
	M. assamensis	*M. a. assamensis*
		M. a. pelops
M. arctoides group	*M. arctoides*	
M. mulatta group	*M. mulatta*	
	M. cyclopis	
	M. fuscata	*M. f. fuscata*
		M. f. yakui

(Continued)

TABLE 1.1 Species Groups, Species and Subspecies of the Genus *Macaca* According to Anandam et al. [8] and Zinner et al. [9]—Cont'd

Species group	Species	Subspecies
M. fascicularis group	*M. fascicularis*	*M. f. fascicularis*
		M. f. philippinensis
		M. f. aureus
		M. f. atriceps
		M. f. condorensis
		M. f. fuscus
		M. f. karimondjawae
		M. f. lasiae
		M. f. tua
		M. f. umbrosus

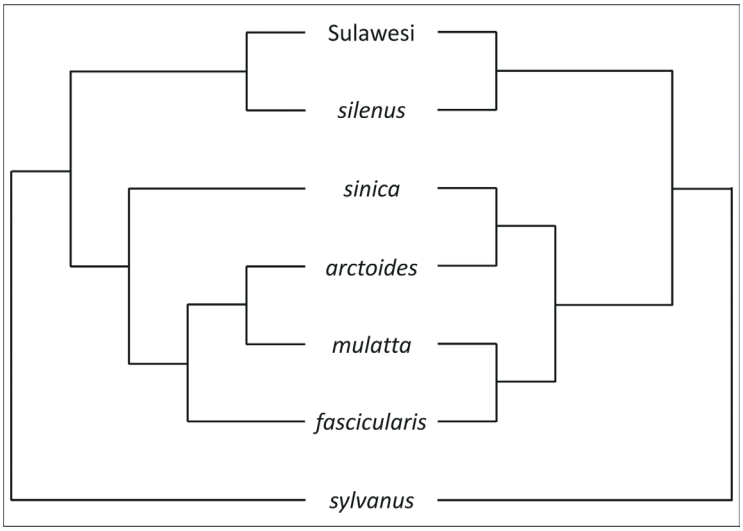


FIGURE 1.2 Phylogenetic relationships among macaque species groups based on mitochondrial (left) and Y chromosomal DNA (right). Autosomal loci show either the left or right branching pattern, depending on the loci studied. Both phylogenies are highly similar but differ in the placement of *Macaca arctoides*, suggesting that this species group is of hybrid origin.

and four polytypic species groups comprising several species (*M. silenus* group, Sulawesi macaques group, *M. sinica* group, and *M. mulatta* group) (Table 1.1; Figure 1.2) [9].

The *M. sylvanus* group includes only the Barbary macaque (*M. sylvanus*) from northwestern Africa and Gibraltar [5,9,33]. With an African origin and diverging from *M. sylvanus* ca. 5.5 Ma, the ancestor of the remaining species invaded Asia [5,47]. The ancestor of extant

VetBooks.ir

Asian macaques initially split into two main clades ca. 4.5 Ma [20,30,35]. One of these clades comprises the *M. silenus* group and the Sulawesi macaques group, which were alternatively combined into one species group [5,47] and even grouped together with *M. sylvanus* [1,2]. The second clade subsumes the remaining species groups (*M. arctoides* group, *M. fascicularis* group, *M. sinica* group, *M. mulatta* group). Earlier arrangements recognized species of the *M. mulatta* group as members of the *M. fascicularis* group [1,2,5,47], and *M. arctoides* was included in either the *M. sinica* group [5,47] or the *M. fascicularis* group [33].

The *M. silenus* group diverged from the Sulawesi macaques group ca. 3 Ma [20,30,35,48]. In the *M. silenus* group, five species are recognized: the lion-tailed macaque (*M. silenus*) from southern India, the Sunda pig-tailed macaque (*M. nemestrina*) from Sundaland, the northern pig-tailed macaque (*M. leonina*) from a wide range of the southeast Asian mainland, the Siberut macaque (*M. siberu*) from Siberut island, and the Pagai macaque (*M. pagensis*) from Sipora and North and South Pagai [9]. Until recently, the latter three species (*M. leonina*, *M. siberu*, *M. pagensis*) were recognized as subspecies of *M. nemestrina* [1,2,5,42,49]. However, the macaques of the Mentawai Islands seem to be paraphyletic; *M. pagensis* forms a sister lineage to all other members of the species group [48]. Accordingly, it was suggested that the Mentawai Islands could have been a rainforest refuge during Pleistocene periods and that all extant members of this species group might have originated there. Among the remaining species of the species group, *M. silenus* and *M. leonina*, as well as *M. siberu* and *M. nemestrina* from Sumatra and the Malaysian Peninsula, share a common ancestor [20,48]. Interestingly, *M. nemestrina* from Borneo clusters mitochondrially with Sulawesi macaques, whereas nuclear data affiliate it with its conspecifics on Sumatra and the Malaysian Peninsula, suggesting ancient hybridization between ancestral *M. nemestrina* and the ancestor of the Sulawesi macaques [20,48].

The ancestor of the Sulawesi macaques invaded the island of Sulawesi ca. 2-3 Ma and diverged rapidly into 6 parapatric species (crested macaque [*M. nigra*], Gorontalo macaque [*M. nigrescens*], Hecks's macaque [*M. hecki*], Tonkean macaque [*M. tonkeana*], moor macaque [*M. maura*], and booted macaque [*M. ochreata*]) [9]. Because of this rapid diversification, phylogenetic relationships among these six species remain largely unresolved [20,48,50]. In almost all contact zones of two species, natural hybridization seems to occur relatively frequently [50].

In the second main clade, the *M. sinica* group diverged first ca. 3.5 Ma [20,30,35]. The species group comprises five species (the toque macaque [*M. sinica*] from Sri Lanka, the bonnet macaque [*M. radiata*] from southern India, the Arunachal macaque [*M. munzala*] from Arunachal Pradesh, India, the Tibetan macaque [*M. thibetana*] from China, and the Assamese macaque [*M. assamensis*], with a wide distribution from Nepal in the west to northern Vietnam in the east). The phylogenetic relationships among these species are also not well understood. However, the few available genetic data suggest that ancient hybridization occurred among several local populations, subspecies, and species [20,51]. Here, further research is needed to fully understand the evolutionary history of this species group.

The *M. arctoides* group includes only the stump-tailed macaque (*M. arctoides*), which has a wide distribution in southeast Asia. This species is most likely a hybrid between ancestors of the *M. sinica* group and the *M. mulatta* group (Figure 1.2). While mitochondrial data link *M. arctoides* with the *M. mulatta* group (split between both ca. 2.6 Ma; [20,30]), Y chromosomal data strongly support a grouping with the *M. sinica* group [20]. Interestingly, autosomal sequence data and retroposon integrations suggest an intermediate position between both parental lineages [35,52], and several morphological traits found in *M. arctoides* are shared with

VetBooks.ir

either the *M. sinica* group or the *M. mulatta* group [1,2,5,47,53]. Thus there is strong evidence that *M. arctoides* is indeed the outcome of bidirectional hybridization that resulted in a mosaic genome.

The *M. mulatta* group consists of three species: the Japanese macaque (*M. fuscata*) from Japan, the Taiwanese macaque (*M. cyclopis*) from Taiwan, and the rhesus macaque (*M. mulatta*). *M. mulatta* has the largest distribution of all macaques, ranging from Afghanistan in the west to eastern China (Figure 1.3). Although numerous subspecies have been described (for an overview see Refs. [33,42,55]), in the most recent classification this species is recognized as monotypic, mainly as a result of confusing intraspecific classification and missing data [9]. According to molecular data, *M. cyclopis* and *M. fuscata* derived from eastern populations of *M. mulatta* ([20]; Roos et al., unpublished data); the disruption of gene flow with the mainland *M. mulatta* populations ca. 1 Ma due to increasing sea levels finally led to allopatric speciation on Japan and Taiwan. In contrast, gene flow among western and eastern *M. mulatta* populations continued until ca. 160,000 years ago [25].

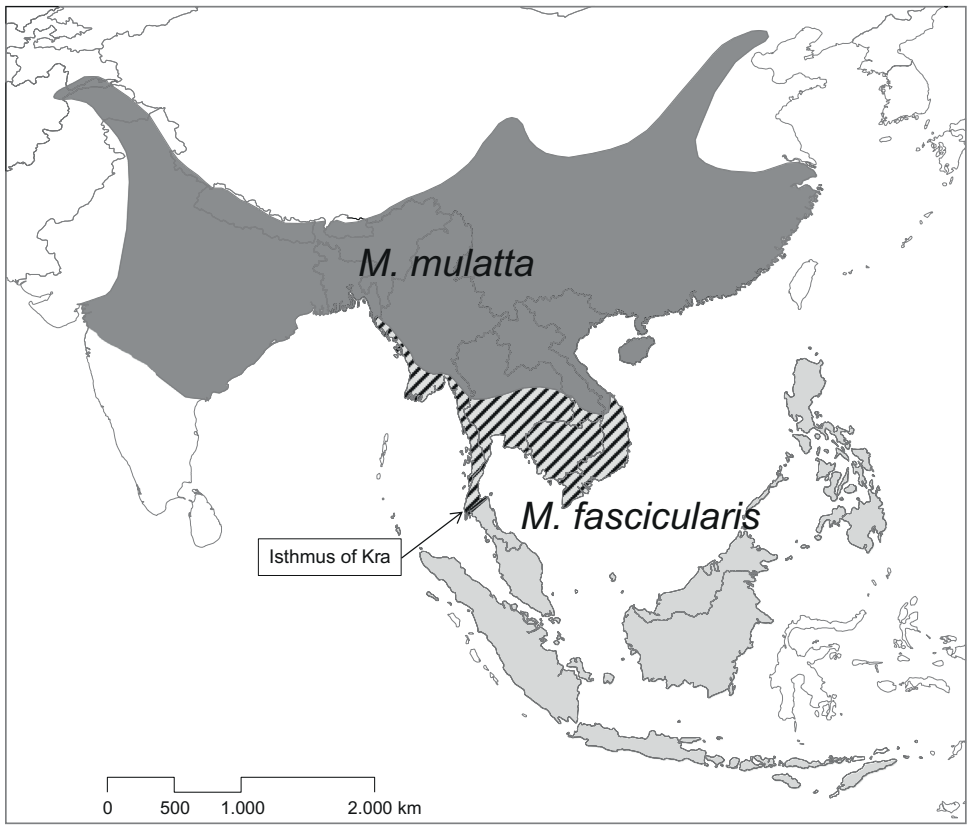


FIGURE 1.3 Map showing the distribution of rhesus macaques (*Macaca mulatta*) and long-tailed macaques (*M. fascicularis*). The hatched region indicates the putative extent of introgression from *M. mulatta* into *M. fascicularis* on the Asian mainland, south to the Isthmus of Kra. *Redrawn from Refs. [2] and [54].*

VetBooks.ir

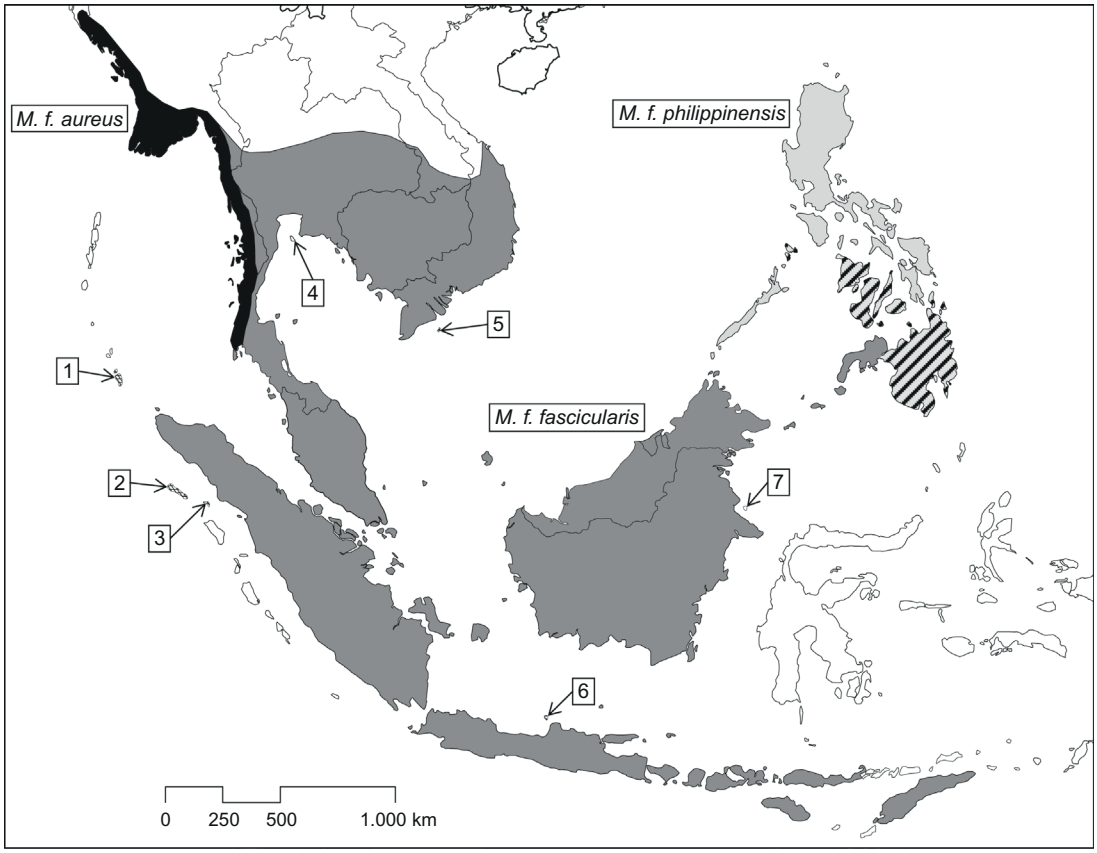


FIGURE 1.4 Map showing the distribution of the 10 currently recognized subspecies of long-tailed macaques (*Macaca fascicularis*). The subspecies *fascicularis*, *philippinensis*, and *aureus* have relatively large distributions and are named on the map. Subspecies on small islands are labeled with numbers: 1 = *M. f. umbrosa*, 2 = *M. f. lasiae*, 3 = *M. f. fusca*, 4 = *M. f. atriceps*, 5 = *M. f. condorensis*, 6 = *M. f. karimondjiwae*, 7 = *M. f. tua*. The hatched region indicates the integration zone between *M. f. fascicularis* and *M. f. philippinensis*. *Adapted from Ref. [56].*

The *M. fascicularis* group is monotypic and contains only the long-tailed, crab-eating or cynomolgus macaque (*M. fascicularis*) [9]. This species lives in the southern part of the southeast Asian mainland and also ranges over wide areas of Sundaland, extending to the Philippines and even to the island of Timor (Figures 1.3 and 1.4). Based mainly on differences in fur coloration, 10 subspecies have been recognized [1,2,8,9,33,56].

TAXONOMIC AND GENETIC DIVERSITY OF RHESUS AND LONG-TAILED MACAQUES

Rhesus and long-tailed macaques are the two most commonly used primate models in biomedical research. The scientific name of the rhesus macaques is *M. mulatta*. The species name *mulatta* derives from the Spanish or Portuguese *mulatto(a)*, meaning "of mixed breed."

VetBooks.ir

The species name of the long-tailed macaque is *fascicularis*, which in Latin means "a small band or stripe." Long-tailed macaques also are called cynomolgus or crab-eating macaques. Cynomolgus derives from the Greek *Kynamolgoi*, which literally means "dog milkers" and was used for members of an ancient African tribe. Why these macaques were named in this way remains obscure. In contrast, the reason these macaques are called long-tailed or crab-eating macaques is obvious: They carry the longest tails of all macaque species and they frequently search for food in and under water and also include crustaceans into their diet.

As mentioned above, both species have relatively large distributions (Figures 1.3 and 1.4) [8,33,49], and genetic variation within species and among local populations is high [20–31]. This, however, is not reflected by the current taxonomic classification. *M. mulatta* is regarded as monotypic [9], meaning that no subspecies are recognized. However, local populations can show extreme differences in morphology, physiology, reproduction, behavior, and genetics [22–25,28,57–61]. Most prominent in this respect are differences in immune response to the simian immunodeficiency virus seen in animals with Indian and Chinese origin [62]. Given the experiences with other widely distributed primate species (e.g., *Papio, Chlorocebus*, and *Semnopithecus*) [63–65], and taking into account the known differences among populations, it is highly likely that rhesus macaques comprise several subspecies or even species.

In contrast, 10 subspecies of *M. fascicularis* are recognized [1,2,8,9,33,56]. However, seven of them are found only on small islands and only three have relatively large distributions (*M. f. fascicularis, M. f. philippinensis, M. f. aureus*) (Figure 1.4) [1,2,8,9,33,56,59]. Actually, the subspecies with the largest distribution is *M. f. fascicularis*, which occurs through most of the species' range (from southern Vietnam, Cambodia, southern Thailand; south to the Malay Peninsula, Sumatra, Borneo, Java, and Bali; and east to Timor, as well as the southern islands of the Philippine Archipelago) (Figure 1.4). Thus, it is not surprising that (genetic) variation within *M. f. fascicularis* is high [26,31,54,66,67] and that the geographic origin of animals used in biomedical and experimental research matters. In fact, there is a deep genetic differentiation between *M. f. fascicularis* from the Asian mainland and the Sunda region, whereas on Sumatra both lineages are found ([54]; Roos et al., unpublished data). In addition to the genetic variation among local long-tailed macaque populations, genetic evidence suggests that *M. fascicularis* on the Asian mainland was introgressed by rhesus macaques [20,68–70]. Around 30% of their genome is of rhesus macaque origin [28]. This ancient hybridization (gene flow) occurred unidirectionally, from rhesus into long-tailed macaques, not vice versa [28,68–70]. Even today, hybridization between both species occurs in a wide hybrid zone running from Vietnam, through Laos and Thailand, and probably into Myanmar [58,71,72].

Because India implemented an export embargo in 1978, rhesus macaques for biomedical research purposes are today mainly imported from China and Vietnam. Given the wide distribution of rhesus macaques in China, the considerable genetic variation found in Chinese rhesus macaques is not surprising. Long-tailed macaques are mainly imported from Vietnam, Indonesia, the Philippines, and China and Mauritius (in the two latter countries the species does not occur naturally). Most of the founder animals in breeding centers in China and Vietnam likely originated from populations on the Asian mainland that are introgressed by rhesus macaques, whereas those from Indonesia, with exception of those from Sumatra, are most likely "pure" and autochthonous. However, because of the large distribution of the species within Indonesia, and in particular because of the isolation of certain island populations, genetic variation among animals is also significant [26,54,66]. Long-tailed macaques from the

Philippines are generally referred to as "Philippine long-tailed macaques," irrespective of the fact that, in the Philippines two subspecies occur (*M. f. philippinensis*, *M. f. fascicularis*). It is very likely that both subspecies are interbred by Philippine breeders, thus mixing up taxonomic and genetic diversity. In contrast, the long-tailed macaque population from Mauritius is comparatively well characterized and homogeneous [67,73–76]. This population derived from a few founding individuals (most likely from Sumatra) that were brought to the island in the 16th or 17th century [54,76].

CONCLUSION

Macaques form a highly diverse group of Old World monkeys, not only in the number of species but also concerning morphology, physiology, reproductive biology, ecology, behavior, and genetics. This diversity is also found within species, mainly in those with large distribution ranges and in particular in the two nonhuman primate species most commonly used in biomedical research: rhesus and long-tailed macaques. The taxonomic and genetic diversity of both species has not yet been fully explored and needs further investigation. So far, the known genetic variation in rhesus and long-tailed macaques is only marginally considered in biomedical experiments, although its influence on such experiments could be tremendous. Thus the biomedical research community, and breeders in particular, should pay more attention to the geographic origin of their test and breeding animals. Importantly, a macaque is not simply a macaque; likewise, a rhesus macaque is not simply a rhesus macaque, and a long-tailed macaque is not simply a long-tailed macaque.

Acknowledgements

The authors are grateful to the editors for giving us the opportunity to contribute to this book.

References

[1] Fooden J. Provisional classification and key to living species of macaques (Primates: *Macaca*). Folia Primatol 1976;25:225–36.
[2] Fooden J. Classification and distribution of living macaques (*Macaca* Lacepede, 1799). In: Lindburg DG, editor. The macaques: studies in ecology, behavior and evolution. New York: Van Nostrand Reinhold; 1980. p. 1–9.
[3] Abegg C, Thierry B. Macaque evolution and dispersal in insular south-east Asia. Biol J Linn Soc 2002;75:555–76.
[4] Thierry B. Unity in diversity: lessons from macaque societies. Evol Anthropol 2007;16:224–38.
[5] Delson E. Fossil macaques, phyletic relationships and scenario of deployment. In: Lindburg DG, editor. The macaques: studies in ecology, behavior and evolution. New York: Van Nostrand Reinhold; 1980. p. 10–29.
[6] Alba DM, Delson E, Carnevale G, Colombero S, Delfino M, Giuntelli P, et al. First joint record of *Mesopithecus* and cf. *Macaca* in the Miocene of Europe. J Hum Evol 2014;67:1–18.
[7] Thierry B. The macaques: a double-layered social organization. In: Campbell CJ, Fuentes A, MacKinnon KC, Bearder SK, Stumpf RM, editors. Primates in perspective. 2nd ed. New York: Oxford University Press; 2011. p. 229–41.
[8] Anandam MV, Bennett EL, Davenport TRB, Davies NJ, Detwiler KM, Engelhardt A, et al. Species accounts of Cercopithecidae. In: Mittermeier RA, Rylands AB, Wilson DE, editors. The handbook of the mammals of the world. vol. 3 primates. Barcelona: Lynx Edicions; 2013. p. 628–753.

VetBooks.ir

[9] Zinner D, Fickenscher G, Roos C. Family Cercopithecidae (Old World monkeys). In: Mittermeier RA, Rylands AB, Wilson DE, editors. The handbook of the mammals of the world. vol. 3 primates. Barcelona: Lynx Edicions; 2013. p. 550–627.
[10] Aykroyd OE, Zuckerman S. Factors in sexual-skin oedema. J Physiol 1938;94:13–25.
[11] Lindburg DG, Shideler S, Fitch H. Sexual behavior in relation to time of ovulation in the lion-tailed macaque. In: Heltne PG, editor. The lion-tailed macaque: status and conservation. New York: Alan R. Liss; 1985. p. 131–48.
[12] Fa JE, Lindburg DG. Evolution and ecology of macaque societies. Cambridge: Cambridge University Press; 1996.
[13] Higham J, Heistermann M, Saggau C, Agil M, Perwitasari-Farajallah D, Engelhardt A. Sexual signalling in female crested macaques and the evolution of primate fertility signals. BMC Evol Biol 2012;12:89.
[14] Melnick DJ, Pearl MC. Cercopithecines in multimale groups: genetic diversity and population structure. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT, editors. Primate societies. Chicago: Chicago University Press; 1987. p. 121–34.
[15] Zumpe D, Michael RP. Female dominance rank and behavior during artificial menstrual cycles in social groups of rhesus monkeys (*Macaca mulatta*). Am J Primatol 1989;17:287–304.
[16] Sterck EHM, Steenbeek R. Female dominance relationships and food competition in the sympatric Thomas langur and long-tailed macaque. Behaviour 1997;139:9–10.
[17] Gumert M, Jones-Engel L, Fuentes A. Monkeys on the edge: ecology and management of long-tailed macaques and their interface with humans. Cambridge: Cambridge University Press; 2011.
[18] Radhakrishna S, Huffman MA, Sinha A. The macaque connection: cooperation and conflict between humans and macaques. New York: Springer; 2013.
[19] IUCN (2013). "IUCN Red List of Threatened Species. Version 2013.2." <www.iucnredlist.org>. Downloaded on 24 March 2014.
[20] Tosi AJ, Morales JC, Melnick DJ. Paternal, maternal, and biparental molecular markers provide unique windows onto the evolutionary history of macaque monkeys. Evolution 2003;57:1419–35.
[21] Kathaswamy S, Smith DG. Effects of geographic origin on captive *Macaca mulatta* mitochondrial DNA variation. Comp Med 2004;54:193–201.
[22] Smith DG. Genetic characterization of Indian-origin and Chinese-origin rhesus macaques (*Macaca mulatta*). Comp Med 2005;55:227–30.
[23] Smith DG, McDonough J. Mitochondrial DNA variation in Chinese and Indian rhesus macaques (*Macaca mulatta*). Am J Primatol 2005;65:1–25.
[24] Ferguson B, Street SL, Wright H, Pearson C, Jia Y, Thompson SL, et al. Single nucleotide polymorphisms (SNPs) distinguish Indian-origin and Chinese-origin rhesus macaques (*Macaca mulatta*). BMC Genomics 2007;8:43.
[25] Hernandez RD, Hubisz MJ, Wheeler DA, Smith DG, Ferguson B, Rogers J, et al. Demographic histories and patterns of linkage disequilibrium in Chinese and Indian rhesus macaques. Science 2007;316:240–3.
[26] Smith DG, McDonough JW, George DA. Mitochondrial DNA variation within and among regional populations of longtail macaques (*Macaca fascicularis*) in relation to other species of the *fascicularis* group of macaques. Am J Primatol 2007;69:182–98.
[27] Ebeling M, Küng E, See A, Broger C, Steiner G, Berrera M, et al. Genome-based analysis of the nonhuman primate *Macaca fascicularis* as a model for drug safety assessment. Genome Res 2011;21:1746–56.
[28] Yan G, Zhang G, Fang X, Zhang Y, Li C, Ling F, et al. Genome sequencing and comparison of two nonhuman primate animal models, the cynomolgus and Chinese rhesus macaque. Nat Biotechnol 2011;29:1019–23.
[29] Satkoski Trask JA, Garnica WT, Smith DG, Houghton P, Lerche N, Kanthaswamy S. Single-nucleotide polymorphisms reveal patterns of allele sharing across the species boundary between rhesus (*Macaca mulatta*) and cynomolgus (*M. fascicularis*) macaques. Am J Primatol 2013;75:135–44.
[30] Liedigk R, Roos C, Brameier M, Zinner D. Mitogenomics of the Old World monkey tribe Papionini. BMC Evol Biol 2014;14:176.
[31] Liedigk R, Kolleck J, Böker KO, Meeijard E, Md-Zain BM, Abdul-Latiff MAB, et al. (subm.) Mitogenomic phylogeny of the common long-tailed macaque (*Macaca fascicularis fascicularis*).
[32] Finstermeier K, Zinner D, Brameier M, Meyer M, Kreuz E, Hofreiter M, et al. A mitogenomic phylogeny of living primates. PLoS One 2013;8:e69504.
[33] Groves CP. Primate taxonomy. Washington, DC: Smithsonian Institution Press; 2001.
[34] Fleagel JG. Primate adaptation and evolution. 3rd ed. New York: Academic Press; 2013.
[35] Perelman P, Johnson WE, Roos C, Seuánez HN, Horvath JE, Moreira MAM, et al. A molecular phylogeny of living primates. PLoS Genet 2011;7:e1001342.

[36] Springer MS, Meredith RW, Gatesy J, Emerling CA, Park J, Rabosky DL, et al. Macroevolutionary dynamics and historical biogeography of primate diversification inferred from a species supermatrix. PLoS One 2012;7:e49521.
[37] Pozzi L, Hodgson JA, Burrell AS, Sterner KN, Raaum RL, Disotell TR. Primate phylogenetic relationships and divergence dates inferred from complete mitochondrial genomes. Mol Phylogenet Evol 2014;75:165–83.
[38] Whitehead PF, Jolly CJ. Old world monkeys. Cambridge: Cambridge University Press; 2000.
[39] Boelsterli UA. Animal models of human disease in drug safety assessment. J Toxicol Sci 2003;28:109–21.
[40] Weinbauer GH, Vogel F. Future trends in primate toxicology and biotechnology. Münster: Waxman; 2011.
[41] Abee CR, Mansfield K, Tardif S, Morris T. Nonhuman primates in biomedical research: biology and management. 2nd ed. London: Academic Press; 2012.
[42] Napier JR, Napier PH. A handbook of living primates. London: Academic Press; 1967.
[43] Jasinska AJ, Schmitt CA, Service SK, Cantor RM, Dewar K, Jentsch JD, et al. Systems biology of the vervet monkey. ILAR J 2013;54:122–43.
[44] Rebellato LM, Verbanac KM, Haisch CE. The current status of xenotransplantation. Curr Surg 2005;62:481–8.
[45] Palermo RE, Tisoncik-Go J, Korth MJ, Katze MG. Old World monkeys and new age science: the evolution of nonhuman primate systems virology. ILAR J 2013;54:166–80.
[46] Zhou Y, Bao R, Haigwood NL, Persidsky Y, Ho WZ. SIV infection of rhesus macaques of Chinese origin: a suitable model for HIV infection in humans. Retrovirology 2013;10:89.
[47] Delson E. The oldest monkeys in Asia. In: International Symposium: evolution of Asian Primates. Inuyama: Freude and Kyoto University Primate Research Institute; 1996. p. 40.
[48] Ziegler T, Abegg C, Meijaard E, Perwitasari-Farajallah D, Walter L, Hodges JK, et al. Molecular phylogeny and evolutionary history of Southeast Asian macaques forming the *M. silenus* group. Mol Phylogenet Evol 2007;42:807–16.
[49] Rowe N. The pictorial guide to the living primates. New York: Pogonias Press; 1996.
[50] Evans BJ, Supriatna J, Andayani N, Melnick DJ. Diversification of Sulawesi macaque monkeys: decoupled evolution of mitochondrial and autosomal DNA. Evolution 2003;57:1931–46.
[51] Chakraborty D, Ramakrishnan U, Panor J, Mishra C, Sinha A. Phylogenetic relationships and morphometric affinities of the Arunachal macaque *Macaca munzala*, a newly described primate from Arunachal Pradesh, northeastern India. Mol Phylogenet Evol 2007;44:838–49.
[52] Li J, Han K, Xing J, Kim HS, Rogers J, Ryder OA, et al. Phylogeny of macaques (Cercopithecidae: *Macaca*) based on Alu elements. Gene 2009;448:242–9.
[53] Fooden J. Complementary specialization of male and female reproductive structures in the bear macaque, *Macaca arctoides*. Nature 1967;214:939–41.
[54] Tosi AJ, Coke CS. Comparative phylogenetics offer new insights into the biogeographic history of *Macaca fascicularis* and the origin of the Mauritian macaques. Mol Phylogenet Evol 2007;42:498–504.
[55] Fooden J. Systematic review of rhesus macaque, *Macaca mulatta* (Zimmermann, 1780). Fieldiana Zool 2000;96:1–180.
[56] Fooden J. Systematic review of southeast Asian longtail macaques, *Macaca fascicularis* (Raffles, 1821). Fieldiana Zool 1995;81:1–206.
[57] Champoux M, Higley JD, Suomi SJ. Behavioral and physiological characteristics of Indian and Chinese-Indian hybrid rhesus macaque infants. Dev Pyschobiol 1997;31:49–63.
[58] Hamada Y, Urasopon N, Hadi I, Malaivijitnond S. Body size and proportions and pelage color of free-ranging *Macaca mulatta* from a zone of hybridization in northeastern Thailand. Int J Primatol 2005;27:497–513.
[59] Fooden J. Comparative review of *fascicularis*-group species of macaques (Primates: *Macaca*). Fieldiana Zool 2006;107:1–43.
[60] Kubisch HM, Falkenstein KP, Deroche CB, Franke DE. Reproductive efficiency of captive Chinese- and Indian-origin rhesus macaque (*Macaca mulatta*) females. Am J Primatol 2012;74:174–84.
[61] Wu SJ, Luo J, Li QQ, Wang YQ, Murphy RW, Blair C, et al. Ecological genetics of Chinese rhesus macaque in response to mountain building: all things are not equal. PLoS One 2013;8:e55315.
[62] Trichel AM, Rajakumar PA, Murphey-Corb M. Species-specific variation in SIV disease progression between Chinese and Indian subspecies of rhesus macaque. J Med Primatol 2002;31:171–8.
[63] Zinner D, Groeneveld LF, Keller C, Roos C. Mitochondrial phylogeography of baboons (*Papio* spp.) - Indication for introgressive hybridization? BMC Evol Biol 2009;9:83.
[64] Haus T, Akom E, Agwanda B, Hofreiter M, Roos C, Zinner D. Mitochondrial diversity and distribution of African green monkeys (*Chlorocebus* Gray, 1870). Am J Primatol 2013;75:350–60.

VetBooks.ir

[65] Osterholz M, Walter L, Roos C. Phylogenetic position of the langur genera *Semnopithecus* and *Trachypithecus* among Asian colobines, and genus affiliations of their species groups. BMC Evol Biol 2008;8:58.
[66] Berry NJ, Marzetta F, Towers GJ, Rose NJ. Diversity of TRIM5α and TRIMCyp sequences in cynomolgus macaques from different geographical origins. Immunogenetics 2012;64:267–78.
[67] Kanthaswamy S, Ng J, Satkoski Trask J, George DA, Kou AJ, Hoffman LN, et al. The genetic composition of populations of cynomolgus macaques (*Macaca fascicularis*) used in biomedical research. J Med Primatol 2013;42:120–31.
[68] Tosi AJ, Morales JC, Melnick DJ. Y-chromosome and mitochondrial markers in *Macaca fascicularis* indicate introgression with Indochinese *M. mulatta* and a biogeographic barrier in the Isthmus of Kra. Int J Primatol 2002;23:161–78.
[69] Bonhomme M, Cuartero S, Blancher A, Crouau-Roy B. Assessing natural introgression in 2 biomedical model species, the rhesus macaque (*Macaca mulatta*) and the long-tailed macaque (*Macaca fascicularis*). J Hered 2009;100:158–69.
[70] Stevison LS, Kohn MH. Divergence population genetic analysis of hybridization between rhesus and cynomolgus macaques. Mol Ecol 2009;18:2457–75.
[71] Fooden J. Rhesus and crab-eating macaques: intergradation in Thailand. Science 1964;143:363–5.
[72] Fooden J. Tail length variation in *Macaca fascicularis* and *M. mulatta*. Primates 1997;38:221–31.
[73] Lawler SH, Sussman RW, Taylor LL. Mitochondrial DNA of the Mauritian macaques (*Macaca fascicularis*): an example of the founder effect. Am J Phys Anthropol 1995;96:133–41.
[74] Bonhomme M, Blancher A, Cuartero S, Chikhi L, Crouau-Roy B. Origin and number of founders in an introduced insular primate: estimation from nuclear genetic data. Mol Ecol 2008;17:1009–19.
[75] Kawamoto Y, Kawamoto S, Matsubayashi K, Nozawa K, Watanabe T, Stanley MA, et al. Genetic diversity of lontail macaques (*Macaca fascicularis*) on the island of Mauritius: an assessment of nuclear and mitochondrial DNA polymorphisms. J Med Primatol 2008;37:45–54.
[76] Satkoski Trask J, George D, Houghton P, Kanthaswamy S, Smith DG. Population and landscape genetics of an introduced species (*M. fascicularis*) on the island of Mauritius. PLoS One 2013;8:e53001.

VetBooks.ir

CHAPTER

2

Evolutionary History and Genetic Variation of *Macaca mulatta* and *Macaca fascicularis*

Betsy Ferguson[*], *David Glenn Smith*[†]

*Oregon Health & Sciences University and Oregon National Primate Research Center, Beaverton, OR, USA

†Department of Anthropology and California National Primate Research Center, University of California, Davis, CA, USA

OUTLINE

Origin and Dispersal of Cynomolgus Macaques 18

Origin and Dispersal of Rhesus Macaques 20

Genetic Diversity in Rhesus and Cynomolgus Macaques 22

Implications of Ancestral Divergence and Disease Susceptibility 23

NHP Genomic Studies 24

Genomic Sequence Analysis and Evolutionary Implications 25

Comparing Linkage Disequilibrium Between Taxa 25

Introgression During Admixture Between Two Taxa 26

Genomic Discovery of Candidate Risk Alleles 27

Functional Allele Comparison in Rhesus and Cynomolgus Macaques 28

G Protein-Coupled Receptor Gene Variation in Rhesus and Cynomolgus Macaques 28

http://dx.doi.org/10.1016/B978-0-12-417144-2.00002-0
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

Genetic Variation and Drug Safety Testing 29
Gene Expression Variation 29
Solute Carriers of Organic Anions Sequence Variation in Cynomolgus Macaques 29
The Macaque Cytochrome P450 Gene Family 30
Immune Response Variation as a Variable in Drug Safety Testing 32
Conclusion 32
References 33

Rhesus (*Macaca mulatta*) and cynomolgus (*M. fascicularis*) macaques are more frequently used as models for human disease in biomedical research than any other nonhuman primate (NHP) species [1]. They share common ancestry with humans from as early as 25 million years ago (mya) [2], and their genomes share approximately 93.5% identity with that of humans [3]. Both species, together with Japanese (*M. fuscata*) and Formosan macaques (*M. cyclopis*), comprise the fascicularis group of macaque species, a monophyletic clade [4,5] that has been diversifying into geographically isolated populations for longer than members of the human genus *Homo* [6]. The geographic distributions of rhesus and cynomolgus macaques exceed those of all other NHP species, encompass regional populations that are genetically subdivided by geographic distance and/or major geographic barriers such as high mountains, wide rivers, and ocean waters, and exhibit moderate levels of population structure.

ORIGIN AND DISPERSAL OF CYNOMOLGUS MACAQUES

Cynomolgus macaques, the least derived of the species of the fascicularis group of macaques, diverged from their nemestrina-like ancestor [7] in Indonesia [8]. As early as 3.5 mya, they had already diversified and dispersed throughout mainland and insular Southeast Asia as far south as Sulawesi [6]. Cynomolgus macaques dispersed eastward as far as Wallace's Line and then northward to the Philippines, probably by rafting [9], since the Philippines were never connected by land bridge to the Sunda shelf [10]. From Sabah, the earliest colonists of the Philippines crossed Palawan to Luzon, where they evolved the dark dorsal pelage characteristic of the subspecies *M. f. philippensis* to which Fooden [11] assigned them. Much later, cynomolgus macaques crossed the Sulu Archipelago to western Mindanoa, where their descendants today closely resemble cynomolgus macaques throughout Malaysia and Indonesia both phenotypically and genotypically. The settlement of the Philippines by rafting necessarily limited the numbers of original colonists, leading to a genetic bottleneck that was more severe in Luzon than in Mindanao, and the loss of genetic heterogeneity [8] due to the extinction of rare alleles.

From Sumatra, the western limit of their distribution in Indonesia and the species' probable homeland [8], cynomolgus macaques dispersed northward over a land bridge connecting Sumatra, Java, Borneo, and the Malay Peninsula [12] to the mainland of Indochina approximately 1 mya [13]. This is demonstrated by the ancestral state of their mitochondrial DNA (mtDNA) relative to that of all other cynomolgus macaques, as illustrated in Figure 2.1.

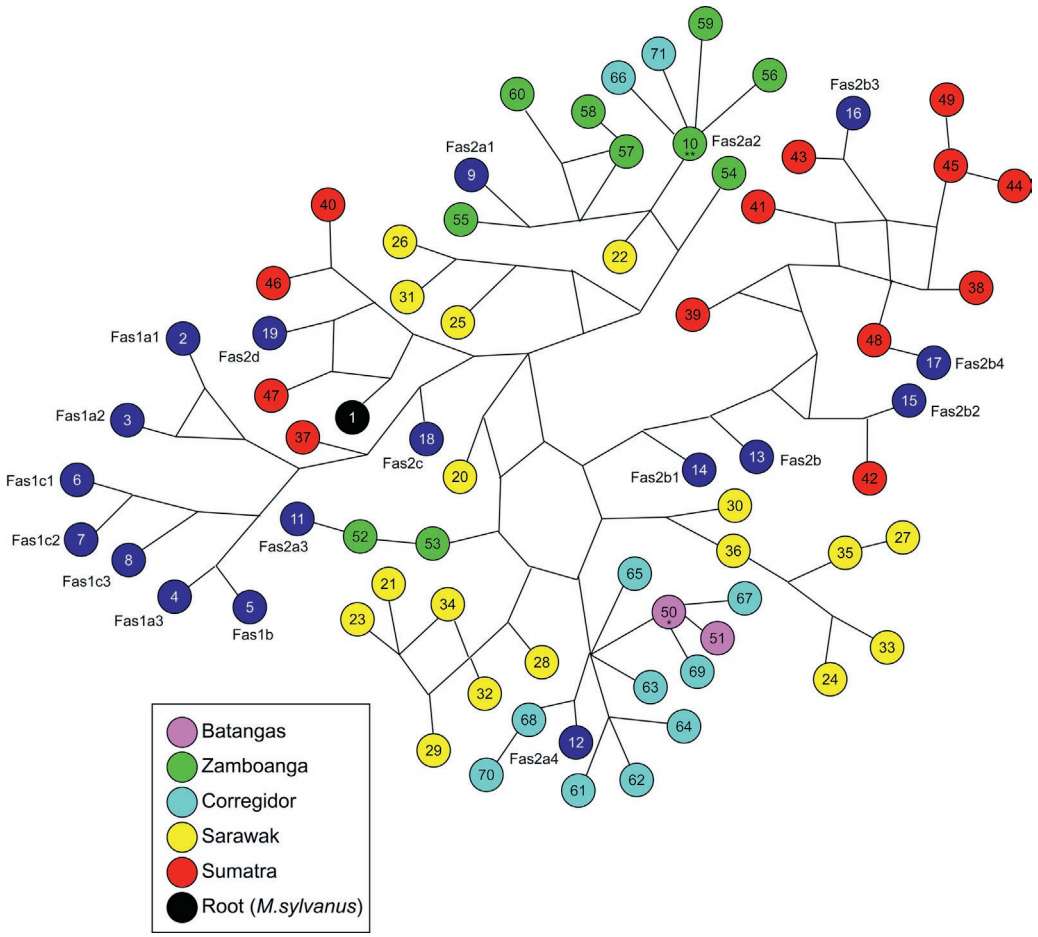


FIGURE 2.1 Median-joining haplotype network based on mitochondrial DNA sequences and rooted with a sequence from *M. sylvanus*. The reference sequences are identified by both their sample numbers and the haplogroup acronym of each reference sample. Note: The sequence of sample number 10 (**) from Zamboanga is identical to the haplogroup reference sequence Fas2a2, and it is shared with Corregidor. The sequence of sample number 50 (*) from Batangas is shared with Corregidor.

Once reaching the mainland, cynomolgus macaques spread throughout Indochina as far west as southern Bangladesh, eastward to the Yellow Sea and northward to the southern boundary of China. Their presence on Mauritius is attributed to their human dispersal by Portuguese sailors who acquired them in Indonesia, probably Sumatra [14], then released them when their sizes and temperaments precluded their value as pets [15,16]. The level of genetic diversity in Mauritian cynomolgus macaques is lower and reflects a more severe founder effect than that of Philippine cynomolgus macaques in Mindanao [17], but it is higher than that in Philippine cynomolgus macaques in the northern island of Luzon, where low genetic diversity rivals that of Japanese macaques [8,17–19]. Both Mauritian and Philippine cynomolgus macaques are equally the most divergent regional populations of cynomolgus macaques [8],

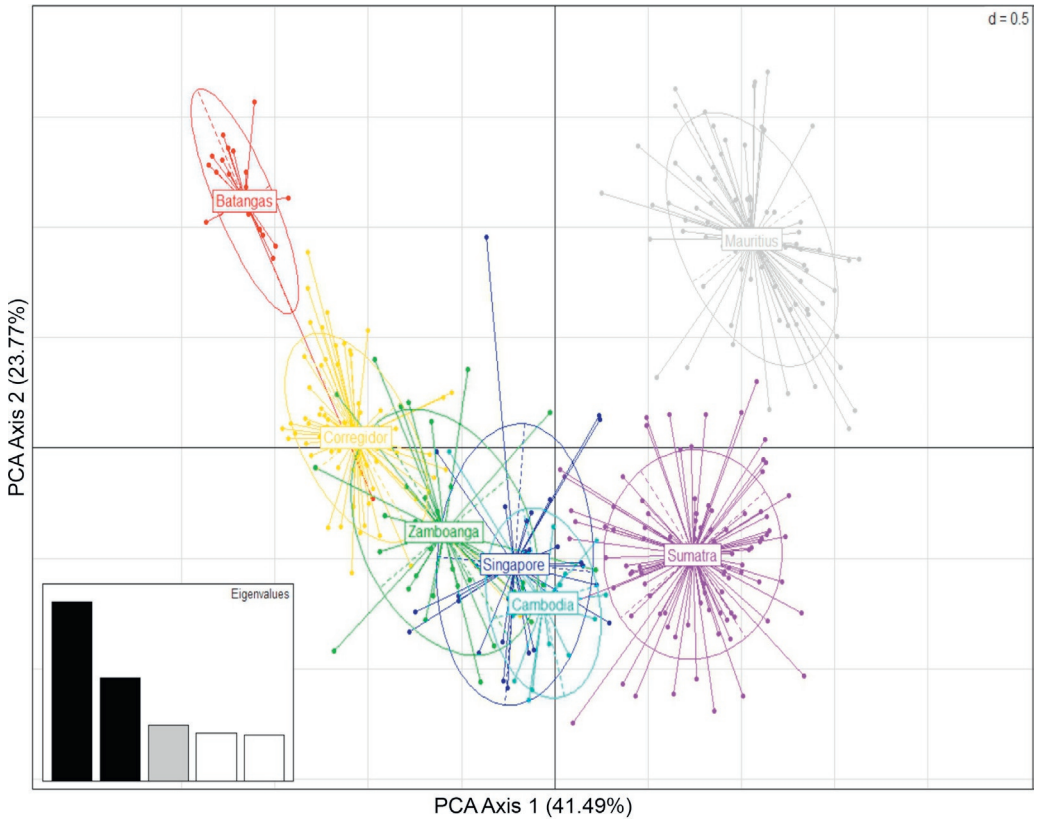


FIGURE 2.2 Principal components analysis (PCA) based on genotypes at 15 short tandem repeat loci in seven regional populations of cynomolgus macaques. Ellipses indicate the 95% confidence limits for each regional population and grid intervals are in units of 0.5. ChiE and ChiW (with subtypes ChiW1, ChiW2 and ChiW3), Ind I and Ind II and Bang I and Bang II refer to the two major mtDNA haplogroups of rhesus macaques in China, India and Bangladesh, respectively.

as illustrated by the principal component analyses (PCA) provided in Figure 2.2. Such divergence resulted more from a drastic decline in effective population size, thereby making the passage of evolutionary time especially evident for Mauritian cynomolgus macaques, the time depth of whose divergence from Indonesian cynomolgus macaques is only a few hundred years [15,16].

ORIGIN AND DISPERSAL OF RHESUS MACAQUES

Rhesus macaques probably diverged from cynomolgus macaque somewhere near a glacial refugium on the northeastern slopes of the Annamite Cordillero [9,20,21] of Vietnam or the Yungui Plateau of southwestern China [9,20–22] soon after the fascicularis-like ancestor of both species reached the mainland. This hypothesis is based on the fact that the

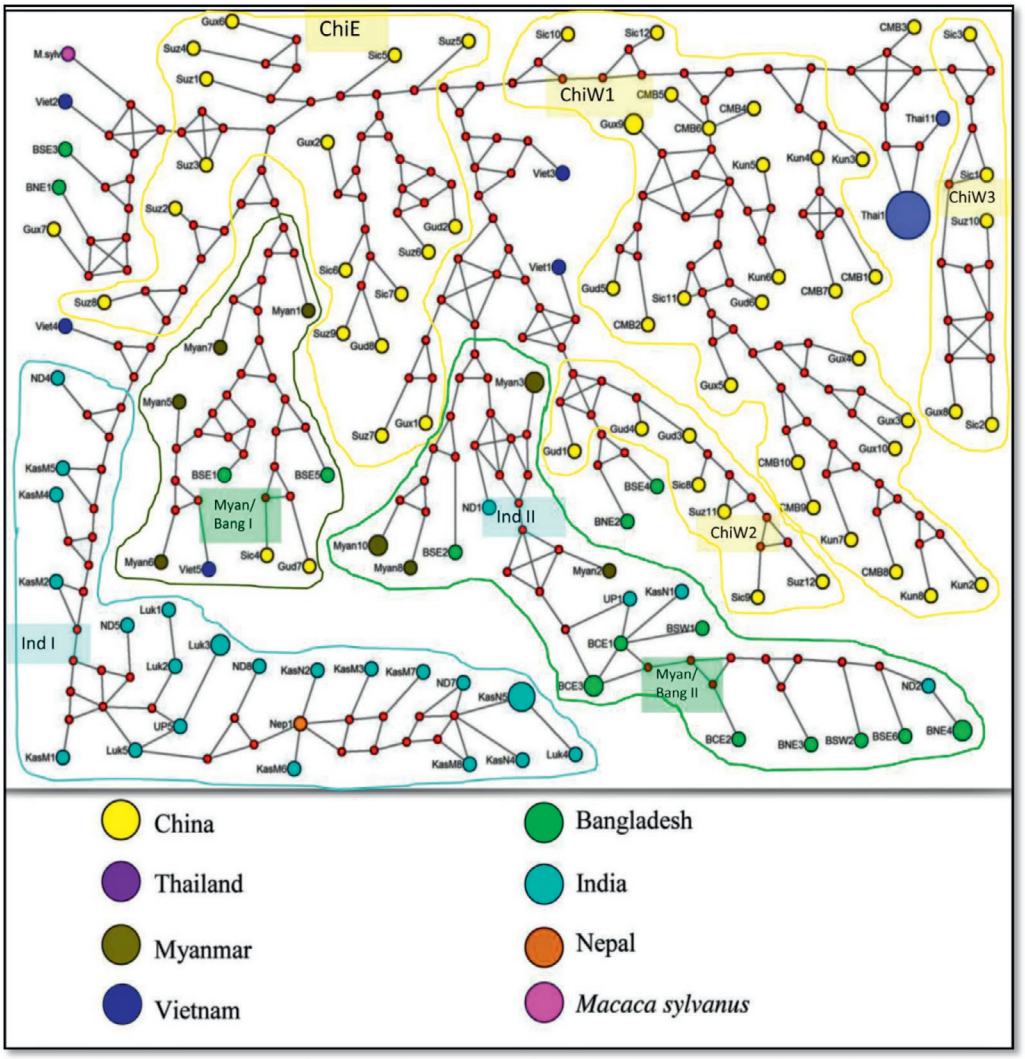


FIGURE 2.3 Median-joining haplotype network rooted with a Macaca sylvanus sequence showing Vietnam–South China rhesus macaque haplotypes as the root for other haplogroups. Name codes of sampling locations used in the network are CMB, China–Myanmar border region; Sic, Sichuan; Kun, Kunming; Gud, Guangdong; Gux, Guanxi; suz, Suzhou in China. Viet, Vietnam; Thi, Thailand; Myan, Myanmar; BSE, Bangladesh Southeast; BNE, Bangladesh Northeast; BCE, Bangladesh Central; BSW, Bangladesh Southwest; Nep, Nepal; ND, New Delhi; KasM, Kashmir Middle; KasN, Kashmir North; UP, Uttar Pradesh, and Luk, Lucknow.

oldest rhesus macaque mtDNA haplotypes reported are found in close proximity to these three hypothesized glacial refugia [20,22], as illustrated by the MJ haplotype network in Figure 2.3. Rhesus macaques then dispersed eastward through southern China along the coast of the Yellow Sea to the eastern plains of China and then northward to the Yellow River. Rhesus macaques established a range north of that of cynomolgus macaques on

VetBooks.ir

the mainland, and eventually they crossed the Formosa Strait to Formosa and the Korean Peninsula to the island of Kyushu, Japan, where they are widely regarded as the separate species *M. cyclopis* and *M. fuscata*, respectively. From Kyushu, Japanese macaques spread westward to Shikoku and northwestward through Honshu as far north as 41°30′ N latitude in Aomori Prefecture and to altitudes exceeding 2,000 meters where they survive −20°C winter temperatures and above 3,000 meters during summer [19]. Because Chinese rhesus macaques are more closely related to both Japanese and Formosan macaques than to Indian rhesus macaques, the latter two should be regarded as regionally derived populations of *M. mulatta* that diverged from Chinese rhesus macaques [18] somewhere between 0.1 and 1.0 mya. Rhesus macaques later re-expanded westward to Myanmar and Bangladesh, southward along the Mekong and Irrawaddy River Valleys through Indochina then westward along the Indo-Ganghetic Plain, beyond the Indus River Valley as far west as Afghanistan then southward to the Tapti River in the west and Krishna River in the east, where the southern boundary of their range meets the northern boundary of the range of bonnet macaques (*M. radiata*) [21].

In the northern parts of Indochina, the ranges of rhesus and cynomolgus macaques currently overlap in a relatively narrow zone of hybridization, but during glacial and interstadial periods of the Pleistocene this zone shifted southward and northward, respectively, resulting in significant levels of interspecies admixture in Indochina [23–27]. Ironically, the mtDNA haplotypes of rhesus macaques from Thailand, where interspecies admixture has been described. Almost the full range of levels of introgression of rhesus macaque genes into cynomolgus macaques in Indochina have been reported [26], suggesting that while some of this admixture occurred relatively recently, it is an ancient phenomenon perhaps dating to or following the last glacial maximum. It may even indicate that at least some of this admixture is ongoing. The westward dispersal of rhesus macaques was late, perhaps dating to the earliest phases of the penultimate interglacial around 160,000 years ago [28], and it reflects a serial founder effect [21] resembling that of Homo sapiens' sustained expansion eastward out of Africa/southwest Asia after 60,000-65,000 years ago [29]. Consequently, Indian rhesus macaques and Japanese macaques, at the western and eastern extremes of the species' geographic range, are highly derived and more genetically homogeneous that other regional populations within the range [18,19].

GENETIC DIVERSITY IN RHESUS AND CYNOMOLGUS MACAQUES

Not including the Japanese macaques' representation of the terminus of the eastward expansion of Chinese rhesus macaques, Indian and Chinese rhesus macaques of the geographic range of *M. mulatta*, at the western and eastern extremes, respectively, are the two most divergent regional populations of rhesus macaques [18]. Rooted mtDNA haplotype networks of regional populations of rhesus macaques, such as that shown in Figure 2.3, reflect little clustering in the eastern range of that species [18]. Presumably, this is due to extensive gene flow fostered by climate changes throughout the last half of the Pleistocene, with haplotypes of most regional populations being interspersed. In contrast, all Indian rhesus macaque haplotypes fall in a cluster furthest from the root, and haplotypes from

VetBooks.ir

Vietnam and southern China fall closest to the root [21] (see Figure 2.3). Somewhat ironically, the mtDNA haplotypes of rhesus macaques from Thailand form a very tight cluster and are highly derived. As might be predicted, the highest levels of genetic heterogeneity are found near the homeland of rhesus macaques in Vietnam and southwestern China, and the lowest levels at the periphery in India, Formosa, and Japan.

Most regional populations of cynomolgus macaques are at least as divergent from each other as those of the Indian and Chinese rhesus macaques [18]. This is largely attributable to the Wahlund effect precipitated by the dispersal of cynomolgus macaques to the mainland and throughout the Sunda shelf during glacial periods when land bridges connected insular and continental Southeast Asia followed by their isolation by water barriers during subsequent interglacials [9]. Rooted mtDNA haplotype networks place Indonesian (specifically, Sumatran) cynomolgus macaques closest to the roots of the networks, and population structure analyses based on short tandem repeat loci reveal three distinct clusters represented by insular, mainland, and Philippine cynomolgus macaques [8]. PCA of these same data, provided in Figure 2.2, exhibit clear evidence of a serial founder effect emanating from Indonesia with opposite termini on the islands of Mauritius and Luzon, Philippines. Cynomolgus macaques in the latter two regions both reflect strong divergence from neighboring populations, resulting from intense founder effects caused by human transplantation from Indonesia and rafting across the deep waters of the Mindoro Strait from Palawan to Mindoro Island off the southern coast of Luzon, respectively. The approximately equal level of divergence of cynomolgus macaque populations of Mauritius and Luzon from other cynomolgus macaque populations occurred recently and long ago, respectively, underscoring the confounding influences of the stochastic effects of drift, accelerated by a dramatic decline in effective population size associated with founder effects, on the genetic structure of populations. In contrast to the insular populations of cynomolgus macaques, mainland cynomolgus macaques exhibit little genetic substructure and instead a high level of genetic diversity augmented by introgression from rhesus macaques [30].

IMPLICATIONS OF ANCESTRAL DIVERGENCE AND DISEASE SUSCEPTIBILITY

Species and regional populations of the same species exhibit a broad range of susceptibility to pathogens, requiring the selection of the single model for biomedical research that best fits the human condition. As with human populations, regional populations of rhesus and/or cynomolgus macaques vary in their susceptibility to simian immunodeficiency virus (SIV), tuberculosis, malaria, cytomegalovirus, and other infectious diseases. Moreover, unknown shared kinship causes unwanted substructure among research subjects of biomedical research, and it can lead to misleading correlations in response to treatment and inflation of the genetic contributions among research subjects to the phenotypic variance. All subjects of any specific experiment in biomedical research should include unrelated members of the same regional population because inbred lineages of NHPs are impractical to develop as research resources. The exception is when research methodologies are specifically designed to exploit known kinship relationships. Unfortunately, the appreciation of this very important and now widely accepted principle of research design postdates an

VetBooks.ir

era during which research subjects were described simply as particular species of monkeys without regard to the subjects' regional ancestry and without concern for varying levels of kinship among research subjects. Consequently, the ancestry and levels of kinship of many captive bred representatives of both species is often mixed and/or unknown.

The Genetics and Genomics Working Group of the National Institutes of Health National Nonhuman Primate Research Consortium developed two panels of 96 single nucleotide polymorphisms (SNPs), one each for identifying/confirming the ancestral (i.e., country of) origin of rhesus macaques and for identifying the parentage of individual rhesus macaques [31,32]. The same resources are now available for cynomolgus macaques, and both can be accessed through the California or Oregon National Primate Research Centers or one of the other six National Primate Research Centers. Moreover, even without the known pedigree relationships available for most captive bred research subjects, levels of kinship among potential research subjects can be relatively accurately estimated using a paternity panel of 96 SNPs, such as in the instance cited above.

NHP GENOMIC STUDIES

Next-generation sequencing technologies have permitted large-scale sequencing of DNA samples and the discovery of millions of SNPs in both Indian [3,33] and Chinese [34] rhesus macaque genomes, and advanced bioinformatics tools have led to comparisons between the two genomes as well as between that of rhesus and cynomolgus macaques [35]. SNP maps constructed from these SNPs have allowed comparative genomic analyses for characterizing interpopulation and interspecies divergence of functional relevance. As expected from their close phylogenetic relationship, the two species exhibit extensive sharing of SNPs in their genomes [36]. Satkoski Trask et al. [37] constructed and sequenced reduced representation libraries from pooled samples of Indian rhesus macaques and created a SNP map comprising 4,040 common SNPs equally dispersed at approximately 0.7-Mb intervals across the rhesus genome. Kanthaswamy et al. [38] compared the human and Indian rhesus macaque genomes and identified approximately 85,000 SNPs homologous between humans and Indian rhesus macaques. These SNPs might mark genes or DNA elements that provided common or, alternatively, divergent adaptations of any two populations or species compared. Ross et al. [39] compared the genomes of Indian and Chinese rhesus macaques and discovered some genomic restructuring involving intrachromosome transpositions and inversions. Some of the highest instances of genomic divergence between the two species studied were associated with genes known to influence SIV or human immunodeficiency virus-1 and represent candidate genes for further study.

The reference genomes of more than a dozen primate species have been, or are in the final stages of being, sequenced. Complete reference genomes are now available for the chimpanzee, bonobo, gorilla, orangutan, rhesus macaque, and cynomolgus macaque, and those for the gibbon, sooty mangabey, vervet (African Green) monkey, baboon (*Papio anubis*), pigtail macaque, sifaka, marmoset, and mouse lemur are now in progress and will be completed soon [40]. The ever-increasing number of reference genomes of species of NHPs becoming available should increase the power of comparative studies to identify adaptive changes through the evolution of primate species responsible for observed phenotypic differences between/among species, particularly those that make humans distinct.

VetBooks.ir

GENOMIC SEQUENCE ANALYSIS AND EVOLUTIONARY IMPLICATIONS

The divergence between the genomes of the Indian rhesus macaque and the Indochinese cynomolgus macaque (0.40%) not only exceeds that between the Chinese and Indian rhesus macaque genomes (0.31%), as expected, but also that between the Chinese rhesus and Indochinese cynomolgus macaque genomes (0.34%) [35]. The highly variable but extensive introgression of rhesus macaque genes into cynomolgus macaques in Indochina, which had already been widely reported [23–27], is at least partially responsible for this closer resemblance of Indochinese cynomolgus macaques to Chinese than to Indian rhesus macaques. Although much greater heterogeneity characterizes the macaque genomes than those found in the human genome [3,33–35], probably due to the macaques' much larger effective population sizes, the plethora of orthologs in all three genomes of human druggable protein domains suggests their functional equivalence and hence utility as animal models for the development of drug therapies [33–35,37].

COMPARING LINKAGE DISEQUILIBRIUM BETWEEN TAXA

Trask et al. [41] genotyped samples of Indian and Chinese rhesus macaques for the 4,040 SNPs on their 0.7-Mb map of equidistant SNPs [37] and used VarLD scores [42] to identify SNPs exhibiting statistically significant between-subspecies differences in linkage disequilibrium (LD). They reasoned that VarLD scores across a chromosome should increase with declining distance from a genomic site influencing major phenotypic differences between the two subspecies, and they should then decrease with increasing distance from that site. Statistically significant VarLD scores were identified at three different locations on rhesus chromosome 4, distant from any annotated gene, and approximately 40 Mb beyond the proximal end of chromosome 5, where GNPDA2 is found, and in three clusters spanning 1.5- to 4-Mb sections of chromosome 11 containing three annotated genes (*CCDC65*, *CG32133-PA*, and *PTPRB*). In addition, an approximately 40-Mb tract (from about 40 to 80 Mb) at the proximal end of chromosome 1 that contained 10 annotated genes exhibited statistically significant VarLD scores. The statistical significance of the scores systematically increased from 0.05 at 40 Mb to 0.0001 at approximately 55 Mb, and then rapidly declining to 0.05 near 58 Mb, reaching nonsignificance beyond 80 Mb. Such distinctive patterns in the genome suggest the locations of selection pressure responsible for phenotypic differences between the taxa being compared, but they might also result from chromosome remodeling. A comparison of the sequence in Indian [3] and Chinese [35] rhesus macaque genomes did indeed reveal several inversions and translocations on chromosome 1 that differentiate the two subspecies but none in or near the 40-Mb tract that could explain extensive LD, suggesting the operation of selection within the region. Further study of the VarLD scores for a much larger number of SNPs in this region could more precisely delimit the region responsible for LD.

In a related study, Ross et al. [39] used the reciprocal smallest distance algorithm to identify amino acid orthologs between the Indian and Chinese rhesus macaque draft genome sequences. They reasoned that orthologs exhibiting the greatest divergence represent candidate genes for selection. The study revealed some genomic restructuring via intrachromosome

VetBooks.ir

transpositions and inversions, sometimes involving entire chromosome blocks. Some of the instances of the highest genomic divergence between the two species were associated with genes known to influence pathogenesis of SIV and, therefore, represent candidate genes for further study.

INTROGRESSION DURING ADMIXTURE BETWEEN TWO TAXA

In a later study, Trask et al. [26] used the same 4,040 rhesus SNPs cited to identify introgression of Chinese rhesus macaque genes into Indochinese cynomolgus macaques using the programs ADMIXTURE [43] and the INTROGRESS package for R software [44]. The admixture proportions of 2,808 of these SNPs that were present in selected samples of Chinese rhesus macaques or Indochinese cynomolgus macaques provided by the two programs were highly correlated ($R^2 = 0.97$) and ranged from 0% to 100% with an average of approximately 29%. Of the 2,808 SNPs, 208 exhibited statistically significant introgression from Chinese rhesus macaques to Indochinese cynomolgus macaques (but were absent in Indonesian cynomolgus macaques) compared with a neutral model of admixture between Chinese rhesus macaques and Indochinese cynomolgus macaques. These 208 SNPs represent candidates for markers for genes acquired by cynomolgus macaques from rhesus macaques that improve fitness and might identify genomics regions of adaptive changes such as those providing increased resistance to local pathogens.

As expected from a population near the end of a serial founder effect, the structure of the genome of Indian rhesus macaque has undergone remodeling through inversion and translocation [39], and the genotypes of Indian-origin rhesus macaques exhibit marked LD throughout their genome, in contrast to those of Chinese rhesus macaques [45]. The higher LD in the Indian rhesus macaque genome probably resulted from a relatively large genetic bottleneck [27] and/or the relatively recent admixture between two very different progenitors that colonized the Indian subcontinent from the east [18,21]. In contrast, the genotypes of Chinese rhesus macaques exhibit approximate linkage equilibrium because of their greater antiquity and demographic stability. One consequence of this difference is that more loci would be required to map genomic locations of genes influencing phenotypes in whole genome association studies of Chinese rather than of Indian rhesus macaques [27].

The genomes of Malaysian [46] and Mauritian [47] cynomolgus macaques have also been sequenced. A total of 2.1 million SNPs and 17,387 orthologs of human genes were identified in the Mauritian cynomolgus macaque genome [46]. By comparing the genomes of Indian and Chinese rhesus macaques with that of the Indochinese (Vietnamese) cynomolgus macaques, Yan et al. [35] identified more than 20 million SNPs, 0.74 million indels, and 217 genomic regions with strong signals of selective sweeps. Surprisingly, the Chinese rhesus macaque genome was almost as closely related to that of Indochinese cynomolgus macaques as to that of Indian rhesus macaques. They attribute this to introgressive hybridization between male Chinese rhesus macaques and female Indochinese cynomolgus macaques and estimate that, on average, approximately 30% of the Indochinese cynomolgus macaque genome is of Chinese rhesus macaque origin.

VetBooks.ir

This estimate is remarkably similar to the estimate by Trask et al. [26] (previously discussed in this chapter), based on their analysis of 2,808 SNPs shared among Chinese rhesus macaques and Indochinese (Cambodian) and Indonesian (Sumatran) cynomolgus macaques. In addition, the 217 genomic regions identified by Yan et al. that exhibit an excessive genetic homogeneity suggestive of a selective sweep, are very close to the number of SNPs (i.e., 208) identified by Trask et al. that exhibit statistically significantly greater levels of introgression of rhesus genes in Indochinese cynomolgus macaques than expected from a neutral model of admixture. Whether the two studies independently identified some of the same genomic regions is a subject for further study, but such regions would be optimal targets for future study. The types of comparative genomic studies discussed have the potential to identify the genetic changes that allowed differential adaptations of NHP species and regional populations that are responsible for the extraordinary level of phenotypic diversity, such as in susceptibility to diseases shared in common with humans, that they now reflect.

GENOMIC DISCOVERY OF CANDIDATE RISK ALLELES

Genome variant discovery studies have not only informed the evolutionary history of macaques but also provided insight into the range of potential functional alleles present within macaque populations. Studies focused on genome-wide characterization of polymorphisms have indicated that the majority of DNA variants fall within intergenic regions [33–35,37,47,48]; however, polymorphisms located within genes or gene promoters are more often implicated in disease risk. Therefore, Yuan et al. [48] focused their variant discovery efforts on gene-linked regions using messenger RNA and H3K4me3-associated DNA derived from the rhesus macaque hippocampus to enrich genic regions expressed in the brain. This approach enabled the detection of nearly 463,000 SNPs in 14 rhesus macaques (11 of Indian origin and 3 Chinese or Chinese–Indian hybrids). By comparing the rhesus macaque variant set to those identified in an equivalent human study, the rhesus macaque was predicted to have nearly three times the SNP density (2.82 SNPs/Kb) as humans (1.07 SNP/Kb). However, when comparing predicted functional effects of the rhesus macaque and human coding SNPs, rhesus have a number of damaging missense and nonsense SNPs (1,571) roughly equivalent to those of humans (1,759) [48].

Genomic sequencing has also been leveraged to identify macaque variants with predicted functional effects that directly implicate disease risk. In a sequencing study of three Indian-origin rhesus macaques, roughly three million SNPs were identified in multiple individuals or were identified using two different sequencing methods [33]. Of these, 4,472 SNPs were predicted to be nonsynonymous, with more than 700 SNPs classified as “possibly” or “probably” damaging based on PolyPhen2 analysis [49]. The potentially functional variants fell within a variety of genes, including those associated with cancer, *BRCA2*, *MSH2*, *FANCM*. Others were located in genes linked to diabetes, asthma, and anemia [33]. This initial study suggests that genomic sequencing can identify novel variants that may contribute risk for a broad range of common diseases among macaques.

VetBooks.ir

FUNCTIONAL ALLELE COMPARISON IN RHESUS AND CYNOMOLGUS MACAQUES

Another genome-wide study compared variants identified in a Chinese rhesus macaque and a cynomolgus macaque of Vietnamese origin, relative to those suggested by the initial genome-wide sequencing of the Indian rhesus macaque genome [34,35]. As described previously in this chapter, the sequence comparison of the rhesus and cynomolgus macaque genomes support a model of asymmetrical gene flow from the Chinese rhesus to the cynomolgus macaque, occurring over an extended period of time. Nonetheless, some predicted functional variants were identified within both Chinese and Indian rhesus macaques that were not evident in cynomolgus macaque, such as a missense variant (R40H) in the ornithine transcarbamylase (*OTC*) gene. A total of 170 variants predicted to create premature stop codons were identified, although a larger sampling of subjects is needed to determine the population prevalence of these variants. Of the novel nonsense variants predicted in this study, 19 fell within genes with a druggable protein domain, including the parathyroid hormone 1 receptor (*PTH1R*), the target of the antiosteoporosis drug teriparatide.

G PROTEIN-COUPLED RECEPTOR GENE VARIATION IN RHESUS AND CYNOMOLGUS MACAQUES

G protein-coupled receptors (GPRCs) are a superfamily of proteins that play central roles in the regulation of the innate immune system, visual and gustatory sensing, and neuronal, hormonal, and cell-cell signaling. Accordingly, the GPRCs are implicated in a broad range of commonly treated afflictions, including cardiovascular disease, depression, and anxiety [50]. To specifically probe the naturally occurring variation present in macaque GPRCs, Goswami et al. [51] used exon-capture technology to sequence GPRC exons in a total of 96 cynomolgus macaques, Indian rhesus macaques, and Chinese rhesus macaques. They identified more than 14,000 nonsynonymous SNPs, of which roughly 1,750 were detected in more than one animal. Nine of the GPCR variants, located in nine different genes, were coincident in both humans and macaques. Based on PolyPhen2 analysis [49], six of these variants also were predicted to be likely deleterious or deleterious to the GPCR proteins FZD6, GPR19, GPR78, GPR98, GPR146, and MRGPRX3. With the exception of the *GPR19* variant, which was detected in both Chinese rhesus and cynomolgus macaques, the other five damaging variants were detected only in a single population (Chinese rhesus, Indian rhesus, or cynomolgus macaques) [51]. The population specificity of these variants may vary as larger numbers of individuals are evaluated. Nonetheless, these human-identical variants provide an outstanding opportunity to characterize their contribution to disease as well as to evaluate their potential effect on therapeutic drug efficacy.

Truncating variants within GPCRs resulting from either frameshift or stop-gain mutations were also detected in both rhesus and cynomolgus macaques. The most common such variants occurred in nine genes, including *CELSR1*, *CELSR2*, *LPHN2*, *CIPR2*, *MRGPRX3*, *HTR5A*, *TAS1RA*, *GHRHR*, and *GRM5*. The population frequencies of these truncating variants range from 1.8% to 35%, with some variants detected only within a single species (rhesus or cynomolgus macaques) and others only within a single population (e.g., present in Chinese

VetBooks.ir

rhesus but not Indian rhesus macaques). The species-specific variants had relatively high minor allele frequencies (MAFs), ranging up to 30% among the single species in which it was identified. The rhesus-specific mutations included some that were prevalent among both Chinese and Indian rhesus (*CELSR1*, 10:90409612, at 30% and 23.3% MAF, respectively) and others that seemed to be exclusive to one population (*CELSR1*, 10:904011842, with 14.3% MAF within Chinese rhesus macaques only) [51].

GENETIC VARIATION AND DRUG SAFETY TESTING

Because of the similar anatomy and physiology as well as the high homology of the molecular transport and metabolizing enzymes to that of humans, macaques have emerged as the gold standard for drug safety testing in preclinical trials [52,53]. Consequently, one important focus in the genetic study of macaques has been to establish the range of variation within proteins involved in the metabolism and clearance of foreign molecules. Such variation can lead to broad-ranging effects that can compromise drug safety and efficacy in both humans and NHPs.

GENE EXPRESSION VARIATION

The liver serves as the most important organ for drug metabolism and the clearance of toxins. Thus there is keen interest in clarifying the range of liver gene expression within macaques. In one study, substantial variation in liver gene expression was identified among 36 cynomolgus macaques of different geographic origin, including those from Mauritius, the Philippines, and Vietnam [47]. Ebeling et al. [47] identified 6.5% (n = 718) of the liver genes tested as having highly variable gene expression. Amongst them were genes that were highly expressed, such as the metallothionein 1B (*MT1B*) and uridine phosphorylase 2 (*UPP2*) genes. Considerable messenger RNA variability (up to 1000-fold) was detected among cytokines, including *IL1*β, *IFN*γ, and *CCL2* genes at baseline time points, suggesting that some animals may be poised for a heightened immune response to therapies tested in preclinical trials. PCA of the gene expression data clearly distinguished Philippine-origin from Chinese- and Mauritius-origin cynomolgus animals [47]. These findings underscore the potential contribution of variable gene expression to disparate toxicity measures among macaque populations.

SOLUTE CARRIERS OF ORGANIC ANIONS SEQUENCE VARIATION IN CYNOMOLGUS MACAQUES

The solute carrier (SLC) protein family consists of 55 gene families with at least 362 protein-coding genes that mediate transport of a wide spectrum of molecules across cells [54]. The solute carriers of organic anions (SLCOs) are of particular importance to drug safety research because they contribute to the transport of organic molecules into the liver for detoxification by cytochrome p450 and other metabolizing enzymes. In comparison with human SLCO protein sequences, the cynomolgus macaque proteins were predicted to have diverged

VetBooks.ir

between 0.75% (SLCO1C1) and 9% (SLCO6A1) from human proteins [47]. This important finding raises the potential of altered substrate specificity or transport efficiency in macaques. Indeed, an example is found in the study of SCLO1B3, which includes two amino acid substitutions (S112A and M233I) that also occur in both the macaque and human genes [47]. In humans, the presence of both variant alleles together abolished substrate binding [55]. Additional study is needed to further identify the extent of macaque SLCO sequence variants on molecule transport efficiency and their contribution to variability in drug clearance studies.

THE MACAQUE CYTOCHROME P450 GENE FAMILY

The cytochrome P450 protein (CYP450) family of proteins encompasses a large number of enzymes that metabolize and detoxify xenobiotic compounds. Among the 57 functional human CYP450 proteins, 9 isoforms in class 1-3 (CYP1A1, CPY1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5/7) are responsible for the metabolism of 75% of all clinical drugs [56,57]. The highly polymorphic nature of some human *CYP450* genes, notably *CYP2B6*, *CYP2C9*, *CYP2C19*, and *CYP2D6*, produce a wide range of enzymatic activities, resulting in individual variation that ranges from poor to intermediate to ultrarapid metabolism [58]. This high variability in enzymatic activity underlies the challenge in establishing safe drug treatment levels. Low CYP450 protein activity can result in accumulation of high levels of a drug, whereas rapid metabolism from a parent compound to an active metabolite can similarly lead to drug toxicity. Thus, identifying the variability in CYP450 protein activities and understanding their implications for drug safety and efficacy testing has been an additional focus.

There have been several recent reports focusing on the characterization of rhesus and cynomolgus macaque *CYP450* genes. A study comparing the genetic variation within a sampling of *CYP450* genes relative to non-*CYP450* (control) genes in rhesus and cynomolgus macaques identified significantly higher population divergence in the *CYP450* gene family (Table 2.1). The *CYP2D6* gene showed the highest interspecies divergence among all of the

TABLE 2.1 Summary of Macaque CYP450 Findings

Gene	Reference
Novel macaque *CYP450* genes	
CYP2C76	59
CYP2C93	60
Lineage-specific variants	
CYP3A5	61
CYP1A2	62
Lineage-specific expression	
CYP2B6	63
CYP3A4	63

VetBooks.ir

loci sequenced [61]. More extensive characterization of one of the major contributors to drug metabolism, *CYP3A5*, among a collection of 222 rhesus (Chinese) and cynomolgus macaques (Indochinese and Indonesian) revealed that the *CYP3A5* region spanning exons 3 to 6 had a high level of genetic differentiation between species but a moderate level of variation within species. A fixed variant that distinguished rhesus from cynomolgus macaques was detected in the first intron; however, the functional consequences of that variant, if any, are unknown. Other *CYP3A5* variants included one nonsense and nine nonsynoymous variants, including two predicted to generate amino acid substitutions (D88E and T230I) within the protein domain that confers substrate binding specificity [61].

A separate study focused on the *CYP1A* gene family, also prominent among drug-metabolizing proteins in humans. The *CYP1A2* gene is expressed exclusively in the liver, whereas *CYP1A1* is in a broad range of tissues, including the liver. In macaques, *CYP1A2* was found to be the more heterogeneous gene, with 5 different null alleles identified among 91 rhesus and cynomolgus macaques. While one null allele was shared between both macaque species, another null allele was detected only in rhesus macaques, and three were detected exclusively in cynomolgus macaques [62]. These studies provide a glimpse into the complexity of *CYP450* gene variation both within and between species and suggest that significantly more work needs to be done to elucidate the full range of genetic heterogeneity in this important gene family.

Also reported are *CYP450* genes that are unique to macaque lineages, with no counterpart in the human genome. Among them, *CYP2C76* was identified in both rhesus and cynomolgus macaques, with some polymorphic variants also shared across species. Of significance, 7 of the 18 nonsynonymous variants identified in *CYP2C76* were predicted to alter substrate recognition sites, and 3 of the variants (L65F, M310L, and N364S) were found to have lower metabolic activity compared with the wild-type protein [59]. While all of the *CYP2C76* nonsynoymous variants detected in Indonesian cynomolgus macaques were also present in Indochinese-origin animals, the converse was not true. Another macaque gene lacking a human ortholog is *CYP2C93*. Functional assays of the CYP2C93 protein product demonstrated functional metabolizing activity of CYP2C substrates. Interestingly, a transcript that produces a functional CYP2C93 protein was detected in rhesus macaques, but it has not yet been identified in cynomolgus macaques, raising the possibility that this isoform may be unique to rhesus macaques [60].

Multiple investigations have also pointed toward variable *CYP450* gene expression or protein levels among macaques. A recent study used microarray analysis to evaluate the expression levels of 14 *CYP450* genes in the livers of rhesus macaques and of cynomolgus macaques derived from Cambodian, Indonesian, and Chinese breeding facilities. In this study, the expression levels did not vary more than 2.5-fold among cynomolgus macaques; however, the levels of *CYP2B6* and *CYP3A4* significantly differed between cynomolgus and rhesus macaques [63]. In a separate study of 31 *CYP450* genes, there was generally low interanimal expression variation; however, six cynomolgus cytochromes (*CYP1B1*, *CYP4X1*, *CYP2D6*, *CYP1A2*, *CYP7A1*, and *CYP2C18*) measured significant variation of at least 100-fold. In addition, the data identified differences in basal expression levels between humans and cynomolgus macaques in nine of the *CYP450* genes [47]. In a complementary approach, immunoblot analysis was used to measure the relative levels of 19 different CYP450 proteins among 27 cynomolgus macaques derived from Indochina and Indonesia. Interestingly,

VetBooks.ir

while many of the proteins varied by 3- to 4-fold between individuals, the CYP2C9/19 proteins, two of the central human drug-metabolizing proteins, varied by 11-fold among the 27 animals studied [64].

Examined together, the macaque CYP450 studies provide insight into the breadth of common genetic variation that may produce a range of protein activities within and between macaque populations. If the challenge to evaluate and to account for this variation is heeded, use of macaques in drug safety and toxicology studies could prove to be extremely valuable, providing important insights into analogous pharmacogenomic effects in human populations. Given the severe impact and cost of adverse drug reactions in human medicine [65,58], a genetic focus on optimizing use of the macaque for drug safety testing seems to be a high priority for future directions.

IMMUNE RESPONSE VARIATION AS A VARIABLE IN DRUG SAFETY TESTING

Variable immune sensitivities among individuals can also contribute to disparate drug tolerance measures in macaques. In extreme cases, super cytokine response can lead to organ failure or fatality [66]. On the other hand, a blunted immune response can mask potential harm that could occur in other individuals. Thus investigations into the range of genetic variation among cytokines, chemokines, and associated regulatory factors have also been an area of emerging interest, with an aim toward understanding the potential contributors to differential immune responses. In one study, polymorphic variants in 49 immune system genes were compared among 40 cynomolgus macaques, 20 of Mauritius origin, and 20 from a Chinese breeding center. Of the 561 SNPs identified, 29 were detected within only one of the two cynomolgus populations., Further studies are needed to confirm the population specificity because most of the population-specific variants detected were rare, with an MAF of <0.06 in the single population. Confirming a common variant (MAF = 0.29) that was detected exclusively among the Chinese-bred animals and predicted to be "probably damaging" to complement component receptor 1 (*CR1*) function will be of particular interest [67]. Potential pathogenic variants that were identified in both population groups included three predicted to produce premature stop codons in (*CR1*, *CTLA-4*, *IFNγR2*). Thus immune-response variation, based on differing gene expression and protein activities, also seems to be an important source of individual and population variation that needs further clarification.

CONCLUSION

This wealth of macaque gene variation underscores an important difference between NHPs and inbred animal models, particularly as it applies to drug development and testing studies. Indeed, the macaque genome variation has the potential to provide substantial advantages for use in preclinical studies because it more closely recapitulates the diversity and complexity of the human genome. The challenge ahead is to more fully characterize the natural variation within and between macaque species and to leverage the information to genetically

define macaque models that closely parallel human disease. The advancement of such defined models will enable the development and testing of pharmacogenetically designed drug therapies. Similarly, if the genetic variables that alter drug clearance rates and inflammatory response are defined, the macaque model could provide an entry into developing genetically based dosage studies that ultimately improves both drug safety and treatment outcomes.

References

[1] Research Resources Information Center. Demands for rhesus macaques in biomedical research: a workshop report. ILAR J 2003;44:222–38.
[2] Kumar S, Hedges SB. A molecular timescale for vertebrate evolution. Nature 1998;392:917–20.
[3] Rhesus Macaque Genome Sequencing and Analysis Consortium, Gibbs RA, Rogers J, Katze MG, Bumgarner R, Weinstock GM, et al. Evolutionary and biomedical insights from the rhesus macaque genome. Science 2007;316:222–34.
[4] Delson E. Fossil macaques, phyletic relationships and a scenario of deployment. New York: Van Nostrand-Reinhold; 1980.
[5] Fooden J. Comparative review of fascicularis-group species of macaques (primates: Macaca). Fieldiana Zool 2006;107:1–43.
[6] Perelman P, Johnson WE, Roos C, Seuznez HN, Horvath JE, Moreira MAM, et al. A molecular phylogeny of living primates. PLoS Genet 2011;7:e1001342.
[7] Li J, Han K, Xing J, Kim H-S, Rogers J, Ryder O, et al. Phylogeny of the macaques (Cercopithecidae: *Macaca*) based on Alu elements. Gene 2009;448:242–9.
[8] Smith DG, Ng J, George DA, Satkoski Trask JA, Houghton P, Singh B, et al. A Genetic Comparison of Two Alleged Subspecies of Philippine Cynomolgus Macaques. Am J Phys Anthropol 2014;155(1):136–48.
[9] Abegg C, Thierry B. *Macaca* evolution and dispersal in insular south-east Asia. Biol J Linn Soc 2002;75:555–76.
[10] Sathiamurth E, Voris HK. Pleistocene sea level maps for the Sunda Shelf. Chicago, IL: The Field Museum; 2006.
[11] Fooden J. Systematic review of Philippine Macaques (Primates, Cercopithecidae: *Macaca fascicularis* subspp.). Field Museum of Natural History, Field Zool 1991;64:1–44.
[12] Voris HK. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J Biogeogr 2000;27:1153–67.
[13] Osada N, Hashimoto K, Kameoka Y, Hirata M, Tanuma R, Uno Y, et al. Large-scale analysis of Macaca fascicularis transcripts and inference of genetic divergence between *M. fascicularis* and *M. mulatta*. BMC Genom 2008;9:90.
[14] Tosi AJ, Coke CS. Comparative phylogenetics offer new insights into the biogeographic history of Macaca fascicularis and the origin of the Mauritian macaques. Mol Phylog Evol 2007;42:498–504.
[15] Sussman R, Tattersal I. Behavior and ecology of *Macaca fascicularis* in Mauritius: a preliminary study. Primates 1981;22:192–205.
[16] Sussman RW, Tattersal I. Distribution, abundance, and putative ecological strategy of *Macaca fascicularis* on the island of Mauritius, southwestern Indian Ocean. Folia Primatol 1986;46:28–43.
[17] Lawler SH, Sussman RW, Taylor LL. Mitochondrial DNA of the Mauritian macaques (*Macaca fascicularis*): an example of the founder effect. Am J Phys Anthropol 1995;96:133–41.
[18] Smith DG, McDonough JW, George DA. Mitochondrial DNA variation within and among regional populations of longtail macaques (*Macaca fascicularis*) in relation to other species of the fascicularis group of macaques. Am J Primatol 2007;69:182–98.
[19] Kawamoto Y. Modes of differentiation in Japanese macaques: perspective from population genetics. In: Nakagawa N, Nakamichi M, Sugiura H, editors. The Japanese macaque. Tokyo: Springer; 2010. p. 53–78.
[20] Eudey AA. Pleistocene glacial phenomena and the evolution of Asian macaques. In: Lindburg DG, editor. The macaques: studies in ecology, behavior and evolution. New York: Van Nostrad Reinhold; 1980. p. 52–83.
[21] Hasan MK, Mostafa Feeroz MM, Jones-Engel L, Engel GA, Liu X, Smith DG. Origin and dispersal routes of rhesus macaque (*Macaca mulatta*). Intern J Primatol 2014;76(11):1094–104.
[22] Qiu Y-X, Fu C-X, Comes HP. Plant molecular phylogeography in China and adjacent regions: Tracing the genetic imprints of Quaternary climate and environmental change in the world's most diverse temperate flora. Mol Phylog Evol 2011;59:225–44.

VetBooks.ir

[23] Tosi AJ, Morales JC, Melnick DJ. Y-chromosome and mitochondrial markers in *Macaca fascicularis* indicate introgression with Indochinese *M. mulatta* and a Biogeographic Barrier in the Isthmus of Kra. Int J Primatol 2002;23:161–78.
[24] Kanthaswamy S, Satkoski J, George DA, Kou A, Erickson BJ, Smith DG. Hybridization and stratification of nuclear genetic variation in *Macaca mulatta* and *Macaca fascicularis*. Int J Primatol 2008;29:1295–311.
[25] Stevison LS, Kohn MH. Divergence population genetic analysis of hybridization between rhesus and cynomolgus macaques. Molec Ecol 2009;18:2457–75.
[26] Trask JS, Smith DG, Houghton P, Lerche N, Kanthaswamy S. Single Nucleotide Polymorphisms Reveal Patterns of Allele Sharing across a Species Boundary in *Macaca mulatta* and *M. fascicularis*. Am J Primatol 2013;75:135–44.
[27] Bonhomme M, Cuartero S, Blancher A, Ceouau-Roy. Assessing natural introgression in 2 biomedical species, the rhesus macaque (*Macaca mulatta*) and the longtail macaque (*Macaca fascicularis*). J Heredity 2009;100:158–69.
[28] Hernandez RD, Hubisz MJ, Wheeler DA, Smith DG, Ferguson B, Rogers J, et al. Demographic Histories and Patterns of Linkage Disequilibrium in Chinese and Indian Rhesus Macaques. Science 2007;316:240–3.
[29] Ramachandran S, Deshpande O, Roseman CC, Rosenberg NA, Feldman MW, Cavalli-Sforza LL. Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. Proc Natl Acad Sci U S A 2005;104:15942–7.
[30] Shiina T, Tanaka K, Katsuyama Y, Otabe K, Sakamoto K, Kurata M, et al. Mitochondrial DNA diversity among three subpopulations of cynomolgus macaques (*Macaca fascicularis*) originating from the Indochinese region. Exp Anim 2010;59:567–78.
[31] Kanthaswamy S, Capitanio JP, Dubay C, Ferguson B, Folks T, Ha JC, et al. Resources for Genetic Management and Genomics Research on Non-human Primates at the National Primate Research Centers. J Med Primatol 2009;38(Suppl.1):17–23.
[32] Kanthaswamy S, Johnson Z, Trask JS, Ramakrishnan R, Bahk J, Ng J, et al. Development and validation of a SNP-based assay for inferring the genetic ancestry of rhesus macaques (*Macaca mulatta*). Am J Primatol 2014;76(11):1105–13.
[33] Fawcett GL, Raveendran M, Deiros DR, Chen D, Yu F, Harris RA, et al. Characterization of single-nucleotide variation in Indian-origin rhesus macaques (*Macaca mulatta*). BMC Genom 2011;12:311.
[34] Fang X, Zhang Y, Zhang R, Yang L, Li M, Yen K, et al. Genome sequence and global sequence variation map with 5.5 million SNPs in Chinese rhesus macaque. Genome Biol 2011;12:R63.
[35] Yan G, Zhang G, Fang X, Zhang Y, Li C, Ling F, et al. Genome sequencing and comparison of two nonhuman primate animal models, the cynomolgus and Chinese rhesus macaques. Nat Biotechnol 2011;29(11):1019–23.
[36] Street S, Keyes RC, Grant R, Ferguson B. Single nucleotide polymorphisms (SNPs) are highly conserved in rhesus macaques (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. BMC Genomics 2007;8:480.
[37] Satkoski Trask JS, Garnica WT, Kanthaswamy S, Malhi RS, Smith DG. 4040 SNPs for genomic analysis in the rhesus macaque (*Macaca mulatta*). Genomics 2011;98:352–8.
[38] Kanthaswamy S, Ng J, Ross CT, Trask JS, Smith DG, Buffalo VS, et al. Identifying human-rhesus macaque gene orthologs using heterospecific SNP probes. Genomics 2013;101:30–75.
[39] Ross, CT, Roodgar M, Smith DG. Evolutionary distance of amino acid sequence orthologs across macaque subspecies: identifying candidate genes for SIV resistance in chinese rhesus macaques. PLOS ONE, In press.
[40] Norgren RB. Improving genome assemblies and annotsations for nonhuman primates. ILAR 2013;54:144–53.
[41] Trask JS, Garnica WT, Malhi RS, Kanthaswamy S, Smith DG. High-throughput single nucleotide polymorphisms discovery and the search for candidate genes for long term SIV_{mac} nonprogression in Chinese rhesus macaques (*Macaca mulatta*). J Med Primatol 2011;40:224–32.
[42] Teo YY, et al. Genome-wide comparisons of variation in linkage disequilibrium. Genome Res 2009;19:1849–60.
[43] Alexander DH, Novembre FJ, Lange K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res 2009;19:1655–64.
[44] Gompert Z, Buerkle CA. INTROGRESS: a software package for mapping components of isolation in hybrids. Molec Ecol Res 2010;10:378–84.
[45] Ng J, Trask JS, Houghton P, Smith DG, Kanthaswamy S. Estimates of Linkage Disequilibrium Based on Genome-wide Heterospecific Single Nucleotide Polymorphisms (SNPs) in Two Biomedically-relevant Macaque Species; 2014, In preparation.
[46] Higashino A, Sakate R, Kameoka Y, Takahashi I, Hirata M, Tanuma R, et al. Whole-genome sequencing and analysis of the Malaysian cynomolgus macaque (*Macaca fascicularis*) genome. Genome Biol 2012;13:R58.

[47] Ebeling M, Kueng E, See A, Broger C, Steiner G, Berrera M, et al. Genome-based analysis of the non-human primate *Macaca fascicularis* as a model for drug safety assessment. Genome Res 2011;21:1746–56.
[48] Yuan Q, Zhou Z, Lindell SG, Higley JD, Ferguson B, Thompson RC, et al. The rhesus macaque is three times as diverse but more closely equivalent in damaging coding variation as compared to the human. BMC Genet 2012;13:52.
[49] Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7(4):248–9.
[50] Zhang R, Xie X. Tools for GPCR drug discovery. Acta Pharmacol Sin 2012;33(3):372–84.
[51] Goswami DB, Ogawa LM, Ward JM, Miller GM, Vallender EJ. Large-scale polymorphism discovery in macaque G-protein coupled receptors. BMC Genomics 2013;14:703.
[52] Porsolt RD. The usefulness of non-human primates in central nervous system safety pharmacology. J Pharmacol Toxicol Methods 2013;68(1):23–9.
[53] Authier S, Vargas HM, Curtis MJ, Holbrook M, Pugsley MK. Safety pharmacology investigations in toxicology studies: an industry survey. J Pharmacol Toxicol Methods 2013;68(1):44–51.
[54] Hagenbuch B, Meier PJ. The superfamily of organic anion transporting polypeptides. Biochim Biophys Acta 2003;1609(1):1–18.
[55] Hamada A, Sissung T, Price DK, Danesi R, Chau CH, Sharifi N, et al. Effect of SLCO1B3 haplotype on testosterone transport and clinical outcome in caucasian patients with androgen-independent prostatic cancer. Clin Cancer Res 2008;14(11):3312–8.
[56] Bertz RJ, Granneman GR. Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. Clin Pharmacokinet 1997;32(3):210–58.
[57] Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999;286(5439):487–91.
[58] Johansson I, Ingelman-Sundberg M. Genetic polymorphism and toxicology–with emphasis on cytochrome p450. Toxicol Sci 2011;120(1):1–13.
[59] Uehara S, Murayama N, Yamazaki H, Uno Y. CYP2C76 non-synonymous variants in cynomolgus and rhesus macaques. Drug Metab Pharmacokinet 2012;27(3):344–8.
[60] Uno Y, Uehara S, Kohara S, Iwasaki K, Nagata R, Fukuzaki K, et al. Newly identified CYP2C93 is a functional enzyme in rhesus monkey, but not in cynomolgus monkey. PLoS One 2011;6(2):e16923.
[61] Osada N, Uno Y, Mineta K, Kameoka Y, Takahashi I, Terao K. Ancient genome-wide admixture extends beyond the current hybrid zone between *Macaca fascicularis* and *M. mulatta*. Mol Ecol 2010;19(14):2884–95.
[62] Uno Y, Osada N. CpG site degeneration triggered by the loss of functional constraint created a highly polymorphic macaque drug-metabolizing gene, CYP1A2. BMC Evol Biol 2011;11:283.
[63] Ise R, Nakanishi Y, Kohara S, Yamashita H, Yoshikawa T, Iwasaki K, et al. Expression profile of hepatic genes in cynomolgus macaques bred in Cambodia, China, and Indonesia: implications for cytochrome P450 genes. Drug Metab Pharmacokinet 2012;27(3):307–16.
[64] Uehara S, Murayama N, Nakanishi Y, Zeldin DC, Yamazaki H, Uno Y. Immunochemical detection of cytochrome P450 enzymes in liver microsomes of 27 cynomolgus monkeys. J Pharmacol Exp Ther 2011;339(2):654–61.
[65] Eichelbaum M, Ingelman-Sundberg M, Evans WE. Pharmacogenomics and individualized drug therapy. Annu Rev Med 2006;57:119–37.
[66] Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med 2006;355(10):1018–28.
[67] Wu H, Adkins K. Identification of polymorphisms in genes of the immune system in cynomolgus macaques. Mamm Genome 2012;23(7–8):467–77.

VetBooks.ir

VetBooks.ir

CHAPTER

3

Genetic Variation of the Major Histocompatibility Complex in *Macaca mulatta* and *Macaca fascicularis*

Lutz Walter

Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

OUTLINE

General Aspects of the Major Histocompatibility Complex 37
Discovery of the Major Histocompatibility Complex 37
Structure, Function, and Polymorphism of the MHC 38
The MHC of M. *mulatta* and M. *fascicularis* 40
Mamu and Mafa MHC Class I Genes 41
Mamu and Mafa MHC Class II Genes 41
Genetic Variability of MHC Genes of Macaques from Different Geographic Origins 42
MHC Disease Associations 45
Conclusion 46
Acknowledgments 46
References 46

GENERAL ASPECTS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Discovery of the Major Histocompatibility Complex

The earliest description of several genetic factors regulating graft rejection in mice dates back to Little and Tyzzer [1,2] at the beginning of the twentieth century. The discovery of the major histocompatibility complex (MHC), however, is attributed to Peter Gorer [3–6], who

http://dx.doi.org/10.1016/B978-0-12-417144-2.00003-2
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

also worked on tumor transplantation models and on blood groups in inbred mice. Gorer's pioneering studies showed that susceptibility to tumor (i.e., no rejection of transplanted tumor cells) is mediated by a single antigenic determinant, which he recognized is also present on erythrocytes. Gorer called it "antigen II." A century later, geneticist George Snell established congenic mice strains, which he used for the identification of genes involved in tissue rejection. Snell established the congenic mice strains by repeated (n>10) backcrossing of offspring carrying the donor phenotype (in this case the donor *MHC*) with the recipient strain. By this means, Snell [7] identified that certain genes, which he named histocompatibility or *H* genes, mediate rejection. These *H* genes can have a major or minor influence that result in either acute or chronic rejection. According to Gorer's studies, Snell named the first *H* gene with a major influence in his mice strains *H-2*.

Snell realized that rejection was not mediated by a single gene, but rather by several closely linked genes. Therefore, he introduced the term *H-2 complex*. The general term *major histocompatibility complex* was introduced after the discovery of similar gene complexes in the chicken by Briles et al. [8] in 1950 and eight years later in humans (human leukocyte antigen [HLA]) by Dausset [9], Payne and Rolfs [10], and van Rood et al. [11]. The designation of the *MHC* in individual species follows a nomenclature proposal by Klein et al. [12], stating that the first two digits of the Latin name of a species should be used. For example, the MHC of *Macaca mulatta* is called *Mamu*. For historical reasons, the designations for the *MHC* of the mouse (the present-day syntax is *H2*) and human (*HLA*) were kept. Soon after these discoveries, several researchers found out that responses to immunizations also underlie genetic control and the responsible genes were therefore called immune response (*Ir*) genes. Eventually, *Ir* genes were included as part of the *MHC* [13,14]. George Snell, Jean Dausset, and Baruj Benacerraf were awarded the Nobel Prize for Physiology or Medicine in 1980 for these achievements.

Structure, Function, and Polymorphism of the MHC

The *MHC* in mammals encompasses roughly 4 Mb and is divided into three subregions, designated *MHC class I*, *class II*, and *class III* regions [15–18] (Figure 3.1). The *class I* and *class II* regions contain the *MHC class I* and *class II* genes; whereas, the *class III* (or central) *MHC* region does not contain genes of a dominating type. The *class I* and *class II* genes have expanded in number and, in particular, the *class I* genes frequently display species-specific expansions and contractions [18]. Depending on the expression pattern, polymorphism, and function, both *MHC class I* and *class II* genes are distinguished as classical and nonclassical.

The MHC class I proteins consist of the polymorphic *MHC*-encoded heavy chain and the noncovalently bound nonpolymorphic beta-2-microglobulin, which is not encoded in the *MHC*. The MHC class I protein is expressed on the cell surface and displays immunoglobulin (Ig)-like domains in the extracellular part of the polypeptide. The heavy chain contains three Ig-like domains that are designated α1, α2, and α3. The α1 and α2 domains form a cleft, which binds small peptides of 8-9 amino acids length, whereas the α3 domain interacts with beta-2-microglobulin and stabilizes the conformation of the molecule, providing the binding site for the CD8 molecule [19,20]. The peptides usually stem from degradation of cellular (self- or pathogen-derived) proteins in the proteasome protein complex and

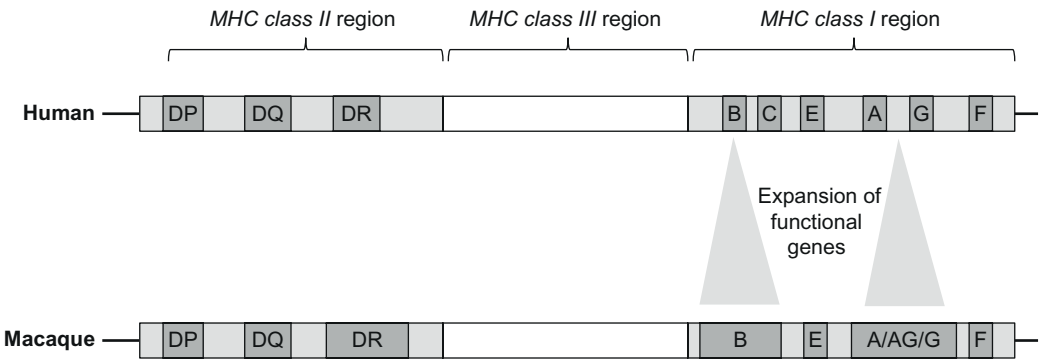


FIGURE 3.1 Schematic map of the human and macaque major histocompatibility complex (*MHC*). Individual regions are indicated. Boxes represent the different types of *MHC* genes. The map is not drawn to scale. Copy number variations are known for *DRB* genes of both human and macaque *MHC* haplotypes. Gene expansions are particularly found for the *A*, *B*, and *AG* types of *MHC class I* genes of macaques.

are translocated by the TAP1/TAP2 heterodimeric peptide transporter into the lumen of the endoplasmic reticulum (ER) [21]. The small peptides then are loaded into the peptide-binding cleft of the MHC class I proteins, which are subsequently released from the ER and transported via the Golgi apparatus to the cell surface, where they present the bound peptides to CD8+ cytotoxic T-lymphocytes. In addition to presentation of antigenic peptides, another important immunological function of the MHC class I proteins is to serve as ligands for receptors of natural killer (NK) cells. These lymphocytes express both inhibitory and stimulatory receptors that can recognize both classical and nonclassical MHC class I proteins on the cell surface of target cells [22]. A delicate balance of inhibitory and stimulatory signaling regulates the activation of NK cells [23,24]. Self-tolerance against healthy cells is achieved by interaction of inhibitory NK cell receptors with MHC class I molecules. In contrast, infections frequently lead to upregulation of certain nonclassical MHC class I proteins ("stress signals") and to downregulation of certain classical MHC class I proteins (to evade recognition by CD8+ T cells). This perturbed balance of receptor signals results in strong activation of NK cells (i.e., killing and cytokine production). Recent studies indicate that a change in the repertoire of presented peptides influences the recognition of MHC class I proteins by NK cell receptors [25] and that pressure by NK cells obviously leads to selection of mutations in viral peptides. These mutations result in higher avidity binding of inhibitory NK cell receptors [26,27].

Three types of classical MHC class II proteins have been described in primates: DP, DQ, and DR [18]. The respective molecules are heterodimers, which are made from noncovalently bound alpha and beta chains that both contain two Ig-like domains and are encoded by respective *DPA*, *DPB* (and so on). The α1 and the β1 domains form a peptide-binding cleft that accommodates peptides 13-18 amino acids in length [28]. The expression of the MHC class II proteins are usually restricted to professional antigen-presenting cells such as dendritic cells, macrophages, B cells, and thymic epithelium. Class II

VetBooks.ir

proteins interact with another polypeptide, the invariant chain. This interaction prevents the loading of peptides in the ER. Due to a respective amino acid motif in the invariant chain, the complex can exit the ER and locates to the endosomal/lysosomal compartment (also called the MHC class II loading compartment), where the invariant chain is degraded with the exception of a small peptide called CLIP (class II-associated invariant peptide). After uptake, exogenous antigen degrades into peptides, which are loaded in the MHC class II loading compartment on the MHC class II proteins after the removal of CLIP by the nonclassical MHC class II protein DM [29]. Subsequently, the peptide-loaded MHC class II proteins are located to the cell surface and present the antigen to CD4+ T-lymphocytes.

The hallmark of the *MHC* is the enormous extent of allelic polymorphism of both *class I* and *class II* genes (see the Immuno Polymorphism Database at http://www.ebi.ac.uk/ipd/). Sequence comparisons of alleles from both classes indicate that the allelic differences are not evenly distributed over the entire gene, but are confined to particular regions of the gene. Seminal studies of solving the three-dimensional structure of peptide-bound MHC class I polypeptides by Bjorkman et al. [19,20] showed that the polymorphic codons are those that are involved in the binding of peptides. Similar findings were obtained for the *MHC class II* gene codons [28]. In accordance with these structural findings, Hughes and Nei [30,31] discovered a much higher rate of nonsynonymous (amino acid changing) than synonymous (silent) substitutions in codons involved in peptide binding. These polymorphisms essentially determine peptide binding and, therefore, allotypes differ in their peptide-binding repertoire [32]. While it is obvious that the presence of pathogens and their selective pressure on the host are responsible for the observed polymorphism of *MHC* genes in populations, the mechanisms contributing to the maintenance of polymorphisms include overdominant selection, frequency-dependent selection, and gene conversion [30,33,34].

THE MHC OF *M. MULATTA* AND *M. FASCICULARIS*

Dissenting data have been published on the chromosomal assignment of the rhesus macaque *MHC* (*Mamu*). While earlier publications mapped the rhesus macaque *MHC* to either chromosome 2 or 5 [35] or to chromosome 4 (public databases such the UCSC Genome Browser), the *Mamu* complex was clearly assigned to chromosome 6q24 [36] by thorough cytogenetic mapping using fluorescence in situ hybridization of cosmid clones combined with RBA and DA/DAPI-like banding patterns. Complete genome sequences are available for the rhesus [37] and long-tailed [38] macaque, but the *MHC* region was not in "finished quality" in these projects because of the substantial gene duplications that are observed in this genomic region. However, before the release of the macaque genome sequences, the rhesus macaque *MHC* was already covered by BAC clonal contigs from a single individual, and this contig was completely sequenced [39].

The Immuno Polymorphism Database contains updated data on the number of genes/alleles as well as sequence retrieval and comparisons of *Mamu* and *Mafa class I* and *class II*, and it is accessible at http://www.ebi.ac.uk/ipd/mhc/nhp/index.html.

VetBooks.ir

Mamu and *Mafa* MHC Class I Genes

The overall organization of the *Mamu* and *Mafa* genomic regions is very similar to the human *HLA* complex (Figure 3.1), with few exceptions. These exceptions are mainly found in the *MHC class I* region and significantly contribute to length differences between *Mamu* (5.3 Mb) and *HLA* (3.7 Mb). The reported *Mamu* sequence is a composite of both haplotypes (composition of alleles from different genes on the same chromosome) from this animal, and it contains the following types of *class I* genes: *Mamu-A* (n = 2, *Mamu-A1*, *Mamu-A2*), *Mamu-AG* (n = 4), *Mamu-B* (n = 19), *Mamu-E* (n = 1), *Mamu-F* (n = 1), and *Mamu-G* (n = 4). Notably, all four *Mamu-G* and five of the *Mamu-B* genes are nonfunctional and represent pseudogenes [39]. The *Mamu-G* pseudogenes are thought to be functionally substituted by the *Mamu-AG* genes [40,41]. The so-called *Mamu-I* genes are a subtype of the *Mamu-B* genes and were previously named *Mamu-B*09* [42].

Rhesus macaques have been analyzed for the content of *Mamu* class I genes of the *Mamu-A* and *Mamu-B* types. In a series of pioneering papers, Otting et al. [43–45] and Doxiadis et al. [46,47] described differential gene contents for both the *Mamu-A* (*A1* to *A7*) and *Mamu-B* gene types in rhesus macaques. It should be noted that the assignment of sequences to individual genes is well established for *Mamu-A* but not for *Mamu-B* genes. More recent developments include the use of next-generation sequencing, allowing for parallel molecular typing of both *MHC-A* and *MHC-B* type genes [48,49].

There are several layers of genetic complexity in *Mamu-A* and *Mamu-B* genes that involve (1) allelic variability, (2) differential gene content (which are also referred to in this context as *polymorphism* and *diversity*, respectively), and (3) varying strengths of gene expression. Whereas the *A2-A7* genes show substantial diversity (i.e., differences in presence/absence of the gene); the *Mamu-A1* gene is present on almost every *Mamu* haplotype [46]. In contrast to *A2-A7*, the *Mamu-A1* gene is polymorphic (i.e., has multiple alleles). It is notable that the extent of allelic polymorphism in the *Mamu-A* and *Mamu-B* regions is clearly below that of the corresponding human *HLA-A* and *HLA-B/C* regions, and the genetic variability of macaques is more often found at the level of haplotype diversity [46,47]. In addition, not all *Mamu-A* or *Mamu-B* genes are transcribed at the same level. Some genes such as *Mamu-A1*, *A7*, *B*01*, *B*030*, or *B*047* display a high level of transcription ("majors"), whereas others such as *A2*, *A4*, *B*017*, or *B*038* are classified as "minors" because of a low level of transcription [43,45]. Interestingly, some of the long-tailed macaque *MHC class I* genes are differentially transcribed in leukocyte subsets such as *Mafa-B*134*. This allele is expressed in monocytes, but transcripts are virtually absent in CD4+ T cells [50]. Different levels of protein expression of transfected *Mamu-A* and *Mamu-B* genes can be found as well [51]. While such differential expression might be compatible with the concept of classical and nonclassical MHC class I genes (refer to the previous section), the biological significance of this variation still needs to be addressed and might be evident for more than just *Mafa-B*134* or in other tissue/cell types or disease/developmental states.

Mamu and *Mafa* MHC Class II Genes

The overall organization of the *MHC class II* region is quite similar between macaques and humans [39,52]. There are three types of classical *MHC class II* genes, including *DP*, *DQ*, and

VetBooks.ir

DR. Differences to the *HLA* complex were found in the macaque *DQ* region, where only single copies of the *DQA* and *DQB* genes are present in both *Mamu* and *Mafa* [39,52]. There have been several recent reports of the genetic variability of *MHC class II* genes of both macaque species [53–61]. Similar to the diversity of *MHC class I* genes and haplotypes (see Section *Mamu* and *Mafa* MHC Class I Genes), the level of *MHC class II* allelic variability is rather low, whereas the haplotype diversity (gene configuration) is very high in both macaque species [47]. This diversity is particularly displayed by the *DRB* genes, and up to six *DRB* genes per haplotype have been described. These different haplotype configurations are thought to stem from non-homologous recombination events. Comparisons of primate *MHC-DRB* sequences suggest that peptide-binding motifs have been maintained for long evolutionary distances and may predate human and macaque speciation. Sharing of peptide-binding motifs between rhesus macaque and human DRB proteins also has been reported. However, more recent studies indicate that *MHC-DRB* genes are rather dynamic and that peptide-binding motifs can be reshuffled between different (expanded) *DRB* genes [62].

The variability of *MHC class II* genes obviously has also a strong effect on interindividual differences in the number of helper T cells and, hence, the proportion of CD4+/CD8+ T-cell numbers [63]. The essential role of MHC class II proteins in the differentiation of CD4+ T-cells in the thymus may explain the findings. This biomedically relevant data were exemplified in long-tailed macaques.

Genetic Variability of MHC Genes of Macaques from Different Geographic Origins

Macaques are generally well known for genetic variability that is dependent on their geographic origin. *MHC class I* (Table 3.1) and *class II* (Table 3.2) genes of both long-tailed and rhesus macaques have been extensively studied in animals originating from different geographic regions. The most prominent difference evident in rhesus macaques is probably the *Mamu-A1*001* allele, which is well known for its association with delayed progression to acquired immunodeficiency syndrome in experimental simian immunodeficiency virus (SIV) infection. This allele is present in Indian and Chinese rhesus macaques, but it was thus far not detected in animals from Myanmar [80]. Furthermore, this study reported a total of 73 alleles that were detected among 100 Burmese rhesus macaques, but only 4 and 25 alleles were previously reported in Indian and Chinese-origin rhesus macaques, respectively. Thus, the sharing of alleles is obviously not very common. The data further suggests that Burmese rhesus macaques might be more closely related to Chinese than to Indian animals. Interestingly, this geographic difference was not obvious for *Mamu-B* gene sequences [80]. Contrasting this high variability of the *Mamu-A1* gene from rhesus macaques of different geographic origin is a certain extent of sharing of gene region configurations [46], which is also observed for *DRB* haplotypes [47]. In general, Indian rhesus macaques seem to have a lower genetic diversity at *MHC* loci compared with Myanmar or Chinese animals [47]. Likewise, long-tailed macaques from different geographic origins show similar variability within their *MHC* genes [54]. The reasons for such intra- and interspecies differences lie in large distribution ranges that lead to accumulation and manifestation of genetic differences, dramatic climate changes, or introgression and hybridization. For a more detailed overview of such factors, see Chapter 1 by Roos and Zinner in this book.

VetBooks.ir

TABLE 3.1 Overview of Reports on *MHC Class I* Gene Variability of Macaques from Different Geographic Regions

***MHC* region or gene**	**Geographic origin of macaques**	**Reference**
Long-tailed macaque:		
A, B	Mauritius, China, Vietnam	[64]
A	Mauritius, Philippines, Vietnam, China	[65]
A, B	Mauritius	[66]
A, B	?	[44]
A2	Indonesia, Vietnam	[67]
B	Mauritius	[68]
A, B	Mauritius	[48]
A, B, I	Mauritius	[69]
A, B, I, AG	Indonesia, Malaysia, Philippines	[70]
B	Vietnam	[56]
Rhesus macaque:		
I	?	[42]
A, B	India	[71]
A, B	?	[72]
A, B	India, China, Myanmar	[43]
A, B	Nepal	[73]
A, B	Myanmar, Laos	[74]
A	India	[65]
A, B	India, China	[44]
A, B	India	[75]
A, B, I	China	[76]
A, B	India	[77]
A, B	China	[78]
A2	China	[67]
B	China	[45]
A	China	[79]
A, B	India, China	[48]
A, B, I	Myanmar, Laos	[80]
A, B	China	[81]

(*Continued*)

VetBooks.ir

TABLE 3.1 Overview of Reports on *MHC Class I* Gene Variability of Macaques from Different Geographic Regions—Cont'd

MHC region or gene	Geographic origin of macaques	Reference
A, B	Myanmar, China	[82]
A	India, China, Myanmar	[46]
A	China	[83]
A, B	China	[84]
A, B	India, Myanmar	[47]
B	China	[85]

TABLE 3.2 Overview of Reports on *MHC Class II* Gene Variability of Macaques from Different Geographic Regions

MHC gene or genomic region	Geographic origin of macaques	Reference
Long-tailed macaque:		
DRB	Mauritius, China	[86]
DRA, DRB, DQA, DQB, DPA, DPB	Mauritius	[87]
DRB	Mauritius	[66]
DRB	China	[88]
DRB	Malaysian/Phillipine islands, Indochina (south of Kra Isthmus)	[89]
DRB	Mauritius, Indonesia	[58]
DRB	Malaysian/Indonesian islands, Indochina	[57]
DRB	Phillipines	[63]
DRB	Vietnam	[55]
DRB	Mauritius, Phillipines, Java, Vietnam	[54]
DRB, DQB1	Vietnam	[56]
DRA, DRB	Indochina, Indonesian Islands	[90]
DRA,DQA, DPA, DOA	Vietnam	[59]
DRA	Mauritius, Phillipines, Vietnam, Indonesia	[91,92]
DRB, DQA, DQB, DPA, DPB	Philippines	[53]
Rhesus macaque:		
DP	India, Myanmar	[93]
DQ, DR	India	[94]
DRB	mostly India, some from China and Myanmar	[95]
DRB	mostly India	[96]

VetBooks.ir

TABLE 3.2 Overview of Reports on *MHC Class II* Gene Variability of Macaques from Different Geographic Regions—Cont'd

MHC gene or genomic region	Geographic origin of macaques	Reference
DQB	India	[97]
DQA, DQB, DPB	India, Myanmar, China	[98]
DR	India	[99]
DR, DQ, DP	India, Myanmar, China	[100]
DRB	India, Myanmar, China	[101]
DRB	India	[60]
DQB	China	[102]
DRB, DQA, DQB, DPB	India, Myanmar	[47]
DRA, DQA, DPA	China	[61]

MHC DISEASE ASSOCIATIONS

Because of their essential role in the presentation of antigen, alleles of human *HLA class I* and *class II* genes are associated with susceptibility to a variety of infectious, autoimmune, and other diseases [103]. Macaques serve as important models of these diseases, particularly of infectious diseases [104]. There have been numerous reports of the infection of rhesus macaques with SIV and the role of *MHC* variability (for reviews, see refs. [105–108]). For example, the following rhesus macaque *MHC class I* alleles were reported to be associated with SIV control: *Mamu-A1*001* [71,72,109–111], *Mamu-A3*13:03* [71], *Mamu-B*008* [112], *Mamu-B*017* [113]. Similarly, the MHC of long-tailed macaques determines susceptibility to experimental SIV infection [114]. Because of their restricted genetic variability, Mauritian-origin long-tailed macaques represent an exceptionally informative model of the influence of the *MHC* on SIV disease susceptibility.

In addition to single genes, the contribution of genetic differences of *MHC* haplotypes to disease susceptibility was studied. The general effect of heterozygosity at the *MHC* was demonstrated, for example, using long-tailed macaques of Mauritian origin that differ in *MHC* zygosity. The *MHC* homozygous animals showed viral loads 80 times higher than the *MHC* heterozygous animals in the chronic phase of infection and a higher rate of virus escape mutations [115]. This study further supports the so-called *MHC* heterozygote advantage hypothesis of Nobel Laureates Doherty and Zinkernagel almost 40 years ago [116]. In particular, *MHC* haplotypes *M3* and *M6* turned out to control infection with SHIVsbg and SIVmac251, respectively [117,118]. In a study involving Mauritian long-tailed macaques, haplotypes *H2* and *H6* were reported to be associated with control and haplotype *H4* with susceptibility to SIVmac251 infection [119]. Along the same lines, *MHC* haplotypes of Indian rhesus macaques were reported to be associated with rapid, medium, or slow progression to acquired immunodeficiency syndrome in experimental SIV infection models [77]. Despite their genetic and immunologic differences, both Indian and Chinese rhesus macaques are used for experimental SIV infection (for a review, see Zhou et al. [120]). While early studies suggested that Indian rhesus macaques might be the superior model of SIV infection [121], many studies now also use Chinese rhesus macaques.

VetBooks.ir

CONCLUSION

Rhesus and long-tailed macaques serve as important animal models for many human diseases because of their closer phylogenetic relationship compared to rodent models. This close similarity is also evident in many aspects of the *MHC*, which is the most polymorphic region in vertebrate genomes. However, the exceptional genetic variability is also responsible for differences between macaques and human *MHC*. This is particularly seen in the *MHC class I* region, where the human *MHC* is banking on allelic variability and a fixed gene content and variability of the macaque *MHC* is displayed in gene configurations. Geographic origins of both rhesus and long-tailed macaques also have influenced the allelic and haplotypic repertoire of the *MHC*. Notably, such biogeographic differences play essential roles in disease models in which the *MHC* is a major determinant of disease outcomes and must be considered in the experimental design.

Acknowledgments

The author thanks the editors for providing the opportunity to contribute to this book.

References

[1] Little CC, Tyzzer EE. Further experimental studies on the inheritance of susceptibility to a Transplantable tumor, Carcinoma (J. W. A.) of the Japanese waltzing Mouse. J Med Res 1916;33:393–453.
[2] Tyzzer EE. A Study of Inheritance in Mice with Reference to their Susceptibility to transplantable Tumors. J Med Res 1909;21:519–73.
[3] Gorer PA. The detection of a hereditary antigenic difference in the blood of mice by means of human group a serum. J Genet 1936;32:17–31, Springer India.
[4] Gorer PA. The detection of antigenic differences in mouse erythrocytes by the employment of immune sera. Br J Exp Pathol 1936;17:42–50.
[5] Gorer PA. The genetic and antigenic basis of tumour transplantation. J Pathol Bacteriol 1937;47:231–52.
[6] Gorer PA. The antigenic basis of tumour transplantation. J Pathol Bacteriol 1938;47:231–52.
[7] Snell GD. Methods for the study of histocompatibility genes. J Genet 1948;49:87–108.
[8] Briles WE, McGibbon WH, Irwin MR. On multiple alleles effecting cellular antigens in the chicken. Genetics 1950;35:633–52.
[9] Dausset J. Iso-leuco-anticorps. Acta Haematol 1958;20:156–66.
[10] Payne R, Rolfs MR. Fetomaternal leukocyte incompatibility. J Clin Invest 1958;37:1756–63.
[11] van Rood JJ, Eernisse JG, van Leeuwen A. Leucocyte antibodies in sera from pregnant women. Nature 1958;181:1735–6.
[12] Klein J, Bontrop RE, Dawkins RL, Erlich HA, Gyllensten UB, Heise ER, et al. Nomenclature for the major histocompatibility complexes of different species: a proposal. Immunogenetics 1990;31:217–9.
[13] McDevitt HO, Chinitz A. Genetic control of the antibody response: relationship between immune response and histocompatibility (H-2) type. Science 1969;163:1207–8.
[14] Ellman L, Green I, Martin WJ, Benacerraf B. Linkage between the poly-L-lysine gene and the locus controlling the major histocompatibility antigens in strain 2 guinea pigs. Proc Natl Acad Sci U S A 1970;66:322–8.
[15] Dressel R, Walter L, Günther E. Genomic and funtional aspects of the rat MHC, the RT1 complex. Immunol Rev 2001;184:82–95.
[16] Günther E, Walter L. Comparative genomic aspects of rat, mouse and human MHC class I gene regions. Cytogenet Cell Genet 2000;91:107–12.
[17] Trowsdale J. "Both man & bird & beast": comparative organization of MHC genes. Immunogenetics 1995;41:1–17.

[18] Kelley J, Walter L, Trowsdale J. Comparative genomics of major histocompatibility complexes. Immunogenetics 2005;56:683–95.
[19] Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. Nature 1987;329:506–12.
[20] Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature 1987;329:512–8.
[21] Trowsdale J, Hanson I, Mockridge I, Beck S, Townsend A, Kelly A. Sequences encoded in the class II region of the MHC related to the "ABC" superfamily of transporters. Nature 1990;348:741–4.
[22] Lanier LL. NK cell recognition. Annu Rev Immunol 2005;23:225–74.
[23] Long EO. Regulation of immune responses through inhibitory receptors. Annu Rev Immunol 1999;17:875–904.
[24] Raulet DH, Vance RE. Self-tolerance of natural killer cells. Nat Rev Immunol 2006;6:520–31.
[25] Fadda L, Borhis G, Ahmed P, Cheent K, Pageon SV, Cazaly A, et al. Peptide antagonism as a mechanism for NK cell activation. Proc Natl Acad Sci U S A 2010;107:10160–5.
[26] Alter G, Heckerman D, Schneidewind A, Fadda L, Kadie CM, Carlson JM, et al. HIV-1 adaptation to NK-cell-mediated immune pressure. Nature 2011;476:96–100.
[27] Jost S, Altfeld M. Evasion from NK cell-mediated immune responses by HIV-1. Microbes Infect 2012;14:904–15.
[28] Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, et al. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 1993;364:33–9.
[29] Denzin LK, Cresswell P. HLA-DM induces CLIP dissociation from MHC class II alpha beta dimers and facilitates peptide loading. Cell 1995;82:155–65.
[30] Hughes AL, Nei M. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. Nature 1988;335:167–70.
[31] Hughes AL, Nei M. Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. Proc Natl Acad Sci U S A 1989;86:958–62.
[32] Falk K, Rötzschke O, Rammensee HG. Cellular peptide composition governed by major histocompatibility complex class I molecules. Nature 1990;348:248–51.
[33] Takahata N, Nei M. Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. Genetics 1990;124:967–78.
[34] Ohta T. Role of diversifying selection and gene conversion in evolution of major histocompatibility complex loci. Proc Natl Acad Sci U S A 1991;88:6716–20.
[35] Hirai M, Takahashi E, Ishida T, Hori T. Chromosomal localization of the major histocompatibility complex (MHC) in the rhesus monkey and chimpanzee by fluorescence in situ hybridization. Cytogenet Cell Genet 1991;57:204–5.
[36] Huber I, Walter L, Wimmer R, Pasantes JJ, Günther E, Schempp W. Cytogenetic mapping and orientation of the rhesus macaque MHC. Cytogenet Genome Res 2004;103:144–9.
[37] Gibbs RA, Rogers J, Katze MG, Bumgarner R, Weinstock GM, Mardis ER, et al. Evolutionary and biomedical insights from the rhesus macaque genome. Science 2007;316:222–34, 80 authors.
[38] Ebeling M, Küng E, See A, Broger C, Steiner G, Berrera M, et al. Genome-based analysis of the nonhuman primate *Macaca fascicularis* as a model for drug safety assessment. Genome Res 2011;21:1746–56.
[39] Daza-Vamenta R, Glusman G, Rowen L, Guthrie B, Geraghty DE. Genetic divergence of the rhesus macaque major histocompatibility complex. Genome Res 2004;14:1501–15.
[40] Boyson JE, Iwanaga KK, Golos TG, Watkins DI. Identification of a novel MHC class I gene, Mamu-AG, expressed in the placenta of a primate with an inactivated G locus. J Immunol 1997;159:3311–21.
[41] Slukvin II, Lunn DP, Watkins DI, Golos TG. Placental expression of the nonclassical MHC class I molecule Mamu-AG at implantation in the rhesus monkey. Proc Natl Acad Sci U S A 2000;97:9104–9.
[42] Urvater JA, Otting N, Loehrke JH, Rudersdorf R, Slukvin II, Piekarczyk MS, et al. Mamu-I: a novel primate MHC class I B-related locus with unusually low variability. J Immunol 2000;164:1386–98.
[43] Otting N, Heijmans CMC, Noort RC, de Groot NG, Doxiadis GGM, van Rood JJ, et al. Unparalleled complexity of the MHC class I region in rhesus macaques. Proc Natl Acad Sci U S A 2005;102:1626–31.
[44] Otting N, de Vos-Rouweler AJM, Heijmans CMC, de Groot NG, Doxiadis GGM, Bontrop RE. MHC class I A region diversity and polymorphism in macaque species. Immunogenetics 2007;59:367–75.
[45] Otting N, Heijmans CMC, van der Wiel M, de Groot NG, Doxiadis GGM, Bontrop RE. A snapshot of the Mamu-B genes and their allelic repertoire in rhesus macaques of Chinese origin. Immunogenetics 2008;60:507–14.

[46] Doxiadis GGM, de Groot N, Otting N, Blokhuis JH, Bontrop RE. Genomic plasticity of the MHC class I A region in rhesus macaques: extensive haplotype diversity at the population level as revealed by microsatellites. Immunogenetics 2011;63:73–83.
[47] Doxiadis GGM, de Groot N, Otting N, de Vos-Rouweler AJM, Bolijn MJ, Heijmans CMC, et al. Haplotype diversity generated by ancient recombination-like events in the MHC of Indian rhesus macaques. Immunogenetics 2013;65:569–84.
[48] Wiseman RW, Karl JA, Bimber BN, O'Leary CE, Lank SM, Tuscher JJ, et al. Major histocompatibility complex genotyping with massively parallel pyrosequencing. Nat Med 2009;15:1322–6.
[49] O'Leary CE, Wiseman RW, Karl JA, Bimber BN, Lank SM, Tuscher JJ, et al. Identification of novel MHC class I sequences in pig-tailed macaques by amplicon pyrosequencing and full-length cDNA cloning and sequencing. Immunogenetics 2009;61:689–701.
[50] Greene JM, Wiseman RW, Lank SM, Bimber BN, Karl JA, Burwitz BJ, et al. Differential MHC class I expression in distinct leukocyte subsets. BMC Immunol 2011;12:39.
[51] Rosner C, Kruse PH, Lübke T, Walter L. Rhesus macaque MHC class I molecules show differential subcellular localizations. Immunogenetics 2010;62:149–58.
[52] Watanabe A, Shiina T, Shimizu S, Hosomichi K, Yanagiya K, Kita YF, et al. A BAC-based contig map of the cynomolgus macaque (*Macaca fascicularis*) major histocompatibility complex genomic region. Genomics 2007;89:402–12.
[53] Blancher A, Aarnink A, Yamada Y, Tanaka K, Yamanaka H, Shiina T. Study of MHC class II region polymorphism in the Filipino cynomolgus macaque population. Immunogenetics 2014;66:219–30.
[54] Blancher A, Aarnink A, Tanaka K, Ota M, Inoko H, Yamanaka H, et al. Study of cynomolgus monkey (*Macaca fascicularis*) Mhc DRB gene polymorphism in four populations. Immunogenetics 2012;64:605–14.
[55] Li W, Wang T, Ling F, Zhao H, Wei L, Zhuo M, et al. Identification of MhcMafa-DRB alleles in a cohort of cynomolgus macaques of Vietnamese origin. Am J Primatol 2012;74:958–66.
[56] Ling F, Zhuo M, Ni C, Zhang G-Q, Wang T, Li W, et al. Comprehensive identification of high-frequency and co-occurring Mafa-B, Mafa-DQB1, and Mafa-DRB alleles in cynomolgus macaques of Vietnamese origin. Hum Immunol 2012;73:547–53.
[57] Doxiadis GGM, de Groot N, de Groot NG, Rotmans G, de Vos-Rouweler AJM, Bontrop RE. Extensive DRB region diversity in cynomolgus macaques: recombination as a driving force. Immunogenetics 2010;62:137–47.
[58] Mee ET, Murrell CK, Sauermann U, Wilkinson RC, Cutler K, North D, et al. The Mhc class II DRB genotype of *Macaca fascicularis* does not influence infection by simian retrovirus type 2. Tissue Antigens 2008;72:369–78.
[59] Xiang R, Zhang H, Deng Q, Yue R, Tang H, Zhang Y, et al. Comprehensive identification of high-frequency and co-occurring Mafa-DPA1, Mafa-DQA1, Mafa-DRA, and Mafa-DOA alleles in Vietnamese cynomolgus macaques. Immunogenetics 2013;65:667–74.
[60] Giraldo-Vela JP, Rudersdorf R, Chung C, Qi Y, Wallace LT, Bimber B, et al. The major histocompatibility complex class II alleles Mamu-DRB1*1003 and -DRB1*0306 are enriched in a cohort of simian immunodeficiency virus-infected rhesus macaque elite controllers. J Virol 2008;82:859–70.
[61] Deng Q, Zhang H, Xiang R, Zhang Z, Ling F, Zhuo M, et al. Identification of Mamu-DPA1, Mamu-DQA1, and Mamu-DRA alleles in a cohort of Chinese rhesus macaques. Immunogenetics 2013;65:901–4.
[62] Doxiadis GGM, de Groot N, de Groot NG, Doxiadis IIN, Bontrop RE. Reshuffling of ancient peptide binding motifs between HLA-DRB multigene family members: old wine served in new skins. Mol Immunol 2008;45:2743–51.
[63] Aarnink A, Garchon H-J, Puissant-Lubrano B, Blancher-Sardou M, Apoil P-A, Blancher A. Impact of MHC class II polymorphism on blood counts of CD4+ T lymphocytes in macaque. Immunogenetics 2011;63:95–102.
[64] Krebs KC, Jin Z, Rudersdorf R, Hughes AL, O'Connor DH. Unusually high frequency MHC class I alleles in Mauritian origin cynomolgus macaques. J Immunol 2005;175:5230–9.
[65] Lafont BAP, McGraw CM, Stukes SA, Buckler-White A, Plishka RJ, Byrum RA, et al. The locus encoding an oligomorphic family of MHC-A alleles (Mane-A*06/Mamu-A*05) is present at high frequency in several macaque species. Immunogenetics 2007;59:211–23.
[66] Wiseman RW, Wojcechowskyj JA, Greene JM, Blasky AJ, Gopon T, Soma T, et al. Simian immunodeficiency virus SIVmac239 infection of major histocompatibility complex-identical cynomolgus macaques from Mauritius. J Virol 2007;81:349–61.

[67] Wu J, Bassinger S, Montoya GD, Chavez L, Jones CE, Holder-Lockyer B, et al. Allelic diversity within the high frequency Mamu-A2*05/Mane-A2*05 (Mane-A*06)/Mafa-A2*05 family of macaque MHC-A loci. Tissue Antigens 2008;72:29–38.
[68] Mee ET, Badhan A, Karl JA, Wiseman RW, Cutler K, Knapp LA, et al. MHC haplotype frequencies in a UK breeding colony of Mauritian cynomolgus macaques mirror those found in a distinct population from the same geographic origin. J Med Primatol 2009;38:1–14.
[69] Budde ML, Wiseman RW, Karl JA, Hanczaruk B, Simen BB, O'Connor DH. Characterization of Mauritian cynomolgus macaque major histocompatibility complex class I haplotypes by high-resolution pyrosequencing. Immunogenetics 2010;62:773–80.
[70] Saito Y, Naruse TK, Akari H, Matano T, Kimura A. Diversity of MHC class I haplotypes in cynomolgus macaques. Immunogenetics 2012;64:131–41.
[71] Mühl T, Krawczak M, Haaft ten P, Hunsmann G, Sauermann U. MHC class I alleles influence set-point viral load and survival time in simian immunodeficiency virus-infected rhesus monkeys. J Immunol 2002;169:3438–46.
[72] O'Connor DH, Mothé BR, Weinfurter JT, Fuenger S, Rehrauer WM, Jing P, et al. Major histocompatibility complex class I alleles associated with slow simian immunodeficiency virus disease progression bind epitopes recognized by dominant acute-phase cytotoxic-T-lymphocyte responses. J Virol 2003;77:9029–40.
[73] Kyes RC, Jones-Engel L, Chalise MK, Engel G, Heidrich J, Grant R, et al. Genetic characterization of rhesus macaques (*Macaca mulatta*) in Nepal. Am J Primatol 2006;68:445–55.
[74] Tanaka-Takahashi Y, Yasunami M, Naruse T, Hinohara K, Matano T, Mori K, et al. Reference strand-mediated conformation analysis-based typing of multiple alleles in the rhesus macaque MHC class I Mamu-A and Mamu-B loci. Electrophoresis 2007;28:918–24.
[75] Kaizu M, Borchardt GJ, Glidden CE, Fisk DL, Loffredo JT, Watkins DI, et al. Molecular typing of major histocompatibility complex class I alleles in the Indian rhesus macaque which restrict SIV $CD8^+$ T cell epitopes. Immunogenetics 2007;59:693–703.
[76] Karl JA, Wiseman RW, Campbell KJ, Blasky AJ, Hughes AL, Ferguson B, et al. Identification of MHC class I sequences in Chinese-origin rhesus macaques. Immunogenetics 2008;60:37–46.
[77] Sauermann U, Siddiqui R, Suh Y-S, Platzer M, Leuchte N, Meyer H, et al. Mhc class I haplotypes associated with survival time in simian immunodeficiency virus (SIV)-infected rhesus macaques. Genes Immun 2008;9:69–80.
[78] Ouyang D, Xu L, Dai Z, Shi H, Zhang G, Zheng Y, et al. Identification of major histocompatibility complex class I alleles in Chinese rhesus macaques. Acta Biochim Biophys Sin (Shanghai) 2008;40:919–27.
[79] Ma X, Tang LH, Qu LB, Ma J, Chen L. Identification of 17 novel major histocompatibility complex-A alleles in a population of Chinese-origin rhesus macaques. Tissue Antigens 2009;73:184–7.
[80] Naruse TK, Chen Z, Yanagida R, Yamashita T, Saito Y, Mori K, et al. Diversity of MHC class I genes in Burmese-origin rhesus macaques. Immunogenetics 2010;62:601–11.
[81] Wambua D, Henderson R, Solomon C, Hunter M, Marx P, Sette A, et al. SIV-infected Chinese-origin rhesus macaques express specific MHC class I alleles in either elite controllers or normal progressors. J Med Primatol 2011;40:244–7.
[82] Otting N, Mörner A, Bontrop RE. Novel major histocompatibility complex class I alleles extracted from two rhesus macaque populations. Tissue Antigens 2011;77:79–80.
[83] Li A, Wang X, Liu Y, Zhao Y, Liu B, Sui L, et al. Preliminary observations of MHC class I A region polymorphism in three populations of Chinese-origin rhesus macaques. Immunogenetics 2012;64:887–94.
[84] Karl JA, Bohn PS, Wiseman RW, Nimityongskul FA, Lank SM, Starrett GJ, et al. Major histocompatibility complex class I haplotype diversity in Chinese rhesus macaques. G3 (Bethesda) 2013;3:1195–201.
[85] Liu Y, Li A, Wang X, Sui L, Li M, Zhao Y, et al. Mamu-B genes and their allelic repertoires in different populations of Chinese-origin rhesus macaques. Immunogenetics 2013;65:273–80.
[86] Leuchte N, Berry N, Köhler B, Almond N, LeGrand R, Thorstensson R, et al. MhcDRB-sequences from cynomolgus macaques (*Macaca fascicularis*) of different origin. Tissue Antigens 2004;63:529–37.
[87] O'Connor SL, Blasky AJ, Pendley CJ, Becker EA, Wiseman RW, Karl JA, et al. Comprehensive characterization of MHC class II haplotypes in Mauritian cynomolgus macaques. Immunogenetics 2007;59:449–62.
[88] Wei H, Wang H, Hou S, Hu S, Fan K, Fan X, et al. DRB genotyping in cynomolgus monkeys from China using polymerase chain reaction -sequence-specific primers. Hum Immunol 2007;68:135–44.
[89] de Groot N, Doxiadis GGM, de Vos-Rouweler AJM, de Groot NG, Verschoor EJ, Bontrop RE. Comparative genetics of a highly divergent DRB microsatellite in different macaque species. Immunogenetics 2008;60:737–48.

[90] Doxiadis GGM, de Vos-Rouweler AJM, de Groot N, Otting N, Bontrop RE. DR haplotype diversity of the cynomolgus macaque as defined by its transcriptome. Immunogenetics 2012;64:31–7.
[91] Aarnink A, Bonhomme M, Blancher A. Positive selection in the major histocompatibility complex class III region of cynomolgus macaques (*Macaca fascicularis*) of the Philippines origin. Tissue Antigens 2013;81:12–8.
[92] Bonhomme M, Blancher A, Jalil MF, Crouau-Roy B. Factors shaping genetic variation in the MHC of natural non-human primate populations. Tissue Antigens 2007;70:398–411.
[93] Slierendregt BL, Otting N, Kenter M, Bontrop RE. Allelic diversity at the Mhc-DP locus in rhesus macaques (*Macaca mulatta*). Immunogenetics 1995;41:29–37.
[94] Khazand M, Peiberg C, Nagy M, Sauermann U. Mhc-DQ-DRB haplotype analysis in the rhesus macaque: evidence for a number of different haplotypes displaying a low allelic polymorphism. Tissue Antigens 1999;54:615–24.
[95] Doxiadis GG, Otting N, de Groot NG, Noort R, Bontrop RE. Unprecedented polymorphism of Mhc-DRB region configurations in rhesus macaques. J Immunol 2000;164:3193–9.
[96] Otting N, de Groot NG, Noort MC, Doxiadis GG, Bontrop RE. Allelic diversity of Mhc-DRB alleles in rhesus macaques. Tissue Antigens 2000;56:58–68.
[97] Vigon N, Sauermann U. Sequence-based typing techniques for rhesus macaque MhcMamu-DQB1 allow the identification of more than 35 alleles. Tissue Antigens 2002;59:88–94.
[98] Doxiadis GGM, Otting N, de Groot NG, de Groot N, Rouweler AJM, Noort R, et al. Evolutionary stability of MHC class II haplotypes in diverse rhesus macaque populations. Immunogenetics 2003;55:540–51.
[99] de Groot N, Doxiadis G, de Groot N, Otting N, Heijmans C, Rouweler A, et al. Genetic Makeup of the DR Region in Rhesus Macaques: gene Content, Transcripts, and Pseudogenes. J Immunol 2004;172:6152.
[100] Penedo MCT, Bontrop RE, Heijmans CMC, Otting N, Noort R, Rouweler AJM, et al. Microsatellite typing of the rhesus macaque MHC region. Immunogenetics 2005;57:198–209.
[101] Doxiadis GGM, de Groot N, Claas FHJ, Doxiadis IIN, van Rood JJ, Bontrop RE. A highly divergent microsatellite facilitating fast and accurate DRB haplotyping in humans and rhesus macaques. Proc Natl Acad Sci U S A 2007;104:8907–12.
[102] Qiu C-L, Yang G-B, Yu K, Li Y, Li X-L, Liu Q, et al. Characterization of the major histocompatibility complex class II DQB (MhcMamu-DQB1) alleles in a cohort of Chinese rhesus macaques (*Macaca mulatta*). Hum Immunol 2008;69:513–21.
[103] Thorsby E. Invited anniversary review: HLA associated diseases. Hum Immunol 1997;53:1–11.
[104] Gardner MB, Luciw PA. Macaque models of human infectious disease. ILAR J 2008;49:220–55.
[105] Goulder PJR, Watkins DI. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. Nat Rev Immunol 2008;8:619–30.
[106] Nehete PN, Singh S, Sastry KJ. Lessons on Non-Progression of HIV Disease from Monkeys. Front Immunol 2013;4:64.
[107] Misra A, Thippeshappa R, Kimata JT. Macaques as model hosts for studies of HIV-1 infection. Front Microbiol 2013;4:176.
[108] Nomura T, Matano T. Association of MHC-I genotypes with disease progression in HIV/SIV infections. Front Microbiol 2012;3:234.
[109] Pal R, Venzon D, Letvin NL, Santra S, Montefiori DC, Miller NR, et al. ALVAC-SIV-gag-pol-env-based vaccination and macaque major histocompatibility complex class I (A*01) delay simian immunodeficiency virus SIVmac-induced immunodeficiency. J Virol 2002;76:292–302.
[110] Zhang Z-Q, Fu T-M, Casimiro DR, Davies M-E, Liang X, Schleif WA, et al. Mamu-A*01 allele-mediated attenuation of disease progression in simian-human immunodeficiency virus infection. J Virol 2002;76:12845–54.
[111] Mothé BR, Weinfurter J, Wang C, Rehrauer W, Wilson N, Allen TM, et al. Expression of the major histocompatibility complex class I molecule Mamu-A*01 is associated with control of simian immunodeficiency virus SIVmac239 replication. J Virol 2003;77:2736–40.
[112] Loffredo JT, Maxwell J, Qi Y, Glidden CE, Borchardt GJ, Soma T, et al. Mamu-B*08-positive macaques control simian immunodeficiency virus replication. J Virol 2007;81:8827–32.
[113] Yant LJ, Friedrich TC, Johnson RC, May GE, Maness NJ, Enz AM, et al. The high-frequency major histocompatibility complex class I allele Mamu-B*17 is associated with control of simian immunodeficiency virus SIVmac239 replication. J Virol 2006;80:5074–7.

[114] Putkonen P, Warstedt K, Thorstensson R, Benthin R, Albert J, Lundgren B, et al. Experimental infection of cynomolgus monkeys (*Macaca fascicularis*) with simian immunodeficiency virus (SIVsm). J Acquir Immune Defic Syndr 1989;2:359–65.
[115] O'Connor SL, Lhost JJ, Becker EA, Detmer AM, Johnson RC, Macnair CE, et al. MHC heterozygote advantage in simian immunodeficiency virus-infected Mauritian cynomolgus macaques. Sci Transl Med 2010;2:22ra18.
[116] Doherty PC, Zinkernagel RM. Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. Nature 1975;256:50–2.
[117] Mee ET, Berry N, Ham C, Sauermann U, Maggiorella MT, Martinon F, et al. Mhc haplotype H6 is associated with sustained control of SIVmac251 infection in Mauritian cynomolgus macaques. Immunogenetics 2009;61:327–39.
[118] Mee ET, Berry N, Ham C, Aubertin A, Lines J, Hall J, et al. Mhc haplotype M3 is associated with early control of SHIVsbg infection in Mauritian cynomolgus macaques. Tissue Antigens 2010;76:223–9.
[119] Aarnink A, Dereuddre-Bosquet N, Vaslin B, Le Grand R, Winterton P, Apoil P-A, et al. Influence of the MHC genotype on the progression of experimental SIV infection in the Mauritian cynomolgus macaque. Immunogenetics 2011;63:267–74.
[120] Zhou Y, Bao R, Haigwood NL, Persidsky Y, Ho W-Z. SIV infection of rhesus macaques of Chinese origin: a suitable model for HIV infection in humans. Retrovirology 2013;10:89.
[121] Trichel AM, Rajakumar PA, Murphey-Corb M. Species-specific variation in SIV disease progression between Chinese and Indian subspecies of rhesus macaque. J Med Primatol 2002;31:171–8.

VetBooks.ir

VetBooks.ir

CHAPTER

4

Genetic Variations in the *Macaca fascicularis* Genome Related to Biomedical Research

Tobias Heckel[*], *Anjali Singh*[*], *Andreas Gschwind*[†], *Alexandre Reymond*[†], *Ulrich Certa*[*]

[*]F. Hoffmann–La Roche Ltd, Pharma Research & Early Development, Basel, Switzerland

[†]Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

OUTLINE

Introduction: Animal Models and Drug Testing 53

Copy Number Variations in the Genome of M. *fascicularis* 55

Conclusion 61

References 62

INTRODUCTION: ANIMAL MODELS AND DRUG TESTING

Prior to use in human studies, regulatory authorities require a clearly defined set of experiments performed *in vitro* and *in vivo* using appropriate animal models. *In vitro* tests include standardized tests such the Ames test for genotoxicity or the micronucleus assay for detection of drug-induced chromosomal instability (The Organization for Economic Co-operation and Development Guidelines for the Testing of Chemicals: Test Guideline 471 "Bacterial Reverse Mutation Test." [1997], and Test Guideline 473 "In Vitro Mammalian Chromosomal Aberration Test" [2012]). *In vivo* testing must be performed in two species, commonly a rodent and a

http://dx.doi.org/10.1016/B978-0-12-417144-2.00004-4
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

nonrodent, that show pharmacological response to the drug under investigation [1]. This requirement is made on the understanding that known species variation makes reliance on tests in one species insufficient for approval of clinical trials in humans [2]. The rat is the preferred rodent model for studies determining dose ranges, acute and chronic toxicity, or life-span carcinogenicity, among other readouts for drug safety. The dog, the miniature pig, and the cynomolgus monkey (*Macaca fascicularis* or the long-tailed macaque) are the most common and best studied nonrodent models [3,4]. In the past decade full genome sequences of all relevant animal models of toxicology testing became publicly available. Whereas the genomes of the mouse [5] and the rat [6] have been sequenced in large consortia over more than a decade using Sanger sequencing, next-generation sequencing added within a few years and with relatively small teams the genomes of the cynomolgus monkey [7,8], the Beagle dog [9], and the miniature pig [9]. These genomes enable the systematic comparison of orthologous sequences of DNA, mRNA, and proteins across multiple species at varying evolutionary distances based on the assumption that evolutionary conservation of sequences correlate with conservation of function [10]. Such ortholog comparisons have proven useful, mainly in the fields of pharmacology but also in toxicology, because they enable the study of translational aspects such as the cross-reactivity of novel drugs. Equally important in this context is the possibility of producing genome-based tools with species specificity such as polymerase chain reaction assays or microarrays for transcript profiling. A first generation of cynomolgus monkey-specific microarrays has been used for an entire genome-wide expression analysis in liver tissues of macaques originating from the Philippines, a Chinese breeding center (Southeast Asia), and Mauritius [7]. This study revealed for the first time origin-specific differences in gene expression affecting about 5% of the transcriptome, mainly involving the genes for sugar, lipid, and amino acid metabolism. In addition, genome sequencing led to the discovery of single nucleotide polymorphisms (SNPs) in each of these genomes. This knowledge now allows for genome-wide association studies in animals to identify genetic factors contributing to a phenotype-like susceptibility to disease or differences in drug response. For example, certain dog breeds, including the Beagle, are prone to developing spontaneously latent diseases. This fact is important for toxicologists to differentiate findings of spontaneous diseases from drug-induced injuries, especially in cases where a genetic predisposition may influence the outcome of a study. In this context, a recent genome-wide association study has been very successful in identifying five genomic loci containing SNPs associated with steroid responsive meningitis-arteritis, which is also known as Beagle pain syndrome [11]. Such loci can serve as predictive genetic biomarkers so that dog cohorts can be tested and stratified regarding disease predisposition. For cynomolgus monkeys, genetic background is at least equally if not even more important than for dog breeds because these macaques are caught in the wild in many different locations within peninsular Southeast Asia, the Philippines, and Mauritius, and they are used to found and continuously refresh breeding programs. Therefore the genetic character of all these cynomolgus monkeys varies as a reflection of the wild cynomolgus populations from which the parents were derived [12]. Furthermore, variability may arise from human influence if animals of differing geographic subpopulations and genetic backgrounds are intermixed. Thus the genetic background of cynomolgus monkeys is an experimental variable that can affect the outcome of biomedical studies. An example has been published by Liu et al. [13]. Their data showed that SNPs of the CD3epsilon T-cell coreceptor in cynomolgus monkeys result in variable binding affinities of CD3epsilon-targeting biologics. This confounded the determination of the lowest

VetBooks.ir

observed effect level, resulting in a major impact on the interpretation of the data [13]. Thus, genotyping before animal experiments has the potential to eliminate experimental variation that can affect drug response. It is of note that side effects, especially in primate studies, are taken seriously by regulatory authorities. A plausible explanation that addresses the question of whether these reactions are drug related and have the potential to translate to humans is required.

COPY NUMBER VARIATIONS IN THE GENOME OF M. *FASCICULARIS*

DNA copy number variations (CNVs) are genetic differences in the normal population displayed as microscopically invisible deletions or amplifications of stretches of genomic DNA ranging from 1 kb and up to the megabase scale [14]. CNV of genomic segments are commonly found in the genomes of humans [15] and model organisms such as the mouse, the rat, or the *Drosophila melanogaster* fly [16]. In humans, more than 2.3 million different CNVs mapping to approximately 200,000 genomic regions have been identified so far [17]. They significantly contribute to genetic variation, covering more nucleotide content per genome than SNPs (e.g., approximately 0.8% of the length of the human genome differs between two human individuals) [18]. Since CNVs can reside in genomic regions harboring genes, it is obvious that CNVs in the form of homozygous deletions act like gene knockout mutants, resulting in a complete loss of gene expression and function. Beyond that, however, it has been shown that CNVs not only affect the expression of genes varying in copy number (amplifications or heterozygous deletions in comparison to a reference) but also impact tissue transcriptomes on a global scale by modifying the level and timing of expression of genes that localize within the CNV [16,19] and on its flanks [20–24], an effect that can extend over the entire length of the affected chromosome [25]. These effects of CNVs are difficult to understand and are not necessarily predictable. Nevertheless, understanding the full impact of CNVs on gene expression regulation helps provide a mechanistic understanding of diseases associated with CNVs either through the dosage of a single gene, such as AIDS susceptibility [26], glomerulonephritis in rats and humans [27], and psoriasis [28], or through expression changes of a contiguous set of genes, such as Williams-Beuren syndrome and infantile spasms [29,30], DiGeorge syndrome [31], Smith-Magenis syndrome [32], Potocki-Lupski syndrome [33], and autism spectrum disorders [34–36]. Moreover, CNV effects on gene expression impart an understanding and association of interindividual variability in pharmacological responses, with CYP2D6 CNVs as the most prominent case example. Such CNVs have been shown to result in humans in nonresponsiveness to antidepressant therapy, an apparently higher incidence of suicides, and toxicity following treatment with the analgesic codeine [37].

Because of the importance of CNVs in biomedical research, we designed the first cynomolgus monkey whole-genome tiling array containing 4.2 million 60-mer probes that interrogate the entire genome at 600 base pair intervals, on average. Excluded from the genome scan are repetitive regions at centromeres and the Y chromosome that have not yet been sequenced. As the CNV reference, we used pooled DNA isolated from 25 cynomolgus males of Mauritian origin. Using male DNA as a baseline has the advantage in that the female chromosomes can be used as a reference for gene duplications and array performance. In Figure 4.1, we show an

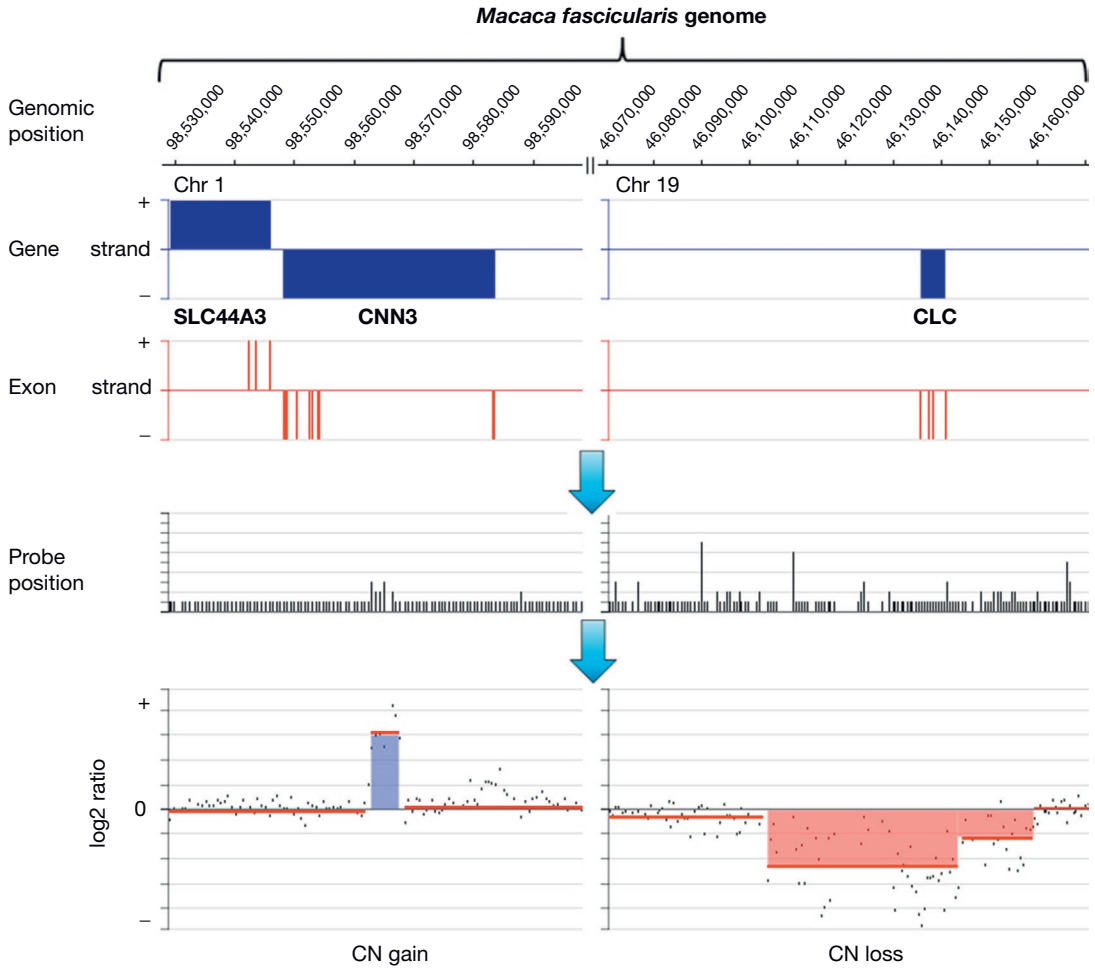


FIGURE 4.1 Detection of copy number variations (CNVs) in one cynomolgus monkey from Mauritius. A 60-kb region on chromosome 1 and a 90-kb region on chromosome 19 were selected as examples for CNV detection in one Mauritian cynomolgus monkey (top). Schematic representations of gene and exon locations within these genomic regions are shown in the middle, together with the genomic positions of the tiling probes. Gaps not covered by probes are either repetitive or not unique, and the bar height per probe represents an increase in a potential ambiguity of the probe. CNV signals in comparison to a reference standard are displayed as log2 ratio (black dots) showing a CNV gain (blue) and loss (red) detected by the SegMNT algorithm (red lines).

example of the tiling probe density across a 60-kb region on chromosome 1 with a CNV gain and a 90-kb region on chromosome 19 with a signal for a CNV loss, both in a male macaque from Mauritius. The amplification effects an intron of the gene calponin 3 (*CNN3*) required for trophoblastic cell fusion [38], whereas the deletion contains the entire locus encoding the Charcot-Leyden crystal galectin (*CLC*). In analogy to a CNV for intronic regulatory elements of the cystic fibrosis transmembrane conductance regulator, the amplified segment could affect transcript splicing and therefore gene expression [39]. The CNV loss in this example will

VetBooks.ir

most likely affect the expression levels of the *CLC* gene, which is associated with a susceptibility to allergic rhinitis [40].

As a next step, we examined CNVs among a group of individual cynomolgus macaques. We chose three females originating from a Chinese breeding center (Southeast Asia) and Mauritius. Based on a low-resolution chromosome view of all data points, we pointed out, as an example, two CNV regions on chromosomes 4 and 13 that show significant CNV loss and one CNV region on chromosome 7 showing a CNV gain in all individuals (Figure 4.2). At the same scale, we detected CNV gains or losses that occur only in one of the individuals, which are marked by red triangles in Figure 4.2. We extended this genome-wide CNV analysis to 24 cynomolgus monkeys and determined that the average number of CNVs per genome is around 800, with a mean length of about 12.5 kb per CNV locus. These numbers are quite comparable with observations made for CNVs in normal human populations, where the majority of CNVs are small (<10-30 kb) [17,41] and where any human individual carries on average about 670 CNV segments [41]. In addition, we analyzed comprehensively the frequency of CNV regions within these 24 monkeys and discovered "private" and common CNVs (Figure 4.3). To this effect, we found that cynomolgus monkey CNVs are nonrandomly distributed throughout the genome, indicating a concentration at chromosomal "hotspots" where CNVs are likely to occur either de novo or throughout evolution. This finding suggests stringent selective mechanisms underlying CNV genesis or maintenance.

Next, we asked the question of whether CNV profiles cluster according to the geographic origin of the primates. Based on a relatively small set of SNPs, Street et al. found that at least some of these variations are indeed restricted to the geographic origin of the monkeys [42]. More relevant for biomedical research is the fact that half of these SNPs are shared with the rhesus macaque [42]. This implies that the "private" cynomolgus SNPs are useful in detecting genomic ingression of rhesus sequences as a result of interspecies hybridization. These observations make it likely that CNVs as indicators of genetic recombination would also cluster according to geographic origin, which was the case (Figure 4.4). The major autosomal CNV profiles of 22 of 24 cynomolgus monkeys cluster according to origin (the Philippines, Southeast Asia, or Mauritius). This implies again that chromosomal target loci for CNVs are nonrandomly distributed over the entire genome and that many CNV loci are "fixed" within a geographical subpopulation and within families of cynomolgus monkeys, which is consistent with the SNP data. When we now take into consideration that the de novo generation rate of SNPs is around 69 new SNPs per offspring genome in humans [43] and that the de novo generation rate of human CNVs is around 1.2 new CNVs per 100 offspring genomes [44], then we can assume that the de novo generation rate of CNVs is rather low in cynomolgus monkeys. This in turn led us to the conclusion that genetic adaptation could drive copy number maintenance at these particular loci. An initial case of adaptive CNV alleles under positive selection was recently uncovered in humans for changes in the gene dosage of the alpha-amylase 1A gene (*AMY1A*). This gene encodes a protein that catalyzes the first step in digestion of dietary starch and glycogen. The copy number of *AMY1A* is three times higher in humans compared to chimpanzees, suggesting that humans were favored in the gene dosage because of a concomitant increase of starch consumption [45]. Thus, it is plausible that genetic adaptation of distinct cynomolgus monkey CNVs can influence gene expression levels, providing a selective advantage to environmental conditions such as nutrition. We tested this hypothesis by analyzing a 2-Mb locus

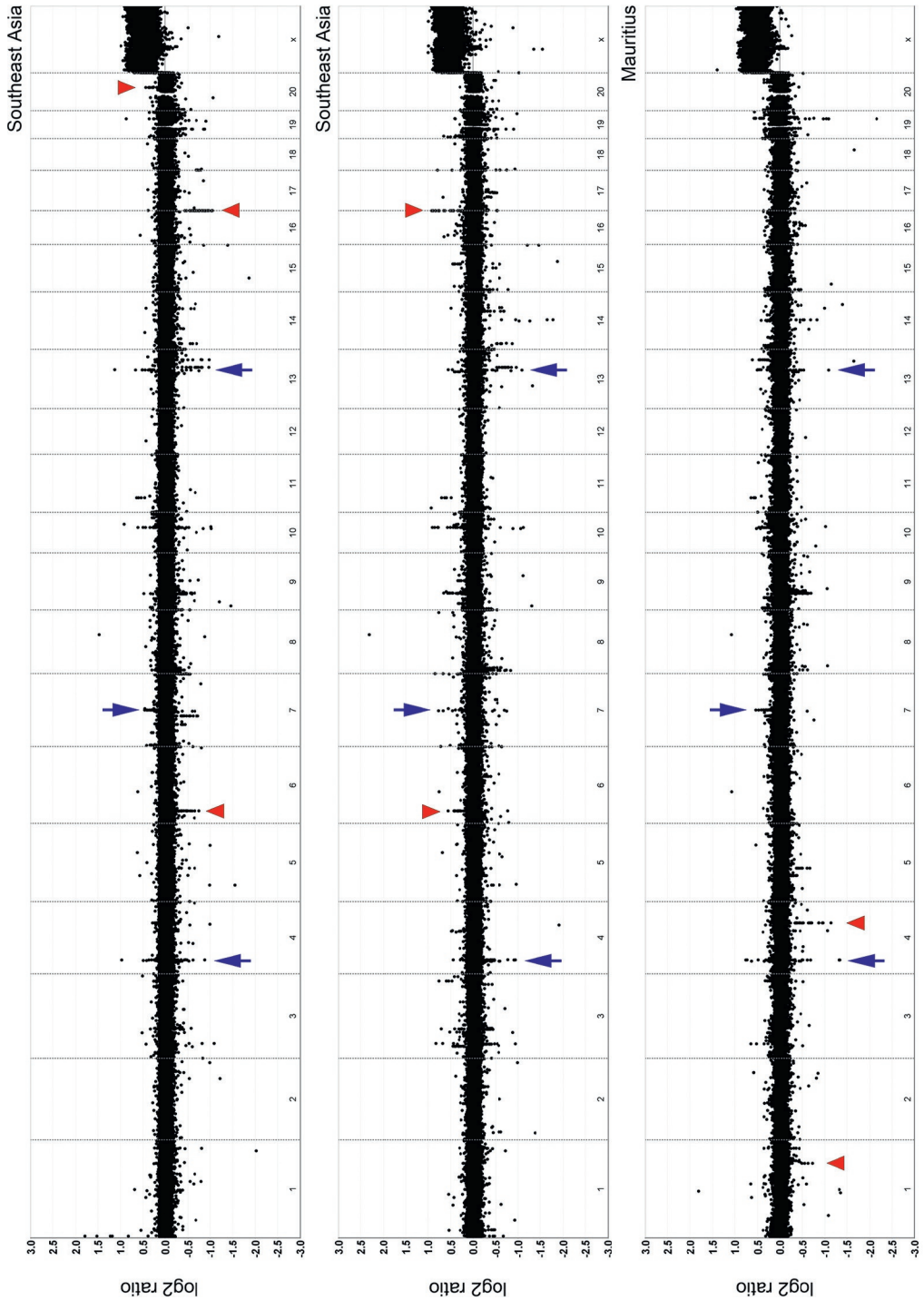


FIGURE 4.2 Genome-wide display of CNV profiles in cynomolgus monkeys. Blue arrows denote conserved CNV gains or losses present in all three individuals. Red triangles denote "private" CNVs. The geographic origin is indicated for each monkey. Note the X chromosome shift at the right end of the display as a result of the comparison of the three female monkeys with a male reference standard.

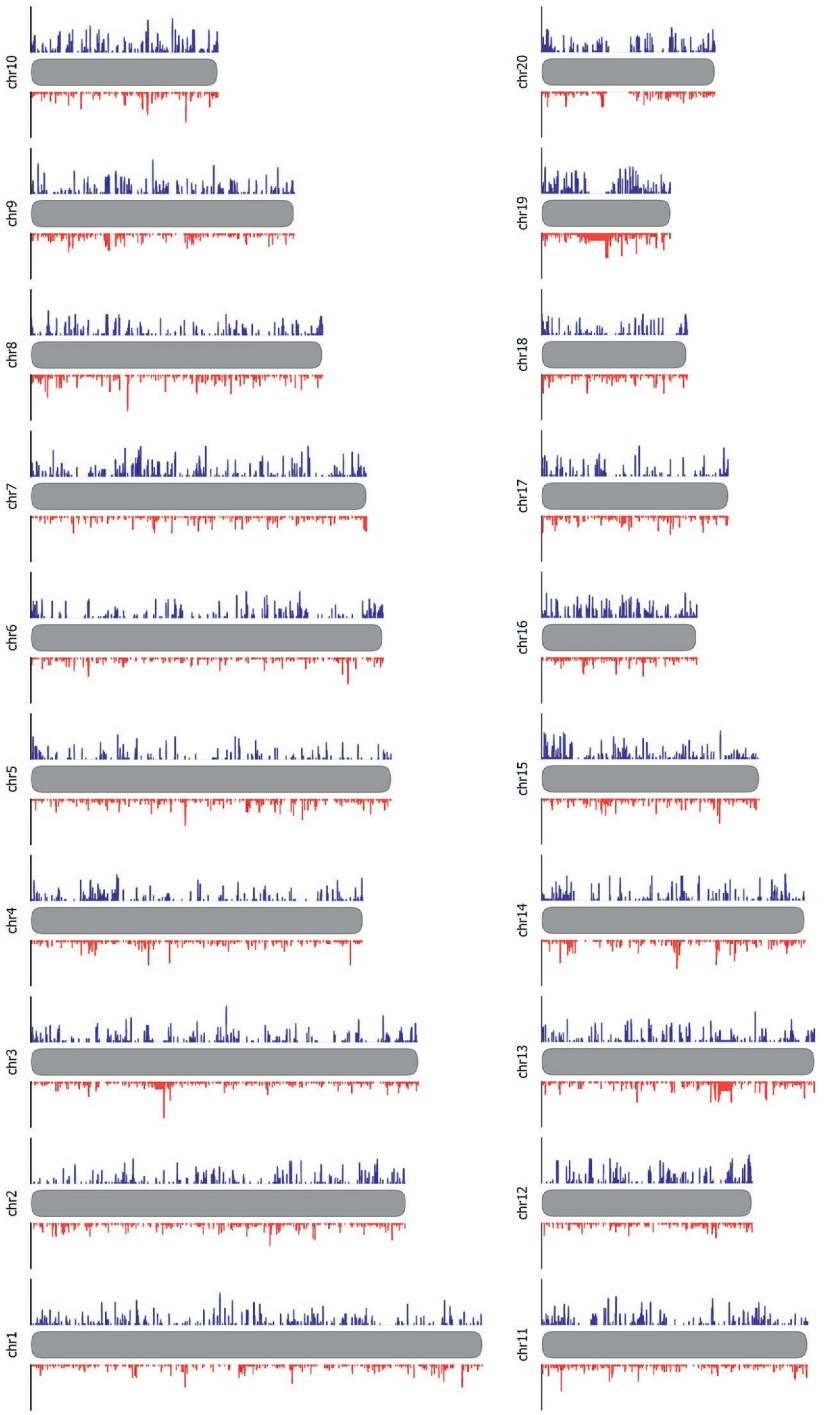


FIGURE 4.3 Ideograms of CNV regions in cynomolgus monkeys. The ideograms show CNV regions and CNV frequencies for 24 cynomolgus monkeys at chromosomal resolution. Common CNVs are reflected by long bars and "private" CNVs by short bars. CNV gains are blue and losses red.

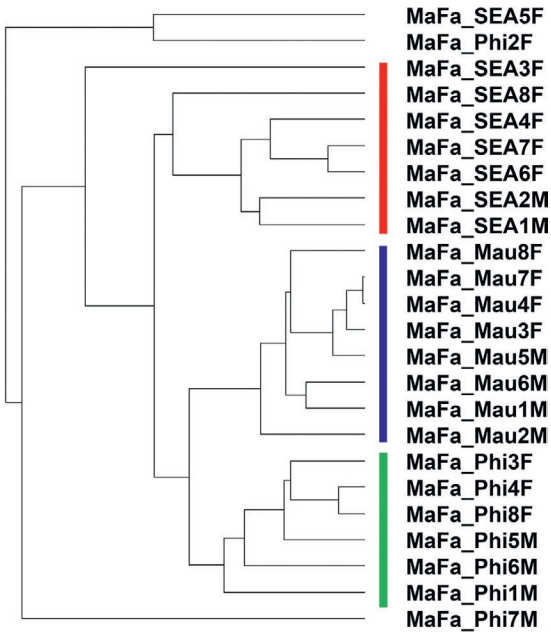


FIGURE 4.4 Geographic clustering according to CNV profiles. The dendrograms show the relationship of CNV clusters found in autosomes of 24 individual cynomolgus monkeys. Gender is indicated by M for male and F for female, and the color bars indicate origin (red = Southeast Asia; blue = Mauritius; green = the Philippines).

on chromosome 19, where we found an approximately 13-kb deletion in two cynomolgus monkeys from Southeast Asia, which affects an intergenic region on this chromosome. We had gene expression microarray data available for the heart, kidney, liver, lung, and spleen of six wild-type animals for this locus in addition to the two animals with the copy number loss. Surprisingly, the copy number loss of the intergenic chromosome segment modulated statistically significantly the expression levels of neighboring genes in the kidney and the heart but not in the other tissues. For example, the mRNA levels of the transcriptional activators FOXA3, MEIS3, NPAS1, and SIX5 were increased in the kidney and the heart, whereas the ubiquitin ligase IRF2BP1, the SUMO-activating enzyme SAE1, the scaffold protein STRN4, the amino acid transporter SLC1A5, the Rho-GTPase-activating protein p190RhoGAP (GRLF1), and the vesicle fusion regulator NAPA were downregulated compared to unaffected animals (Figure 4.5). Interestingly enough, the deletion affects expression of about 45 genes that are encoded up to 1 Mb up- and downstream of this CNV (data not shown). Furthermore, there seems to be a tendency of downmodulating genes closer to the copy number loss, whereas genes farther away seem to be upregulated. The expression levels of genes outside of this 2-Mb locus show relatively high interindividual variation, complicating data interpretation. At this point we can only speculate about the mechanism behind the chromosome-wide effects of the deleted DNA stretch. Such mechanisms could include physical dissociation of transcriptional activators or repressors, alteration of chromatin structure, or repositioning of a genomic region within the nucleus.

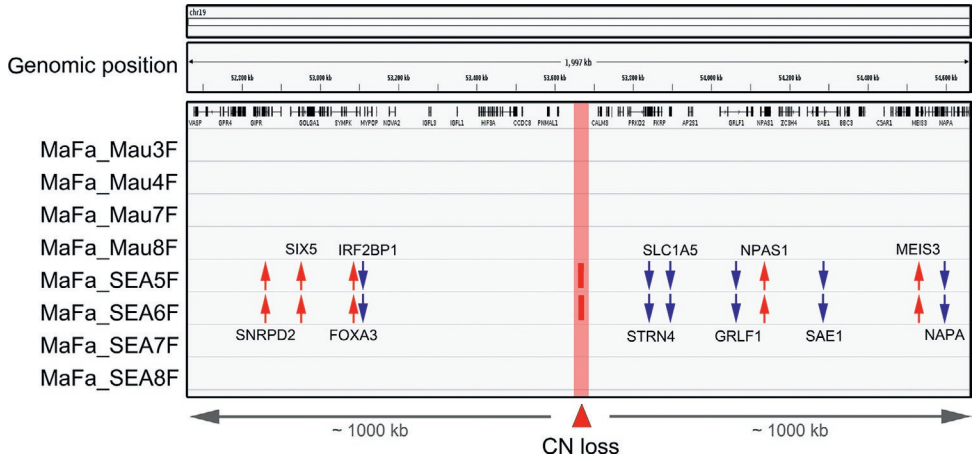


FIGURE 4.5 Relation between CNV and gene expression. Shown is a 2-Mb region on chromosome 19 containing more than 45 gene loci. Two of eight cynomolgus monkeys carry an approximately 13-kilobase deletion in the center of this genomic region. The change of gene expression is indicated for genes strongly affected by the CNV in the carrier compared with noncarrier monkeys based on microarray data for the heart and the kidney. Red arrows indicate gene expression upregulation and blue arrows downregulation. The codes of the individual cynomolgus monkeys appear on the *y*-axis.

CONCLUSION

In this chapter we present the first data on CNV in *M. fascicularis*. We used a cynomolgus monkey-specific comparative genomic hybridization (CGH) array for genome-wide CNV detection. This technology has the advantage that CGH arrays generally show better signal-to-noise ratios compared with SNP arrays, probably as a consequence of longer probes on CGH array platforms, that CGH arrays have greater sensitivity to detect small differences in copy number, and that CGH array probes are evenly distributed across all chromosomes, allowing a more precise characterization of CNV breakpoints, the start and end coordinates of a CNV [46]. Nevertheless, SNP arrays can also be used for CNV genotyping but are especially not feasible in cynomolgus monkeys because the SNPs known so far are not evenly distributed across the genome and therefore give rise to many regions lacking SNPs, which in turn confounds the accuracy of CNV breakpoint determination and the false-negative rate of CNV calling in general.

Although the data set on cynomolgus monkey CNVs is relatively small, we can draw some conclusions of relevance to biomedical research. First, occurrence of CNVs per animal is much lower than the number of SNPs, with an estimate of approximately 800 CNVs per animal across all autosomes in comparison to approximately 2 million SNPs per animal [7]. Second, similar to SNPs, the CNV loci cluster according to geographic origin likely is due to limited genetic recombination between the peninsular Southeast Asian and insular macaque populations. Third, we found that CNVs alter the expression levels of genes over a distance of up to 1 Mb in drug-naïve cynomolgus monkeys, as seen for a deletion on chromosome 19 and an amplification on chromosome 14 (data not shown). Because of the CNVs' impact on genes within the CNV [16,19] and on its flanks, an effect that can extend

VetBooks.ir

over a thousand kilobases from the breakpoints [20–25], the effects of copy number gains or losses are difficult to predict. Although one would expect a priori increased expression levels of amplified and decreased expression levels of heterozygous deleted genes, experimental validation of CNV effects is still necessary, especially when it comes to the long range effects of CNVs.

With respect to genes relevant for pharmacology and toxicology, CNVs can serve as a biomarker to understand the genetic contribution to interindividual variability in gene expression and as a consequence to variability in drug response. For pharmacokinetic genes in humans, there have been some notable findings developing from candidate gene approaches. The *CYP2D6* gene, which encodes an enzyme for the phase I metabolism of a large number of drugs, has occurred in CNVs of 0-13 copies [47]. These CNVs in turn drastically affect the plasma concentrations of drugs so that guidelines for dose adjustments or recommendations of alternative agents on the basis of the genotype have been formulated for antidepressants [48]. For the phase II family of drug-conjugating enzymes, pharmacogenomic studies have highlighted a CNV-based mechanism for the sulfotransferase SULT1A1 that may lead to increased risk for chemical carcinogenesis and adverse drug reactions [49–51].

These findings as well as our own work in cynomolgus monkeys demonstrate that CNVs represent an additional genetic element apart from SNPs or epigenetic modifications that can alter gene expression levels. They also indicate that CNVs can serve as expression quantitative trait loci. This characteristic of CNVs highlights the necessity of incorporating copy number analysis to elucidate the genetic underpinnings for interindividual differences in the outcomes of drug treatments. Inclusion of CNV data in retrospective analyses of outlier animals in pharmacokinetic or toxicological studies can provide a rationale for explaining the observed phenotypic differences. For example, detection of a CNV in the outlier animal that causes an expression difference of the drug target or of drug-metabolizing enzymes in comparison to the rest of the test animals can provide a mechanistic link to explain the phenotypic variation. Such CNVs can then be used for prescreening and stratification of animals in follow-up experiments by simple blood testing. In addition, our results further suggest indirectly that CNV markers for a specific geographic origin can be identified, selected, and used to exclude monkeys with different CNV profiles and origins. Such an analysis might be useful in Mauritian cynomolgus monkey populations since all founders were imported to the island by trading ships 400-500 years ago [42].

We are currently expanding our CNV data set using data from other geographic origins such as Cambodia and Vietnam. This data set is part of a large exome-sequencing project, and we will use exon frequency as a surrogate for losses or gains of CNV genes. As pointed out in this chapter, this approach may miss variations residing in noncoding regions. Therefore, the use of dedicated CNV microarrays is probably the most reliable and affordable technology for CNV detection with the shortest time to result.

References

[1] Zbinden G. The concept of multispecies testing in industrial toxicology. Regul Toxicol Pharmacol 1993;17:85–94.
[2] Animal-Procedures-Committee. The use of primates under the Animals (Scientific Procedures) Act (1986); 2002.
[3] Helke KL, Swindle MM. Animal models of toxicology testing: the role of pigs. Expert Opin Drug Metab Toxicol 2013;9:127–39.
[4] Quigley M. Non-human primates: the appropriate subjects of biomedical research? J Med Ethics 2007;33:655–8.

[5] Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal R, et al. Initial sequencing and comparative analysis of the mouse genome. Nature 2002;420:520–62.
[6] Gibbs RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, Scherer S, et al. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature 2004;428:493–521.
[7] Ebeling M, Kung E, See A, Broger C, Steiner G, Berrera M, et al. Genome-based analysis of the nonhuman primate *Macaca fascicularis* as a model for drug safety assessment. Genome Res 2011;21:1746–56.
[8] Yan G, Zhang G, Fang X, Zhang Y, Li C, Ling F, Cooper DN, et al. Genome sequencing and comparison of two nonhuman primate animal models, the cynomolgus and Chinese rhesus macaques. Nat Biotechnol 2011;29:1019–23.
[9] Vamathevan JJ, Hall MD, Hasan S, Woollard PM, Xu M, Yang Y, et al. Minipig and beagle animal model genomes aid species selection in pharmaceutical discovery and development. Toxicol Appl Pharmacol 2013;270:149–57.
[10] Searls DB. Pharmacophylogenomics: genes, evolution and drug targets. Nat Rev Drug Discov 2003;2:613–23.
[11] Wilbe M, Jokinen P, Truve K, Seppala EH, Karlsson EK, Biagi T, et al. Genome-wide association mapping identifies multiple loci for a canine SLE-related disease complex. Nat Genet 2010;42:250–4.
[12] Stevison LS, Kohn MH. Determining genetic background in captive stocks of cynomolgus macaques (*Macaca fascicularis*). J Med Primatol 2008;37:311–7.
[13] Liu YY, Wang Z, Thomas J, Goodwin KJ, Stavrou S, Neville Jr. DM. Polymorphisms of CD3epsilon in cynomolgus and rhesus monkeys and their relevance to anti-CD3 antibodies and immunotoxins. Immunol Cell Biol 2007;85:357–62.
[14] Scherer SW, Lee C, Birney E, Altshuler DM, Eichler EE, Carter NP, et al. Challenges and standards in integrating surveys of structural variation. Nat Genet 2007;39:S7–15.
[15] Redon R, Ishikawa S, Fitch KR, Feuk G, Perry GH, Andrews TD, et al. Global variation in copy number in the human genome. Nature 2006;444:444–54.
[16] Henrichsen CN, Chaignat E, Reymond A. Copy number variants, diseases and gene expression. Hum Mol Genet 2009;18:R1–8.
[17] MacDonald JR, Ziman R, Yuen RK, Feuk L, Scherer SW. The Database of Genomic Variants: a curated collection of structural variation in the human genome. Nucleic Acids Res 2014;42:D986–92.
[18] Conrad DF, Pinto D, Redon R, Feuk L, Gokcumen O, Zhang Y, et al. Origins and functional impact of copy number variation in the human genome. Nature 2010;464:704–12.
[19] Chaignat E, Yahya-Graison EA, Henrichsen CN, Chrast J, Schutz F, Pradervand S, et al. Copy number variation modifies expression time courses. Genome Res 2011;21:106–13.
[20] Henrichsen CN, Vinckenbosch N, Zollner S, Chaignat E, Pradervand S, Schutz F, et al. Segmental copy number variation shapes tissue transcriptomes. Nat Genet 2009;41:424–9.
[21] Cahan P, Li Y, Izumi M, Graubert TA. The impact of copy number variation on local gene expression in mouse hematopoietic stem and progenitor cells. Nat Genet 2009;41:430–7.
[22] Merla G, Howald C, Henrichsen CN, Lyle R, Wyss C, Zabot MT, et al. Submicroscopic deletion in patients with Williams-Beuren syndrome influences expression levels of the nonhemizygous flanking genes. Am J Hum Genet 2006;79:332–41.
[23] Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 2007;315:848–53.
[24] Orozco LD, Cokus SJ, Ghazalpour A, Ingram-Drake L, Wang S, van Nas A, et al. Copy number variation influences gene expression and metabolic traits in mice. Hum Mol Genet 2009;18:4118–29.
[25] Ricard G, Molina J, Chrast J, Gu W, Gheldof N, Pradervand S, et al. Phenotypic consequences of copy number variation: insights from Smith-Magenis and Potocki-Lupski syndrome mouse models. PLoS Biol 2010;8:e1000543.
[26] Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, Catano G, et al. The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. Science 2005;307:1434–40.
[27] Aitman TJ, Dong R, Vyse TJ, Norsworthy J, Johnson MD, Smith J, et al. Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. Nature 2006;439:851–5.
[28] Hollox EJ, Huffmeier U, Zeeuwen PL, Palla R, Lascorz J, Rodijk-Olthuis D, et al. Psoriasis is associated with increased beta-defensin genomic copy number. Nat Genet 2008;40:23–5.
[29] Bayes M, Magano LF, Rivera N, Flores R, Perez Jurado LA. Mutational mechanisms of Williams-Beuren syndrome deletions. Am J Hum Genet 2003;73:131–51.

[30] Marshall CR, Young EJ, Pani AM, Freckmann ML, Lacassie Y, Howald C, et al. Infantile spasms is associated with deletion of the MAGI2 gene on chromosome 7q11.23-q21.11. Am J Hum Genet 2008;83:106–11.
[31] Baldini A. DiGeorge syndrome: an update. Curr Opin Cardiol 2004;19:201–4.
[32] Bi W, Yan J, Stankiewicz P, Park SS, Walz K, Boerkoel CF, et al. Genes in a refined Smith-Magenis syndrome critical deletion interval on chromosome 17p11.2 and the syntenic region of the mouse. Genome Res 2002;12:713–28.
[33] Potocki L, Chen KS, Park SS, Osterholm DE, Withers MA, Kimonis V, et al. Molecular mechanism for duplication 17p11.2 – the homologous recombination reciprocal of the Smith-Magenis microdeletion. Nat Genet 2000;24:84–7.
[34] Gilman SR, Iossifov I, Levy D, Ronemus M, Wigler M, Vitkup D. Rare de novo variants associated with autism implicate a large functional network of genes involved in formation and function of synapses. Neuron 2011;70:898–907.
[35] Levy D, Ronemus M, Yamrom B, Lee H, Leotta A, Kendall J, et al. Rare de novo and transmitted copy-number variation in autistic spectrum disorders. Neuron 2011;70:886–97.
[36] Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, et al. Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 2011;70:863–85.
[37] Johansson I, Ingelman-Sundberg M. Genetic polymorphism and toxicology – with emphasis on cytochrome p450. Toxicol Sci 2011;120:1–13.
[38] Shibukawa Y, Yamazaki N, Kumasawa K, Daimon E, Tajiri M, Okada Y, et al. Calponin 3 regulates actin cytoskeleton rearrangement in trophoblastic cell fusion. Mol Biol Cell 2010;21:3973–84.
[39] Rowntree RK, Vassaux G, McDowell TL, Howe S, McGuigan A, Phylactides M, et al. An element in intron 1 of the CFTR gene augments intestinal expression in vivo. Hum Mol Genet 2001;10:1455–64.
[40] Bryborn M, Hallden C, Sall T, Cardell LO. CLC – a novel susceptibility gene for allergic rhinitis? Allergy 2010;65:220–8.
[41] Park H, Kim JI, Ju YS, Gokcumen O, Mills RE, Kim S, et al. Discovery of common Asian copy number variants using integrated high-resolution array CGH and massively parallel DNA sequencing. Nat Genet 2010;42:400–5.
[42] Street SL, Kyes RC, Grant R, Ferguson B. Single nucleotide polymorphisms (SNPs) are highly conserved in rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. BMC Genomics 2007;8:480.
[43] Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, et al. Rate of de novo mutations and the importance of father's age to disease risk. Nature 2012;488:471–5.
[44] Itsara A, Wu H, Smith JD, Nickerson DA, Romieu I, London SJ, et al. De novo rates and selection of large copy number variation. Genome Res 2010;20:1469–81.
[45] Perry GH, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, et al. Diet and the evolution of human amylase gene copy number variation. Nat Genet 2007;39:1256–60.
[46] Pinto D, Darvishi K, Shi X, Rajan D, Rigler D, Fitzgerald T, et al. Comprehensive assessment of array-based platforms and calling algorithms for detection of copy number variants. Nat Biotechnol 2011;29:512–20.
[47] Johansson I, Lundqvist E, Bertilsson L, Dahl ML, Sjoqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. Proc Natl Acad Sci U S A 1993;90:11825–9.
[48] Hicks JK, Swen JJ, Thorn CF, Sangkuhl K, Kharasch ED, Ellingrod VL, et al. Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. Clin Pharmacol Ther 2013;93:402–8.
[49] Hebbring SJ, Moyer AM, Weinshilboum RM. Sulfotransferase gene copy number variation: pharmacogenetics and function. Cytogenet Genome Res 2008;123:205–10.
[50] Palli D, Rizzolo P, Zanna I, Silvestri V, Saieva C, Falchetti M, et al. SULT1A1 gene deletion in BRCA2-associated male breast cancer: a link between genes and environmental exposures? J Cell Mol Med 2013;17:605–7.
[51] Daniels J, Kadlubar S. Sulfotransferase genetic variation: from cancer risk to treatment response. Drug Metab Rev 2013;45:415–22.

VetBooks.ir

PART II

COMPARATIVE PHYSIOLOGY OF NONHUMAN PRIMATE SPECIES USED IN NONCLINICAL SAFETY ASSESSMENT AND DRUG DEVELOPMENT

VetBooks.ir

VetBooks.ir

CHAPTER

5

Basic Physiology of *Macaca fascicularis*

Iris D. Bolton
University of Texas Medical Branch, Galveston, TX, USA

OUTLINE

History and Introduction	67	Hepatic and Urinary	78
Appearance and Morphology	69	Other Organ Systems	80
Hematology	70	Endemic Diseases and Susceptibility	81
Serum Chemistry	73	Behavior	82
Reproduction and Development	75	References	83
Cardiovascular	77		

HISTORY AND INTRODUCTION

Throughout the last century, the Indian-origin rhesus monkey (*Macaca mulatta*) was the nonhuman primate model of choice in biomedical research. On March 31, 1978, the Indian government banned the export of rhesus monkeys. The effect on biomedical research was extensive. Several breeding colonies in the United States existed and could supply limited numbers of this historically important model. However, even under the best of circumstances, the limited colonies available could not meet the demands of biomedical research. This forced researchers to seek a more cost-effective and readily available nonhuman primate for use in biomedical research.

The cynomolgus monkey (*Macaca fascicularis*, synonymous with *Macaca irus* and *Macaca cynomolgus*, the long-tailed macaque or the crab-eating macaque) is a close relative of the

http://dx.doi.org/10.1016/B978-0-12-417144-2.00005-6
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

rhesus monkey. It has the largest geographic dispersal of all nonhuman primates. The natural range of the cynomolgus monkey includes Southern Indochina, Myanmar (Burma), Indonesia, the Philippines, and the Nicobar Islands. Since the Division of Global Migration and Quarantine at the Centers for Disease Control and Prevention (CDC) began tracking nonhuman primate importations by country in 2004, cynomolgus monkeys have represented a significant percentage of the nonhuman primates imported into the United States. Figure 5.1, CDC Import Data, is a graphical representation of the cumulative information shared annually at the Association of Primate Veterinarians Workshop from 2004 to 2013 by the CDC [G. Galland, personal communication, November 18, 2013]. It is significant that the cynomolgus monkeys imported most often originate from China or Mauritius without being indigenous to either region. This significance is explored in greater depth later in this section. Over time the number of cynomolgus monkeys imported from areas where they are indigenous is a small and declining proportion of the overall number.

The cynomolgus monkey is protected under the Convention on International Trade in Endangered Species of Wild Fauna and Flora and is listed in its Appendix II. Appendix II includes species not necessarily threatened with extinction but affected in such a manner that the trade of these animals may need to be controlled to avoid utilization that is incompatible with their survival [1]. Although some of the insular constituents of the species may be more threatened than others, the species is highly adaptable to the presence of humans, a wide variety of food sources, and to geographic locations, and it is not threatened with extinction.

The Mauritius-origin or "African" cynomolgus monkey was introduced to Mauritius by the shipping trade approximately 400 years ago [2] along with Java deer from Indonesia [3]. The deer were imported as a potential source of protein for the long trip around the horn. The monkeys were most likely brought along as either pets or as an alternative food source and accidentally left behind. Both the deer and the monkeys have been very successful on Mauritius. The founder population of Indonesian cynomolgus monkeys that contributed to the now large population found in Mauritius must have been relatively healthy resulting in a nonhuman primate source that is believed to be free of the most commonly screened for viruses [4]. In addition, the genetic diversity of the population of cynomolgus monkeys found on Mauritius is limited. It has been speculated that the original founding stock may have been 15 or fewer animals [5]. These factors have made the Mauritius-origin cynomolgus monkey a highly desirable colony from both an occupational health and a genetic research perspective.

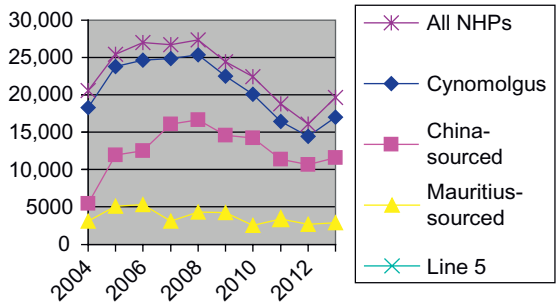


FIGURE 5.1 CDC import data as presented at the annual Association of Primate Veterinarians Workshop, 2004–2013, demonstrating the proportion of nonhuman primate imports into the United States that are cynomolgus monkeys.

VetBooks.ir

The cynomolgus monkey indigenous to China is a curious animal. Under the influence of "paramount leader" Deng Xiaoping from 1978 to 1992, China saw an economic reform that became known as a "socialist market economy." This movement effectively opened China to international trade and invited investors [6]. In part because of China's emergence into the world marketplace and in response to the shifting demand for an alternative to the Indian-origin rhesus, China became a leading supplier of rhesus monkeys. In addition, cynomolgus monkey breeding stock was acquired and multiple monkey breeding farms were established to secure a portion of that market [7]. Part of what makes this a curious animal to study is sorting out the genetic makeup of the Chinese-origin cynomolgus monkey and recognizing the impact their natural history could have in the context of regulated and controlled research.

The cynomolgus monkey species can loosely be divided into three geographic subgroups consisting of the mainland or Indochinese cynomolgus, the Mauritius-origin cynomolgus, and the insular Asian cynomolgus monkeys. This determination is based on mitochondrial DNA analysis [8,9]. The Isthmus of Kra seems to be a natural boundary dividing the cynomolgus as a species, as is the case with other plants and animals [10]. It has been demonstrated that cynomolgus monkeys originating from sites north of the Isthmus of Kra are highly influenced genetically by the Chinese rhesus monkey [9–12]. The Mauritius cynomolgus monkey population is believed to have originated from 15 or fewer founder animals that were introduced to the island from Indonesia [2,5]. The insular Asian cynomolgus monkey is believed to be free of rhesus monkey influence [9] and probably is representative of the original cynomolgus monkey. The Malaysian and Indonesian cynomolgus monkey population seems to exhibit the greatest diversity, whereas the Mauritius-origin cynomolgus population exhibits the least [8]. Once one accepts the geographic division of the species, accounts for the evolutionary pressures placed on each population over time, and considers the implication of industry-mediated selection pressure, it is difficult to ignore the potential diversity found among cynomolgus monkeys, even from geographically close but "closed" facilities. Failure to account for these differences has the potential for severe consequences in research and the reproducibility/reliability of study results [13]. The goal of this chapter is to provide basic information about the cynomolgus monkey and provide a resource for more definitive information based on the needs of the reader.

APPEARANCE AND MORPHOLOGY

The Pictorial Guide to the Living Primates was published by Pegonias Press in 1996 [14]. A "coffee table" style book owned by many primatologists, it contains pictures and normative data on nearly all of the nonhuman primates. It serves as a simple, cursory reference for basic information about nonhuman primate species in addition to providing photographs. The cynomolgus monkey is described as:

> The body of long-tailed macaques varies from gray to reddish brown, with lighter underparts. The hair on the crown of the head grows directly backward, often resulting in a pointed crest. The face is pinkish. Males have cheek whiskers and a mustache; females have a beard. Infants are born black. [14]

This is an excellent description of the prototypical cynomolgus monkey, but it does not capture all of the variations observed in this species. Villano et al. demonstrated that Singaporean

VetBooks.ir

and Bintan cynomolgus monkeys have darker pelage than their Vietnamese counterparts, as determined by color reflectometry [15]. This characterization supports Gloger's rule, which states that warm-blooded animals develop more melanin pigment, resulting in a darker skin and coat the closer they live to the equator [16]. It has also been shown that the Vietnamese cynomolgus monkey is larger and heavier but shorter in body length than those found in island populations [15]. This observation follows Bergmann's rule that warm-blooded animals from warmer regions are smaller than those from colder regions [16]. The influence of the larger-bodied rhesus monkey [10] on the Vietnamese cynomolgus monkey might account for this variation in size. Differences in tail length and coloration [17,18] also point to the rhesus monkey influence on the mainland Asian cynomolgus monkey. It has been reported that up to 30% of the Chinese cynomolgus genome is Chinese rhesus monkey in origin [12].

Some generalizations about morphometry can be drawn from the literature. Male cynomolgus monkeys are larger than females in all geographic regions [19,20] as expected. The cynomolgus monkey from Mauritius is larger than the cynomolgus monkey from the Philippines [19]. Figure 5.2 lists indices of normal cynomolgus monkey information with relevant information about origin as available.

HEMATOLOGY

Blood is commonly collected from the cephalic, saphenous, or femoral veins in cynomolgus monkeys [21]. This can be accomplished with or without anesthesia.

In planning safety assessment studies utilizing the cynomolgus monkey, having basic reference information regarding hematology is important. There is no shortage of referenced hematology values in the literature, which is available on many suppliers' Websites [22] or even within a given testing facility based on their own historical results. Figure 5.3 is provided to demonstrate mean hematology values reported for cynomolgus monkeys. This list is not meant to be exhaustive but to demonstrate some of the variables for consideration and the variability in "normal" from facility to facility that might affect the suitability for use of this data in certain populations of animals. Without complete knowledge of the variables surrounding the blood collection, processing, and interpretation of data, caution must be exercised before readily accepting published values. Some variables for consideration include the number of animals used, age, gender, colony and country of origin, health status, diet, geographic environment, method of restraint, location of sampling, device used to process samples, and statistical methods used in reporting data.

Because the variables surrounding reported hematologic indices are so diverse, it precludes absolute statements even though some trends and conclusions can be observed. The lifespan of the erythrocyte is approximately 100 days and reticulocyte counts are low [23]. Monocytes are larger than lymphocytes and are also present in low numbers [23]. Lymphocyte numbers generally exceed neutrophil numbers [19,20,23–25]. Sugimoto et al. report an inversion in neutrophil to lymphocyte numbers in animals aged 5-10 years, [26] but because the country of origin of the animals is not stated, this should be interpreted with caution because this may not apply to all cynomolgus monkeys. Changes relative to age are an initial decrease in packed cell volume, mean corpuscular volume (MCV), and hemoglobin (Hgb) seen between 1 and 3 months of age [23]; Hgb, MCV, and mean corpuscular hemoglobin content continue to decline slightly over

Reference	21	22	25	50	51	51	51	51
Age			2 years	3-9 years	25 - 36 months	25 - 36 months	37 - 48 months	37-48 months
Gender				Male	Male	Female	Male	Female
Origin			Mauritius	Indonesian	Chinese	Chinese	Chinese	Chinese
PT (s)	9.9-11.1		13.1-13.9					
APTT (s)	18.2-22.6		25.7-34.2					
D-dimer (ug/mL)			0.40-1.68					
TAT complex (ug/L)			3.5-14.6					
Oral dose volume (mL/kg)		5						
Subcutaneous dose volume (mL/kg)		2						
Intramuscular dose volume (mL/kg)		0.25						
Intravascular dose volume - bolus (mL/kg)		2						
Intravascular infusion (mL/kg/24 h)		2.5						
Total infusion volume (mL/24 h)		60						
Cardiothoracic ratio (%)				0.55	0.59	0.58	0.58	0.56

FIGURE 5.2 Normal data demonstrating indices reported in the literature. Blank cells indicate values not provided in the cited reference.

Reference	20	20	20	20	26	26	27	27	32	32	32	32	32	32
Age	3-5 years	3-5 years	3-5 years	3-5 years	2 years	2 years	3 - 7 yrs	3 - 7 yrs	< 1 year	1 - 4 years	> 5 years	< 1 year	1 - 4 years	> 5 years
Gender	Male	Male	Female	Female	Male	Female	Male	Female	Male	Male	Male	Female	Female	Female
Origin	Chinese	Chinese	Chinese	Chinese	Mauritius	Mauritius	China	China						
Fasted?	No	No	No	No					Yes	Yes	Yes	Yes	Yes	Yes
Anesthetized?	Yes	No	Yes	No					Yes	Yes	Yes	Yes	Yes	Yes
Collection site	Cephalic	Cephalic	Cephalic	Cephalic			femoral	femoral	femoral	femoral	femoral	femoral	femoral	femoral
Analyzer	*a*	*a*	*a*	*a*			*b*	*b*	*c*	*c*	*c*	*c*	*c*	*c*
AST (IU/L)	64.86	41.03	50.83	36.8	43	45	34.38	29.90	57.8	60.90	40.20	41.70	49.00	47.90
ALT (IU/L)	52.17	40.16	72.48	42.49	38	34	37.74	45.79	28.0	25.70	23.20	15.50	26.90	32.50
AlkPhos (IU/L)	1829.2	1923.0	479.0	524.19	2731	2134	1230.4	784.8	913.0	610.0	226.00	973.0	538.00	230.00
BUN (mg/dL)	18.33	17.67	20.06	15.16	48	40	22.17	19.04	42.40	51.20	38.80	38.70	46.00	49.50
Creatinine (mg/dL)	0.79	0.85	0.84	0.84	0.85	0.83	0.6409	0.5466	1.01	0.94	1.25	0.73	1.03	1.03
Glucose (mg/dL)	63.68	83.63	73.61	87.41	73	80	83.82	80.07	63.20	57.30	62.2	82.00	61.00	53.60
Cholesterol (mg/dL)	196.86	126.13	137.64	129.71	132	121	115.6	140.5	207.6	144.30	133.5	181.00	142.90	149.80
Total Protein (g/dL)	7.43	7.53	7.73	7.86	7.9	8.0	7.435	7.615	7.50	7.60	8.30	7.40	8.00	8.20
Albumin (g/dL)	4.45	4.60	4.39	4.51	4.89	4.99								
Globulin (g/dL)					3.0	3.0								
CPK (IU/L)	732.16	194.68	236.50	126.31	252	171	310.0	186.4	503.00	405.00	320.00	281.0	447.00	360.00
Ca (mg/dL)	0.41	2.91	9.35	9.51	11.6	12.9	10.136	10.057						
P (mg/dL)	6.03	7.02	4.11	5.85	8.4	4.5	6.052	5.198						
Na (meq/L)	147.47	152.47	147.75	152.69	157.0	160								
Cl (meq/L)	108.84	106.68	105.75	107.63										
K (meq/L)	4.68	5.20	4.81	5.30	6.28	6.13								

Values presented are the reported means. Values may have been converted for comparison purposes. For additional information please see cited reference.

a. Shimadzu CL-7200; Shimadzu Co., Kyoto, Japan & 644 NA/K/Cl Analyzer; Ciba-Corning, Medfield, MA, USA

b. BM-8 autoanalyzer; JEOL, Tokyo, Japan

c. R.A. 1000 Technicon Co.

FIGURE 5.3 Hematologic Indices are provided to give examples of these indices reported in the literature. Cited references can be used for comparison based on the demographics and other factors affecting a given cohort of cynomolgus monkeys. Blank cells indicate values not provided in the cited reference.

VetBooks.ir

VetBooks.ir

time [27]. Cynomolgus monkeys from Mauritius are often reported to have higher red blood cell (RBC) and lower white blood cell numbers than Asian cynomolgus [19,23,27].

A physiologic leukocytosis characterized by a lymphocytosis with or without neutrophilia has been reported in samples collected from unanesthetized and untrained animals [23]. Other sources report that lymphocyte numbers are lower in sedated monkeys [20,27,28]. Regardless of whether the difference between awake and anesthetized lymphocyte counts is precipitated by stress or secondary to the anesthetic of choice, the study director needs to be aware if anesthesia will be consistently used or not during the predose, dosing, and postdose phase of the studies.

Regardless of country of origin, mature male monkeys demonstrate higher values in erythrocytes (RBC), Hgb, and hematocrit [26,27,29] than females. A generalization cannot be made because of conflicting reports regarding the effect of gender on MCV [23,30]. Menstruating females exhibit variable hematologic indices [23,30], making stage of cycle an important consideration in sexually mature females.

SERUM CHEMISTRY

Drug development and safety assessment test systems are routinely subjected to serum chemistry analysis as a means to assess test article impacts on organ systems, particularly those involved with the metabolism and excretion of the test article [24]. Figure 5.4 is provided to demonstrate a representative sampling of serum biochemical values reported in the literature. As with hematology, there are a number of variables that, if not disclosed and accounted for, have the potential to impart confounding of data generated during the course of a study. Among these are sex, country of origin, anesthesia, fed state, age, equipment used to generate data, and the statistical means of generating the data to be reported [19,20,24,25,29–32].

Conclusions that can be drawn from the work of Drevon-Gaillot et al. are that cynomolgus monkeys from Mauritius can be anticipated to have higher serum calcium (Ca) and lower serum phosphorus (P) than cynomolgus monkeys from either the Philippines or Vietnam [19]. Cynomolgus monkeys indigenous to the Philippines demonstrate lower creatine phosphokinase (CPK) values and the lowest alanine aminotransferase values compared with the Mauritius-origin or the Vietnamese cynomolgus monkey [19]. Vietnamese-origin cynomolgus have lower serum alkaline phosphatase (ALP) and aspartate aminotransferase. The Vietnamese cynomolgus monkeys were, in general, younger than the Philippine and Mauritius cynomolgus evaluated. Because ALP has been shown to decline with age [29], it would seem intuitive that the ALP of the Vietnamese cynomolgus monkeys would have been higher, yet it is not. This is an excellent example of how the country of origin of a cohort of animals might introduce confusion regarding results.

The significance of gender on serum biochemical values seems somewhat inconsistent in the literature. This may be in part that the menstrual cycle of females influences their serum chemistry indices. Perretta et al. and Bourges-Abella et al. both report that differences in serum biochemical vales observed by gender are not significant [27,30], whereas others have reported that not only is gender an important variable for consideration [29], but that there are at least 13 commonly assessed or reported biochemical indices that vary in a predictable way by gender. Creatinine, albumin, albumin-to-globulin ratio, gamma-glutamyl

Reference	**19**	**19**	**19**	**19**	**19**	**19**	**20**	**20**	**24**	**24**	**25**	**25**	**26**	**26**	**27**	**27**	**29**
Age	30.3 mos	30.3 mos	30.7 mos	30.7 mos	24.8 mos	24.8 mos	3 - 5 yrs	3 - 5 yrs	Adult	Adult	Adult	Adult	2 years	2 years	3 - 7 yrs	3 - 7 yrs	2 - 4 yrs
Gender	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	both
Origin	Mauritius	Mauritius	Phillipines	Phillipines	Vietnam	Vietnam	China	China			Chinese	Chinese	Mauritius	Mauritius	China	China	All
Fasted																	Yes
Anesthetized?							Yes	Yes	Yes	Yes							Yes
Collection site							cephalic	cephalic							femoral	femoral	femoral
Analyzer							*c*	*c*	*a*	*a*					*d*	*d*	*b*
RBC x 10^6/mL	6.53	6.44	6.91	6.64	5.82	5.58	5.26	5.13	6.3	6.16	5.39	5.17	7.21	6.86	5.49	5.44	6.13
Hgb (g/dL)							12.65	12.24	39.8	37	40.2	39.1	50.2	49.3	13.2	12.93	41.7
Hct (%)	13.1	12.6	12.9	12.3	13.4	13.5	47.46	46.99	13.6	12.6	12.6	12.3	14.3	13.7	42.88	42.2	12.15
MCV (fL)	68.4	68.5	65.5	65.7	77.4	78.2	90.3	91.67	63.7	60.8	74.7	75.8	70	72	78.21	77.62	68.3
MCH (pg)	20.1	19.7	18.8	18.4	23.1	24.2	24.04	23.86	21.57	20.31	23.5	23.8	19.9	20	24.05	23.78	19.8
MCHC (5)							26.64	26.02	34.09	34.15	31.5	31.4	28.6	27.7	30.77	30.67	29
WBC x 10^3/mL	9.44	10.3	13.79	11.15	11.02	12.4	13.05	11.06	9.75	8.03	11.5	11.2	7	10	12.92	11.92	12.6
Neutrophils (%)	36.5	47.3	31.5	37.5	35.2	46	26	28.84	65.38	68.15			25.8	25.9	31.95	32.16	
Lymphocytes (%)	56.4	45.5	59.9	52.3	59.5	48.7	67.3	63.3	31.04	27.92			68.3	64.6	60.4	59.53	
Eosinophils (%)	1.2	1.2	3.5	4.5	0.9	1.1	0.88	1.63	1.33	0.85			0.6	1.5	2.32	3.189	
Basophils (%)							4.27	2.91	0.05	0			0.3	0.3	0.92	0.6977	
Monocytes (%)							3.4	3.78	1.95	1.61			4.3	6.6	3.615	3.522	

Values presented are the reported means. Values may have been converted for comparison purposes. For additional information please see cited reference.

a. 530/550 cellular counter, CELM™ Cia Equipadora de Laboratorios Modernos

b. Sysmex KX-21 Analyzer, Kobe, Japan

c. Advia 120 Hematology System, Bayer, New York, NY

d. Advia 120 automated blood cell counter, Bayer Medical, Tokyo, Japan

FIGURE 5.4 Serum chemistry Indices are provided to give examples of these indices reported in the literature. Cited references can be used for comparison based on the demographics and other factors affecting a given cohort of cynomolgus monkeys. Blank cells indicate values not provided in the cited reference.

VetBooks.ir

transpeptidase, CPK, amylase, Ca, P, and serum sodium are generally higher in males than in females, whereas globulin, total bilirubin, triglycerides, and serum chloride are generally lower in males than in females [33]. It is worth alerting readers that the country of origin of the cynomolgus monkeys assessed in this last citation was not provided so caution is indicated before broadly translating this information to all cynomolgus monkeys. There could be other variables contributing to differences in serum biochemical analysis that are not yet appreciated [34].

It is commonly accepted that the fed state of animals has an effect on serum biochemical values [30]. Even in humans, we anticipate an 8-12 h fast before blood collection. It is also understood that age has an effect on serum biochemical values [30,32]. When consulting published reference ranges, the fed state of animals is often not clearly stated. In addition, safety assessment studies are often carried out in immature animals [19]. The effects of anesthesia and age on serum biochemical values have been reported repeatedly [20,28,30,35,36]. Figure 5.4 includes information regarding the variables surrounding blood collection, if they are known, that might affect results. Recognizing that the significance of differences observed between populations can be influenced by the selection of the statistical test used to evaluate the results is also important. Many statistical tests assume that data generated will exhibit normal distribution but many serum biochemical parameters do not [25].

REPRODUCTION AND DEVELOPMENT

The *Laboratory Nonhuman Primate* [33] is an excellent resource for basic information not necessarily required for safety assessment studies but for issues that inevitably come up in nonhuman primate colony management. Predicted values for gestational sac, length, biparietal diameter, and femur length measurements in utero are provided in that text. It is assumed that the breeding date and ultimately gestational age of a fetus are known in the drug development and safety assessment arena. However, this book should be consulted in the event that an unexpected pregnancy occurs [33]. Occasionally, there is a need to assess or confirm an animal's age. Evaluation of dentition is the most common means of determining age in veterinary medicine. Figure 5.5 includes limited information about maxillary dental eruption dates to assist the clinician or research staff in determining or verifying age [33].

Cynomolgus monkeys are not seasonal breeders [33,37,38] and females generally exhibit a roughly monthly menstrual cycle [14,37–39] analogous to humans. Menarche can be expected to occur between the second and third years of life and no evidence exists that ovarian cycle synchrony occurs in the female cynomolgus monkey [39]. Spermarche can be expected as early as 3.9 years in Asian-origin cynomolgus males and as early as 2.8 years in Mauritius-origin males [40].

Safety assessment studies often require sexually mature animals to determine the test article effect on the reproductive system. Weinbauer et al. recommends that ovarian cycle monitoring in female cynomolgus monkeys should be available for three to five cycles prior to study initiation in animals where sexual maturity is required [39]. Although it might seem simple enough to select female cynomolgus monkeys for study for which this information has already been captured, the process of randomizing females into dose groups and/or establishing social pairs has been shown to have a significant impact on the ovarian cycle [39].

Reference	14	19			20	21	*a*	22
Age if provided		30.3 mos	24.8 mos	30.7 mos	3 - 5 years			
Origin if provided		Mauritius	Vietnamese	Phillipines	Chinese			
Head and Body (female) mm	385 - 503							
Head and body (male) mm	412 - 648						430 - 580	
Tail length (female) mm	400 -550							
Tail length (male) mm	435 - 655							
Weight (female) kg	2.5 - 5.7kg	2.647	2.196	2.474	2.73	2 - 6		
Weight (male) kg	4.7 - 8.3	3.054	2.270	2.729	2.86	4 - 8	5 - 8	
Respiratory Rate/min						30 - 54		
Heart Rate/min						115 - 243		
Rectal Temp °C						37 - 39		
Daily water intake/mL						350 - 950		
Daily food intake/g						350 - 550		
Urine excreted - mL/day						150 - 550		
Blood Volume - mL/kg						55 - 75		65
Life span (years)	37.1							

a. 24. Taylor, K. (2010). Clinical veterinarian's perspective of non-human primate (NHP) use in drug safety studies. J Immunotoxicol.7(2):114-119.

FIGURE 5.5 Reproductive and developmental indices are provided to give examples of these indices reported in the literature. Cited references can be used for comparison based on the demographics and other factors affecting a given cohort of cynomolgus monkeys. Blank cells indicate values not provided in the cited reference.

VetBooks.ir

Social reorganization has been demonstrated to cause aberrations in cycle that persist in the short-term and usually return to baseline by approximately 6 months [39]. Ideally, studies relying on menstrual data as an indication of sexual maturity should only include already established pairs or groups of females with at least 6 months of prior social history [39].

Ovarian cycle monitoring in female cynomolgus monkeys is best accomplished by daily vaginal smears for the simple assessment of the presence of vaginal blood [39]. Unlike other nonhuman primate species that exhibit exuberant changes in sex skin throughout their cycle, cynomolgus monkeys are more difficult to assess by this method if at all [39]. Animals can be trained to present their perineum for vaginal swabbing. Anecdotal evidence and personal experience suggest that cynomolgus monkeys from China are easier to train for vaginal swabbing than those from Mauritius [K. McGrew, personal communication, April 1, 2014; I. Bolton, personal experience].

Weinbauer et al. describe assessing sexual maturity in the male cynomolgus by utilizing rectal probe electrostimulation to obtain semen for analysis [41]. This is the method of choice as efforts to establish correlations between male sexual maturity/ejaculatory ability and age, weight, and testes size have not proven reliable [42]. Based on the work of Luetjens et al., it has been demonstrated that the presence of sperm in ejaculate provides unequivocal proof of sexual maturity [40]. Leutjens et al. also found that Mauritius-origin male cynomolgus monkeys achieve testicular maturity approximately 2 years earlier than mainland Asian-origin male cynomolgus monkeys.

Figure 5.5 includes age ranges for the stages of development described in several references. Some of these stages overlap or are used synonymously to varying degrees. It is important to be cognizant of which age range an author is actually describing when talking about developmental age to fully appreciate whether information can be translated to other studies or not.

CARDIOVASCULAR

A number of authors have described background lesions or incidental findings in cynomolgus monkeys [19,43,44] from various origins. Special attention should be paid to country of origin as there does seem to exist a difference in at least histologic presentation between Mauritius-origin cynomolgus monkeys and cynomolgus monkeys from other locations [45].

With respect to cardiovascular and respiratory systems, a literature review reveals few references related to normal physiology. This could be an indication that there is little unique information related to the cynomolgus monkey. Gauvin et al. provide a prospective look at electrocardiogram and blood pressure over 22 h in the cynomolgus monkey [46]. The study utilizes both Mauritius and Vietnamese-origin cynomolgus monkeys but does not differentiate data by origin. This study also utilizes animals of approximately 1 year of age and less than 2.5 kg, so care must be taken in extrapolating to older animals.

The most common finding reported in the heart of cynomolgus monkeys is inflammatory cell infiltration [19,44,45]. This finding is reported in numerous organ systems but with dubious significance. The challenge is often determining when infiltration becomes inflammation. Assigning inflammation when degeneration or necrosis of parenchymal tissue is present or when fibrin and edema are present is one strategy [44] but consensus should be

VetBooks.ir

the goal. One report suggests that the overall degree of inflammatory infiltrates is lower in the Mauritius-origin cynomolgus monkey than in the Asian-origin cynomolgus monkey [19]. This observation does not seem to extend to the heart. Vidal et al. report an overall incidence of myocardial degeneration/necrosis in vehicle control Mauritius-origin cynomolgus in 13% of the animals, subendocardial hemorrhage in 30% of the animals, and inflammatory cell infiltration of the myocardium in 40% of the animals [45]. This is in comparison to an earlier work in Indonesian-origin cynomolgus monkeys where the incidence of myocardial degeneration/necrosis was 4-5% [47]. In addition to the myocardial degeneration/necrosis, Vidal et al. report a cascade of additional findings that might present challenges in sorting out the difference between test-article-related cardiac toxicity and background findings [45]. There may be a temperament component to these findings that is discussed further in the "Behavior" section of this chapter.

The ratio of the heart at its maximum width to the inside of the thoracic cavity at its maximum width is referred to as the cardiothoracic ratio (CtR). This measurement is made on regular anterior-posterior X-ray films. The CtR is a standard measure of cardiac size in humans and is useful from a prognostic standpoint in the ongoing assessment of both congenital and acquired heart diseases [48,49]. Screening radiographs have been adapted for use in nonhuman primates as a means of screening for pulmonary pathology (i.e., Tuberculosis), but they have the potential for use in screening animals for subclinical heart disease as well. A CtR >0.5 is considered indicative of cardiac enlargement in humans [50]. In the Indonesian male cynomolgus monkey, a CtR of 0.55 is normal [50] whereas the Chinese-origin cynomolgus monkey typically demonstrates a higher CtR [51] that is more similar to that of rhesus monkeys [52]. Changes in CtR are not significantly correlated with age [51]. Males consistently present with higher CtR than females of the same age range [51]. Figure 5.6 includes CtR values for consideration. Figure 5.6 also includes other miscellaneous data that might be useful when investigating baseline indices for coagulation parameters and dosing parameters.

HEPATIC AND URINARY

Liver and kidney function are of utmost importance in drug development and safety assessment as these organs play such an important role in the metabolism and excretion of the test article. The assessment of these systems is usually accomplished via the evaluation of changes in serum biochemistry values. Behind the heart, the second most likely anatomic location to find inflammatory cell infiltrates is in the kidney [44] although they are commonly identified in the liver as well [19,44]. In the kidney, the cortex is most often affected. Inflammatory cell infiltrates tend to be lower in cynomolgus monkeys of Mauritius-origin [19]. Other common findings are glycogen accumulation in the centrilobular region in the liver, cytoplasmic inclusions in the kidney, and multinucleated giant cells in the medullary collecting ducts [19]. Ectopic adrenal cortical tissue is occasionally identified within the capsule of the kidney [44].

Multinucleated hepatocytes (MNHs) are observed in cynomolgus monkeys from multiple origins [53]. Novilla et al. gathered information about the occurrence of MNHs to better characterize the incidence and nature of the finding in order to make better judgments about potential test article effect in drug development and safety assessment studies [53]. In

Reference	14	19	21	24	37	38	39	40	40	*b*	*b*
Origin								Mauritius	China or Vietnam	Indonesia	Indochina
Gestation (days)	160-170					155-165					
Menstrual Cycle (days)	28				28	28-32	28-32				
Follicular Phase (days)							12-14				
Periovulatory Phase (days)							3				
Luteal Phase (days)							14-16				
Female sexual maturity					46 mos	3.0-3.4 yrs					
Male sexual maturity					42-60 mos	3 - 4 yrs					
Male Birth Weight (g)					340-400	345*a*					
Female Birth Weight (g)					260-310	345*a*					
Baby (months)				<6							
Infant (months)	6-12			7-18							
Weaning (months)	14										
Juvenile (months)	12-42			19-31							
Puberty (months)				32-44							
Adolescent (months)		32-44									
Menarche (months)							24-36				
Spermarche - min (years)								2.8	3.9		
Subadult (months)	42-54										
Female maturity (months)	51.6	4 years*									
Male maturity (months)	50.4	6 years*									
Adults/mature (months)				45-192							
Age at first birth (months)										39.2	
Age at first birth (months)											54.8
Maxillary I^1 eruption (months)			30								
Maxillary I^2 eruption (months)			32								
Maxillary C eruption (months)			41								
Maxillary PM1 eruption (months)			42								
Maxillary PM2 eruption (months)			42								
Maxillary M1 eruption (months)			20								
Maxillary M2eruption (months)			41								
Maxillary M3 eruption (months)			69								

a. value reported without gender specified

b. 48. Izard, M. K. and Smith, D. G. (2010). Reproduction in Indonesian vs. Indochinese cynomolgus macaques revisited. Am J Primatol 51(1):63-64.

FIGURE 5.6 Miscellaneous Indices are provided to give examples of these indices reported in the literature. Cited references can be used for comparison based on the demographics and other factors affecting a given cohort of cynomolgus monkeys. Blank cells indicate values not provided in the cited reference.

VetBooks.ir

Cambodian-, Chinese-, Indonesian-, and Mauritius-origin cynomolgus monkeys, there was a 61% incidence of MNHs overall, which did not correlate with country of origin or clinical signs in the animals [53]. The presence of MNH was slightly higher in males than in females: 70% and 56%, respectively [53]. MNHs did not seem to be significant [53].

OTHER ORGAN SYSTEMS

As is the case with other organ systems in the cynomolgus monkey, the most common incidental finding is varying degrees of inflammatory cell infiltrates on a continuum of mild to moderate inflammation in the absence of other indicators of illness [19,43,44]. Other incidental findings might include the presence of ectopic thymus in the thyroid and parathyroid gland [44], splenic lymphoid hyperplasia [19,44], and hemosiderin pigment in the spleen [19].

Most incidental findings of the skin are attributable to trauma or treatment sites [44]. One exception is the occurrence of condyloma acuminatum (human papilloma virus-induced wart-like masses) of the penile shaft in Mauritius-origin cynomolgus monkeys [54]. This observation is consistent with the observation reported elsewhere of pseudocarcinomatous hyperplasia of the prepuce and penis [44]. This lesion is occasionally identified in other locations suggestive of papilloma virus. Efforts to identify a viral ideology in the cynomolgus monkey lesion have proven fruitless [54]. The genital lesion described is basically exclusive to the Mauritius-origin cynomolgus monkey [I. Bolton, personal experience].

As for other background findings, Sasaki et al. report the identification of a 7-year-old treatment-naïve Chinese cynomolgus monkey with severe bilateral cataracts [55] similar to hypermature cortical cataracts that might be seen in humans. No inflammation was identified in any portion of the eyes [55]. The author speculates that genetic factors may have contributed to the development of cataracts in this monkey [55], but ocular pathology does not seem to be a common background finding in cynomolgus monkeys.

When assessing reviews of incidental findings in the cynomolgus monkey, it is important to consider the source of the animals surveyed when generating the report. Reference may be strongly biased toward either age [19] or country of origin [43,44,47].

The digestive system warrants special consideration because so many drug candidates are administered orally. Diarrhea is the most common clinical sign reported in captive nonhuman primates although clear cause is not often identified [37]. Understanding normal gastrointestinal function is very important in avoiding unexpected outcomes related to absorption and clearance of the test article. Multiple citations by Kondo et al. describe the effect of the fed state on gastric emptying rate, orocecal transit times, and gastric pH [56–58] in cynomolgus monkeys. In general, gastric emptying time is significantly delayed when animals are not fasted, but orocecal transit is unaffected [56] and the volume of an administered liquid dose does not significantly affect gastric emptying time or orocecal transit [57]. The gastric pH of fasted cynomolgus monkeys is 1-3 [58]. This value is consistent with that seen in fasted people. When monkeys were fed the gastric pH increased to a range of 5-7 [58]. Although this is also consistent with the change observed in humans, this rise in pH is only transient in humans, whereas it lasts for approximately 9 h in the cynomolgus monkey. This factor needs to be considered as it may influence the dissolution and bioavailability of some drugs and undermine the integrity of the cynomolgus model for some studies [58].

VetBooks.ir

Subclinical gastritis appears to be widespread in cynomolgus monkeys from Mauritius, Vietnam, and the Philippines with a high incidence of *Helicobacter* spp. [19,44]. Inflammatory infiltrates in the stomach are lower in Mauritius-origin cynomolgus monkeys than in Asian-origin animals [19]. This finding persists in the colon [19]. *Balantidium coli* is identified more commonly in Asian-origin than in Mauritius-origin cynomolgus monkeys, and *Trichuris* spp. is only reported in animals from Vietnamese origin [19].

ENDEMIC DISEASES AND SUSCEPTIBILITY

The Mauritius-origin cynomolgus has long been appreciated for its apparent pathogen free status with respect to common viral diseases of interest in macaques [4]. This also seems to hold true for infections with *Plasmodium* spp. [59]. There are other examples of varying disease susceptibility by country of origin. These should be considered when planning prestudy screening and in the interpretation of clinical signs on study.

There are numerous species of malaria known to infect the cynomolgus monkey including *Plasmodium knowlesi*, *Plasmodium cynomolgi*, *Plasmodium inui*, *Plasmodium fieldi*, and *Plasmodium coatneyi* [60]. There is a great deal of variability in disease susceptibility even among cynomolgus of Asian origin. The natural host of *P. knowlesi* is the Asian-origin cynomolgus monkey [61]; however, the cynomolgus monkey from the Philippines seems to be less susceptible to disease caused by *P. knowlesi* than the Malaysian-origin cynomolgus [62]. Mainland Asian-origin and Philippine cynomolgus monkeys are known to survive infections with *P. coatneyi* [59], whereas Mauritius-origin cynomolgus monkeys and rhesus monkeys are highly susceptible to disease and death [59,61]. An average prevalence of malaria in cynomolgus monkeys has been reported as 3.4% in Guangdong and 3.6% in Guangxi provinces in southern China where many cynomolgus breeding farms are located with a reported incidence range of 0-5.8% [63]. However, these results are based on seropositivity for *Plasmodium vivax*, a species not known to naturally infect cynomolgus monkeys [60], so further investigation and awareness is necessary when ruling out or excluding malaria infections in cynomolgus monkeys.

Because of their close phylogenetic relationship to humans, it is important to be aware of the potential for the presence of zoonotic or accidental infection caused by agents known to cause disease in humans. In an effort to identify a small animal model for Hepatitis B virus infection, a survey was conducted that positively identified the virus in Mauritius-origin cynomolgus monkeys [34]. Of 120 Mauritius cynomolgus monkeys tested, 25.8% were seropositive and 42% were liver tissue positive for DNA by PCR for a closely human-related virus. It is easy to imagine how this virus could have been originally transmitted from humans to cynomolgus monkeys by what would have been close proximity inherent to shipboard life when the monkey was originally introduced to the island. Also, cynomolgus monkeys can serve as accidental hosts for diseases much the same way that humans do as in a report of sparganosis (*Spirometra erinaceicuropai*) in a Mauritius-origin cynomolgus [64]. These cases illustrate the ongoing need to protect our primate resource from possible human diseases.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* or *Mycobacterium bovis*, is historically and remains extremely important in the nonhuman primate biomedical research model. Although the human incidence of TB remains significantly higher in Asian countries than in Mauritius [65], between the years of 1990 and 1994, 90% of the cases of TB in imported

VetBooks.ir

nonhuman primates were identified in cynomolgus monkeys and 94% of the infected cynomolgus monkeys were from Mauritius [66]. The significance of these observations increases when one takes into account that nonhuman primates imported from Mauritius represented only 16% of all nonhuman primate imports at that time. In addition, 70% of cases were not identified until after monkeys had been released from quarantine [66], which seriously undermines the confidence we can have in the traditional skin test to identify the presence of infection. Rhesus monkeys and cynomolgus monkeys seem to experience differing disease courses; rhesus are more likely to present with clinical signs of coughing and inappettance [67] and cynomolgus monkeys are more likely to be asymptomatic [67,68]. The outbreak at Stanford University described by Garcia et al. was first suspected in the rhesus based on clinical signs but found in the cynomolgus colony by surveillance. Only one of 19 cynomolgus monkeys diagnosed with TB exhibited clinical signs [67]. Cynomolgus monkeys are believed to exhibit a course of infection more similar to latent infections in humans [67], meaning they could go for extended times without identification. Panarella et al. describe an outbreak of TB in a cohort of China-origin cynomolgus monkeys where the diagnosis was made completely from skin test results but all animals were asymptomatic and had negative TB tests 2 weeks previous [68]. Also cynomolgus monkeys vaccinated with bacillis Calmette-Guerin vaccine are protected during a TB challenge whereas rhesus monkeys are only minimally less susceptible [69], further demonstrating a different susceptibility.

In May 1992, increased TB surveillance and eradication efforts in Mauritius resulted in a dramatic decrease in the incidence of TB in the Mauritius-origin cynomolgus [66]. These efforts included better exclusion of the Java deer herds that are known to carry *M. bovis* [3], the causative agent identified in both the Stanford University outbreak [67] and the outbreak described by Panarella et al. [68]. Despite improvements in the pre-export screening processes prior to leaving the country of origin, clinicians and researchers alike must always be vigilant about the possibility of TB occurring in cynomolgus monkeys.

Ebola Virus was first identified in the Sudan and in Zaire in 1976 [70]. During November 1989, a shipment of cynomolgus monkeys originating from the Philippines presented with a hemorrhagic disease that was ultimately diagnosed as Ebola-Reston [70,71]. This incident represented the first naturally occurring case of Ebola in monkeys and the first time that Ebola was diagnosed in the continental United States [70]. This occurred again in 1996. This was a case of an African virus occurring in an Asian sourced monkey. Although agents such as Ebola should not be high on the list of differentials when considering disease status, these events should serve as a reminder to be ever vigilant for new or emerging diseases in the nonhuman primate populations from which we derive research models.

BEHAVIOR

Anyone who has had the opportunity to work with large groups of cynomolgus monkeys from different geographic origins will confirm that they are behaviorally different. Although they may not be able to tell you how or why, they will have appreciated that some cynomolgus are quieter than others, some are more aggressive and demonstrative than others, and some of them are more vocal than others. Differences in temperament [72], adrenocortical response, motor activity [73], and heart rate [74] by species have been described in the literature. Given

VetBooks.ir

the assumption that the modern cynomolgus monkey is a product of hybridization and geographic isolation, selection, and evolution, it would follow that behavioral differences exist in addition to morphologic and physiologic differences.

One subjective observation was that Indochinese cynomolgus monkeys were more aggressive and active than the insular macaques. Brent and Veira found themselves in a unique position to make observations and record behavioral data from a large mixed cohort of cynomolgus monkeys originating from Indochina, the Philippines, Mauritius, and Indonesia under similar circumstances, and they took advantage of the opportunity to quantify social behavior, record differential behavior by country of origin, and determine changes in behavior over acclimation time [75]. Based on their observations, they were able to demonstrate that cynomolgus monkeys of Indochinese-origin exhibit lower levels of affiliative behavior and that Indochinese-origin male cynomolgus monkeys display higher levels of aggression than other ages/classes of Indochinese monkeys and all other age/sex/class of monkeys of other origins [75]. In 1971, Bernstein reported that cynomolgus monkeys had higher levels of social behavior than rhesus or pigtailed macaques [76]. Is it possible that the differences in behaviors observed in the Indochinese cynomolgus monkey [75] could be attributable to the genetic influence of the rhesus monkey [9–12].

Anecdotal evidence, research, and the personal experiences of people involved in acclimating and conditioning the training of monkeys [75; I. Bolton, personal experience; K. McGrew, personal communication, April 1, 2014] suggest that Chinese-origin cynomolgus monkeys are easier to train and acclimate. During pole and collar adaptation in both genders and acclimation to vaginal swabbing for menses detection in females, the Chinese-origin monkeys seem more nervous at first but struggle less and accept handling more quickly than their Mauritius-origin counterparts [K. McGrew, personal communication, April 1, 2014]. Other authors have suggested that Mauritius-origin cynomolgus monkeys respond differently to stress than their mainland counterparts [6,77].

Some of the observations made about cardiac changes in the heart of the Mauritius-origin cynomolgus monkey reported by Vidal et al. [45] include myocardial degeneration and necrosis as well as subendocardial hemorrhage. Myocardial degeneration and necrosis and subendothelial hemorrhage have been described in a number of species in association with increased catecholamines [78–80], and myocardial degeneration and necrosis have been induced in rhesus monkeys by infusion of norepinephrine [81]. These changes are remarkably consistent with those reported by Vidal et al. [45]. It would be interesting to know if the Mauritius-origin cynomolgus monkey simply operates at a higher sympathetic tone and responds to the environmental stress imposed by the research environment differently than cynomolgus monkeys from other origins.

References

[1] The CITES Appendices. Retrieved from: http://www.cites.org/eng/app/index.php; 2014.
[2] Sussman RW, Tattersall I. Distribution, abundance, and putative ecological strategy of *Macaca fascicularis* on the island of Mauritius, Southwestern Indian Ocean. Folia Primatol 1986;46:28–43.
[3] Jori F, Godfroid J, Michel AL, Potts AD, Jaumally MR, Sauzier J, et al. An assessment of zoonotic and production limiting pathogens in Rusa Deer (*Cervis timorensis rusa*) from Mauritius. Transbound Emerg Dis 2013;0(0):1–12.
[4] Matsubayashi K, Gotoh S, Kawamoto Y, Watanabe T, Nozawa K, Takasaka M, et al. Clinical examinations on crab-eating macaques in Mauritius. Primates 1992;33:281–8.
[5] Bonhomme M, Blancher A, Cuartero S, Chikhi L, Crouau-Roy B. Origin and number of founders in an introduced insular primate: estimation from nuclear genetic data. Mol Ecol 2008;17:1009–19.

[6] Socialist Market Economy. Retrieved from: http://en.wikipedia.org/wiki/Socialist_market_economy; 2014.
[7] Hsu CK. China as a resource for NHP [lecture]. Retrieved from National Research Council (US) Institute for Laboratory Animal Research. Animal Research in a Global Environment: Meeting the Challenges: Proceedings of the November 2008 International Workshop. Washington (DC): National Academies Press (US). International Coordination of Nonhuman Primates. Available from: http://www.ncbi.nlm.nih.gov/books/NBK91512/; 2011.
[8] Glenn Smith D, McDonough JW, George DA. Mitochondrial DNA variation within and among regional populations of longtail macaques (*Macaca fascicularis*) in relation to other species of the *Fascicularis* group of macaques. Am J Primatol 2007;69:182–98.
[9] Stevison LS, Kohn MH. Determining genetic background in captive stocks of cynomolgus macaques (*Macaca fascicularis*). J Med Primatol 2008;37:311–7.
[10] Tosi AJ, Morales JC, Melnick DJ. Y-chromosome and mitochondrial markers in *Macaca fascicularis* indicate introgression with Indochinese *M. mulatta* and a biographic barrier in the Isthmus of Kra. Int J Primatol 2002;23(1):161–78.
[11] Bonhomme M, Cuartero S, Blancher A, Crouau-Roy B. Assessing natural introgression in 2 biomedical model species, the rhesus macaque (*Macaca mulatta*) and the long-tailed macaque (*Macaca fascicularis*). J Hered 2009;100(2):158–69.
[12] Yan G, Zhang G, Fang X, Zhang Y, Li C, Ling F, et al. Genome sequencing and comparison of two nonhuman primate animal models, the cynomolgus and Chinese rhesus macaques. Nat Biotechnol 2011;29(11):1019–23.
[13] Williams-Blangero S, VandeBerg JL, Dyke B. Genetic management of nonhuman primates. J Med Primatol 2002;31:1–7.
[14] Rowe N. The pictorial guide to the living primates. New York: Pegonias Press; 1996.
[15] Villano JS, Ogden BE, Yong PP, Lood NM, Sharp PE. Morphometrics and pelage characterization of longtailed macaques (*Macaca fascicularis*) from Pulau Bintan, Indonesia; Singapore; and Southern Vietnam. J Am Assoc Lab Anim Sci 2009;48(6):727–33.
[16] Goldschmidt RB, Goldschmidt R. The material basis of evolution. New Haven, CT: Yale University Press; 1982.
[17] Fooden J. Rhesus and crab-eating macaques: intergradation in Thailand. Science 1964;143:363–5.
[18] Fooden J. Tail length variation in *Macaca fascicularis* and *M. mulatta*. Primates 1997;38(3):221–31.
[19] Drevon-Gaillot E, Perron-Lepage MF, Clement C, Burnett R. A review of background findings in cynomolgus monkeys (Macaca fascicularis) from three different geographical origins. Exp Toxicol Pathol 2006;58(2–3):77–88.
[20] Kim CY, Lee HS, Han SC, Heo JD, Kwon MS, Ha CS, et al. Hematological and serum biochemical values in cynomolgus monkeys anesthetized with ketamine hydrochloride. J Med Primatol 2005;34(2):96–100.
[21] Diehl K-H, Hull R, Morton D, Pfister R, Rabidemampianina Y, Smith D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 2001;21:15–23.
[22] Nonhuman Primates. Retrieved from: https://www.criver.com/source/home; 2014.
[23] Barnhart K. Hematology of laboratory primates. In: Weiss DJ, Wardrop KJ, editors. Schalm's veterinary hematology. Ames, IA: Blackwell-Wiley; 2010. p. 869–87.
[24] Bonfanti U, Lamparelli D, Colombo P, Bernardi C. Hematology and serum chemistry parameters in juvenile cynomolgus monkeys (*Macaca fascicularis*) of Mauritius origin: comparison between purpose-bred and captured animals. J Med Primatol 2009;38:228–35.
[25] Koga T, Kanefuji K, Nakama K. Individual reference intervals of hematological and serum biochemical parameters of cynomolgus monkeys. Int J Toxicol 2005;24:377–85.
[26] Sugimoto Y, Hanari K, Narita H, Honjo S. Normal hematologic values in the cynomolgus monkeys aged from 1 to 18 years. Exp Anim 1986;35(4):443–7.
[27] Bourges-Abella N, Geffre A, Moureaux E, Vincenti M, Braun JP, Trumel C. Hematologic reference intervals in cynomolgus (*Macaca fascicularis)* monkeys. J Med Primatol 2014;43:1–10.
[28] Venkatesan R, Nagarajan P, Rajaretnam RS, Majumdar SS. Hematologic and serum biochemical values in aged female bonnet macaques (*Macaca radiata*) anesthetized with ketamine hydrochloride. J Am Assoc Lab Anim Sci 2006;45(2):45–8.
[29] Xie L, Xu F, Liu S, Ji Y, Zhou Q, We Q, et al. Age-and sex-based hematological and biochemical parameters for *Macaca fascicularis*. PLoS One 2013;8(6):e64892.
[30] Perretta G, Violante A, Scarpulla M, Beciana M, Monaco V. Normal serum biochemical and hematological parameters in *Macaca fascicularis*. J Med Primatol 1991;20:345–51.
[31] Andrade MCR, Ribeiro CT, da Silva VF, Molinaro EM, Goncalves MAB, Pereira MA, et al. Biologic Data of *Macaca mulatta, Macaca fascicularis*, and *Saimiri sciureus* used for research at the Fiocruz Primate Center. Mem Inst Oswaldo Cruz 2004;99(6):581–9.

[32] Schuurman H-J, Smith HT. Reference values for clinical chemistry and clinical hematology parameters in cynomolgus monkeys. Xenotransplantation 2005;12:72–5.
[33] Fortman JD, Hewett TA, Bennett TB. The laboratory nonhuman primate. Boca Raton: CRC Press; 2002.
[34] Dupinay T, Gheit T, Roques P, Cova L, Chevallier-Queyron P, Tasahsu S-I, et al. Discovery of naturally occurring transmissable chronic hepatitis B virus infection among *Macaca fascicularis* from Mauritius Island. Hepatology 2013;58(5):1610–20.
[35] Lugo-Roman LA, Rico PJ, Sturdivant R, Burks R, Settle TL. Effects of serial anesthesia using ketamine or ketamine/medetomodine on hematology and serum biochemistry values in rhesus macaques (*Macaca mulatta*). J Med Primatol 2010;39:41–9.
[36] Woodward RA, Weld KP. A comparison of ketamine, ketamine-acepromazine, and tiletamine-zolazepam on various hematologic parameters in rhesus monkeys (*Macaca mulatta*). Contemp Top Lab Anim Sci 1997;36(3):55–7.
[37] Fox JG, Anderson LA, Loew FM, Quimby FW, editors. Laboratory animal medicine. 2nd ed. New York: Academia Press; 2002.
[38] Hendrickx AG, Dukelow RW. Reproductive biology. In: Bennett BT, Abee CR, Henrickson R, editors. Nonhuman primates in biomedical research, biology and management. San Diego: Academic Press; 1995.
[39] Weinbauer GF, Niehoff M, Niehaus M, Srivastav S, Fuchs A, Van Esch E, et al. Physiology and endocrinology of the ovarian cycle in macaques. Toxicol Pathol 2008;36(7S):7S–23S.
[40] Luetjens CM, Weinbauer GF. Functional assessment of sexual maturity in male macaques (*Macaca fascicularis*). Regul Toxicol Pharmacol 2012;63:391–400.
[41] Weinbauer GF, Limberger A, Behre HM, Nieschlag E. Can testosterone alone maintain the gonadotropin-releasing hormone antagonist-induced suppression of spermatogenesis in the non-human primate? J Endocrinol 1994;142:485–95.
[42] Ku WW, Pagliusi F, Foley G, Roesler A, Zimmerman T. A simple orchidometric method for the preliminary assessment of maturity status in male cynomolgus monkeys (*Macaca fascicularis*) used for nonclinical safety studies. J Pharmacol Toxicol 2010;61:32–7.
[43] Sato J, Doi T, Kanno T, Wako Y, Tsuchitani M, Narama I. Histopathology of incidental findings in cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. J Toxicol Pathol 2012;25:63–101.
[44] Chamanza R, Marxfeld HA, Blanco AI, Naylor SW, Bradley AE. Incidences and range of spontaneous findings in control cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. Toxicol Pathol 2010;38(4):642–57.
[45] Vidal JD, Drobatz LS, Holliday DF, Geiger LE, Thomas HC. Spontaneous findings in the heart of Mauritian-origin cynomolgus macaques (*Macaca fascicularis*). Toxicol Pathol 2010;38:297–302.
[46] Gauvin DV, Tilley LP, Smith Jr. FWK, Baird TJ. Electrocardiogram, hemodynamics, and core body temperatures of the normal freely moving cynomolgus monkey by remote radiotelemetry. J Pharmacol Toxicol Methods 2006;53:140–51.
[47] Keenan CM, Vidal JD. Standard morphologic evaluation of the heart in the laboratory dog and monkey. Toxicol Pathol 2006;34:67–74.
[48] Hammermeister KE, Chikos PM, Fisher L, Dodge HT. Relationship of cardiothoracic ratio and plain film heart volume to late survival. Circulation 1979;59:89–95.
[49] Hubbell FA, Greenfield S, Tyler JL, Chetty K, Wyle FA. The impact of routine admission chest x-ray films on patient care. N Engl J Med 1985;312:209–13.
[50] Schillaci MA, Lischka AR, Karamitsos AA, Engel GA, Paul N, Ramoul R, et al. Radiographic measurement of the cardiothoracic ratio in a feral population of long-tailed macaques (*Macaca fascicularis)*. Radiography 2010;16:163–6.
[51] Xie L, Zhou Q, Liu S, Wu Q, Ji Y, Zhang L, et al. Normal thoracic radiographic appearance of the cynomolgus monkey (*Macaca fascicularis*). Plos One 2014;9(1):e84599.
[52] Ji Y, Xie L, Liu S, Cheng K, Xu F, Li X, et al. Correlation of thoracic radiograph measurements with age in adolescent Chinese rhesus macaques (*Macaca mulatta*). J Am Assoc Lab Anim Sci 2013;52(1):78–82.
[53] Novilla MN, Jackson MK, Reim DA, Jacobson SB, Nagata RA. Occurrence of multinucleated hepatocytes in cynomolgus monkeys (*Macaca fascicularis*) from different geographical regions. Vet Pathol 2014;51(6):1183–6.
[54] Gordon HP, Reim DA, McClain SA. Condyloma acuminatum in a cynomolgus monkey (*Macaca fascicularis*). Contemp Top Lab Anim Sci 2000;39(2):30–3.
[55] Sasaki Y, Kodama R, Iwashige S, Fujishima J, Yoshikawa T, Kamimura Y, et al. Bilateral cataract in a cynomolgus monkey. J Toxicol Pathol 2011;24:69–73.

VetBooks.ir

[56] Kondo H, Watanabe T, Yokohama S, Watanabe J. Effect of food on gastrointestinal transit of liquids in cynomolgus monkeys. Biopharm Drug Dispos 2003;24(4):141–51.
[57] Kondo H, Takahashi Y, Watanabe T, Yokohama S, Watanabe J. Gastrointestinal transit of liquids in unfed cynomolgus monkeys. Biopharm Drug Dispos 2003;24(3):131–40.
[58] Kondo H, Shinoda T, Nakashima H, Watanabe T, Yokohama S. Characteristics of the gastric pH profiles of unfed and fed cynomolgus monkeys as pharmaceutical product development subjects. Biopharm Drug Dispos 2003;24(1):45–51.
[59] Migot-Nabias F, Ollomo B, Dubreuil G, Morelli A, Domarle O, Nabias R, et al. *Plasmodium coatneyi:* differential clinical and immune responses of two populations of *Macaca fascicularisI* from different origins. Exp Parasitol 1999;91:30–9.
[60] Elmore DB. *Plasmodium vivax* seroprevalence in bred cynomolgus monkeys, China. Emerg Infect Dis 2011;17(10):1978–9.
[61] Butcher GA, Mitchell GH, Cohen S. *Plasmodium knowlesi* infections in a small number of non-immune natural hosts (*Macaca fascicularis*) and in rhesus monkeys (*M. mulatta*). Trans R Soc Trop Med Hyg 2010;104:75–7.
[62] Schmidt LH, Fradkin R, Harrison J, Rossan RN. Differences in the virulence of *Plasmodium knowlesi* for *Macaca irus (fascicularis)* of Philippine and Malayan origins. Am J Trop Med Hyg 1977;26(4):612–22.
[63] Li H-L, Liu Z-Y, Li J, Ai L, Zhou D-H, Yuan Z-G, et al. *Plasmodium vivax* seroprevalence in bred cynomolgus monkeys, China. Emerg Infect Dis 2011;17(5):928–9.
[64] Bauchet A-l, Joubert C, Helies J-M, Lacour SA, Bosquet N, Le Grand R, et al. Disseminated sparganosis in a cynomolgus macaque (*Macaca fascicularis*). J Comp Pathol 2013;148:294–7.
[65] Tuberculosis Country Profiles. Retrieved from: http://www.who.int/tb/country/en/; 2014.
[66] Tuberculosis in imported nonhuman primates—United States, June 1990–May 1993. MMWR 1993;42(29).
[67] Garcia MA, Bouley DM, Larson MJ, Lifland B, Moorhead R, Simkins MD, et al. Outbreak of *Mycobacterium bovis* in a conditioned colony of rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. Comp Med 2004;54(5):578–84.
[68] Panarella ML, Bimes RS. A naturally occuring outbreak of tuberculosis in a group of imported cynomolgus monkeys (*Macaca fascicularis*). J Am Assoc Lab Anim Sci 2010;49(2):221–5.
[69] Langermans JA, Anderson P, van Soolingen D, Vervenne RA, Frost PA, van der Laan T, et al. Divergent effect of bacillus Calmette-Guerin (BCG) vaccination on *Mycobacterium tuberculosis* infection in highly related macaque species: implications for primate models in tuberculosis vaccine research. Proc Natl Acad Sci USA 2001;98:11497–502.
[70] Ebola virus infection in imported primates—Virginia, 1989. MMWR 1989;38(48):831–2, 837–8.
[71] DeMarcus TA, Tipple MA, Ostrowski SR. US policy for disease control among imported nonhuman primates. J Infect Dis 1999;179(Suppl. 1):S281–2.
[72] Clarke AS, Boinski S. Temperament in nonhuman primates. Am J Primatol 1995;37:103–25.
[73] Clarke AS, Mason WA, Moberg GP. Differential behavioral and adrenocortical responses to stress among three macaque species. Am J Primatol 1988;14:37–52.
[74] Clarke AS, Mason WA, Mendoza SP. Heart rate patterns under stress in three species of macaques. Am J Primatol 1994;33:133–48.
[75] Brent L, Veira Y. Social behavior of captive Indochinese and insular long-tailed macaques (*Macaca fascicularis*) following transfer to a new facility. Int J Primatol 2002;23(1):147–59.
[76] Bernstein IS. Activity profiles in primate groups. In: Schrier AM, Stollnitz F, editors. Behavior of nonhuman primates, modern research trends. New York: Academic Press; 1971.
[77] Krebs KC, Jin Z, Rudersdorf R, Hughes AL, O'Connor DH. Unusually high frequency MHC class I alleles in Mauritian origin cynomolgus macaques. J Immunol 2005;175:5230–9.
[78] Cowan MJ, Giddens Jr. WE, Reichenbach DD. Selective myocardial cell necrosis in nonhuman primates. Arch Pathol Lab Med 1983;107:34–9.
[79] Samuels MA. The brain-heart connection. Circulation 2007;116:77–84.
[80] Corley KC, Shiel FO, Mauck HP, Greenhoot J. Electrocardiographic and cardiac morphological changes associated with environmental stress in squirrel monkeys. Psychosom Med 1973;35(4):361–4.
[81] Khullar M, Datta BN, Wahi PL, Chakravarti RN. Catecholine-induced experimental cardiomyopathy—a histological, histochemical and ultrastructural study. Indian Heart J 1989;41:307–13.

VetBooks.ir

CHAPTER

6

Basic Physiology of *Macaca mulatta*

Anne D. Lewis, Kamm Prongay

Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

OUTLINE

General Biology 88

Gastrointestinal Tract and Nutrition 89
- *Natural Diet and Anatomic Specializations* 89
- *Feeding in Captivity* 90
- *Stomach Volume, Passage Time, and Typical Administration Routes* 90

Cardiovascular System 91
- *Vascular Access and Sampling* 91
- *Hematologic Parameters* 92
- *Cardiovascular Evaluation* 93
  - Blood Pressure 93
  - Heart Rate 93
  - Electrocardiography 93
  - Echocardiography 95

Respiratory System 96
- *Structure and Function* 96
  - Unique Anatomy of Lungs, and Trachea 96
- *Respiratory Rate, Tidal Volume, and Monitoring* 97

Neurologic System and Special Senses 98
- *Neurologic Evaluation and Body Temperature* 98
- *Special Sense: Eye* 100

Development and Reproduction 100
- *Breeding* 100
- *Female Reproduction* 101
  - Development 101
  - Pregnancy 102
  - Lactation 103
  - Senescence 103
- *Male Reproduction* 104
  - Development 104
  - Semen Collection and Evaluation 104

Urinary System 105

Lymphatic System 107

Endocrine System 108

References 108

http://dx.doi.org/10.1016/B978-0-12-417144-2.00006-8
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

GENERAL BIOLOGY

Rhesus macaques (*Macaca mulatta*) are Old World nonhuman primates in the diverse Cercopithecidae family. They are one of the approximately 20 species within the genus *Macaca* that are found almost exclusively in Asia. Taxonomists generally recognize five species groups. The *fascicularis* group includes the rhesus macaque, cynomolgus macaque (*M. fasciculata*), and Japanese macaque (*M. fuscata*), all of which are used as laboratory animals. Smaller numbers of animals from the other species have also been used in laboratory settings, including the pigtailed macaque (*M. nemestrina*) and bonnet macaque (*M. radiata*) in the *silenus* group. The *sylvanus* group, composed of a single species, the Barbary macaque (*M. sylvanus*), is found in the Gibraltar region of northern Africa and is the only species found outside of Asia. Macaques are medium-sized monkeys with a robust body type, variable tail length, and moderately elongated noses [1].

Rhesus macaques share classic features with other Old World nonhuman primate species, including tough keratinized ischial callosities overlying and firmly adherent to the underlying ischial tuberosities; catarrhine nasal morphology with forward-facing, closely spaced nares; and a nonprehensile tail. They have nails (rather than claws) on the distal ends of all digits. As with all macaques, rhesus have cheek pouches, which are cavities lined by mucosal epithelium continuous with the oral cavity. There is a cervical air sac that communicates with a central ostium in the pharynx. Their coloration is a medium brown with lighter undersides. The skin may be patchily pigmented, but this pigment is not reflected in the color of the overlying coat.

Metrics for physical characteristics and life history parameters are presented in Table 6.1. Rhesus macaques exhibit moderate sexual dimorphism. The average weight of males is 7.7 kg and the average height is 531 mm; for females, the average weight is 5.34 kg and height is

TABLE 6.1 Physical Characteristics and Life History Parameters for Rhesus Macaques

Weight and length:
- Birth weight: 450 g
- Adult weight: ♀ 5.34 kg; ♂ 7.7 kg
- Adult body length (without tail): ♀ 470-531 mm; ♂ 480-635 mm
- Adult tail length: ♀: 189-284 mm; ♂: 203-305 mm

Dentition:
- Canine length ♀ 6.0 mm; ♂ 8.9 mm

Life history:
- Life span: 25 years
- Weaning age: 270 days
- Puberty: ♀: 2-4 years; ♂: 3-5 years
- Sexual and social maturity: ♀: 3-5 years; ♂: 4-6 years

Reproduction characteristics:
- Multimale-multifemale breeding groups
- Ovarian cycle: 29 days

Gestation:
- Length: 165-170 days
- Litter size: single offspring
- Interbirth interval: 12 months

Adapted from Rowe [119] and Leutenegger and Cheverud [120]

VetBooks.ir

469 mm [2]. In addition to differences in height and weight, adult males have sharp, well-developed, elongated canines and prominent temporal musculature. Under the influence of estrogen, the perineal skin (sex skin) of female rhesus macaques becomes mildly reddened and swollen. Similarly responsive skin is found on the caudal thighs and may extend to the flanks and be present on the brow ridge of the face.

The geographic range of rhesus macaques is unusually broad, extending from Afghanistan to Eastern China and including the northern regions of the Indian subcontinent and northern Indochina. Their habitats are commensurately diverse, including both subtropical and temperate zones, sea level to altitudes of 2000 m and above, and semiarid plains to rain forest. They are found in a variety of environments including agricultural zones, dense forests, and urban settings [1,3]. They are diurnal and adapted for arboreal life but may spend significant time on the ground foraging. They are strong swimmers [1].

In the wild they live in mixed-gender groups of 8-180 individuals. Females generally outnumber males. Males often leave the groups and dwell singly or in small single-gender groups. Dominance hierarchies are maintained separately between the sexes; male hierarchies are frequently less stable. The size of the home range is highly dependent on habitat. Large ranges occur in forests with poor resources, whereas small ranges are found in urban environments with ready access to food, water, and shelter. There is low to moderate territoriality among groups [1].

Rhesus reach adult size at 5-7 years of age; males mature later than females. The average life span in captivity is 25-27 years. Maximum life span may approach 40 years [2,4]. In their use as laboratory subjects, rhesus macaques are generally characterized as either of Indian or Chinese origin. Indian-origin animals in the United States are derived from stock imported before the 1978 ban on rhesus macaque exportation by the Indian government [1]. Animals designated as of Chinese origin are obtained from captive breeding programs in China but are derived from a broad geographic range and tend be more genetically heterogeneous [5]. There are documented differences in temperament, behavior, disease susceptibility, morphology, and physiology [6–8]. Differences in immunology and disease susceptibility and progression are of particular importance in simian immunodeficiency virus research, for which rhesus macaques are a model of major importance [9–12].

The primary uses of the rhesus macaque in biomedical research are in areas of reproductive biology, including assisted reproductive technology, neuroscience, immunology, and infectious disease. They are the principle nonhuman primate model for the study of acquired immunodeficiency syndrome pathogenesis and vaccine development. In addition, they are emerging as a major model of the biology of aging. Rhesus macaques are less commonly used in toxicologic studies because of their larger size and more limited availability compared to cynomolgus macaques. Significant aspects of rhesus macaque physiology are presented here because of their ongoing use in biomedical research and their wider use historically in the study of basic nonhuman primate physiology.

GASTROINTESTINAL TRACT AND NUTRITION

Natural Diet and Anatomic Specializations

The natural diet is composed principally of plants, with proportionally smaller amounts of insects, seeds, and occasional small mammals. The major dietary components, in order,

VetBooks.ir

are carbohydrates, proteins, and lipids. The largest source of ingested energy comes from complex plant carbohydrates, and the oral cavity has several modifications to facilitate their ingestion. Cheek pouches, or pockets in the oral cavity, are used to temporarily store food. Saliva in the cheek pouch contains amylase, active in starch digestion [13]. The dentition has some adaptation for ripping and tearing. Multiple molars facilitate mastication of plant material. The adult dental formula is 2-1-2-3/2-1-2-3 [14].

The second major gastrointestinal tract function, digestion, is carried out in the stomach and intestine. The stomach, which includes a cardia, fundus, body, and pylorus, is the principle site of protein digestion. The small intestine, composed of the duodenum, jejunum, and ileum, is the principle site of lipid digestion and where most nutrient absorption takes place. Digestion of complex carbohydrates occurs primarily in the cecocolic region and is aided by microbial activity and fermentation. Water absorption and storage of waste for defecation occurs in the descending colon.

Feeding in Captivity

The diet should provide calories for maintenance and essential vitamins and minerals should comprise the majority of the diet. Commercial diets, formulated using the National Research Council's Nutrient Requirements of Nonhuman Primates, typically have 75% of metabolizable energy from carbohydrates, 15-22% from protein, and the remainder from lipids [15]. Rations fed in captivity are typically nutritionally dense and balanced. While satisfying nutrient and caloric needs, the ration may not allow for adequate foraging behavior. The addition of fresh fruits and vegetables at various times during the day ensures variety and may be important for gastrointestinal health. The use of puzzle feeders, stocked with high-value foods, can further enhance foraging and exploratory behaviors. Highly desirable foods, such as raisins or marshmallows, are used for training rewards. Enrichment and training foods will be consumed preferentially, so these should be carefully balanced with commercial rations.

Overnutrition can lead to obesity and may negatively impact research [16]. Controlling feed intake in individual cages is straightforward. Feeding in group settings can be problematic because dominant animals may block subordinate animals from feeding stations and become overweight. Multiple feeding stations reduce this tendency. In age-mixed populations, creep feeders stocked with higher-protein chow may benefit weanlings and small juveniles. Older animals may have decreased nutrient absorption and may require vitamin B and caloric supplementation [17]. Dietary sensitivities are uncommon, but gluten sensitivity has been demonstrated [18].

Stomach Volume, Passage Time, and Typical Administration Routes

The stomach is distensible and may accommodate 10-20 mL/kg of fluid or ingesta. Typical volumes of administration for adult rhesus are provided in Table 6.2. Overexpansion of the stomach can cause emesis and should be avoided. Stomach emptying time is 12-16 h [13]. Gut passage time may vary from 16 to 27 h, depending on diet, activity, and stress level [13]. Routes of administration commonly used in humans are easily achieved in the rhesus macaque. Nasogastric administration may be done on sedated infants weighing as little as 300 g.

VetBooks.ir

TABLE 6.2 Dosing Volumes for Adult Rhesus Macaques

Route	Maximum volume	Comments
Capsule	Single 0 capsule	Anatomic size limits capsule size
Nasogastric or orogastric	10 mL/kg	Per dose; two doses, at least 4 h apart, may be given daily
IV bolus (≤3 min)	10 mL/kg	
IV infusion (≤1 h)	15 mL/kg	
IV infusion (1-6 h)	10 mL/kg/h	
IV infusion (6-24 h)	5 mL/kg/h	
IV infusion (>24 h)	4 mL/kg/h	For chronic infusion

Adapted from Walker et al. [19]

Oral drugs, when appropriately flavored, are typically well tolerated. Intake may decline as novelty wanes [19]. Chair restraint or light sedation is required for nasogastric or orogastric administration of compounds. Repeated nasal or oral gastric lavage may cause esophageal trauma. Nasogastric tubes can be left in place in premature infants. Intraperitoneal injection may be performed with ultrasound guidance. Continuous intraperitoneal infusion, when protected by a jacket, is possible. The gastric and colonic lumen of 8 kg adult animals can typically accommodate pediatric endoscopes and colonoscopes used in humans.

Experimental manipulation may cause stress. Weight loss of ≥10% over a 14-day period is not uncommon if animals are repeatedly sedated or have not been adequately habituated to the experimental procedure. Standardizing daily weight and feed times, and prestudy training, may reduce this variability.

CARDIOVASCULAR SYSTEM

Vascular Access and Sampling

The femoral vein is typically selected for venipuncture. Secondary peripheral locations include the saphenous and cephalic veins. Juvenile and adult animals tolerate 22- or 23-gauge needles and catheters. Animals weighing <1 kg may require a 24-gauge needle or catheter. Acute catheterization of femoral and jugular veins is readily achieved. Continuous infusions through either site usually require surgical implantation and must be protected by jacket and tether. Vascular access ports can be placed in the dorsolateral thoracic region and tunneled to an appropriate vein. These ports can, when coupled with a jacket and pump, be used for ambulatory intravenous access. Maximum blood draw amount should not exceed 10% of the body weight, and no more than 15% of the total blood volume should be drawn in a 2-week period [19].

Hand restraint and blood draw can easily be accomplished on infants but alterations in blood gas concentrations and glucose are common. Collection of venous blood from unsedated animals may be performed; however, stress from physical restraint can cause elevated leukocyte counts [20]. Concentrations may normalize following acclimatization [21].

VetBooks.ir

Hematologic Parameters

Information on cell number and size by sedation agent is displayed in Table 6.3 [22]. Detailed information on the rhesus red blood cell proteome, and comparisons with mouse and human cells, has recently been published [23]. Infants from birth to approximately 3 months of age have higher hematocrit, hemoglobin, and red blood cell counts relative to adults [24]. Adult males typically have higher hematocrit, hemoglobin, and red blood cell counts than females [25]. These sex differences have not been reported in juvenile animals. With aging, mean corpuscular volume and mean corpuscular hemoglobin decrease [26,27], while hematocrit, hemoglobin, and red blood cell count increase [27]. Geriatric females may experience decreased hematocrit concentrations in conjunction with a decrease in mean corpuscular volume, as well as increased leukocyte counts [26]. Eosinophil concentrations are higher in adult wild-ranging rhesus macaques than subadults [26]. Female rhesus macaques have higher neutrophil and eosinophil counts than males [25,26].

Anemia is the most common abnormal finding [28]. In captivity, blood loss secondary to excessive phlebotomy is the primary cause [29]. Less common etiologies include endometriosis in older females, chronic enteric loss due to bleeding from tumors such as colonic adenocarcinomas, and loss associated with parturition [30]. Coagulation abnormalities are most often associated with experimental manipulation or experimental infection, although

TABLE 6.3 Normal Hematological and Leukocyte Parameters in Adult Rhesus Macaques by Sedation Type

	Ketamine ($n=53$)	**Pentobarbital ($n=54$)**	**Isoflurane ($n=9$)**
Red blood cells ($\times 10^6$/L)	5.32±0.26	5.4±0.44	5.07±0.31
Hemoglobin (g/dL)	12.55±0.54	12.74±0.89	12.04±0.59
Hematocrit (%)	38.86±1.57	39.35±1.91	36.21±1.85
Mean cellular volume (fL)	73.07±2.07	73.20±2.70	71.36±2.34
Mean cellular hemoglobin (pg)	23.65±0.87	23.66±0.76	23.74±0.93
Mean cellular hemoglobin concentration (g/dL)	32.19±1.23	31.89±1.13	33.27±0.35
White blood cells ($\times 10^3$/L)	6.15±1.98	5.75±2.29	5.51±2.31
Neutrophils (%)	39.54±12.94	41.22±11.46	33.17±11.47
Lymphocytes (%)	55.44±16.16	51.50±11.48	63.33±10.97
Monocytes (%)	2.91±1.41	3.83±1.86	1.44±1.89
Eosinophils (%)	2.18±1.74	2.21±1.86	1.61±1.72
Basophils (%)	0.21±0.22	0.41±1.07	0.11±0.31
Large unstained cells (%)	0.64±0.49	0.86±0.29	Not tested
Platelets ($\times 10^3$/L)	310.49±50.04	303.75±46.98	350.33±62.32

Adapted from Hom et al. [22]

type-3 von Willebrand disease has been reported [31,32]. Thrombosis is rare other than in experimental manipulation [33].

Early red blood cell studies of rhesus macaques contributed to our understanding of blood antigens. The human Rh factor is named for the rhesus monkey. Many of the oligosaccharide antigens on cells (i.e., blood group antigens) have been characterized in the rhesus. Rhesus macaques express the A-B-O, Rh-Hr, M-N, Lewis, and I-I antigen groups, which occur in humans [34]. Of practical significance is that the A-B-O antigens are not expressed on macaque erythrocytes as they are in humans but are only expressed on endothelium, epithelial cells, and exocrine gland secretions. Practical concerns over blood type incompatibility in transfusion are of little significance; however, tissue typing may be of concern in organ transplantation studies [35]. Several methods of determining ABO type have been developed, including a reverse gel system assay [36], multiplex polymerase chain reaction [37], and buccal mucosal immunohistochemistry [38], because standard red blood cell agglutination tests cannot be used.

Cardiovascular Evaluation

The vital organs of the cardiovascular, respiratory, and central nervous systems are key areas of focus in safety pharmacology studies and are outlined in the S7A International Conference on Harmonization guidelines [39]. These guidelines recommend blood pressure, heart rate, and electrocardiography measurements to evaluate effects on the cardiovascular system. Measurements may be obtained directly or from implanted telemetry devices. For studies of longer duration, implanted telemetry is preferred because both chemical and physical restraint will alter values [40].

Blood Pressure

Indirect measurement of systolic, diastolic, and mean arterial pressure can be achieved using most monitors, provided the blood pressure cuff is appropriately sized. Typical sites include the arm or tail. Doppler pressure using the dorsal pedal artery is also possible. Invasive monitoring using implanted telemetry is recommended for longer studies and to avoid restraint- and sedative-induced artifacts. Values for infant, pregnant, and adult animals are shown in Table 6.4. Infant rhesus have lower blood pressure than adults. Ketamine anesthesia has been associated with lower blood pressure in 5-day-old rhesus infants but not in 35-day-old infants. Pregnant rhesus macaques typically have lower blood pressure than nonpregnant adult females. This difference persists in spite of restraint or sedation [40].

Heart Rate

Direct measurement of heart rate is most often done with a stethoscope. The femoral arterial pulse is easily palpated and should be compared with the auscultated rate to confirm each beat is associated with a pulse.

Electrocardiography

Telemetric electrocardiographic evaluation is recommended to more accurately capture conduction and repolarization abnormalities. Normal values and typical lead placement locations are shown in Tables 6.5 and 6.6, respectively. The evaluation of the QT interval is of

TABLE 6.4 Normal Physiologic Cardiovascular Values for Rhesus Macaques

	Adults	Pregnant (GD 122)	Infant (PND 5)	Infant (PDN 35)
Heart rate (bpm)				
Conscious	110±11.51	197±32	219±25	212±34
Ketamine sedation	134±14.72	130±16	158±26	171±22
Oxygen saturation (%)				
Conscious		94±4	96±3	97±2
Ketamine sedation	96.53±1.00	94±4	94±3	94±3
Mean arterial pressure (mmHg)				
Conscious	99±10.3	85±13	63±16	58±16
Ketamine sedation	96±12.97	68±14	47±10	53±12
Systolic blood pressure (mmHg)				
Conscious		112±14	85±19	74±17
Ketamine sedation		87±17	66±10	74±16
Diastolic blood pressure (mmHg)				
Conscious		68±14	54±16	50±15
Ketamine sedation		54±13	38±8	42±10

Adapted from Hotchkiss et al. [24] and Hom et al. [22]

TABLE 6.5 Mean Electrocardiography Intervals, Durations, and Parameters in Rhesus Macaques

P Wave Duration	P-R Interval	QRS Duration	QT Interval	P Wave (mV)	Q Wave (mV)	R Wave (mV)	Mean Electrical Access	Heart Rate (bpm)
40±10	90±10	30±10	200±20	0.20±0.05	Not determined	0.8±0.03	69±16	178±29

Adapted from Gonder et al. [121]

TABLE 6.6 Standard Electrocardiography Lead Placement

Lead	Lead placement
Left arm	Wrist of the left arm
Right arm	Wrist of the right arm
Left leg	Calf of the left leg
Right leg	Calf of the left leg
V1	Fourth intercostal space, 4 cm right from midsternal line
V2	Fourth intercostal space, 4 cm left from midsternal line
V3	Fifth intercostal space, left midaxillary line
V4	Seventh thoracic vertebra

Adapted from Walker et al. [19]

particular importance in toxicology studies [41]. In humans, a drug-induced prolonged QT interval can lead to a life-threatening tachyarrhythmia, torsade de pointes.

Echocardiography

Detailed evaluation of cardiovascular structure and function is achieved using cardiac ultrasonography. Mean values are presented in Tables 6.7–6.9 [42]. Older animals may have decreased heart rate, lower left ventricular diastolic function, and diminished compliance of the pulmonary artery and aorta [43]. Prestudy cardiac ultrasound is recommended. Congenital heart defects, while uncommon, have been reported [44]. Valvular endocarditis secondary to endometritis and systemic bacterial disease can occur [45].

TABLE 6.7 Left Ventricular Chamber Geometry

	Mean	SD	Minimum-maximum
Minor axis left ventricular dimension at end diastole (LVD_{ed}) (cm)	2.23	0.31	1.60-2.90
Minor axis left ventricular dimension at end systole (LVD_{es}) (cm)	1.41	0.25	1.00-2.00
Left ventricular long axis dimension at end diastole (LVL_{ed}) (cm)	3.57	0.44	2.75-4.43
Left ventricular long axis dimension at end systole (LVL_{es}) (cm)	2.99	0.42	2.08-3.87
Left ventricular posterior wall thickness at end diastole ($LVPW_{ed}$) (cm)	0.47	0.07	0.30-0.65
Left ventricular wall thickness at end systole ($LVPW_{es}$) (cm)	0.65	0.09	0.50-0.90
Left ventricular mass (LVM) (g)	18.83	6.81	8.69-37.65
Inferior vena cava diameter (IVC_d) (cm)	0.51	0.12	0.32-0.76
Main pulmonary artery diameter (MPA_d) (cm)	1.54	0.15	1.14-1.79
M-mode left atrium (cm)	1.39	0.24	1.00-1.85
M-mode aortic root diameter (cm)	1.33	0.16	1.10-1.80
M-mode aortic valve excursion (cm)	0.96	0.12	0.70-1.20

Adapted from Korcarz et al. [42]

TABLE 6.8 Left Ventricular Function Values for the Rhesus Macaque

	Mean	SD	Minimum-maximum
Heart rate (beats/min)	142	19	115-200
Left ventricular ejection time (LVET) (s)	0.171	0.024	0.120-0.210
Left ventricular ejection time corrected for heart rate ($LVET_c$)	0.262	0.027	0.202-0.302
%ΔD	37.3	5.4	27.3-45.5
Stroke volume derived from aortic Doppler (V_{cfc}) (circ/s)	2.19	0.39	1.55-2.98
End-systolic left ventricular meridional wall stress (esm) (g/cm^2)	59.6	13.9	36.9-87.8
End-systolic left ventricular circumferential wall stress (esc) (g/cm^2)	161.9	29.4	108.64-218.3

Adapted from Korcarz et al. [42]

TABLE 6.9 Doppler Parameters for the Adult Rhesus Macaque

	Mean	SD	Minimum-maximum
Left ventricular outflow tract diameter (LVOTd) (cm)	0.94	0.11	0.72-1.12
Stroke volume derived from aortic Doppler velocities (SVao) (mL)	9.24	2.57	4.73-13.21
Cardiac output derived from aortic flow (COao) (L/min)	1.36	0.41	0.54-2.07
Main pulmonary artery diameter (MPAd) (cm)	0.95	0.12	0.75-1.26
Stroke volume derived from main pulmonary artery Doppler velocities (SVpa) (mL)	8.61	2.52	4.53-15.38
Cardiac output derived from pulmonary artery flow (COpa) (L/min)	1.23	0.37	0.52-2.04
Systolic BP (mm Hg)	118	17.8	91-145
Diastolic BP (mm Hg)	62.1	20.4	32-107
Mean BP (mm Hg)	83.6	20.9	51-122
Total peripheral resistance (TPR) (dynes-s/cm^3)	5815	3318	2956-17,194
Diastolic filling time (ms)	195.5	37.4	140-288

Adapted from Korcarz et al. [42]

RESPIRATORY SYSTEM

Respiratory function in drug toxicity studies is typically assessed by monitoring respiratory rate and respiratory function. Functional assessment is done measuring tidal volume or oxygen saturation.

Structure and Function

The upper respiratory system includes the nasal cavity, paranasal sinuses, nasopharynx, pharynx, larynx, trachea, and bronchi. The upper respiratory tract filters and moisturizes incoming air. The pharynx and larynx ensure food and liquids are blocked from the trachea. Receptors for the sense of smell are present in the nasal cavity and paranasal sinuses. They also contribute to the sense of smell and taste. Macaques lack a vomeronasal organ. The nasal cavity mucosa includes stratified squamous epithelium, transition epithelium, ciliated pseudostratified respiratory epithelium, and olfactory epithelium overlying nasal-associated lymphoid tissue. Detailed cellular descriptions are presented in other references [46]. Pseudostratified respiratory epithelium lines the trachea and bronchi. Cells in this area include ciliated, mucous, serous, basal, and neuroendocrine cells. The mucosa of the tracheal and bronchial possesses lymphocytes, globular leukocytes, and intraepithelial nerve fibers. The submucosa contains connective tissue, blood vessels, lymph channels, nerves, glands, and occasional focal or diffuse lymphocyte infiltrates [47].

Unique Anatomy of Lungs, and Trachea

The lungs in the rhesus macaque have four lobes on the right and three on the left [46]. The right mainstem bronchus is the first bifurcation and departs distal to the carina. Pulmonary

VetBooks.ir

and bronchial arteries provide arterial blood to the bronchi. Bronchiole branches lack cartilage and submucosal glands. Terminal bronchioles open to the acinus. Each acinus includes a respiratory bronchiole, alveolar duct, alveolar sac, alveoli, and arterial and venous blood supply.

Respiratory Rate, Tidal Volume, and Monitoring

Tidal volume, respiratory rate, and minute volume in the unanesthetized rhesus macaque is similar to that reported for other primate species [48]. There are some differences in pulmonary mechanics between males and females. Mean tidal volume for males is 38.9 mL and mean tidal volume for females is 46 mL. The respiratory rate is similar: 38 breaths per minute for males and 40 for females. Additional values, including minute volumes, are displayed in Table 6.10. Mild differences in pH, pCO2, pO2, and base excess are also reported between males and females and between animals evaluated at different time points [48]. The effects of anesthesia on blood gas values are represented in Table 6.11. Naturally occurring pulmonary disease is uncommon. Systemic inflammatory response syndrome and acute lung injury has been reported in both toxicology studies and viral research studies [31,49].

Intubation is typically done in dorsal recumbency. Infants can be intubated using a small, straight laryngoscope blade (the 00 Miller). A curved Macintosh of appropriate size is generally used for juveniles and adults. Bronchoscopy of the trachea and mainstem bronchi of adults can be achieved using a pediatric scope with 3.8-mm outer diameter. Total and differential cell counts from bronchoalveolar lavage fluid are presented in Table 6.12 [50].

TABLE 6.10 Measurement of Lung Mechanics in Adult Rhesus Macaques

	Wt (kg)	Vt (mL)	*f* (cycles/min)	Respiratory Minute Volume (mL/min)
Males	3.48	38.9	38	1441
Females	3.33	46.0	40	1820
Total mean	3.40	42.5	39	1630

Adapted from Binns et al. [48]

TABLE 6.11 Arterial Blood Gas Values for Rhesus Macaques, by Anesthetic Agent

	Ketamine ($n=33$)	Pentobarbital ($n=36$)	Propofol ($n=7$)	Isoflurane ($n=8$)
pH	7.39±0.03	7.35±0.02	7.34±0.01	7.39±0.02
pCO_2 (mm Hg)	38.19±3.77	47.45±3.51	46.81±2.29	44.07±1.36
pO_2 (mm Hg)	88.06±7.49	85.20±7.64	82.65±4.16	Supplemented
HCO_3 (mmol/L)	23.53±2.65	26.68±2.14	25.70±1.68	26.69±0.86
TCO_2 (mmol/L)	24.71±2.75	28.15±2.23	27.13±1.72	28.05±0.88
O_2 saturation (%)	96.53±1.00	95.60±1.21	95.28±0.55	100±0.00
O_2 concentration (mL/dL)	19.18±0.23	19.01±0.31	18.89±0.12	19.8±0.00

Adapted from Hom et al. [22]

TABLE 6.12 Total and Differential Cell Analysis of Bronchoalveolar Lavage Fluid from Rhesus Macaques

Total count[a] ($\times 10^5$/mL)	Macrophages[a,b] (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
10.2±1.48	98.20±1.61	1.13±1.41	0.60±0.91	0.07±0.26

[a]*Values are mean±standard deviation (n=15).*
[b]*Differential cell analysis presented as a percentage of 200 cells in at least 5 representative fields.*
Adapted from TateRico and Roy [50]

NEUROLOGIC SYSTEM AND SPECIAL SENSES

Neurologic Evaluation and Body Temperature

The neurologic system of the rhesus is similar to that of all primates. Detailed neurophysiology is available in both veterinary and human physiology texts [51,52]. Evaluation of the central nervous system in toxicology studies begins with cage-side observations. The presence of an observer may alter normal behavior, so video monitoring is encouraged. Behavior, including normal postures, is initially assessed. Motor function, including limb placement and balance, may be evaluated by having the animal move from cage floor to perch and back. Gait, and thus cerebellar function, may be assessed by placing the animal in a larger enclosure. A sample grading system is presented in Table 6.13 [19].

Following the initial observation, light sedation facilitates evaluation of muscle mass and balance and cranial nerves. A rough estimate of intracranial pressure can be estimated by

TABLE 6.13 Sample Neurologic Examination Grading System

Posture Grade	Clinical signs
0	Absence of purposeful movement; paraplegia
1	Unable to stand to support; slight movement when supported by tail; severe paraparesis
2	Unable to stand to support; when assisted moves limbs readily but stumbles and falls frequently; moderate paraparesis and ataxia
3	Can stand to support but frequently stumbles and falls; mild paraparesis and ataxia
4	Can stand to support; minimal paraparesis and ataxia
5	Normal strength and coordination
Patellar Reflexes	**Clinical signs**
0	No reflex
1	Hyporeflexic
2	Normal
3	Hyperreflexic
4	Colonic/tonic

Adapted from Walker et al. [19]

VetBooks.ir

performing a fundoscopic examination. Body temperature should also be measured. Rectal temperatures can be acquired with a digital rectal thermometer. Esophageal monitors or rectal probes can also be used. Temperature elevation of 1-2°C is common following capture or restraint.

Cerebral spinal fluid may be collected from the cisterna magna or lumbosacral region. Concentrations of various components are shown in Table 6.14 [53].

TABLE 6.14 Enzyme Activity, Amino Acid Level, and Normal Values of Cerebrospinal Fluid in Rhesus Macaques

	Animals	Samples	Mean	SE	SD	Range
Enzyme activity (U/I)						
Alkaline phosphatase	13	37	18.7	3.2	19.4	1.7-39.7
Creatine phosphokinase	13	37	9.9	1.5	9.2	0.3-35.0
Glutamine oxaloacetate transaminase (aspartate aminotransferase)	13	43	13.7	0.6	4.1	6.6-20.0
Glutamine pyruvate transaminase (alanine aminotransferase)	13	44	9.2	0.7	4.9	3.8-28.3
γ-Glutamyl transpeptidase	13	36	3.1	0.5	2.7	1.0-16.7
α-Hydroxybutyrate dehydrogenase	13	44	33.0	2.3	15.2	7.3-67.3
Lactate dehydrogenase	13	44	47.2	3.2	20.9	8.5-101.6
Sorbitol dehydrogenase	13	29	3.9	0.4	2.3	0.6-10.4
Amino acid levels (mmol/L)						
Alanine	13	39	0.04	0	0.01	0.02-0.07
Glutamate	13	41	0.07	0	0.02	0.02-0.14
Glutamine+serine+threonine	13	40	0.31	0.01	0.09	0.13-0.68
Glycine	13	38	0.13	0.3	0.16	0.02-0.53
Histidine	13	41	0.03	0	0.01	0.03-0.07
Lysine	13	37	0.03	0	0.01	0.02-0.05
Ornithine	13	34	0.03	0	0.01	0.02-0.06
Valine	13	39	0.05	0	0.01	0.02-0.06
CSF fluid constituents						
Copper (μg/dL)	11	32	2.6	0.2	1.10	1.1-6.0
Iron (μg/dL)	11	29	35.9	2.8	15.1	12-65
Zinc (μg/dL)	11	35	1.0	0.06	0.38	0.1-1.7
Sodium (mEq/L)	13	42	154.5	5.6	36.2	94-254

(*Continued*)

TABLE 6.14 Enzyme Activity, Amino Acid Level, and Normal Values of Cerebrospinal Fluid in Rhesus Macaques—Cont'd

	Animals	Samples	Mean	SE	SD	Range
Potassium (mEq/L)	13	42	1.57	0.04	0.23	1.12-1.54
Calcium (mmol/L)	13	42	1.55	0.07	0.45	0.95-2.85
Magnesium (mmol/I)	13	42	1.16	0.03	0.21	0.78-1.79
Creatinine (mg/dL)	13	40	0.49	0.02	0.15	0.16-0.8
Triglycerides (mg/dL)	6	11	32.3	7.9	26.1	15.8-109.6
Total protein (mg/dL)	8	30	66.4	0.09	49.7	14-200
Urea nitrogen (mg/dL)	12	31	25.7	1.9	11.1	2.5-46.6

Adapted from Hambelton et al. [53]

Special Sense: Eye

The boney orbit and brow ridge are prominent, and the globe is recessed relative to many other mammals. These features can make measurement of intraocular pressure (IOP) difficult. The mean IOP of ketamine-sedated adult animals (4-21-years old) is 14.9±2.1 mm Hg, and the mean IOP of infants and juveniles (0.6-3-years old) is 15.7±2.0 mm Hg. Time of day variations in IOP are reported [54]. The sclera is largely obscured by the palpebral conjunctiva. The anterior chamber, iris, and ciliary bodies are readily visualized with an ophthalmoscope. Assessment of the vitreous and fundus, including the optic disc, macula, and retina, is aided by a topical mydriatic. Detailed assessment of the retinal vessels is achieved with fluorescein angiography. Electroretinography and ocular ultrasonography may also be performed.

DEVELOPMENT AND REPRODUCTION

Breeding

Unlike cynomolgus macaques and common marmosets, rhesus macaques are seasonal breeders. In the wild, ovarian cycling occurs predominately in the fall and winter. An anovulatory period with irregular menses occurs during the spring and summer. Infants are born primarily in the spring and early summer (Figure 6.1). Males also exhibit a seasonal reduction in fertility. Similar seasonal patterns are observed in captive rhesus macaques managed outdoors in northern latitudes [55,56]. This marked seasonality is abated somewhat in animals maintained indoors in artificial light cycles, but within a population of macaques, both female and male fertility is reduced during the summer months [57–59].

In the wild, mating and rearing of infants occurs in mixed-gender groups in which females outnumber males two- to fourfold. Mating occurs preferentially among higher ranking members of the troop. In captivity, a variety of breeding strategies are used, primarily involving variably sized mixed-sex social groups in which females outnumber males. Commonly used infrastructures are semi-free-ranging islands, outdoors corrals up to 1 acre, intermediate-sized outdoor group housing, and indoor or outdoor harem/corn crib type housing. To provide

VetBooks.ir

FIGURE 6.1 Rhesus macaques are seasonal breeders; the majority of infants are born in the late spring (ONPRC/Bill Sutton).

pregnant animals or offspring for experimental studies year round, timed mating breeding protocols may be used. Pregnancies of known gestational age can be obtained by housing males with receptive females for 4 days beginning 10 days after the onset of first day of menses.

Fecundity is low in this species. Female rhesus undergo puberty at 2-4 years of age. A primiparous dam may deliver her first offspring as early as 3 years of age; 4-5 years of age is more common. Peak fertility is between 7 and 10 years, after which there is general decline in fertility [60]. Female macaques routinely deliver a single offspring (Figure 6.2). Twinning is rare and often associated with adverse fetal outcomes. Rates of fetal loss in all pregnancies are relatively high, ranging from 5% to 23% among different age groups and colonies. Factors influencing higher rates of fetal loss include nulliparity of the dam, increasing age of the dam, and housing conditions [60–62]. The interbirth interval is 1 year but can be influenced by multiple factors such as loss of the prior pregnancy and the age and condition of the dam [60]. In one large study, the average number of pregnancies per dam was 7.9, with a range of 1-19 live births over the dam's lifetime [60].

Female Reproduction

Development

At birth, circulating levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are high, reaching a peak at approximately 20 weeks of age. Between this period and the onset of puberty, FSH and LH concentrations remain low. Puberty occurs between 2 and

VetBooks.ir

FIGURE 6.2 Female rhesus macaques usually deliver a single infant. Twinning is extremely rare (ONPRC/Bill Sutton).

4 years in females. It is marked by pulsatile gonadotropin-releasing hormone release and accompanied by steady increased LH concentrations. Menarche (first incidence of endometrial bleeding) precedes regular cycling by 12-18 months. Irregular and prolonged menstrual cycles with limited ovulation occur during this interval [63].

OVARIAN CYCLE

The ovarian cycle biology is typical of Old World primates and has been recently reviewed [59,64–66]. The cycle comprises a follicular phase of 12-14 days, a periovulatory phase of approximately 3 days, and luteal phase of 14-16 days. In the absence of pregnancy, the luteal phase is concluded with regression of the corpus luteum and menses. A single, dominant follicle develops. Ovulation is limited to a single oocyte in the vast majority of cycles. Cycle length generally ranges from 23 to 34 days; the average is reported as approximately 27-29 days [55,59]. Longer and more irregular cycle intervals are associated with seasonal reproductive quiescence and nonovulatory cycles [55]. Ovulation occurs from either ovary in a random fashion [55]. Lactational suppression of ovulation occurs in rhesus macaques and is mediated through suckling and elevated prolactin concentrations [67,68].

Detection of ovarian cycles can be evaluated in rhesus macaques using a variety of methods. Rhesus macaques have an overt menses readily detected by the presence of fresh blood on the vulva, perineum, and thighs. In the research setting, females may be taught through positive reinforcement training to present their posteriors for visual observation. Hormone profiles may be obtained through daily or targeted interval blood; measurement of urinary estrone conjugates (E1C), progesterone metabolites and LH, and ultrasonographic evaluation of ovarian size and structures [66,69,70].

Pregnancy

The rhesus macaque reproductive tract morphology consists of a simplex uterus and an unusually tortuous cervix. Implantation occurs at gestational day (GD) 13 [71]. The

VetBooks.ir

molecular changes in the primate endometrium at implantation have recently been reviewed [72]. Macaques exhibit a unique and characteristic epithelial proliferative response of the superficial endometrium, termed *epithelial plaque*, during early implantation [73]. Pregnancy can be detected using a variety of methods. Assays for the presence of chorionic gonadotropin in urine have been developed for use in nonhuman primates, including rhesus macaques [74–76]. Bimanual palpation may be used to detect pregnancy through the assessment of changes in the size and consistency of the uterus from GD 16 onward, although this method is most accurate from GD 25 [59]. Abdominal ultrasound can be used for early pregnancy diagnosis by detecting changes in the endometrial cavity echo as early as GDs 14-16, which correspond to epithelial plaque formation [77], and by identifying the gestational sac at GDs 16-18 [78].

Excellent descriptions of the ultrasonographic features of the developing fetus are available [59,79]. Organogenesis occurs between GDs 20 and 50 [44]. Maximum growth rate of the brain and kidneys occurs between GDs 100 and 125 [80]. Maximum growth of general body weight, placenta, and the majority of organs occurs between GDs 125 and 150 [80]. Pancreatic growth is fastest during late gestation. Postnatal growth is characterized by the continuous proportionate growth of most organs with increasing age and body weight until approximately 5 years of age. The notable exception is the brain, for which the majority of postnatal growth occurs by the end of the first year [80]. Specific details regarding development of gonadal, adrenal, and lymphoid tissue development are discussed separately.

The average gestation length is 165 days, with a range of around 160-175 days for normal pregnancies. Delivery usually occurs at night. The rhesus placenta is bidiscoid in the majority of pregnancies, although approximately 10% of normal full-term pregnancies may have a monodiscoid placenta [81]. The umbilical cord is inserted centrally or slightly eccentrically on the primary disc, which is generally larger than the secondary disc. The placental blood circulation is hemochorial, as is typical of humans and other nonhuman primates. In addition to its major function as the interface for transfer of nutrients to the fetus and transfer of waste to the dam, the placenta plays a significant role in endocrine function during pregnancy by producing estrogens, progesterone, chorionic gonadotropin, and other peptide hormones. Transfer of endogenous antibody across the placenta occurs predominately late in gestation, particularly in the last 2 weeks [82,83].

Lactation

Rhesus milk is similar to human milk in nutrient composition and caloric density [84,85]. It is considered dilute compared with that of other mammal groups [86]. Over the postpartum period of lactation, milk fat, protein, and caloric value increase [87]. Specific components of composition are dependent on numerous factors. Individual variations between mothers can be affected by parity, body condition, and sex of the offspring [87,88]. Natural weaning occurs at approximately 220 days of age but is influenced by postnatal growth and attainment of threshold weight [89].

Senescence

The occurrence of menopause in nonhuman primates is the subject of much interest as a natural model of menopause in women. Macaques undergo a reproductive decline at about 24 years of age, which approximates menopause in women [90,91]. It is associated with irregular menstrual cycling and reduced urinary excretion of the estrogen metabolite

VetBooks.ir

E1C and irregular progesterone concentrations as measured by pregnane-3-glucoronide [92]. However, other endocrine markers of menopause in women, such as urinary FSHb and circulating concentrations of inhibin, do not mimic the endocrine pattern observed in women [93].

Male Reproduction

Development

As in the female rhesus macaque, the development of the male reproductive system is characterized by initially high concentrations of gonadotropins, which drop abruptly at approximately 20 weeks of age [94]. During this period of infancy, there is a moderately high rate of Sertoli cell proliferation and steady low level of spermatogonial proliferation that continues through juvenile development [95]. The juvenile hiatal period is characterized by low concentrations of gonadotropins. The onset of puberty is at 3.5-4 years of age and is marked by a rise in gonadotropins and increase in testicular weight [80]. In the early pubertal period (45-51 months of age), initial testicular development is characterized by a rapid proliferation and differentiation of Sertoli cells [96]. Proliferation of undifferentiated spermatogonia continues at a steady rate throughout the pubertal period. Descent of the testes to a scrotal location does not occur until late in puberty in most rhesus; infant and juvenile testes are generally in the inguinal region. Sexual maturity occurs at 5-6 years of age.

Seasonal breeding variations are present in the male as well as female rhesus. Testicular size and serum testosterone concentrations are low in summer and peak in autumn and winter corresponding to the breeding season [58,97]. In one study, testosterone concentrations in the breeding season range from 10 to 20 nmol/L and dropped to 5 to 10 nmol/L in the late winter and spring. These findings were irrespective of exposure to female macaques [97].

Staging of the seminiferous tubule cycle may be accomplished through histologic evaluation. Spermatogenesis is described as occurring in 12 stages of germ cell differentiation, initially described by Clermont and Leblond [83]. The duration of the spermatogenic cycle is reported as 10.42 days (range, 10.25-10.62 days) [98], and the duration of spermatogenesis is 48 days [99]. Maturation of sperm occurs within the epididymis with maturity during passage through the epididymis.

Semen Collection and Evaluation

Semen may be collected by several methods including rectal probe electroejaculation and penile vibrostimulation. Current practices recommend penile band electrostimulation of conditioned male macaques, which produces a higher volume of semen and a larger number of sperm. This method requires training and conditioning the subjects to both the procedure and short periods of chair restraint. Semen quality is influenced by interindividual variation, time of collection, and handling after collection [100]. Macaque sperm requires capacitation with dibutryl cyclic AMP and caffeine [100]. Washing sperm is required to prevent decapacitation by intrinsic factors in the semen sample. Numerous studies have attempted to optimize the parameters of freezing macaque sperm for later use in assisted reproductive technology techniques [97,101–105]. Sperm quality can be evaluated by direct observation and computer-assisted sperm motion analysis [106]. The

VetBooks.ir

ability to penetrate the cervical mucus or a hyaluronic acid gel substitute may also be used [103]. Although post-thaw motility may be enhanced by modifications of freezing media and handling and freezing rates before and after thawing, ultimately the preservation of sperm for use in assisted reproductive technology techniques has been challenging. Artificial vaginal insemination with frozen-thawed semen has not been successful in producing pregnancies in rhesus macaques [103]. The tortuosity of the rhesus female cervix precludes the use of many standard transcervical procedures in the nongravid macaque, such as artificial insemination and nonsurgical embryo transfer.

URINARY SYSTEM

Urinary physiology of the rhesus macaque is similar to that of other primates. Captive rhesus may exhibit polydipsia without other abnormal behavior [107]. Water consumption, urine volume, and typical urinalysis values of Chinese rhesus are presented in Table 6.15. Normal serum chemistry values including electrolyte values are presented in Table 6.16. Ultrasound evaluation of the kidney, ureter, and bladder is easily achieved. Sterile urine samples for sample analysis may be collected via ultrasound-guided cystocentesis or urinary catheterization. Hyposthenuria is common in captive animals [108].

TABLE 6.15 Water Volume, Urine Volume, and Urinalysis of Chinese-Origin Rhesus Macaques

	Mean±standard deviation	Males	Females
Water consumption (mL)	726.70±308.50	750.00±378.59	680.00±197.99
Urine volume (mL)	538.70±376.60	562.00±330.78	532.00±394.73
Urine color	Yellow	Negative	Negative
Clarity	Clear	Negative	Negative
Glucose	Negative	Negative	Negative
Bilirubin	Negative	Negative	Negative
Ketones	Negative	Negative	Negative
Specific gravity	1.013±0.00	1.013±0.00	1.013±0.00
RBC	Negative	Negative	Negative
pH	7.89±0.99	7.50±1.47	7.98±0.84
Protein	Negative	Negative	Negative
Urobilirubin	0.2±0.0	0.2±0.2	0.3±0.4
Nitrite	Negative	Negative	Negative
Leukocyte	Negative	Negative	Negative/trace

Values are from 70 (n = 20 males and n = 41 females) Chinese-origin rhesus macaques.
Adapted from Lee et al. [108]

VetBooks.ir

TABLE 6.16 Normal Serum Chemistry Values for Rhesus Macaques

Serum chemistry value	Males and females ($n=36$)	Males ($n=18$)	Females ($n=18$)
Glucose (mmol/L)	5.72±1.20	5.26±1.13	6.19±1.10
Alanine aminotransferase (IU/L)	53.42±25.26	59.06±22.36	47.78±27.32
Aspartate aminotransferase (IU/L)	38.67±9.62	42.50±9.92	34.83±7.82
AST/ALT	0.83±0.32	0.81±0.32	0.85±0.32
Total protein (g/L)	78.05±3.59	76.49±3.30	79.60±3.23
Albumin (g/L)	53.87±2.67	53.43±2.60	54.31±2.73
Globulin (g/L)	24.18±2.70	23.06±1.83	25.29±3.00
A/G	2.25±0.27	2.33±0.21	2.18±0.31
Indirect bilirubin (μmol/L)	2.11±0.68	2.07±0.73	2.16±0.64
Total serum bile acids (μmol/L)	18.85±15.02	24.94±16.74	12.76±10.29
Urea nitrogen (mmol/L)	7.83±1.24	8.47±1.21	7.18±0.90
Creatinine (μmol/L)	69.64±10.24	69.73±11.51	69.56±9.13
Uric acid (μmol/L)	5.30±5.33	5.81±5.83	4.78±4.90
Triglyeride (mmol/L)	0.84±0.22	0.87±0.24	0.81±0.20
Cholesterol (mmol/L)	2.95±0.52	2.85±0.61	3.06±0.40
High-density lipoprotein cholesterol (mmol/L)	1.28±0.30	1.29±0.31	1.28±0.31
Low-density lipoprotein cholesterol (mmol/L)	1.91±0.39	1.77±0.42	2.05±0.32
Alkaline phosphatase (IU/L)	546.53±171.43	563.61±187.22	529.44±157.60
Gamma glutamyl transferase (IU/L)	73.67±15.73	76.50±16.89	70.83±14.39
Creatine kinase (IU/L)	207.36±98.96	240.33±118.75	174.39±61.31
Lactate dehydrogenase (IU/L)	513.58±187.34	597.11±212.44	430.06±111.15
Hydroxybutyrate dehydrogenase (IU/L)	382.31±120.31	432.33±139.66	332.28±70.69
Electrolytes			
Sodium (mmol/L)	151.72±3.82	149.71±3.07	153.74±3.47
Potassium (mmol/L)	4.78±0.69	4.67±0.62	4.90±0.76
Chloride (mmol/L)	107.14±3.31	105.72±3.04	108.57±3.01
Calcium (mmol/L)	2.67±0.16	2.63±0.13	2.72±0.17
Magnesium (mmol/L)	0.92±0.07	0.89±0.48	0.94±0.9

TABLE 6.16 Normal Serum Chemistry Values for Rhesus Macaques—Cont'd

Serum chemistry value	Males and females ($n=36$)	Males ($n=18$)	Females ($n=18$)
Iron (μmol/L)	27.98±5.31	27.66±4.18	28.31±6.41
Inorganic phosphorus (mg/L)	2.22±0.50	2.14±0.51	2.29±0.49
Carbon dioxide (mmol/L)	13.94±3.77	14.90±3.71	12.97±3.68
Anion gap (mmol/L)	35.43±5.68	33.76±5.22	37.10±1.36

Adapted from Chen et al. [25,26].

LYMPHATIC SYSTEM

The lymphoid organs include the thymus, spleen, bone marrow, lymph nodes, and mucosa-associated lymphoid tissue of the gastrointestinal, respiratory, and urogenital tracts. Fetal development of the immune system has been described [109]. The thymus develops from the third pharyngeal pouch during the fourth week of gestation and migrates to the anterior mediastinum by week 7. The cortex and medulla are identifiable by GD 65 and populated primarily by CD3 lymphocytes. Hassall's corpuscles are visible by GD 80. Increasing numbers of B cells are present from GD 65 onward. Similar to humans, the thymus increases in size postnatally and then involutes over time. In the rhesus, the thymus reaches maximal size at approximately 2-3 years of age and then steadily declines through adulthood. When plotted against body weight, thymic mass declines steadily from late gestation through postnatal life [110]. In the aged rhesus, the thymus is macroscopically undetectable within the mediastinal adipose tissue. Fetal development of the spleen involves its initial population by CD20 B cells at GD 75. Red and white pulp can be distinguished by GD 80, at which time T- and B-cell populations are roughly equivalent.

Immune function has been well characterized in the rhesus macaque, in part because of the use of this species as a model of human immunodeficiency virus-1 infection [111,112]. Many of the reagents used to characterize the immune system in humans are suitable for use in the rhesus. Immune system function is comprised of innate and adaptive immune responses. Cell types considered part of the innate immune system include natural killer (NK) cells, myeloid and plasmacytoid dendritic cells, macrophages, and neutrophils. For the most part, all of these cells have similar cellular markers and function in rhesus macaques and humans [111]. The most notable difference occurs in NK cells; unlike in humans, rhesus NK cells produce abundant CD8-α [113].

The cells of the adaptive immune system are B- and T-lymphocytes. As in humans, rhesus B-lymphocytes can be identified by their pattern of CD27 and immunoglobulin D expression. Naïve B cells express immunoglobulin D but not CD27, whereas memory B cells express only CD27. A transitional subset of B cells in circulation expresses both markers. T-lymphocytes are comprised of αβ-CD4 and CD8-positive T cells and a smaller population of ϒδ T cells. CD8 (cytotoxic) T cells recognize foreign peptides in the context of host major histocompatability complex (MHC) class I molecules. CD4 (helper) T cells recognize foreign antigens bound to host MHC class II molecules. Whereas humans have three MHC class I loci—human

leukocyte antigen (HLA)-A, HLA-B, and HLA-C, with a single allele of each locus inherited from each parent—the rhesus has two loci (Mamu) A and B [114]. Because of the effect of the accumulation of genetic events (duplication, deletion, and rearrangement) in this region, rhesus may have multiple alleles of each locus on each chromosome. Both humans and rhesus have three MHC class II loci: DP, DQ, and DR [114].

ENDOCRINE SYSTEM

The fetal adrenal gland, as mentioned earlier, plays an important role in supplying precursors for the endocrine function of the placenta. The fetal adrenal gland grows rapidly during late gestation [80]. The neonatal adrenal gland is comprised of a definitive zone, transitional zone dense band, and fetal zones [115,116]. The transition to adult morphology is characterized by regression of the fetal zone and maturation of the zona reticularis. Adrenarche is defined by the secretion of adrenal androgens from the zona reticularis. In humans, this occurs just before the onset of puberty. Recent studies of the rhesus macaque indicate that a process similar to that in humans occurs in the macaque by 3 months of age [117,118].

References

[1] Nowak RM. Walker's primates of the world. Baltimore, MD: The Johns Hopkins University Press; 1999. p. 140–8.
[2] Cawthon Lang K. Primate factsheets: rhesus macaques (Macaca mulatta) taxonomy, morphology & ecology 2005, http://pin.primate.wisc.edu/factsheets/entry/rhesus_macaque.
[3] Smith DG. Taxonomy of nonhuman primates used in biomedical research. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. Nonhuman primates in biomedical research: biology and management, Vol. 1. 2nd ed. San Diego, CA: Academic Press; 2012. p. 57–85.
[4] Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 2009;325:201–4.
[5] Satkoski J, George D, Smith DG, Kanthaswamy S. Genetic characterization of wild and captive rhesus macaques in China. J Med Primatol 2008;37:67–80.
[6] Champoux M, Higley JD, Suomi SJ. Behavioral and physiological characteristics of Indian and Chinese-Indian hybrid rhesus macaque infants. Dev Psychobiol 1997;31:49–63.
[7] Lowenstine LJ. A primer of primate pathology: lesions and nonlesions. Toxicol Pathol 2003;31(Suppl.):92–102.
[8] Clarke MR, O'Neil JA. Morphometric comparison of Chinese-origin and Indian-derived rhesus monkeys (Macaca mulatta). Am J Primatol 1999;47:335–46.
[9] Trichel AM, Rajakumar PA, Murphey-Corb M. Species-specific variation in SIV disease progression between Chinese and Indian subspecies of rhesus macaque. J Med Primatol 2002;31:171–8.
[10] Joag SV, Stephens EB, Adams RJ, Foresman L, Narayan O. Pathogenesis of SIVmac infection in Chinese and Indian rhesus macaques: effects of splenectomy on virus burden. Virology 1994;200:436–46.
[11] de Groot NG, Heijmans CM, Koopman G, Verschoor EJ, Bogers WM, Bontrop RE. TRIM5 allelic polymorphism in macaque species/populations of different geographic origins: its impact on SIV vaccine studies. Tissue Antigens 2011;78:256–62.
[12] Viray J, Rolfs B, Smith DG. Comparison of the frequencies of major histocompatibility (MHC) class-II DQA1 and DQB1 alleles in Indian and Chinese rhesus macaques (*Macaca mulatta*). Comp Med 2001;51:555–61.
[13] Lambert JE. Primate digestion: interactions among anatomy, physiology, and feeding ecology. Evol Anthropol 1998;7:8–20.
[14] Marshall J. The teeth. In: Hartmann CG, editor. The anatomy of the rhesus monkey. New York, NY: Hafner; 1961. p. 22–7.

[15] Nutrient Requirements of Nonhuman Primates: Second Revised Edition. Committee on Animal Nutrition, Ad Hoc Committee on Nonhuman Primate Nutrition, National Research Council. Washington, D. C.: The National Academies Press; 2003. p. 191-258.
[16] Bauer SA, Arndt TP, Leslie KE, Pearl DL, Turner PV. Obesity in rhesus and cynomolgus macaques: a comparative review of the condition and its implications for research. Comp Med 2011;61:514–26.
[17] Power M, Toddes B, Koutos L. Nutrient requirements and dietary husbandry principles for captive nonhuman primates. In: Abee C, Mansfield K, Tardiff S, Morris T, editors. Nonhuman primates in biomedical research: biology and management, Vol. 1. 2nd ed. San Diego, CA: Academic Press; 2012. p. 269–84.
[18] Sestak K, Conry L, Aye P, Smriti M, Doxiadis GG, Kaushal D. Improved xenobiotic metabolism and reduced susceptibility to cancer in gluten-sensitive macaques upon introduction of a gluten-free diet. PLoS One 2011;6(4):e18648.
[19] Walker M, Nelson J, Bernal J, Baskin G, Gad S. Primates: pathology. In: Gad S, editor. Animal models in toxicology. 2nd ed. Boca Raton, FL: CRC Press; 2007. p. 665–723.
[20] Loomis M, Henrickson R, Anderson J. Effects of ketamine hydrochloride on the hemogram of rhesus monkeys (*M. mulatta*). Lab Anim Sci 1980;30:851–3.
[21] Hassimota M, Harada T. Changes in hematology, biochemical values, and restraint ECG of rhesus monkeys (*M. mulatta*) following 6-month laboratory acclimation. J Med Primatol 2004;33:175–86.
[22] Hom GJ, Bach TJ, Carroll D, Forrest MJ, Mariano MA, Trainor CE, et al. Comparison of cardiovascular parameters and/or serum chemistry and hematology profiles in conscious and anesthetized rhesus monkeys (*Macaca mulatta*). Contemp Top Lab Anim Sci 1999;38:60–4.
[23] Pasini E, Kirkengaard M, Mortensen P, Mann M, Thomas A. Deep-coverage rhesus red blood cell proteome: a first comparison with the human and mouse red blood cell. Blood Transfus 2010;8:126–39.
[24] Hotchkiss C, Wang C, Slikker W. Effect of prolonged ketamine exposure on cardiovascular physiology in pregnant and infant rhesus monkeys (*M. mulatta*). J Am Assoc Lab Anim Sci 2007;46:21–48.
[25] Chen Y, Qin S, Ding Y, Wei L, Zhang J, Li H, et al. Reference values of clinical chemistry and hematology parameters in rhesus monkeys (*M. mulatta*). Xenotransplantation 2009;16:496–501.
[26] Kessler M, Rwalins R, London W. The hemogram, serum biochemistry, and electrolyte profile of aged rhesus monkeys (*M. mulatta*). J Med Primatol 1983;12:382–8.
[27] Smuncny D, Allison D, Ingram D, Roth G, Kemnitz J, Kohama S, et al. Changes in blood chemistry and hematology variables during aging in captive rhesus macaques (*M. mulatta*). J Med Primatol 2004;30:161–73.
[28] Hall R, Everds N. Factors affecting the interpretation of canine and nonhuman primate clinical pathology. Toxicol Pathol 2003;31:6–10.
[29] Mandell C, George J. Effect of repeated phlebotomy on the iron status of rhesus monkeys (*M. mulatta*). Am J Vet Res 1991;52:728–33.
[30] Story L, Kennedy S. Animal studies in endometriosis: a review. ILAR J 2004;45:132–8.
[31] Hukkenen R, Liggitt H, Murnane R, Frevert C. Systemic inflammatory response syndrome in nonhuman primates culminating in multiple organ failure, actue lung injury, and disseminated intravascular coagulation. Toxicol Pathol 2009;37:799–804.
[32] Patterson M, Jackson L, Brooks M, Catalfamo J. Type-3 von Willebrand's disease in a rhesus monkey (*M. mulatta*). Comp Med 2002;52:368–71.
[33] Lin C, Cooper D, Dorling A. Coagulation dysregulation as a barrier to xenotransplantation in the primate. Transpl Immunol 2009;21:75–80.
[34] Socha WW, Moor-Jankowski J, Ruffie J. Blood groups of primates: present status, theoretical implications and practical applications: a review. J Med Primatol 1984;13:11–40.
[35] Socha WW, Marboe CC, Michler RE, Rose EA, Moor-Jankowski J. Primate animal model for the study of ABO incompatibility in organ transplantation. Transplant Proc 1987;19:4448–55.
[36] Chen S, Wei Q, Li J, Xiang Y, Guo H, Ichim TE, et al. A simple and reliable method to blood type monkeys using serum samples. Transpl Int 2009;22:999–1004.
[37] Premasuthan A, Kanthaswamy S, Satkoski J, Smith DG. A simple multiplex polymerase chain reaction to determine ABO blood types of rhesus macaques (*Macaca mulatta*). Tissue Antigens 2011;77:584–8.
[38] Busch J, Specht S, Ezzelarab M, Cooper DK. Buccal mucosal cell immunohistochemistry: a simple method of determining the ABH phenotype of baboons, monkeys, and pigs. Xenotransplantation 2006;13:63–8.
[39] ICH. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Safety pharmacology studies for human pharmaceuticals, vol. S7A. 2000. p. 5–6.

[40] Sasseville V, Hotchkiss C, Lavesque P, Mankowski J. Hematopoietic, cardiovascular, lymphoid and mononuclear phagocyte systems of nonhuman primates. In: Abee C, Mansfield K, Tardiff S, Morris T, editors. Nonhuman primates in biomedical research: diseases, Vol. 2. 2nd ed. San Diego, CA: Academic Press; 2012. p. 357–76.
[41] Ando K, Hombo T, Kanno A, Ikeda H, Imaizumi M, Shimizu N, et al. QT PRODACT: in vivo QT assay with a conscious monkey for assessment of the potential for drug-induced QT interval prolongation. J Pharmacol Sci 2005;99:487–500.
[42] Korcarz CE, Padrid PA, Shroff SG, Weinert L, Lang RM. Doppler echocardiographic reference values for healthy rhesus monkeys under ketamine hydrochloride sedation. J Med Primatol 1997;26:287–98.
[43] Tang H, Wang L, Cheng G, Wang L, Li S. Evaluation of the cardiovascular function of older adult Rhesus monkeys by ultrasonography. J Med Primatol 2008;37:101–8.
[44] Brandt DJ, Canfield DR, Peterson PE, Hendrickx AG. Persistent truncus arteriosus in a rhesus monkey (*Macaca mulatta*). Comp Med 2002;52:269–72.
[45] Cantrell C, Baskin G, Garcia R. Endometritis and valvular endocarditis in a rhesus monkey. J Am Vet Med Assoc 1986;189:1221–2.
[46] Scott GBD. Comparative primate pathology. Oxford: Oxford University Press; 1992.
[47] Lowenstine L, Osborn K. Respiratory system diseases of nonhuman primates. In: Abee C, Mansfield K, Tardiff S, Morris T, editors. Nonhuman primates in biomedical research: diseases, Vol. 2. 2nd ed. San Diego, CA: Academic Press; 2012.
[48] Binns R, Clark G, Simpson C. Lung function and blood gas characteristics in the rhesus monkey. Lab Animal 1972;6:189–98.
[49] Chalifoux LV, Simon MA, Pauley DR, MacKey JJ, Wyand MS, Ringler DJ. Arteriopathy in macaques infected with simian immunodeficiency virus. Lab Invest 1992;67:338–49.
[50] TateRico M, Roy C. Comparative study of lung cytologic features in normal rhesus (*Maccaca mulatta*), cynomolgus (*Macacca fasicularis*), and African green (*Chlorocebus aethiops*) nonhuman primates by use of bronchoscopy. Comp Med 2004;54:393–6.
[51] Klein B. Neurophysiology. In: Cunningham's textbook of veterinary physiology. 5th ed. St Louis, Missouri: Elsevier Saunders; 2013.
[52] Hall J, Guyton A. The nervous system: a. general principles and sensory physiology. In: Textbook of medical physiology. 12th ed. Philadelphia, PA: Saunders Elsevier; 2011. p. 543–92.
[53] Hambelton P, Baskervill A, Wade J, Bailey N. Some normal clinical chemistry values for cerebrospinal fluid of the rhesus monkey (*Macaca mulatta*). Lab Animal 1981;15:181–6.
[54] Bito LZ, Merritt SQ, DeRousseau CJ. Intraocular pressure of rhesus monkey (*Macaca mulatta*). I. An initial survey of two free-breeding colonies. Invest Ophthalmol Vis Sci 1979;18:785–93.
[55] Ghosh D, Sengupta J. Patterns of ovulation, conception and pre-implantation embryo development during the breeding season in rhesus monkeys kept under semi-natural conditions. Acta Endocrinol (Copenh) 1992;127:168–73.
[56] Du Y, Fan TY, Tan Y, Xiong Z, Wang Z. Seasonal changes in the reproductive physiology of female rhesus macaques (*Macaca mulatta*). J Am Assoc Lab Anim Sci 2010;49:289–93.
[57] Walker ML, Gordon TP, Wilson ME. Menstrual cycle characteristics of seasonally breeding rhesus monkeys. Biol Reprod 1983;29:841–8.
[58] Wickings EJ, Nieschlag E. Seasonality in endocrine and exocrine testicular function of the adult rhesus monkey (*Macaca mulatta*) maintained in a controlled laboratory environment. Int J Androl 1980;3:87–104.
[59] Tardif S, Carville A, Elmore D, Williams LE, Rice K. Reproduction and breeding of nonhuman primates. In: Abee C, Mansfield K, Tardif S, Morris T, editors. Nonhuman primates in biomedical research: biology and management, Vol. 1. 2nd ed. San Diego, CA: Academic Press; 2012. p. 197–249.
[60] Gagliardi C, Liukkonen JR, Phillippi-Falkenstein KM, Harrison RM, Kubisch HM. Age as a determinant of reproductive success among captive female rhesus macaques (*Macaca mulatta*). Reproduction 2007;133:819–26.
[61] Westergaard GC, Izard MK, Drake JH. Reproductive performance of rhesus macaques (*Macaca mulatta*) in two outdoor housing conditions. Am J Primatol 2000;50:87–93.
[62] Hendrie TA, Perterson P, Amela E, Short JJ, Tarantal AF, Rothgarn E, et al. Frequency of prenatal loss in a macaque breeding colony. Am J Primatol 1996;40:41–53.
[63] Resko JA, Goy RW, Robinson JA, Norman RL. The pubescent rhesus monkey: some characteristics of the menstrual cycle. Biol Reprod 1982;27:354–61.

[64] Stouffer RL. Progesterone as a mediator of gonadotrophin action in the corpus luteum: beyond steroidogenesis. Hum Reprod Update 2003;9:99–117.
[65] Zeleznik AJ, Pohl CR. Control of follicular development, corpus luteum function, the maternal recognition of pregnancy, and the neuroendocrine regulation of the menstrual cycle in higher primates. In: Neill JD, editor. 3rd ed. Knobil and Neill's physiology of reproduction. vol. 2. San Diego, CA: Academic Press; 2006. p. 2449–510.
[66] Weinbauer GF, Niehoff M, Niehaus M, Srivastav S, Fuchs A, Van Esch E, et al. Physiology and endocrinology of the ovarian cycle in macaques. Toxicol Pathol 2008;36:7S–23S.
[67] Schallenberger E, Richardson DW, Knobil E. Role of prolactin in the lactational amenorrhea of the rhesus monkey (*Macaca mulatta*). Biol Reprod 1981;25:370–4.
[68] Ordog T, Chen MD, O'Byrne KT, Goldsmith JR, Connaughton MA, Hotchkiss J, et al. On the mechanism of lactational anovulation in the rhesus monkey. Am J Physiol 1998;274:E665–76.
[69] Monfort SL, Hess DL, Shideler SE, Samuels SJ, Hendrickx AG, Lasley BL. Comparison of serum estradiol to urinary estrone conjugates in the rhesus macaque (*Macaca mulatta*). Biol Reprod 1987;37:832–7.
[70] Morgan PM, Hutz RJ, Kraus EM, Cormie JA, Dierschke DJ, Bavister BD. Evaluation of ultrasonography for monitoring follicular growth in rhesus monkeys. Theriogenology 1987;27:769–80.
[71] Ghosh D, Dhara S, Kumar A, Sengupta J. Immunohistochemical localization of receptors for progesterone and oestradiol-17 beta in the implantation site of the rhesus monkey. Hum Reprod 1999;14:505–14.
[72] Nimbkar-Joshi S, Rosario G, Katkam RR, Manjramkar DD, Metkari SM, Puri CP, et al. Embryo-induced alterations in the molecular phenotype of primate endometrium. J Reprod Immunol 2009;83:65–71.
[73] Cline JM, Wood CE, Vidal JD, Tarara RP, Buse E, Weinbauer GF, et al. Selected background findings and interpretation of common lesions in the female reproductive system in macaques. Toxicol Pathol 2008;36:142s–63s.
[74] Hodgen GD, Niemann WH, Tullner WW. Duration of chorionic gonadotropin production by the placenta of the rhesus monkey. Endocrinology 1975;96:789–91.
[75] Shimizu K, Lohstroh PN, Laughlin LS, Gee NA, Todd H, Shideler SE, et al. Noninstrumented enzyme-linked immunosorbant assay for detection of early pregnancy in macaques. Am J Primatol 2001;54:57–62.
[76] Hodgen GD, Ross GT. Pregnancy diagnosis by a hemagglutination inhibition test for urinary macaque chorionic gonadotropin (mCG). J Clin Endocrinol Metab 1974;38:927–30.
[77] Tarantal AF. Ultrasound imagining in rhesus (*Macaca mulatta*) and long-tailed (*Macaca fascicularis*) macaques: reproductive and research applications. In: Woolfe-Coote S, editor. The laboratory primate. Amsterdam: Elsevier Academic Press; 2005. p. 317–52.
[78] Tarantal AF, Hendrickx AG. Use of ultrasound for early pregnancy detection in the rhesus and cynomolgus macaque (*Macaca mulatta* and *Macaca fascicularis*). J Med Primatol 1988;17:105–12.
[79] Hendrickx AG, Dukelow WR. Breeding. In: Bennett BT, Abee CR, Henrickson R, editors. Nonhuman primates in biomedical research. Biology and management, Vol. 1. New York, NY: Academic Press; 1995. p. 335–76.
[80] Kerr GR, Allen JR, Scheffler G, Couture J. Fetal and postnatal growth of rhesus monkeys (*M. mulatta*). J Med Primatol 1974;3:221–35.
[81] Bunton TE. Incidental lesions in nonhuman primate placentae. Vet Pathol 1986;23:431–8.
[82] Coe CL, Kemnitz JW, Schneider ML. Vulnerability of placental antibody transfer and fetal complement synthesis to disturbance of the pregnant monkey. J Med Primatol 1993;22:294–300.
[83] Clermont Y, Leblond CP. Differentiation and renewal of spermatogonia in the monkey, Macacus rhesus. Am J Anat 1959;104:237–73.
[84] Osthoff G, Hugo A, de Wit M, Nguyen TP, Seier J. Milk composition of captive vervet monkey (*Chlorocebus pygerythrus*) and rhesus macaque (*Macaca mulatta*) with observations on gorilla (*Gorilla gorilla gorilla*) and white handed gibbon (*Hylobates lar*). Comp Biochem Physiol B Biochem Mol Biol 2009;152:332–8.
[85] Lonnerdal B, Keen CL, Glazier CE, Anderson J. A longitudinal study of rhesus monkey (*Macaca mulatta*) milk composition: trace elements, minerals, protein, carbohydrate, and fat. Pediatr Res 1984;18:911–4.
[86] Kunz C, Lonnerdal B. Protein composition of rhesus monkey milk: comparison to human milk. Comp Biochem Physiol Comp Physiol 1993;104:793–7.
[87] Hinde K, Power ML, Oftedal OT. Rhesus macaque milk: magnitude, sources, and consequences of individual variation over lactation. Am J Phys Anthropol 2009;138:148–57.
[88] Hinde K, Foster AB, Landis LM, Rendina D, Oftedal OT, Power ML. Daughter dearest: sex-biased calcium in mother's milk among rhesus macaques. Am J Phys Anthropol 2013;151:144–50.

VetBooks.ir

[89] Bowman JE, Lee PC. Growth and threshold weaning weights among captive rhesus macaques. Am J Phys Anthropol 1995;96:159–75.
[90] Wu JM, Zelinski MB, Ingram DK, Ottinger MA. Ovarian aging and menopause: current theories, hypotheses, and research models. Exp Biol Med (Maywood) 2005;230:818–28.
[91] Schramm RD, Paprocki AM, Bavister BD. Features associated with reproductive ageing in female rhesus monkeys. Hum Reprod 2002;17:1597–603.
[92] Gilardi KV, Shideler SE, Valverde CR, Roberts JA, Lasley BL. Characterization of the onset of menopause in the rhesus macaque. Biol Reprod 1997;57:335–40.
[93] Shideler SE, Gee NA, Chen J, Lasley BL. Estrogen and progesterone metabolites and follicle-stimulating hormone in the aged macaque female. Biol Reprod 2001;65:1718–25.
[94] Plant TM. The role of KiSS-1 in the regulation of puberty in higher primates. Eur J Endocrinol 2006;155(Suppl. 1):S11–6.
[95] Plant TM, Ramaswamy S, Simorangkir D, Marshall GR. Postnatal and pubertal development of the rhesus monkey (*Macaca mulatta*) testis. Ann N Y Acad Sci 2005;1061:149–62.
[96] Simorangkir DR, Ramaswamy S, Marshall GR, Roslund R, Plant TM. Sertoli cell differentiation in rhesus monkey (*Macaca mulatta*) is an early event in puberty and precedes attainment of the adult complement of undifferentiated spermatogonia. Reproduction 2012;143:513–22.
[97] Herndon JG, Bein ML, Nordmeyer DL, Turner JJ. Seasonal testicular function in male rhesus monkeys. Horm Behav 1996;30:266–71.
[98] Rosiepen G, Arslan M, Clemen G, Nieschlag E, Weinbauer GF. Estimation of the duration of the cycle of the seminiferous epithelium in the non-human primate *Macaca mulatta* using the 5-bromodeoxyuridine technique. Cell Tissue Res 1997;288:365–9.
[99] de Rooij DG, van Alphen MM, van de Kant HJ. Duration of the cycle of the seminiferous epithelium and its stages in the rhesus monkey (*Macaca mulatta*). Biol Reprod 1986;35:587–91.
[100] VandeVoort CA. High quality sperm for nonhuman primate ART: production and assessment. Reprod Biol Endocrinol 2004;2:33.
[101] Dong Q, Hill D, VandeVoort CA. Interactions among pre-cooling, cryoprotectant, cooling, and thawing for sperm cryopreservation in rhesus monkeys. Cryobiology 2009;59:268–74.
[102] Dong Q, Rodenburg SE, Huang C, VandeVoort CA. Effect of pre-freezing conditions on semen cryopreservation in rhesus monkeys. Theriogenology 2008;70:61–9.
[103] Tollner TL, Dong Q, VandeVoort CA. Frozen-thawed rhesus sperm retain normal morphology and highly progressive motility but exhibit sharply reduced efficiency in penetrating cervical mucus and hyaluronic acid gel. Cryobiology 2011;62:15–21.
[104] Yang S, Ping S, Ji S, Lu Y, Niu Y, Wang H, et al. The positive effects of seminal plasma during the freezing process on cryosurvival of sperm with poor freezability in the rhesus macaque (*Macaca mulatta*). J Reprod Dev 2011;57:737–43.
[105] Yang S, Ping S, Si W, He X, Wang X, Lu Y, et al. Optimization of ethylene glycol concentrations, freezing rates and holding times in liquid nitrogen vapor for cryopreservation of rhesus macaque (*Macaca mulatta*) sperm. J Vet Med Sci 2011;73:717–23.
[106] Maree L, van der Horst G. Quantification and identification of sperm subpopulations using computer-aided sperm analysis and species-specific cut-off values for swimming speed. Biotech Histochem 2013;88:181–93.
[107] Oikawa H, Yamashita T, Muto M, Sawai M. Water intake and urinary output in rhesus (*Macaca mulatta*) and cynomolgus monkeys (Macaca fascicularis). Jikken Dobutsu 1982;31:279–86.
[108] Lee J-IL, Shin J-S, Lee J-E, Jung W-Y, Lee G, Kim M-S, et al. Reference values of hematology, chemistry, electrolytes, blood gas, coagulation time, and urinalysis in the Chinese rhesus macaques (*Macaca mulatta*). Xenotransplantation 2012;19:244–8.
[109] Hendrickx AG, Makori N, Peterson P. The nonhuman primate as a model of developmental immunotoxicity. Hum Exp Toxicol 2002;21:537–42.
[110] Healy DL, Bacher J, Hodgen GD. A method of thymectomy in macaques. J Med Primatol 1983;12:89–100.
[111] Messaoudi I, Estep R, Robinson B, Wong SW. Nonhuman primate models of human immunology. Antioxid Redox Signal 2011;14:261–73.
[112] Pitcher CJ, Hagen SI, Walker JM, Lum R, Mitchell BL, Maino VC, et al. Development and homeostasis of T cell memory in rhesus macaque. J Immunol 2002;168:29–43.

VetBooks.ir

[113] Webster RL, Johnson RP. Delineation of multiple subpopulations of natural killer cells in rhesus macaques. Immunology 2005;115:206–14.
[114] Wiseman RW, Karl JA, Bohn PS, Nimityongskul FA, Starrett GJ, O'Connor DH. Haplessly hoping: macaque major histocompatibility complex made easy. ILAR J 2013;54:196–210.
[115] McNulty WP, Novy MJ, Walsh SW. Fetal and postnatal development of the adrenal glands in *Macaca mulatta*. Biol Reprod 1981;25:1079–89.
[116] Mapes S, Tarantal AF, Parker CR, Moran FM, Bahr JM, Pyter L, et al. Adrenocortical cytochrome b5 expression during fetal development of the rhesus macaque. Endocrinology 2002;143:1451–8.
[117] Nguyen AD, Mapes SM, Corbin CJ, Conley AJ. Morphological adrenarche in rhesus macaques: development of the zona reticularis is concurrent with fetal zone regression in the early neonatal period. J Endocrinol 2008;199:367–78.
[118] Conley AJ, Moeller BC, Nguyen AD, Stanley SD, Plant TM, Abbott DH. Defining adrenarche in the rhesus macaque (*Macaca mulatta*), a non-human primate model for adrenal androgen secretion. Mol Cell Endocrinol 2011;336:110–6.
[119] Rowe N. In: The pictorial guide to the living primates. Charlestown, RI: Pogonias Press; 1996. p. 126–7.
[120] Leutenegger W, Cheverud J. Correlates of sexual dimorphism in primates: ecological and size variables. Intl J Primatol 1982;3:387–403.
[121] Gonder JC, Card EA, Lott NE. Electrocardiograms of nine species of nonhuman primates sedated with ketamine. Am J Vet Res 1980;41:972–5.

VetBooks.ir

VetBooks.ir

CHAPTER

7

Basic Physiology of *Callithrix jacchus*

Kerstin Mätz-Rensing[*], *Sven Korte*[†]

[*]German Primate Center, Göttingen, Germany

[†]Covance Laboratories GmbH, Münster, Germany

OUTLINE

General Biology 115

Gastrointestinal Tract and Nutrition 117

Complete Blood Count 119

Cardiovascular System 122
- *Blood Pressure and Heart Rate* 122
- *Electrocardiography* 122
- *Core Body Temperature* 123

Respiratory System 123
- *Structure and Function* 123

Development and Reproduction 123
- *Ovarian Cycles* 124
- *Subordinate Females* 126
- *Pregnancy and Lactation* 126
- *Senescence* 127
- *Male Primate Reproduction* 128
- *Spermatogenesis* 128

Conclusion 129

References 129

GENERAL BIOLOGY

The common marmoset *Callithrix jacchus* is a representative of the family Callithrichidae, which belongs to the parvorder *Platyrrhini*, a native to South America (Figure 7.1). The family Callithrichidae is formed by marmosets of the two genera *Cebuella* and *Callithrix*, tamarins of the two genera *Saguinus* and *Leontopithecus*, and by the genus *Callimico*. The common marmoset belongs to the genus *Callithrix*. All six known Atlantic marmoset species are similar

http://dx.doi.org/10.1016/B978-0-12-417144-2.00007-X
© 2015 Elsevier Inc. All rights reserved.

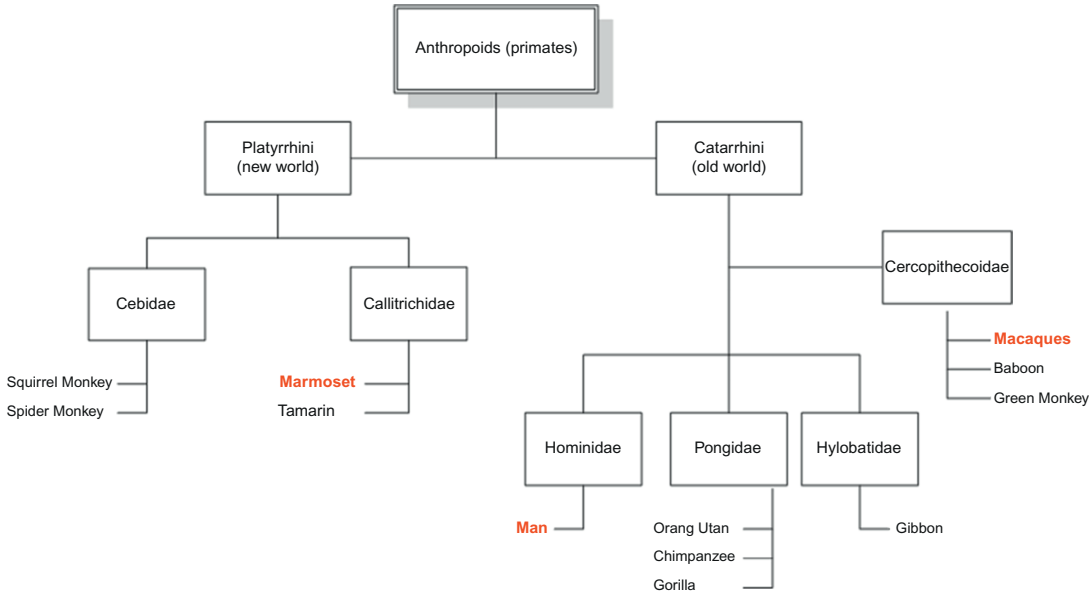


FIGURE 7.1 Taxonomy. The common marmoset, Parvorder: Patyrrhini; Family: Callithrichidae; Scientific name: *Callithrix jacchus*. *Figure modified by Covance Laboratorie GmbH, Münster, 2009.*

in size and habits but are distinguishable by pelage coloration. Their close relatives are the Amazonian marmosets of the genus *Mica*, which are comparable in morphology and behavior but distinguished by their zone of distribution within the Amazon basin. Most species have tufts of hair alongside the ears and a long ringed tail [1]. The common marmoset is the only species of this family that has been widely used in pharmacologic and toxicologic studies over the past 20 years.

Marmosets are arboreal diurnal New World monkeys domiciled in northeast and central Brazil. The natural habitat of this species is restricted to Brazil. Their dentition and large oval nostrils located laterally and separated by a broad internarial septum make them distinct from Old World monkeys. Sometimes they are referred to as "clawed New World monkeys" because of their claw-shaped nails on all digits except for the big toe.

Common marmosets are marbled black, gray, and yellow above, with transverse stripes on the lower back and a heavily ringed tail. The head is a dark brown with characteristic white fan-shaped, approximately 3-cm-long tufts around the ears. On the forehead is a prominent patch, and the crown is dark brown (Figure 7.2). There is no conspicuous gender dimorphism [1]. The adult body weight ranges between 320 and 450 g. Marmosets reach adult size at about 12 months of age and adult weight and full social and sexual maturity between 18 and 24 months. Their lifespan is 8-20 years, but reproductive performance deteriorates after 8-10 years [2] (Table 7.1). Marmosets live in small family groups with well-defined territories. Specific anatomic peculiarities of marmosets are prominent cutaneous scent glands of the apocrine type on the chest or perineum that are used to mark their territories. The family group is based on one breeding pair that shares caring for the young with sibling juveniles and related adults.

VetBooks.ir

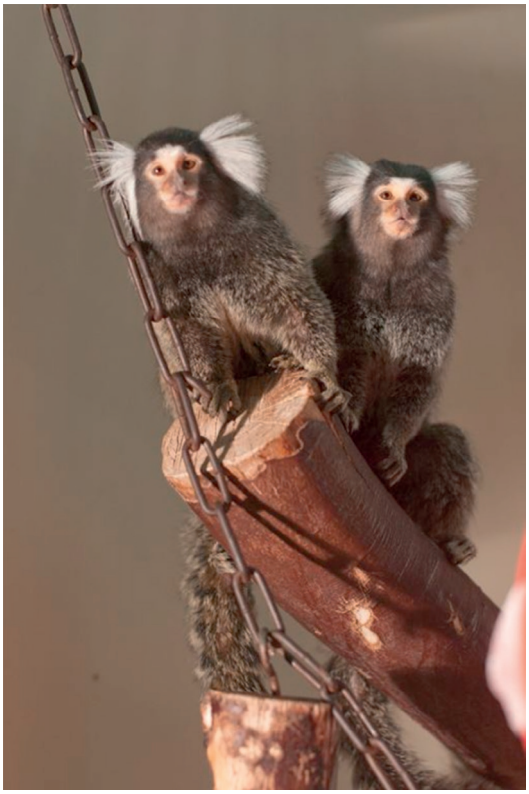


FIGURE 7.2 Taxonomy. Class: Mammalia; Order: Primates; Suborder: Haplorrhini; Parvorder: Patyrrhini; Family: Callithrichidae; Scientific name: *Callithrix jacchus*. *Picture DPZ, A. Säckel.*

GASTROINTESTINAL TRACT AND NUTRITION

Common marmosets are omnivorous, consuming both insects and plant items such as flowers and fruits. They are specialist gum feeders (gummivory). Their dentition shows some adaptions to this special nutrition. They have three premolars and their dental formula is 2-1-3-2/2-1-3-2. The crowns of the lower canines are barely longer than the adjacent incisors and premolars. *Callithrix* are therefore called short-tusked marmosets [4]. The incisors are relatively long with rodent-like enamel to bore holes into tree trunks to extract tree exudates such as saps and gums. Sap and gum form a large portion of their natural diet and provide high-energy food source in seasons when fruits are unavailable.

Morphologic adaption associated with tree gouging and gum feeding can also be found in their mandibular articulation, the distinct yaw muscle, and the missing cheek pouches. Anatomic peculiarities of the intestine include a relatively short small intestine and a larger colon and cecum adapted to enable cecal fermentation. The cecum possesses large folds and sacculations, and it acts as the fermentation chamber for the gum with a special microbial flora. A sigmoid flexure and taenia, or haustra, are missing in the colon [1,5].

Gum Arabic provides marmosets with a source of complex polysaccharides, carbohydrates and minerals, especially calcium and tannin. Marmosets are well adapted to their natural diet, and many efforts have been undertaken to develop an appropriate diet plan.

TABLE 7.1 Physiological Data

Weight and length
- Birth weight: 25-35 g
- Weaning weight: 60-150 g
- Adult weight: 320-450 g
- Adult body length (without tail): ♀: 17-20 cm; ♂: 16-21 cm
- Adult tail length: ♀: 24-30 cm; ♂: 25-31 cm
- Cranial circumference: ♀: 13.3-135 cm; ♂: 13.2-14 cm

Dentition
- Specialized lower incisors
- Short canines

Life history
- Life span: 8-20 years
- Weaning age: 40-120 days
- Puberty: 8-12 months
- Sexual and social maturity: 18-24 months
- Age at first reproduction: 18-20 months

Reproduction characteristics
- Monogamous
- Ovarian cycle: 28 days (polyestrus/multiovular)
- Follicular phase: 8 days
- Luteal phase: 20 days

Gestation: 141-145 days
- Litter size: 1-3, usually twins (30-70%), triplets (20-52%)
- Interbirth interval: 6 months
- Parents/F1: rear and carry new offspring

Source: [1–3].

Because of their shorter gut-passage time compared with anthropoids, callitrichids require a diet high in nutritional quality and energy [6]. The dietary ascorbic acid (vitamin C) requirement is several times higher than that of humans [7] and increases when the animals are stressed [8]. Common marmosets that are maintained without access to natural or ultraviolet (UV) B light have a high requirement for dietary vitamin D_3. The serum concentrations of 1.25-dihydroxyvitamin D in the marmoset are extremely high, probably because of end-organ resistance, which is greater for vitamin D_2 than for D_3 [9,10]. Most commercially available marmoset diets contain about 300 µg vitamin D_3/kg diet, although there is still some disagreement as to the suitability of this level. Because vitamin D_3 is potentially toxic, excessive supplementation should be avoided. Although commercial diets can provide all nutritional requirements, marmosets need variety in their diet.

Dietary requirements and preferences are rather complex. Therefore, successful breeding and maintenance requires a sophisticated food plan. A standard feeding regime in many colonies is based on a cafeteria style, including a highly caloric, vitamin-rich porridge for breakfast, vegetables and a protein source for lunch, and insects in the afternoon. Additional food that can be cooked includes rice, noodles, potatoes, hardboiled eggs, cottage cheese, boiled chicken, raisins, sunflower seeds, dried dates, grasshoppers, and mealworms [3]. The addition of gum to the diet slows the gut-passage rate without depressing the digestive efficiency [11].

TABLE 7.2 Weekly Bill of Fare for *Callithrix jacchus*

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
am	Curd pulp	Curd pulp	Curd pulp	Curd pulp	Curd pulp	Curd pulp	Curd pulp
pm	Pellets Gum arabic Fruits Vegetable Eggs Carob pods	Pellets Gum arabic Fruits Vegetable Chickpea	Pellets Gum arabic Fruits Vegetable Rice Sunflower seeds	Pellets Gum arabic Fruits Vegetable Potatoes Mealworm	Pellets Gum arabic Fruits Vegetable Rice	Pellets Gum arabic Fruits Vegetable Cottage cheese Carob pods	Pellets Gum arabic Fruits Vegetable Crispbread Mealworm

Curd pulp: *bananas, curd, yogurt natural, vitamin C, safflower oil, carrot juice, protevit, yeast, vitamin D3, minerals, rice meal;* Fruits: *banana, apple, kiwi, melon, grapes, pineapple, pear;* Vegetables: *carrot, fennel, pepper, tomatoes, celery, radish, beetroot;* Pellets: *Ssniff (Ger), Altromin (Ger), SDS (UK); eggs, chickpeas, rice, and potatoes are cooked.*
Source: German Primate Center.

Cat food should be avoided because of the high iron content. The feeding intervals should be 4.5-6.5 h. An example of a weekly diet plan for a marmoset housing colony is provided in Table 7.2.

COMPLETE BLOOD COUNT

The site most commonly selected for venopuncture is the femoral vein. The volume of blood obtained should be limited to 10% of the calculated normal blood volume, which is estimated to be 10% of body weight [12]. Without close monitoring, repeated sampling for kinetic profiles should not exceed 2 mL/week. Volumes of 0.3 mL whole blood per occasion can be regarded as standard volumes to estimate normal blood chemistry [13]. Ethylenediamine tetraacetic acid is the most commonly used and acceptable anticoagulant. Normal values for hematology [2,14–16] and serum biochemistry [2,15,17,18] parameters of captive marmosets have been publish. Most of these data were published nearly 30 years ago. They vary slightly depending on animal source, housing conditions, collection technique, and laboratory variables and values, and they should be regarded as standard values. Standard hematologic and chemistry values are summarized in Tables 7.3 and 7.4. These values should not be regarded as the benchmark. Optimal values can only be received by comparing an animal to itself using different health status values before starting an experimental study.

Values for routinely examined parameters are generally similar to those of other primate species. However, there are some indications of species differences that include a lower prothrombin time [16] and lower gamma-glutamyl transpeptidase, and alanine aminotransferase values in marmosets when compared with other species [13].

Furthermore, there are some age and sex-related differences. Glucose, urea nitrogen, alkaline phosphatase, lactate dehydrogenase, inorganic phosphor, and amylase are reported to be higher in juvenile animals. Adult males have higher values for cholesterol and triglycerides. Hemoglobin and hematocrit are higher in adults, whereas platelet concentration tends to decrease from juvenile to adult marmosets [2]. In one study a higher platelet count in females was reported [16], but this could not be confirmed by other studies [19]. Fibrinogen seems

TABLE 7.3 Standard Hematologic Values in the Common Marmoset

Parameter	Value	Adult Marmosets
WBC, leucocytes	G/L	2.72-10.85
RBC, erythrocytes	T/L	3.47-7.88
MCV	fL	53.32-83.45
MCH	fmol	1.21-1.54
MCHC	mmol/L	18.33-21.24
Band neutrophils	G/L	0.00-0.18
	%	0.00-4.00
Segmented neutrophils	G/L	1.04-15.39
	%	28.25-78.05
Lymphocytes	G/L	0.49-4.4
	%	14.00-62.70
Monocytes	G/L	0.07-1.63
	%	2.00-17.05
Eosinophils	G/L	0.00-0.71
	%	0.00-13.75
Basophils	G/L	0.00
	%	0.00
Thrombocytes	G/L	17.6-788.6
Hemoglobin	mmol/L	3.45-10.67
Packed cell volume	I/L	0.28-0.54

Source: Kuehnel et al. [15].

to be higher in female than in male marmosets [16]. Alkaline phosphatase concentrations decrease with age and young males have higher plasma concentrations than young females, but in older animals the sex differences no longer exist. Concentrations of gamma-glutamyl transpeptidase and sorbitol dehydrogenase were higher in older males than in younger females. High plasma iron concentrations were found in males with increasing age [17]. Male marmosets have significantly higher total and low-density lipoprotein cholesterol concentrations than females, whereas the mean corpuscular volume and the mean corpuscular hemoglobin were significantly lower than in females [15]. No age or gender-related differences were found for aspartate aminotransferase or alanine aminotransferase [20]. During pregnancy, serum biochemical and hematological parameters are predominantly within the normal range, but pregnant females show significantly lower glucose, aspartate aminotransferase, and urea values [21]. Transport and housing stress might influence the animal's hematologic and clinical chemistry parameters. Cortisol determination in feces and saliva is a suitable and

VetBooks.ir

TABLE 7.4 Standard Chemistry and Lipid Metabolism in the Common Marmoset

Parameter	Value	Adult Marmosets
Glucose	mmol/L	4.01-11.89
Creatinine	µmol/L	14.00-48.28
Urea	mmol/L	3.37-18.67
Calcium	mmol/L	1.78-2.96
Phosphorus	mmol/L	0.47-2.43
Sodium	mmol/L	155.50-174.00
Potassium	mmol/L	2.30-4.65
Chloride	mmol/L	101.85-119.90
Iron	µmol/L	17.63-39.48
Magnesium	mmol/L	0.60-1.03
Total proteins	g/L	41.10-74.80
Albumin	g/L	10.77-57.88
Aspartate aminotransferase, AST	U/L	51.24-316.21
Alanine aminotransferase, ALT	U/L	0.79-45.33
Gamma-glutamyl-transferase, GGT	U/L	0.24-13.50
Glutamate dehydrogenase, GLDH	U/L	4.00-61.33
Total bilirubin	µmol/L	0.03-4.24
Amylase	U/L	617.40-1243.20
Alk. phosphatase, AP	U/L	44.00-426.25
Lactate dehydrogenase, LDH	U/L	134.77-1119.69
Creatine kinase, CK	U/L	76.90-1801.84
Lipase	U/L	10.80-58.80
Total cholesterol	mmol/L	2.28-5.09
Total triglyceride	mmol/L	0.56-2.63
HDL chol	mmol/L	0.55-2.36
HDL tg	mmol/L	0.15-0.50
LDL chol	mmol/L	1.34-3.09
LDL tg	mmol/L	0.32-1.24
VLDL chol	mmol/L	0.02-0.45
VLDL tg	mmol/L	0.07-1.13

Source: Kuehnel et al. [15].

noninvasive method to measure stress levels, and this method has been established for the common marmoset [22–24]. Under stress conditions, an increase in cortisol measured as fecal cortisol and a decrease of the lymphocytes can be observed [15].

In May-Grünwald-Giemsa-stained blood films, neutrophils appear as regular cells containing pink-red granula within their cytoplasm and well-formed nuclei with three to seven lobes. Lymphocytes are mostly of intermediate size. Eosinophils contain irregular spherical granulas with a bluish tinge and trilobed nuclei. It could be difficult to distinguish them from basophils, which have small granulas and stain midblue [14].

CARDIOVASCULAR SYSTEM

Blood Pressure and Heart Rate

The normal heart rate (HR) in unrestrained marmosets ranges from 215 to 265/min. Blood pressure can be measured directly with an indwelling arterial catheter connected to an external pressure transducer, by telemetry, or indirectly by the tail-cuff method. Restraint during investigation causes excitement that can increase HR, blood pressure, and respiratory rate [25]. Whereas most anesthetics cause a decrease in HR, ketamine increases HR and pulmonary blood pressure [26].

Consistent diurnal variations of blood pressure measured as mean arterial pressure (MAP), HR, and motor activity are observed using telemetry in unrestrained animals. Normal diurnal rhythms in marmosets are characterized by significantly lower parameters during nighttime. The lowest values of MAP and HR are measured at midnight. Both parameters rise rapidly in the morning with a daytime peak in the morning and the afternoon, and they fall rapidly when the lights are turned off (Table 7.5) [27].

Electrocardiography

Echocardiography (ECG) is a noninvasive method used to investigate cardiac morphology and function. ECG uses standard two-dimensional, three-dimensional, and Doppler ultrasound to create images of the heart. Two-dimensional ECG provides a visualization of the morphology of the heart. M-mode ECG allows heart function to be monitored. Pulsed Doppler ECG is the method of choice to measure blood flows. ECG in marmosets is an established method, and a database of major echocardiographic parameters is available [28]. It is a

TABLE 7.5 Blood Pressure and Heart Rate Values in Unrestrained Marmosets

Day Time	Mean Arterial Pressure	Heart Rate
Midnight	82±2 mmHg	153±4 beats/min
6 am	99±2 mmHg	259±6 beats/min
Midday	93±2 mmHg	220±5 beats/min
6 pm	98±1 mmHg	238±4 beats/min

Source: Schnell et al. [27].

noninvasive technique well suited for longitudinal studies of cardiac function, and it should be regarded as a key component in primate-based cardiovascular studies. Furthermore, it allows monitoring of drug effects on the cardiac function in pharmacology or toxicology studies [29].

Core Body Temperature

The normal body temperature ranges from 36.8 to 38.6 °C. Temperature logger systems or neuro logger systems measure core body temperature. Using the neuro logger system, marmosets displayed a pronounced daily rhythm in core body temperature, with a difference of about 3 °C between light and dark periods. There is a sharp difference between female and male marmosets that parallels the differences in activity pattern. The body temperature of the females began to decrease 2 h earlier than that of the males before reaching stable nighttime levels. In the first 2 h after lights-on, body temperature increases gradually to a stable daytime level of approximately 39 °C [30].

RESPIRATORY SYSTEM

Structure and Function

The respiratory system includes the nasal cavities, the paranasal sinuses, pharynx, larynx trachea, bronchi, bronchioli, and lung alveoli. Marmosets lack a typical laryngeal diverticula or air sack as found in other primate species. The nasal cavities are lined by stratified squamous epithelium, transitional epithelium, ciliated pseudo-respiratory epithelium, olfactory epithelium, and lymphoepithelium. The trachea and bronchi are lined by a pseudo-stratified respiratory epithelium that includes ciliated, mucous, and nonciliated cells. The trachea has a diameter of 1000-1150 µm, the diameter of the bronchus principalis dexter is a little larger (800-1050 µm) than the left counterpart (650-800 µm), the bronchi vary between 250 and 700 µm in diameter [31]. The lower respiratory system is composed of the lungs, which are divided into six lobes, two on the left side (lobus craniali and lobus caudalis) and four on the right side (lobus cranialis, lobus medius, lobus caudalis, lobus accessorius).

Healthy marmosets show an average minute respiratory volume value of 24.9±2.39 mL/min, a respiratory frequency of 26.9±1.87/min, a tidal volume of 0.98±0.11 mL, an esophageal pressure of 3.90±0.36 cm H_2O, a midexpiratory flow of 3.64±0.26 mL/s, dynamic compliance of 0.40±0.05 mL/cm H_2O, and a lung resistance of 0.28±0.03 cm H_2O s/mL (data are means±standard errors of the mean; $n=10$). Data were obtained after a lung function test using plethysmography [32].

DEVELOPMENT AND REPRODUCTION

Marmoset reproductive features are substantially different from that of macaques and humans in many instances. In the wild, marmosets exhibit a variety of mating systems, but monogamy is the predominant form. Females form social hierarchies in which only the dominant female breeds. Common marmoset males are biparental nonhuman primates that show

VetBooks.ir

extensive paternal behavior. Paternal testosterone levels seem to be regulated by the infant's scent. Infant scent leads to a decrease in the father's serum testosterone, which may have an acute effect on paternal care [33]. The breeding male and older offspring in the group similarly participate in the care of the new offspring, which is associated with elevated circulating concentrations of prolactin. Older offspring will not reproduce as long as the dominant mated pair is intact.

In general, marmosets have a high fecundity. They are nonseasonal breeders. Callithrichidae are the only anthropoid primates with a high and variable number of ovulations, ranging from one to four follicles per cycle. Therefore marmoset litter sizes typically vary in captivity from singletons to quadruplets [34]. Sexual maturity, as defined by the occurrence of ovulation and ejaculation of motile sperm, occurs at 11-13 months, and social maturity is reached within 18-24 months. Male age at sexual maturity is 382 days, whereas female age at sexual maturity is 477 days [35] (Table 7.1).

Ovarian Cycles

Female marmosets lack external signs of ovulation. They do not menstruate and do not show signs of a sex skin. Marmoset reproduction can be monitored only by endocrinological evaluation of urine and serum for cycle relevant hormones such as estrogens, luteinizing hormone (LH), and progesterone. A combination with vaginal cytology defined on the basis of the karyopyknotic index over time may aid in predicting the date of ovulation [36]. The ovarian cycle or ovarian triad is a sequence of follicular growth and development, leading to ovulation of mature oocytes and the formation of a functional corpus luteum. A typical cycle generally consists of the follicular or proliferative phase, ovulation, and the luteal or secretory phase. During the luteal phase, the corpus luteum (CL) is established. Subsequent corpus luteum regression is not followed by menstruation as it is in other primate species. Marmosets and tamarins are the only anthropoid primate species with multiple ovulations [37]. Dominant female marmosets have an ovarian cycle of 28 days, with a range of 15-28 days [38]. The follicular phase of 8-10 days is relatively short, and the luteal phase lasts 18-20 days [39]. The follicular phase is characterized by low concentrations of circulating progesterone (≤10 ng/mL) and increasing concentrations of estradiol stemming from the growing Graafian follicle [40–42]. Estradiol concentrations peak on day 8, followed by a prominent preovulatory LH surge. Estradiol concentrations decline following ovulation. The luteal phase is characterized by increasing concentrations of progesterone (10-120 ng/mL), which rise rapidly at the end of the ovulatory phase and reach a maximum at the midpoint of the luteal phase [40]. The relaxin concentration is low in the follicular phase but increases after ovulation around day 13 or 14, and it remains elevated during the luteal phase, following the pattern for progesterone [43].

The pattern of inhibin secretion seems to resemble that of progesterone. Mean values for inhibin are 5465 and 4972 U/L during the early and late follicular phase. Concentrations rise during the luteal phase to 8431, 12,246, and 12,557 U/L for the early, mid-, and late luteal phase, respectively [44]. The marmoset follicle-stimulating hormone (FSH) profile is unusual and characterized by three peaks: the first on day 2 of the follicular phase, a second on day 6, and a third around the time of ovulation [45].

Marmoset ovaries contain a high number of small antral follicles (averaging 68 per ovarian pair) that represent the vast majority of follicles during all stages of the cycle. In the luteal

VetBooks.ir

phase, only small antral follicles (≤1.0 mm) are found. Multiple follicles >1.0 mm appear in the follicular phase, and preovulatory follicles (>2.0 mm) occur only at the end of the follicular phase (days 7-9). Follicular growth beyond the 1-mm threshold seems to be initiated at the start of the follicular phase and corresponds to the first FSH peak. A small number of medium-sizes (1-1.5 mm) follicles appear on days 1-2. Until day 7 of the follicular phase, two or three preovulatory follicles emerge corresponding to the second FSH peak and rising estradiol concentrations. Ovulation usually occurs between days 8 and 12 of the cycle and follows a preovulatory LH peak [34,45–47].

Ultrasonography provides a reliable noninvasive method to examine the ovarian cycle. Mean follicle size increases from 2.1 mm on day 7 to 3.2 mm before they disappear [34]. Follicular development can be monitored by measurement of plasma estradiol; a detectable increase in this hormone occurs about 3 days before ovulation [39]. The measurement of unconjugated progestins and, to a lesser extent, estrogen metabolites such as estrone-3-sulfate in feces is a reliable method for monitoring ovarian cycles in marmosets [48]. The excretion of hydroxypregnanolone, the main progesterone metabolite, shows low concentrations during the follicular phase and high concentrations in the luteal phase of the ovulatory cycle. The excretion of the metabolite 5α-pregnane-3α,7α-diol-20-one is especially high in the marmoset [49].

Multiple follicles are released at each ovulation, resulting in twins in 30-70% of pregnancies (Figure 7.3) and in triplets in 20-25% of pregnancies [50]. Many long-established colonies report that triplets are the most common litter size. Ovulation number and litter size are positively related to maternal weight [51]. Twinning presents the opportunity to use

FIGURE 7.3 Reproduction. Twinning is a frequently observed phenomena in marmosets (*Callithrix jacchus*). *Picture DPZ, A. Säckel.*

genetically identical animals assigned to different experimental conditions within the same study. Implantation begins on days 11-12 after ovulation [52,53]. Atretic follicles undergo luteinization, forming accessory CLs or intestinal glands. These glands are steroidogenic and may contribute to the extremely high concentration of circulating progesterone characteristic of New World monkeys. Marmoset CLs secrete substantial amounts of E_2 and inhibin, and they have progesterone concentrations 10- to 30-fold higher than those in Old World primates [40,48].

Subordinate Females

Another characteristic of marmosets housed in groups is that only the socially most dominant female reproduces. Interaction between the dominant female and other female group members induces suppression of ovulation among the subordinate females. This immediate and precise behavioral control of ovulation is unique among anthropoid primates [54,55]. After puberty, ovulation can be suppressed for years under certain conditions. Inhibitory effects seem to be mediated by visual, olfactory, and behavioral signals. In subordinate female marmosets, anovulation is associated with suppressed pituitary LH secretion and reduced pituitary LH response to gonadotropin-releasing hormone (GnRH). Basal plasma LH concentrations are commonly <2 IU/L. Plasma estrogen concentrations are similarly low (maximum 0.62 nmol/L) and plasma progesterone concentrations <30 nmol/L characterize the infertile condition. Subordinate females reestablish ovulation quickly after they are removed from their social groups. Under altered conditions, plasma LH (maximum 140.0 IU/L) and estrogen (maximum 7.84 nmol/L) concentrations increase preceding ovulation [56,57].

Pregnancy and Lactation

Marmosets have an uterus simplex. The age of the first parturition is 1.44 years. Average gestation length is 148 ± 3-4 days and the interbirth interval approximates 6 months [37,58,59]. Postpartum ovulation at day 9-11 following parturition is typical for marmosets, and it can result in conception and successful delivery. Lactation lasts for 65-90 days and does not influence ovulation. Lactation-induced anovulation is not observed.

The placentation form is unique among nonhuman primates. The placenta consists of two discoid parts connected by vascular anastomoses (placenta bidiscoidales) and is permeable to antibodies (placenta haemochorialis). Marmoset embryos establish vascular anastomoses between their placentae and share a common chorionic cavity by day 30 of gestation. In the case of a twin birth of the opposite sex, animals are blood chimeric, containing XX and XY karyotype lymphocytes [60].

Pregnancy can be diagnosed by measuring the presence of chorionic gonadotropin (CG), which is excreted throughout pregnancy. Maximum concentrations can be detected between the eighth and ninth week of gestation [52,61]. During early pregnancy, relaxin produced by uterus and placenta increases significantly, making relaxin and CG early pregnancy markers. Relaxin concentrations of approximately 1 ng/mL in serum can be measured in early pregnancy, which increases up to 15 ng/mL in the second trimester at a time when progesterone concentrations have declined. In the third trimester, relaxin decreases and progesterone increases again [43]. Pregnancy can also be diagnosed by ultrasound around the 30th

VetBooks.ir

gestational day by the presence of a hypoechoic area between the uterus walls and a fully developed gestational sac. Heart beats are detectable around day 50 [35].

The patterns of the reproductive hormones, including estradiol-17β, estrone, progesterone, testosterone, androstenedione, LH/CG, and inhibin in serum or plasma during gestation are well-characterized [62,63]. Concentrations of LH/CG, androstenedione, and testosterone increase in early pregnancy to maximum values at 6-10 weeks. Thereafter, LH/CG concentrations decline and androstenedione and testosterone concentrations remain at plateau values or decline until term. At 12 weeks of gestation, when LH/CG are declining, progesterone and estradiol start to rise above luteal-phase concentrations. Serum estradiol rises slowly to a mean concentration of about 2 ng/mL by week 13 of pregnancy and then climb to a level of 12 ng/mL, which persists until just before delivery. Serum progesterone rises to a concentration between 80 and 100 ng/mL, remains at that level throughout more than half of the pregnancy, and then rises further to >200 ng/mL 2 weeks before parturition [64]. The pattern of progesterone is similar to that in women, in which luteal concentrations are maintained for the first quarter of gestation followed by a gradual increase. Progesterone is secreted by the corpus luteum until at least day 40. At this time there is also placental secretion [63]. Inhibin concentrations remain increased throughout gestation [44]. Relaxin is not detectable until the fourth week of pregnancy and then rises to a peak of about 10 ng/mL by week 11 or 12, slowly declining thereafter. The relaxin pattern differs from that of other primates resembling more closely that seen in many nonprimate mammalian species such as rodents, carnivores, and equids [64].

During the last week of pregnancy, plasma steroid hormone concentrations of progesterone (229 ng/mL), estradiol (91 ng/mL), estrone (23 ng/mL), androstenedione (7 ng/mL), and testosterone (1 ng/mL) can be found [62]. An elevated concentration of the progesterone metabolite hydroxypregnanolone in urine measured during pregnancy reflects the progesterone profile. Maximum hydroxypregnanolone excretion is observed between days 7 and 20, followed by a decline of 30-40% compared to the initial value. After day 75, the concentration of the gestagene metabolite increases steadily with the highest concentrations 7-10 days before delivery. Measurement of hydroxypregnanolone can be used to monitor the persistence of pregnancy [49].

The pattern of maternal circulating corticotropin-releasing hormone (CRH) is characterized by a rapid rise starting at 50 days after conception and a peak at approximately 70 days after conception. Approximately 110 days after conception, CRH concentrations plateau at a significantly lower value. Females gestating triplets have higher values than females gestating twins [65].

Senescence

Similarly to most other mammals, marmosets show a decline in the total number of antral follicles with advancing age. Reproductive decline in this species is not a gradual process; it occurs rather abruptly. Females begin to display anovulation associated with follicular depletion at a point relatively close to the maximum life span. They continue to display significant steroidogenic activity in the ovary until the end of their life span. Only small age effects on litter size and interbirth intervals were observed [66]. The average life span for breeding females is around 6 years [37].

VetBooks.ir

Male Primate Reproduction

Male reproduction stands under the regulation of the hypothalamic-pituitary-testicular axis. The interrelationship of these tissues is essential for neonatal and juvenile development and controls the spermatogenesis in the adult. GnRH secreted in the hypothalamus stimulates the cells in the anterior pituitary to release LH and FSH. LH stimulates the testicular Leydig cells to secrete testosterone. FSH stimulates the Sertoli cells that release androgen-binding protein, inhibin, and activin. Negative feedback mechanisms from testosterone and estradiol control the GnRH amplitude and frequency. Hormone-specific receptors within the tissues regulate the interaction. Marmosets differ from Old World monkeys in their LH receptor. They do not express the β-subunit of LH, but the β-subunit of CG in the pituitary, which is the only gonadotropin with LH activity expressed in this species [67,68]. The action of CG in the marmoset is mediated by a modified LH receptor isoform lacking exon 10 in its messenger RNA [69], indicating the possibility that marmosets' Leydig cells are controlled by CG rather than by LH and offering a new route of testosterone production in the marmoset [70].

Male reproductive development leading to puberty has three important endocrine phases, starting with neonatal activation of the pituitary-gonadal axis up to 16 weeks of age. During this time, males exhibit a neonatal testosterone surge [50]. This phase is followed by a quiescent phase up to 35 weeks of age. Reactivation of the pituitary-gonadal axis leads to the onset of puberty between the 40th and 52nd week. At that time, lumen in the tubules appears and all advanced cell types including spermatozoa in the testis are present [71]. This process is accompanied by an increase in serum testosterone concentrations from values <10 to values >80 nmol/L [50,72]. Full sexual maturity is reached at week 70 [71].

Spermatogenesis

Marmoset spermatogenesis is highly efficient and shares many organizational similarities with that of humans [73,74]. The testicular development starts with first testicular differentiation in the late embryo stage (approximately gestation day 75). At birth, tubules contain many genocytes that are not in contact with the basement membrane and develop into spermatogonial stem cells. Spermatogenesis involves the multiplication and proliferation of spermatogonial stem cells, recombination of genetic material during meiosis, and differentiation and maturation of spermatids into sperm. Prespermatogenesis starts in juveniles. First adult spermatogonia are seen during puberty. After puberty, males start producing fully mature spermatozoa [75]. Spermatogenesis occurs within the seminiferous epithelium and involves nine stages of cell differentiation during the seminiferous epithelium cycle [75,76]. The six-stage scheme for human spermatogenesis could also be successfully applied to a proportion of marmoset tubules [77]. Up to five cell stages could be observed within individual cross-sections of seminiferous tubules (multistage tubule type) [73,75]. Therefore, the marmoset seminiferous epithelium and organization of seminiferous epithelial stages closely resemble the situation in humans. The length of the spermatogenic cycle is 10 days and spermatogenesis requires 37 days. Four mitotic divisions occur during spermatogonia [73]. Only a few germ cells are lost during spermatid maturation. The conversion of type A to type B spermatogonia is several-fold higher than that reported for other primates. Haploid germ cell apoptosis is less than 2% [77]. The morphology of marmoset sperm follows the general primate pattern.

VetBooks.ir

The sperm head is ovoid in outline and bilaterally flattened, and the tail is inserted slightly off center [78]. The mean total length is 58.31 µm, the mean head length is 5.47 µm, and its width is 3.95 µm. Marmosets display an acrosome maturation profile. Maturation and storage of sperm occurs within the epididymis. The characteristics of the marmoset ejaculate depend on the method of achievement. Repeated collection of semen from the same individual is usually performed using rectal probe electro-ejaculation or vaginal washing after natural copulation [79]. Both methods have disadvantages: The first is invasive and therefore requires anesthesia. The second yields ejaculates inevitably contaminated with mucus from the female genital tract. Penile vibrostimulation is a third repeatable noninvasive method of semen sampling [80]. Using penile vibrostimulation, a mean ejaculate volume of 31.9 µL, a mean total sperm number of 34.2×10^6/ejaculate, a mean concentration of 1154.2×10^6 sperm/mL, a percentage of live sperm (74.6%) and motile sperm (59.6%) can be obtained [80]. The pH of normal sperm is 7.51 ± 0.22. There is considerable inter- and intramale variation in all seminal characteristics. Of sperm, 49% exhibits normal morphology (range, 24-81%). Tail defects are commonly noted (median, 50%; range, 17-76%), whereas head defects are a relatively rare event (median, 4.5%; range, 0-24%) [78]. Stress reduces sperm quality. Lower semen volume and sperm concentrations and a significantly higher percentage of abnormally shaped sperm heads were observed under stress conditions [81]. Marmoset spermatogenesis cannot effectively be suppressed by common hormonal regimes using testosterone undecanoate or norethisterone enanthate [82].

CONCLUSION

In recent years, common marmosets have become a suitable animal model in many research fields. The advantage over other nonhuman primate models include their small size, easy handling and husbandry, early sexual maturity, and short gestation time, which makes them attractive for toxicology and reproductive biology. Nevertheless, there are some endocrine peculiarities that have to be considered. Gonadotropins and steroids are specifically different from those of other primates [67,75]. Marmosets exhibit extremely high circulating cortisol concentrations and a hyperfunctional hypothalamic-pituitary-adrenal axis.

Moreover, extremely high concentrations of vitamin D metabolites are observed and a generalized steroid resistance is suggested [10,83,84]. Marmosets show a different response to regimens of male contraception from macaques or humans and have to be considered as a problematic model for preclinical trials of male hormonal contraception.

References

[1] Rylands AB, Mittermeier RA. Familiy Callitrichidae (marmosets and tamarins). In: Mittermeier RA, Rylands AB, Wilson DE, editors. Handbook of the mammals of the world. 3. Primates, vol. 3. Spain: Lynx Edicions Barcelona; 2013. p. 262–321.

[2] Yarbrough LW, Tollet JL, Montrey RD, Beattie RJ. Serum biochemical, hematological and body measurement data for the common marmosets (*Callithrix jacchus*). Lab Anim Sci 1984;34:276–80.

[3] Rensing S, Oerke AK. Husbandry and management of new world species: marmosets and tamarins. In: Wolfe-Coote S, Bullock G, Petrusz P, editors. The laboratory primate. Amsterdam: Elsevier, Academic Press; 2005. p. 145–62.

VetBooks.ir

[4] Ankel-Simons F. Primate anatomy: an introduction. Primate Dentition, San Diego: Academic Press; 1983 p. 231–48.
[5] Caton J. Diet and food processing in the common marmoset. Aust Primatol 1994;8:13.
[6] Garber PA. Nectar feeding in two species of Peruvian tamarins (*Saguinus mystax* and *Saguinus fuscicollis*). Am J Phys Anthropol 1986;69:202.
[7] Flurer CI, Kern M, Rambeck WA. Ascorbic acid requirement and assessment of ascorbate status in the common marmoset (*Callithrix jacchus*). Ann Nutr Metab 1987;31:245–52.
[8] Flurer CI, Zucker H. Ascorbic acid in a New World monkey family: species difference and influence of stressors on ascorbic acid metabolism. Z Ernahrungswiss 1989;28:49–55.
[9] Marx SJ, Jones G, Weinstein RS. Differences in mineral metabolism among nonhuman primates receiving diets with only vitamin D3 or only vitamin D2. J Clin Endocrinol Metab 1989;69:1282–90.
[10] Shinki T, Shiina Y, Takahashi N, Tanioka Y, Koizumi H, Suda T. Extremely high circulating levels of 1 alpha,25-dihydroxyvitamin D3 in the marmoset, a new world monkey. Biochem Biophys Res Commun 1983;114:452–7.
[11] Power ML, Oftedal OD. Differences among captive callitrichids in the digestive responses to dietary gum. Am J Primatol 1996;40:131–44.
[12] McGuill MW, Rowan AN. Biologic effects of blood loss: implications for sampling volumes and techniques. ILAR News 1989;31:5–20.
[13] Zühlke U, Weinbauer G. The common marmoset (*Callithrix jacchus*) as a model in toxicology. Toxicol Pathol 2003;31(Suppl.):123–7.
[14] Hawkey CM, Hart MG, Jones DM. Clinical hematology of the common marmoset *Callithrix jacchus*. Am J Primatol 1982;3:179–99.
[15] Kuehnel F, Grohmann J, Buchwald U, Koeller G, Teupser D, Einspanier A. Parameters of haematology, clinical chemistry and lipid metabolism in the common marmoset and alterations under stress conditions. J Med Primatol 2012;41:241–50.
[16] McIntosh GH, Lawson CA, Rodgers SE, Lloyd JV. Haematological characteristics of the common marmoset (*Callithrix jacchus jacchus*). Res Vet Sci 1985;38:109–14.
[17] Davy CW, Jackson MR, Walker J. Reference intervals for some clinical chemical parameters in the marmoset (*Callithrix jacchus*): effect of age and sex. Lab Anim 1984;18:135–42.
[18] McNees DW, Lewis RW, Ponzio BJ. Blood chemistry of the common marmoset (*Callithrix jacchus*) maintained in an indoor-outdoor environment: primate comparisons. Primates 1984;25:103–9.
[19] Evans GO, Smith DE. Platelet measurements in the common marmoset, *Callithrix jacchus*. J Comp Pathol 1986;96:343–7.
[20] Cowie JR, Evans GO. Plasma aminotransferase measurements in the marmoset (*Callithrix jacchus*). Lab Anim 1985;19:48–50.
[21] Casetti R, Taglioni A, Perreta G. Baseline blood values in the marmoset (*Callithrix jacchus*): pregnancy related changes. Primate Report 1995;42:16.
[22] Cross N, Pines MK, Rogers LJ. Saliva sampling to assess cortisol levels in unrestrained common marmosets and the effect of behavioral stress. Am J Primatol 2004;62:107–14.
[23] Heistermann M, Palme R, Ganswindt A. Comparison of different enzyme immunoassays for assessment of adrenocortical activity in primates based on fecal analysis. Am J Primatol 2006;68:257–73.
[24] Sousa MBC, Ziegler TE. Diurnal variation on the excretion patterns of fecal steroids in common marmoset (*Callithrix jacchus*) females. Am J Primatol 1998;46:105–17.
[25] Hassimoto M, Harada T. Use of telemetry system to examine recovery of the cardiovascular system after excitement induced by handling stress in a conscious cynomolgus monkey (*Macaca fascicularis*). J Med Primatol 2003;32:346–52.
[26] Bergmann SA. Ketamine: review of its pharmacology and its use in pediatric anesthesia. Anesth Prog 1999;46:10–20.
[27] Schnell CR, Wood JM. Measurement of blood pressure and heart rate by telemetry in conscious, unrestrained marmosets. Am J Physiol 1993;264:509–16.
[28] Hanton G, Eder V, Bonnet P, Rochefort GY. Echocardiography in marmosets: a non-invasive method for the assessment of cardiovascular toxicology and pharmacology. In: Weinbauer GF, Vogel F, editors. Novel approaches towards primate toxicology. Münster/New York: Waxmann Publishing Co; 2006. p. 27–45.
[29] Hanton G, Sobry S, Dagues N, Rochefort GY, Bonnet P, Eder V. Cardiovascular toxicity of minoxidil in the marmoset. Toxicol Lett 2008;180:157–65.

[30] Hoffmann K, Coolen A, Schlumbohm C, Meerlo P, Fuchs E. Remote long-term registrations of sleep-wake rhythms, core body temperature and activity in marmoset monkeys. Behav Brain Res 2012;235:113–23.
[31] Seidel V, Hoffmann R, Braun A, Seehase S, Knauf S, Kaup F-J, et al. Distribution and morphology of Clara cells in common marmosets (*Callithrix jacchus*). J Med Primatol 2013;42:79–88.
[32] Curths C, Wichmann J, Dunker S, Windt H, Hoymann HG, Lauenstein HD, et al. Airway hyper-responsiveness in lipopolysaccharide-challenged common marmosets (*Callithrix jacchus*). Clin Sci 2014;126:155–62.
[33] Prudom SL, Broz CA, Schultz-Darken N, Ferris CT, Snowdon C, Ziegler TE. Exposure to infant scent lowers serum testosterone in father common marmosets (*Callithrix jacchus*). Biol Lett 2008;4:603–5.
[34] Oerke AK, Einspanier A, Hodges JK. Noninvasive monitoring of follicle development, ovulation, and corpus luteum formation in the marmoset monkey (*Callithrix jacchus*). Am J Primatol 1996;39:99–113.
[35] Tardif S, Carville A, Elmore D, Williams LE, Rice K. Reproduction and breeding of nonhuman primates. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. Nonhuman primates in biomedical research: biology and management. American College of Laboratory Animal Medicine Series, vol. 1. Amsterdam: Academic Press; 2012. p. 197–249.
[36] Gluckman TL, Walz SE, Schultz-Darken N, Bolton ID. Cytologic assessment of the vaginal epithelium in the common marmoset (*Callithrix jacchus*): a preliminary new approach to reproductive screening. Contemp Top Lab Anim Sci 2004;43:28–31.
[37] Tardif SD, Smucny DA, Abbott DH, Mansfield K, Schultz-Darken N, Yamamoto ME. Reproduction in captive common marmosets (*Callithrix jacchus*). Comp Med 2003;53:364–8.
[38] Fraser HM, Lunn SF. Nonhuman primates and female reproductive medicine. In: Weinbauer GF, Korte R, editors. Reproduction in nonhuman primates—a model system for human reproductive physiology and toxicology. Münster: Waxmann; 1999. p. 27–59.
[39] Hodges JK, Cottingham PG, Summers PM, Yingnan L. Controlled ovulation in the marmoset monkey (*Callithrix jacchus*) with human chorionic gonadotropin following prostaglandin-induced luteal regression. Fertil Steril 1987;48:299–304.
[40] Harding RD, Hulme MJ, Lunn SF, Henderson C, Aitken RJ. Plasma progesterone levels throughout the ovarian cycle of the common marmoset (*Callithrix jacchus*). J Med Primatol 1982;11:43–51.
[41] Harlow CR, Gems S, Hodges JK, Hearn JP. The relationship between plasma progesterone and the timing of ovulation and early embryonic development in the marmoset monkey (*Callithrix jacchus*). J Zool (Lond) 1983;201:273–82.
[42] Harlow CR, Hearn JP, Hodges JK. Ovulation in the marmoset monkey: endocrinology, prediction and detection. J Endocrinol 1984;103:17–24.
[43] Einspanier A, Nubbemeyer R, Schlote S, Schumacher M, Ivell R, Fuhrmann K, et al. Relaxin in the marmoset monkey: secretion pattern in the ovarian cycle and early pregnancy. Biol Reprod 1999;61:512–20.
[44] Smith KB, Lunn SF, Fraser HM. Inhibin secretion during the ovulatory cycle and pregnancy in the common marmoset monkey. J Endocrinol 1990;126:489–95.
[45] Gilchrist RB, Wicherek M, Heistermann M, Nayudu PL, Hodges JK. Changes in follicle-stimulating hormone and follicle populations during the ovarian cycle of the common marmoset. Biol Reprod 2001;64:127–35.
[46] Tardif SD, Lacker HM, Feuer M. Follicular development and ovulation in the marmoset monkey as determined by repeated laparoscopic examination. Biol Reprod 1993;48:1113–9.
[47] Nubbemeyer R, Heistermann M, Oerke AK, Hodges JK. Reproductive efficiency in the common marmoset (*Callithrix jacchus*): a longitudinal study from ovulation to birth monitored by ultrasonography. J Med Primatol 1997;26:139–46.
[48] Heistermann M, Tari S, Hodges JK. Measurement of faecal steroids for monitoring ovarian function in New World primates, Callitrichidae. J Reprod Fertil 1993;99:243–51.
[49] Heger W, Neubert D. Determination of ovulation and pregnancy in the marmoset (*Callithrix jacchus*) by monitoring of urinary hydroxypregnanolone excretion. J Med Primatol 1987;16:151–64.
[50] Abbott DH, Hearn JP. Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. J Reprod Fertil 1978;53:155–66.
[51] Tardif SD, Jaquish CE. Number of ovulations in the marmoset monkey (*Callithrix jacchus*): relation to body weight, age and repeatability. Am J Primatol 1997;42:323–9.
[52] Hearn JP, Hodges JK, Gems S. Early secretion of chorionic gonadotrophin by marmoset embryos in vivo and in vitro. J Endocrinol 1988;119:249–55.
[53] Moore HDM, Gems S, Hearn JP. Early implantation stages in the marmoset monkey (*Callithrix jacchus*). Am J Anat 1985;172:265–78.

[54] Abbott DH, McNeilly AS, Lunn SF, Hulme MJ, Burden FJ. Inhibition of ovarian function in subordinate female marmoset monkeys (*Callithrix jacchus jacchus*). J Reprod Fertil 1981;63:335–45.
[55] Barrett J, Abbott DH, George LM. Sensory cues and the suppression of reproduction in subordinate female marmoset monkeys (*Callithrix jacchus*). J Reprod Fertil 1993;97:301–10.
[56] Abbott DH, Hodges JK, George LM. Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). J Endocrinol 1988;117:329–39.
[57] Tardif SD, Hyde K, Digby L. Evidence for suppression of ovulation in singly-housed female common marmosets (*Callithrix jacchus*). Lab Primate Newsl 1994;33:1–4.
[58] Hearn JP, Lunn SF. The reproductive biology of the marmoset monkey, *Callithrix jacchus*. Lab Anim Handbks 1975;6:191–202.
[59] Hearn JP. The embryo maternal dialogue during early pregnancy in primates. J Reprod Fertil 1986;76:809–19.
[60] Haig D. What is a marmoset? Am J Primatol 1999;49:285–96.
[61] Hobson BM, Hearn JP, Lunn SF, Flockhart JH. Urinary excretion of biologically active chorionic gonadotrophin by the pregnant marmoset (*Callithrix jacchus jacchus*). Folia Primatol 1977;28:251–8.
[62] Chambers PL, Hearn JP. Peripheral plasma levels of progesterone, oestradiol-17β, oestrone, testosterone, androstenedione and chorionic gonadotrophin during pregnancy in the marmoset monkey, *Callithrix jacchus*. J Reprod Fertil 1979;56:23–32.
[63] Hodges JK, Henderson C, Hearn JP. Relationship between ovarian and placental steroid production during early pregnancy in the marmoset monkey (*Callithrix jacchus*). J Reprod Fertil 1983;69:613–21.
[64] Steinetz BG, Randolph C, Mahoney JC. Patterns of relaxin and steroids in the reproductive cycle of the common marmoset (*Callithrix jacchus*): effects of prostaglandin F2 on relaxin and progesterone secretion during pregnancy. Biol Reprod 1995;53:834–9.
[65] Power ML, Bowman ME, Smith R, Ziegler TE, Layne DG, Schulkin J, et al. Pattern of maternal serum corticotropin-releasing hormone concentration during pregnancy in the common marmoset (*Callithrix jacchus*). Am J Primatol 2006;68:181–8.
[66] Tardif SD, Araujo A, Arruda MF, French JA, Sousa MB, Yamamoto ME. Reproduction and aging in marmosets and tamarins. Interdiscip Top Gerontol 2008;36:29–48.
[67] Luetjens CM, Weinbauer GF, Wistuba J. Primate spermatogenesis: new insights into comparative testicular organisation, spermatogenic efficiency and endocrine control. Biol Rev Camb Philos Soc 2005;80:475–88.
[68] Müller T, Simoni M, Pekel E, Luetjens CM, Chandolia R, Amato F, et al. Chorionic gonadotrophin beta subunit mRNA but not luteinising hormone beta subunit mRNA is expressed in the pituitary of the common marmoset (*Callithrix jacchus*). J Mol Endocrinol 2004;32:115–28.
[69] Gromoll J, Wistuba J, Terwort N, Godmann M, Müller T, Simoni M. A new subclass of the luteinizing hormone/chorionic gonadotropin receptor lacking exon 10 messenger RNA in the New World monkey (*Platyrrhini*) lineage. Biol Reprod 2003;69:75–80.
[70] Wistuba J, Gromoll J, Weinbauer GF, Luetjens CM. Comparative analysis of the primate testis: size, endocrine regulation and sperm competition. In: Weinbauer GF, editor. New developments and challenges in primate toxicology. New York: Waxman; 2008. p. 73–85.
[71] Chandolia RK, Luetjens CM, Wistuba J, Yeung CH, Nieschlag E, Simoni M. Changes in endocrine profile and reproductive organs during puberty in the male marmoset monkey (*Callithrix jacchus*). Reproduction 2006;132:355–63.
[72] Wistuba J, Luetjens CM, Wesselmann R, Nieschlag E, Simoni M, Schlatt S. Meiosis in autologous ectopic transplants of immature testicular tissue grafted to *Callithrix jacchus*. Biol Reprod 2006;74:706–13.
[73] Millar MR, Sharpe RM, Weinbauer GF, Fraser HM, Saunders PT. Marmoset spermatogenesis: organizational similarities to the human. Int J Androl 2000;23:266–77.
[74] Wistuba J, Schrod A, Greve B, Hodges JK, Aslam H, Weinbauer GF, et al. Organization of seminiferous epithelium in primates: relationship to spermatogenic efficiency, phylogeny, and mating system. Biol Reprod 2003;69:582–91.
[75] Li LH, Donald JM, Golub MS. Review on testicular development, structure, function, and regulation in common marmoset. Birth Defects Res Part B Dev Reprod Toxicol 2005;74:450–69.
[76] Holt WV, Moore HD. Ultrastructural aspects of spermatogenesis in the common marmoset (*Callithrix jacchus*). J Anat 1984;138:175–88.
[77] Weinbauer GF, Aslam H, Krishnamurthy H, Brinkworth MH, Einspanier A, Hodges JK. Quantitative analysis of spermatogenesis and apoptosis in the common marmoset (*Callithrix jacchus*) reveals high rates of spermatogonial turnover and high spermatogenic efficiency. Biol Reprod 2001;64:120–6.

[78] Cui KH, Flaherty SP, Newble CD, Guerin MV, Napier AJ, Matthews CD. Collection and analysis of semen from the common marmoset (*Callithrix jacchus*). J Androl 1991;12:214–20.
[79] Morrell JM, Kuederling I, Hodges JK. Influence of semen collection method on ejaculate characteristics in the common marmoset, *Callithrix jacchus*. J Androl 1996;17:164–72.
[80] Kuederling I, Schneiders A, Sonksen J, Nayudu PL, Hodges JK. Non-invasive collection of ejaculates from the common marmoset (*Callithrix jacchus*) using penile vibrostimulation. Am J Primatol 2000;52:149–54.
[81] Cui KH. The effect of stress on semen reduction in the marmoset monkey (*Callithrix jacchus*). Hum Reprod 1996;11:568–73.
[82] Wistuba J, Luetjens CM, Ehmcke J, Redmann K, Damm OS, Steinhoff A, et al. Experimental endocrine manipulation by contraceptive regimen in the male marmoset (*Callithrix jacchus*). Reproduction 2013;145:439–51.
[83] Lipsett MB, Chrousos GP, Tomita M, Brandon DD, Loriaux DL. The defective glucocorticoid receptor in man and nonhuman primate. Recent Prog Horm Res 1985;41:199–247.
[84] Pryce CR, Palme R, Feldon J. Development of pituitary-adrenal endocrine function in the marmoset monkey: infant hypercortisolism is the norm. J Clin Endocrinol Metab 2002;87:691–9.

VetBooks.ir

VetBooks.ir

CHAPTER

8

Comparative Physiology, Growth, and Development

Wayne R. Buck
AbbVie, Inc., North Chicago, IL, USA

OUTLINE

Introduction 136
Comparative Genetics 136
Comparative Growth and Development 139
Oocyte to Parturition 139
Hormonal Control of Gonadotropin Release 139
The Ovarian Cycle 142
Placentation 143
Hormonal Maintenance of Pregnancy 144
Fetal Growth 144
Neonatal and Juvenile Growth 145
Physiological Parameters 145
Hormonal Changes in Infancy 146
Growth 146
Lactation and Early Nutrition 146
Sexual Maturation 147
Skeletal and Dental Maturation 149
Comparative Cardiovascular Physiology 151
Heart and Anatomy 151
Control of Contraction 151
Torsades de Pointes 152
Electrocardiography 153
Echocardiography 154
Peripheral Vasculature 154
Comparative Cardiac Gene Expression 154
Comparative Renal Physiology 155
Comparative Respiratory Physiology 156
Anatomy 156
Development 157
Physiology 158
Neurobiology 159
Visual System 161
References 162

http://dx.doi.org/10.1016/B978-0-12-417144-2.00008-1
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

Understanding the comparative physiology of the nonhuman primate species used in toxicology studies enables the toxicologist to better determine the human relevance of any toxicologic findings, design studies of the appropriate developmental stage, realize limitations of the animal test system for predicting human toxicity, better evaluate toxic changes when control sets are small, and raise awareness of confounding factors that may unintentionally impact study end points. The rhesus macaque (*Macaca mulatta*) has been the most studied nonhuman primate, with over 150 years of scientific investigation reported in the literature [1,2]. However, trade restrictions in 1978 eliminated export of rhesus macaques from India, increasing dependence on existing colonies in laboratory animal facilities, and export from China. The use of the cynomolgus monkey (*Macaca fascicularis*, also known as the crab-eating macaque, long-tailed macaque, or irus macaque), particularly for pharmaceutical testing, has increased because of both these factors and the preference for a slightly smaller, more docile, nonseasonal breeding model. Both rhesus and cynomolgus monkeys originate from wide geographic areas. Subpopulations that have slightly different biological characteristics that potentially affect experimental outcomes have been identified (see also Chapters 5 and 6; and references [3,4]). Cynomolgus monkeys originating from Mauritius represent an isolated nonnative population frequently used in biomedical research and differ in hematology, for example, from *M. fascicularis* imported from the Philippines and Vietnam [5]. The marmoset (*Callithrix jacchus*), as a New World primate, has physiological attributes that are more distinct from those of humans, but its small size and twice-yearly reproductive cycle in purpose-bred research colonies has made it an attractive alternative to the larger primate species [6,7].

COMPARATIVE GENETICS

The genetic information encoded by the DNA of the nucleus and mitochondrion, along with a limited repertoire of epigenetic molecular modifications, is believed to account for species differences in form and function. Early efforts to understand genetic similarity between species focused on microscopic characterization of chromosome banding and number. Chromosome number in humans (46 diploid chromosomes, which includes the 2 sex chromosomes) is different from those of great apes (48 chromosomes), and gibbons (44 chromosomes), macaques, and marmosets (Table 8.1) [8]. However, banding patterns of chromosomes after Giemsa staining permitted early proposal of scenarios for chromosomal modifications that could provide a continuous progression of genetic change from primate ancestors [16]. Microsatellite analysis [17], chromosomal painting [18], and then whole-genome sequencing [9,10,19] have progressively refined our understanding of the genetic differences between primate species and allow us to identify regions of homology between humans and other primates (Tables 8.2 and 8.3). Comparisons between rhesus and cynomolgus macaques using chromosomal banding techniques suggested complete homology except for a possible paracentric inversion on the long arm of chromosome 5 [20]. While mitochondrial genetic analysis indicates that cynomolgus monkeys diverged from rhesus and other macaques about 1.5-3.0 million years ago [21], whole-genome sequence analysis indicates introduction of Chinese rhesus DNA, likely through immigration and cross-breeding of male Chinese rhesus

VetBooks.ir

TABLE 8.1 Comparative Genetics of Nonhuman Primates Used in Toxicology Research

	Callithrix jacchus	*Macaca fascicularis*	*Macaca mulatta*	*Homo sapiens*
Diploid chromosome number [8]	46	42	42	46
Average sequence identity to human [9]	NF	93%	93%	100%
Total genome length [9–11, 260]	2.51 Gb	2.85 Gb	2.84-2.87 Gb	3.2 Gb
Estimated time from common ancestor with humans [12]	48 mya	23 mya	23 mya	—
Mitochondrial genome [13–15, 261]	16,499 bp	16,571 bp	16,561 bp	16,569 bp

Numbers in parentheses refer to the data sources, which are listed in the chapter's reference list.
mya, million years ago; bp, base pairs; Gb, gigabases; NF, not found.

TABLE 8.2 Chromosomal Homology Between *Macaca mulatta* and *Homo sapiens*

Rhesus chromosome number	Homologous human chromosome number
1	1
2	3
3	7/21
4	6
5	4
6	5
7	14/15
8	8
9	10
10	20/22
11	12
12	2q
13	2p
14	11
15	9
16	17
17	13
18	18
19	19
20	16

Data source: Ref. [17]

VetBooks.ir

TABLE 8.3 Chromosomal Homology Between *Callithrix jacchus* and *Homo sapiens*

Marmoset chromosome number	Homologous human chromosome number
1	13/9/22
2	5
3	4
4	6
5	17/20
6	2/15
7	1/10
8	7
9	12
10	14/15
11	11
12	10/16
13	8/18/13
14	2
15	3
16	8
17	3
18	1
19	1
20	16
21	21
22	19

Data source: Ref. [18]

into cynomolgus colonies. This process is credited with introducing additional diversity into the cynomolgus genome [10]. Indeed, experimental production of rhesus/cynomolgus hybrids results in viable offspring [22]. The degree of isolation and potential cross-breeding of cynomolgus colonies with rhesus monkeys is believed to explain differences in the genetic diversity of this species based on geographic location [23]. The mitochondrial genome has been sequenced and reported for rhesus [13] and cynomolgus monkeys [14] and the common marmoset [261]. The mechanisms for genetic change seem to be somewhat different between Old World monkeys and apes. Retrotransposons, which are elements derived from ancient retroviruses, are able to insert into DNA from RNA intermediates and create a mechanism for rapid gene duplication and expansion of genome size [24]. Repeat elements account for about half of the genome of primates and other mammals [25]. This mechanism for the creation of

VetBooks.ir

genetic diversity seems to have been particularly active in macaques compared with great apes, in which nonallelic homologous recombination has played a greater role in evolution [26]. Because of the piecemeal approach to sequencing, the high rate of natural sequence duplication, and errors in sequencing methods, a complete and accurate understanding of a genome requires refinement, which sometimes proceeds long after its initial publication as a draft [27]. Nonetheless, it is clear that an understanding of the genetic differences between nonhuman primates and humans can better characterize model systems for drug target function and metabolism and could possibly lead to better selection of individual animals before testing [28]. Identification of genes with evolutionarily modified function between monkey models and humans will also help clarify the translational relevance of transcriptomic data, which is increasingly used to predict toxicity and investigate molecular mechanisms of toxicity.

One subject of note with regard to marmosets is the high incidence of microchimerism present in animals due to the high incidence of twinning with placental vascular anastomoses [29]. Microchimerism is the presence of a population of cells within a tissue, such as the blood, that originates from a different individual; these cells are able to renew and sustain themselves for an extended period of time. Demonstration of chimerism is most easily performed by detecting Y chromosomes by polymerase chain reaction methodology in females who are chimeric for male twin hematopoietic stem cells. Quantitative investigation of the extent of chimerism in marmosets and tamarins across multiple tissues was consistent with hematopoietic stem cells as the predominant and possibly sole source of chimerism in these species [30]. Approximately 10% of blood DNA sequences originate from chimeric co-twin origin [260]. Some reports have suggested that chimerism may contribute to the pathogenesis of autoimmune or other diseases and has a role in transplant rejection [31]; however, further work is needed.

COMPARATIVE GROWTH AND DEVELOPMENT

Oocyte to Parturition

Table 8.4 compares selected developmental milestones between the marmoset, macaques, and humans. A number of sources can be referenced for further reading on the reproductive biology of nonhuman primates, including Chapter 28 within this volume, as well as other reviews and books [35,40,46,49,55,63–66].

Hormonal Control of Gonadotropin Release

The neurohormonal control of the ovarian cycle in macaques is very similar to that of humans. Neurons in the mediobasal hypothalamus and arcuate nucleus synthesize and release gonadotropin-releasing hormone (GnRH) in a frequency- and amplitude-coded manner; this GnRH is delivered through the pituitary portal circulation to trigger release of two gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in macaques and humans, from the anterior pituitary. GnRH is a decapeptide that exists as two forms, GnRH-I and GnRH-II, which are expressed in discrete neuronal populations within the brain. Both seem to be capable of stimulating gonadotropic hormone release through the GnRH receptor-1 [67]. It has been hypothesized that the GnRH-I neuron population is

TABLE 8.4 Developmental Milestones

	Callithrix jacchus	*Macaca fascicularis*	*Macaca mulatta*	*Homo sapiens*
Embryos/term (n)	1 (5%), 2 (49%), ≥3 (46%) [35] (litters are smaller in the wild)	1	1 (≥99%), 2 (0.34%) [36]	1 (96%), 2 (3.3%), ≥3 (0.12%) [37]
Implantation (gestational day)	12 [38]	9 [39]	9 [40]	7.5 [40]
Placental blood supply established (gestational day)	45 [41]	12 [39]	11 [40]	9 [40]
Birth weight (g)	27 [42]	345 [42]	475 [42]	2500-4000 [37]
Placental mass at term (g)	4.87 [38]	150 [39]	NF	513±102 [43]
Embryonic development equivalent to human 50-day embryo (gestational day)	80 [38]	NF	45 [38]	50 [38]
Initiation of ossification (gestational day)	80 [35], 106[44]	NF	56-60 [44]	~63 [45]
Birth (gestational day)	144 [35]	155-165 [42]	165-178 [42]	280 [39]
Newborn solid food consumption	28 days [42]	28 days [42]	46-91 days [42]	0.5 year
Weaning	70 days [35]	375 days [46]	279 days [46]	3 years [47]
Eruption of deciduous teeth (start to completion) [48]	Before birth to 38 days	14-198 days	16-160 days	230 days-2.3 years
Eruption of permanent teeth (start to completion) [48]	113-321 days	1.4-5.8 years	1.4-6.0 years	6.2-20.5 years
Onset of puberty	M: 213 days [49] F: 167 days [49]	M: 3-4 years [50] F: 2-3 years [51]	M: 2.7 years [52] F: 1.7-2.5 years [53]	M: 14 years [54] F: 13 years [54]
Onset of pubertal growth spurt (years)	1 [49]	NF	M: 3.7 [54] F: 3.0 [54]	M: 10.9 [54] F: 9.3 [54]
Menarche (years)	—	2 [55]	2 [56]	13.7 [56]
Ovarian cycle length (days)	28.7±6.3 [55]	28-32 [42]	26-30 [42]	21-35

TABLE 8.4 Developmental Milestones—Cont'd

	Callithrix jacchus	*Macaca fascicularis*	*Macaca mulatta*	*Homo sapiens*
Sexual maturity (years)	M: 1.25 [49] F: 1.25 [49]	M: 4.23 [46] F: 3.39 [46]	M: 5.50 [46] F: 3.37 [46]	15 [47]
Age at first parturition (years)	1.6 [49]	3.9	3.75	19 [47]
Age at last physeal closure (years)	1.79 [57] (proximal humerus is the last to close)	M: 6.5 [58] F: 5.75 [58] (distal ulna is the last to close)	6.4 [59] (for most physes, females fuse 4-6 months earlier than males)	23 [60]
Adult body weight	~350 g [61]	M: 4-8 kg [42] F: 2-6 kg [42]	M: 6-11 kg [42] F: 4-9 kg [42]	M: 73 kg [62] F: 60 kg [62]
Lifespan (years)	15 [32]	30	30 [33]	M: 69 [34] F: 75 [34]

Numbers in parentheses refer to the data sources, which are listed in the chapter's reference list.
F, female; M, male; NF, not found.

responsible for pulsatile release of FSH and LH throughout most of the ovarian cycle, whereas GnRH-II neurons are responsible for the LH surge at ovulation [67].

In marmosets, a second GnRH receptor, the GnRH-II receptor, exists and seems to respond specifically to the GnRH-II ligand. Activation of the GnRH-II receptor seems to stimulate sexual behavior [67]. The GnRH-II receptor is not present in humans or other Old World monkeys.

The action of GnRH hormones on the pituitary results in the pulsatile release of two gonadotropic hormones. Humans and Old World monkeys respond to GnRH receptor activation by the release of FSH and LH. These two peptide hormones then act on their receptors (FSH-R and LH-R, respectively) in multiple cell types to coordinate reproductive tissue functions. Here again, a difference in the neotropical primates can be found. Marmosets and other New World primates have a deletion of the LH-R in exon 10, which renders the receptor insensitive to LH while it remains sensitive to chorionic gonadotropin (CG). As an apparent compensatory mechanism, these animals express CG from the pituitary (as well as the placenta), and therefore seemed to have substituted CG for LH compared to humans [49,55]. The control of CG release by GnRH is not entirely homologous to that of LH release, and it has been proposed that the inhibition of reproduction in nondominant, group-housed female marmosets may be mediated by separate neural control of CG release [68].

Molecular feedback from the pituitary gland and gonads regulates the release of GnRH by acting on two distinct populations of neurons upstream from the GnRH-containing neurons [67]. In both cases, Kisspeptin (Kiss1) acts as a peptide neurotransmitter to stimulate the release of GnRH from GnRH-containing neurons. First, neurons in the anteroventral periventricular nucleus are sensitive to steroids in the female and may mediate positive feedback on GnRH secretion (increased Kiss1 release). Second, neurons in the arcuate nucleus are sensitive to sex steroids in all species and mediate negative feedback on Kiss1 release and,

VetBooks.ir

subsequently, GnRH secretion. Both of these neuronal populations, as well as neurons in the periventricular nucleus and anterodorsal preoptic nucleus, express Kiss1, which acts through its receptor (GPR54) to stimulate GnRH release from GnRH-containing neurons [69,70]. Kiss1 is also expressed in the placenta, testes, pancreas, liver, and intestine, and its expression is modulated by leptins and ghrelin, which may serve as the link between sensing body composition and the onset and maintenance of reproductive cycles [70]. Estrogen may also have more immediate effects on ion channels that affect the excitability and activity of GnRH neurons [71], and a number of neurotransmitter systems have been implicated in the control of GnRH release [72].

The Ovarian Cycle

The ovarian cycle in cynomolgus macaques is reported to be 30.4±4.7 days (mean±standard deviation) in duration [55] and is composed of three principal stages: a 12- to 14-day follicular phase, a 3-day periovulatory phase, and a 14- to 16-day luteal phase (unless pregnancy is established after ovulation). The marmoset ovarian cycle is shorter, with a follicular phase approximately 8 days and a luteal phase around 20 days, for a total cycle duration of approximately 28.7 days [55]. During the follicular phase, estrogen feedback on the hypothalamus causes suppression of the amplitude of GnRH release, which subsequently suppresses FSH and LH release. Then, at the periovulatory phase, estrogen feedback switches to an opposite, positive effect and induces increased FSH and LH release, creating a positive feedback loop of increased estrogen and further increases in FSH and LH, driving ovulation. This feedback is thought to occur at both the hypothalamic and pituitary levels. The marmoset seems to always ovulate at least two oocytes, although fetal death may result in singleton pregnancies [73]. The marmoset has multiple ovulations, but these are not necessarily synchronous [73]. Following ovulation, estrogen decreases rapidly while progesterone increases. At this stage, the frequency, but not amplitude, of FSH and LH release is suppressed at the level of both the hypothalamus and pituitary [74]. Two peptide hormones, inhibin A and inhibin B, have distinct profiles during the ovarian cycle, with inhibin B concentrations high in the early follicular phase and inhibin A concentrations highest during the luteal phase [75]. As progesterone levels decrease, with the involution of the corpus luteum, the ovary returns to the follicular phase.

Luteolysis precedes menstruation by 2-4 days. Following luteolysis, a wave of follicular development resumes. Granulosa cells are fundamental to the control and progression of follicular development, both pioneering differentiation of the theca layer [55] and initiating atresia through familiar apoptotic mechanisms [76]. As granulosa cells surrounding primordial follicles enlarge under the influence of FSH release, they stimulate differentiation of surrounding endometrial stromal cells to become the thecal layer [77]. A number of factors have been implicated in this process: insulin-like growth factor (IGF)-1, stem cell factor, basic fibroblast growth factor, transforming growth factor (TGF)-β family members (including activin and inhibin, bone morphogenetic proteins, growth differentiation factor 9, TGF-β1, and TGF-β2), and the hedgehog pathway [77], although some species differences are seen; the function of these mediators remains to be defined specifically for primates. As thecal cells differentiate, they become responsive to LH, which induces steroidogenic enzymes and transporters (steroidogenic acute regulatory protein, which transports cholesterol esters into the mitochondria; cytochrome P450 11A, localized in mitochondria; cytochrome P450 17, a pregnenolone

VetBooks.ir

17 alpha-hydroxylase; and 3-β-hydroxysteroid dehydrogenase), enabling the production of dehydroepiandrosterone (DHEA) and testosterone. These androgens then are converted to estradiol in the granulosa cell by P450 aromatase. The effects of endocrine disruptors, often of environmental toxicity concern, may alter follicular maturation [78]. Vascular endothelial growth factor is produced by granulosa and theca cells to stimulate vascularization of the developing follicle and is necessary for corpus luteum development. Inhibitors of vascular endothelial growth factor signaling result in impaired corpora lutea development in the cynomolgus macaque [79] and prevent pregnancy in marmosets [80]. Immediately leading up to ovulation, a shift from estrogen and androgen production to progesterone occurs within the follicular wall; this prevents atresia and promotes oocyte maturation [81]. Other factors, including lipids, catecholamines, and vasoactive factors, interact to cause follicular rupture and release of the oocyte and may be unintended targets in toxicity. In rhesus monkeys, for example, indomethacin inhibits oocyte release apparently through the inhibition of prostaglandin production [82]. Within a few hours of follicular rupture, the granulosa cells undergo luteinization and produce large amounts of progesterone, an event that is LH dependent [83]. The prevention of other sites of follicular development during the luteal phase may be due to altered FSH activity [84] and inhibin A production [85].

In the absence of pregnancy, the corpora lutea produces progesterone, estrogens, inhibin A, relaxin, and oxytocin for a period of 14-16 days before regressing. Compared with humans and macaques, New World primates have much higher concentrations of estrogen and progesterone during this phase of the cycle [72]. Fertilization is presumed to occur within the oviduct [86]. Of the millions of sperm introduced into the female reproductive tract at copulation, one estimate in rhesus monkeys was that fewer than 50 sperm reach the oviduct where fertilization occurs [87]. Hendrickx and Sawyer [86] reviewed embryology in the rhesus with comparisons to humans and baboons, including photomicrographs of the embryo from fertilization to 20 days. A more contemporary review described some of the molecular interactions at implantation [88]. Implantation stages of the marmoset have been described by Moore and colleagues [89].

Placentation

Placentation of *M. fascicularis* has been extensively studied as a model for human placentation, and a review of macaque placentation has been published [65]. In macaques, the villous structure, interhemal barrier, and circulation within the intervillous space resemble those of the human [64]. The macaque blastocyst invades less deeply into the endometrium than it does in humans, and the exposed blastocyst interacts with the opposing endometrium in the formation of the bidiscoid placenta of the macaque, in contrast to the discoid placenta of the human [90]. The macaque placenta forms a lacunar stage before the formation of villous anchoring and maternal blood communication [65], unlike the marmoset, in which the placenta initially forms a trabecular attachment and lacks the lacunar stage [91]. The marmoset placenta is relatively late in the formation of blood-filled spaces relative to other species [41]. Notable differences between humans and macaques include a more sharply demarcated trophoblastic shell in the macaque, the paucity of interstitial trophoblast invasion in the macaque, earlier invasion of the spiral arteries and establishment of maternal placental circulation in the macaque, and differences in the decidual response between humans and macaques [65].

VetBooks.ir

Maternofetal placental transfer of immunoglobulins (Igs) during pregnancy is an important consideration for fetal exposure to biologics. In *M. fascicularis*, fetal concentrations of 34 Fc-containing biopharmaceuticals were reported [92]. In *M. fascicularis*, maternal antibody administration was associated with higher fetal concentrations after gestation day 100, compared with dosing before gestation day 50, but fetal concentrations remained below maternal concentrations for 30 of 34 biologics administered. Monkey transfer of IgG1 seemed to be more efficient than with other isotypes, although maternal concentrations of IgG2 or IgG4 biologics were generally higher [92]. Human fetal IgG concentrations show a smooth linear increase beginning as early as 13 weeks of gestation, exceeding maternal concentrations by term. Human fetal concentrations of IgG1 and IgG4 both exceed maternal concentrations in the newborn [93]. Neither human nor macaque Igs substantially enter the fetal circulation by absorption of milk, although IgA antibodies in milk are important for the protection of infants from enteric disease [94]. In contrast, New World monkeys are reported to have <50% of adult concentrations of IgG at birth [95].

Hormonal Maintenance of Pregnancy

In primates, pituitary LH secretion decreases in the absence of pregnancy, and the loss of this trophic factor leads to luteolysis. CG mimics pituitary LH and ensures the continued function of the ovarian corpus luteum to maintain pregnancy [96]. Maternal production of LH and FSH are negligible during gestation [97]. In marmosets, CG concentrations are detectable in maternal blood and urine by gestation day 17 [49]. The placenta also contributes to progesterone production to maintain pregnancy. Whereas in the follicle theca cells produce androgens, which are converted to estrogens by granulosa cells, in the fetus the fetal adrenal gland produces DHEA-sulfate, which is converted within the syncytiotrophoblast into estradiol. It is the fetal zone of the adrenal that produces these androgens, and it occupies the inner four-fifths of the cortex. Estradiol acts through the estrogen receptor expressed in the placenta to induce the functional differentiation of syncytiotrophoblast and, in particular, the expression of $P450_{scc}$, which is necessary for the production of progesterone from cholesterol. In midgestation, maternal cortisol suppresses development of the fetal adrenal cortex though the inhibition of adrenocorticotropic hormone release from the pituitary. During later gestation, estrogen-driven increases in 11β-hydroxysteroid dehydrogenase production within the placenta convert an increasing proportion of cortisol to its less active metabolite cortisone, which permits maturation of the fetal adrenal gland and the fetal production of cortisol, which is necessary for maturation of the lung [72].

Fetal Growth

As a general rule across species, fetal growth is regulated by the placenta. The placenta senses oxygen tension and nutrition levels and regulates fetal growth through the release of IGFs and IGF binding proteins, which have opposing effects on fetal growth [98]. An extensive array of transporter proteins function in the placenta to support growth [99]. Physical growth of macaques and marmosets have been reported previously and are presented in Figure 8.1 for comparative purposes [38,42,46,100,103,104]. Unique to marmosets and tamarins, anastomoses normally develop in fetal placental circulation between multiple fetuses by gestation day 30, with the chorionic cavity fusing between days 19 and 29 [89]. It is notable that, in the marmoset, fetal growth proceeds slowly up to about midgestation, when

VetBooks.ir

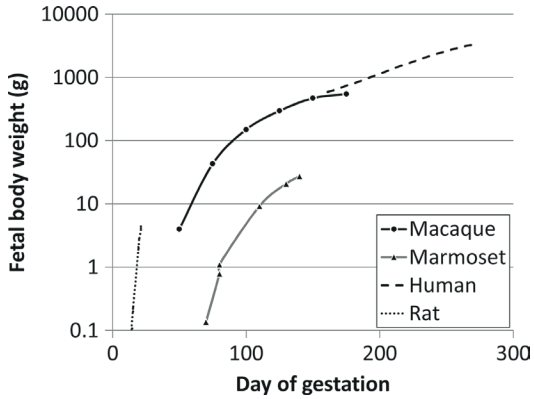


FIGURE 8.1 Fetal growth curves of the rat, common marmoset, rhesus monkey, and human. The early delay in marmoset fetal growth is evident as the right shift compared with other primates. *Data from Refs [38,100–102].*

it resumes at a pace matching other primate species [38]. This is evident in Figure 8.1 as the gap between parallel growth curves for marmosets and other species. Body weight, arterial oxygen saturation, blood glucose, blood lactate, and arterial blood pressure were measured in fetal monkeys from gestation days 115 to 158. Arterial oxygen saturation was between 51% and 77%, glucose concentrations averaged 29 mg/dL (range, 20-44 mg/dL), and blood pressure gradually rose from about 35 mmHg at gestation day 115 to 50 mmHg at gestation day 158 [103]. Marmosets are distinct from other primates, except perhaps tamarins, in their ability to resorb fetal deaths in late pregnancy without stillbirth and also in the incidence of late fetal death. In the second half of gestation, 20-30% mortality was reported in fetal marmosets that were monitored longitudinally by ultrasound, whereas rate of human fetal deaths in late gestation is only 10% [105].

Neonatal and Juvenile Growth

Physiological Parameters

Dawes and colleagues [103] report a number of physiological parameters for the newborn rhesus macaque. The neonatal rhesus monkey has an arterial oxygen saturation of approximately 94% and blood glucose concentration of 49 mg/dL, which are both increased relative to the fetal state (approximately 58% and 29 mg/dL, respectively). The oxygen carrying capacity of the neonate, 15-20 mL oxygen/100 mL blood, does not different significantly from late gestation to adulthood. Oxygen consumption is dependent on temperature, with the thermoneutral (least energy expenditure) point at approximately 35 °C. Oxygen consumption in the neonatal rhesus macaque is approximately 14.8 mL oxygen/kg/min. The mean arterial blood pressure at birth is 55 mmHg. The mean body surface area at birth was directly measured to be 0.122 m^2/kg. In contrast, an adult rhesus weighing 5.56 kg has a measured body surface are of 0.275 m^2, or 0.049 m^2/kg. Neonatal heart rate is 200-250 beats/min. Normally, no murmur is heard on auscultation, indicating that closure of the ductus arteriosus has occurred. A comparison of infant rhesus macaque metabolism using nuclear magnetic resonance metabolomic analysis of serum and urine found that 65% of urine metabolite concentrations and 40% of serum metabolite concentrations were similar between the rhesus macaques and humans, supporting the general validity of the rhesus macaque as a model of human infancy [106].

VetBooks.ir

Hormonal Changes in Infancy

As discussed above, during pregnancy the fetal zone of the adrenal cortex provides androgens that are converted by the placenta to produce estrogen. After birth, the fetal zone of the adrenal undergoes involution. Compared with humans, where involution occurs rapidly within the first two postnatal weeks [107], macaques have a more gradual postnatal involution of the fetal zone, occurring over 6 months [108]. This is accompanied by a period of adrenal activation in the infant with increase release of DHEA, DHEA-sulfate, and androstenedione to about 3.5 months while the zona reticularis develops [109]. Involution of the infant marmoset adrenal fetal zone resembles that of the human in timing, but its functional replacement, the zona reticularis, does not form [110]. As the fetal zone regresses the infant marmoset develops high concentrations of cortisol (1900 µg/dL) at 1 week of age, which decline sharply by 2 months of age to <200 µg/dL and then drift lower to reach adult concentrations of 150 µg/dL around 6 months of age [111].

Growth

The rate of growth following birth decreases sharply with age in primates [56,112]. At birth, rhesus macaques have a sitting height of approximately 195 mm and typically weigh 475 g [42,56]. Cynomolgus monkeys have a birth weight of approximately 345 g and marmosets of 27 g [42]. Gavan and Swindler [56] compared rhesus growth with chimpanzees and chimpanzees with humans using a logarithmic transformation of sitting height growth rate. They found that the initial newborn macaque growth rate of $e^{1.263}$ (3.5-fold change per 3-month period) was modestly higher than the chimpanzee at $e^{0.968}$ (2.63-fold change per 3-month period). Both species undergo rapid reductions in growth rate, with the macaque maintaining the higher growth rate of the two until about 5 years of age, when growth rates equalize. The chimpanzee continues growing for a longer period of time than the macaque, and the human growth period outlasts the chimpanzee. Thus, the larger primates achieve their larger size by growing somewhat slower, but for a longer period of time, relative to smaller primates [112]. For infant rhesus monkeys born to normally fed mothers, size at birth was an indicator of future growth rate relative to the population mean; smaller infants remained on the low side of the population growth curve [113].

Lactation and Early Nutrition

Infant weight gain is dependent on the quantity of nutrition received from the milk of the dam [113]. Primate milk is generally considered to have dilute energy content compared with that of other mammals. An evaluation of milk from 114 different rhesus macaques found an energy density of about 1.0 kcal/g [114], which is similar to the energy density of 0.76 kcal/g reported in marmosets [115]. Primiparous rhesus dams produced a smaller volume of milk than multiparous dams [114], but weaning size and age seem to be mostly dependent on total milk energy consumed [116]. Milk also plays an important role in establishing a healthy gut flora. Currently, it is believed that macrophages carrying a select set of healthy gut flora migrate from the intestine into the milk through the mammary gland for this purpose. Evaluation of aerobic bacteria species present in rhesus milk found strains from five genera: *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, and *Bacillus* [117]. The first three of these are also represented in milk from human mothers. Lactation represents the greatest energy expenditure the mother makes in the development of her offspring [118]. Solid food consumption begins

VetBooks.ir

around 28 days in marmosets and cynomolgus monkeys and around 46 days in rhesus macaques [42]. Primates all require vitamin C because of the absence of L-gulonolactone oxidase activity. Vitamin D is only a dietary requirement in primates when access to ultraviolet (UV) B light is limited. Vitamin D3 is synthesized in light-exposed skin from 7-hydrocholesterol to provitamin D3 and then vitamin D3. Vitamin D3 is converted enzymatically to its most active form, 1,25-dihydroxy vitamin D3 (calcitriol). When access to UVB is limited, dietary supplementation is necessary. Two forms of vitamin D, ergocalciferol (vitamin D_2, plant origin) and cholecalciferol (vitamin D_3, animal form), are available. New World monkeys utilize vitamin D_2 poorly and therefore require either UVB exposure or vitamin D_3 supplementation to avoid metabolic bone disease [119].

Sexual Maturation

The exact definition of the characteristics defining infancy, puberty, and adulthood for primates vary within the literature. Furthermore, these stages can vary significantly between individuals of the same species, such as in rhesus macaques, where hormonal evidence of the onset puberty varied from 2.5 to 5.5 years of age [120]. Therefore, printed values of maturation milestones must be regarded as guidelines and can vary considerably between sources. See Table 8.5 for an example of developmental staging by age for marmosets, macaques, and humans. In a population of cynomolgus macaques from Indonesia, menarche (the first bleeding of the menstrual cycle) began at 1.75 years of age, with the latest monkeys cycling by 4 years of age and 50% of the population achieving cycling by about 2.38 years [122]. In the rhesus macaque, a mean age at menarche of 2.25 ± 0.28 years was described [54]. The sexual maturation of rhesus and cynomolgus macaques are chronologically similar, although the rhesus breeds only seasonally, between October and February in the northern hemisphere [121].

In the newborn male macaque, FSH concentrations at birth are generally low (<400 pg/mL) but then quickly jump over the following 2 weeks to concentrations between 500 and 1000 pg/mL. FSH concentrations hold at this level for about 4 weeks before declining from about 1.5 to 8 months of age [123]. In male human infants, FSH concentrations also increase,

TABLE 8.5 Developmental Stages and Approximate Ages

	Callithrix jacchus [49]	Macaques [121]	*Homo sapiens* [47]
Infancy	0-3 months	0-1.5 years	0-3 years
Juvenile	3-12 months	1.5-3.5 years	3-7 years
Subadult (pubertal period)	13-18 months	Male: 3.5-5.5 years Female: 3.5-4.5 years	7-10 years (child) 10-18 years (adolescent)
Adult	>18 months	4.5-5.5 years	> 18 years
Fully mature adult	—	>5.5 years	—

Sexual maturity in males can be defined as the appearance of spermatozoa in ejaculate, whereas in females the onset of menstruation is often the criterion used for sexual maturity for the purposes of toxicology.
Numbers in parentheses refer to the data sources, which are listed in the chapter's reference list.

VetBooks.ir

peaking around 2 weeks and trailing off by 3 months [97]. In the newborn female macaque, FSH concentrations increase during the first month and remain elevated until 3 months of age, when they decrease to undetectable concentrations by 7 months of age [124]. In female human infants, FSH concentrations rise through the first month postpartum and are sustained for a period of time before falling to detectable steady concentrations from 3 to at least 5 months of age [97]. Although FSH concentrations have been reported in the adult female marmoset, description of neonatal FSH concentrations is lacking. LH concentrations increase more dramatically in male infants than in females for both humans and castrated macaques, but otherwise they show the same general trend as FSH [97,109].

A burst in adrenal androgen production occurs in humans around the time of puberty, which is termed *adrenarche*. Adrenal androgens result in the development of some characteristics of adolescence such as the growth of axillary and pubic hair and acne. Adrenarche starts around 6-7 years of age in humans, with adrenal androgen production peaking in the 20s and slowly declining thereafter. Regulation of adrenarche seems to be independent of the GnRH-pituitary-gonadal axis [72,125]. Adrenarche seems unique to humans and apes and is not observed in macaques or marmosets [126,127].

Gonadarche is the "reawakening" of the reproductive system after a period of very low gonadotropin concentrations through the juvenile period and initiates puberty in the macaques and marmoset. Control of the resumption of GnRH release is clearly tied to body condition, although not body size [128]. This has led the "somatometer" hypothesis, which predicts one or more factors that sense body condition and report this to the hypothalamus. Among candidate factors are leptin and IGF-1, which are increased in macaques eating a high-calorie diet and are associated with precocious puberty [128,129]. In the rhesus, neural control of GnRH release is clearly dependent on signaling by the neuropeptide Kiss1 [130]. Complex interactions of steroid hormones, IGF-1, and seasonal melatonin concentrations are integrated in the brain to trigger GnRH release, but much remains to be understood. In female rhesus macaques, starting at 20-30 months, puberty progress through an initial activation of LH cycling, characterized initially by nocturnal increases in the pulsatile release of LH, which leads to menarche, the initiation of menstruation. In the following 20 months, LH concentrations continue to increase and retain their circadian rhythm. Finally, at 50-60 months of age, ovulation occurs and LH concentrations decline and become evenly distributed throughout the 24-hour period [53]. Starting during puberty and maturing into adulthood, the uterus responds to the cyclical production of ovarian steroid hormones. Estrogen induces expression of both the nuclear receptors for estrogen and progesterone. Immediately following menstruation, exposure to estrogen upregulates its own receptor throughout the glands and stromal cells, leading to the growth of the endometrium. Following ovulation, the ovary increases progesterone production and estrogen decreases quickly. When progesterone concentrations exceed 1 ng/mL, the endometrium steadily loses estrogen and progesterone receptor expression. This effectively decreases sensitivity to the trophic effects of progesterone, and if pregnancy is not established, waning concentrations of progesterone lead to sloughing of the endometrium, that is, menstruation [63].

In infant male rhesus macaques, LH concentrations increase, with lesser increases in FSH, which stimulate testicular interstitial cell proliferation and induce their capacity to produce testosterone. Following 4 days of exposure to LH, the gonad responds to LH with a pulsatile release of testosterone [125]. When the infant pulse of adrenal androgens, gonadotropins, and

VetBooks.ir

testosterone occurs, the Sertoli and testicular interstitial cells proliferate fourfold and sixfold, respectively. During the intervening juvenile period, when gonadotropins are lacking, Sertoli cells remain mainly static while undifferentiated spermatogonia continue to proliferate and the testosterone-producing interstitial cells slightly regress in number. Spermatogonial cell numbers increase approximately 1000-fold from birth to adulthood. Finally, with the pubertal surge in LH and FSH, Sertoli and interstitial cells proliferate to achieve final numbers of 2,000 million Sertoli cells and 100 million interstitial cells per testis [125]. Under the influence of LH, interstitial cells produce testosterone, which induces spermatogonia to initiate the spermatogenic cycle. In adult male macaques, the germ cells form 4 nested layers, which are morphologically classified into 12 stages. The spermatogenic cycle, which is the time to transit all 12 stages, is 10.5 days. This is the time period to advance one layer apically into the spermatogenic tubule. The total time required for a spermatogonial cell to become a mature sperm cell is 42 days. For a review of the morphology of spermatogenesis, see the work by Dreef et al. [131].

From a toxicologic perspective, toxicities with nuclear receptor agonists such as phytoestrogens and polychlorinated biphenyls are implicated in the abnormal timing of puberty [72]. Around the time of puberty in some primate species, a growth spurt occurs. In male rhesus monkeys, the growth spurt occurs following the maturation of the testes and increased testosterone production, with the implication that androgens are partly responsible for the growth [54]. Conversely, in the female rhesus, onset of menstruation precedes the growth spurt by approximately 1.5 years and occurs when the monkey has achieved only 30% of adult size. In humans, menarche occurs after reaching about 70% of adult size [54]. In the marmoset, a corresponding growth spurt has been proposed to occur around 12-14 months of age [49].

For the purpose of toxicological assessments of experimental compounds on reproductive function, sexual maturity in males should be considered the presence of sperm in the ejaculate (not testosterone concentrations, testicular size, body weight, or age). The presence of menstrual bleeding in female macaques is considered sufficient evidence of sexual maturity, although early ovarian cycles may be irregular and not ovulatory. In the female marmoset, menstruation does not occur and sexual maturity would be defined by the occurrence of ovulation, which has no reliable outward indicators but can be monitored by urinary pregnandiol-3-glucuronide concentrations [132]. Marmosets at least 18 months old are generally considered sexually mature [6,7], although social housing conditions can result in significantly delayed reproductive activity [133]. A review of nonhuman primates in developmental and reproductive toxicity is available in Chapter 28 and elsewhere [134].

Skeletal and Dental Maturation

An extensive comparative review of primate growth, including body weight, height, physical measurements, ossification center appearance, physeal plate closure, cranial capacity, exceptional drawings of skull, foot, and hand morphology, and dental eruption timing for 7 to 35 species, including macaques and humans but limited data for marmosets, was compiled by Schultz [135]. Dental formulae are provided in Table 8.6. A contemporary review of deciduous and permanent dental eruption timing and patterns in 33 species of primates was complied by Smith and colleagues [48]. Early interest was originally partly to enable the age determination of wild specimens but continues to have utility in comparative evaluation of

TABLE 8.6 Skeleton and Dentition

	Callithrix	*Macaca*	*H. sapiens*
Vertebrae [136]			
Cervical	7	7	7
Thoracic	13 (12–13)	12 (12–13)	12 (11–13)
Lumbar	6 (5–7)	7 (6–8)	5 (4–6)
Sacral	3 (2–3)	3 (2–5)	5 (4–7)
Caudal	27 (25–30)	17 (5–28)	4 (2–5)
Decidual dentition [48]			
Upper arcade	$i^1\ i^2\ c\ p^2\ p^3\ p^4$	$i^1\ i^2\ c\ p^3\ p^4$	$i^1\ i^2\ c\ p^3\ p^4$
Lower arcade	$i_1\ i_2\ c\ p_2\ p_3\ p_4$	$i_1\ i_2\ c\ p_3\ p_4$	$i_1\ i_2\ c\ p_3\ p_4$
Permanent dentition [48]			
Upper arcade	$I^1\ I^2\ C\ P^2\ P^3\ P^4\ M^1\ M^2$	$I^1\ I^2\ C\ P^3\ P^4\ M^1\ M^2\ M^3$	$I^1\ I^2\ C\ P^3\ P^4\ M^1\ M^2\ M^3$
Lower arcade	$I_1\ I_2\ C\ P_2\ P_3\ P_4\ M_1\ M_2$	$I_1\ I_2\ C\ P_3\ P_4\ M_1\ M_2\ M_3$	$I_1\ I_2\ C\ P_3\ P_4\ M_1\ M_2\ M_3$

Vertebral numbers given as mode and range of observed. Number of cervical, thoracic, lumbar, and sacral vertebrae combined is almost always 29. Numbers in square parentheses refer to the data sources, which are listed in the chapter bibliography.

effects of compounds on bone or tooth growth. Epiphyseal union schedules are described for the rhesus [59], cynomolgus [58], and marmoset [44,57,137]. For comparison of physeal closure across 10 different species, see the work by Kilborn et al. [138]. Additional references on dentition are also available for rhesus macaques [59] and marmosets [139]. Physical measurement data for marmosets and excellent photography of the skeletal elements can be found in the work by Casteleyn et al. [140]. An extensive compendium of tables of growth and physiological data are also available for the rhesus macaque [141]. Table 8.6 lists the segmentation of vertebrae between cervical, thoracic, lumbar, and sacral compartments. Schultz [136] compiled tables of the frequency of variants in vertebral number.

The use of nonhuman primates in models of skeletal diseases such as osteoporosis has been recently reviewed [142]. Castrated marmosets serve as a model of osteoporosis, and decreases in bone mineral density are associated with approximately fivefold elevations in serum alkaline phosphatase activity [143], but middle-aged female marmosets did not show the same correlation. The bone mineral content as a percentage of bone weight in the marmoset was determined to evaluate its suitability as a model of bone mineral loss; for the femur this was found to be 0.506 (mg mineral/mg dry bone weight), which compared favorably with the human femur at 0.557 (mg/mg) [144]. Intracortical bone turnover in the midshaft femur, estimated by histomorphometry of bone microstructure, found that bone formation in the femur of macaques >4 years of age was about one-third of the bone formation in adult humans. The opposite was seen in the rib, where bone formation was higher in macaques than humans [145]. These findings may indicate that toxicities relating to bone turnover may be easier to detect in macaque ribs than femurs and that the location of bony lesions may differ between species.

COMPARATIVE CARDIOVASCULAR PHYSIOLOGY

VetBooks.ir

Heart and Anatomy

Selected cardiovascular parameters are given in Table 8.7 and electrocardiographic parameters in Table 8.8. Overall, the cardiovascular system of monkeys and humans is similar. A morphometric description of 28 female and 19 male rhesus monkey hearts ranging in age from prenatal stillbirth to >24 years was reported by Swindle and colleagues [172]. Measuring ratios of anatomic measurements, they found that the proportions of the heart remain remarkably similar, and the only sex difference was that the mitral/tricuspid annulus circumference ratio was greater in males than females. Morphometric study of the marmoset heart found heart weight as a proportion of body weight (0.51 ± 0.38%) consistent with that of other mammals, and there was no significant sex difference [173]. Anatomic study of the carotid vasculature and circle of Willis revealed only minor differences compared with humans, although these may have implications for models of cerebrovascular infarction [174]. An anatomic study of the conduction system of the rhesus has been reported [175], and immunohistochemical markers of autonomic nerve fibers and the conducting system of the adult cynomolgus heart have been described [176]. The comparative anatomy of the autonomic innervation of the primate heart compared with the human heart shows no difference in vagal innervation and minor craniad shifts in the sympathetic ganglia and a more definite thoracic cardiac nerve in apes [177,178]. In the neonatal rhesus monkey, heart rate is controlled by dominant sympathetic tone, whereas in the adult, parasympathetic tone controls the heart rate [179], with logical consequences for the effects of pharmacologic treatment that would alter the function of these two neurotransmitter systems.

Control of Contraction

The role of ion channels in excitation-contraction coupling and autonomic control of heart beat frequency has been recently reviewed [180]. Compared with humans, differences in the duration of the cardiomyocyte action potential, expression of myosin heavy chain subtypes,

TABLE 8.7 Selected Cardiovascular Physiology Parameters

	C. jacchus	*M. fascicularis*	*M. mulatta*	*H. sapiens*
Heart rate beats/min	192-242 [42] (telemetry)	115-243 [42] (telemetry)	98-122 [42] (telemetry)	63-93 [146]
Blood volume mL/kg	65 mL/kg [147]	55-75 mL/kg [42]	50-96 mL/kg [42]	65-73 mL/kg [62]
Mean arterial pressure	95 ± 9 mmHg [148]	120 ± 14 mmHg [149]	113 ± 13 mmHg [141]	63-93 mmHg [146]
Cardiac Output	25.9 ± 3.5 (SEM) mL/min/g [150]	0.375 L/min [151]	1.3 L/min [141]	5.9-6.5 L/min [62]
Stroke volume	0.093 ± 0.007 (SEM) mL/g [150]	2.9 mL [151]	7.1 mL [141]	102 mL [62,146]

Some values given as mean ± standard deviation. Data from [146] derive from young adult males only. Other human data based on 60 kg female or 73 kg male.
Numbers in parentheses refer to the data sources, which are listed in the chapter bibliography.

VetBooks.ir

TABLE 8.8 Electrocardiographic Parameters [152]

	C. jacchus ($n=6$)	*M. fascicularis* ($n=124$)	*M. fascicularis* ($n=100$)	*M. mulatta* ($n=35$)	*H. sapiens*
Beats/min	224 (206–245)	238±26.7	203±29	150±29	60-80
Duration P-wave (ms)	25 (21–29)	NF	30±10	40±1	120
Duration QRS complex (ms)	24 (20–29)	49±7	30±10	45±1	80
Duration T-wave (ms)	101 (91–112)	NF	NF	10±5	20
Duration PR interval (ms)	57 (57–62)	70±8	80±10	75±1	200
Duration QT interval (ms)	117 (88–156)	170±10	190±20	140-200	34
Heart axis (degrees)	NF	NF	+65±22	+50±100	+30-110
P-wave Amplitude (mV)	NF	0.21±0.05	0.16±0.05	0.005±0.005	0.1-0.3
QRS complex Amplitude (mV)	NF	0.36±0.28	0.7±0.3	1±0.01	1
T-wave Amplitude (mV)	NF	0.18±0.06	NF	0.15±0.01	0.02-0.06

Marmosets were anesthetized with pentobarbital (64 mg/kg), rhesus monkeys were anesthetized with ketamine (10 mg/kg) and in left lateral recumbency, cynomolgus monkeys were either manually restrained by a technician ($n=124$) or anesthetized with ketamine (10 mg/kg).
Abbreviations: NF, not found.
Data source: Ref. [152].

and expression of structural protein isoforms have been described in rodents [181], for example. These fundamental differences alter the function of the heart between species, presumably as an adaptation to unique physiological demands. The susceptibility of the heart to pharmacological or toxic effects on ion channels can vary across species, and nonhuman primates have sometimes been show to predict risk for arrhythmia better than dogs [182]. Conversely, while the autonomic control of cardiac function varies during development, in adults the influence of respiration on heart rate seems to be a common mechanism across a wide range of species [183].

Torsades de Pointes

The safety assessment of candidate drugs for adverse cardiovascular effects has been reviewed elsewhere [184]. The interaction of some small-molecule drugs with certain ion channels may predispose patients to life-threatening arrhythmia. One example of such an arrhythmia is a polymorphic ventricular tachycardia termed *torsades de pointes* (TdP), pronounced \tȯr-säd(z)-də-ˈpwant\. The name of the arrhythmia derives from the

VetBooks.ir

electrocardiographic trace, consisting of a series of ventricular contraction potentials with a sinusoidally varying amplitude, resembling a twisted strip of paper, like a party streamer. TdP is associated with a lengthening of the QT interval, and so precise measurement of drug effects on the QT interval has led to standardization of this measurement and calculation of a normalized QT interval, termed the corrected QT [185]. The need for standardization can be appreciated in that diurnal variability in marmosets has been recorded for corrected QT of healthy animals [186]. Using ideal electrode placements and measurements, QT effects of drugs can reliably be detected in dogs, cynomolgus monkeys, mini pigs, marmosets, guinea pigs, and humans [185]. The most frequently implicated ion channel in drug-induced QT prolongation is HERG (human ether-a-go-go-related gene), which conducts the rapid I_{Kr} current (delayed rectifier potassium current) [187,188]. A prototypical drug causing this effect is dofetilide, an atrial antiarrhythmic drug [189]. A specific case of TdP in a cynomolgus monkey has been published [190]. Mice and rats are not suitable models of these effects because of considerable divergence in the expression and function of the ion channels controlling cardiac excitation [191]. The dog has been the dominant in vivo model in cardiovascular safety evaluation in drug development, but nonhuman primates may be necessary when canine drug metabolism, binding activity in the case of biologics, or other differences invalidate the use of the dog.

Although uncertain, the mechanism by which HERG channel inhibition predisposes to TdP may be due to regional differences in the ion conductance properties of cardiomyocytes, particularly under the influence of adrenergic stimulation [192]. It has been proposed that cardiomyocytes in the middepth ventricular myocardium are particularly prone to prolongation of the myocardial action potential and that these cells might be responsible for triggering the ventricular premature beats that are characteristic of TdP. While present efforts in drug screening are focused on detection of QT prolongation, this hypothesis suggests that only those compounds that cause increased variability in depolarization properties between epicardial and midmyocardial muscle cells are at risk of initiating TdP [192]. A molecular basis for this proposed regional difference in electrical activity might lie in the spatially different expression of the gap junction protein connexin 43 [193]. Although there are differences in male and female myocardial responses to autonomic stimulation [192], they are replicated in the rhesus monkey [194].

Electrocardiography

Telemeterized electrocardiography for drug safety assessment may be coupled with respiratory physiology measurements [195] and automated blood sampling [196] and has improved the sensitivity of this risk assessment method. A study of the incidence of arrhythmia in chronically telemeterized cynomolgus monkeys most frequently observed daytime supraventricular premature contraction (in 10.7% of animals in 24 hours), escape beats (3.8%), sinus node pause (2.8%), and ventricular premature beats (VPB; 25.4%). At night, a significantly different profile is observed, with sinus node pause (32.6%), escape beats (18.5%), and VPB (11.7%) predominating [197]. By comparison, 39% of humans were reported to have at least one VPB in a 24-hour period [198], and a prospective study of arrhythmia was not predictive of future coronary artery events [199]. It was suggested that prestudy monitoring of individual animals could generate baseline data to control for individual animal variability in frequency of spontaneous

VetBooks.ir

arrhythmias [197]. A study of electrocardiographic values in chemically restrained black-tufted marmosets (*Callithrix penicillata*) reported normal parameter ranges which did not differ by sex [200]. A comparative study of electrocardiotopography in rhesus macaques found that they had a shorter activation time (61 ms) than humans (97 ms), which was only partly explained by the difference between the species' resting heart rates [194]. The implication is that rhesus myocardiocytes may be more rapidly excitable than the human myocardium. Otherwise, effects of age and sex on parameters paralleled those observed in humans.

Echocardiography

Echocardiography is a noninvasive, translatable method that allows functional characterization of the myocardium and is relatively inexpensive and easy to perform. Sleeper et al. [201] summarized two-dimensional and M-mode imaging parameters for 118 female and 199 male healthy cynomolgus monkeys under sedation. Tsusaki and colleagues [202] described the use of three-dimensional echocardiography to quantify end-diastolic volume, end-systolic volume, and ejection fraction in control monkeys, and Kito [203] described the application of this technology to safety assessment.

Peripheral Vasculature

Nonhuman primates have been used to model the progression of peripheral vascular disorders including hypertension and atherosclerosis (see Chapter 13 for an in-depth discussion of nonhuman primate models of disease). With age, arteries lose elasticity and become more rigid, with implications for pulse propagation (pulse wave velocity) and the ability of vessels to dilate in response to physiological demands for blood flow (flow-mediated dilatation). Schroeder and colleagues [204] reported their findings of the negative correlation of flow-mediated dilatation and positive correlation of pulse wave velocity with age in a cohort of 14 male and 7 female cynomolgus macaques. The mechanism for age-related increases in vascular stiffness is an area of active investigation. While initial evidence indicated matrix deposition may contribute, Qui and colleagues [205] found sex-specific and age-related regulation of gene expression in the monkey aorta and proposed a cellular contribution to changes in vascular compliance. Follow-up studies of cynomolgus aortas showed that a 200% increase in vascular stiffness with age was correlated with a 200% increase in the elastic modulus of vascular smooth muscle cells and further support the existence of a potentially reversible active regulation of vascular distensibility [206].

Comparative Cardiac Gene Expression

Similarities of gene expression patterns between species are further evidence for the translatability of findings between species. Zhao and colleagues [207] performed transcriptomic analysis of heart messenger RNA from humans, rhesus macaques, rats, and mice and reported that rhesus macaques had the highest similarity to humans ($r = 0.58$), as might be expected based on phylogenetic relationships. Differentially expressed genes included energy metabolism and peptide hormone receptor sets. Analysis of microRNAs in the heart of rats, dogs, and cynomolgus monkeys found a number of microRNAs in the valve and myocardial

tissue [208]. Of these, miR-1 was myocardium enriched, and miR-125b-5p and miR-204 were enriched in the valve in rats, cynomolgus monkeys, and dogs.

VetBooks.ir

COMPARATIVE RENAL PHYSIOLOGY

Numbers of publications related to the basic physiology of renal function in nonhuman primates peaked in the 1970s, and the basics of human renal physiology are widely available and translate well. Table 8.9 presents a selection of basic renal physiologic parameters for marmosets, macaques, and humans. In a study of six immature and seven mature rhesus macaques (age and sex not specified), inulin clearance was remarkably consistent: 2.18±0.680 mL/min/kg (mean±standard deviation) for immature animals and 1.96±0.445 mL/min/kg for adults [217]. Inulin clearance is considered the best estimation of glomerular filtration rate; inulin clearance in human women is 1.75±0.22 mL/min/kg [218]. Creatinine clearance from the same study was 3.08±0.939 mL/min/kg, and clearance of para-aminohippurate, an estimator

TABLE 8.9 Selected Renal Physiology Parameters

	C. jacchus	*M. fascicularis*	*M. mulatta*	*H. sapiens*
Blood volume mL/kg	65 mL/kg [147]	55-75 mL/kg [42]	50-96 mL/kg [42]	65-73 mL/kg [62]
Daily water intake	11.7±10.2 mL [209]	350-950 mL [42]	400-600 mL [42]	1960-2600 mL [62]
Daily food intake	20 g+fruit [42] (adult)	350-550 g [42]	400-600 g [42]	1800-2800 kcal/day [62]
Urine production	12.6±7.1 mL/day [209]	150-550 mL/day [42]	70-80 mL/kg/day [141]	20-22 mL/kg/day [62]
Metabolic rate	205-350 kJ/kg$^{0.75}$ [210]	NF	653 kcal/m^2/24 hr [141] ($n=4$)	752-859 kcal/m2/24 hr [62]
Creatinine clearance	3.6±0.6 (SEM) mL/min/kg [211], 3.25±0.39 mL/kg/min [212]	1.84-2.53 mL/kg/min [213],	3.08 mL/kg/min [141]	1.52 mL/kg/min [211]
Renal plasma flow	21±2 mL/kg/min [214]	16.25±3.61 mL/kg/min [212]	9.5-14.4 mL/kg/min [141]	9.6-10.5 mL/kg/min [62]
Glomerular filtration rate	4.8±0.6 mL/kg/min [171]	2.71±0.37 mL/kg/min [212]	0.93-3.30 mL/kg/min [141]	1.71 mL/kg/min [62]
Total body water	M: 65.3% [215] F: 68.0% [215]	62-64% [216]	62-72% [141]	53-60% [62]
Extracellular fluid	NF	NF	208 mL/kg [141] (121-295 mL/kg)	3.7-3.9 mL/kg [62]

Values given as mean±standard deviation except where indicated otherwise.
Numbers in parentheses refer to the data sources, which are listed in the chapter bibliography.
Abbreviations: M, male; F, female; NF, not found.

VetBooks.ir

of total renal plasma flow, was 8.06±1.220 mL/min/kg. These results indicate that creatinine is partially excreted in the rhesus monkey. Liu [218] reported several renal physiological parameters in female adult rhesus monkeys, including total renal plasma flow (557±153 mL/min/m^2), total renal blood flow (809±217 mL/min/m^2), renal oxygen consumption (1010±433 mL/hr/m^2), and tubular maximal reabsorption of glucose (67.4±14.3 mg/min/m^2). Values adjusted to body surface area were generally more comparable to human values than when adjusted by weight. Liu and Higbee [219] provided direct measurements of macaque body surface area to arrive at the best fit equation for determining body surface area in the macaque for crown-to-rump or head-to-heel measurements.

$$\mathrm{BSA}\left(\mathrm{cm}^2\right) = W^{0.6046}\left(\mathrm{kg}\right) \times L^{0.1862}\left(\text{cm, head to anus}\right) \times 514 \tag{1}$$

or

$$\mathrm{BSA}\left(\mathrm{cm}^2\right) = W^{0.6184}\left(\mathrm{kg}\right) \times L^{0.1447}\left(\text{cm, head to heel}\right) \times 565 \tag{2}$$

In a study of 20 rhesus macaques evaluated for urine output and water intake, urine voiding occurred most commonly between 7 and 8 AM, approximately 1.5 hours before feeding [220]. Water consumption was typically distributed across the early afternoon. Water intake and urine output were not different between sexes. Water intake was 83±31 mL/kg/24 hr and urine output was 51±19 mL/kg/24 hr. A more contemporary measurement of creatinine clearance was provided for the adult female cynomolgus monkey: 1.84-2.53 mL/kg/min [213]. Renal weight and volume were remarkably homogeneous across adult rhesus macaques without correction for body weight [221]; male kidneys were about 0.8 g heavier than in females, and left kidneys were about 0.5 g heaver than right kidneys. On average, the left kidney of the male rhesus macaque weighed 13.7 g and had a volume of 13.5 mL. Renal gene expression for cyclooxygenase (Cox)-1 and -2 was examined by immunohistochemistry and in situ hybridization in humans, cynomolgus monkeys, dogs, and rats, with a remarkably different distribution of Cox-2, but not Cox-1, between species. Humans and monkeys express Cox-2 in the glomerulus and renal vasculature, whereas dogs and rats express it in the thick ascending limb of the loop of Henle, the macula densa, and within the interstitium [222]. This was proposed as the basis for the difference in species susceptibility for renal papillary necrosis when dosed with Cox inhibitors. Detailed anatomy and physiology observations for the rhesus kidney have been published [223].

COMPARATIVE RESPIRATORY PHYSIOLOGY

Anatomy

The gross anatomy of the rhesus macaque respiratory tract has been well described [224,225]. In the nasal cavity there is no concha comparable to the human superior concha; the monkey's superior concha is equivalent to the human middle concha. A maxillary sinus is present, but a sphenoidal sinus is not. Harkema [226] described the distribution of squamous, transitional, respiratory, olfactory, and lymphoepithelial mucosa from a comparative perspective. Like humans, macaques have a much-reduced olfactory epithelial surface. The volume of the nasal cavity has been reported to be 8 mL in the rhesus [227], which Harkema compared with two estimates of human nasal cavity volume ranging from 16 to 28 mL. The number of mast cells

VetBooks.ir

in the olfactory mucosa has been studied in rhesus monkeys, and this cell population varied in females, peaking during ovulation, whereas in males it was described as moderately abundant [228]. Interestingly, electron microscopic examination of the olfactory epithelium of female rhesus monkeys demonstrated variation during the ovulatory cycle, with changes in the sustentacular cells, which were suggested to be related to changes in olfaction related to sex hormone production [229]. In New World primates, including marmosets, computed tomography and histologic study of maxillary pneumatization was examined [230]. Marmosets, but not macaques or humans, retain the presence of a vomeronasal organ, although its size is reduced compared with prosimians. Details of vomeronasal organ anatomy have been published [231]. In the larynx of macaques, a laryngeal air sac that lies on the midline between the thyroid cartilage and the hyoid bone exists and opens through the caudal part of the epiglottis, slightly cranial to the ventricular folds [224]. It was believed that these sacs function as resonating chambers; however, surgical removal did not alter the frequency spectrum of sound production [232]. Therefore, the function of these structures remains uncertain. The lung of the rhesus differs from the human in its lobulation pattern: the left side has three lobes, whereas the human has two; the right side has cranial, middle, caudal, and accessory lobes, whereas the human does not have an accessory lobe [225]. A histological study of the airway epithelium of adult rhesus monkeys detected four cell types: ciliated, mucous goblet, small mucous granule, and basal [233]. Clara cells, a kind of nonciliated bronchiolar epithelial cell, were restricted to respiratory bronchioles. Compared with human Clara cells, *Macaca radiata* Clara cells share the same electron microscopic characteristics: abundant membrane-bound granules in the apical cytoplasm and abundant granular endoplasmic reticulum with no smooth endoplasmic reticulum [234]. The anatomy, ultrastructure, and morphometry of the marmoset lung has been described [235]. The alveolar surface are of the adult marmoset was reported as $7662 \pm 1647\,cm^2$. Capillary surface area was $6000 \pm 1549\,cm^2$ and lung capillary volume was $1.01 \pm 0.34\,mL$. The estimated oxygen diffusion capacity of $0.0299 \pm 0.0134\,mL$ oxygen/sec/mmHg in marmosets was considered high in relation to large rodents.

Development

In a study of the development of the trachea [236], maturation of the lining epithelium incorporated an increasing complexity of cell types over time. Fetuses younger than 46 days of gestation had a single columnar, nonciliated cell type. From 46 days of gestation, ciliated cells were present. By 120 days, all cell components were present, including ciliated, mucous goblet, small mucous granule, and basal cells. At 134 days of gestation, the tracheal lining resembles that of the adult. The development of tracheal glands begins with submucosal projections of columns of cells at 80 days of gestation, with differentiation of mucous cells preceding serous cells and continuing into the postnatal period [237]. The growth of the infant larynx was studied in rhesus and *Macaca nemestrina* monkeys aged 1 to 51 days, but dimensions varied between animals without a clear dependence on age [238]. The development of the rhesus lung was studied in fetuses of 50, 75, 100, 125, and 150 days' gestation by Kerr and colleagues [239]. Primitive bronchioles grew from 50 to 75 days but were replaced as they developed into bronchioles, alveolar ducts, and alveoli until they disappeared completely at 150 days. Alveoli were first present at 100 days of age and composed half of the lung volume by 150 days of gestation. In the adult, alveoli account for two-thirds of the lung

VetBooks.ir

volume. Type I and type II pneumocytes are distinct by 150 days of gestation. Maturation of lung phospholipids was investigated by Perelman and colleagues [240]. They found that decreased concentrations of phosphatidylethanolamine and sphingomyelin were accompanied by increases in phosphatidylcholine at around 162 days of gestation, which coincide with decreases in glycogen content. Although the role of cortisol is established in the maturation of the human lung [241], this study was unable to document a relationship between fetal cortisol and the parameters of lung development studied. Immunohistochemistry for Clara cell secretory protein, a protective antioxidant and anti-inflammatory protein, was performed in the lungs of rhesus monkeys aged 1 week to 3 years [242]. The neonatal lung had little Clara cell secretory protein at 1 month, but this increased to near adult concentrations by 3 months, after which it remained unchanged. This indicated that a critical development period of lung airways occurs between 1 and 3 months of age. Plopper and colleagues [243] reported acceleration of alveolar type II pneumocyte differentiation in the fetal rhesus macaque after administration of epidermal growth factor, implicating it as a possible mediator of lung maturation. This was accompanied by increases in surfactant apoprotein A but not alterations in overall lung growth. Stereological assessment of alveolar number during postnatal growth of the lung of the rhesus monkey from 2 to 13 years of age revealed the increase in size of the lung was due to expansion of existing alveoli and that no new alveoli were formed during this growth [244].

Physiology

Standard respiratory physiological parameters are compared in Table 8.10. Binns and colleagues [245] reported a range of respiratory physiological parameters for the rhesus and compared them with cynomolgus and baboon values. Other reports of respiratory physiology parameters include those from Liu and KeLauter [250]. Kosch and colleagues measured maximum expiratory flow volume in adult rhesus and bonnet monkeys (*M. radiata*) and found that rhesus monkeys had expiratory flow rates equivalent to young adult humans [251]. In addition, the chest wall compliance of rhesus and bonnet macaques was about four times as stiff as that in dogs, whereas their lungs were twice as compliant. This resulted in a high functional residual capacity of 64%, about double that of dogs [251]. Pare and colleagues [252] corroborated the assessment that rhesus macaques have rigid chest walls.

Interest in the use of the nonhuman primates to model asthma motivated investigation of the similarities in the response to pharmacology between rhesus and cynomolgus monkeys. Histamine aerosol administration caused up to a 200% increase in airway resistance and a decreased lung compliance of 30% to 80% [253]. Both monkeys responded identically to various bronchodilators (prostaglandin, terbutaline, isoproterenol, and aminophylline), indicating that cynomolgus and rhesus pulmonary physiology is very similar. The use of lung slice cultures originating from macaques, marmosets, and humans allowed comparative characterization of the responses to bronchoconstricting agents [254]. Histamine had the greatest effect in macaques but only about half the degree of bronchoconstriction in the marmoset. Leukotriene D_4 did not cause bronchoconstriction in marmosets and rhesus but caused 50% airway constriction in baboons and cynomolgus monkeys, which more closely resembled the human response. Therefore it was concluded that the cynomolgus monkey may be a better predictor of human bronchoconstriction than the rhesus. Asgharian and

VetBooks.ir

TABLE 8.10 Selected Respiratory Physiology Parameters

	C. jacchus	*M. fascicularis*	*M. mulatta*	*H. sapiens*
Respiratory Rate	19-25/min [171]	30-54/min [42]	35-50/min [42]	12-14/min [62]
Temperature	36.8-38.6 °C [42] (telemetry)	37-39°C [42]	37-39°C [42]	37 °C
Tidal volume	0.98±0.11 mL [171]	53±6 mL [245]	42.5±10.2 mL [141]	460-750 mL [62]
Breaths/min	26.9±1.87/min [171]	39±7/min [245]	39±8/min [141]	12-14/min [62]
Respiratory minute volume	24.9±2.38 mL/min [171]	2039±299/min [245]	1630±465 mL/min [141]	6500-9000 mL/min [62]
Average pulmonary resistance	0.28±0.03 cm H_2O·s/mL [171]	0.019±0.005 cm H_2O·s/mL [245]	0.021±0.009 cm H_2O·s/mL [141]	1.58±0.11 cm H_2O·s/L [246]
Arterial pO_2	95.2±2.6 mmHg [247]	100.7±4.2 mmHg [248]	100.8±7.0 mmHg [248]	80 mmHg [249]
Arterial pCO_2	34.3±0.8 mmHg [247]	38.8±2.7 mmHg [248]	42.6±1.0 mmHg [248]	40 mmHg [249]
Arterial pH	7.38±0.01 [247]	7.45±0.02 [248]	7.46±0.04 [248]	7.40 [249]
Metabolic rate	205-350 kJ/$kg^{0.75}$ [210]	NF	653 kcal/m^2/24 hr [141] (n=4)	752-859 kcal/m^2/24 hr [62]

Some values given as mean±standard deviation
Numbers in parentheses refer to the data sources, which are listed in the chapter bibliography.
Abbreviation: NF, not found.

colleagues [255] described a geometric model that predicts comparatively lower 10-μm particle pulmonary deposition in infant and young adult rhesus macaques compared with humans. A similar model that extended estimation for airways from the nose to the lung was reported. Comparative evaluation of monkey and human parameters revealed that monkeys have higher nasal extraction and lower pulmonary deposition than humans [256], in agreement with the results of Asgharian et al.

NEUROBIOLOGY

Brain development, anatomy, and function in nonhuman primates are empirically similar to that in humans, which has been the basis for their use as models in neuroscience. Surprisingly, some selected behaviors, such as sex differences in toy preference among macaques and vervet monkeys, mirror findings in humans [257,258]. A neurobehavioral assessment test battery for the macaque has been described to measure the adverse neurological effects of drugs and is similar to tests used in rodents [259]. A cognitive test battery evaluating *M. fascicularis* against apes found that chimpanzees had greater intelligence for spatial understanding and tool use but no superiority in sociocognitive tasks, whereas human children excel in the sociocognitive domain [153]. The differences between primates in particular functional domains undoubtedly have molecular and anatomic bases, but these details have only begun to be understood.

VetBooks.ir

Current understanding of the molecular and anatomic differences that underlie the cognitive differences between humans and nonhuman primates has been recently reviewed by Geschwind and Rakic [154]. Sites of high levels of gene duplication and rearrangement include those areas where ancestral ape chromosomes have fused and inverted, as well as on telomeres [155]. A comparison between humans and nonhuman primates showed more than 600 genes with accelerated evolution, more than 500 deletions in highly conserved regions, more than 400 regions with differential histone methylation, at least 8 families of stably duplicated genes, 24 regions of highly transcribed intergenic regions with possible regulatory functions, at least 10 new microRNAs, more than 300 enhancer regions specific to the cortex, and 54 genes uniquely upregulated in the prefrontal cortex [154]. For example, the transcription factor FoxP2 has a two-amino acid change, which directs altered function in striatal circuitry, affecting sensorimotor integration; it may also modify craniofacial development and neural progenitor proliferation [154]. Another human-specific example is SRGAP2C, a truncated form of SRGAP2A, which alters dendritic spine density and shaft length in transgenic mice to resemble the human phenotype [156]. Analysis of coexpressed sets of genes in the human frontal lobe has increased complexity in the regulation of genes controlling neuronal morphology and synaptic function compared with chimpanzees and macaques [157]. Thus abundant molecular differences exist between humans and nonhuman primates and could drive the differences in cognitive ability.

Comparative neuroanatomy has increased our understanding of the essential neurobiological differences between nonprimate mammals, nonhuman primates, and humans [158]. In early development, the radial unit hypothesis explains cortical growth as a two-step process: First, neural stem cells proliferate in the ventricular zone of the developing neural tube, which determines overall cortical surface area. Second, the proliferation and radial migration of neural progenitors endures to form the laminae of the neocortex [159]. The duration of cortical neurogenesis in mice is approximately 6 days, in macaques about 60 days (embryonic days 40-100), and in humans about 100 days (embryonic days 43-120), which allows for a neocortical surface ratio of 1:100:1000, respectively [159]. Alterations in area-specific neocortical populations, such as the primate-specific fourfold increase in the thickness of primary visual cortex layer IV neurons (which receive afferent signals), have been proposed to occur by area-specific molecular control of the second phase of progenitor proliferation during neurogenesis [160]. A number of gene expression gradients across the neocortex during prenatal development have been identified; these differ between humans and macaques and may provide the molecular signals that direct formation of brain areas unique to humans, such as the Broca and Wernicke language centers. Later, after adolescence, gene expression again diverges between macaques and humans, with greater complexity in humans [161].

Following these initial phases of neurogenesis and neuronal migration, which construct the neocortex, interconnections between neurons proliferate (synaptogenesis). Unique to primates, synaptic density reaches a maximum during childhood (a 2-year-old human has approximately twice as many synapses as the adult), maintains this level for a prolonged period, then undergoes a "pruning" of synapses around the time of sexual maturity [162]. An excellent review of the comparative postnatal development of the rat, macaque, and human brain is presented by Watson and colleagues [163]. Understanding

VetBooks.ir

the relative periods of these developmental events is important for selecting an animal safety model and dosing schedule that allow for comparable periods of drug exposure in nonclinical safety testing.

VISUAL SYSTEM

Humans have two remarkable specializations of the visual system in comparison to many other mammals: trichromatic color vision and the fovea centralis. The fovea centralis is a focal increased density of cone photoreceptors with exclusion of rods, nonphotoreceptor cell bodies, and vasculature, which appears as an approximately 0.5 mm diameter pit in the retina. The fovea is present in the retina of all primates with the exception of prosimians and the nocturnally adapted owl monkey (*Aotus* sp.). Neurotransmission circuits in the fovea have one-to-one connections between photoreceptors, bipolar cells, and ganglion cells, which allow for maximal acuity [164]. Evolution has created similarly concentrated photoreceptors in other animals, such as falcons, which have two distinct retinal foveae for binocular or monocular acuity [165], and the visual streak or area centralis of other mammals [164]. The macaque eye is very similar to that of humans in terms of photoreceptor density and spectral sensitivity [166], and it has been a predominant model in vision research. The macaque fovea has a photoreceptor density within the range reported for humans (*M. mulatta*: 140,500 cones/mm^2; *M. fascicularis*: 100,400 cones/mm^2; humans: 199,000 cones/mm^2, with a range of 98,000 to 324,000 cones/mm^2) [167]. Interestingly, the foveal photoreceptor density of New World monkeys is generally similar across species, and foveal diameter does not change with body size; the photoreceptor density in the marmoset fovea is reported to range from 152,000 to 211,000 cones/mm^2. The limitation of nutrient and oxygen diffusion to the photoreceptors of the avascular fovea has been suggested as the limiting factor in the evolution of its diameter. Therefore the visual angle of the fovea decreases with increasing eye size: it is 7.8° in marmosets and <5° in humans [167].

Maximum spectral sensitivity of cones in macaques occurs at 430, 530, and 561 nm for the short (blue), medium (green), and long (red) wavelength-sensitive cones and 491 nm for rods. Human red and green cones have spectral sensitivity essentially identical to that of the cynomolgus macaque [166]. The detailed molecular genetics of visual pigments are surprisingly complex. While the short-wavelength cone opsins are autosomal, the medium- (green) and long-wavelength (red) cone opsins are the result of gene duplication on the X chromosome, and recombination or mutation within these genes can lead to an array of opsin expression [168]. Dichromacy (red-green color blindness) affects approximately 1% of Caucasian males [168] but has been described as very rare in *M. fascicularis* [169]. In marmosets, a single locus for rod opsin exists on the X chromosome, in addition to the autosomal short-wavelength opsin. However, three distinct alleles with maximum spectral sensitivity at 543, 556, and 563 nm may be found at this locus [170]. Male marmosets are therefore always dichromatic, but female marmosets may be di- or trichromatic based on the inheritance pattern at this locus. In the female marmoset, the random inactivation of one X chromosome in cone photoreceptors allows for the separate expression of both alleles in different cone cells, resulting in trichromatic vision [170].

VetBooks.ir

References

[1] Johnsen DO, Johnson DK, Whitney Jr. RA. History of the Use of Nonhuman Primates in Biomedical Research. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. 2nd edit. Nonhuman Primates in Biomedical Research: Biology and Management, 1. London: Academic Press; 2012. p. 1–33, 2 vols.

[2] Hill BF. The rhesus monkey (*M. mulatta*): history, management, and scientific investigation. Charles River Digest 1977;16:1–7.

[3] Clarke MR, O'Neil JA. Morphometric comparison of Chinese-origin and Indian-derived rhesus monkeys (*Macaca mulatta*). Am J Primatol 1999;47:335–46.

[4] Groves CP. Primate Taxonomy. Washington DC: Smithsonian Institution Press; 2001.

[5] Bourges-Abella N, Geffre A, Moureaux E, Vincenti M, Braun JP, Trumel C. Hematologic reference intervals in Cynomolgus (*Macaca fascicularis*) monkeys. J Med Primatol 2014;43:1–10.

[6] Orsi A, Rees D, Andreini I, Venturella S, Cinelli S, Oberto G. Overview of the marmoset as a model in nonclinical development of pharmaceutical products. Regul Toxicol Pharmacol 2011;59:19–27.

[7] Zuhlke U, Weinbauer G. The common marmoset (*Callithrix jacchus*) as a model in toxicology. Toxicol Pathol 2003;31(Suppl):123–7.

[8] Chiarelli B. Some new data on the chromosomes of Catarrhina. Experientia 1962;18:405–6.

[9] Gibbs RA, Rogers J, Katze MG, Bumgarner R, Weinstock GM, Mardis ER, et al. Evolutionary and biomedical insights from the rhesus macaque genome. Science 2007;316:222–34.

[10] Yan G, Zhang G, Fang X, Zhang Y, Li C, Ling F, et al. Genome sequencing and comparison of two nonhuman primate animal models, the cynomolgus and Chinese rhesus macaques. Nat Biotechnol 2011;29:1019–23.

[11] SmithHardy CL, Hart LT, Dimopoullos GT, Lambremont EN. *Plasmodium lophurae*: quantitative in vitro incorporation of 14C-1-acetate into lipids. Exp Parasitol 1975;37:193–204.

[12] Kumar S, Hedges SB. A molecular timescale for vertebrate evolution. Nature 1998;392:917–20.

[13] Wu AQ, Ma KS, Yang TW, Sheng DF, Chen L, Li LL, et al. The complete mitochondrial genome of the Chinese rhesus macaques, *Macaca mulatta lasiota*. Mitochondrial DNA 2014. Epub ahead of print.

[14] Li R, Wang H, Yang L, Zhang B, Li Y, Hu J, et al. The whole mitochondrial genome of the Cynomolgus macaque (*Macaca fascicularis*). Mitochondrial DNA 2013. Epub ahead of print.

[15] Hodgkinson A, Idaghdour Y, Gbeha E, Grenier JC, Hip-Ki E, Bruat V, et al. High-resolution genomic analysis of human mitochondrial RNA sequence variation. Science 2014;344:413–5.

[16] Dutrillaux B. Chromosomal evolution in primates: tentative phylogeny from *Microcebus murinus* (Prosimian) to man. Hum Genet 1979;48:251–314.

[17] Rogers J, Garcia R, Shelledy W, Kaplan J, Arya A, Johnson Z, et al. An initial genetic linkage map of the rhesus macaque (*Macaca mulatta*) genome using human microsatellite loci. Genomics 2006;87:30–8.

[18] Sherlock JK, Griffin DK, Delhanty JD, Parrington JM. Homologies between human and marmoset (*Callithrix jacchus*) chromosomes revealed by comparative chromosome painting. Genomics 1996;33:214–9.

[19] McPherson JD, Marra M, Hillier L, Waterston RH, Chinwalla A, Wallis J, et al. A physical map of the human genome. Nature 2001;409:934–41.

[20] Dutrillaux B, Biemont MC, Viegas-Pequignot E, Laurent C. Comparison of the karyotypes of four Cercopithecoidae: *Papio papio*, *P. anubis*, *Macaca mulatta*, and *M. fascicularis*. Cytogenet Cell Genet 1979;23:77–83.

[21] Hayasaka K, Horai S, Gojobori T, Shotake T, Nozawa K, Matsunaga E. Phylogenetic relationships among Japanese, rhesus, Formosan, and crab-eating monkeys, inferred from restriction-enzyme analysis of mitochondrial DNAs. Mol Biol Evol 1988;5:270–81.

[22] Avise JC, Duvall SW. Allelic expression and genetic distance in hybrid macaque monkeys. J Hered 1977;68:23–30.

[23] Kanthaswamy S, Ng J, Satkoski Trask J, George DA, Kou AJ, Hoffman LN, et al. The genetic composition of populations of cynomolgus macaques (*Macaca fascicularis*) used in biomedical research. J Med Primatol 2013;42:120–31.

[24] Deininger PL, Batzer MA. Mammalian retroelements. Genome Res 2002;12:1455–65.

[25] Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860–921.

[26] Gokcumen O, Tischler V, Tica J, Zhu Q, Iskow RC, Lee E, et al. Primate genome architecture influences structural variation mechanisms and functional consequences. Proc Natl Acad Sci U S A 2013;110:15764–9.

[27] Zhang X, Goodsell J, Norgren Jr. RB. Limitations of the rhesus macaque draft genome assembly and annotation. BMC Genomics 2012;13:206.
[28] Ebeling M, Kung E, See A, Broger C, Steiner G, Berrera M, et al. Genome-based analysis of the nonhuman primate Macaca fascicularis as a model for drug safety assessment. Genome Res 2011;21:1746–56.
[29] Benirschke K, Anderson JM, Brownhill LE. Marrow Chimerism in Marmosets. Science 1962;138:513–5.
[30] Sweeney CG, Curran E, Westmoreland SV, Mansfield KG, Vallender EJ. Quantitative molecular assessment of chimerism across tissues in marmosets and tamarins. BMC Genomics 2012;13:98.
[31] Dierselhuis MP, Goulmy E. We are all born as microchimera. Chimerism 2013;4:18–9.
[32] Ash H, Buchanan-Smith HM. Long-term data on reproductive output and longevity in captive female common marmosets (Callithrix jacchus). Am J Primatol 2014;76:1062–73.
[33] Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL, et al. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature 2012;489:318–21.
[34] Raftery AE, Li N, Ševčíková H, Gerland P, Heilig GK. Bayesian probabilistic population projections for all countries. Proc Natl Acad Sci U S A 2012;109:13915–21.
[35] Tardif SD, Smucny DA, Abbott DH, Mansfield K, Schultz-Darken N, Yamamoto ME. Reproduction in captive common marmosets (*Callithrix jacchus*). Comp Med 2003;53:364–8.
[36] Jerome CP. Congenital malformations and twinning in a breeding colony of Old World monkeys. Lab Anim Sci 1987;37:624–30.
[37] Martin JA, Hamilton BE, Osterman MJK, Curtin SC, Mathews TJ. Births: Final data for 2013. National Vital Statistics Reports 2013;62:1–69.
[38] Chambers PL, Hearn JP. Embryonic, foetal and placental development in the Common marmoset monkey (*Callithrix jacchus*). J Zool (Lond) 1985;207:545–61.
[39] Dimitriadis E, Robb L, Liu YX, Enders AC, Martin H, Stoikos C, et al. IL-11 and IL-11Ralpha immunolocalisation at primate implantation sites supports a role for IL-11 in placentation and fetal development. Reprod Biol Endocrinol 2003;1:34.
[40] Luckett WP. Comparative development and evolution of the placenta in primates. Contrib Primatol 1974;3:142–234.
[41] Merker H-J, Bremer D, Csato W, Heger W, Gossrau R. Development of the Marmoset Placenta. In: Neubert D, Merker H-J, Hendrickx AG, editors. Non-Human Primates–Developmental Biology and Toxicology. Berlin: Ueberreuter Wissenschaft; 1988. p. 245–72.
[42] Fortman JD, Hewett TA, Bennett BT. The laboratory nonhuman primate. New York: CRC Press; 2002.
[43] Haeussner E, Schmitz C, von Koch F, Frank HG. Birth weight correlates with size but not shape of the normal human placenta. Placenta 2013;34:574–82.
[44] Phillips IR. Skeletal development in the foetal and neonatal marmoset (*Callithrix jacchus*). Lab Anim 1976;10:317–33.
[45] O'Rahilly R, Gardner E. The timing and sequence of events in the development of the limbs in the human embryo. Anat Embryol (Berl) 1975;148:1–23.
[46] Tardif S, Carville A, Elmore D, Williams LE, Rice K. Reproduction and Breeding of Nonhuman Primates. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. Nonhuman Primates in Biomedical Research: Biology and Management, 1. London: Academic Press; 2012. p. 197–249, 2 vols.
[47] Bogin B, Smith BH. Evolution of the human life cycle. Am J Hum Biol 1996;8:703–16.
[48] Smith BH, Crummett TL, Brandt KL. Ages of Eruption of Primate Teeth: A Compendium for Aging Individuals and Comparing Life Histories. Am J Phys Anthropol 1994;37:177–231.
[49] Abbott DH, Barnett DK, Colman RJ, Yamamoto ME, Schultz-Darken NJ. Aspects of common marmoset basic biology and life history important for biomedical research. Comp Med 2003;53:339–50.
[50] Dang DC, Meusy-Dessolle N. Quantitative study of testis histology and plasma androgens at onset of spermatogenesis in the prepuberal laboratory-born macaque (*Macaca fascicularis*). Arch Androl 1984;12(Suppl):43–51.
[51] Watanabe D, Hoshiya T, Sato J, Yamaguchi Y, Horiguchi K, Nagashima Y, et al. Changes in the Reproductive Organs Depending on Phases of Reproductive Cycle and Aging in Female Cynomolgus Monkeys. J Toxicol Pathol 2006;19:169–77.
[52] Catchpole HR, van Wagenen G. Reproduction in the Rhesus Monkey, *Macaca mulatta*. In: Bourne GH, editor. The Rhesus Monkey, 2. New York: Academic Press; 1975. p. 117–40, 2 vols.

[53] Terasawa E, Bridson WE, Nass TE, Noonan JJ, Dierschke DJ. Developmental changes in the luteinizing hormone secretory pattern in peripubertal female rhesus monkeys: comparisons between gonadally intact and ovariectomized animals. Endocrinology 1984;115:2233–40.
[54] Watts ES, Gavan JA. Postnatal growth of nonhuman primates: the problem of the adolescent spurt. Hum Biol 1982;54:53–70.
[55] Weinbauer GF, Niehoff M, Niehaus M, Srivastav S, Fuchs A, Van Esch E, et al. Physiology and Endocrinology of the Ovarian Cycle in Macaques. Toxicol Pathol 2008;36:7S–23S.
[56] Gavan JA, Swindler DR. Growth rates and phylogeny in primates. Am J Phys Anthropol 1966;24:181–90.
[57] Schultea TD, Smallwood JE, Stott GG. Radiographic evaluation of appendicular skeletal maturation in the common marmoset (*Callithrix jacchus*). J Med Primatol 1983;12:8–29.
[58] Fukuda S, Cho F, Honjo S. Bone growth aud development of secondary ossification centers of extremities in the cynomolgus monkey (*Macaca fascicularis*). Jikken Dobutsu 1978;27:387–97.
[59] Cheverud JM. Epiphyseal union and dental eruption *Macaca mulatta*. Am J Phys Anthropol 1981;56: 157–67.
[60] White TD, Black MT, Folkens PA. *Human Osteology*. 3rd edit. Elsevier: New York; 2012.
[61] Araujo A, Arruda MF, Alencar AI, Albuquerque F, Nascimento MC, Yamamoto ME. Body weight of wild and captive common marmosets (*Callithrix jacchus*). Int J Primatol 2000;21:317–24.
[62] Annon. Basic anatomical and physiological data for use in radiological protection: reference values. A report of age- and gender-related differences in the anatomical and physiological characteristics of reference individuals. ICRP Publication 89. Ann ICRP 2002;32:5–265.
[63] van Esch E, Buse E, Weinbauer GF, Cline JM. The Macaque Endometrium, with Special Reference to the Cynomolgus Monkey (*Macaca fascicularis*). Toxicol Pathol 2008;36:67S–100S.
[64] Carter AM. Animal models of human placentation–a review. Placenta 2007;28(Suppl A):S41–7.
[65] de Rijk EPCT, van Esch E. The Macaque Placenta–A Mini-Review. Toxicol Pathol 2008;36:108S–18S.
[66] Buse E, Haeger JD, Svensson-Arvelund J, Markert UR, Faas MM, Ernerudh J, et al. The placenta in toxicology. Part I: Animal models in toxicology: placental morphology and tolerance molecules in the cynomolgus monkey (*Macaca fascicularis*). Toxicol Pathol 2014;42:314–26.
[67] Urbanski HF. Differential roles of GnRH-I and GnRH-ii neurons in the control of the primate reproductive axis. Front Endocrinol (Lausanne) 2012;3:20.
[68] Abbott DH, Saltzman W, Schultz-Darken NJ, Smith TE. Specific neuroendocrine mechanisms not involving generalized stress mediate social regulation of female reproduction in cooperatively breeding marmoset monkeys. Ann N Y Acad Sci 1997;807:219–38.
[69] Popa SM, Clifton DK, Steiner RA. The role of kisspeptins and GPR54 in the neuroendocrine regulation of reproduction. Annu Rev Physiol 2008;70:213–38.
[70] Garcia-Galiano D, Pinilla L, Tena-Sempere M. Sex steroids and the control of the Kiss1 system: developmental roles and major regulatory actions. J Neuroendocrinol 2012;24:22–33.
[71] Ronnekleiv OK, Bosch MA, Zhang C. 17beta-oestradiol regulation of gonadotrophin-releasing hormone neuronal excitability. J Neuroendocrinol 2012;24:122–30.
[72] Saltzman W, Tardif SD, Rutherford JN. Hormones and reproductive cycles in primates. In: Norris DO, Lopez KH, editors. Hormones and Reproduction of Vertebrates, vol. 5. London: Elsevier; 2011. p. 291–328.
[73] Tardif SD, Lacker HM, Feuer M. Follicular development and ovulation in the marmoset monkey as determined by repeated laparoscopic examination. Biol Reprod 1993;48:1113–9.
[74] Mizuno M, Terasawa E. Search for neural substrates mediating inhibitory effects of oestrogen on pulsatile luteinising hormone-releasing hormone release in vivo in ovariectomized female rhesus monkeys (*Macaca mulatta*). J Neuroendocrinol 2005;17:238–45.
[75] Fraser HM, Groome NP, McNeilly AS. Follicle-stimulating hormone-inhibin B interactions during the follicular phase of the primate menstrual cycle revealed by gonadotropin-releasing hormone antagonist and antiestrogen treatment. J Clin Endocrinol Metab 1999;84:1365–9.
[76] Matsuda F, Inoue N, Manabe N, Ohkura S. Follicular growth and atresia in mammalian ovaries: regulation by survival and death of granulosa cells. J Reprod Dev 2012;58:44–50.
[77] Young JM, McNeilly AS. Theca: the forgotten cell of the ovarian follicle. Reproduction 2010;140:489–504.
[78] Lee HR, Jeung EB, Cho MH, Kim TH, Leung PC, Choi KC. Molecular mechanism(s) of endocrine-disrupting chemicals and their potent oestrogenicity in diverse cells and tissues that express oestrogen receptors. J Cell Mol Med 2013;17:1–11.

[79] Patyna S, Arrigoni C, Terron A, Kim TW, Heward JK, Vonderfecht SL, et al. Nonclinical safety evaluation of sunitinib: a potent inhibitor of VEGF, PDGF, KIT, FLT3, and RET receptors. Toxicol Pathol 2008;36:905–16.
[80] Fraser HM, Morris KD, Wiegand SJ, Wilson H. Inhibition of vascular endothelial growth factor during the postovulatory period prevents pregnancy in the marmoset. Contraception 2010;82:572–8.
[81] Stouffer RL. Progesterone as a mediator of gonadotrophin action in the corpus luteum: beyond steroidogenesis. Hum Reprod Update 2003;9:99–117.
[82] Duffy DM, Stouffer RL. Follicular administration of a cyclooxygenase inhibitor can prevent oocyte release without alteration of normal luteal function in rhesus monkeys. Hum Reprod 2002;17:2825–31.
[83] Zelinski-Wooten MB, Hutchison JS, Hess DL, Wolf DP, Stouffer RL. A bolus of recombinant human follicle stimulating hormone at midcycle induces periovulatory events following multiple follicular development in macaques. Hum Reprod 1998;13:554–60.
[84] Zeleznik AJ. Follicle selection in primates: "many are called but few are chosen". Biol Reprod 2001;65:655–9.
[85] Molskness TA, Woodruff TK, Hess DL, Dahl KD, Stouffer RL. Recombinant human inhibin-A administered early in the menstrual cycle alters concurrent pituitary and follicular, plus subsequent luteal, function in rhesus monkeys. J Clin Endocrinol Metab 1996;81:4002–6.
[86] Hendrickx AG, Sawyer RH. Embryology of the Rhesus Monkey. In: Bourne GH, editor. The Rhesus Monkey, 2. New York: Academic Press; 1975. p. 141–69, 2 vols.
[87] Marston JH, Kelly WA. Time relationships of spermatozoon penetration into the egg of the rhesus monkey. Nature 1968;217:1073–4.
[88] Sengupta J, Ghosh D. Blastocyst-endometrium interaction at implantation in the rhesus monkey. J Reprod Immunol 2002;53:227–39.
[89] Moore HD, Gems S, Hearn JP. Early implantation stages in the marmoset monkey (*Callithrix jacchus*). Am J Anat 1985;172:265–78.
[90] Enders AC. Implantation in the macaque: expansion of the implantation site during the first week of implantation. Placenta 2007;28:794–802.
[91] Enders AC, Carter AM. Review: The evolving placenta: different developmental paths to a hemochorial relationship. Placenta 2012;33(Suppl):S92–8.
[92] Bowman CJ, Breslin WJ, Connor AV, Martin PL, Moffat GJ, Sivaraman L, et al. Placental transfer of Fc-containing biopharmaceuticals across species, an industry survey analysis. Birth Defects Res B Dev Reprod Toxicol 2013;98:459–85.
[93] Kane SV, Acquah LA. Placental transport of immunoglobulins: a clinical review for gastroenterologists who prescribe therapeutic monoclonal antibodies to women during conception and pregnancy. Am J Gastroenterol 2009;104:228–33.
[94] Lilius EM, Marnila P. The role of colostral antibodies in prevention of microbial infections. Curr Opin Infect Dis 2001;14:295–300.
[95] Coe CL, Lubach GR, Izard KM. Progressive improvement in the transfer of maternal antibody across the order Primates. Am J Primatol 1994;32:51–5.
[96] Zeleznik AJ, Pohl CR. Control of follicular development, corpus luteum function, the maternal recognition of pregnancy, and the neuroendocrine regulation of the menstrual cycle in higher primates. In: Neill JD, editor. Knobil and Neill's Physiology of Reproduction, vol. 2. St Louis: Elsevier; 2006. p. 2449–510.
[97] Kuijper EA, Ket JC, Caanen MR, Lambalk CB. Reproductive hormone concentrations in pregnancy and neonates: a systematic review. Reprod Biomed Online 2013;27:33–63.
[98] Clapp JF. Influence of endurance exercise and diet on human placental development and fetal growth. Placenta 2006;27:527–34.
[99] Desforges M, Sibley CP. Placental nutrient supply and fetal growth. Int J Dev Biol 2010;54:377–90.
[100] Kerr GR, Kennan AL, Waisman HA, Allen JR. Growth and development of the fetal rhesus monkey. I. Physical growth. Growth 1969;33:201–13.
[101] Knox WE, Lister-Rosenoer LM. Timing of gestation in rats by fetal and maternal weights. Growth 1978;42:43–53.
[102] Olsen IE, Groveman SA, Lawson ML, Clark RH, Zemel BS. New intrauterine growth curves based on United States data. Pediatrics 2010;125:e214–24.
[103] Dawes GS, Jacobson HN, Mott JC, Shelley HJ. Some observations on foetal and new-born rhesus monkeys. J Physiol 1960;152:271–98.
[104] Kerr GR, Wallace JH, Chesney CF, Waisman HA. Growth and development of the fetal rhesus monkey. 3. Maturation and linear growth of the skull and appendicular skeleton. Growth 1972;36:59–76.

[105] Jaquish CE, Tardif SD, Toal RL, Carson RL. Patterns of prenatal survival in the common marmoset (*Callithrix jacchus*). J Med Primatol 1996;25:57–63.
[106] O'Sullivan A, He X, McNiven EM, Hinde K, Haggarty NW, Lonnerdal B, et al. Metabolomic phenotyping validates the infant rhesus monkey as a model of human infant metabolism. J Pediatr Gastroenterol Nutr 2013;56:355–63.
[107] Bocian-Sobkowska J. Morphometric study of the human suprarenal gland in the first postnatal year. Folia Morphol (Warsz) 2000;58:275–84.
[108] McNulty WP, Novy MJ, Walsh SW. Fetal and postnatal development of the adrenal glands in *Macaca mulatta*. Biol Reprod 1981;25:1079–89.
[109] Conley AJ, Plant TM, Abbott DH, Moeller BC, Stanley SD. Adrenal androgen concentrations increase during infancy in male rhesus macaques (*Macaca mulatta*). Am J Physiol Endocrinol Metab 2011;301:E1229–35.
[110] Pattison JC, Mapes SM, Pryce CR, Conley AJ, Bird IM. Marmosets express a fetal zone at birth but no ZR in adulthood. Endocr Res 2002;28:675.
[111] Pryce CR, Palme R, Feldon J. Development of pituitary-adrenal endocrine function in the marmoset monkey: infant hypercortisolism is the norm. J Clin Endocrinol Metab 2002;87:691–9.
[112] McKim Jr. D, Hutchinson TC. Phylogenetic implications of comparative primate growth rates. Am J Phys Anthropol 1975;42:495–500.
[113] Coe CL, Shirtcliff EA. Growth trajectory evident at birth affects age of first delivery in female monkeys. Pediatr Res 2004;55:914–20.
[114] Hinde K. Richer milk for sons but more milk for daughters: Sex-biased investment during lactation varies with maternal life history in rhesus macaques. Am J Hum Biol 2009;21:512–9.
[115] Power ML, Oftedal OT, Tardif SD. Does the milk of Callitrichid monkeys differ from that of larger anthropoids? Am J Primatol 2002;56:117–27.
[116] Bowman JE, Lee PC. Growth and threshold weaning weights among captive rhesus macaques. Am J Phys Anthropol 1995;96:159–75.
[117] Jin L, Hinde K, Tao L. Species diversity and relative abundance of lactic acid bacteria in the milk of rhesus monkeys (*Macaca mulatta*). J Med Primatol 2011;40:52–8.
[118] Hinde K, Power ML, Oftedal OT. Rhesus macaque milk: magnitude, sources, and consequences of individual variation over lactation. Am J Phys Anthropol 2009;138:148–57.
[119] Power ML, Toddes B, Koutsos L. Nutrient Requirements and Dietary Husbandry Principles for Captive Nonhuman Primates. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. Nonhuman Primates in Biomedical Research: Biology and Management, 1. London: Academic Press; 2012. p. 269–92, 2 vols.
[120] Bernstein IS, Ruehlmann TE, Judge PG, Lindquist T, Weed JL. Testosterone changes during the period of adolescence in male rhesus monkeys (*Macaca mulatta*). Am J Primatol 1991;24:29–38.
[121] Ehardt CL, Bernstein IS. Matrilineal Overthrows in Rhesus Monkey Groups. Int J Primatol 1986;7:157–81.
[122] Dewi FN, Wood CE, Lees CJ, Willson CJ, Register TC, Tooze JA, et al. Dietary soy effects on mammary gland development during the pubertal transition in nonhuman primates. Cancer Prev Res (Phila) 2013;6:832–42.
[123] Winters SJ, Plant TM. Partial characterization of circulating inhibin-B and pro-alphaC during development in the male rhesus monkey. Endocrinology 1999;140:5497–504.
[124] Pohl CR, deRidder CM, Plant TM. Gonadal and nongonadal mechanisms contribute to the prepubertal hiatus in gonadotropin secretion in the female rhesus monkey (*Macaca mulatta*). J Clin Endocrinol Metab 1995;80:2094–101.
[125] Plant TM, Ramaswamy S, Simorangkir D, Marshall GR. Postnatal and pubertal development of the rhesus monkey (*Macaca mulatta*) testis. Ann N Y Acad Sci 2005;1061:149–62.
[126] Cutler Jr. GB, Glenn M, Bush M, Hodgen GD, Graham CE, Loriaux DL. Adrenarche: a survey of rodents, domestic animals, and primates. Endocrinology 1978;103:2112–8.
[127] Smail PJ, Faiman C, Hobson WC, Fuller GB, Winter JS. Further studies on adrenarche in nonhuman primates. Endocrinology 1982;111:844–8.
[128] Rogol AD. Sex steroids, growth hormone, leptin and the pubertal growth spurt. Endocr Dev 2010;17:77–85.
[129] Terasawa E, Kurian JR, Keen KL, Shiel NA, Colman RJ, Capuano SV. Body weight impact on puberty: effects of high-calorie diet on puberty onset in female rhesus monkeys. Endocrinology 2012;153:1696–705.
[130] Guerriero KA, Keen KL, Millar RP, Terasawa E. Developmental changes in GnRH release in response to kisspeptin agonist and antagonist in female rhesus monkeys (*Macaca mulatta*): implication for the mechanism of puberty. Endocrinology 2012;153:825–36.

[131] Dreef HC, Van Esch E, De Rijk EP. Spermatogenesis in the cynomolgus monkey (*Macaca fascicularis*): a practical guide for routine morphological staging. Toxicol Pathol 2007;35:395–404.
[132] Mustoe AC, Jensen HA, French JA. Describing ovarian cycles, pregnancy characteristics, and the use of contraception in female white-faced marmosets, *Callithrix geoffroyi*. Am J Primatol 2012;74:1044–53.
[133] Abbott DH. Natural suppression of fertility. Symp Zool Soc Lond 1988;60:7–28.
[134] Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicology studies in nonhuman primates. Birth Defects Res B Dev Reprod Toxicol 2009;86:446–62.
[135] Schultz AH. Postembryonic age changes. Primatologia 1956;1:887–964.
[136] Schultz AH. Vertebral column and thorax. Primatologia 1961;4:1–66.
[137] Nassar-Montoya F, Sainsbury AW, Kirkwood JK, du Boulay GH. Age determination of common marmosets (*Callithrix jacchus*) by radiographic examination of skeletal development. J Med Primatol 1992;21:259–64.
[138] Kilborn SH, Trudel G, Uhthoff H. Review of growth plate closure compared with age at sexual maturity and lifespan in laboratory animals. Contemp Top Lab Anim Sci 2002;41:21–6.
[139] Henderson E. Platyrrhine dental eruption sequences. Am J Phys Anthropol 2007;134:226–39.
[140] Casteleyn C, Bakker J, Breugelmans S, Kondova I, Saunders J, Langermans JA, et al. Anatomical description and morphometry of the skeleton of the common marmoset (*Callithrix jacchus*). Lab Anim 2012;46:152–63.
[141] Bourne GH. Collected Anatomical and Physiological Data from the Rhesus Monkey. In: Bourne GH, editor. The Rhesus Monkey, 1. New York: Academic Press; 1975. p. 1–63, 2 vols.
[142] Black A, Lane MA. Nonhuman primate models of skeletal and reproductive aging. Gerontology 2002;48:72–80.
[143] Grohmann J, Kuehnel F, Buchwald U, Koeller G, Habla C, Einspanier A. Analysis of the bone metabolism by quantitative computer tomography and clinical chemistry in a primate model (*Callithrix jacchus*). J Med Primatol 2012;41:1–10.
[144] Tsujio M, Mizorogi T, Kitamura I, Maeda Y, Nishijima K, Kuwahara S, et al. Bone mineral analysis through dual energy X-ray absorptiometry in laboratory animals. J Vet Med Sci 2009;71:1493–7.
[145] Burr DB. Estimated intracortical bone turnover in the femur of growing macaques: implications for their use as models in skeletal pathology. Anat Rec 1992;232:180–9.
[146] Freis ED, Schnaper HW, Johnson RL, Schreiner GE. Hemodynamic alterations in acute myocardial infarction. I. Cardiac output, mean arterial pressure, total peripheral resistance, central and total blood volumes, venous pressure and average circulation time. J Clin Invest 1952;31:131–40.
[147] Korte S, Niggemann B. Reference Control Data for the Common Marmoset (*Callithrix jacchus*): A Comparison with Macaques. Int J Toxicol 2013;32:78, (Abstract P600).
[148] Ludlage E, Mansfield K. Clinical care and diseases of the common marmoset (*Callithrix jacchus*). Comp Med 2003;53:369–82.
[149] Lalayeva N, Bailey N, Kenfield J, Makori N, Beck T, Nagata R. A study in telemetry implanted cynomolgus monkeys to evaluate select cardiovascular parameters in animals restrained in procedure cage or restrain chair (pole and collar). Int J Toxicol 2013;32:65–6.
[150] McLennan PL, Barnden LR, Bridle TM, Abeywardena MY, Charnock JS. Dietary fat modulation of left ventricular ejection fraction in the marmoset due to enhanced filling. Cardiovasc Res 1992;26:871–7.
[151] Tsusaki H, Yonamine H, Tamai A, Shimomoto M, Iwao H, Nagata R, et al. Evaluation of cardiac function in primates using real-time three-dimensional echocardiography as applications to safety assessment. J Pharmacol Toxicol Methods 2005;52:182–7.
[152] Milhaud C-L, Rosolen S-G. Electrocardiogramme Normal du Macaque Rhesus *Macaca mulatta*: Aspects compares de l'E.C.G. des primates. Recueil de Medecine Veterinaire de l'Ecole d'Alfort 1984;160:651–6.
[153] Schmitt V, Pankau B, Fischer J. Old world monkeys compare to apes in the primate cognition test battery. PLoS One 2012;7:e32024.
[154] Geschwind DH, Rakic P. Cortical evolution: judge the brain by its cover. Neuron 2013;80:633–47.
[155] O'Bleness M, Searles VB, Varki A, Gagneux P, Sikela JM. Evolution of genetic and genomic features unique to the human lineage. Nat Rev Genet 2012;13:853–66.
[156] Charrier C, Joshi K, Coutinho-Budd J, Kim JE, Lambert N, de Marchena J, et al. Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. Cell 2012;149:923–35.
[157] Konopka G, Friedrich T, Davis-Turak J, Winden K, Oldham MC, Gao F, et al. Human-specific transcriptional networks in the brain. Neuron 2012;75:601–17.
[158] Kaas JH. The Evolution of Brains from Early Mammals to Humans. Wiley Interdiscip Rev Cogn Sci 2013;4:33–45.

[159] Rakic P. A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. Trends Neurosci 1995;18:383–8.
[160] Lukaszewicz A, Cortay V, Giroud P, Berland M, Smart I, Kennedy H, et al. The concerted modulation of proliferation and migration contributes to the specification of the cytoarchitecture and dimensions of cortical areas. Cereb Cortex 2006;16(Suppl 1):i26–34.
[161] Pletikos M, Sousa AM, Sedmak G, Meyer KA, Zhu Y, Cheng F, et al. Temporal specification and bilaterality of human neocortical topographic gene expression. Neuron 2014;81:321–32.
[162] Johnston MV, Nishimura A, Harum K, Pekar J, Blue ME. Sculpting the developing brain. Adv Pediatr 2001;48:1–38.
[163] Watson RE, Desesso JM, Hurtt ME, Cappon GD. Postnatal growth and morphological development of the brain: a species comparison. Birth Defects Res B Dev Reprod Toxicol 2006;77:471–84.
[164] Provis JM, Dubis AM, Maddess T, Carroll J. Adaptation of the central retina for high acuity vision: cones, the fovea and the avascular zone. Prog Retin Eye Res 2013;35:63–81.
[165] Tucker VA. The deep fovea, sideways vision and spiral flight paths in raptors. J Exp Biol 2000;203:3745–54.
[166] Schnapf JL, Kraft TW, Nunn BJ, Baylor DA. Spectral sensitivity of primate photoreceptors. Vis Neurosci 1988;1:255–61.
[167] Finlay BL, Franco EC, Yamada ES, Crowley JC, Parsons M, Muniz JA, et al. Number and topography of cones, rods and optic nerve axons in New and Old World primates. Vis Neurosci 2008;25:289–99.
[168] Neitz M, Neitz J. Molecular genetics of color vision and color vision defects. Arch Ophthalmol 2000;118:691–700.
[169] Koida K, Yokoi I, Okazawa G, Mikami A, Widayati KA, Miyachi S, et al. Color vision test for dichromatic and trichromatic macaque monkeys. J Vis 2013;13:1.
[170] Tovee MJ, Bowmaker JK, Mollon JD. The relationship between cone pigments and behavioural sensitivity in a New World monkey (*Callithrix jacchus jacchus*). Vision Res 1992;32:867–78.
[171] Curths C, Wichmann J, Dunker S, Windt H, Hoymann HG, Lauenstein HD, et al. Airway hyper-responsiveness in lipopolysaccharide-challenged common marmosets (*Callithrix jacchus*). Clin Sci (Lond) 2014;126:155–62.
[172] Swindle MM, Blum JR, Weiss JL. Morphometric studies of the heart in normal rhesus monkeys (*Macaca mulatta*). J Med Primatol 1986;15:433–40.
[173] Senos R, Benedicto HG, del Rio do Valle CM, del Rio do Valle R, Nayudu PL, Kfoury Junior JR, et al. Gross morphometry of the heart of the Common marmoset. Folia Morphol (Warsz) 2014;73:37–41.
[174] Kumar N, Lee JJ, Perlmutter JS, Derdeyn CP. Cervical carotid and circle of willis arterial anatomy of macaque monkeys: a comparative anatomy study. Anat Rec (Hoboken) 2009;292:976–84.
[175] Qayyum MA. Anatomy and histology of the specialized tissues of the heart of the Indian rhesus monkey, *Macaca mulatta*. Brotéria: Ciéncias Naturais 1974;43:73–94.
[176] Mueller RW, Gill SS, Pulido OM. The monkey (*Macaca fascicularis*) heart neural structures and conducting system: an immunochemical study of selected neural biomarkers and glutamate receptors. Toxicol Pathol 2003;31:227–34.
[177] Kawashima T, Sato K, Akita K, Sasaki H. Comparative anatomical study of the autonomic cardiac nervous system in macaque monkeys. J Morphol 2005;266:112–24.
[178] Kawashima T, Thorington Jr. RW, Whatton JF. Comparative anatomy and evolution of the cardiac innervation in New World monkeys (Platyrrhini, e. Geoffroy, 1812). Anat Rec (Hoboken) 2009;292:670–91.
[179] Goldberg JM, Moberg GP. Autonomic control of heart rate in the neonatal rhesus monkey. J Med Primatol 1985;14:19–27.
[180] Korzick DH. From syncitium to regulated pump: a cardiac muscle cellular update. Adv Physiol Educ 2011;35:22–7.
[181] Milani-Nejad N, Janssen PM. Small and large animal models in cardiac contraction research: advantages and disadvantages. Pharmacol Ther 2014;141:235–49.
[182] Champeroux P, Ouille A, Martel E, Fowler JS, Maurin A, Richard S, et al. A step towards characterisation of electrophysiological profile of torsadogenic drugs. J Pharmacol Toxicol Methods 2011;63:269–78.
[183] Taylor EW, Leite CA, Sartori MR, Wang T, Abe AS, Crossley 2nd DA. The phylogeny and ontogeny of autonomic control of the heart and cardiorespiratory interactions in vertebrates. J Exp Biol 2014;217:690–703.
[184] Leishman DJ, Beck TW, Dybdal N, Gallacher DJ, Guth BD, Holbrook M, et al. Best practice in the conduct of key nonclinical cardiovascular assessments in drug development: current recommendations from the Safety Pharmacology Society. J Pharmacol Toxicol Methods 2012;65:93–101.

[185] Holzgrefe H, Ferber G, Champeroux P, Gill M, Honda M, Greiter-Wilke A, et al. Preclinical QT safety assessment: cross-species comparisons and human translation from an industry consortium. J Pharmacol Toxicol Methods 2014;69:61–101.
[186] Honda M, Komatsu R, Isobe T, Tabo M, Ishikawa T. Involvement of the autonomic nervous system in diurnal variation of corrected QT intervals in common marmosets. J Pharmacol Sci 2013;121:131–7.
[187] Thomas D, Karle CA, Kiehn J. The cardiac hERG/IKr potassium channel as pharmacological target: structure, function, regulation, and clinical applications. Curr Pharm Des 2006;12:2271–83.
[188] Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell 1995;81:299–307.
[189] Aktas MK, Shah AH, Akiyama T. Dofetilide-induced long QT and torsades de pointes. Ann Noninvasive Electrocardiol 2007;12:197–202.
[190] Misner DL, Frantz C, Guo L, Gralinski MR, Senese PB, Ly J, et al. Investigation of mechanism of drug-induced cardiac injury and torsades de pointes in cynomolgus monkeys. Br J Pharmacol 2012;165:2771–86.
[191] Rosati B, Dong M, Cheng L, Liou SR, Yan Q, Park JY, et al. Evolution of ventricular myocyte electrophysiology. Physiol Genomics 2008;35:262–72.
[192] Antzelevitch C. Drug-induced spatial dispersion of repolarization. Cardiol J 2008;15:100–21.
[193] Poelzing S, Rosenbaum DS. Nature, significance, and mechanisms of electrical heterogeneities in ventricle. Anat Rec A: Discov Mol Cell Evol Biol 2004;280:1010–7.
[194] Ruttkay-Nedecky I. Comparative dipolar electrocardiotopographic study of ventricular activation in *Macaca mulatta* monkeys and man. Physiol Res 2004;53:143–7.
[195] Authier S, Haefner P, Fournier S, Troncy E, Moon LB. Combined cardiopulmonary assessments with implantable telemetry device in conscious freely moving cynomolgus monkeys. J Pharmacol Toxicol Methods 2010;62:6–11.
[196] Gien B, Hopper D, Kruzich P, Kurtz S. Automated blood sampling and telemetry recording in awake unrestrained macaques. Drug Metab Rev 2014;45:226.
[197] Chui RW, Derakhchan K, Vargas HM. Comprehensive analysis of cardiac arrhythmias in telemetered cynomolgus monkeys over a 6 month period. J Pharmacol Toxicol Methods 2012;66:84–91.
[198] Kostis JB, McCrone K, Moreyra AE, Gotzoyannis S, Aglitz NM, Natarajan N, et al. Premature ventricular complexes in the absence of identifiable heart disease. Circulation 1981;63:1351–6.
[199] Fleg JL, Kennedy HL. Long-term prognostic significance of ambulatory electrocardiographic findings in apparently healthy subjects greater than or equal to 60 years of age. Am J Cardiol 1992;70:748–51.
[200] Giannico AT, Somma AT, Lange RR, Andrade JNBM, Lima L, Souza AC, et al. Electrocardiographic values in marmosets (*Callithrix penicillata*). Pesqui Vet Bras 2013;33:937–41.
[201] Sleeper MM, Gaughan JM, Gleason CR, Burkett DE. Echocardiographic reference ranges for sedated healthy cynomolgus monkeys (*Macaca fascicularis*). J Am Assoc Lab Anim Sci 2008;47:22–5.
[202] Tsusaki H, Yonamine H, Tamai A, Shimomoto M, Kuwano K, Iwao H, et al. Left ventricular volume and function in cynomolgus monkeys using real-time three-dimensional echocardiography. J Med Primatol 2007;36:39–46.
[203] Kito G. Evaluation of cardiac function in cynomolgus monkeys using real-time three dimensional echocardiography: applications to pharmacological and safety assessment. Nihon Yakurigaku Zasshi 2007;129:437–43.
[204] Schroeder RL, Smith AH, Beebe DA, Sokolowski SA, Huang C, Knight DR. Reductions in Vascular Function Correlate with Age in a Novel Non-Human Primate Model for Assessment of Vascular Health. Circulation 2011;124:A16821 [abstract].
[205] Qiu H, Tian B, Resuello RG, Natividad FF, Peppas A, Shen YT, et al. Sex-specific regulation of gene expression in the aging monkey aorta. Physiol Genomics 2007;29:169–80.
[206] Qiu H, Zhu Y, Sun Z, Trzeciakowski JP, Gansner M, Depre C, et al. Short communication: vascular smooth muscle cell stiffness as a mechanism for increased aortic stiffness with aging. Circ Res 2010;107:615–9.
[207] Zhao Y, Sheng Z, Huang J. A systematic analysis of heart transcriptome highlights divergent cardiovascular disease pathways between animal models and humans. Mol Biosyst 2012;8:504–10.
[208] Vacchi-Suzzi C, Hahne F, Scheubel P, Marcellin M, Dubost V, Westphal M, et al. Heart structure-specific transcriptomic atlas reveals conserved microRNA-mRNA interactions. PLoS One 2013;8:e52442.
[209] Lunn SF. Systems for collection of urine in the captive common marmoset, *Callithrix jacchus*. Lab Anim 1989;23:353–6.
[210] King GJ. Comparative feeding and nutrition in captive, non-human primates. Br J Nutr 1978;40:55–62.

[211] Inada Y, Murakami M, Kaido K, Nakao K. Effects of the new angiotensin II type 1 receptor antagonist KRH-594 on several types of experimental hypertension. Arzneimittelforschung 1999;49:13–21.
[212] Birndorf NI, Lopas H. Effects of red cell stroma-free hemoglobin solution on renal function in monkeys. J Appl Physiol 1970;29:573–8.
[213] Weekley LB, Deldar A, Tapp E. Development of renal function tests for measurement of urine concentrating ability, urine acidification, and glomerular filtration rate in female cynomolgus monkeys. Contemp Top Lab Anim Sci 2003;42:22–5.
[214] Van Gompel JJ, Ottman R, Worrell GA, Marsh R, Wetjen NM, Cascino GD, et al. Use of anterior temporal lobectomy for epilepsy in a community-based population. Arch Neurol 2012;69:1476–81.
[215] Power RA, Power ML, Layne DG, Jaquish CE, Oftedal OT, Tardif SD. Relations among measures of body composition, age, and sex in the common marmoset monkey (*Callithrix jacchus*). Comp Med 2001;51:218–23.
[216] Azar E, Shaw Jr. ST. Effective body water half-life and total body water in rhesus and cynomolgus monkeys. Can J Physiol Pharmacol 1975;53:935–9.
[217] Pickering DE, Sussman HH. Renal function studies in monkeys (*Macaca mulatta*). Am J Vet Res 1962;23:667–72.
[218] Liu CT. Comparison of cardiorenal functions between women and female rhesus macaques. Am J Vet Res 1977;38:1439–42.
[219] Liu CT, Higbee GA. Determination of body surface area in the rhesus monkey. J Appl Physiol 1976;40:101–4.
[220] Feldmahn AL, Smith WK, Leventhal CM. Diurnal patterns of micturition and drinking in rhesus monkeys. Ann N Y Acad Sci 1960;85:828–41.
[221] Hill LR, Hess KR, Stephens LC, Price RE, Gray KN. Correlation of kidney weight and volume and selected skeletal parameters to sex in the adult rhesus monkey (*Macaca mulatta*). J Med Primatol 2001;30:56–60.
[222] Khan KN, Venturini CM, Bunch RT, Brassard JA, Koki AT, Morris DL, et al. Interspecies differences in renal localization of cyclooxygenase isoforms: implications in nonsteroidal antiinflammatory drug-related nephrotoxicity. Toxicol Pathol 1998;26:612–20.
[223] Tisher CC. Structure and Function of the Rhesus Kidney. In: Bourne GH, editor. The Rhesus Monkey, 1. New York: Academic Press; 1975. p. 107–43, 2 vols.
[224] Geist FD. Nasal Cavity, Larynx, Mouth and Pharynx. In: Hartman CG, Straus Jr. WL , editors. The Anatomy of the Rhesus Monkey. Baltimore: Williams & Wilkins Company; 1933. p. 189–209.
[225] Lineback P. The Respiratory, Digestive and Urinary System. In: Hartman CG, Straus Jr. WL , editors. The Anatomy of the Rhesus Monkey. Baltimore: Williams & Wilkins Company; 1933. p. 210–30.
[226] Harkema JR. Comparative aspects of nasal airway anatomy: relevance to inhalation toxicology. Toxicol Pathol 1991;19:321–36.
[227] Schreider JP, Raabe OG. Anatomy of the nasal-pharyngeal airway of experimental animals. Anat Rec 1981;200:195–205.
[228] Saini KD, Breiphol W. Frequency of mast cells in the olfactory mucosa of rhesus monkeys. Cell Tissue Res 1977;178:61–71.
[229] Saini KD, Breipohl W. Surface morphology in the ofactory epithelium of normal male and female rhesus monkeys. Am J Anat 1976;147:433–45.
[230] Smith TD, Rossie JB, Cooper GM, Schmieg RM, Bonar CJ, Mooney MP, et al. Comparative microcomputed tomography and histological study of maxillary pneumatization in four species of new world monkeys: the perinatal period. Am J Phys Anthropol 2011;144:392–410.
[231] Hunter AJ, Fleming D, Dixson AF. The structure of the vomeronasal organ and nasopalatine ducts in *Aotus trivirgatus* and some other primate species. J Anat 1984;138(Pt 2):217–25.
[232] Hilloowala RA, Lass NJ. Spectrographic analysis of laryngeal air sac resonance in rhesus monkey. Am J Phys Anthropol 1978;49:129–31.
[233] Plopper CG, Heidsiek JG, Weir AJ, George JA, Hyde DM. Tracheobronchial epithelium in the adult rhesus monkey: a quantitative histochemical and ultrastructural study. Am J Anat 1989;184:31–40.
[234] Plopper CG, Hill LH, Mariassy AT. Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung. III. A study of man with comparison of 15 mammalian species. Exp Lung Res 1980;1:171–80.
[235] Barbier A, Bachofen H. The lung of the marmoset (*Callithrix jacchus*): ultrastructure and morphometric data. Respir Physiol 2000;120:167–77.
[236] Plopper CG, Alley JL, Weir AJ. Differentiation of tracheal epithelium during fetal lung maturation in the rhesus monkey *Macaca mulatta*. Am J Anat 1986;175:59–71.

[237] Plopper CG, Weir AJ, Nishio SJ, Cranz DL, St George JA. Tracheal submucosal gland development in the rhesus monkey, *Macaca mulatta*: ultrastructure and histochemistry. Anat Embryol (Berl) 1986;174:167–78.
[238] Taylor EM, Sutton D, Lindeman RC. Dimensions of the infant monkey upper airway. Growth 1976;40:69–81.
[239] Kerr GR, Couture J, Allen JR. Growth and development of the fetal Rhesus monkey. VI. Morphometric analysis of the developing lung. Growth 1975;39:67–84.
[240] Perelman RH, Engle MJ, Kemnitz JW, Kotas RV, Farrell PM. Biochemical and physiological development of fetal rhesus lung. J Appl Physiol Respir Environ Exerc Physiol 1982;53:230–5.
[241] Gnanalingham MG, Mostyn A, Gardner DS, Stephenson T, Symonds ME. Developmental regulation of the lung in preparation for life after birth: hormonal and nutritional manipulation of local glucocorticoid action and uncoupling protein-2. J Endocrinol 2006;188:375–86.
[242] Coppens JT, Plopper CG, Murphy SR, Van Winkle LS. Postnatal lung development of rhesus monkey airways: cellular expression of Clara cell secretory protein. Dev Dyn 2009;238:3016–24.
[243] Plopper CG, St George JA, Read LC, Nishio SJ, Weir AJ, Edwards L, et al. Acceleration of alveolar type II cell differentiation in fetal rhesus monkey lung by administration of EGF. Am J Physiol 1992;262:L313–21.
[244] Hyde DM, Tyler NK, Putney LF, Singh P, Gundersen HJ. Total number and mean size of alveoli in mammalian lung estimated using fractionator sampling and unbiased estimates of the Euler characteristic of alveolar openings. Anat Rec A: Discov Mol Cell Evol Biol 2004;277:216–26.
[245] Binns R, Clark GC, Simpson CR. Lung function and blood gas characteristics in the rhesus monkey. Lab Anim 1972;6:189–98.
[246] Bhansali PV, Irvin CG, Dempsey JA, Bush R, Webster JG. Human pulmonary resistance: effect of frequency and gas physical properties. J Appl Physiol Respir Environ Exerc Physiol 1979;47:161–8.
[247] Bush M, Custer R, Smeller J, Bush LM. Physiologic measures of nonhuman primates during physical restraint and chemical immobilization. J Am Vet Med Assoc 1977;171:866–9.
[248] Munson ES, Gillespie JR, Wagman IH. Respiratory blood gases and pH in two species of unanesthetized monkeys. J Appl Physiol 1970;28:108–9.
[249] Trulock EP. Arterial Blood Gases. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edit. Boston: Butterworths; 1990.
[250] Liu CT, KeLauter RD. Pulmonary functions in conscious and anesthetized rhesus macaques. Am J Vet Res 1977;38:1843–8.
[251] Kosch PC, Gillespie JR, Berry JD. Respiratory mechanics in normal bonnet and rhesus monkeys. J Appl Physiol Respir Environ Exerc Physiol 1979;46:166–75.
[252] Pare PD, Boucher R, Michoud MC, Hogg JC. Static lung mechanics of intact and excised rhesus monkey lungs and lobes. J Appl Physiol Respir Environ Exerc Physiol 1978;44:547–52.
[253] O'Neil RM, Ashack RJ, Goodman FR. A comparative study of the respiratory responses to bronchoactive agents in rhesus and cynomolgus monkeys. J Pharmacol Methods 1981;5:267–73.
[254] Seehase S, Schleputz M, Switalla S, Matz-Rensing K, Kaup FJ, Zoller M, et al. (2011). Bronchoconstriction in nonhuman primates: a species comparison. J Appl Physiol 1985;111:791–8.
[255] Asgharian B, Price O, McClellan G, Corley R, Einstein DR, Jacob RE, et al. Development of a rhesus monkey lung geometry model and application to particle deposition in comparison to humans. Inhal Toxicol 2012;24:869–99.
[256] Corley RA, Kabilan S, Kuprat AP, Carson JP, Minard KR, Jacob RE, et al. Comparative computational modeling of airflows and vapor dosimetry in the respiratory tracts of rat, monkey, and human. Toxicol Sci 2012;128:500–16.
[257] Alexander GM, Hines M. Sex differences in response to children's toys in nonhuman primates (*Cercopithecus aethiops sabaeus*). Evol Hum Behav 2002;23:467–79.
[258] Hines M, Alexander GM. Monkeys, girls, boys and toys: a confirmation Letter regarding "Sex differences in toy preferences: striking parallels between monkeys and humans". Horm Behav 2008;54:478–9 author reply 480–1.
[259] Gauvin DV, Baird TJ. A functional observational battery in non-human primates for regulatory-required neurobehavioral assessments. J Pharmacol Toxicol Methods 2008;58:88–93.
[260] The Marmoset Genome Sequencing and Analysis Consortium. The common marmoset genome provides insight into primate biology and evolution. Nat Genet 2014;46:850–7.
[261] Wang W, Liu JY, Wang HF, Yang MY, Liu QY, Ding MX. The complete mitochondrial genome of white-tufted-ear marmoset, Callithrix jacchus (Primates: Callitrichinae). Mitochondrial DNA 2014; Epub ahead of print.

VetBooks.ir

VetBooks.ir

CHAPTER

9

Implications and Limitations of Reference Values and Historical Data for the Interpretation of Nonhuman Primate Toxicology Studies

Harry Yang[*], *Laura Richman*[*], *Robert Hall*[†]

[*]Translational Sciences, MedImmune, Gaithersburg, MD, USA

[†]Covance Laboratories Inc, Madison, WI, USA

OUTLINE

Introduction 173

Utility of Reference Intervals for Nonhuman Primate Safety Studies 174

Limitations of Reference Intervals for Safety Assessment 178

Conclusion 182

References 182

INTRODUCTION

The use of nonhuman primates for nonclinical safety studies is highly regulated by animal welfare regulations and faces increasing ethical scrutiny. Therefore, toxicology studies with nonhuman primates are usually conducted with few animals per dose group and of each sex. In addition, animal-to-animal variability in nonhuman primates is usually considerably greater compared to small rodent studies using in-bred strains. These unique features often make it difficult to assess the biological significance of an observed effect by comparing the

http://dx.doi.org/10.1016/B978-0-12-417144-2.00009-3
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

data of the treated animal groups with the data from the control animals. To overcome this limitation in study design, reference values or historical control data are frequently used to justify whether an observed effect is considered related to treatment or within the normal variability within the species. This chapter discusses the utility and the limitations of the use of reference values and/or historical control values to aid in the interpretation of data derived from safety studies using nonhuman primates.

UTILITY OF REFERENCE INTERVALS FOR NONHUMAN PRIMATE SAFETY STUDIES

Harry Yang and Laura Richman

Translational Sciences, MedImmune, Gaithersburg, MD, USA

Nonhuman primates are phylogenetically and physiologically similar to humans. As such, they often are used in the assessment of the safety of new drugs. However, despite their predictive value, studies involving nonhuman primates are both costly and often constrained by animal availability. In addition, ethical considerations further prevent liberal use of nonhuman primates. Although lacking in scientific and statistical justification, three to five primates per sex/group have been conventionally used in preclinical safety assessment. Because the intersubject variability in nonhuman primates is conceivably large, data from small studies alone tend to render a limited chance to detect differences between the test article and control groups. Therefore, using all available data, including those from previous experiments, is fundamental in evaluating safety—even more so when the incidence rates of potential toxicity related to the test article are low. Leveraging knowledge and data from historical studies is the key to more reliable safety assessment.

One source of information that can be used to help data interpretation is the reference intervals for safety parameters. The concept of using population-based reference values for clinical diagnosis was first introduced by Gräsbeck in 1969. Not until 1978 did the application of the concept to animal populations begin [1]. Ever since it has gained wide acceptance and become one of the most commonly used tools in both human and animal safety studies. Reference intervals are usually developed by applying statistical treatment to historical data and are expressed as numerical limits. The limits often are determined such that they contain 95% of results from the healthy population. To this end, statistical methods, both parametric and nonparametric, are used. The parameter methods rely on the assumption that the test results are normally distributed, whereas nonparametric approaches require relatively larger sample sizes. When the normality assumption of the underlying data distribution does not hold, data are usually transformed to achieve approximate normality before the reference intervals are constructed. In the case where acquisition of sufficient historical controls is challenging and the normality assumption cannot be met regardless of data transformation, a nonparametric method called bootstrapping can be used to achieve reliable reference intervals that are not sensitive to distributional assumptions [2]. The technique uses samples repeatedly drawn from an empirical distribution of the available data to construct point and interval estimates [3]. Other robust methods may be used to compensate for the issue of small sample sizes [18]. Once determined, reference intervals provide the baseline against which

VetBooks.ir

test article safety is assessed. Subjects with safety measures falling within the intervals are deemed to have no adverse reactions. Conversely, values outside of the interval corroborate possible adverse findings.

Historical data can also be directly used to aid data interpretation. As previously pointed out, preclinical studies tend to be small, and even more so for nonhuman primate trials. With three to five animals per sex/group, there is hardly any statistical power to detect any deleterious effects related to treatment unless they are much more pronounced. Therefore formal statistical inferences benefit from the synthesis of information from all sources of available data. Combining data from both concurrent and historical controls is an effective and efficient way to accurately estimate baseline measurements, thus enhancing the chance of detecting differences between the control and treatment groups. Certainly, care must be taken to ensure comparability between historical and concurrent controls. In fact, even if the two sets of controls are not comparable, there are statistical methodologies that can be used to synthesize information from all available data. For example, statistical meta-analysis such as random effect modeling can be conducted to tease out the effects caused by the aforesaid variables when assessing the effect induced by the test article. The value and scientific advantages of historical data have been fully recognized by the clinical laboratory community [4–6,19]. Various applications of statistical methods using historical controls for safety assessment have been reported by investigators [7–9,19]. Sutton et al. discuss in detail statistical methods combining results from different sources, including historical controls [10]. Appropriate applications of these methods enable researchers to conduct safety assessment in the presence of additional sources of uncertainties.

A statistical technique called Bayesian analysis has increasingly been used to incorporate historical controls in the interpretation of preclinical study data [11]. A noted example is given by Dempster et al. [7]). By considering historical controls together with concurrent controls as a random sample from a hypothetical superpopulation, a method allowing for compromise between complete pooling of data and ignoring historical controls was developed. The degree of pooling is determined by experiment-to-experiment variability in the historical data. The method was successfully used to analyze data from several preclinical animal studies. In general, unlike the conventional statistical methods, Bayesian analysis views safety parameters of interest as random variables. Control data from historical studies can be used to provide understanding of the distributions of the variables, which often are referred to as "priors." The more consistent and uniform the studies are, the tighter those statistical distributions are, and the greater confidence one has in the historical control. Taken together with data from the control arm of the current study, posterior distributions of the safety measures are obtained, as are the estimates of means and intervals of safety measures. The mean estimates are averages of historical and concurrent controls, weighted by variability of the data. So, if study-to-study variability is large, the total variability in the historical data is large. As a result, the Bayesian estimates would be weighted more toward that of the current controls. The means and intervals estimated from the Bayesian analysis provide better comparators for the test article.

Historical controls become more valuable when they are collected from the same laboratory, when the same strain of animals is used in repeated experiments, and when standardized procedures and husbandry practices implemented. A control chart consisting of a mean value and lower and upper limits covering 95% of the healthy population can be constructed and used to detect any anomalies in concurrent controls possibly caused by deviations in sample collection,

VetBooks.ir

handling, and testing. It can also be used as a tool to select animals for new studies. Animals whose measures fall within the controls' limits are deemed proper candidates for the studies, whereas animals with measures outside of the limits are considered unsuitable. As cautioned by Hall in Chapter 9, however, exclusion of an animal from a study cannot be solely based on one or two results that are outside of the reference intervals. In fact, because there are usually more than 40 clinical pathology tests performed for each animal, the probability of at least one measured result being out of its corresponding reference interval is greater than 87.1% ($1–0.95^{40}$). Therefore, an outlying result needs to be interpreted in relation to other safety measures.

It is also worth pointing out that historical data from laboratories implementing the same or similar standards may be pooled to further enhance the quality and reliability of control data and reference intervals developed from the data. In addition, there is another benefit of reference intervals that is underappreciated. Specifically, because nonhuman primate studies are, in general, small in size, detecting difference between test and control arms is difficult. Knowing that reference intervals based on historical data are usually wider than those constructed using concurrent controls, statistical significance can be claimed if a result from the test article arm is outside of the range; the outlying result would exceed the limits of the reference interval based on the concurrent controls, which are presumably narrower. In this case, the additional sources of variations in the reference intervals in effect enhance confidence in the adverse finding.

However, because many factors such as supplier, origin, husbandry, and test methods may influence the quality and reliability of reference intervals, use of reference intervals and historical data in toxicological assessment has for years been a topic of vigorous debate. Some support reference intervals as a reliable tool; some suggest their use with caution; and others either dismiss or discount the utility of reference intervals [12]. The primary argument of the opposing view is that differences in study populations make reference intervals virtually irrelevant to the interpretation of data from the current study. While recognizing those limiting factors, we believe that reference intervals, along with historical data, contain valuable information that can be used advantageously to improve the accuracy of safety assessment. This is especially true when relevant and sufficient knowledge of the historical controls is readily available, making it possible to either reestablish the reference intervals based on a subclass of more homogeneous controls or incorporate the partitioning factors as covariates in appropriate statistical methods. Therefore the point of debate should not be whether historical data and reference intervals are useful in aiding data interpretation of the current study. Instead it should be how to maximize the use of control data from previous experiments. With applications of appropriate statistical techniques, the issue can be adequately addressed. For instance, to set reference intervals that are affected less by study-to-study variability, a well-developed statistical method called variance component analysis can be performed. The method apportions total variation of the historical control data into inter- and intrastudy variability. In doing so, one has the ability to reestablish new reference intervals after removing interstudy variation. As an alternative, one may use statistical methods to assess the comparability of control data from various previous studies so that reference intervals are established using data only from studies that are deemed comparable. In statistical literature, many methods are available for this purpose. For example, the above-mentioned variance component analysis provides a formal test to assess whether

VetBooks.ir

interstudy variability is statistically significantly different from zero. This analysis relies on the assumption that the data under evaluation are normally distributed. When this assumption does not hold, one can either perform the analysis after transferring the data or use nonparametric methods. For example, the Wilcoxon test can be used to assess compatibility between the distributions of two data sets. These tests can be readily performed with many commercially available statistical software packages such as SAS [13]. Likewise, the same statistical treatment can be applied to historical data consisting of data from several laboratories so that both study-to-study and lab-to-lab variations can be taken out before setting reference intervals for a specific study. In addition, statistical methods including Bayesian analysis, which are discussed earlier in this chapter, can be effectively applied to aid safety assessment using combined data from the current and previous experiments.

Recognizing that many factors may influence reference intervals, over the years significant efforts have been made to develop guidelines for determining reference intervals in veterinary species following scientific and statistically sound methods and study designs [14,15,18]. The guidelines put forth by a working group commissioned by the American Society for Veterinary Clinical Pathology provides a uniform and consistent approach to the selection of reference individuals, sample collection and testing, and using statistically sound methods to set reference intervals for a variety of animal species [14]. The method, largely modeled after the CLSI document [18], includes both practical and statistical considerations. It also provides recommendations for determining multicenter reference intervals and for transferring and validating reference intervals from other sources when acquiring a large amount of reference data is challenging. In addition, the use of subject-based reference intervals when intrasubject variation is less than intersubject variability is discussed and suggested. Collectively, these efforts make reference intervals more and more useful for animal safety assessment.

As pointed out by many researchers, while important, historical control data should be viewed as one of many sources of information that contribute to the overall "weight of evidence" in preclinical safety assessment [16,17]. A result outside of the reference interval does not necessarily signify an important safety concern because it might be caused by either animal biological variability or analytical variations. Correlative measures need to be assessed, and a clinical decision should be made only in light of the totality of the data. In doing so, one may minimize the chance of false-positive findings that might inadvertently cause early termination of otherwise promising drugs and increase the probability of detecting unacceptable toxicity possibly related to the test article.

In conclusion, the use of historical data and reference intervals is a critical component of preclinical safety assessment. Given the limiting factor of the small size of most nonhuman primate safety studies, full utilization of historical data is crucial for reliable safety assessment. With proper applications of statistical methods, useful information can be synthesized from historical controls and combined with concurrent controls to provide a baseline for toxicological evaluations. In addition, compliance with industry guidelines in collecting reference individuals would enhance the ability of clinical laboratories to use a relevant subpopulation of historical controls to establish reference intervals for studies at hand. With all measures taken, judicious use of historical controls and reference intervals is not only possible but advantageous.

VetBooks.ir

LIMITATIONS OF REFERENCE INTERVALS FOR SAFETY ASSESSMENT

Robert Hall

Covance Laboratories Inc, Madison, WI, USA

Reference intervals (also referred to as reference ranges, normal ranges, or historical control data) have been used as a tool in preclinical safety assessment for as long as numerical data have been generated by participating laboratories. Reference intervals are, however, only one tool of many for data interpretation, and their value is heavily influenced by the manner of their construction and characteristics of the safety assessment study in question. Reference intervals are perhaps most valuable for assessing the relative health or normalcy of animals before administering the test article and for providing perspective concerning normal inter-animal variability for different clinical pathology parameters and other physiological end points. They also have the less recognized benefit of serving as a form of quality control for sample collection and handling procedures, instruments and assays, and the animals themselves. However, reference intervals are generally an inappropriate tool for identifying test article—related effects and can be a misleading tool for determining the toxicologic importance of test article—related effects.

Reference intervals for any given parameter typically define the central 95% of expected results, bounded by upper and lower limits, in animals deemed to be healthy. The reference population of healthy animals is defined by specific partitioning criteria (e.g., species, strain, sex, age), but many other factors influence reference intervals, including, but not limited to, the animal supplier, country of origin, husbandry practices, diet, fasting status, sample collection and handling procedures, sample matrix, and laboratory methods. For reference intervals to have value, the partitioning criteria defining the reference population must closely match the animals and study in question, and that is usually problematic.

Detailed guidelines for constructing clinical pathology reference intervals in veterinary medicine have been published [14] and mirror the CLSI recommendations for human medicine [15]. Ideally, a minimum of 120 reference animals are recommended to determine upper and lower reference interval limits by nonparametric methods with 90% confidence intervals. If the reference animal sample size is smaller, other statistical methods can be used, but the amount of uncertainty in the estimation of reference limits increases and the value of the reference interval decreases. For nonhuman primates, there is a good news/bad news aspect to the construction and use of reference intervals. The good news is that (1) many animals are available for a reference population by taking samples and measurements from animals before initiating the study, and (2) animal age is less critical than for rodents, which change considerably during even a short study. A standard preclinical study with 3-5 animals per sex/group represents 12-20 potential reference animals of each sex. It is relatively easy for a large company or institution that does a fair amount of preclinical safety assessment work to obtain data from a large number of cynomolgus monkeys of each sex for construction of reference intervals. The bad news is that once a study is initiated, study-related procedures introduce many new variables (i.e., additional potential partitioning criteria) that did not affect animals in the prestudy reference population. Variables that cause prestudy reference intervals to lose value when interpreting data for any given study include factors such as daily handling and dosing; unusual vehicle control articles; changes in housing and/or pen mates; anesthesia

VetBooks.ir

for procedures such as ophthalmic examinations; multiple blood collections for toxicokinetics, antidrug antibodies, pharmacodynamic end points, and/or novel biomarkers; telemetry instrumentation; and indwelling intravenous catheters. Because these variables often differ considerably from one study to the next, it would take a long time to construct more appropriate reference intervals from control animals handled and dosed in the same manner, administered the same vehicle control article, bled a similar volume a similar number of times, and so on. While lumping together control animal data from different studies increases the size of the reference population, it also broadens reference intervals and further reduces their relevance for data interpretation. Ultimately, that is why studies include control groups and why prestudy data for individuals and data from concurrent control animals are far more valuable than reference intervals in the identification of test article—related effects.

Published reference intervals for clinical pathology test results serve virtually no purpose for preclinical safety assessment data interpretation because of all the variables previously mentioned that generally differ from one institution to another. As important, it is almost certain that published test results were not determined using the same instruments and reagents by technologists with the same skill sets following the same standard operating procedures as those used in any other laboratory. Although published reference intervals can serve as a rough guide to differences between species (or animal origin within a single species) and what one may expect from their own laboratory if they have limited or no experience with a certain species, they are an inappropriate substitute for reference intervals established within one's own laboratory.

If an institution has a study volume large enough to establish reference intervals based on a large number of animals and does so, it is critical that scientists in that institution understand their appropriate use and inherent limitations for data interpretation. Perhaps most important, in-house reference intervals provide appropriate context for animal selection before initiating a study. Animals with results well outside the reference interval, especially if several parameters are affected or there is a pattern that signals a clear health concern, should not be included in the study, if possible, until their issues are resolved. However, one or two results slightly outside the reference interval should not be the sole basis for eliminating a study candidate. Given that reference intervals are constructed such that 5% (1 of 20) of all results from the reference population of healthy animals are outside reference limits, it should be expected that several animals will have at least one atypical result out of the ≥40 clinical pathology tests usually performed. It was once relatively common to see study protocols that stated animals should not be used if they had test results outside of "historical controls" or "normal ranges." That requirement was, and is, nearly impossible to satisfy and should never be included in a study protocol.

An underappreciated aspect of a well-constructed reference interval is that it serves a quality control function. If a shipment of animals has test values that are clearly different from what has come to be expected, three possibilities exist: either the animals are somehow different, whether due to health, origin, husbandry, or some other factor; a standard procedure for acclimation or sample collection and handling has changed or was not followed (e.g., animals were not fasted); or the assay is somehow different, whether due to a technical problem, instrument or reagent change, or some other factor. Regardless of the reason, the reference interval serves as an alert that something is different. It could be a significant problem that needs to be corrected, or it could simply be that an unrecognized change was made. If the

VetBooks.ir

latter occurred (e.g., a reagent change), reestablishing the reference interval may be necessary. The frequency with which new reference intervals should be established is up to the institution and depends not only on the amount and kind of change that has taken place but also on how their reference intervals are used. Considering the many caveats associated with using reference intervals for data interpretation, it is not critical that very current reference intervals be strictly maintained for that purpose. Without software and/or processes developed specifically for that purpose, this can be a laborious task with a poor cost-to-benefit ratio. If required for some reason, however, one option is to periodically add newly acquired reference animal data while concurrently eliminating a similar amount of the oldest data. This method of maintaining current reference intervals would be inappropriate if wholesale changes were made to procedures or assays, but such large changes are uncommon in most large institutions.

Once dosing is initiated in a preclinical safety assessment study, reference intervals are of limited value because they have little relevance to the two most important questions to be answered concerning differences observed between control and treated animals. Are the differences real? That is, do they represent true effects of the test article? And if real, are they bad? That is, are they toxicologically important or adverse? The first problem with using reference intervals to help answer either question was alluded to previously. Reference animals used for establishing reference intervals rarely match animals in any given study because they have not undergone study-related procedures such as daily handling, dosing, vehicle administration, blood collection, anesthesia for special examinations, and instrumentation for telemetry or intravenous dose administration. Each of these can affect the test results of study animals and in some cases cause results to move outside reference limits. Hematology parameters are especially susceptible to study-related procedures. Iatrogenic anemia is a common occurrence in nonhuman primate preclinical studies because of the number of blood samples collected for a myriad of tests. Even if the calculated volume of blood taken does not seem excessive, it does not account for rebleeding and blood loss at venipuncture sites once animals are returned to their pens. Evidence of inflammation such as increased leukocyte counts and fibrinogen concentration and decreased albumin-to-globulin ratio (caused by decreased albumin, a negative acute-phase protein, and increased globulin) is commonplace in studies using indwelling catheters for intravenous infusion. Decreased platelet counts also are frequently observed in individual animals dosed by continuous intravenous infusion. Stress associated with procedures can affect clinical pathology parameters, most notably decreased absolute lymphocyte and eosinophil counts, in some studies. On the other hand, once animals become more accustomed to frequent handling and blood collection procedures during the course of a study, they are less likely to have the "fight or flight" catecholamine response that some animals experience when bled before study initiation. In other words, total white blood cell counts and glucose concentrations are often higher for the reference animal population than for animals that become veterans of various procedures. Marked increases in creatine kinase activity and smaller increases in aspartate and alanine aminotransferase activities occur following intramuscular ketamine injection and can be difficult to avoid when trying to fit multiple end points into the final days of a short study. Creatine kinase activity may remain elevated in some individuals for 4 or 5 days. Finally, some vehicle control articles (e.g., corn oil) can also cause changes. All of these examples demonstrate the reality that the reference animal population used to

VetBooks.ir

construct reference intervals is often a poor reflection of animals in any given preclinical safety study. Subsequently, those reference intervals are much less valuable for data interpretation than each study animal's respective baseline data and data from concurrent control animals. Even if the reference animal population closely resembles animals in a study with respect to all the variables mentioned, the small number of animals in the study still will not have results that perfectly reflect the reference interval. That is, results from study animals unaffected by vehicle or a test article will not have the same means or distributions as the reference interval from a large reference population; their results may fall at either end of the reference interval or even be slightly outside.

Reference intervals, even well-constructed ones, should not be used to determine whether a difference between control and treated animals is real or related to the test article. Whether results of treated animals fall inside or outside a reference interval should not influence interpretation. Effects of small magnitude tend to be the rule for clinical pathology test results if dose selection is done well, and many, if not most, results fall within reference limits. Conversely, results outside reference limits do not automatically mean that a difference from control is related to the test article. It is entirely possible, and often the case, that study-related procedures (e.g., blood collections) move results for all animals, including controls, to one end or the other of a reference interval. Results for some treated animals can easily fall outside the reference interval as an incidental finding. When determining whether differences between control and treated animals are related to the test article, many factors should be considered in a weight of evidence approach. These factors include, but are not limited to, the size of the difference, consistency over time, consistency between sexes (acknowledging sex differences exist), dose dependence (less predictable for large molecules), correlative clinical observations or anatomic pathology findings, a pattern of related changes, statistical significance, differences before initiation of dosing, timing of the change after dosing, normal inter- and intra-animal variability for the affected analyte, normal analytical variability for the affected analyte, number of animals tested, possible effects of study-related procedures, possible effects of the test article based on prior studies or drug class knowledge, and even the appearance of reversibility during recovery.

If a difference between control and treated animals is determined to be real, then how is its toxicologic importance determined? It seems intuitive that reference intervals (historically called normal ranges) would help determine what is abnormal. Although it is true that reference intervals have more relevance to this question than the first, reference intervals are sometimes used erroneously. It is faulty reasoning to conclude differences between control and treated groups are not adverse or toxicologically important simply because values for the treated group fall within a reference interval. It is just as inappropriate to conclude differences between control and treated groups are adverse or toxicologically important because values for the treated group fall outside a reference interval. A minor, test article—related, nonadverse reduction in red cell mass (i.e., red blood cell count, hemoglobin concentration, and hematocrit) associated with decreased food consumption and weight loss could easily result in values outside of the reference interval if animals were bled multiple times during the dosing phase. On the other hand, a similar minor reduction in red cell mass related to the test article could occur 1 to 2 weeks after administration of a test article that destroys the bone marrow without any of the results dropping below the lower reference limits because not enough time had passed or animals were slightly dehydrated. Many factors are used to determine

VetBooks.ir

the adverseness of a test article—related effect, and a weight of evidence approach is again needed. Most important is the presence of correlative findings indicating a deleterious effect on organ function, tissue integrity, overall health, or survival. While it is true that results far outside reference limits are more likely to be toxicologically important, they are nearly always associated with significant correlative findings if the effect is adverse or a marker of an adverse effect. The mechanism responsible for an effect, if known, is another critical factor to consider. Increases in urea and creatinine concentrations can be larger for animals mildly dehydrated due to emesis or liquid feces than for animals with renal tubular degeneration and necrosis, but the latter condition is of far greater toxicologic importance.

CONCLUSION

Reference intervals have a role in preclinical safety assessment, but that role is limited. Published reference intervals serve only as a guide to species differences, interanimal variability for different parameters, and what a laboratory might expect in the initial stages of obtaining data from a species not previously studied. In-house reference intervals are most relevant for evaluating data obtained before the initiation of dosing and can provide a quality control function. Once dosing begins, the reference animal population used to construct the intervals is rarely a good match for animals being studied. Concurrent control animals are the truest sample of the population to which treated animals belong, and prestudy data can function as each animal's own control. A weight of evidence approach is necessary for determining (1) whether differences between control and treated animals are related to the test article and (2) whether test article—related differences are toxicologically important or adverse. Reference intervals are the wrong tool for the former determination and a relatively poor tool for the latter.

References

[1] Lumsden JH, Mullen K. On establishing reference values. Can J Comp Med 1978;42:293–301.
[2] Dimauro CN, Macciotta NP, Rassu SP, Patta C, Pulina G. A bootstrap approach to estimate reference intervals of biochemical variables in sheep using reduced sample sizes. Small Ruminant Res 2009;83:34–41.
[3] Efron B, Tibshirani RJ. An Introduction to Bootstrapping. New York: Chapman & Hall; 1994.
[4] Blankenship B, Skaggs Hollie. Findings in historical control Harlan RCCHanTM: WIST rats from 4-, 13-, 26-week studies. Toxicol Pathol 2013;41:537–47.
[5] Deschl U, Kittel B, Rittinghausen S, Morawietz G, Kohler M, Mohr Ulrich, et al. The Value of Historical Control Data—Scientific Advantages for Pathologists, Industry and Agencies. Toxicol Pathol 2002;30:80–7.
[6] Vojta A, et al. Hematological and biochemical reference intervals in Dalmatian pramenka sheep estimated from reduced sample size by bootstrap resampling. Veterinarski Arhiv 2011;81(1):25–33.
[7] Dempster AP, Selwyn MD, Weeks BJ. Combining historical and randomized controls for assessing trends in proportions. J Am Stat Assoc 1983;78:221–7.
[8] Tarone RE. The use of historical control information in testing for a trend in proportion. Biometrics 1982;38:215–20.
[9] Hoel DG, Yanagawa T. Incorporating historical controls in testing for a trend in proportions. J Am Stat Assoc 1986;81:1095–9.
[10] Sutton AJ, et al. Methods for Meta-Analysis in Medical Research. Chichester: John Wiley & Sons; 2000.
[11] Salburg DS. Statistics for Toxigologists. New York and Basel: Marcel Dekker; 1986.
[12] Hall R. Lies, damn lies, and reference intervals (or hysterical control values for clinical pathology data). Toxicol Pathol 1997;25:647–9.
[13] SAS (2014). SAS/STAT User's Guide. http://www.math.wpi.edu/saspdf/stat/pdfidx.htm.

[14] Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, et al. ASVCP Reference Interval Guidelines: Determination of de novo Reference Intervals in Veterinary Species and Other Related Topics. Vet Clin Pathol 2012;41:441–53.
[15] Horowitz, G.L., Altaie, S., Boyd, J.C., et al. Clinical and Laboratory Standards Institute (CLSI). (2008). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - 3rd Edition. CLSI document C28-A3.
[16] Hall R. Reference intervals for safety assessment: implications and limitations. In: Bluemel J, Korte S, Schenck E, Weinbauer G, editors. The Nonhuman Primate in Drug Development and Safety Assessment. Massachusetts: Elsevier; 2014.
[17] Keenan C, Elmore S, Francke-Carroll S, Kemp R, Kerlin R, Peddada S, et al. Best practices for use of historical control data of proliferative rodent lesions. Toxicol Pathol 2009;37:679–93.
[18] CLSI. EP28-A3c Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline. 3rd ed. CLSI document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
[19] Haseman JK. Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ Health Perspect 1984;58:385–92.

VetBooks.ir

VetBooks.ir

Part III

PATHOPHYSIOLOGY OF NONHUMAN PRIMATE SPECIES USED IN NONCLINICAL SAFETY ASSESSMENT AND DRUG DEVELOPMENT

VetBooks.ir

VetBooks.ir

CHAPTER

10

Nonhuman Primate Diseases of Relevance in Drug Development and their Impact on the Interpretation of Study Findings

Chandrassegar Saravanan, Vito G. Sasseville, Keith G. Mansfield

Novartis Institutes for Biomedical Research, Inc., Cambridge, MA, USA

OUTLINE

Introduction 188

Viral Infections 190
- *Simian Betaretroviruses (Simian Type D Retroviruses)* 190
- *Measles (Rubeola) Virus* 191
- *Macacine Herpesvirus-1 (Herpes B Virus)* 192
- *Cytomegalovirus* 194
- *Macacine Herpesvirus 4 (Rhesus Lymphocryptovirus)* 195
- *Simian Adenoviruses* 196
- *Simian Virus 40* 198
- *Simian Parvovirus* 198

Bacterial Infections 199
- *Mycobacterium tuberculosis* 199
- *Moraxella catarrhalis* 200
- *Helicobacter pylori* 200

Parasitic Infections 200
- *Malaria* 200
- *Trypanosoma cruzi* 201
- *Toxoplasma gondii* 202
- *Balantidium coli, Trichomonas spp., and Cryptosporidium muris-Like Protozoa* 202

Fungal Infections 203
- *Candida albicans* 203
- *Pneumocystis carinii* 204
- *Pneumonyssus simicola* 204

Conclusion 205

References 205

http://dx.doi.org/10.1016/B978-0-12-417144-2.00010-X
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

Nonhuman primates (NHPs), primarily cynomolgus (*Macaca fascicularis*) and less frequently rhesus macaques (*Macaca mulatta*), have been increasingly used in preclinical safety assessment studies because of their high specificity for biopharmaceuticals and relevant pharmacology [1–3]. The common marmoset (*Callithrix jacchus*), a New World species, has been used infrequently [4]. Unlike other laboratory animal species, elimination of infectious diseases from NHP colonies has proven to be difficult, and several pathogens remain a significant threat to animal and colony health [5–8]. Infectious diseases may adversely affect the health of study animals and confound study findings.

In recent decades, much progress in the husbandry of NHPs maintained in research facilities has been made to prevent major infectious disease outbreaks and at times eliminate specific pathogens from captive colonies [9–14]. However, the increased demand for macaques in biomedical research has resulted in the shortage of these animals [15]. Thus, in addition to the well-established Mauritius-origin cynomolgus monkeys, importation of macaques from China and Southeast Asia has increased substantially. The importation of NHPs from different geographic areas with varying background susceptibility [16–19] to primary and opportunistic pathogens may predispose them to new or novel infections or serve as the nidus of the spread of infectious agents to other indigenous or specific pathogen-free (SPF) research colonies, potentially affecting study outcomes in addition to posing a risk to the human populations when exposed to zoonotic pathogens [20,21].

We now understand that host-pathogen coevolution allows the propagation and persistence of microbes within a species, often with a limited detrimental effect on the host [22,23]. In contrast, when the host's normal immune response is impaired—either from environmental stress, experimental or natural infection with immunosuppressive viruses, or preclinical testing of immunomodulatory agents—the host-pathogen balance is impaired and may cause potentially confounding disease [5,6,24,25]. For example, nearly all Old World and New World NHPs harbor infectious agents such as cytomegaloviruses (CMVs) and lymphocryptoviruses (LCVs) as lifelong persistent infections. During immunosuppression, these latent viruses may reactivate, causing progressive cytolytic or lymphoproliferative diseases, respectively. In some viral infections, the clinicopathologic presentations of primary infection differ from the reactivation of latent infection, confounding accurate diagnosis and proper interpretation of study findings. For instance, central nervous system (CNS) manifestation of primary simian polyomavirus (simian virus 40 [SV40]) infection in immunosuppressed rhesus macaques is associated with meningoencephalitis as opposed to progressive multifocal leukoencephalopathy (PML) in recrudescent infection [26]. Clearly, infectious diseases may adversely affect research programs and the interpretation of study findings. However, elimination of all infectious agents from NHP colonies is not possible because of husbandry challenges and ethical reasons [9]. Therefore, the present strategies for microbial quality control in NHPs include appropriate prepurchase evaluation of source colonies and animals, strict quarantine measures, infectious disease preventive health programs, and eradication and control of specific infectious agents, as well as occupational health and safety programs for the animal handlers [9–14].

This chapter describes the common primary and opportunistic viral, bacterial, parasitic, and fungal infections that may affect animal health or confound the interpretation of study

VetBooks.ir

TABLE 10.1 List of Common Primary and Opportunistic Infections Affecting Macaques

	Primary infections	Opportunistic infections
Viral	Simian type D retroviruses Measles virus Herpes B virus Hepatitis A virus Simian immunodeficiency virus (SIV) Simian T-cell leukemia viruses (STLVs)	Cytomegalovirus Lymphocryptovirus Simian adenovirus Simian virus 40 Simian parvovirus Papillomavirus Rhesus rhadinovirus Simian varicella virus
Bacterial	*Mycobacterium tuberculosis* *Moraxella catarrhalis* *Helicobacter* spp. *Shigella flexneri* *Campylobacter coli* *Campylobacter jejuni* *Yersinia enterocolitica*	*Rhococcus equi* *Mycobacterium avium* complex Enteropathogenic *Escherichia coli*
Parasitic	*Sarcocystis* spp. *Schistosoma* spp. *Trichuris trichiura* *Strongyloides fuelleborni* *Crytosporidium muris*-like [7,27] *Pneumonyssus simicola*	*Plasmodium* spp. *Toxoplasma gondii* *Trichomonas* spp. *Balantidium coli* *Acanthamoeba* spp. *Cryptosporidium parvum* *Enterocytozoon bieneusi*
Fungal	-	*Candida albicans* *Pneumocystis carinii* *Aspergillus fumigatus* *Histoplasma capsulatum* *Cryptococcus neoformans*

Adapted from Sasseville and Mansfield [6].

findings involving macaques (Table 10.1). For more comprehensive reviews, readers are referred to other sources (reviewed in refs [28–31]). As detailed below, the diagnosis of agent(s) that could potentially affect the outcome/interpretation of drug discovery and development studies is based on the constellation of epizootology; clinical, gross, and histologic findings; and common molecular localization tools, such as immunohistochemistry and in situ hybridization techniques. These diagnostic techniques are inherently biased and limited in scope, defining only a fraction of microbial pathogens.

New nucleic acid sequencing technologies, referred to as next-generation sequencing (NGS), have opened up opportunities to define the metagenome and diagnose infectious diseases in humans and NHPs in an unbiased fashion [32–34]. The deep-sequencing technologies are cost-effective, computationally straightforward, and can be applied on a variety of biological samples, including whole blood, plasma, urine, cerebrospinal fluid, feces, and tissues. Such technologies may be helpful to establish the role of infectious agents in potentially confounding the interpretation of study results. Newer molecular techniques could be of immense value in identifying uncommon or novel infectious agents that could cause disease in NHPs and confound the interpretation of study data.

VetBooks.ir

VIRAL INFECTIONS

Simian Betaretroviruses (Simian Type D Retroviruses)

Simian betaretroviruses are members of the subfamily *Orthoretrovirinae* and family *Retroviridae* [29]. Endogenous and exogenous forms are common among simian betaretroviruses [35–37]. Asian macaques are the predominant hosts for the exogenous viruses [29,38]. Seven distinct simian retrovirus (SRVs) serotypes (SRV-1 to -7) have been reported based on virus neutralization studies and genetic divergence [28,39,40]. Cynomolgus and pig-tailed macaques (*Macaca nemestrina*) are predominantly infected with SRV-2, while in rhesus macaques SRV-1 is common. However, macaques housed in the same environment may carry any of the serotypes. SRV-seropositive rates depend on husbandry practices and the geographic origin of macaques. For example, Mauritius-origin cynomolgus macaques seem to be free of SRV infection, whereas those of Indonesian origin have a prevalence of up to 50% [40]. New World NHPs are resistant to SRV infection [41]. Biting with inoculation of saliva or blood seems to be the most common mode of horizontal transmission [42,43]. The virus can also be transmitted vertically [44]. Virus-positive seronegative animals play a significant role in viral persistence within colonies.

SRV infects $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, macrophages, epithelial cells, and cells of the choroid plexus [45–47]. SRV infection may result in clearance, a subclinical carrier state, or a rapidly fatal immunosuppressive disease [40]. The asymptomatic carriers may have generalized lymphadenopathy with varying proportions of follicular and paracortical lymphoid hyperplasia. During immunosuppressive disease there is marked lymphoid depletion within the spleen, lymph nodes, and thymus. The lymphocytes and plasma cells within the paracortex of the affected nodes are replaced by histiocytes and contain hyalinized arterioles [48]. The bone marrow may be hypoplastic or hyperplastic, with resultant anemia and granulocytopenia. There may be suppressed T- and B-cell function, altered immunoglobulin synthesis, change in cytokine/chemokine profiles, and increased susceptibility to infection and tumors. Nevertheless, the mechanism of lymphoid depletion is not currently understood.

The hallmark of the acquired immunodeficiency induced by SRV is opportunistic infections. SRV viremic animals are more susceptible to infection by pyogenic bacteria such as *Corynebacterium equi*. Noma (cancrum oris), a rapidly progressive necrotizing stomatitis and gingivitis, is caused by a polymicrobial opportunistic infection and is often secondary to SRV-related immune deficiency. Other common opportunistic infections include disseminated CMV, adenovirus, candidiasis, and cryptosporidiosis [49]. In addition to susceptibility to a plethora of opportunistic infections, SRV-2 has been associated with a mesenchymal proliferative disease called retroperitoneal fibromatosis in combination with retroperitoneal fibromatosis-associated herpesvirus infection [50]. Particularly in SRV-infected cynomolgus macaques, rare B-cell lymphomas have been reported [51]. The presence of virus in the tissues can be identified by either immunohistochemistry or in situ hybridization and localized in immune and epithelial cells [47]. In subclinical infection, cells of the choroid plexus harbor the virus without any immune response [52].

Simian betaretroviruses are considered the most important cause of dampened immune response in macaques, thereby imposing a high risk to colony health and a confounding

VetBooks.ir

variable in research. Although there are only a few studies reported in the literature as being affected by SRV infection [53,54], because of the high prevalence of the virus in some populations of cynomolgus monkeys, it is possible that other studies were affected in years past. In these referenced cases, SRV infection in study animals was established retrospectively using archived specimens. Such compromised studies were associated with increased costs, delays in drug development, and animal replacement. Since SRV screening by serology alone is not adequate because antibody-negative/antigen-positive animals would be missed, polymerase chain reaction (PCR) coupled with serological methods of detection [55,56] is now the optimum procedure used by primate research facilities. Subsequent to the successful strategies used to establish SRV-free breeding colonies, SRV outbreaks in biomedical research and pharmaceutical companies are a rare event. The risk of zoonotic transmission of SRV is not completely elucidated but is considered quite low.

Measles (Rubeola) Virus

Measles virus belongs to genus *Morbillivirus*, family *Paramyxoviridae*. Humans are the only natural host of measles [28,29]. Measles virus infection typically spreads to NHPs from infected human handlers, and the virus can spread rapidly within an NHP colony through the aerosolized route, contact, and fomites. In fact infected animals are capable of aerosol transmission before the onset of clinical signs [21,57]. Most if not all NHP species are susceptible to the infection.

Measles virus infection can result in marked thymic atrophy and lymphoid depletion, resulting in clinically significant immunosuppression [58]. Lymphoid necrosis and depletion and giant cells lacking viral inclusions (Warthin-Finkeldey cells) are common in lymphoid organs. Immunosuppression may last for months following resolution of overt clinical signs and interfere with immunology-based assays, including tuberculin testing [59]. The degree of immunosuppression may be severe enough to allow the occurrence of opportunistic infections such as CMV and adenovirus.

Characteristic maculopapular exanthema induced by measles viral infection is particularly evident on the face and sparsely haired skin of the ventrum. This may progress to a dry or scaly desquamative dermatitis. Dermatitis might represent an Arthus-type (antibody-antigen complex) reaction because it correlates with rising neutralizing antibodies in the circulation. Histologically, affected skin shows mild erythema and parakeratotic hyperkeratosis with multinucleated syncytial cells containing up to 20 nuclei [60]. Necrosis of hair follicles is characteristic. Koplik's spot (small, raised, white spots on the oral mucosa) are considered pathognomonic but are inconsistent findings. Disease progression leads to infection of cells in the lung and gastrointestinal tract. Pulmonary lesions consist of proliferative and necrotizing bronchointerstitial pneumonia with the presence of large syncytial cells and intracytoplasmic and/or intranuclear eosinophilic viral inclusions within the syncytial cells, type II pneumocytes, and histiocytes. The lesion may be complicated by a secondary bacterial pneumonia due to epithelial damage and immune suppression. Viral inclusions may be found in a variety of organs, including the salivary gland, pancreatic duct, thyroid, liver, and brain. Rare cases of abortion and neurologic signs have been associated with measles [61,62]. In New World NHPs, these pathologic changes are accompanied by a necrotizing and proliferative gastroenteritis. Recent publications report disease associated with canine distemper virus infection

VetBooks.ir

of macaques [63,64]. This related morbillivirus was associated with cutaneous, respiratory, and gastrointestinal signs in cynomolgus macaques and clinically must be differentiated from measles virus infection. Further data are needed to confirm these findings and determine the source of infection and preventive measures. Viral isolation from nasopharyngeal secretions, whole blood, or buffy coat preparations is valuable in definitive diagnosis. Molecular localization of viral antigen in the syncytial, epithelial, and immune cells using a commercially available antibody against the measles matrix protein augments the diagnosis of measles viral infection [65].

In general, infant macaques should be vaccinated at 3 months of age or older with a modified live measles vaccine designed for human use, with a booster dose given at 6 weeks. However, a single-valent measles virus vaccine is no longer available in the United States. Adequacy of vaccination programs can be assessed periodically using serologic assays. NHPs exposed to measles virus have lifelong immunity, and vaccination can be effective in the prevention of the disease. Measles still commonly affects humans and NHPs in developing countries, such as Indochina and the Indo-Pacific region, from where most macaques are imported [21,66]. Therefore importation of infected animals remains a major concern and may pose a serious threat to naïve NHP colonies and human handlers in the United States and Europe. A high clinical index of suspicion should be maintained for measles virus infection in animals recently imported from Southeast Asia.

Macacine Herpesvirus-1 (Herpes B Virus)

Herpes B virus is a member of the genus *Simplexvirus* and subfamily *Alphaherpesvirinae* [67,68]. Herpes B virus is highly endemic as a latent infection in most populations of macaques [67–69]. Factors contributing to recrudescence of infection are poorly understood, but stress related to changing social dynamics, transportation or relocation, fever, ultraviolet light, tissue or nerve damage, and immunosuppression may play a role. Though it rarely causes disease in the natural host, accidental infection in humans and nonmacaque primates have been reported to cause fatal disseminated infection. Macaques periodically shed the virus via oral, ocular, and genital secretions. While sexual contact is an important means of virus transmission, other routes such as biting, fomites, overcrowding, and unsanitary conditions might also contribute to viral infection [70,71].

Infections of Asian macaques are usually mild and self-limiting. Vesicles and ulcerations may occur in oral and genital mucosa and may resolve in 10-14 days. Disseminated viral infection is rare in macaques, but if it occurs it is usually fatal. The clinical course of disseminated viral infection can be peracute to slowly progressive, and herpes B virus infection as an underlying causative agent may not be suspected. In such cases the risk of human exposure may be increased. Herpes B virus infection of humans is characterized by ascending paralysis and a high mortality rate. Common sites of viral infection include buccal, gingival, conjunctival, anal, and genital mucosa [72]. In these sites, infection can be accompanied by erythema, vesicles, and ulcerations. Histologically, there is ballooning degeneration of keratinocytes with progression to vesiculations accompanied by inflammatory cells (Figure 10.1). Multinucleated syncytial cells with eosinophilic to basophilic intranuclear viral inclusions may be prominent. Endothelial cell necrosis with intranuclear viral inclusions may be seen. In disseminated disease there is widespread, multifocal, hemorrhagic necrosis within the liver,

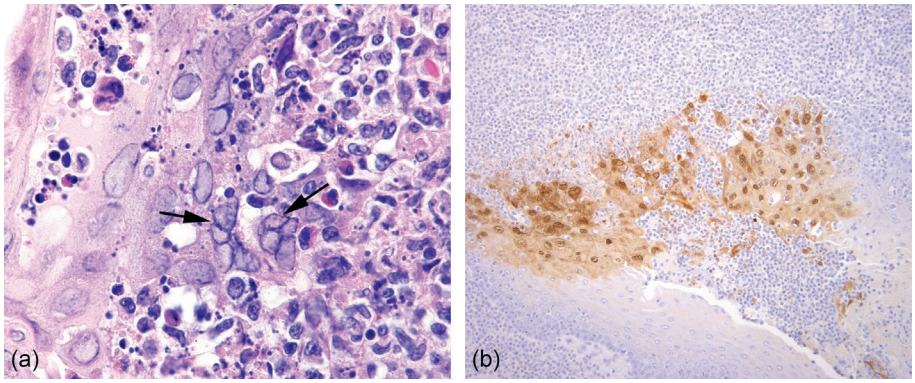


FIGURE 10.1 Herpes B virus infection. Viral infection causes epithelial cell necrosis within the oral mucosa, with infiltration of neutrophils. Note the presence of multinucleated syncytial cells with intranuclear inclusions (arrows in (a)). Immunohistochemistry demonstrates the viral antigen within the epithelial cells (b).

lung, brain, adrenal gland, and lymphoid organs [67–69]. Herpes B viral infection should be included as a differential diagnosis in primate diseases with multifocal necrotizing hepatitis. Seroconversion occurs soon after the primary infection and is often associated with the resolution of clinical signs. False-negative results and latent infection may complicate the interpretation of serological assays [73,74]. Because of infrequent viral shedding, PCR-based detection of viral DNA is of limited value for diagnosis. Immunohistochemistry using a commercially available rabbit polyclonal antibody against the closely related human herpesvirus-1 can be used to demonstrate viral antigen in equivocal lesions [65,75] (Figure 10.1). However, this antibody detects viral glycoproteins shared by all simplex viruses and therefore is not able to definitively confirm herpes B viral infection. Molecular tools or viral isolation are required if definitive identification is desired. The samples can be submitted to the National B Virus Resource Laboratory (Georgia State University, Atlanta, Georgia) for viral culture and molecular diagnosis.

Reactivation of herpes B virus with clinical disease or death has been reported in captive macaques with immunosuppression from natural viral infection (e.g., SRV infection) or those administered immunosuppressive therapeutics [76,77]. For instance, 3 of 14 cynomolgus macaques given the highest dose of an immunosuppressive drug in a 6-month toxicology study developed herpes B virus oral lesions after 3 months of dosing [76]. This resulted in early removal of all high-dose monkeys from the study because of zoonotic concerns. In another study [77] two adult cynomolgus monkeys that were housed together died from fatal disseminated herpes B virus infection due to immunosuppression caused by SRV infection. In addition to a propensity to cause fatal disease in immunosuppressed macaques, there is high zoonotic risk associated with herpes B virus. Therefore every effort should be made to use SPF animals in models that are likely to result in immunosuppression. Determination of herpes B virus status in an individual animal can be difficult because both serological assays and viral isolation have limitations. Thus SPF status can be ascertained only by knowing the virologic history of the animal under consideration and the history of all its contacts. For these reasons, treating all macaque species as if they are potentially infected with herpes B virus and taking appropriate precautions is advisable.

Cytomegalovirus

CMVs are ubiquitous viruses that cause asymptomatic persistent infection in humans and NHPs [78–84]. CMVs are members of the *Betaherpesvirinae* subfamily and are slowly cytolytic, causing enlargement of the cytoplasm (cytomegaly) and nuclei (karyomegaly) of infected cells [28,78]. Viruses within this group are generally believed to have a narrow host range, but interspecies transmission is possible. CMV infection is common; seroprevalence in adult macaques approaches 100% in most colonies [85,86]. CMV persists as a latent infection and is periodically shed in bodily secretions, including oral secretions, breast milk, and urine [78,87]. Macaques may be infected within the first year of life, and prior immunity does not prevent the infection.

There are no clinical signs in immunocompetent animals. Latent infections occur in glandular tissues, lymphoreticular cells, and kidneys rather than in neurons, as seen in members of the *Alphaherpesvirinae* [88,89]. Pneumonitis, encephalomyelitis, gastroenteritis, orchitis, neuritis, and vasculitis have all been described in immunosuppressed animals, wherein lung is the most frequently affected organ. Histologically, neutrophilic inflammation is commonly observed with cytomegaly and the presence of intracytoplasmic and intranuclear viral inclusions (often described as "owl's eyes" inclusion bodies) (Figure 10.2). Exposure to CMV can be determined by serology or PCR. Kamperschroer et al. [90] recently developed an interferon-γ enzyme-linked immunosorbent spot in vitro assay measuring T-cell responses against LCV and CMV in cynomolgus macaques, which may be applied to nonclinical testing of immunomodulatory therapeutics. Immunohistochemistry is of value in increasing diagnostic

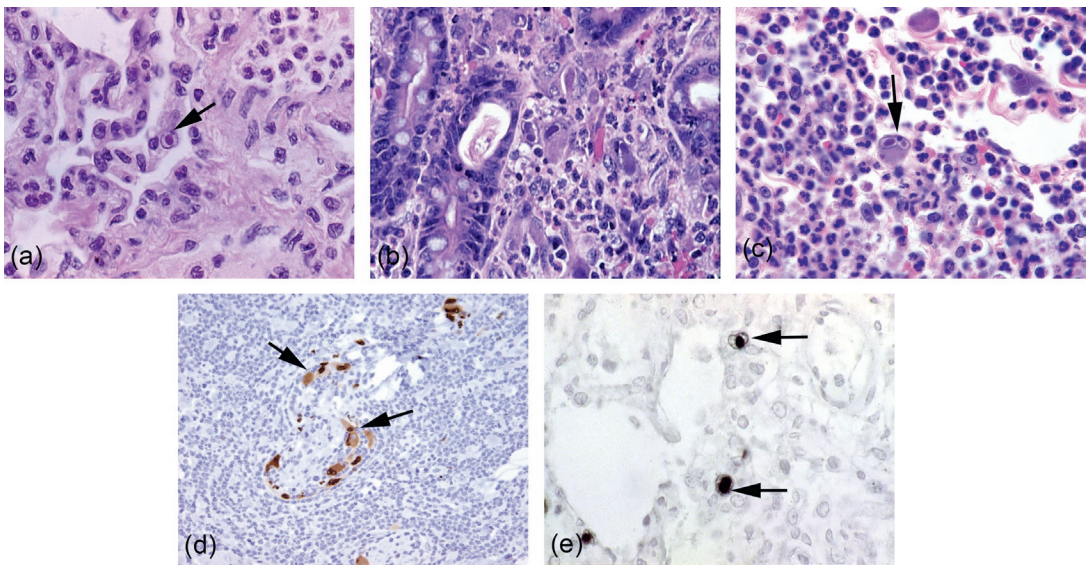


FIGURE 10.2 Cytomegalovirus infection. H&E -stained sections of lung and colon demonstrating CMV infection accompanied with neutrophilic infiltrates ((a) and (b)). Note that CMV infection causes cytomegaly, with the presence of large viral inclusions (owl's eyes inclusion bodies; arrows in (a) and (c)). Infection can be confirmed by immunohistochemistry (as demonstrated by arrows in (d) within infected peripheral nerves) or by in situ hybridization (as shown by arrows in (e) within infected lungs).

VetBooks.ir

sensitivity [65]. An antibody directed against the early intermediate viral protein of rhesus macaque CMV (RhCMV) has been developed [91] and detects macaque CMVs in stromal, endothelial, and inflammatory cells such as macrophages and neutrophils (Figure 10.2).

CMV is one of the most common opportunistic pathogens encountered with profound immunosuppression [92] and is a particular issue with human transplantation immunomodulatory therapies [93,94]. In macaques, CMV is also a common opportunistic infection in natural or experimental viral infection (e.g., simian immunodeficiency virus (SIV), SRV, and measles virus) [28,78]. Likewise, CMV-associated disease has been observed in immunomodulatory pharmacology models (e.g., anti-CD40 or anti-major histocompatibility complex II) and occasionally in chronic toxicology studies with immunomodulatory therapeutics [95,96]. Macaques infected with RhCMV are sensitive to disruption in immunologic functions, leading to the emergence of RhCMV-disseminated and often fatal disease. Prophylactic therapy with ganciclovir, foscarnet, and benzimidazole nucleosides may be coupled with immunosuppressive regimens to prevent severe fatal disease [78,97].

Macacine Herpesvirus 4 (Rhesus Lymphocryptovirus)

NHP LCVs show genomic sequence and disease similarities with the Epstein-Barr virus (EBV; human herpesvirus-4) that infects humans [28,95]. Two different rhesus LCV types (rhesus macaque LCV [RhLCV]-1 and -2) have been defined [98], but strain-specific differences in pathology have not been recognized. Orthologous LCVs have been identified in both Old World and New World NHPs [99,100]. As the maternal antibodies wane at approximately 4-6 months of age, juvenile macaques are susceptible to infection and quickly seroconvert. By 2 years of age, nearly all animals harbor the virus [101,102]. Infection occurs via oral secretions. The virus infects B lymphocytes and persists for life, usually in a latent form. Reactivation and transition to a lytic cycle of viral replication occurs with periodic shedding of viruses.

Primary infection of immunocompetent macaques with RhLCV is typically asymptomatic. In contrast, macaques rendered immunodeficient by SIV infection develop an epithelial proliferative disorder similar to oral hairy leukoplakia in humans and a B-cell malignant lymphoma similar to the non-Hodgkin's lymphoma seen in human immunodeficiency virus-infected patients with AIDS [96,103]. LCV-induced leukoplakia occurs in the oral mucosa and esophagus and, less commonly, in haired skin [104,105]. Histologically, it is characterized by focal areas of parakeratotic hyperkeratosis with or without acanthosis, epithelial ballooning degeneration, and basophilic intranuclear brick-shaped viral inclusions. Harari et al. [106] described a genital condyloma-like lesion that was associated with macaque LCV DNA in cynomolgus macaques from the island of Mauritius. Histology of this lesion is characterized by eosinophilic, neutrophilic, and lymphoplasmacytic penile and vulvar inflammation, epidermal hyperplasia with acanthosis, and increased collagenous stroma. Lymphomas associated with LCV infection are most often B cell (CD20 positive) in origin and are classified as large cell, immunoblastic, or Burkitt-like [107,108]. These neoplasms usually develop in extranodal locations including the nasal cavity, periorbital tissues, gastrointestinal tract, urogenital tract, myocardium, and CNS (Figure 10.3). T-cell immunity is thought to be critical in the prevention of virus-induced lymphoproliferation [103,109–111]. Immunosuppressive regimens to prevent organ rejection after transplantation have been linked to the reactivation of

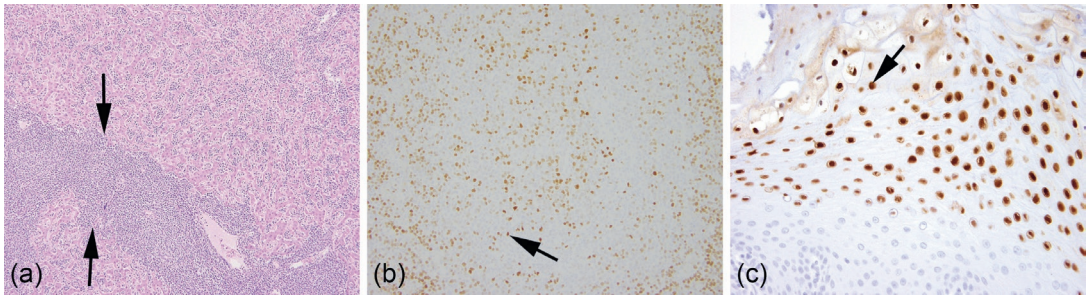


FIGURE 10.3 Rhesus lymphocryptovirus infection. LCV may cause lymphoproliferative disorders in hosts receiving immunosuppressive therapy, with involvement of extranodal sites such as the liver (arrows in (a)). The presence of neoplastic cells in the sinusoids can be identified by immunohistochemistry using antibodies against EBNA2 (arrow in (b)). LCV may cause an epithelial cell proliferative disorder termed *hairy leukoplakia*. The presence of viral antigen is evident within the intranuclear inclusions in the affected epithelium (arrow in (c)).

latent LCV, leading to B-cell lymphoma in cynomolgus macaques [112]. This posttransplant lymphoproliferative disorder reflects the condition in human transplant patients. Diagnosis can be assisted by localizing viral proteins or nucleic acids within lesions. A commercially available monoclonal antibody against the EBV nuclear antigen-2 (EBNA2) detects latently infected cells (Figure 10.3) and an antibody against EBV BZLF1 detects lytically infected cells [65]. In situ hybridization using an RNA probe directed against RhLCV-EBER (EBER-Epstein-Barr Virus encoded RNA) demonstrates increased sensitivity for viral detection. PCR detection of EBERs in peripheral blood cells demonstrates persistent infection.

RhLCV-associated lymphoproliferative disorders and lymphomas have occurred in macaque studies with various immunomodulators and infection with immunosuppressive viral infection such as SRV [113]. Because seroprevalence is high in juvenile and adult macaques, obtaining RhLCV-naïve macaques for routine studies is extremely difficult. Thus, to control for the potential of LCV reactivation complicating the study outcome, it is recommended that macaques subjected to immunomodulatory regimens be screened for LCVs before initiating the study to ensure uniform viral-positive status among vehicle and test article treatment groups. If testing is not possible before initiating the study, pre- and intrastudy serum samples should be stored for possible future analysis in the event of a putative LCV reactivation. Although NHP LCVs seem to share the biology of human EBV [95], there are challenges in the use of NHPs as a preclinical model to determine the potential of new immunomodulatory compounds to produce EBV-induced lymphomas in humans. For example, at present, the methodology for measuring LCV viral load and LCV-specific T-lymphocyte responses in cynomolgus macaques are not sufficiently standardized [90,114]. In contrast to CMV, prophylactic use of antivirals during immunosuppression does not prevent LCV-associated lymphoproliferative disease [95].

Simian Adenoviruses

Adenoviruses are commonly associated with infections of the respiratory and gastrointestinal tract in NHPs. Simian adenoviruses are members of the *Adenoviridae* family and the *Mastadenovirus* genus [28]. In general adenoviruses have narrow host ranges, but in some

VetBooks.ir

cases closely related species may be infected. More than 50 serotypes have been isolated from NHP species [115–119]. In a recent report using a NGS approach, Handley et al. [34] identified several novel adenoviruses in a captive macaque colony, but the pathogenicity of these novel adenoviruses has not been established. Aerosol and fecal-oral routes are significant modes of transmission of the virus because the host may persistently harbor the virus in the tonsils and intestinal epithelium, and nearly 36-90% of NHPs maintained in captivity shed the virus in stool [117]. In immunocompetent animals, the infection is usually self-limiting and establishes persistent infections [25,28], whereas more severe forms of the disease, including gastroenteritis, hepatitis, nephritis, or pancreatitis, may develop in immunosuppressed animals secondary to immunomodulatory drug therapy or during the course of concomitant infections with immunosuppressive viruses, such as measles virus, SIV, and SRV [120–124].

Neonatal animals are more susceptible to clinically apparent infections than adults [125–127]. In diseased animals, respiratory airways and lungs are commonly affected, with necrosis of epithelial cells of the trachea, bronchi, bronchioles, and alveoli and infiltration by variable numbers of neutrophils and macrophages. Keratoconjunctivitis may accompany respiratory infections. In the gastrointestinal tract, lesions are often segmental, and the regions affected are dependent on the viral genotype. In the affected segments, areas of erosion or ulceration are common. In some tissues, such as pancreas, viral infection may produce a chronic, active inflammatory response with considerable pancreatic acinar cell necrosis (Figure 10.4) and multifocal or locally extensive fibrosis. Intraepithelial intranuclear basophilic viral inclusions surrounded by a prominent halo are common [25,28]. Ultrastructural microscopy shows prominent crystalline arrays of virions. Less commonly recognized forms of the disease include necrotizing hepatitis, hemorrhagic cystitis, and tubulointerstitial nephritis. Immunohistochemistry may be helpful to distinguish simian adenoviral infections from other viral infections causing intranuclear inclusions, including CMV and SV40. In this regard, a commercially available antibody against the viral antigen Adeno3 can be used to

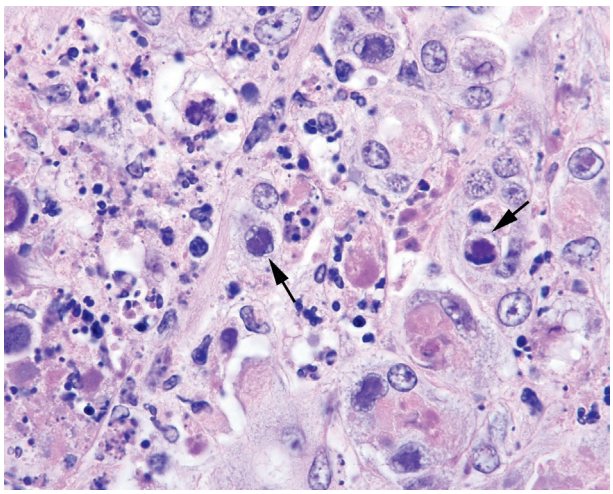


FIGURE 10.4 Simian adenovirus infection. Simian adenovirus may cause necrotizing pancreatitis with intranuclear basophilic inclusions (arrows), which may progress to fibrosing pancreatitis.

VetBooks.ir

identify all adenoviral serotypes [34]. To further identify adenoviral serotypes, viral isolation or PCR and sequencing technologies are required.

Adenovirus-associated disease is an important confounding variable in research, particularly during the course of immunosuppressive protocols [6,24,25]. There are no specific antiviral drugs available to treat the infection. Vaccines are not available, and preventive measures to control or eliminate adenovirus infection in NHP colonies must be directed toward minimizing the exposure of susceptible populations to potentially infected aerosols or feces [28].

Simian Virus 40

SV40 and the closely related cynomologus polyomavirus (CPV) are common latent infections of Asian macaques [28,128]. Serologic evidence suggests that asymptomatic SV40 infection of healthy captive macaques is common. The virus remains latent in renal tissue, and urinary excretion may play a role in viral transmission. In contrast, SV40 infection causes clinical disease in immunosuppressed animals secondary to SIV infection [129] and in animals receiving immunosuppressive drugs for solid-organ transplant [130]. Clinical signs are slowly progressive and relate to pulmonary, renal, or CNS involvement.

In the brain, SV40 infects oligodendrocytes, resulting in multifocal demyelination within the white matter and subependymal region [129]. Large, basophilic, intranuclear viral inclusions are evident in the oligodendroglia and astrocytes. As the lesion progresses, gliosis and astrocytosis may develop. These lesions resemble features of PML in immunocompromised humans. In general PML-type lesions occur following recrudescence of latent infection. Interstitial nephritis with typical viral inclusions may be found primarily within the renal tubules. Pulmonary lesions with proliferative interstitial pneumonia develop less frequently. A distinct SV40-induced meningoencephalitis without demyelination has been recognized in SIV-infected macaques [26]. It was postulated that meningoencephalitis can be a primary manifestation of SV40 in conjunction with interstitial nephritis and pneumonitis. Immunohistochemistry using a commercially available antibody against the SV40 T antigen or to viral protein 1 (VP1) may augment the SV40-associated disease diagnosis because the molecular localization tools often reveal far more infected cells than routine histological analysis [65,129]. Ultrastructural demonstration of polyoma virions in typical lesions is also diagnostic.

CPV-associated tubulointerstitial nephritis, hepatitis, and pneumonitis have been described in cynomolgus monkeys after immunosuppressive protocols in renal transplantation studies [130,131]. There is no specific treatment or prophylactic options available for polyomavirus infection. The monkeys can be screened serologically before study assignments if polyomaviruses are considered to be a potential confounder.

Simian Parvovirus

Simian parvoviruses are related to the human B19 virus [28,132] and show high tropism for cells of erythroid lineage. Infection is asymptomatic in healthy macaques, but disease is severe in immunosuppressed monkeys, characterized by normocytic, normochromic, nonregenerative anemia compounded by other effects of immunosuppression. Bone marrow shows erythroid and myeloid dysmaturation with megakaryocytic hyperplasia and eosinophilic intranuclear viral inclusions. Ultrastructurally, 24-nm viral particles arranged in small

VetBooks.ir

arrays can be demonstrated. In situ hybridization often establishes more infected cells than routine stains [65,133,134].

Vigilance of simian parvovirus infection is particularly important in macaques undergoing solid-organ transplantation drug regimes. Screening animals before initiating studies involving immunosuppressive protocols may reduce such a confounding variable. Since both parvoviral viremic antibody-negative and nonviremic antibody-positive states have been recognized, a combination of PCR and serologic testing must be used [135]. There are no specific antiviral agents available for parvoviruses, but corticosteroids may help in acute hemolytic crisis.

BACTERIAL INFECTIONS

Mycobacterium tuberculosis

Although there have been improvements in tuberculosis (TB) testing and husbandry practices, TB caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis* remains a concern in NHPs because these agents are communicable, have zoonotic potential, and are able to establish latent infection [11,13,30,136,137]. A large survey conducted by the Centers for Disease Control and Prevention revealed a low prevalence of TB in imported NHPs; the majority of animals were asymptomatic and had negative tuberculin skin tests [138]. Such asymptomatic and test-negative animals may precipitate epizootics once introduced into the captive research colonies. Mycobacteria can also be introduced into colonies from infected animal handlers. It is estimated that 32% of the world's human population is infected with *M. tuberculosis*, with the highest incidence recorded in India, Asia, and Africa [139]. Notably, these regions represent a major source for importation of NHPs to the United States [10,13]. Aerosol transmission is the major route of transmission, but bacilli can be acquired by ingestion, direct contact, and fomites, including contaminated thermometers [140] and tattooing needles [141].

Immunosuppression or inadequate host immune responses may permit infected macrophages to migrate from the primary site (usually lungs) and seed granulomas in distant organs. Clinical findings, if present, are often nonspecific. Unexplained weight loss in macaques should prompt a clinical suspicion of TB. Gross lesions may include caseous granulomas ("tubercles") in the hilar lymph nodes and lung that may become confluent, liquefy, or cavitate. Granulomas in the liver, spleen, and other viscera are common in the disseminated disease [142]. Histologically, granulomas have central areas of caseous necrosis surrounded by epithelioid macrophages, Langhans-type giant cells, and lymphocytes. A rim of lymphocytes and fibrous connective tissue surrounds the mature lesions. Acid-fast bacilli may be found within the caseous core or within the surrounding macrophages. Multiple sections and granulomas often must be examined to identify the pathogen [143].

The difficulty in maintaining a TB-free colony is partly due to the fact that there is the lack of availability of convenient, sensitive, and specific antemortem diagnostic tests for TB. The skin tuberculin test with intradermal injection of mammalian old tuberculin and subjective visual scoring of delayed-type hypersensitivity remains the standard means to screen animals and colonies. The skin test is insensitive to latently infected animals, leading to false-negative results; in such cases the test should be periodically repeated to identify newly acquired or reactivated infections [13,144]. Two additional commercially available diagnostic kits are

VetBooks.ir

available for use in NHPs and when used with the skin test may help to resolve equivocal results. The PRIMIGAM kit (Prionics AG, Zurich, Switzerland) measures interferon-γ production in the whole-blood leukocytes in response to mycobacterial exposure, whereas PrimaTB STAT-PAK (Chembio Diagnostic Systems, Medford, NY) detects antibodies to several mycobacterial proteins in serum [145,146]. False-positive results due to presumptive exposure to environmental atypical mycobacteria are common [147]. Immunosuppressive agents or comorbidities such as measles virus infection may cause false-negative results because these three tests all depend on the functional immune system. Culture of the mycobacteria remains the gold standard for diagnosis. PCR may also help establish the diagnosis, but the sensitivity and specificity of this assay has not been rigorously determined.

Confined epizootics can occur in well-maintained research colonies. For example, in a regulated toxicological study, eight animals housed in two different rooms were infected through aerosol transmission and affected study outcomes [24]. Vigilance is essential in screening for TB in NHPs, despite the current reduced incidence of the disease.

Moraxella catarrhalis

Moraxella catarrhalis (formerly *Branhamella catarrhalis*) is a diplococcus [30] found as normal flora in the nasopharynx of NHPs and humans, but it has been implicated in outbreaks of epistaxis (bloody nose syndrome) in both immunocompetent and immunosuppressed macaques [148,149]. The disease is usually mild and self-limiting, but severe disease can occur with the use of immunomodulatory drugs. Careful monitoring of animals for the onset of *Moraxella*-induced epistaxis is important in toxicological evaluation of immunomodulatory agents. Treatment with amoxicillin, clavulonic acid, extended-spectrum cephalosporins, newer macrolides (azithromycin and clarithromycin), trimethoprim-sulfa, tetracyclines, and fluoroquinolones may clear the infection [150]. Differential diagnoses for epistaxis in NHPs include simian hemorrhagic fever and Ebola-Reston infection.

Helicobacter pylori

Helicobacter pylori is a spiral or curved, flagellated bacterium found in the gastric mucosa of humans and some NHPs [30]. Both *H. pylori* and *Helicobacter heilmannii* have been associated with gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphomas in humans [151]. Natural infection of macaques with *H. pylori* is often asymptomatic with epithelial hyperplasia, lymphoplasmacytic infiltrates, and erosions in the antral portion of the stomach [152]. The incidence of antral gastritis may differ based on the geographic ancestry of macaques [153,154]. These lesions may confound experimental studies [6].

PARASITIC INFECTIONS

Malaria

Malaria, caused by protozoa of the genus *Plasmodium*, is one of the most frequent parasitic diseases of NHPs imported from malaria-endemic regions [24,155]. Malarial prevalence as high as 43% has been reported in imported cynomolgus monkeys [156], but natural infection is

VetBooks.ir

uncommon in rhesus macaques, marmosets, and owl monkeys [157,158]. At least 26 *Plasmodium* species have been known to infect NHPs in their natural habitats [31]. Among them, *P. brasilianum*, *P. cynomolgi*, *P. inui*, *P. knowlesi*, and *P. simium* can cause diseases in human [20,159–161]. The common species that infect humans include *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* [162].

The life cycle of malarial parasites begins when an infected female anopheles mosquito inoculates sporozoites into the vertebrate host's peripheral circulation [31]. The sporozoites invade hepatocytes, where exoerythrocytic development occurs. Erythrocytic schizogeny within erythrocytes results in intravascular hemolysis, causing anemia and hemoglobinuric nephrosis with a high parasitic burden [5]. The parasitized erythrocytes are removed by the reticuloendothelial system, resulting in extravascular hemolysis, anemia, and splenomegaly. Microscopically, Kupffer cells and splenic macrophages contain intracytoplasmic granular brown to black malarial pigment (hemozoin). The diagnosis of malaria is established by routine examination of thick and thin blood smears and demonstration of the organisms in the erythrocytes. Chronic infection in cynomolgus macaques poses more diagnostic difficulty because of the lower parasitic burdens and the cyclic rhythm of schizogeny. Several daily consecutive smears may be necessary to establish the diagnosis. Quantitative PCR (qPCR) assays are commercially available to detect *Plasmodium* spp. in the blood [163,164]. However, there are no tests available to detect dormant exoerythrocytic organisms.

Cynomolgus macaques control infection well and are often subclinically infected [165,166]. If immunocompromised by stress or as a part of an experimental protocol, monkeys may develop fulminant recrudescent disease. In a preclinical toxicity study, an immunomodulatory antibody targeting activated T-cell and macrophage adhesion was reported to cause recrudescence of malaria in cynomolgus macaques [113]. In addition, an increase in the number of reticulocytes is thought to play a role in plasmodial recrudescence [167], but this hypothesis is not yet verified in macaques. With the increased use of NHPs from China and Southeast Asia, where malaria is endemic, infection could affect drug safety studies, in particular with those with immunomodulatory compounds. Many pharmaceutical companies incorporate disease-free monkeys in their studies based on the results of qPCR assays (KGM, personal communication). Chloroquine and primaquine can be used to treat malaria in NHPs [31]. Effective mosquito control is essential to prevent the transmission of malaria in laboratory animal colonies. With increased zoonotic evidence of *Plasmodium knowlesi* in Southeast Asia [161], NHP handlers are at risk of acquiring infection through the blood-borne route.

Trypanosoma cruzi

Cardiac findings of unknown cause and progression in purpose-bred macaques can be relatively common and have been interpreted to be background and incidental changes [168–170]. However, pathogens such as *Sarcocystis* spp. [171,172], *Trypanosoma cruzi* [173,174], *Toxoplasma gondii* [175–177], *Encephalotozoon cuniculi* [178,179], and encephalomyocarditis virus [180] have been associated with myocardial changes either in spontaneous or in immunocompromised situations and should be always be on the list of differential diagnoses. In this regard, two recent studies [173,174] based on PCR and serology revealed higher incidence rates of *T. cruzi* in cynomolgus macaque research colonies from Texas. In fact, one of these studies [174] demonstrated that 17 of the 19 archived specimens (89%) with lymphocytic myocarditis were PCR-positive for *T. cruzi*.

VetBooks.ir

Chagas disease, or American trypanosomiasis, caused by a hemoflagellate protozoan, *T. cruzi*, is a tropical parasitic disease affecting humans and a wide range of animals, including NHPs [31,181]. The disease is endemic in Central and South America. Natural transmission occurs through blood-sucking reduviids (genus *Triatoma*). Trypomastigotes deposited at the site of the insect bite circulate in the blood and enter into macrophages or myocytes, forming amastigotes. Acute and chronic forms of the disease have been recognized with predominant cardiac and gastrointestinal manifestations [182]. Histologically, there is chronic lymphoplasmacytic myocarditis, mild necrosis, extensive loss of myocardial fibers, and marked fibrosis [182,183]. The infected myofibers may contain amastigotes within the pseudocysts that are round to oval, 1.5-4 µm in diameter, and contain a nucleus and a rodlike kinetoplast parallel to the nucleus. Although the mechanism of myocardial damage caused by the protozoa is not understood, it is proposed that direct damage, immune-mediated effects, microvascular disturbances, and destruction of the cardiac autonomic nervous system (neurologic theory) have a role in pathogenesis [8,184,185].

Since there is a high incidence of *T. cruzi* infection in Chagas-endemic areas, screening monkeys for *T. cruzi* infection before initiating drug safety studies may reduce this confounding variable. There is no specific treatment available for this disease. Control is based on the elimination of the insect vectors. Because *T. cruzi* causes serious disease in humans, proper care and use of NHPs should be exercised to prevent accidental exposure to the protozoa.

Toxoplasma gondii

Toxoplasmosis, caused by the apicomplexan parasite *T. gondii*, remains a clinically important opportunistic infection in immunodeficient humans and macaques [31,186–188]. Marmosets are very susceptible and may die within 5-6 days after contracting the disease [189]. Felids serve as the definitive hosts for the protozoa. The oocysts passed in feline feces are infectious to other mammals and cats [31,187]. Infection with *Toxoplasma* can also occur via transplacental transmission and consumption of tissue cysts. Disease among New World NHPs can be severe, with neurologic manifestations, diarrhea, fever, cough, abortion, or sudden death [186]. Infection in Old World NHPs is often mild or asymptomatic [190]. The infection is associated with necrosis and accompanied by neutrophilic and histiocytic infiltrates in the lungs, liver, CNS, gastrointestinal tract, skeletal muscle, and lymphoid tissue. In affected tissues, tachyzoites can be recognized as intracellular, crescent-, or banana-shaped structures that measure 4-8 µm by 2-4 µm [191] (Figure 10.5). Giemsa and periodic acid-Schiff (PAS) may be used to highlight the organisms. Immunohistochemistry and electron microscopy may help to distinguish between *Sarcocystis* spp. and *Neospora* spp.

Preventive strategies include exclusion of rodent vectors from the animal facilities; they may serve as intermediate hosts and may be eaten by the NHPs. Similarly, cockroaches may serve as important mechanical vectors. Avoiding feed and bedding material contaminated with feline fecal material is advisable.

Balantidium coli, *Trichomonas* spp., and *Cryptosporidium muris*-Like Protozoa

Balantidium coli is a large (50-130 µm long by 20-70 µm wide), ciliated protozoa that inhabits the large intestines of normal New and Old World NHPs. The parasitic load and incidence of

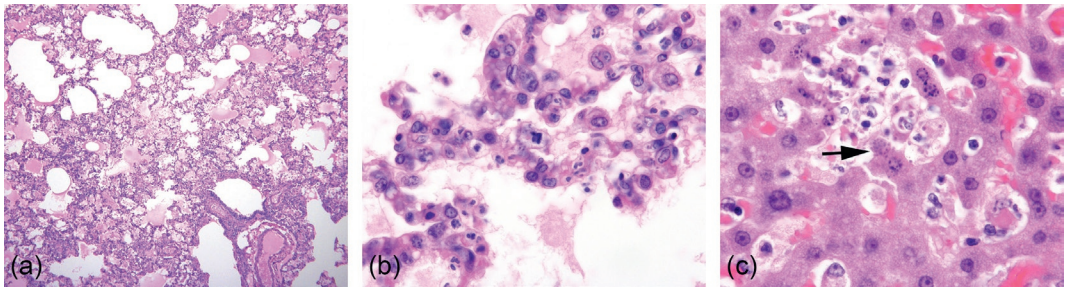


FIGURE 10.5 Toxoplasmosis. *Toxoplasma gondii* causes necrosis, with infiltration of neutrophils and histiocytes in many organs including the lungs ((a) and (b)) and liver (c). In the affected tissues, tachyzoites can be recognized as intracellular, crescent-, or banana-shaped basophilic structures that measure 4-8×2-4μm (arrow in (c)).

B. coli vary according to the source of macaques [153]. For example, the incidence in Mauritian cynomolgus monkeys was reported to be lower (13%) than in those from the Philippines (75%) and Vietnam (65%). Though there is a similar incidence, the parasitic load was higher in Philippine than in Vietnamese monkeys. Another study reported an incidence of 12% in rhesus macaques [127]. In these studies, *B. coli* infection was not usually associated with clinical disease or morphological changes in the intestine. In our perspective, the increased loads of *B. coli* in animals with gastrointestinal diseases are secondary to inflammation or alterations in normal gut transit time. The protozoal invasion of the gut wall is considered a rare event but occurs secondary to mucosal ulceration.

Trichomonads are common commensal flagellates found in the small and large intestine of macaques. Similar to *B. coli*, in most instances infection is not associated with disease. In SIV-associated immunodeficient macaques, disease has occasionally been observed with parasitic invasion into the lamina propria [192,193].

C. muris-like protozoa have been observed in the microvillus border of the upper one-third of gastric fundic glands in both naïve and immunosuppressed cynomolgus macaques [7,27]. Protozoa are approximately 3-8μm in diameter, representing various stages of development, including trophozoites and immature and mature schizonts. Ultrastructurally, parasites are located in parasitophorous vacuoles and attach to host cells with a prominent feeder organelle similar to that described for *C. muris* that parasitizes the gastric glands of rodents [27]. Epithelial cells of the affected glands can have an increased amount of pale, staining mucus with or without an inflammatory reaction. PCR has shown that this organism clusters with *C. muris* and *Cryptosporidium andersoni* [7].

FUNGAL INFECTIONS

Candida albicans

Candidiasis caused by *Candida albicans* is the most frequently occurring mycotic disease in NHPs [30]. *Candida* spp. are ubiquitous dimorphic fungi that normally inhabit the alimentary and genital mucosa as well as the skin of animals and humans [194–197]. Localized and systemic infections occur in young, immunocompromised, or debilitated

VetBooks.ir

animals. Predisposing factors for the development of candidiasis in NHPs include antibiotic therapy, recent importation, age, concomitant mycobacterial infection, naturally occurring or experimental retroviral infection, or parasitism [48,197]. The localized form of candidiasis is called thrush and appears as pale tan to white plaques (pseudomembrane) that adhere to the epithelium of the affected mucosa [30]. These plaques comprise masses of pseudohyphae and blastospores (3-5 µm in diameter) mixed with sloughed epithelial cells and neutrophils. The morphology of the organisms may be best revealed by PAS or Gomori methenamine silver (GMS) stains. Candida infection of the gastrointestinal tract is rare, which may be accompanied by diarrhea and anorexia [198]. Systemic infection accompanied by fever and hypotensive shock may occur in debilitated animals treated with antibiotics or in association with concurrent disease. Fluconazole has been the preferred treatment for candidiasis in humans [30]. Because of the ubiquitous nature of the organism, prevention is difficult.

Pneumocystis carinii

Pneumocystis carinii is an extracellular obligate fungal pathogen, but colonization has been reported commonly in immunosuppressed NHPs [30,199–201]. Transmission occurs via the aerosol route. Infection in neonates is asymptomatic, and the animals may clear the infection or become carriers [202]. In the lung, recognized life cycle stages include a 1- to 4-µm-diameter, thin-walled vegetative or trophic form that attaches to type 1 pneumocytes and appears to develop through three consecutive sporocyte stages to produce a 3- to 8-µm-diameter, thick-walled cyst form (asci). Development of clinical disease implies impairment of either macrophage function or cell-mediated immunity. During periods of immunosuppression, disease may occur through reactivation of suppressed latent *Pneumocystis* or acquisition of new infection from contacts [203]. Clinical signs in immunosuppressed animals are associated with pneumonitis and include dyspnea and tachypnea [204]. Clinical diagnosis may be aided by PCR amplification of DNA and cytology of bronchoalveolar lavage. Lesions vary from multifocal to coalescing interstitial pneumonitis to multifocal gray to white nodules. The organisms may be recognized as pale, pink foamy material in alveolar spaces. GMS stain or immunohistochemistry can be used for better visualization of the yeast. Inflammatory infiltrates are mild.

Prevention of *Pneumocystis* pneumonia in NHPs is difficult. Separation of immunosuppressed animals from neonates may assist in prevention, and trimethoprim-sulfa drugs may be used prophylactically [203].

Pneumonyssus simicola

Pulmonary acariasis is caused by *Pneumonyssus simicola* in Old World NHPs. Historically, *P. simicola* was found in the lungs of 100% of imported rhesus macaques and could be transmitted among animals raised in a laboratory setting [31,205]. Histologically, there is eosinophilic and granulomatous inflammation centered on and around the airways with birefringent, golden-brown mite pigment. Sections of mites are present within the affected areas [206]. However, antihelminthic treatments and improved husbandry practices have virtually eliminated this pathogen for domestic NHP colonies [31].

VetBooks.ir

CONCLUSION

Infectious diseases remain a serious threat to NHP colonies and can adversely impact drug development programs. Infectious agents may directly impact animal and colony health, causing overt morbidity and mortality. Alternatively, nonpathogenic agents may affect the animal's responses to experimental manipulation or cause zoonotic disease in human contacts. Microbes may also cause limited disease in the immunologically normal host but become pathogenic secondary to immunodysfunction. Furthermore, establishing whether infectious disease outbreaks during the course of a study are effects related to the test article or non-test article is often quite challenging. Therefore it is essential that toxicologists and pathologists involved in the drug discovery and development studies be familiar with the disease presentation caused by these agents to ensure colony health, intelligent study design, appropriate animal selection, and proper interpretation of clinical and microscopic findings. Eliminating all the infectious agents from NHP colonies may not be possible, but enhanced levels of bioexclusion to produce SPF colonies can be adapted to minimize an untoward effect of these infectious agents on the colonies, as well as zoonotic risk to the personnel handling animals. In summary, all NHP facilities should implement comprehensive microbial quality control programs and should seek methods for disease surveillance that can be standardized and bridged across facilities.

References

[1] Bussiere JL. Species selection considerations for preclinical toxicology studies for biotherapeutics. Expert Opin Drug Metab Toxicol 2008;4:871–7.
[2] Kronenberg S, Baumann A, de Haan L, Hinton HJ, Moggs J, Theil FP, et al. Current challenges and opportunities in nonclinical safety testing of biologics. Drug Discov Today 2013;18:1138–43.
[3] van Meer PJ, Kooijman M, van der Laan JW, Moors EH, Schellekens H. The value of non-human primates in the development of monoclonal antibodies. Nat Biotechnol 2013;31:882–3.
[4] 't Hart BA, Abbott DH, Nakamura K, Fuchs E. The marmoset monkey: a multi-purpose preclinical and translational model of human biology and disease. Drug Discov Today 2012;17:1160–5.
[5] Bailey C, Mansfield K. Emerging and reemerging infectious diseases of nonhuman primates in the laboratory setting. Vet Pathol 2010;47:462–81.
[6] Sasseville VG, Mansfield KG. Overview of known non-human primate pathogens with potential to affect colonies used for toxicity testing. J Immunotoxicol 2010;7:79–92.
[7] Sasseville VG, Mansfield KG, Mankowski JL, Tremblay C, Terio KA, Matz-Rensing K, et al. Meeting report: spontaneous lesions and diseases in wild, captive-bred, and zoo-housed nonhuman primates and in nonhuman primate species used in drug safety studies. Vet Pathol 2012;49:1057–69.
[8] Sasseville VG, Mankowski JL, Baldessari A, Harbison C, Laing S, Kaliyaperumal S, et al. Meeting report: emerging respiratory viral infections and nonhuman primate case reports. Vet Pathol 2013;50:1145–53.
[9] Mansfield KG, Kemnitz JW. Introduction: challenges in microbial quality control for nonhuman primate. ILAR J 2008;49:133–6.
[10] Morton WR, Agy MB, Capuano SV, Grant RF. Specific pathogen-free macaques: definition, history, and current production. ILAR J 2008;49:137–44.
[11] Roberts JA, Andrews K. Nonhuman primate quarantine: its evolution and practice. ILAR J 2008;49:145–56.
[12] Simmons JH. Development, application, and quality control of serology assays used for diagnostic monitoring of laboratory nonhuman primates. ILAR J 2008;49:157–69.
[13] Lerche NW, Yee JL, Capuano SV, Flynn JL. New approaches to tuberculosis surveillance in nonhuman primates. ILAR J 2008;49:170–8.
[14] Taylor K. Clinical veterinarian's perspective of non-human primate (NHP) use in drug safety studies. J Immunotoxicol 2010;7:114–9.

VetBooks.ir

[15] Patterson JL, Carrion Jr R. Demand for nonhuman primate resources in the age of biodefense. ILAR J 2005;46:15–22.
[16] Blancher A, Aarnink A, Tanaka K, Ota M, Inoko H, Yamanaka H, et al. Study of cynomolgus monkey (*Macaca fascicularis*) Mhc DRB gene polymorphism in four populations. Immunogenetics 2012;64:605–14.
[17] Yan G, Zhang G, Fang X, Zhang Y, Li C, Ling F, et al. Genome sequencing and comparison of two nonhuman primate animal models, the cynomolgus and Chinese rhesus macaques. Nat Biotechnol 2011;29:1019–23.
[18] Huh JW, Kim YH, Park SJ, Kim DS, Lee SR, Kim KM, et al. Large-scale transcriptome sequencing and gene analyses in the crab-eating macaque (*Macaca fascicularis*) for biomedical research. BMC Genomics 2012;13:163.
[19] Ebeling M, Kung E, See A, Broger C, Steiner G, Berrera M, et al. Genome-based analysis of the nonhuman primate Macaca fascicularis as a model for drug safety assessment. Genome Res 2011;21:1746–56.
[20] Lee KS, Divis PC, Zakaria SK, Matusop A, Julin RA, Conway DJ, et al. Plasmodium knowlesi: reservoir hosts and tracking the emergence in humans and macaques. PLoS Pathog 2011;7:e1002015.
[21] Schillaci MA, Jones-Engel L, Engel GA, Kyes RC. Exposure to human respiratory viruses among urban performing monkeys in Indonesia. Am J Trop Med Hyg 2006;75:716–9.
[22] Brinkworth JF, Pechenkina K. Primates, pathogens and evolution: an introduction. In: Barrett LS, editor. Developments in primatology: progress and prospects. New York: Springer; 2013. p. 1–14.
[23] Greenwood EJD, Schmidt F, Heeney JL. The evolution of SIV in primates and the emergence of the pathogen of AIDS. In: Barrett L, editor. Primates, pathogens, and evolution. New York: Springer; 2013.
[24] Sasseville VG, Diters RW. Impact of infections and normal flora in nonhuman primates on drug development. ILAR J 2008;49:179–90.
[25] Wachtman LM, Mansfield KG. Opportunistic infections in immunologically compromised nonhuman primates. ILAR J 2008;49:191–208.
[26] Simon MA, Ilyinskii PO, Baskin GB, Knight HY, Pauley DR, Lackner AA. Association of simian virus 40 with a central nervous system lesion distinct from progressive multifocal leukoencephalopathy in macaques with AIDS. Am J Pathol 1999;154:437–46.
[27] Dubey JP, Markovits JE, Killary KA. *Cryptosporidium muris*-like infection in stomach of cynomolgus monkeys (Macaca fascicularis). Vet Pathol 2002;39:363–71.
[28] Wachtman LM, Mansfield KG. Viral diseases of nonhuman primates. In: Abee CR, Mansfield KG, Tardif S, editors. Nonhuman primates in biomedical research, volume 2: diseases. Canada: Elsevier; 2012.
[29] Voevodin AF, Marx PA. Simian virology. Iowa, USA: Wiley-Blackwell; 2009.
[30] Simmons J, Gibson S. Bacterial and mycotic diseases of nonhuman primates. In: Abee CR, Mansfield K, Tardif S, editors. Nonhuman primates in biomedical research. Canada: Elsevier; 2012. p. 105–72.
[31] Strait K, Else JG, Eberhard ML. Parasitic diseases of nonhuman primates. In: Abee CR, Mansfield K, Tardif S, editors. Nonhuman primates in biomedical research, vol. 2. Canada: Elsevier; 2012. p. 197–297.
[32] Capobianchi MR, Giombini E, Rozera G. Next-generation sequencing technology in clinical virology. Clin Microbiol Infect 2013;19:15–22.
[33] Didelot X, Bowden R, Wilson DJ, Peto TE, Crook DW. Transforming clinical microbiology with bacterial genome sequencing. Nat Rev Genet 2012;13:601–12.
[34] Handley SA, Thackray LB, Zhao G, Presti R, Miller AD, Droit L, et al. Pathogenic simian immunodeficiency virus infection is associated with expansion of the enteric virome. Cell 2012;151:253–66.
[35] Heberling RL, Barker ST, Kalter SS, Smith GC, Helmke RJ. Oncornavirus: isolation from a squirrel monkey (*Saimiri sciureus*) lung culture. Science 1977;195:289–92.
[36] Todaro GJ, Benveniste RE, Sherr CJ, Schlom J, Schidlovsky G, Stephenson JR. Isolation and characterization of a new type D retrovirus from the Asian primate, *Presbytis obscurus* (spectacled langur). Virology 1978;84:189–94.
[37] van der Kuyl AC, Mang R, Dekker JT, Goudsmit J. Complete nucleotide sequence of simian endogenous type D retrovirus with intact genome organization: evidence for ancestry to simian retrovirus and baboon endogenous virus. J Virol 1997;71:3666–76.
[38] Chopra HC, Mason MM. A new virus in a spontaneous mammary tumor of a rhesus monkey. Cancer Res 1970;30:2081–6.
[39] Montiel NA. An updated review of simian betaretrovirus (SRV) in macaque hosts. J Med Primatol 2010;39:303–14.
[40] Lerche NW. Simian retroviruses: infection and disease—implications for immunotoxicology research in primates. J Immunotoxicol 2010;7:93–101.

[41] Diehl WE, Stansell E, Kaiser SM, Emerman M, Hunter E. Identification of postentry restrictions to Mason-Pfizer monkey virus infection in New World monkey cells. J Virol 2008;82:11140–51.
[42] Lerche NW, Osborn KG, Marx PA, Prahalada S, Maul DH, Lowenstine LJ, et al. Inapparent carriers of simian acquired immune deficiency syndrome type D retrovirus and disease transmission with saliva. J Natl Cancer Inst 1986;77:489–96.
[43] Wilkinson RC, Murrell CK, Guy R, Davis G, Hall JM, North DC, et al. Persistence and dissemination of simian retrovirus type 2 DNA in relation to viremia, seroresponse, and experimental transmissibility in *Macaca fascicularis*. J Virol 2003;77:10751–9.
[44] Tsai CC, Follis KE, Snyder K, Windsor S, Thouless ME, Kuller L, et al. Maternal transmission of type D simian retrovirus (SRV-2) in pigtailed macaques. J Med Primatol 1990;19:203–16.
[45] Maul DH, Zaiss CP, MacKenzie MR, Shiigi SM, Marx PA, Gardner MB. Simian retrovirus D serogroup 1 has a broad cellular tropism for lymphoid and nonlymphoid cells. J Virol 1988;62:1768–73.
[46] Lackner AA, Schiodt M, Armitage GC, Moore PF, Munn RJ, Marx PA, et al. Mucosal epithelial cells and Langerhans cells are targets for infection by the immunosuppressive type D retrovirus simian AIDS retrovirus serotype 1. J Med Primatol 1989;18:195–207.
[47] Lackner AA, Rodriguez MH, Bush CE, Munn RJ, Kwang HS, Moore PF, et al. Distribution of a macaque immunosuppressive type D retrovirus in neural, lymphoid, and salivary tissues. J Virol 1988;62:2134–42.
[48] Osborn KG, Prahalada S, Lowenstine LJ, Gardner MB, Maul DH, Henrickson RV. The pathology of an epizootic of acquired immunodeficiency in rhesus macaques. Am J Pathol 1984;114:94–103.
[49] Lowenstine LJ. Type D retrovirus infection, macaques. In: Jones TC, Mohr U, Hunt RD, editors. Nonhuman primates I. Berlin and New York: Springer-Verlag; 1993. p. 20–32.
[50] Tsai CC, Warner TF, Uno H, Giddens Jr WE, Ochs HD. Subcutaneous fibromatosis associated with an acquired immune deficiency syndrome in pig-tailed macaques. Am J Pathol 1985;120:30–7.
[51] Paramastri YA, Wallace JM, Salleng KJ, Wilkinson LM, Malarkey DE, Cline JM. Intracranial lymphomas in simian retrovirus-positive *Macaca fascicularis*. Vet Pathol 2002;39:399–402.
[52] Lackner AA, Marx PA, Lerche NW, Gardner MB, Kluge JD, Spinner A, et al. Asymptomatic infection of the central nervous system by the macaque immunosuppressive type D retrovirus, SRV-1. J Gen Virol 1989;70(Pt 7):1641–51.
[53] Schroder MA, Fisk SK, Lerche NW. Eradication of simian retrovirus type D from a colony of cynomolgus, rhesus, and stump-tailed macaques by using serial testing and removal. Contemp Top Lab Anim Sci 2000;39:16–23.
[54] Lerche NW, Osborn KG. Simian retrovirus infections: potential confounding variables in primate toxicology studies. Toxicol Pathol 2003;(31 Suppl.):103–10.
[55] Lerche NW, Yee JL, Jennings MB. Establishing specific retrovirus-free breeding colonies of macaques: an approach to primary screening and surveillance. Lab Anim Sci 1994;44:217–21.
[56] Guzman RE, Kerlin RL, Zimmerman TE. Histologic lesions in cynomolgus monkeys (*Macaca fascicularis*) naturally infected with simian retrovirus type D: comparison of seropositive, virus-positive, and uninfected animals. Toxicol Pathol 1999;27:672–7.
[57] Willy ME, Woodward RA, Thornton VB, Wolff AV, Flynn BM, Heath JL, et al. Management of a measles outbreak among Old World nonhuman primates. Lab Anim Sci 1999;49:42–8.
[58] Schneider-Schaulies S, Schneider-Schaulies J. Measles virus-induced immunosuppression. Curr Top Microbiol Immunol 2009;330:243–69.
[59] Choi YK, Simon MA, Kim DY, Yoon BI, Kwon SW, Lee KW, et al. Fatal measles virus infection in Japanese macaques (*Macaca fuscata*). Vet Pathol 1999;36:594–600.
[60] Hall WC, Kovatch RM, Herman PH, Fox JG. Pathology of measles in rhesus monkeys. Vet Pathol 1971;8:307–19.
[61] Renne RA, McLaughlin R, Jenson AB. Measles virus-associated endometritis, cervicitis, and abortion in a rhesus monkey. J Am Vet Med Assoc 1973;163:639–41.
[62] Steele MD, Giddens Jr. WE, Valerio M, Sumi SM, Stetzer ER. Spontaneous paramyxoviral encephalitis in nonhuman primates (*Macaca mulatta* and *M. nemestrina*). Vet Pathol 1982;19:132–9.
[63] Sakai K, Nagata N, Ami Y, Seki F, Suzaki Y, Iwata-Yoshikawa N, et al. Lethal canine distemper virus outbreak in cynomolgus monkeys in Japan in 2008. J Virol 2013;87:1105–14.
[64] Qiu W, Zheng Y, Zhang S, Fan Q, Liu H, Zhang F, et al. Canine distemper outbreak in rhesus monkeys, China. Emerg Infect Dis 2011;17:1541–3.
[65] Mansfield KG, Sasseville VG, Westmoreland SV. Molecular localization techniques in the diagnosis and characterization of nonhuman primate infectious diseases. Vet Pathol 2013;51(1):110–26.

VetBooks.ir

[66] Global control and regional elimination of measles, 2000-2011. MMWR Morb Mortal Wkly Rep 2013;62:27–31.
[67] Hilliard J. Monkey B virus. In: Arvin A, Campadelli-Fiume G, Mocarski E, Moore PS, Roizman B, Whitley R, Yamanishi K, editors. Human herpesviruses: biology, therapy, and immunoprophylaxis. Cambridge: Cambridge University Press; 2007.
[68] Simmons JH. Herpesvirus infections of laboratory macaques. J Immunotoxicol 2010;7:102–13.
[69] Elmore D, Eberle R. Monkey B virus (Cercopithecine herpesvirus 1). Comp Med 2008;58:11–21.
[70] Weigler BJ, Roberts JA, Hird DW, Lerche NW, Hilliard JK. A cross sectional survey for B virus antibody in a colony of group housed rhesus macaques. Lab Anim Sci 1990;40:257–61.
[71] Weigler BJ, Hird DW, Hilliard JK, Lerche NW, Roberts JA, Scott LM. Epidemiology of cercopithecine herpesvirus 1 (B virus) infection and shedding in a large breeding cohort of rhesus macaques. J Infect Dis 1993;167:257–63.
[72] Anderson DC, Swenson RB, Orkin JL, Kalter SS, McClure HM. Primary Herpesvirus simiae (B-virus) infection in infant macaques. Lab Anim Sci 1994;44:526–30.
[73] Ward JA, Hilliard JK. B virus-specific pathogen-free (SPF) breeding colonies of macaques: issues, surveillance, and results in 1992. Lab Anim Sci 1994;44:222–8.
[74] Ward JA, Hilliard JK. Herpes B virus-specific pathogen-free breeding colonies of macaques: serologic test results and the B-virus status of the macaque. Contemp Top Lab Anim Sci 2002;41:36–41.
[75] Simon MA, Daniel MD, Lee-Parritz D, King NW, Ringler DJ. Disseminated B virus infection in a cynomolgus monkey. Lab Anim Sci 1993;43:545–50.
[76] Chellman GJ, Lukas VS, Eugui EM, Altera KP, Almquist SJ, Hilliard JK. Activation of B virus (Herpesvirus simiae) in chronically immunosuppressed cynomolgus monkeys. Lab Anim Sci 1992;42:146–51.
[77] Carlson CS, O'Sullivan MG, Jayo MJ, Anderson DK, Harber ES, Jerome WG, et al. Fatal disseminated cercopithecine herpesvirus 1 (herpes B infection in cynomolgus monkeys (*Macaca fascicularis*). Vet Pathol 1997;34:405–14.
[78] Yue Y, Barry PA. Rhesus cytomegalovirus a nonhuman primate model for the study of human cytomegalovirus. Adv Virus Res 2008;72:207–26.
[79] Eizuru Y, Tsuchiya K, Mori R, Minamishima Y. Immunological and molecular comparison of simian cytomegaloviruses isolated from African green monkey (*Cercopithecus aethiops*) and Japanese macaque (*Macaca fuscata*). Arch Virol 1989;107:65–75.
[80] Elkington R, Shoukry NH, Walker S, Crough T, Fazou C, Kaur A, et al. Cross-reactive recognition of human and primate cytomegalovirus sequences by human CD4 cytotoxic T lymphocytes specific for glycoprotein B and H. Eur J Immunol 2004;34:3216–26.
[81] Hutto EH, Anderson DC, Mansfield KG. Cytomegalovirus-associated discrete gastrointestinal masses in macaques infected with the simian immunodeficiency virus. Vet Pathol 2004;41:691–5.
[82] Lorenz D, Albrecht P. Susceptibility of tamarins (*Saguinus*) to measles virus. Lab Anim Sci 1980;30:661–5.
[83] Rangan SR, Chaiban J. Isolation and characterization of a cytomegalovirus from the salivary gland of a squirrel monkey (*Saimiri sciureus*). Lab Anim Sci 1980;30:532–40.
[84] Ambagala AP, Marsh A, Chan J, Pilon R, Fournier J, Mazzulli T, et al. Isolation and characterization of cynomolgus macaque (*Macaca fascicularis*) cytomegalovirus (CyCMV). Virology 2011;412:125–35.
[85] Andrade MR, Yee J, Barry P, Spinner A, Roberts JA, Cabello PH, et al. Prevalence of antibodies to selected viruses in a long-term closed breeding colony of rhesus macaques (*Macaca mulatta*) in Brazil. Am J Primatol 2003;59:123–8.
[86] Vogel P, Weigler BJ, Kerr H, Hendrickx AG, Barry PA. Seroepidemiologic studies of cytomegalovirus infection in a breeding population of rhesus macaques. Lab Anim Sci 1994;44:25–30.
[87] Barry PA, Lockridge KM, Salamat S, Tinling SP, Yue Y, Zhou SS, et al. Nonhuman primate models of intrauterine cytomegalovirus infection. ILAR J 2006;47:49–64.
[88] Klotman ME, Henry SC, Greene RC, Brazy PC, Klotman PE, Hamilton JD. Detection of mouse cytomegalovirus nucleic acid in latently infected mice by in vitro enzymatic amplification. J Infect Dis 1990;161:220–5.
[89] Sinclair J, Sissons P. Latency and reactivation of human cytomegalovirus. J Gen Virol 2006;87:1763–79.
[90] Kamperschroer C, O'Donnell LM, Schneider PA, Li D, Roy M, Coskran TM, et al. Measuring T-cell responses against LCV and CMV in cynomolgus macaques using ELISPOT: potential application to non-clinical testing of immunomodulatory therapeutics. J Immunotoxicol 2014;11:35–43.
[91] Assaf BT, Mansfield KG, Westmoreland SV, Kaur A, Oxford KL, Diamond DJ, et al. Patterns of acute rhesus cytomegalovirus (RhCMV) infection predict long-term RhCMV infection. J Virol 2012;86:6354–7.

[92] Gandhi MK, Khanna R. Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. Lancet Infect Dis 2004;4:725–38.
[93] Cytomegalovirus. Am J Transplant 2004;4(Suppl. 10):51–8.
[94] Humar A, Snydman D. Cytomegalovirus in solid organ transplant recipients. Am J Transplant 2009;9(Suppl. 4):S78–86.
[95] Carville A, Mansfield KG. Comparative pathobiology of macaque lymphocryptoviruses. Comp Med 2008;58:57–67.
[96] Moghaddam A, Rosenzweig M, Lee-Parritz D, Annis B, Johnson RP, Wang F. An animal model for acute and persistent Epstein-Barr virus infection. Science 1997;276:2030–3.
[97] North TW, Sequar G, Townsend LB, Drach JC, Barry PA. Rhesus cytomegalovirus is similar to human cytomegalovirus in susceptibility to benzimidazole nucleosides. Antimicrob Agents Chemother 2004;48:2760–5.
[98] Cho YG, Gordadze AV, Ling PD, Wang F. Evolution of two types of rhesus lymphocryptovirus similar to type 1 and type 2 Epstein-Barr virus. J Virol 1999;73:9206–12.
[99] Ishida T, Yamamoto K. Survey of nonhuman primates for antibodies reactive with Epstein-Barr virus (EBV) antigens and susceptibility of their lymphocytes for immortalization with EBV. J Med Primatol 1987;16:359–71.
[100] Cho Y, Ramer J, Rivailler P, Quink C, Garber RL, Beier DR, et al. An Epstein-Barr-related herpesvirus from marmoset lymphomas. Proc Natl Acad Sci U S A 2001;98:1224–9.
[101] Fujimoto K, Honjo S. Presence of antibody to Cyno-EBV in domestically bred cynomolgus monkeys (*Macaca fascicularis*). J Med Primatol 1991;20:42–5.
[102] Jenson HB, Ench Y, Gao SJ, Rice K, Carey D, Kennedy RC, et al. Epidemiology of herpesvirus papio infection in a large captive baboon colony: similarities to Epstein-Barr virus infection in humans. J Infect Dis 2000;181:1462–6.
[103] Rivailler P, Carville A, Kaur A, Rao P, Quink C, Kutok JL, et al. Experimental rhesus lymphocryptovirus infection in immunosuppressed macaques: an animal model for Epstein-Barr virus pathogenesis in the immunosuppressed host. Blood 2004;104:1482–9.
[104] Baskin GB, Roberts ED, Kuebler D, Martin LN, Blauw B, Heeney J, et al. Squamous epithelial proliferative lesions associated with rhesus Epstein-Barr virus in simian immunodeficiency virus-infected rhesus monkeys. J Infect Dis 1995;172:535–9.
[105] Kutok JL, Klumpp S, Simon M, MacKey JJ, Nguyen V, Middeldorp JM, et al. Molecular evidence for rhesus lymphocryptovirus infection of epithelial cells in immunosuppressed rhesus macaques. J Virol 2004;78:3455–61.
[106] Harari A, Wood CE, Van Doorslaer K, Chen Z, Domaingue MC, Elmore D, et al. Condylomatous genital lesions in cynomolgus macaques from Mauritius. Toxicol Pathol 2013;41:893–901.
[107] Baskin GB, Cremer KJ, Levy LS. Comparative pathobiology of HIV- and SIV-associated lymphoma. AIDS Res Hum Retroviruses 2001;17:745–51.
[108] Fortgang IS, Srivastav SK, Baskin GB, Schumacher PM, Levy LS. Retrospective analysis of clinical and laboratory factors associated with lymphoma in simian AIDS. Leuk Lymphoma 2004;45:161–9.
[109] Fogg MH, Garry D, Awad A, Wang F, Kaur A. The BZLF1 homolog of an Epstein-Barr-related gammaherpesvirus is a frequent target of the CTL response in persistently infected rhesus macaques. J Immunol 2006;176:3391–401.
[110] Johannessen I, Crawford DH. In vivo models for Epstein-Barr virus (EBV)-associated B cell lymphoproliferative disease (BLPD). Rev Med Virol 1999;9:263–77.
[111] Rooney CM, Smith CA, Ng CY, Loftin SK, Sixbey JW, Gan Y, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood 1998;92:1549–55.
[112] Schmidtko J, Wang R, Wu CL, Mauiyyedi S, Harris NL, Della Pelle P, et al. Posttransplant lymphoproliferative disorder associated with an Epstein-Barr-related virus in cynomolgus monkeys. Transplantation 2002;73:1431–9.
[113] Hutto DL. Opportunistic infections in non-human primates exposed to immunomodulatory biotherapeutics: considerations and case examples. J Immunotoxicol 2010;7:120–7.
[114] Kawabata T, Weaver J, Thomas D, Rowe M, Wang F, Kamperschroer C, et al. Summary of roundtable discussion meeting: non-human primates to assess risk for EBV-related lymphomas in humans. J Immunotoxicol 2012;9:121–7.
[115] Bullock G. An association between adenoviruses isolated from simian tonsils and episodes of illness in captive monkeys. J Hyg (Lond) 1965;63:383–7.

VetBooks.ir

[116] Davis KJ, Hubbard GB, Soike KF, Butler TM, Lipscomb TP. Fatal necrotizing adenoviral hepatitis in a chimpanzee (*Pan troglodytes*) with disseminated cytomegalovirus infection. Vet Pathol 1992;29:547–9.
[117] Roy S, Vandenberghe LH, Kryazhimskiy S, Grant R, Calcedo R, Yuan X, et al. Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog 2009;5:e1000503.
[118] Kim CS, Sueltenfuss ES, Kalter SS. Isolation and characterization of simian adenoviruses isolated in association with an outbreak of pneumoenteritis in vervet monkeys (*Cercopithecus aethiops*). J Infect Dis 1967;117:292–300.
[119] Shroyer EL, Kelley ST, Taylor PC, Vanderloo P, Lester TL. Three serologic types of adenovirus infections of owl monkeys. Am J Vet Res 1979;40:532–6.
[120] Martin BJ, Dysko RC, Chrisp CE. Pancreatitis associated with simian adenovirus 23 in a rhesus monkey. Lab Anim Sci 1991;41:382–4.
[121] McClure HM, Chandler FW, Hierholzer JC. Necrotizing pancreatitis due to simian adenovirus type 31 in a rhesus monkey. Arch Pathol Lab Med 1978;102:150–3.
[122] Baskin GB, Soike KF. Adenovirus enteritis in SIV-infected rhesus monkeys. J Infect Dis 1989;160:905–7.
[123] Ochs HD, Morton WR, Tsai CC, Thouless ME, Zhu Q, Kuller LD, et al. Maternal-fetal transmission of SIV in macaques: disseminated adenovirus infection in an offspring with congenital SIV infection. J Med Primatol 1991;20:193–200.
[124] Chandler FW, Callaway CS, Adams SR. Pancreatitis associated with an adenovirus in a rhesus monkey. Vet Pathol 1974;11:165–71.
[125] Boyce JT, Giddens Jr. WE, Valerio M. Simian adenoviral pneumonia. Am J Pathol 1978;91:259–76.
[126] Stuker G, Oshiro LS, Schmidt NJ, Holmberg CA, Anderson JH, Glaser CA, et al. Virus detection in monkeys with diarrhea: the association of adenoviruses with diarrhea and the possible role of rotaviruses. Lab Anim Sci 1979;29:610–6.
[127] Sestak K, Merritt CK, Borda J, Saylor E, Schwamberger SR, Cogswell F, et al. Infectious agent and immune response characteristics of chronic enterocolitis in captive rhesus macaques. Infect Immun 2003;71:4079–86.
[128] Simon MA. Polyomaviruses of nonhuman primates: implications for research. Comp Med 2008;58:51–6.
[129] Horvath CJ, Simon MA, Bergsagel DJ, Pauley DR, King NW, Garcea RL, et al. Simian virus 40-induced disease in rhesus monkeys with simian acquired immunodeficiency syndrome. Am J Pathol 1992;140:1431–40.
[130] Maki T, Carville A, Stillman IE, Sato K, Kodaka T, Minamimura K, et al. SV40 infection associated with rituximab treatment after kidney transplantation in nonhuman primates. Transplantation 2008;85:893–902.
[131] van Gorder MA, Della Pelle P, Henson JW, Sachs DH, Cosimi AB, Colvin RB. Cynomolgus polyoma virus infection: a new member of the polyoma virus family causes interstitial nephritis, ureteritis, and enteritis in immunosuppressed cynomolgus monkeys. Am J Pathol 1999;154:1273–84.
[132] Simon MA. Simian parvoviruses: biology and implications for research. Comp Med 2008;58:47–50.
[133] O'Sullivan MG, Anderson DC, Fikes JD, Bain FT, Carlson CS, Green SW, et al. Identification of a novel simian parvovirus in cynomolgus monkeys with severe anemia. A paradigm of human B19 parvovirus infection. J Clin Invest 1994;93:1571–6.
[134] O'Sullivan MG, Anderson DK, Goodrich JA, Tulli H, Green SW, Young NS, et al. Experimental infection of cynomolgus monkeys with simian parvovirus. J Virol 1997;71:4517–21.
[135] Schroder C, Pfeiffer S, Wu G, Azimzadeh AM, Aber A, Pierson 3rd RN, et al. Simian parvovirus infection in cynomolgus monkey heart transplant recipients causes death related to severe anemia. Transplantation 2006;81:1165–70.
[136] Lin PL, Flynn JL. Tuberculosis research using nonhuman primates. In: Abee CR, Mansfield K, Tardif S, editors. Nonhuman primates in biomedical research. Canada: Elsevier; 2012. p. 173–96.
[137] Wilbur AK, Engel GA, Jones-Engel L. TB infection in the nonhuman primate biomedical model: tip of the iceberg? Med Hypotheses 2012;79:365–7.
[138] Centers for Disease Control and Prevention (CDC). Tuberculosis in imported nonhuman primates—United States, June 1990-May 1993. MMWR Morb Mortal Wkly Rep 1993;42:572–6.
[139] Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA 1999;282:677–86.
[140] Riordan JT. Rectal tuberculosis in monkeys from the use of contaminated thermometers. J Infect Dis 1943;73:221–3.

[141] Allen AM, Kinard RF. Primary cutaneous inoculation tuberculosis in the *Macaca mulatta* monkey. Am J Pathol 1958;34:337–47.
[142] Langermans JA, Andersen P, van Soolingen D, Vervenne RA, Frost PA, van der Laan T, et al. Divergent effect of bacillus Calmette-Guerin (BCG) vaccination on *Mycobacterium tuberculosis* infection in highly related macaque species: implications for primate models in tuberculosis vaccine research. Proc Natl Acad Sci U S A 2001;98:11497–502.
[143] Capuano 3rd. SV, Croix DA, Pawar S, Zinovik A, Myers A, Lin PL, et al. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. Infect Immun 2003;71:5831–44.
[144] Lin PL, Yee J, Klein E, Lerche NW. Immunological concepts in tuberculosis diagnostics for non-human primates: a review. J Med Primatol 2008;37(Suppl. 1):44–51.
[145] Garcia MA, Yee J, Bouley DM, Moorhead R, Lerche NW. Diagnosis of tuberculosis in macaques, using whole-blood in vitro interferon-gamma (PRIMAGAM) testing. Comp Med 2004;54:86–92.
[146] Lyashchenko KP, Greenwald R, Esfandiari J, Greenwald D, Nacy CA, Gibson S, et al. PrimaTB STAT-PAK assay, a novel, rapid lateral-flow test for tuberculosis in nonhuman primates. Clin Vaccine Immunol 2007;14:1158–64.
[147] Wachtman LM, Miller AD, Xia D, Curran EH, Mansfield KG. Colonization with nontuberculous mycobacteria is associated with positive tuberculin skin test reactions in the common marmoset (*Callithrix jacchus*). Comp Med 2011;61:278–84.
[148] Bowers LC, Purcell JE, Plauche GB, Denoel PA, Lobet Y, Philipp MT. Assessment of the nasopharyngeal bacterial flora of rhesus macaques: moraxella, Neisseria, haemophilus, and other genera. J Clin Microbiol 2002;40:4340–2.
[149] VandeWoude SJ, Luzarraga MB. The role of *Branhamella catarrhalis* in the "bloody-nose syndrome" of cynomolgus macaques. Lab Anim Sci 1991;41:401–6.
[150] Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. Clin Infect Dis 2009;49:124–31.
[151] Nakamura M, Murayama SY, Serizawa H, Sekiya Y, Eguchi M, Takahashi S, et al. "Candidatus *Helicobacter heilmannii*" from a cynomolgus monkey induces gastric mucosa-associated lymphoid tissue lymphomas in C57BL/6 mice. Infect Immun 2007;75:1214–22.
[152] Reindel JF, Fitzgerald AL, Breider MA, Gough AW, Yan C, Mysore JV, et al. An epizootic of lymphoplasmacytic gastritis attributed to Helicobacter pylori infection in cynomolgus monkeys (*Macaca fascicularis*). Vet Pathol 1999;36:1–13.
[153] Drevon-Gaillot E, Perron-Lepage MF, Clement C, Burnett R. A review of background findings in cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins. Exp Toxicol Pathol 2006;58:77–88.
[154] Doi SQ, Kimbason T, Reindel J, Dubois A. Molecular characterization of Helicobacter pylori strains isolated from cynomolgus monkeys (*M. fascicularis*). Vet Microbiol 2005;108:133–9.
[155] Le Bras J, Larouze B, Geniteau M, Andrieu B, Dazza MC, Rodhain F. Malaria, arbovirus and hepatitis infections in *Macaca fascicularis* from Malaysia. Lab Anim 1984;18:61–4.
[156] Donovan JC, Stokes WS, Montrey RD, Rozmiarek H. Hematologic characterization of naturally occurring malaria (*Plasmodium inui*) in cynomolgus monkeys (*Macaca fascicularis*). Lab Anim Sci 1983;33:86–9.
[157] Lehner NDM. Biology and diseases of Cebidae. In: Fox BG, Cohen BJ, Loew FM, editors. Laboratory animal medicine. Orlando, FL: Academic Press; 1984. p. 321–53.
[158] Marinkelle CJ, Grose ES. Plasmodium brasilianum in Colombian monkeys. Trop Geogr Med 1968;20:276–80.
[159] Coatney GR, Chin W, Contacos PG, King HK. *Plasmodium inui*, a quartan-type malaria parasite of Old World monkeys transmissible to man. J Parasitol 1966;52:660–3.
[160] Bray RS, Garnham PC. The life-cycle of primate malaria parasites. Br Med Bull 1982;38:117–22.
[161] Antinori S, Galimberti L, Milazzo L, Corbellino M. *Plasmodium knowlesi*: the emerging zoonotic malaria parasite. Acta Trop 2013;125:191–201.
[162] Aravind L, Iyer LM, Wellems TE, Miller LH. Plasmodium biology: genomic gleanings. Cell 2003;115:771–85.
[163] http://www.criver.com/files/pdfs/research-models/rm_ld_c_rads_us_a.aspx.
[164] http://www.vrlsat.com/catalog/.
[165] Collins WE, Skinner JC, Broderson JR, Filipski VK, Morris CM, Stanfill PS, et al. Susceptibility of *Macaca fascicularis* monkeys from Mauritius to different species of *Plasmodium*. J Parasitol 1992;78:505–11.
[166] Matsumoto J, Kawai S, Terao K, Kirinoki M, Yasutomi Y, Aikawa M, et al. Malaria infection induces rapid elevation of the soluble Fas ligand level in serum and subsequent T lymphocytopenia: possible factors

VetBooks.ir

responsible for the differences in susceptibility of two species of *Macaca* monkeys to *Plasmodium coatneyi* infection. Infect Immun 2000;68:1183–8.
[167] Chang KH, Tam M, Stevenson MM. Modulation of the course and outcome of blood-stage malaria by erythropoietin-induced reticulocytosis. J Infect Dis 2004;189:735–43.
[168] Chamanza R, Marxfeld HA, Blanco AI, Naylor SW, Bradley AE. Incidences and range of spontaneous findings in control cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. Toxicol Pathol 2010;38:642–57.
[169] Chamanza R, Parry NM, Rogerson P, Nicol JR, Bradley AE. Spontaneous lesions of the cardiovascular system in purpose-bred laboratory nonhuman primates. Toxicol Pathol 2006;34:357–63.
[170] Vidal JD, Drobatz LS, Holliday DF, Geiger LE, Thomas HC. Spontaneous findings in the heart of Mauritian-origin cynomolgus macaques (*Macaca fascicularis*). Toxicol Pathol 2010;38:297–302.
[171] Hernandez-Jauregui P, Silva-Lemoine E, Giron-Rojas H. A case of sarcocystic myocarditis in a rhesus monkey. Light and electron microscopic study. Arch Invest Med (Mex) 1983;14:139–44.
[172] Lane JH, Mansfield KG, Jackson LR, Diters RW, Lin KC, MacKey JJ, et al. Acute fulminant sarcocystosis in a captive-born rhesus macaque. Vet Pathol 1998;35:499–505.
[173] Pisharath H, Zao CL, Kreeger J, Portugal S, Kawabe T, Burton T, et al. Immunopathologic characterization of naturally acquired Trypanosoma cruzi infection and cardiac sequalae in cynomolgus macaques (*Macaca fascicularis*). J Am Assoc Lab Anim Sci 2013;52:545–52.
[174] Mubiru JN, Yang A, Dick Jr EJ, Owston M, Sharp RM, Vandeberg JF, et al. Correlation between presence of Trypanosoma cruzi DNA in heart tissue of baboons and cynomolgus monkeys, and lymphocytic myocarditis. Am J Trop Med Hyg 2014;90:627–33.
[175] Anderson DC, McClure HM. Acute disseminated fatal toxoplasmosis in a squirrel monkey. J Am Vet Med Assoc 1982;181:1363–6.
[176] Cunningham AA, Buxton D, Thomson KM. An epidemic of toxoplasmosis in a captive colony of squirrel monkeys (*Saimiri sciureus*). J Comp Pathol 1992;107:207–19.
[177] Pertz C, Dubielzig RR, Lindsay DS. Fatal Toxoplasma gondii infection in golden lion tamarins (*Leontopithecus rosalia rosalia*). J Zoo Wildl Med 1997;28:491–3.
[178] Guscetti F, Mathis A, Hatt JM, Deplazes P. Overt fatal and chronic subclinical *Encephalitozoon cuniculi* microsporidiosis in a colony of captive emperor tamarins (*Saguinus imperator*). J Med Primatol 2003;32:111–9.
[179] Juan-Salles C, Garner MM, Didier ES, Serrato S, Acevedo LD, Ramos-Vara JA, et al. Disseminated encephalitozoonosis in captive, juvenile, cotton-top (*Saguinus oedipus*) and neonatal emperor (*Saguinus imperator*) tamarins in North America. Vet Pathol 2006;43:438–46.
[180] Masek-Hammerman K, Miller AD, Lin KC, MacKey J, Weissenbock H, Gierbolini L, et al. Epizootic myocarditis associated with encephalomyocarditis virus in a group of rhesus macaques (*Macaca mulatta*). Vet Pathol 2012;49:386–92.
[181] Rassi Jr. A, Rassi A, Marcondes de Rezende J. American trypanosomiasis (Chagas disease). Infect Dis Clin North Am 2012;26:275–91.
[182] Carvalho CM, Andrade MC, Xavier SS, Mangia RH, Britto CC, Jansen AM, et al. Chronic Chagas' disease in rhesus monkeys (*Macaca mulatta*): evaluation of parasitemia, serology, electrocardiography, echocardiography, and radiology. Am J Trop Med Hyg 2003;68:683–91.
[183] Andrade MC, Dick Jr. EJ, Guardado-Mendoza R, Hohmann ML, Mejido DC, VandeBerg JL, et al. Nonspecific lymphocytic myocarditis in baboons is associated with Trypanosoma cruzi infection. Am J Trop Med Hyg 2009;81:235–9.
[184] Chuenkova MV, Pereiraperrin M. Neurodegeneration and neuroregeneration in Chagas disease. Adv Parasitol 2011;76:195–233.
[185] Fazan VP, Lachat JJ. Qualitative and quantitative morphology of the vagus nerve in experimental Chagas' disease in rats: a light microscopy study. Am J Trop Med Hyg 1997;57:672–7.
[186] Sasseville VG, Pauley DR, MacKey JJ, Simon MA. Concurrent central nervous system toxoplasmosis and simian immunodeficiency virus-induced AIDS encephalomyelitis in a Barbary macaque (*Macaca sylvana*). Vet Pathol 1995;32:81–3.
[187] Munoz M, Liesenfeld O, Heimesaat MM. Immunology of *Toxoplasma gondii*. Immunol Rev 2011;240:269–85.
[188] Hoffmann C, Ernst M, Meyer P, Wolf E, Rosenkranz T, Plettenberg A, et al. Evolving characteristics of toxoplasmosis in patients infected with human immunodeficiency virus-1: clinical course and *Toxoplasma gondii*-specific immune responses. Clin Microbiol Infect 2007;13:510–5.

[189] Benirschke K, Richart R. Spontaneous acute toxoplasmosis in a marmoset monkey. Am J Trop Med Hyg 1960;9:269–73.
[190] Wong MM, Kozek WJ. Spontaneous toxoplasmosis in macaques: a report of four cases. Lab Anim Sci 1974;24:273–8.
[191] Dubey JP, Lindsay DS, Speer CA. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev 1998;11:267–99.
[192] Blanchard JL, Baskin GB. Trichomonas gastritis in rhesus monkeys infected with the simian immunodeficiency virus. J Infect Dis 1988;157:1092–3.
[193] Kondova I, Simon MA, Klumpp SA, MacKey J, Widmer G, Domingues HG, et al. Trichomonad gastritis in rhesus macaques (*Macaca mulatta*) infected with simian immunodeficiency virus. Vet Pathol 2005;42:19–29.
[194] Wikse SE, Fox JG, Kovatch RM. Candidiasis in simian primates. Lab Anim Care 1970;20:957–63.
[195] Al-Doory Y. Fungal and bacterial diseases. In: Fiennes RNT-W, editor. Pathology of simian primates. Basel: Karger; 1972. p. 206–41.
[196] Hunt RD, Anderson MP, Chalifoux LV. Spontaneous infectious diseases of marmosets. Primates Med 1978;10:239–53.
[197] Migaki G, Schmidt RE, Toft 2nd. JD, Kaufmann AF. Mycotic infections of the alimentary tract of nonhuman primates: a review. Vet Pathol Suppl 1982;19(Suppl. 7):93–103.
[198] Patterson DR, Wagner JE, Owens DR, Ronald NC, Frisk CS. *Candida albicans* infections associated with antibiotic and corticosteroid therapy in spider monkeys. J Am Vet Med Assoc 1974;164:721–2.
[199] Furuta T, Fujita M, Mukai R, Sakakibara I, Sata T, Miki K, et al. Severe pulmonary pneumocystosis in simian acquired immunodeficiency syndrome induced by simian immunodeficiency virus: its characterization by the polymerase-chain-reaction method and failure of experimental transmission to immunodeficient animals. Parasitol Res 1993;79:624–8.
[200] Gigliotti F, Harmsen AG, Haidaris CG, Haidaris PJ. *Pneumocystis carinii* is not universally transmissible between mammalian species. Infect Immun 1993;61:2886–90.
[201] Norris KA, Wildschutte H, Franko J, Board KF. Genetic variation at the mitochondrial large-subunit rRNA locus of *Pneumocystis* isolates from simian immunodeficiency virus-infected rhesus macaques. Clin Diagn Lab Immunol 2003;10:1037–42.
[202] Demanche C, Wanert F, Barthelemy M, Mathieu J, Durand-Joly I, Dei-Cas E, et al. Molecular and serological evidence of *Pneumocystis* circulation in a social organization of healthy macaques (*Macaca fascicularis*). Microbiology 2005;151:3117–25.
[203] Vogel P, Miller CJ, Lowenstine LL, Lackner AA. Evidence of horizontal transmission of *Pneumocystis carinii* pneumonia in simian immunodeficiency virus-infected rhesus macaques. J Infect Dis 1993;168:836–43.
[204] Board KF, Patil S, Lebedeva I, Capuano 3rd S, Trichel AM, Murphey-Corb M, et al. Experimental *Pneumocystis carinii* pneumonia in simian immunodeficiency virus-infected rhesus macaques. J Infect Dis 2003;187:576–88.
[205] Knezevich AL, McNulty Jr. WP. Pulmonary acariasis (*Pneumonyssus simicola*) in colony-bred *Macaca mulatta*. Lab Anim Care 1970;20:693–6.
[206] Andrade MC, Marchevsky RS. Histopathologic findings of pulmonary acariasis in a rhesus monkeys breeding unit. Rev Bras Parasitol Vet 2007;16:229–34.

VetBooks.ir

VetBooks.ir

CHAPTER

11

Consequences and Indicators of Test Article-Related Immune Suppression in Nonhuman Primates

Jacintha M. Shenton[*], *Kristin L. Henson*[†], *Marlon C. Rebelatto*[‡]

[*]Novartis Institutes for Biomedical Research, Cambridge, MA, USA

[†]Novartis Institutes for Biomedical Research, East Hanover, NJ, USA

[‡]Biologics Safety Assessment, MedImmune, Gaithersburg, MD, USA

OUTLINE

Introduction 216

Selection of the NHP as the Nonclinical Safety Species for Immunomodulatory Drugs 218

Immunologically Relevant End Points in Standard Toxicology Studies of NHPs 219

Clinical Pathology 220

Hematology 220

Serum Chemistry 221

Gross Pathology and Histopathology 222

Specialized Immunologic End Points in NHPs 222

Differential Diagnosis of On-Target, Off-Target, and Stress-Related Effects on the Immune System 224

Diagnosis of Stress-Related Effects on the Immune System 224

Diagnosis of Suspected Infections and/or the Presence of Infectious Agent(s) and Establishing the Role of Test Article-Related Immune Suppression 225

Identifying Hyperplastic Lesions and Tumors and Establishing the Role of Test Article-Related Immune Suppression 226

http://dx.doi.org/10.1016/B978-0-12-417144-2.00011-1

© 2015 Elsevier Inc. All rights reserved.

Challenges for Diagnosing Test Article-Related Immune Suppression in Lymphoid Tissue 226
Role of Immune Suppression in Decreased Serum Globulins 227
Use of Bone Marrow Smears for Mechanistic Insight into Test Article-Related Changes to the Hemogram and/or Bone Marrow 227
Role of Immune Function Assays in Differential Diagnosis and Risk Assessment 227
Clinically Apparent Manifestations of Immune Suppression in NHPs Observed in Nonclinical Safety Studies: Case Examples 228
Identification of a Risk for Clinically Adverse Immune Suppression: Impact on Further Development 231
Conclusion 232
References 232

INTRODUCTION

Nonhuman primates (NHPs), particularly cynomolgus monkeys (*Macaca fascicularis*), are sometimes used as a nonrodent species to assess the nonclinical safety of small-molecule drugs [1,2] and are routinely the only species used to assess the nonclinical safety of large-molecule drugs (biotherapeutics) [3]. In many cases these drugs are intended immunomodulators for the treatment of diseases in which aberrant or unwanted immune responses are a principal or contributing factor [4]. Thus the nonclinical investigator experienced with the safety evaluation of immunomodulatory drugs will undoubtedly also become experienced in the consequences of immune suppression in NHPs.

In this chapter the term immune suppression is used to refer to any alteration in the immune system that could potentially decrease the host's resistance to infectious agents or aberrant cells. The possible consequences of immune suppression observed in NHPs are similar to the spectrum of consequences observed in humans: opportunistic infections, recrudescence of latent infections, and virally induced cancers, i.e., clinically apparent disease [5]. Because of the redundancy of the immune system, drug-related phenotypic (e.g., decreased B-cell counts) or functional changes (e.g., reduced B-cell responses to mitogen) in the immune system indicate only the potential for increased susceptibility to developing clinically apparent disease. Within the confines of toxicity studies using NHPs, however, clinically apparent consequences of immune suppression may not be observed because of a lack of challenge or because of the short duration of the study. Thus, recognizing indicators of immune suppression observable in standard toxicity studies—as described in the International Conference on Harmonisation (ICH) S8 guidance on Immunotoxicity Studies for Human Pharmaceuticals [6]—and discriminating whether these findings are on/off target or stress related are important.

The ICH S8 guidance and the United States Food and Drug Administration (US FDA) guidance on Immunotoxicology Evaluation of Investigational New Drugs [7] are useful references for both small- and large-molecule drugs (these guidance documents are discussed in Chapter 27, "Immunotoxicology Testing of Monoclonal Antibodies in Cynomolgus Monkeys: From Manufacturing to Preclinical Studies" as well). Although the scope of ICH S8

VetBooks.ir

specifically excludes biotechnology-derived pharmaceutical products and other biologicals and is restricted to unintended immunomodulators, the principles and studies described are generally applicable to both small and large molecules intended for use as immunomodulators. A comment in the European Public Assessment Report on the small-molecule Janus kinase (JAK) inhibitor tofacitinib (Xeljanz®) illustrates this point: "Studies outlined in ICH S8 do not apply since tofacitinib is intended to modulate the immune response. However, parameters that relate to immune toxicity were collected in some of the toxicology studies (hematology/immunophenotyping, lymphoid organ weights, and histology)" [8]. The ICH S6 (R1) guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals does not provide specific guidance on immune system evaluations but notes that immunotoxicologic evaluation may require screening studies followed by mechanistic studies to clarify immunosafety-related issues, which is in line with the principles of ICH S8 [9].

Specifically, ICH S8 describes a tiered approach to immunotoxicity assessment. That is, the initial screen for potential immunotoxicity derives from routine parameters assessed in standard toxicity studies, namely clinical signs, clinical pathology, organ weight, and anatomic pathology. A weight-of-evidence review—including differential diagnosis—defines whether a cause for concern exists and whether additional immunotoxicity studies should be conducted [6]. These additional studies could involve stand-alone studies and/or the addition of specialized immunologic assessments to ongoing or planned studies. For intended immunomodulators, a tiered approach may emerge when the breadth and depth of the intended pharmacology is greater than expected or when there are potential immunotoxicologic findings with unknown relevance to the intended pharmacology. These unexpected findings may warrant specialized immunologic assessments. Prospectively including specialized immunologic assessments in standard toxicity studies may also be appropriate to demonstrate pharmacodynamic (PD) effects and/or to characterize the breadth and depth of the expected pharmacology. This is especially the case for NHP studies to avoid unnecessary use of animals.

Once test article-related immune suppression is suspected or confirmed in NHPs, the risk assessment for clinically adverse immune suppression is generally focused on the breadth and/or depth of the immunologic changes observed (Figure 11.1), reversibility of these changes following withdrawal of the test article, consideration of the dose-response in relation to clinical doses, the duration of clinical dosing, the clinical ability to monitor for the finding, and characteristics of the proposed patient population. Based on the anticipated risk in the clinical population, establishing a risk management plan based on exclusion criteria, vaccination protocols, and/or additional immune system monitoring may be possible. Nonclinical findings of immune suppression are important and can impact development even when a significant clinical database has been accrued (see Section "Clinically Apparent Manifestations of Immune Suppression in NHPs Observed in Nonclinical Safety Studies: Case Examples" later in this chapter). Nonclinical immune suppression may also be described on the product label (for an example, refer to the label for abatacept [ORENCIA] [10]).

The goals of this chapter are to discuss the challenges of using NHPs to evaluate the nonclinical safety of intended immunomodulators, outline indicators of immune suppression observable from both routine parameters included in standard toxicity studies and specialized immunologic assessments, provide considerations for differential diagnosis, describe clinically apparent manifestations of immune suppression in NHPs that have been observed within the confines of nonclinical safety studies, and highlight the possible impact of immune

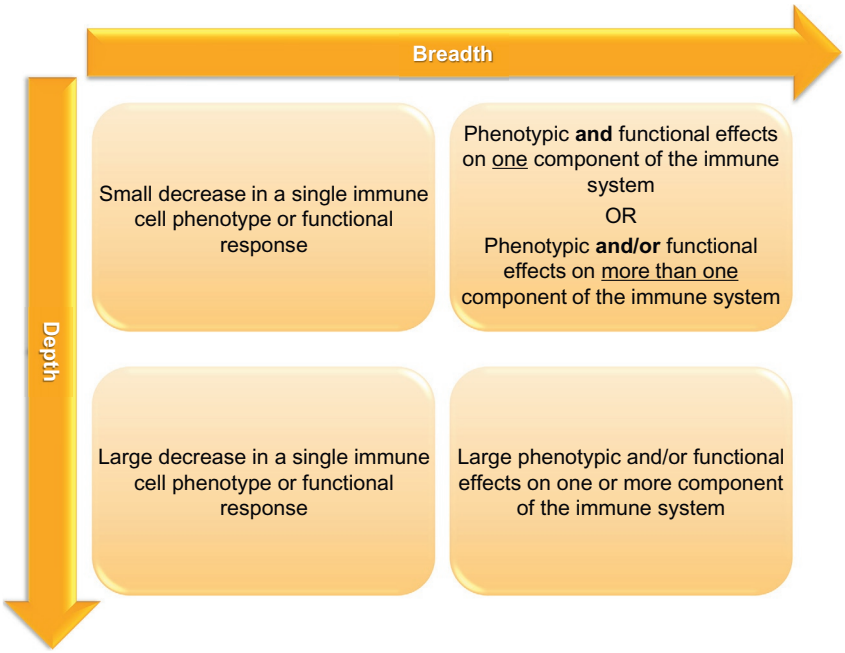


FIGURE 11.1 The breadth and depth of the effects on the immune system should be considered in the overall risk assessment. The effects on the immune system may be considered based on breadth, i.e., the number of components affected and whether there are combined phenotypic and functional effects, and depth, i.e., the magnitude of the changes. For instance, evidence of drug-induced neutropenia (narrow) would be considered differently than pancytopenia (broad), neutropenia in combination with impaired neutrophil function (broad), or agranulocytosis (deep).

suppression on the clinical use of the drug under investigation. Many of the principles discussed in this chapter are drawn from experience with intended immunomodulators; however, the same principles can generally be applied following the observation of unintended immunological effects in nonclinical safety studies using NHPs. Because the cynomolgus monkey is the most common NHP used for nonclinical safety assessment, this chapter focuses on this species of monkey.

SELECTION OF THE NHP AS THE NONCLINICAL SAFETY SPECIES FOR IMMUNOMODULATORY DRUGS

NHPs, especially cynomolgus monkeys, are often used for the nonclinical safety evaluation of immunomodulatory drugs. Nonclinical safety evaluation of biotherapeutics is often limited to NHPs because of species specificity (i.e., the NHP is the only nonclinical species that is pharmacologically relevant [3,9]) and/or a reduced potential for immunogenicity [11].The consequences of immunomodulation may also be more readily observed and evaluated in NHPs than in dogs—the more commonly used large animal species for small-molecule drugs [12,13]. Many specialized immunologic assessments are feasible and have been developed for application in cynomolgus monkeys (see Chapter 27, "Immunotoxicology Testing of Monoclonal Antibodies

VetBooks.ir

in Cynomolgus Monkeys: From Manufacturing to Preclinical Studies" and Lebrec [14]). Of note, cross-reactivity of many antihuman cluster of differentiation (CD) markers to cynomolgus monkeys facilitates immunophenotyping assessments. As such, the cynomolgus monkey may also be selected in part or wholly because of the intended immunomodulatory effects of the drug under investigation.

Although the rhesus monkey (*Macaca mulatta*) is commonly used for academic research of immune modulation, for example, as a model of simian immunodeficiency virus (SIV) or organ transplant rejection, rhesus monkeys are rarely used for nonclinical safety assessment of drugs. This is, in part, because rhesus monkeys are seasonal breeders, limiting the ability to conduct studies of development and reproduction, which are required for most drugs; NHPs may be the only species available in which to conduct development and reproduction studies of large molecules. Marmosets (*Callithrix jacchus*), a New World monkey, are also less commonly used than cynomolgus monkeys partly because of their small size (250-450 g) and smaller blood volumes, which hamper the conduct of specialized immunologic assessments such as immunophenotyping [15].

Despite the phylogenetic similarity of cynomolgus monkeys to humans and the array of immunologic assessments that can be conducted in this species, there are also differences between the cynomolgus monkey and human immune systems, data gaps that must be considered and challenges to be faced in the design and interpretation of nonclinical studies [16] (Table 11.1). The small number of animals being studied and genetic diversity may contribute to a low frequency of immunologic changes that are more difficult to interpret compared with studies of rodents. NHP pathogen load can also challenge the interpretation of NHP studies (see Chapter 10, "Nonhuman Primate Diseases of Relevance for Drug Development and its Impact on Interpretation of Study Findings"). However, illness due to opportunistic infections, recrudescence of latent infections, and virally induced cancers can identify excessive immune suppression. The lack of such clinically apparent disease in NHPs following dosing with immunomodulatory drugs has been leveraged to argue against excessive immune suppression (see the Section "Clinically Apparent Manifestations of Immune Suppression in NHPs Observed in Nonclinical Safety Studies: Case Examples" later in this chapter).

IMMUNOLOGICALLY RELEVANT END POINTS IN STANDARD TOXICOLOGY STUDIES OF NHPs

As described in ICH S8, immune-relevant end points in standard toxicity studies are considered tier 1 tests and include alterations in clinical pathology (peripheral blood leukocyte counts, changes in serum globulins) and/or gross pathology and histopathology (macroscopic changes in lymphoid organs, alterations in lymphoid organ weights, and microscopically observed changes in lymphoid tissue) [6]. These routine parameters are considered structural or morphological assessments rather than functional immune tests. In addition, increased incidence of infection or tumor occurrence in nonclinical studies may indicate decreased immune surveillance as a consequence of test article-related immune suppression (see Chapter 10 "Nonhuman Primate Diseases of Relevance for Drug Development and its Impact on Interpretation of Study Findings"). In fact such clinically apparent disease may be the key indicator of immune suppression in cases where function of a given cell type is affected (e.g., macrophage phagocytosis) without any alterations to cell morphology or cell numbers in blood and tissues. The immune-relevant end points in standard toxicity studies are considered next.

TABLE 11.1 Human and NHP Immune Systems: Known Differences and Data Gaps Relevant to Nonclinical Safety Assessment

Known difference or data gap	Consequences
CD56 in macaques is expressed not just by NK cells but also by monocytes [17]	Other phenotypic markers such as CD159a or CD16 should be considered
Cynomolgus monkeys have two distinct populations of B cells rather than one ($CD20^{high}CD40^{low}CD21^{-}$ and $CD20^{low}CD40^{high}CD21^{+}$) [18]	• Analysts must be aware of this because CD20 is used as a B-lymphocyte marker when immunophenotyping cynomolgus monkeys • Challenges with extrapolating results from cynomolgus monkeys to humans when targeting B cells, e.g., B-cell depleting agents such as rituximab (RITUXAN®), an anti-CD20 mAb [18]
• NHPs have different FcγR expression patterns. For example, cynomolgus monkeys have one CD16 (FcγRIII) gene homologous to human CD16a, whereas humans also express CD16b. CD16a expression is more restricted in cynomolgus monkeys [19] • Comparative biology between NHP Fc-FcγR or human Fc-NHP FcγR interactions and human Fc-FcγR interactions is not well characterized. This also extends to Fc interactions with the complement component C1q [20]	• For biotherapeutics targeting human FcγR for therapy, justifying the NHP as a pharmacologically relevant species may be challenging • For biotherapeutics with Fc-mediated pharmacology, e.g., antibody-dependent cellular cytotoxicity, human Fc-NHP FcγR interactions may not predict human Fc-human FcγR interactions [21]
NHP T cells express inhibitory signaling molecules (Siglecs) [22]	NHP T cells give weaker proliferative responses upon activation via the T-cell receptor [22]. This may make NHPs less likely to manifest certain immunologic effects
Macaque $CD4^{+}$ effector memory T cells lack CD28 expression [23]	Macaques failed to predict the cytokine storm observed in humans with TGN1412 (CD28 superagonist mAb). $CD4^{+}$ effector memory T cells were a key source of cytokines in humans; thus the lack of CD28 expression on these cells in macaques may have contributed to the lack of predictivity [23]

Clinical Pathology

Clinical pathology evaluation not only yields information on the potential for test article-related immune suppression but can provide a time course assessment if samples are collected serially within a study. Hematological assessment of leukocytes and serum chemistry evaluation of globulin concentrations can also provide clinically relevant and translatable data because these samples are routinely collected during clinical trials.

Hematology

Neutrophils and lymphocytes in NHPs generally occur in similar proportions, with a trend toward neutrophil predominance in rhesus and African green monkeys, chimpanzees, and baboons and lymphocyte predominance in adult cynomolgus and New World monkeys [24].

VetBooks.ir

Leukocyte numbers may vary because of handling-associated stress (see Section "Differential Diagnosis of On-Target, Off-Target, and Stress-Related Effects on the Immune System"), and interanimal variability can make comparison between control and treated animals difficult. Thus, appropriate acclimation to minimize handling-associated stress and comparison with individual animal pretest (i.e., before the first dose) values are important. Hematology changes suggestive of test article-related immune suppression include decreased total leukocyte counts (white blood cell counts), with associated decreases in granulocytes (neutrophils/eosinophils) and/or lymphocytes. Causes of decreased granulocyte and lymphocyte absolute counts include suppression of hematopoiesis/lymphopoiesis due to test article cytotoxicity or alterations in hemato/lymphopoietic cytokines or growth factors, changes in leukocyte trafficking (e.g., sphingosine 1-phosphate receptor modulators such as fingolimod [GILENYA] [2,25]), intended deletion of immune cells (e.g., B-cell-depleting agents such as rituximab [18]), or increased utilization of granulocytes secondary to infection or severe inflammation. Since significant decreases in neutrophil numbers (neutropenia) increase the risk for life-threatening opportunistic infections and sepsis, guidelines are available for neutropenia monitoring and intervention in clinical trials and chemotherapeutic therapy in veterinary medicine using a severity grading scale of neutropenia based on human- and canine-specific threshold values [26,27]. Guidelines for NHPs have not been published; however, similar rules of thumb for assessing neutropenia in NHPs in nonclinical studies may be considered. While thresholds for neutropenia and subsequent interventions should utilize facility- or laboratory-specific reference intervals for the NHP species in question, in cynomolgus monkeys a consistent reduction in circulating neutrophils to $<1.0\times10^9$/L warrants monitoring for fever and adverse gastrointestinal signs (vomiting, diarrhea). For neutrophil counts $<0.4\times10^9$/L, discontinuation of test article administration and institution of antibiotic and fluid therapy, in addition to careful monitoring for fever/gastrointestinal signs/sepsis should be considered [28]. Acute reductions in lymphocytes (lymphopenia) are typically not associated with life-threatening complications; however, marked reductions in lymphocytes over the course of a study should trigger careful monitoring for evidence of infection, such as inappetence, fever, skin lesions, and pulmonary or gastrointestinal signs.

Serum Chemistry

Serum globulin concentrations comprise the alpha, beta, and gamma globulin fractions based on protein electrophoretic fractionation. Acute-phase proteins, complement, and fibrinogen migrate in the alpha and beta fractions and immunoglobulins (Igs), primarily IgG, migrate in the gamma globulin fraction. Test article-related decreases in lymphocyte numbers or suppression of lymphocyte function may manifest as decreased serum globulin concentrations due to decreased Ig production [29]. A decreased serum globulin concentration may also be associated with an increased albumin-to-globulin (A:G) ratio and decreased total protein concentration, depending on the severity of the globulin change. Since measurement of serum globulin concentrations provides an overall assessment of circulating Igs, changes in specific classes or subclasses may not be detected through biochemistry profiling. Serum IgM, IgA, or IgG or NHP-specific IgG subclasses (IgG1, IgG2, IgG3) may be considered as a specialized immunologic end point for evaluation in routine toxicity studies, particularly if test article-related modulation of Ig production is anticipated or suspected [29,30].

VetBooks.ir

Gross Pathology and Histopathology

Macroscopic (including lymphoid organ weights and gross pathologic changes) and microscopic evaluation of lymphoid tissue is a key component of assessing the immunosuppressive potential of a therapeutic compound. NHP lymphoid organs are generally similar to humans and characterized by a bilobed thymus located in the anterior thoracic cavity, a spleen composed of distinct lymphoid compartments, lymph nodes with large numbers of well-developed germinal centers, and mucosal-associated lymphoid tissue (gut, bronchial, nasal) [31,32]. Appropriate pathological examination of the immune system includes spleen and thymus organ weights; macroscopic review of the spleen, thymus, and lymph nodes; and histological evaluation of the thymus, spleen, bone marrow in situ, Peyer's patches, lymph nodes draining the site of administration (i.e., draining lymph nodes) and at least one other lymph node, mucosal lymphoid tissue, and any gross lesions of lymphoid organs [32,33]. Regarding draining lymphoid tissue/lymph node collections, Peyer's patches and mesenteric lymph nodes are considered the draining lymphoid tissue for orally administered compounds. For cutaneously, subcutaneously, or intradermally administered compounds, the most proximal regional lymph node closest to the application site should be collected, e.g., axillary for applications in the prescapular area or inguinal for applications in the rear limbs. For inhalation compounds, lung-associated lymph nodes, in addition to mucosal-associated lymphoid tissue (bronchial, nasal), represent the most proximal draining lymphoid tissue [31,33]. For lymph nodes arranged in chains (i.e., mesenteric), two or more nodes in the chain should be collected, with the addition of nodes draining specific gastrointestinal sections, depending on the mechanism of action or findings from earlier studies. Lymphoid tissues should be evaluated using semiquantitative descriptions of changes within each compartment supporting a specific immune function with the use of descriptive, rather than interpretative, terms. For example, "thymus, cortex, decreased lymphocytes, marked" is preferable to "thymic involution" [33]. The pathologist should provide the appropriate pathological and biological interpretation of immune system changes that are deemed to be related to the test article in the context of other study findings [33].

Test article-related changes in gross or pathological evaluation of lymphoid tissue suggestive of immune suppression include decreased spleen or thymus weight; decreased tissue cellularity, which may be generalized within an organ or confined to a tissue compartment; shifts in the proportions of cells within lymphoid or bone marrow tissues; or changes in cellular localization due to alterations in trafficking. If the mechanism of action of a drug alters or inhibits egress of lymphocytes from lymphoid tissues, increased cellularity due to accumulation of lymphocytes may be observed.

SPECIALIZED IMMUNOLOGIC END POINTS IN NHPs

Specialized immunologic assessments in NHPs are most often included in standard toxicity studies rather than dedicated immunotoxicity studies because of animal use considerations. These assessments should be included in standard toxicity studies if the pharmaceutical is intended to modulate the immune system or if a cause for concern is identified in in vitro, pharmacology, or early toxicity studies. Specialized immunoassays can add to the weight of

VetBooks.ir

evidence for an immunologic effect observed in routine parameters, indicate the severity and dose dependency of the immunologic effect, confirm and monitor a PD effect, and assess the time to recovery following withdrawal of the test article. The specific assessments selected for evaluation depend on the cause for concern or the anticipated pharmacology. As described in ICH S8, specialized immunologic tests are considered tier 2 tests and include structural or morphological evaluations such as immunophenotyping of blood or tissue lymphoid cells using flow cytometry or immunohistochemistry, measurement of serum cytokine/chemokine concentrations, and determination of serum Igs [6]. Inclusion of at least one functional immune test is recommended by ICH S8 [6]. Functional assessment(s) should be selected based on the target, mechanism of action, or findings from earlier studies and may include in vivo and/or ex vivo cell-based assays.

The most commonly performed functional assay is the T-cell-dependent antibody response (TDAR). The TDAR is an assay involving in vivo challenge with a recall or neoantigen and ex vivo measurement of the subsequent antigen-specific antibody responses. The TDAR provides a broad functional assessment of the immune system; the test involves several interrelated steps in the immune response: antigen processing and presentation, T-cell regulation, antibody production, and Ig class switching. The application of the TDAR in NHPs and considerations for interpretation and clinical translation of results have been recently reviewed [34,35]. The most common T-cell-dependent antigen used in NHPs is keyhole limpet hemocyanin (KLH). Decreased TDAR—observed, for example, as decreased anti-KLH IgM concentrations following immunization with this antigen in animals treated with a test article relative to controls—has been shown to be a good indicator of immune suppression, but because of the animal-to-animal variation in NHPs and the typical small group size in NHP toxicity studies, TDAR tests seem to detect only "fairly large treatment effects" [34]. Although less common, T-cell-independent antigens can also be used as immunogens to evaluate T-cell-independent antibody responses (TIARs) in cases where there is a cause for concern (see Section "Role of Immune Function Assays in Differential Diagnosis and Risk Assessment").

The delayed-type hypersensitivity (DTH) assay is an in vivo assay used to evaluate cell-mediated immune responses following an intradermal antigen challenge [36]. Of note, the DTH assay is much less commonly used than the TDAR and has faced significant challenges in NHPs. Ex vivo functional assays can be used to assess lymphocyte responses and proliferation, natural killer (NK) cell activity, and neutrophil/macrophage function [12,14,37,38]. Details on the application and methodology of specialized immunologic assays are discussed in Chapter 27, "Immunotoxicology Testing of Monoclonal Antibodies in Cynomolgus Monkeys: From Manufacturing to Preclinical Studies." Available reagents that cross-react with NHP CD markers suitable for flow cytometry and immunohistochemistry (IHC) can be found at http://nhpreagents.bidmc.harvard.edu/NHP/literature.aspx.

Immunologically relevant end points in standard toxicity studies (tier 1) and specialized morphological and functional immunologic assays (tier 2) can provide informative data on the potential for test article-related immune suppression. Although tier 3 testing using host defense assays is described in ICH S8, these are not typically performed in NHPs because of the specialized facilities needed to house infected animals and the requirement of large group sizes to obtain statistically significant results [12]. Interpretation of tier 1 and tier 2 immunologic tests can be difficult because of inherent interanimal variability in the immune response and small animal numbers in NHP preclinical studies. In addition, there is no

VetBooks.ir

consensus on the degree of change in an immune assessment that translates to a biologically significant effect on immune function or risk for immunotoxicity in a clinical setting [39]. It has been suggested that changes in some immunological parameters exhibit threshold characteristics, requiring more than a statistically significant effect to result in biologically significant immune suppression [40]. All available information, including the mechanism of action, nonclinical data, the predicted extent and duration of clinical exposure, and the patient population, must be evaluated together to inform the clinical risk management plan [39].

DIFFERENTIAL DIAGNOSIS OF ON-TARGET, OFF-TARGET, AND STRESS-RELATED EFFECTS ON THE IMMUNE SYSTEM

Differential diagnosis is part of the weight-of-evidence review when an indicator of immune suppression is observed. Proactively responding to clinical findings associated with immune suppression observed in life or at necropsy (e.g., collection of additional blood samples and/or tissue samples for culture or specialized microscopy) may help with differential diagnosis. In addition, incorporating certain specialized immunologic parameters into future in vivo NHP studies and/or conducting hypothesis-driven mechanistic studies such as ex vivo NHP cell-based assays should be considered.

Mechanisms of drug-related immune suppression can be direct (primary) or indirect (secondary). Direct mechanisms of immune suppression, as the term implies, are directly related to the action of the test article and can be categorized as on-target or off-target mechanisms. On-target effects are related to the mechanism of action and PD of the drug, which affect the intended target or biological pathway. On-target effects can be relatively easy to demonstrate, for example, in cases of therapeutic monoclonal antibodies that deplete cells. A classic example is rituximab, which depletes $CD20^+$ B cells. Demonstration of dose-related decreased numbers of B cells in circulation and in tissues following treatment can prove the expected, on-target PD effect of the compound [41]. Another example of small-molecule, on-target—albeit unintended—immune suppression is the myelotoxic effects of anti-proliferative agents used for the treatment of cancer. Off-target effects are the result of modulation of other targets, which can be related (biologically) or not to the intended target.

Diagnosis of Stress-Related Effects on the Immune System

Both animal handling and test article-related toxicity can induce stress within nonclinical safety studies. In fact, the most common indirect mechanism for drug-related immune suppression is stress. The biology, impact, and assessment of stress in routine toxicity studies have been comprehensively reviewed by Everds et al. [42], and the reader is highly encouraged to consult this work. Needless to say, direct test article-related immune suppression (on or off target) must be differentiated from stress-related immune suppression. Indicators of immune suppression that may be due to stress are decreased peripheral blood lymphocytes (occurring as part of a stress "leukogram," i.e., decreased lymphocytes and eosinophils with increased neutrophils) and decreased thymus and spleen weight (due to lymphocyte depletion). Other findings commonly associated with stress include decreased body weight or body weight gain, decreased food consumption, deceased activity, increased adrenal gland weight (due

VetBooks.ir

to cortical hypertrophy), and reproductive effects (e.g., testicular atrophy). However, stress responses vary depending on species, strain, and physiologic state of the animal, as well as the type, magnitude, frequency, and duration of the stressful event, so not all of these findings may be observed in a given toxicity study. Stress-related changes to immunological end points in routine toxicity studies may be differentiated from direct immune suppression by a weight-of-evidence approach [42]. Basically, the more parameters known to be linked to stress that are observed in the study, the stronger the evidence the alterations were caused by stress. The evidence for stress is stronger if the biology of the test article and its target is not related to the effects of stress that typically occur. The magnitude of the response should be proportional to the stress level, duration, and frequency, and stress-related changes do not generally occur at doses at which there are no test article-related changes. Stress-related effects do not typically follow a dose-response curve, whereas direct immunologic effects generally do [40]. In general, altered circulating leukocyte counts and increased adrenal gland and decreased thymus weights are the most sensitive parameters that indicate stress; the less sensitive indicators of stress (e.g., reproductive effects) do not occur in the absence of the more sensitive indicators.

With the typical small group sizes in NHP studies, stress effects are more likely to show a wider variation between animals in the same group than direct immunologic effects. In addition, group or social housing has become a common practice in NHP toxicity studies, and in such environments, despite being overall less stressful than single housing, animals often form dominance hierarchies, which elicit stress for individuals [43]. The ICH S8 guidelines warn that "thymic weight decreases in the presence of clinical signs, such as decreased body weight and physical activity, are too often attributed to stress. These findings on their own should not be considered sufficient evidence of stress-related immunotoxicity" [6]. However, the interpretation of alterations in thymus weights of NHPs in relation to stress, immunotoxicity, and normal physiology is complicated by the high degree of variability between animals [44] because thymic involution occurs naturally with age and follows an individual time course (see Section "Challenges for Diagnosing Test Article-Related Immune Suppression in Lymphoid Tissue").

Diagnosis of Suspected Infections and/or the Presence of Infectious Agent(s) and Establishing the Role of Test Article-Related Immune Suppression

Clinical observations that suggest immune suppression are difficult to ascertain, manifesting as nonspecific observations of general distress such as lethargy, inappetence, and decreased body weight, or decreased body weight gains. Signs associated with infections (clinical signs vary with the type of agent) may indicate immune suppression. In the case of suspected infection, collection of diagnostic samples such as blood and tissue swabs for culture or tissue and serum for retrospective testing for pathogens may be helpful in identifying a causative agent. Planned or ad hoc clinical pathology testing can also help to demonstrate that the animal(s) are responding to an infection, e.g., evidence of an increase in circulating leukocytes (to be differentiated from test article-related inflammation secondary to organ/tissue toxicity). Histopathology (and other ancillary pathology tests) can be helpful in identifying specific causative agents of disease, if present. Identification of an opportunistic (weak pathogen) infectious agent in tissues is highly suggestive of immune suppression. It

VetBooks.ir

is important to remember, however, that NHPs have numerous background infections and infestations that may have existed before the study started and may not be related to the treatment (see Chapter 10, "Nonhuman Primate Diseases of Relevance in Drug Development and Their Impact on the Interpretation of Study Findings"). If an active infection or opportunistic infectious agent is identified and considered to be a potential consequence of test article-related immune suppression, then elucidating a mechanism for the increased susceptibility to infection is important (see Section "Clinically Apparent Manifestations of Immune Suppression in NHPs Observed in Nonclinical Safety Studies: Case Examples"). For example, if more bacterial infections are observed without a significant decrease in concentrations of blood neutrophils, ex vivo tests to evaluate the function of neutrophils (e.g., phagocytosis assays) may be considered.

Identifying Hyperplastic Lesions and Tumors and Establishing the Role of Test Article-Related Immune Suppression

Increased incidence of tumors can be viewed as a sign of immune suppression in the absence of other plausible causes such as genotoxicity, hormonal effects, or liver enzyme induction [6]. Lymphomas or other solid tumors and hyperplastic, preneoplastic morphologic changes, such as lymphoid hyperplasias, have been observed within the confines of NHP nonclinical safety studies, mostly due to recrudescence of endogenous, oncogenic viruses (see Section "Clinically Apparent Manifestations of Immune Suppression in NHPs Observed in Nonclinical Safety Studies: Case Examples"). Retrospective analysis of serum for viral load and inspection of tumor tissue by light microscopy for viral inclusions may point to recrudescence of an oncogenic virus. If possible, based on the available tissue, transmission electron microscopy (TEM) as well as polymerase chain reaction (PCR) may help with a viral diagnosis. For example, when lymphomas and lymphoid hyperplasia were identified in a chronic toxicity study of NHPs with the immunomodulatory small-molecule tofacitinib (see Section "Clinically Apparent Manifestations of Immune Suppression in NHPs Observed in Nonclinical Safety Studies: Case Examples"), several assessments were added to the study [1]. Using immunohistochemical methods, lymphoproliferative tissues were evaluated for lymphocyte subtype (CD3, CD4, CD20, CD79a) to discriminate a B- or T-cell origin, for retroviruses by TEM (simian retrovirus-1, -2, and -5; SIV; and simian T-cell lymphotrophic virus), and for human and cynomolgus monkey Epstein-Barr virus (EBV) [i.e., lymphocryptovirus (LCV)] by immunohistochemistry, in situ hybridization, and PCR. Serum was also tested for titers to LCV [1].

Challenges for Diagnosing Test Article-Related Immune Suppression in Lymphoid Tissue

Histologic changes in lymphoid organs may be part of normal variation or may be considered incidental, spontaneous, or background findings in NHPs; therefore they should not always be interpreted as signs of immune suppression (see Chapter 12, "Toxicologic Pathology and Background Lesions of NHPs"). Consideration of age, sex, species, and origin (caught in the wild versus bred in captivity) are important in the evaluation and interpretation of test article-related lymphoid changes [32]. As an illustration, thymic involution becomes more

VetBooks.ir

apparent as NHPs become older, resulting in decreases in thymic weight. Decreased thymic weight must be interpreted in conjunction with histological findings to differentiate between age-related thymic involution, stress-related thymic atrophy, and test article-related immune suppression. Correlation of histopathological findings in conjunction with in-life observations, clinical pathology, and pharmacokinetic (PK)/PD data are needed to fully assess the immunosuppressive potential of the test article.

The incidence and dose-relatedness of the effects on the lymphoid tissue help determine the relationship to the test article. Specialized immunologic end points can be useful for further characterizing those effects. For example, lymphoid tissue immunophenotyping using flow cytometry can be helpful in characterizing specific cell types that may be affected by the test article. Immunohistochemistry and immunofluorescence can also be used to further characterize and quantify changes of specific immune cells in tissues.

Role of Immune Suppression in Decreased Serum Globulins

Although decreases in serum globulins can be an indicator of immune suppression, these changes can also be unrelated to the immune system and instead be secondary to decreased liver function or increased loss associated with renal dysfunction. Thus alterations in serum globulin concentrations should be correlated with other clinical pathology parameters for the most accurate interpretation.

Use of Bone Marrow Smears for Mechanistic Insight into Test Article-Related Changes to the Hemogram and/or Bone Marrow

Cytological evaluation of bone marrow smears may be considered to further define alterations observed in the hemogram and/or histopathology of the bone marrow. For example, in the presence of decreased neutrophil numbers, no identifiable source of inflammation (which could increase the utilization of granulocytes), and histological findings of normal to hypercellular bone marrow, a marrow smear can differentiate between test article-related effects on neutrophil maturation (increase in the immature granulocytic pool) and delayed release of marrow neutrophils (increase in the mature granulocytic pool). Bone marrow smear evaluation, including preparation, indications for inclusion in preclinical studies, and method of evaluation have been recently described [45].

Role of Immune Function Assays in Differential Diagnosis and Risk Assessment

Immune function assays can aid in demonstrating an effect on the immune system as well as in characterizing the nature of immune effects. In cases where blood and tissue indicators of immune suppression were not observed in standard toxicity studies but the animals developed clinically apparent disease indicative of immune suppression, functional assays may confirm test article-related immune suppression. In cases where morphologic and structural effects on the immune system indicate test article-related immune suppression, functional assays may help characterize the impact of these changes. For example, despite the targeted effect on the lymphoid marginal zone, BR3-Fc, a soluble B-cell-activating factor antagonist, had no effect on either T-cell-dependent (TDAR) or T-cell-independent (TIAR) antibody response

VetBooks.ir

suggesting that the clinical risk of opportunistic infections in patients given BR3-Fc should be low, as reviewed by Danilenko and Wang [46]. Finally, immune function assays may be used to help confirm the intended pharmacology in NHPs. Dose-response data, in conjunction with other data, can be useful for human PK/PD modeling to help select human doses.

CLINICALLY APPARENT MANIFESTATIONS OF IMMUNE SUPPRESSION IN NHPs OBSERVED IN NONCLINICAL SAFETY STUDIES: CASE EXAMPLES

The small-molecule immunomodulatory drug tofacitinib and the frequently cited case of the large-molecule immunomodulatory drug alefacept (Amevive®) [5,47] provide examples of nonclinical safety studies in NHPs where active infections or virally induced cancers have been observed. The JAK inhibitor tofacitinib is approved in the United States for the treatment of adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response or intolerance to methotrexate [48]. Inhibition of JAKs decreases inflammatory cytokine release, which in turn decreases lymphocyte activation and proliferation [1].

In the 1-month oral toxicity study using cynomolgus monkeys at dosages ≥50 mg/kg/day, active bacterial and viral infections associated with mortality were reported and considered to be a consequence of immune suppression [8]. Infection was observed in multiple organs and is described in the US FDA Center for Drug Evaluation and Research Summary Basis of Approval: Pharmacology Review(s) [1]. Indicators of immune suppression were observed in both routine parameters and by specialized immunologic assessments in this and shorter-term toxicity studies (Table 11.2). Specifically, in a 14-day oral toxicity study, decreases in lymphocytes (up to 92% reduced from pretest); lymphoid depletion of thymus, spleen, and mesenteric lymph node; and/or depletion of the bone marrow were observed at dosages ≥50 mg/kg/day. In the 1-month study, decreases in lymphocytes, specifically T-helper lymphocytes ($CD4^+CD3^+$), cytotoxic/suppressor T-lymphocytes ($CD8^+CD3^+$), and natural killer (NK) cells ($CD16^+CD3^-$) were observed at dosages ≥10 mg/kg/day, as was lymphoid depletion of the spleen and lymph node at dosages ≥50 or 100 mg/kg/day, respectively [8]. In addition, loose, mucoid stools with a bloodlike substance, observed at dosages ≥10 mg/kg/day, were considered to be related to infections resulting from immune suppression [8]. In the clinic, an association between significant lymphopenia and an increased incidence of serious and opportunistic infections was found [8].

The high dosage in a chronic (9-month) oral toxicity study in cynomolgus monkeys was limited to 10 mg/kg/day—the low dose in the 1-month study and corresponding to 1.5 times the human exposure. Indicators of immune suppression—decreases in specific immune cell phenotypes—were observed at dosages ≥0.5 mg/kg/day [8]. Maximal reductions in T-lymphocytes and NK cells occurred at 10 mg/kg/day and were generally in the range of 40-60% of those in the control group [1]. Three animals had lymphomas at scheduled ($n=2$) or unscheduled necropsy ($n=1$). Two of the lymphomas were identified to be of B-cell origin, and one was identified to be of T-cell origin, following additional investigations [1]. The B-cell lymphomas were found in animals positive for LCV [1,8]. Of note, all

VetBooks.ir

TABLE 11.2 Repeat-Dose Oral Toxicity Studies of Tofacitinib in Cynomolgus Monkeys [1,8]

		Indicators of immune suppression	
Duration of dosing	**Dosage (mg/kg/day)**	**Routine parameters**	**Specialized immunologic end points**
2 weeks	0, 20/500[a], 50, 200	≥50 mg/kg/day: decreased lymphocytes; lymphoid depletion of thymus, spleen, and mesenteric lymph node; and/or depletion of the bone marrow.	None described
4 weeks (1 month)	0, 10, 50, 100	≥10 mg/kg/day: decreased lymphocytes; loose, mucoid stools with bloodlike substance ≥50 mg/kg/day: lymphoid depletion of the spleen; mortality attributed to bacterial infection Active bacterial and viral infections in multiple organs 100 mg/kg/day: lymphoid depletion of the mesenteric lymph node	≥10 mg/kg/day: decreases in T-helper lymphocytes, cytotoxic/suppressor T-lymphocytes, and NK cells
39 weeks (9 months)	0, 0.5, 2, 10	≥0.5 mg/kg/day: decreases in lymphocytes; lymphoid hyperplasia in lymphoid tissues 10 mg/kg/day: reduced lymphoid organ weight (thymus[b], spleen) Mortality due to an infiltrative lymphoma ($n = 1$); additional lymphomas ($n = 2$) discovered at necropsy	≥0.5 mg/kg/day: decreases in T-helper lymphocytes and/or cytotoxic/suppressor T-lymphocytes and NK cells

[a]*On day 8, the 20-mg/kg/day dosage group was escalated to 500 mg/kg/day.*
[b]*A reviewer commented on the challenge of interpreting the reductions in thymus weight (see Section "Differential Diagnosis of On-Target, Off-Target, and Stress-Related Effects on the Immune System").*

animals tested positive for LCV, indicating that LCV was endemic in the study animals [1]. However, only three animals developed clinically apparent tumors. LCV in NHPs has similar characteristics to EBV in humans, as described in more detail in Chapter 10, "Nonhuman Primate Diseases of Relevance in Drug Development and Their Impact on the Interpretation of Study Findings" [49]. At a 2010 roundtable discussion held in Silver Spring, Maryland, sponsored and organized by the Immunotoxicology Technical Committee of the International Life Sciences Institute and Health and Environmental Sciences Institute on "NHPs to Assess Risk for EBV-Related Lymphomas in Humans," it was agreed that there were significant challenges to the use of NHPs to assess the risk for EBV-associated lymphomas in humans [49]. Vahle et al. [47] also indicate that no comprehensive evaluation has been conducted to ascertain the utility of cancer incidence data from chronic toxicity studies using animals with various background rates of latent viral infections for informing cancer risk assessment. In the case of tofacitinib, EBV-associated posttransplant lymphoproliferative disorder was observed at an increased rate in patients undergoing renal transplant and treated with tofacitinib and concomitant immunosuppressive medications [48].

VetBooks.ir

Interestingly, the antibody response to KLH immunization was evaluated only in a study using juvenile monkeys and not studies using adult monkeys. In the juvenile animals, the TDAR to KLH was significantly reduced at 10 mg/kg/day (the anti-KLH IgM response was reduced and the anti-KLH IgG response was completely inhibited), but no effects were observed at 2 mg/kg/day following immunization during week 27 of the 9-month (39-week) study [1]. Effects on the TDAR are expected based on the mechanism of action of tofacitinib and provide a functional assessment of the structural and morphologic changes observed. When vaccine responses were evaluated clinically, the percentage of satisfactory responders to the influenza vaccine and pneumococcal vaccine were similar or slightly lower, respectively, than placebo-treated patients after short-term exposure. These differences were not readily apparent after longer-term exposure [8].

Alefacept is a CD2-directed leukocyte function antigen (LFA)-3/Fc fusion protein indicated for the treatment of adult patients with moderate to severe chronic plaque psoriasis [50]. Alefacept interferes with lymphocyte activation by inhibiting the interaction between CD2 on T-lymphocytes and leukocyte LFA-3 on antigen-presenting cells. At the end of 2011, Astellas Pharma US, Inc. chose to cease promotion, manufacturing, distribution, and sales of alefacept for business reasons [51]. As reviewed extensively by others [5,47], during a 1-year toxicity study using high doses of alefacept, B-cell lymphoma in a single monkey was noted after 28 weeks of dosing at 20 mg/kg/week. Additional monkeys in this study, receiving both 1 and 20 mg/kg/week, developed B-cell hyperplasia of the spleen and lymph nodes but not lymphoma. Similar to the NHP in the tofacitinib chronic toxicity study, all animals in the study were positive for LCV [50]. Interestingly, the majority of NHPs used in the 1-year toxicity study for abatacept (ORENCIA®), a CTLA4Ig fusion protein that blocks the CD28 costimulation pathway to prevent T-cell activation, also tested positive for LCV [52]; however, despite clear indicators of immune suppression (for example, minimal transient decreases in serum IgG and minimal to severe lymphoid depletion of germinal centers in the spleen and/or lymph nodes [10]), no evidence of lymphomas or preneoplastic morphologic changes were observed [10]. Abatacept inhibits T-lymphocyte activation by binding to CD80 and CD86, thereby blocking interaction with CD28. This interaction provides a costimulatory signal necessary for full activation of T-lymphocytes [10].

Additional case examples have been reviewed elsewhere. For instance, Price [53] presents a case study of the occurrence of different types of bacterial infections observed in NHPs dosed with three closely related immunomodulatory small molecules. This case example highlights how the infections presented clinically, the diagnostic steps taken, and the strategies used for treatment or intervention, as well as the investigative efforts to characterize the drug-related effects on the immune system that were increasing susceptibility to infection. Hutto [5] provides a case example for recrudescence of plasmodium in NHPs following dosing with an immunomodulating monoclonal antibody. The authors of this chapter have also observed opportunistic infections in NHPs following dosing with immunomodulators. For instance, cryptosporidia and adenovirus were observed (Figure 11.2) in a NHP toxicity study. As mentioned throughout this chapter, although many animals had clinically occult evidence of infection, only one developed clinically apparent disease. It is anticipated that these findings will be published separately [54].

VetBooks.ir

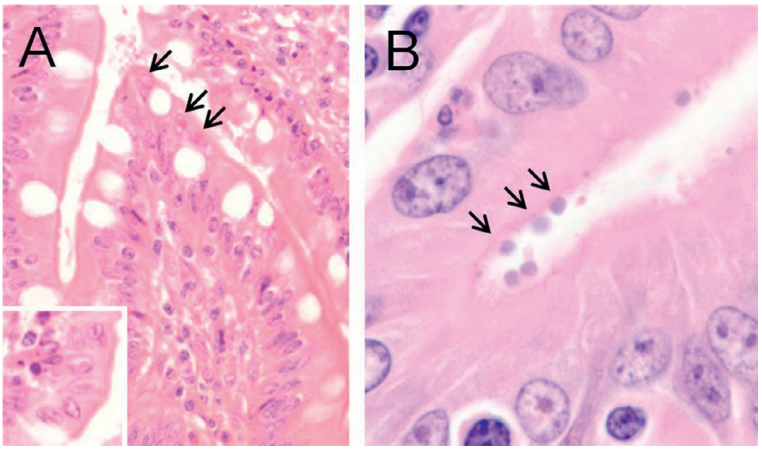


FIGURE 11.2 Histopathologic evidence of opportunistic infections. (a) Adenoviral intranuclear inclusions (arrows) present within enterocytes of the villous tip (hematoxylin and eosin (H&E) stain, small intestine). (b) *Cryptosporidium parvum* (arrows) localized to the luminal surface of enterocytes (H&E stain, small intestine). *Images kindly provided by Keith G. Mansfield, Preclinical safety: discovery and investigative safety, Novartis Institutes for Biomedical Research, Cambridge, MA.*

IDENTIFICATION OF A RISK FOR CLINICALLY ADVERSE IMMUNE SUPPRESSION: IMPACT ON FURTHER DEVELOPMENT

When the risk assessment indicates the potential for clinically adverse immune suppression, these findings may have varying impact on future development of the drug. The findings should be considered in relation to other attributes of the drug, its position in the company pipeline and within the overall competitive landscape, and the magnitude of the unmet medical need. For a drug in an already crowded disease area with a low tolerance for risk, possibly combined with other negative attributes in relation to the competition, a finding of immune suppression observed in a nonclinical safety study(ies) may result in the termination of development, especially for an unintended immunomodulator. Alternatively, the risk for clinically adverse immune suppression can be incorporated into a risk management plan that will help to guide the design of the clinical trial protocol(s). Risk management may encompass exclusion/inclusion and stopping criteria as well as additional monitoring. For example, trial participants may need to be vaccinated against certain pathogens in advance of dosing. In the case example published by Price [53], in which NHPs succumbed to bacterial infections following dosing with small-molecule immunomodulators during nonclinical safety studies, regulatory acceptance to proceed with clinical trials was granted on the basis of appropriate diagnosis and treatment of the bacterial infections, specialized immunologic assessments being conducted to assess any mechanism for decreased resistance, and characterization of the kinetics of the reversibility of the immune effects. In addition, strict monitoring of infection was implemented in clinical trials with associated inclusion/exclusion and stopping criteria [53]. Although clinical data become more relevant to the overall safety profile of a drug later

VetBooks.ir

in development, the nonclinical safety data continue to play a role, and major findings such as clinically overt evidence of immune suppression will be documented in the product label [10,48,50]. While tofacitinib was approved by the US FDA in 2012, the European Committee for Medicinal Products for Human Use refused to provide marketing authorization in 2013 because the benefits of treatment did not outweigh significant and unresolved concerns about safety [55]. One of the grounds provided for this refusal relates to the serious and opportunistic infections observed with tofacitinib in clinical studies; it was remarked that "relevant information from the toxicological program was not adequately followed up in the clinical development program leading to uncertainties in mechanistic understanding" [8]. The FDA-approved label for tofacitinib contains a black box warning for serious infections and malignancy [48].

CONCLUSION

NHPs are often used for nonclinical safety evaluation of immunomodulatory drugs. The possible consequences of immune suppression observed in NHPs include opportunistic infections, recrudescence of latent infections, and virally induced cancers, but these outcomes may not be observed within the confines of toxicity studies using NHPs because of a lack of challenge or the short duration of the study. However, indicators of immune suppression are generally observable in routine parameters included in standard toxicity studies. Specialized immunologic end points may help further characterize unexpected or anticipated effects on the immune system. In NHPs specialized immunologic end points are generally incorporated into routine toxicity studies—either prospectively to evaluate anticipated effects or retrospectively to investigate a cause for concern—rather than conducting stand-alone studies. Although NHPs have proven to be useful models for assessing the risk of clinically adverse immune suppression, there remain challenges with their use, including differences in the NHP and human immune systems and the small numbers of animals being studied. Discriminating between direct test article-related effects (on target or off target) and indirect effects on the immune system requires a careful assessment of the totality of the data and exclusion of alternative causes. Although not within the scope of this chapter, data from other sources, in addition to the nonclinical safety studies using NHPs, must be considered (e.g., the literature, in vitro assays, competitor programs, and pharmacology studies) in the overall risk assessment. When a potential for clinically adverse immune suppression is identified, there can be various impacts on further development. The more complete the understanding of the potential risk, the easier it is to define a path forward.

References

[1] XELJANZ® (tofacitinib) United Stated Food and Drug Administration, Center for Drug Evaluation and Research. Pharmacology Review(s) 2012. Available online at http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203214Orig1s000PharmR.pdf.

[2] Novartis. GILENYA™ (fingolimod) United States Product Information. 2014. Available online at http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022527s009lbl.pdf.

[3] de Haan L, Henderson S, McFarlane M, Scott A, Shenton J. Species selection. Eur Biopharm Rev 2011;(Summer). Available online at, http://www.samedanltd.com/magazine/12/issue/155/article/2976.

[4] Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. Nat Rev Drug Discov 2010;9:767–74.

[5] Hutto DL. Opportunistic infections in non-human primates exposed to immunomodulatory biotherapeutics: considerations and case examples. J Immunotoxicol 2010;7:120–7.
[6] ICH. S8 immunotoxicity studies for human pharmaceuticals. 2005. Available online at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S8/Step4/S8_Guideline.pdf.
[7] United States Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry on immunotoxicology evaluation of investigational new drugs. 2002. Available online at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079239.pdf.
[8] European Medicines Agency Assessment Report XELJANZ (tofacitinib). 2013. Available online at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002542/WC500154697.pdf.
[9] ICH. S6(R1) preclinical safety evaluation of biotechnology-derived pharmaceuticals. 2011. Available online at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S6_R1/Step4/S6_R1_Guideline.pdf.
[10] Bristol-Myers Squibb. ORENCIA® (abatacept) United States Product Information. 2013. Available online at http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125118s171lbl.pdf.
[11] Bussiere JL. Species selection considerations for preclinical toxicology studies for biotherapeutics. Expert Opin Drug Metab Toxicol 2008;4:871–7.
[12] Grote-Wessels S, Frings W, Smith CA, Weinbauer GF. Immunotoxicity testing in nonhuman primates. Methods Mol Biol 2010;598:341–59.
[13] Lebrec H, O'Lone R, Freebern W, Komocsar W, Moore P. Survey: immune function and immunotoxicity assessment in dogs. J Immunotoxicol 2012;9:1–14.
[14] Lebrec HN. Regulatory forum opinion piece*: immunotoxicology assessments in nonhuman primates—challenges and opportunities. Toxicol Pathol 2013;41:548–51.
[15] Frings W. Practical considerations for testing biopharmaceuticals in nonhuman primates. In: Weinbauer GF, Friedhelm V, editors. Critical contributions of primate models for biopharmaceutical drug development. New York, NY: Waxman Publishing Co.; 2008. p. 161–74.
[16] Muller PY, Brennan FR. Safety assessment and dose selection for first-in-human clinical trials with immunomodulatory monoclonal antibodies. Clin Pharmacol Ther 2009;85:247–58.
[17] Carter DL, Shieh TM, Blosser RL, Chadwick KR, Margolick JB, Hildreth JE, et al. CD56 identifies monocytes and not natural killer cells in rhesus macaques. Cytometry 1999;37:41–50.
[18] Vugmeyster Y, Howell K, Bakshl A, Flores C, Canova-Davis E. Effect of anti-CD20 monoclonal antibody, rituxan, on cynomolgus monkey and human B cells in a whole blood matrix. Cytometry A 2003;52:101–9.
[19] Rogers KA, Scinicariello F, Attanasio R. IgG Fc receptor III homologues in nonhuman primate species: genetic characterization and ligand interactions. J Immunol 2006;177:3848–56.
[20] Idusogie EE, Presta LG, Gazzano-Santoro H, Totpal K, Wong PY, Ultsch M, et al. Mapping of the C1q binding site on rituxan, a chimeric antibody with a human IgG1 Fc. J Immunol 2000;164:4178–84.
[21] Warncke M, Calzascia T, Coulot M, Balke N, Touil R, Kolbinger F, et al. Different adaptations of IgG effector function in human and nonhuman primates and implications for therapeutic antibody treatment. J Immunol 2012;188:4405–11.
[22] Nguyen DH, Hurtado-Ziola N, Gagneux P, Varki A. Loss of Siglec expression on T lymphocytes during human evolution. Proc Natl Acad Sci U S A 2006;103:7765–70.
[23] Eastwood D, Findlay L, Poole S, Bird C, Wadhwa M, Moore M, et al. Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells. Br J Pharmacol 2010;161:512–26.
[24] Barnhart K. Hematology of laboratory primates. In: Weiss DJ, Wardrup KJ, editors. Schalm's veterinary hematology. 6th ed. Ames, IA: Wiley-Blackwell; 2010. p. 869–87.
[25] Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 2002;296:346–9.
[26] Veterinary Cooperative Oncology Group. Veterinary cooperative oncology group—common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. Vet Comp Oncol 2011;(July 20).
[27] United States Department of Health and Human Services: National Institutes of Health (NIH), National Cancer Institute (NCI). Common terminology criteria for adverse events (CTCAE), v. 4.03; 2010. Available online at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5×7.pdf.
[28] Elizabeth Skuba, Novartis Institutes of Biomedical Research, personal communication 2014.
[29] Tizard I. Antibodies: soluble antigen receptors. In: Tizard I, editor. Veterinary immunology: an introduction. 8th ed. St. Louis, MO: Saunders; 2009. p. 170–80.

VetBooks.ir

[30] Haggerty HG, Proctor SJ. Chronic administration of belatacept, a T-cell costimulatory signal blocker, in cynomolgus monkeys. Toxicol Sci 2012;127:159–68.
[31] Haley PJ. Species differences in the structure and function of the immune system. Toxicology 2003;188:49–71.
[32] Haley P. Lymphoid system. In: Sahota JA, Popp JF, Hardisty JF, Gopinath C, editors. Toxicologic pathology: nonclinical safety assessment. Boca Raton, FL: CRC Press; 2013. p. 517–60.
[33] Haley P, Perry R, Ennulat D, Frame S, Johnson C, Lapointe JM, et al. STP position paper: best practice guideline for the routine pathology evaluation of the immune system. Toxicol Pathol 2005;33:404–7, discussion 408.
[34] Lebrec H, Cowan L, Lagrou M, Krejsa C, Neradilek MB, Polissar NL, et al. An inter-laboratory retrospective analysis of immunotoxicological endpoints in non-human primates: T-cell-dependent antibody responses. J Immunotoxicol 2011;8:238–50.
[35] Lebrec H, Molinier B, Boverhof D, Collinge M, Freebern W, Henson K, et al. The T-cell-dependent antibody response assay in nonclinical studies of pharmaceuticals and chemicals: study design, data analysis, interpretation. Regul Toxicol Pharmacol 2014;69:7–21.
[36] Córdoba F, Wieczorek G, Preussing E, Bigaud M. Modeling of delayed type hypersensitivity (DTH) in the non-human primate (DTH). Drug Discov Today Dis Models 2008;5:63–71.
[37] Kamperschroer C, Kaur A, Lebrec H. A summary of meeting proceedings for 'measuring immune responses in non-human primates for drug development-opportunities and challenges for predicting human efficacy and immunotoxicity'. J Immunotoxicol 2012;9:108–20.
[38] Siemsen DW, Malachowa N, Schepetkin IA, Whitney AR, Kirpotina LN, Lei B, et al. Neutrophil isolation from nonhuman species. Methods Mol Biol 2014;1124:19–37.
[39] Brennan FR, Morton LD, Spindeldreher S, Kiessling A, Allenspach R, Hey A, et al. Safety and immunotoxicity assessment of immunomodulatory monoclonal antibodies. MAbs 2010;2:233–55.
[40] Luster MI, Portier C, Pait DG, Rosenthal GJ, Germolec DR, Corsini E, et al. Risk assessment in immunotoxicology. II. Relationships between immune and host resistance tests. Fundam Appl Toxicol 1993;21:71–82.
[41] Ryan AM, Sokolowski SA, Ng CK, Shirai N, Collinge M, Shen AC, et al. Comparative nonclinical assessments of the proposed biosimilar PF-05280586 and Rituximab (MabThera(R)). Toxicol Pathol 2014;42(7):1069–81.
[42] Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, et al. Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. Toxicol Pathol 2013;41:560–614.
[43] Sapolsky RM. The influence of social hierarchy on primate health. Science 2005;308:648–52.
[44] Spoor MS, Radi ZA, Dunstan RW. Characterization of age- and gender-related changes in the spleen and thymus from control cynomolgus macaques used in toxicity studies. Toxicol Pathol 2008;36:695–704.
[45] Reagan WJ, Irizarry-Rovira A, Poitout-Belissent F, Bolliger AP, Ramaiah SK, Travlos G, et al. Best practices for evaluation of bone marrow in nonclinical toxicity studies. Vet Clin Pathol 2011;40:119–34.
[46] Danilenko DM, Wang H. The yin and yang of immunomodulatory biologics: assessing the delicate balance between benefit and risk. Toxicol Pathol 2012;40:272–87.
[47] Vahle JL, Finch GL, Heidel SM, Hovland Jr DN, Ivens I, Parker S, et al. Carcinogenicity assessments of biotechnology-derived pharmaceuticals: a review of approved molecules and best practice recommendations. Toxicol Pathol 2010;38:522–53.
[48] Pfizer. XELJANZ® (tofacinib) United States Product Information. 2014. Available online at http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/203214s007lbl.pdf.
[49] Kawabata T, Weaver J, Thomas D, Rowe M, Wang F, Kamperschroer C, et al. Summary of roundtable discussion meeting: non-human primates to assess risk for EBV-related lymphomas in humans. J Immunotoxicol 2012;9:121–7.
[50] Astellas Pharma. AMEVIVE® (alefacept) United States Product Information. 2012. Available online at http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125036s0144lbl.pdf.
[51] Astellas Pharma AMEVIVE® (alefacept) Physician Letter. 2011 Available online at http://www.amevive.com/.
[52] Gonchoroff DG, Abbott M, Bigwarfe T, Phelps L, Devona A, Testorf T, et al. Immunological assessments in one-year intravenous toxicity study in cynomolgus monkeys with abatacept, a selective costimulation modulator. Toxicologist 2008;102:439.
[53] Price KD. Bacterial infections in cynomolgus monkeys given small molecule immunomodulatory antagonists. J Immunotoxicol 2010;7:128–37.
[54] Novartis personal communication (2014).
[55] European Medicines Agency About XELJANZ (tofacitinib). 2013. Available online at http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002542/human_med_001662.jsp&mid=WC0b01ac058001d124.

CHAPTER

12

Toxicologic Pathology and Background Lesions of Nonhuman Primates

Daniel J. Patrick[*], *Marlon C. Rebelatto*[†]

***MPI Research, Mattawan, MI, USA**

†MedImmune, Biologics Safety Assessment, Gaithersburg, MD, USA

OUTLINE

Introduction 236

The Art and Practice of Toxicologic Pathology 236

Utility of Organ Weight in Microscopic Evaluation 237

Impact of Blinding 238

Identifying, Naming, and Recording Histopathology Findings 239

Thresholds 239

Terminology 240

Origin and Interpretation of Microscopic Findings 241

Microscopic Historical Control Data 242

Quality Control: Pathology Peer Review 243

Common Background Microscopic Changes in NHPs 244

Old World Primates: Cynomolgus Macaques (M. fascicularis) and Rhesus Macaques (M. mulatta) 245

Mononuclear Cell Infiltrates 245

Cardiovascular Lesions 248

Gastrointestinal and Hepatic Lesions 249

Pulmonary Lesions 250

Renal Lesions 251

Skeletal Muscle 252

Reproductive Lesions 252

Lymphoid Lesions 253

Marmosets (C. jacchus) 254

Conclusion 254

References 255

http://dx.doi.org/10.1016/B978-0-12-417144-2.00012-3

© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

Toxicologic pathologists play a critical role in the safety assessment of nonhuman primates (NHPs) used in drug development and are uniquely qualified to perform this assessment because of their extensive medical and anatomical pathology training. The primary question they must answer is: Is the test article causing any microscopic changes in tissue? To answer this question they need to be able to identify when microscopic changes are present in tissues and determine whether the changes are related to the test article or merely incidental. Incidental changes not related to the test article are often referred to as "background" changes, and these changes usually arise spontaneously. Background changes can occur either before or during the safety study and can be affected by the nature of the genetic variations within NHPs (Chapter 2), the variable environmental conditions they are exposed to before they reach the testing facility, exposure to infectious or parasitic agents (Chapter 10), or simply the "wear and tear" of aging. Furthermore, most findings that are typically recognized as background in NHPs can be induced or exacerbated by treatment with xenobiotics. The accurate discrimination between background findings and true test article-related findings can be challenging.

The first part of this chapter provides a broad overview of the art and practice of toxicologic pathology, primarily focusing on histopathology, and the second part focuses on some of the more common NHP background microscopic lesions encountered in safety assessment studies. As part of the overall evaluation of NHPs, it is extremely important for pathologists to attempt to describe these changes within the context of pathogenesis, biological and toxicological significance, and adversity. For these and other reasons, toxicologic pathology has relied heavily on the collective practical experiences of the toxicologic pathology community, and the practice of toxicologic pathology can be appropriately considered an "art" almost as much as it is considered a science. As with any area of expertise that relies heavily on experience, there are different approaches and opinions related to several aspects of this art, some of which are presented in this chapter and are nonetheless biased by our opinions. However, the pathology community represented in colleges (e.g., American College of Veterinary Pathologists, European College of Veterinary Pathologists) and societies (e.g., Society of Toxicologic Pathology, British Society of Toxicological Pathology) have continuously produced best practices documents and consensus opinion articles addressing many aspects of the art of toxicologic pathology, and they are used and referenced in this chapter as applicable. Of note, the reader is advised to consult the Society of Toxicologic Pathology (STP) "Best Practices Guideline: Toxicologic Histopathology" [1], which provides the basic recommendations for histopathologic evaluations of tissues in toxicology studies.

THE ART AND PRACTICE OF TOXICOLOGIC PATHOLOGY

The accurate identification of test article-related microscopic findings from background findings begins with a carefully designed study, which is covered in Chapters 21 and 26. The study pathologist needs to have a clear understanding of the study design, which is detailed in the study protocol and amendments. They should also have knowledge of the nature, class,

VetBooks.ir

mechanism of action, metabolism, and pharmacokinetics of the test material; the clinical observations and other ancillary tests (e.g., electrocardiography, ophthalmoscopic examination); and clinical pathology and other bioassay data (e.g., flow cytometry data, hormone and cytokine data). A careful evaluation of the macroscopic observations made during necropsy, and organ weight values, individually and by group, alerts the toxicologic pathologist of potential microscopic alterations to be identified. Next, the pathologist ensures that standard sections of tissues and organs (and their critical elements) are present on the slides and are suitable for diagnosis. Tissues are typically evaluated animal by animal or organ by organ, depending on the preference of the pathologist or goal of the review, usually starting with tissues from concurrent control (untreated) animals, followed by the animals receiving the highest dose of the test article. The evaluation of the concurrent control animals is one of the best initial means of understanding the breadth and incidence of the various background changes that might be encountered in the treated animals, since all of the animals in a study are generally of similar age and geographic origin and have been exposed to similar environments and handling. The initial evaluation is typically done in an "unblinded" fashion, followed by a blinded evaluation as needed. During examination, the pathologist encounters morphologic alterations in tissues that are worthy of recording, chooses and records appropriate terms that characterize each finding, and records a severity grade (e.g., minimal, mild, moderate, or severe). At the end of the histopathology evaluation and data entry, summary tables are generated that capture the incidence and severity of findings in different dose groups, which aid the pathologist in identifying findings that were related to treatment and discriminate them from spontaneous or incidental findings. An interpretative report that focuses on any relationships of the finding to the test article mechanism of action, possible pathogenesis, and a determination of adversity is then written. It is the systematic and careful completion of this process that permits a pathologist to identify findings as spontaneous/background or incidental. Each of these key steps is discussed in greater detail below.

Utility of Organ Weight in Microscopic Evaluation

Weighing particular organs and assessing for dose-related changes are important steps in the overall pathology evaluation for test article effects, and background/spontaneous tissue changes also can have a substantial impact on the values and interpretation. There are two primary goals in weighing organs: (1) to help the pathologist detect a subtle atrophy/hypocellularity in a tissue, and (2) to help the pathologist detect a subtle hypertrophy/hyperplasia in a tissue. In other words, these quantitative measurements increase the pathologist's sensitivity to these types of test article-induced microscopic changes. The procedure generally involves collecting the tissue during necropsy in a manner that is consistent across all animals, weighing the tissue on a digital scale, and statistically analyzing group means. In general, organ weight changes that are <20-30% are difficult to detect microscopically. The STP has published a list of recommended organs for weighing in all multidose general toxicology studies [2]. Recommended organ to be weighed in NHP studies include the liver, heart, kidneys, brain, adrenal glands, thyroid gland, pituitary gland, and testes. The spleen and thymus may also be weighed. Since reproductive organ weights often vary greatly due to age, sexual maturity, and stage of the menstrual cycle, reproductive organ weights can be complicated and/or of limited value.

VetBooks.ir

Weighing other organs should be considered on a case-by-case basis, and factors such as the compound class, mechanism of action, and overall data set for the entire study should be considered. In addition, differences in organ weights between groups needs to be interpreted with great care because statistically significant differences that are not biologically meaningful may occur, whereas other nonstatistically significant differences may be meaningful, and both of these scenarios can be more frequently observed in NHP studies with small group sample sizes. Unfortunately, despite recommendations for particular organ weights by professional societies such as the STP, there continues to be considerable variability between regulatory guidelines regarding organ weight recommendations, and a single global standard for organ weights is lacking. Study designs need to include weights of particular organs depending on the applicable regulatory agency that will be reviewing the nonclinical study, for example, the US Environmental Protection Agency, the Center for Food Safety and Applied Nutrition of the US Food and Drug Administration, Organization for Economic Co-operation and Development, Japanese Ministry of Agriculture, Forestry and Fisheries, European Economic Community, and Japan's Ministry of Economy, Trade, and Industry and Ministry of Health, Labor, and Welfare. Recognizing these differences, the STP convened a working group to examine available regulatory guidelines and assess current practices, the results of which were published and provide an excellent reference [3].

Impact of Blinding

Blinding is a procedure wherein the animal's treatment (e.g., control vs. low dose vs. mid dose vs. high dose) is concealed. Probably because blinding is used to reduce bias in clinical research, some nonclinical researchers also propose that the individuals collecting data in animal studies, including pathologists, be blinded to treatment. These individuals are invariably nonpathologists, and the interest in blinding is usually abandoned after the many negatives are pointed out. In general, a blinded slide examination during the initial evaluation can decrease the quality of the information obtained from the evaluation and unnecessarily increases both time and labor. Numerous documents have been published on this topic, including those supported by the STP [1,4–10]. Blinding can greatly increase the difficulty in separating treatment-related changes from normal variation/background changes. In addition, there is concern that blinding during the initial evaluation may result in nonrecognition of subtle lesions. Study pathologists should evaluate slides with an awareness of the treatment group assignment, which favors the identification of all treatment-related effects and enhances the overall accuracy of the histopathological evaluation. It is especially important to note that after the unblinded initial review of the slides is completed and tissues with potential test article-related findings are identified (i.e., potential target organs), most toxicologic pathologists often reevaluate these tissues in a blinded, or masked, fashion. This is accomplished by the pathologist reviewing particular tissues from all animals in a random fashion without looking at the slide label; there may be several rounds of masked reevaluations before the study pathologist is comfortable that the diagnoses can be consistently repeated without bias caused by knowing the treatment group. This masked reevaluation helps ensure consistent use of diagnostic criteria and terminology, uniform application of severity scores, and appropriate determination of a no observable effect level.

VetBooks.ir

Identifying, Naming, and Recording Histopathology Findings

A histopathology finding could be loosely defined as any alteration in the expected morphology or components of the normal histology of the tissue under examination. General pathology provides the bulk of the terminology that can be used to succinctly describe and record the alteration observed in the tissue. Examples include degenerative changes (vacuolar degeneration); cell death (necrosis, apoptosis); developmental abnormalities; cell growth and proliferation (hypertrophy, hyperplasia, or neoplasia); inflammation; and hemodynamic alterations (edema, hemorrhage, thrombosis, infarction, etc.). Special pathology terms provide morphologies and diagnoses that are specific to certain organs, such as gliosis (central nervous system pathology), hyperostosis (bone pathology), and glomerulopathy (renal pathology). Morphologic terms can be simply descriptive, such as vacuolation, increased cellularity, and tubular dilatation. Morphologic terms can be used to record "normal" features, such as a stage of the estrous cycle. Morphologic terms can be used to describe endogenous and exogenous deposits and pigments, foreign materials, and organisms (bacteria, fungi, nematodes, etc.). The pathologist also must be knowledgeable about potentially procedural-related alterations (e.g., the damage caused by a needle), postmortem alterations (e.g., autolysis, congestion), and alterations induced by the processes required to generate a paraffin-embedded, hematoxylin and eosin-stained tissue section on a glass slide, the standard specimen for histopathology evaluation. Variations in tissue fixation, processing, embedding, sectioning, and staining can all induce artificial alterations that were not present in vivo. These alterations can be noted as comments in the pathology database if the pathologist desires but should not be considered histopathology findings.

Thresholds

Virtually every tissue has a feature that could be considered a histopathologic "finding" at the cellular or tissue level when observed by light microscopy. However, the objective of a histopathologic evaluation in a toxicity study is not to absolutely identify every possible alteration no matter how subtle; this can unnecessarily increase the background "noise," confounding the overall goal of accurately identifying what changes the test article is causing. A tissue could have a few inflammatory cells (cellular infiltration or inflammation) or a cell that is larger than expected (hypertrophy), or a few cells could be pyknotic (apoptosis). These findings, however, as observed at a certain level, can be considered a normal part of tissue dynamics and homeostasis. For this reason, it has been suggested that a histopathologic finding is any alteration that the pathologist considers worthy of recording. The main tool pathologists use in this process is the use of thresholds. These thresholds can be defined objectively but are more often assessed subjectively. For example, alveolar macrophages are a normal finding in the lungs of primates, and a pathologist may choose to define a threshold based on their experience and/or the levels observed in control animals, recording an observation only when it is above this threshold. Thresholds can be quantitative (e.g., above a certain number of cells per high-power field) or qualitative (e.g., a morphologic feature such as "clustering" of cells). It is recommended that such thresholds be defined in advance, ideally for consistency within a laboratory or institution, but at least by the study pathologist. Including such criteria in the definition of the observation, which is standard in most pathology reports, can also sometimes be helpful. In some cases, when a tissue change that could be induced by the

VetBooks.ir

test substance is identified, a masked or blinded examination of the target organ that applies the predefined threshold criteria helps ensure the consistency and accurate identification of potentially test article-related effects. The use of thresholds is a valuable tool to help identify true treatment-related alterations. If not applied diligently, however, identification of subtle treatment-related effects may be more difficult.

Terminology

Once a histopathologic finding is identified, the pathologist needs to record the most appropriate term that accurately describes it. The choice of terms is not as simple as it seems. For many histopathologic findings, there are many synonyms that have been used interchangeably by toxicologic pathologists. For example, to describe a small thymus with a decreased number of cells in the cortex, terms such as *atrophy*, *involution*, *lymphoid depletion*, and *decreased lymphocytes* have been used. Traditionally, pathologists are trained to interpret findings to identify a disease diagnosis, and for this reason they have a tendency to choose "interpretative terms." In the example above, the term *involution* implies an interpretation that the thymus is undergoing the age-related process of involution. In toxicologic pathology, however, choosing descriptive terms and objectively identifying threshold and severity levels is more appropriate. This greatly helps the pathologist interpret the findings as treatment-related or background. In addition, toxicologic pathologists are often faced with multiple histopathologic features that represent a continuum or a spectrum of the same pathologic process. In these instances, identifying a single term that represents that spectrum and "lumping" the different features into this term is possible. For example, an ulcer of the skin (i.e., a break in the epithelium that exposes the underlying dermis) is often accompanied by epidermal cell degeneration and necrosis, hemorrhage, serocellular exudate, inflammatory changes, and epidermal hyperplasia. All these terms could be summarized by recording these findings under one term, *ulcer*. Combination (or "lumping") terms can also be used to represent a spectrum of findings. As in the example above, epidermal cells may be morphologically in different stages of cell damage—degeneration (reversible cell swelling) or necrosis (irreversible cell death). These terms could be combined into a single diagnosis of "degeneration/necrosis, epidermal cell." Lumping findings can be very helpful in identifying treatment-related differences because the final incidence table of microscopic findings is not cluttered by multiple terms that represent the same process (hence, that are not independent of each other). On the other hand, similar to the issues with identifying thresholds, deciding between using one term (lumping) or multiple ("splitting") to capture the histopathologic finding(s) can be difficult, and the choice can potentially mask identification of subtle treatment-related effects or the root cause of the findings, that is, the pathogenesis. In the example above of a skin ulcer, the root cause was likely the degeneration and necrosis of epidermal cells, and these terms should be recorded. Some pathologists lump particular observations during their initial evaluation to look for potential dose-related trends. For instance, the term *chronic inflammation* could be used as a lumping term to record when some or all of the following changes are present in a tissue: lymphocytes, plasma cells, macrophages, and fibrosis. If after review of the dose group summary tables there is a potential test article-related trend, the pathologist may decide to review the tissue again, splitting out and recording separately the various components. The study pathologist must decide whether it makes more sense to lump or

VetBooks.ir

split various observations to maximize the detection and understanding of test article-related changes before finalizing the pathology data.

An additional nomenclature challenge that pathologists face while evaluating tissues in toxicity studies is that, by the nature of these studies (i.e., the fact that the animals are being exposed to new molecules), novel lesions may be identified. In these cases, the pathologist has to create new terms (and definitions) to record the finding. The same is true for spontaneous findings in NHPs. There is always a chance that a new finding with no relationship to a treatment is identified that has not been reported.

The challenge of identifying the most appropriate terms to record histopathologic findings in toxicity studies has long been recognized by the toxicologic pathology community, which has been consistently working toward global harmonization. The obvious challenge of harmonizing nomenclature across the globe has taken years of continuous evolution, culminating recently in a series of International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) guidelines, led by the STP, European Society of Toxicologic Pathology, and the Registry of Industrial Toxicology Animal-data. Currently there are active organ system working groups for all organ systems in the rodent, and various organ systems are being intermittently released for publication. INHAND also has recently been expanded to include nonrodent species. Although no such guideline for lesions in NHPs is yet available, a majority of the principles and diagnostic criteria outlined in these guidelines [11] can be applied to NHP studies.

Origin and Interpretation of Microscopic Findings

Once histopathologic findings are identified and appropriate nomenclature, modifiers, and severity grades are recorded, the pathologist needs to interpret the findings in the context of the study. It is during this step that a distinction between treatment-related effects and spontaneous, incidental, or background findings is made. To aid in this process, it helps to identify whether the finding in question is pathological or physiological. Physiological changes are part of the normal variation of the tissues, such as changes seen as part of the estrous cycle in female NHPs, which are not considered treatment-related. Similarly, glycogen accumulation in hepatocytes of the liver is a normal, physiologic function of the liver. However, even in such cases, it is possible that a given treatment may cause alterations in the estrous cycle or changes in glycogen accumulation in the liver. Another distinction is between a pathological alteration and an "agonal" (near death) event and postmortem artifacts. When animals are killed in extremis, there are often changes from the damage to tissue integrity as a result of hypoxia or caloric restriction from inappetence. Examples of such changes include congestion of organs, focal hemorrhage, and apoptosis of cells (e.g., pancreas). Postmortem artifacts are generally easy to recognize by the homogeneous lysis of cells. Artifacts can also be induced during tissue fixation, processing, embedding, sectioning, and staining, and some of these artifacts may be confused with pathologic alterations. Excessive time in alcohols, for example, can cause artificial vacuolation in central nervous tissues. Other alterations that are considered pathologic develop antemortem, yet are not due to the treatment but to a technical procedure. These "iatrogenic" findings are commonly seen, for example, with venipuncture for collection of specimens for clinical pathology (hemorrhage and inflammation at collection site) or with oral gavage (damage to esophageal mucosa).

VetBooks.ir

An understanding of the biology of a histopathologic finding also helps in relating it to a test article effect (or not). For example, developmental abnormalities, such as ectopic tissues (e.g., an adrenal tissue in the kidney) or embryonic remnants cannot be induced by a test article and are considered spontaneous. In addition, a finding that is known to take weeks to months to develop (e.g., mineralized fibrotic areas) cannot be induced by a treatment in an acute toxicity study and is considered a preexisting finding.

Despite current efforts to use relatively "disease-free" NHPs in toxicity assessment, they can sometimes be infected with a viral, bacterial, or protozoal diseases that can also sometime elude screening attempts. These diseases are covered in Chapter 10 and must be taken into consideration when identifying lesions that can be induced by these agents because they are usually not related to treatment. However, they may be secondarily related to treatment if true toxicity is inducing severe stress-related immunosuppression in the animals or if the treatment is directly impairing the immune system. The impact of stress-related events in toxicity studies has been extensively reviewed [12] and is also discussed in Chapter 11.

Microscopic Historical Control Data

Another tool that pathologists can use to separate background changes from treatment effects is historical control data [13]. Typically, within an institution, pathology data collected from control animals in previous studies can be tabulated, and the prevalence of histopathologic findings can be determined and compared with the study of interest, provided that the studies used to generate the database are similar to the latter. However, historical control databases are often burdened with drawbacks. Despite the longstanding efforts in harmonizing nomenclature, there are still no established guidelines. Even in institutions that attempt harmonization in nomenclature, changes in personnel or approaches overtime, and even minor variations in "thresholding" and lumping versus splitting among the different pathologists who contributed to the database, affect the quality of the database. Also, when searching the database for the prevalence of a finding, the pathologist needs to include all possible synonyms of the terminology of interest. For these reasons, historical control databases may be useful but only to support the overall evaluation, not to make definitive conclusions about the relevance of the finding of interest.

In summary, to decide whether a given finding is related to the test article or is background/spontaneous, the pathologist needs to carefully and systematically utilize a weight-of-evidence approach, which may include the following questions:

- Is the finding likely to be preexisting (e.g., developmental abnormality), within normal variation, an artifact, a postmortem or agonal change, or a well-established/reported background finding in the NHP?
- Is there a dose-related increase in the incidence and/or severity of the findings?
- Is the finding present only in the test article-treated animals or is it present in controls as well?
- Does the finding correlate with other end points (organ weight, clinical pathology)?
- Is the finding expected for the pharmacology of the compound or biology of the target?
- Is the finding secondary to a clinical event or to another histopathologic finding?
- Is the biology of the finding consistent with a test article effect during the study?
- Is the finding caused by a study protocol procedure?
- Is the finding within the historical control data range?

VetBooks.ir

The challenges outlined above lend further support for the argument that there is an "artistic" element inherent in the science of toxicologic pathology. These challenges are overcome by a careful and methodical effort by an experienced toxicologic pathologist, by using the collective experiences within an institution, and by effective peer-review processes to ensure that the data that are captured accurately reflect the study findings. This systematic approach also ensures the accurate identification of treatment-related findings and their differentiation from background, spontaneous, or incidental findings in NHPs.

Quality Control: Pathology Peer Review

Pathology peer review is an optional quality control measure where the anatomic pathology data and interpretation, usually at an unfinalized stage (i.e., unsigned or draft status), is reviewed by a second pathologist. The protocol should state whenever a peer review is to be conducted; however, the protocol does not need to include detailed methods regarding the peer review. The decision on whether to include a pathology peer review should be made on a case-by-case basis and is sometimes related to study time constraints, study type, compound class, the culture of the organization conducting the study, experience/skill of the study pathologist, and regulatory requirements. Contrary to what some nonpathologists may think, most pathologists enthusiastically participate in peer reviews and promote them whenever possible.

For NHP studies, the pathology peer review usually consists of (1) an examination of all of the protocol organs in at least half of the animals treated with a high dose in the treatment phase (minimum of two per sex); (2) a review of all of the potential target organs from affected sexes in all control animals, all animals in affected dose groups, and all animals in the highest dose group that lacks the finding; and (3) a review of unscheduled animal deaths to confirm the cause of death. Examination of control animal tissues is usually performed at the discretion of the peer-review pathologist.

Any differences of opinion between the study pathologist and peer-review pathologist are discussed so that the two pathologists agree on the overall study data and interpretations and the finalized pathology report reflects consensus. It is important to note that peer review does not produce a new set of observations or a new pathology report, and the study pathologist is the sole point of accountability for the histopathology data and its interpretation. Any differences of opinion between the peer-review pathologist and the study pathologist may be resolved through consultation with other pathologists or subject matter experts or by convening a pathology working group. Pathology working groups are often assembled with members from different institutions, and extensive experience, often with specific expertise in the target organ of interest, may be needed for a definitive interpretation of the findings. The study pathologist's signature on the pathology report is certification that the pathology report is their professional scientific interpretation, and the signed pathology report comprises the raw data regarding histopathology findings. The peer-review pathologist then prepares, signs, and dates a peer-review memo, which lists the materials, methods, and conduct of the peer-review process and their general agreement with the study pathologist's pathology report. This memo is then archived and/or included in the study pathologist's report or the final study report but is not considered raw data. Preliminary diagnoses, interim notes, and worksheets of the study pathologist and peer-review pathologist, and draft versions of the study pathologist's reports, are also

VetBooks.ir

not raw data, and retaining or archiving these documents after the final study report is signed is not necessary. For an excellent and more thorough review of the currently accepted best practice for pathology peer review in the context of nonclinical safety studies, readers are strongly encouraged to refer to "Recommendations for Pathology Peer Review" [14], which has been endorsed by all major toxicologic pathology organizations, including the American STP, the European Society of Toxicologic Pathology, the Japanese Society of Toxicologic Pathology, the British Society of Toxicologic Pathologists, the French Society of Toxicologic Pathology, the Italian Society of Toxicologic and Experimental Pathology, the Society of Toxicologic Pathology—India, the Korean Society of Toxicologic Pathology, the Latin American Society of Toxicologic Pathology, and the American College of Veterinary Pathologists.

COMMON BACKGROUND MICROSCOPIC CHANGES IN NHPs

Probably the most important factor in accurately determining test article-related effects in the safety assessment of NHPs studies is having a solid understanding of the common spontaneous microscopic changes in NHP species that are commonly used in drug development. As previously mentioned, these spontaneous changes can also be referred to as "incidental" or "background" changes. Determining the relationship of the test article to these changes often relies on the experience of the pathologist, the features and biology of the effect, how the magnitude and incidence of the change varies across the groups, other study data, and historical control data. The importance of historical control data in NHP studies cannot be underestimated, especially since these studies typically have small numbers of animals (usually three or four) in each group, which increases the possibility that a background change will be present only in the treated animals and absent in controls. A commonly encountered dilemma is that a background change often is present only in the high-dose group, which can greatly confound the interpretation of the study and the accurate determination of spontaneous versus drug-induced findings. The following sections provide a short description of some of the more notable background lesions in by far the most common NHP species used in preclinical research, the cynomolgus macaque. Both cynomolgus macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*) are Old World primates and have similar background changes. The common marmoset (*Callithrix jacchus*), a New World primate, is also sometimes used in toxicology, and their small size is especially attractive in nonclinical toxicity testing if only small amounts of a test article are available. Some of the more common background changes in marmosets, including those that are similar to macaques as well as others that are unique to marmosets, are provided near the end of this section. It is important to note that there is no generic NHP, and background lesions can vary greatly between species, strain, and geographic origin. NHPs used in nonclinical research are generally young (1-5 years old), purpose bred, and raised in barrier conditions where infectious disease is kept to a minimum, which can impact the general types of background lesions encountered. The number of possible spontaneous alterations that can occur in NHPs is vast, and the respective prevalence within a given population changes over time and varies between different laboratories, making simply capturing them all in a single "atlas" or a table impractical. A lengthy dissertation covering all of the potential background lesions in NHPs is beyond the intent of this chapter; however,

VetBooks.ir

we refer the reader to several particularly excellent references on this topic: Sato et al. [15], Chamanza [16], Chamanza et al. [17], Kaspareit et al. [18], and David et al. [19].

Old World Primates: Cynomolgus Macaques (*M. fascicularis*) and Rhesus Macaques (*M. mulatta*)

Mononuclear Cell Infiltrates

Mononuclear cell infiltrates, also sometimes referred to as lymphocytic or lymphoplasmacytic infiltrates, are by far the most common background lesion in cynomolgus macaques. These infiltrates typically comprise focal aggregates of lymphocytes, plasma cells, and macrophages within a tissue, with little or no evidence of damage to the surrounding parenchyma (such as renal tubules, gastric glands, cardiac myocytes, or hepatocytes). Because of the typical lack of associated parenchymal damage, it is highly unlikely that these infiltrates would be detectable with current commonly available biomarkers. It has been demonstrated that serum cardiac troponin I (cTnI) concentrations are not increased in cynomolgus monkeys with myocardial mononuclear cell infiltrates, and thus serum cTnI does not seem to be a biomarker that can be used to screen cynomolgus macaques for this spontaneous cardiac finding [20]. The mononuclear cell infiltrates are typically observed in many cynomolgus macaque tissues; the most commonly affected sites are the liver (61%), kidneys (29%), heart (26%), salivary glands (21%), and stomach (12%) [17] (Figures 12.1–12.5). There are variations in the degree of mononuclear cell infiltrates between cynomolgus monkeys from different sources. When compared with Mauritian monkeys, cynomolgus monkeys from Southeast Asia (the Philippines and Vietnam) had increased mononuclear cell infiltrates that correlated with an increased white blood cell count [21]. Mononuclear cell infiltrates are also present in the uvea of the eyes of cynomolgus macaques in approximately 25% of drug safety evaluation studies

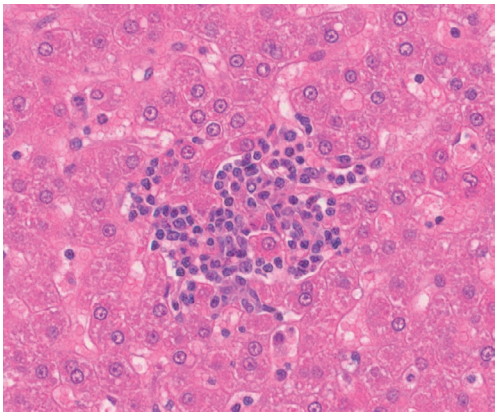


FIGURE 12.1 Cynomolgus monkey, liver, mononuclear cell infiltrates, original magnification 400×. Mononuclear cell infiltrates are sometimes referred to as lymphocytic or lymphohistiocytic infiltrates and are by far the most common background lesion in cynomolgus macaques. These infiltrates typically comprise focal aggregates of lymphocytes, plasma cells, and macrophages within a tissue, with little or no evidence of damage to the surrounding parenchymal cells.

VetBooks.ir

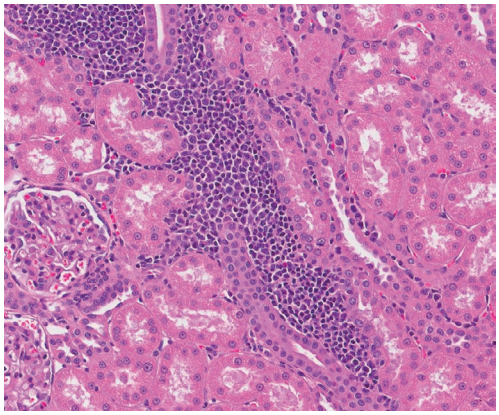


FIGURE 12.2 Cynomolgus monkey, kidney, mononuclear cell infiltrate, original magnification 200×.

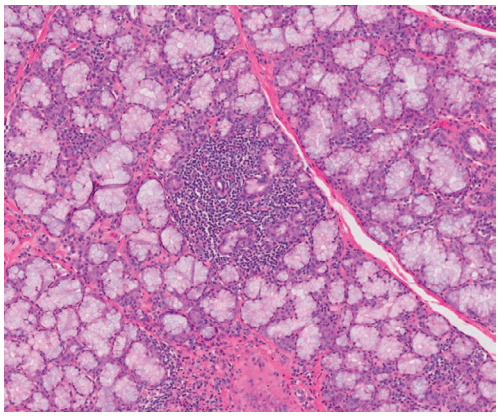


FIGURE 12.3 Cynomolgus monkey, sublingual salivary gland, mononuclear cell infiltrate, original magnification 100×.

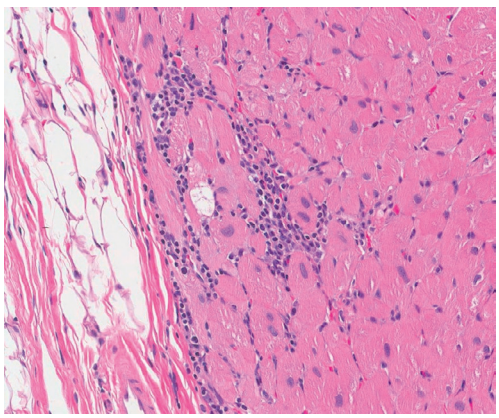


FIGURE 12.4 Cynomolgus monkey, heart, mononuclear cell infiltrate, original magnification 200×.

VetBooks.ir

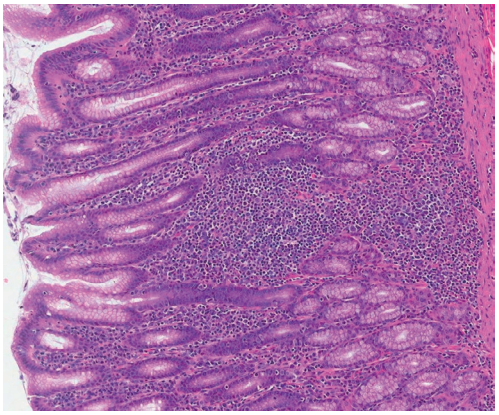


FIGURE 12.5 Cynomolgus monkey, stomach, mononuclear cell infiltrate, original magnification 100×.

and are not associated with ocular histopathologic or ophthalmoscopic findings [17,22]. The most common site of mononuclear cell infiltrates is the liver, and these infiltrates can be present diffusely throughout the parenchyma or within periportal regions. The liver infiltrates are usually minimal in magnitude and can occasionally be associated with minimal focal hepatocellular necrosis. Clinical chemistry changes (e.g., increased alanine aminotransferase [ALT] or aspartate aminotransferase [AST]) are typically not associated with hepatic mononuclear cell infiltrates [15,17]. Mononuclear cell infiltrates in the heart can be of particular concern since they can be more difficult to distinguish from inflammation, can be associated with myocyte injury, and are one of the primary histopathologic lesions associated with *Trypanosoma cruzi* infection (Chagas disease) (Figures 12.6 and 12.7). *T. cruzi* protozoal amastigotes are often difficult to detect histologically, precluding a definitive microscopic diagnosis. Mononuclear cell infiltrates within the stomach (sometimes also referred to as chronic gastritis) is one of the most consistently recorded background findings in cynomolgus macaques, with incidences of 100% affected animals reported in nearly half of studies. These lesions are not usually associated with clinical signs, and *Helicobacter pylori*- and *Helicobacter heilmannii*-like organisms are frequently observed in both sections with mononuclear cell infiltrates and

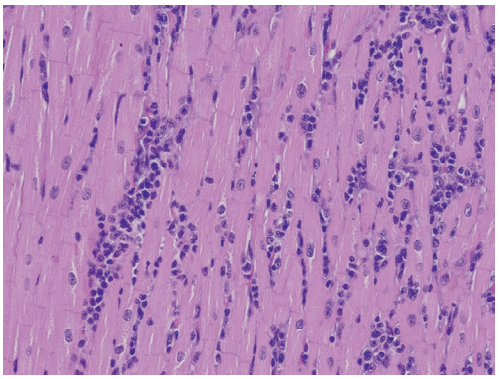


FIGURE 12.6 Cynomolgus monkey, heart, mononuclear cell infiltration, *Trypanosoma cruzi* (organisms often not in same plane of section), original magnification 100×.

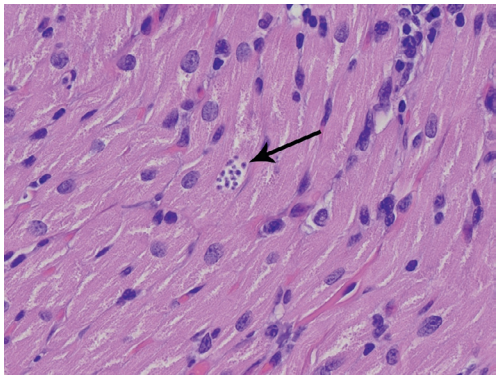


FIGURE 12.7 Cynomolgus monkey, heart, *Trypanosoma cruzi* amastigotes (arrow) within a cardiomyocyte, original magnification 400×.

in normal-appearing sections [15,21,23]. There is always the potential that the frequency and/or severity of these infiltrates could be altered by an administered xenobiotic, especially one that can stimulate or suppress the immune system.

Cardiovascular Lesions

Various myocardial background lesions in cynomolgus macaques, such as degeneration/necrosis, hemorrhage, and fibrosis, can mimic test article-related cardiac toxicity. Cardiomyopathy is characterized by some or all of the following: cardiomyocyte cytoplasmic changes that can include hypertrophy, disarray, pallor, and vacuolation, increased nuclear size (karyomegaly), necrosis, mineralization, fibrosis, and inflammatory cell infiltration (Figure 12.8). The disrupted cardiomyocytes are immunoreactive to desmin and cTnI antibodies and have a normal cross-striation pattern by phosphotungstic acid hematoxylin stain, indicating the chronic cardiomyopathy is not associated with active cardiomyocyte damage [24]. Myocardial degeneration and necrosis, which in advanced cases is also associated with inflammation (myocarditis) and fibrosis or mineralization, has also been reported as an idiopathic cardiac background lesion in cynomolgus macaques [17,21,25,26]. A higher incidence and severity of myocardial degeneration/necrosis as well as subendocardial hemorrhage with

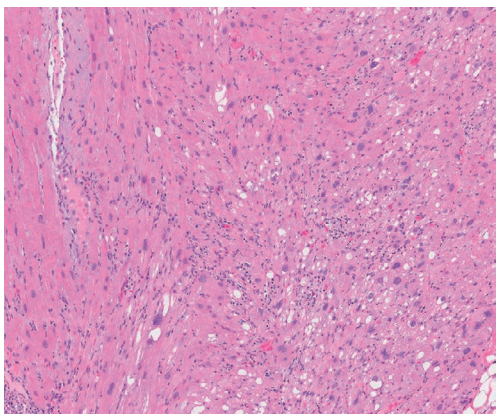


FIGURE 12.8 Cynomolgus monkey, heart, cardiomyopathy, original magnification 80×. The presence of cardiomyocyte changes such as increased cell size (hypertrophy), disarray, pallor, vacuolation, increased nuclear size (karyomegaly), and necrosis associated with fibrosis and mixed inflammatory cell infiltrates is seen.

VetBooks.ir

hemosiderin, myocardial fibrosis, and arterial medial degeneration/hemorrhage has been reported in Mauritian-source cynomolgus macaques [27]. Background vascular lesions include accumulation of mucopolysaccharides in the intima of the aorta, subendocardial areas of the base of the heart, and coronary arteries (most commonly without lipid accumulation, although atherosclerotic lesions have occasionally been observed). Vasculocentric inflammation, which involves either the wall of vessels (vasculitis, arteritis) or tissues surrounding vessels (perivascular inflammation, perivasculitis, periarteritis), can be observed spontaneously in cynomolgus macaques and is usually only observed in one or a few tissues; the most common sites are the heart, intestines, and epididymis [15,17,25] (Figure 12.9). A spontaneous vasculitis that involves more tissues and includes necrosis of the vessel, resembling polyarteritis nodosa in humans, has been observed in cynomolgus macaques [28,29]. Background vasculocentric lesions can be extremely problematic in toxicology because similar-appearing drug-induced vascular lesions are an incredibly serious toxicity and can cast significant doubt on the advancement of the compound to humans. Periarteritis in the lungs, usually with edema, can be associated with continuous intravenous infusions. Vasculocentric and glomerular changes associated with biopharmaceuticals have been reviewed by Rojko et al. [30].

Gastrointestinal and Hepatic Lesions

Acute inflammation of the stomach (gastritis) occurs at a much lower incidence than chronic mononuclear cell inflammatory infiltrates and is often associated with hemorrhage, erosions, ulceration, and/or glandular microabscesses [17]. Gastric ulceration is uncommon, usually subclinical, and can be associated with *Helicobacter* infection; however, when the ulceration is severe, the animals may present with anorexia, weight loss, lethargy, and vomiting blood. Upper gastrointestinal tract hemorrhage can be associated with vomiting, regenerative anemia, and elevated blood urea nitrogen. Idiopathic chronic colitis, presents with microscopic changes that include a lymphoplasmacytic infiltration, crypt atrophy, microabscesses, mucosal hyperplasia, and microherniation into the gut-associated lymphatic tissue [16]. Other background lesions in the intestine are uncommon but can occasionally be associated with diarrhea and diarrhea-related clinical pathology such as dehydration, electrolyte disturbances, hemoconcentration, and, if associated with hemorrhage, hypoproteinemia and

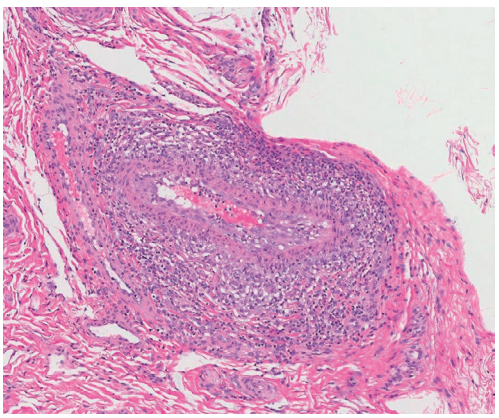


FIGURE 12.9 Cynomolgus monkey, epididymis, perivasculitis, original magnification 100×. The presence of mixed inflammatory cells (primarily lymphocytes, plasma cells, and neutrophils) surrounding a vessel is seen.

VetBooks.ir

anemia. It is important to note that the cause of diarrhea in cynomolgus macaques is usually unknown [17,23]. Amyloid can be deposited within the intestines (amyloid enteropathy) or systemically throughout numerous tissues, most commonly the liver and spleen. Intestinal amyloidosis can cause protein loss into the intestinal lumen (protein losing enteropathy) and can be associated with hypoalbuminemia.

Pulmonary Lesions

The most commonly observed background findings in the lung include mononuclear infiltrates within the perivascular and peribronchial interstitium, pleuritis/fibrosis with or without adhesions, alveolar macrophages (Figure 12.10), and foreign body granulomas. Yellow-brown pulmonary pigment is usually perivascular and located within macrophages (Figure 12.11). This pigment is iron positive (hemosiderin), suggestive of previous hemorrhage or congestion caused by some abnormality of vessels, cardiac function, or blood coagulation [15]. Fine black pigment that is usually within the interstitium around bronchi can be due to inhalation of carbon particles (anthracosis or pneumoconiosis) (Figure 12.12). Lung mites (*Pneumonyssus simicola*) and lung mite pigment are uncommonly encountered in NHPs

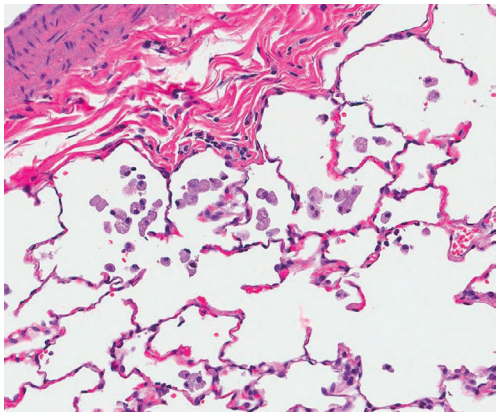


FIGURE 12.10 Cynomolgus monkey, lung, alveolar macrophages, original magnification 200×.

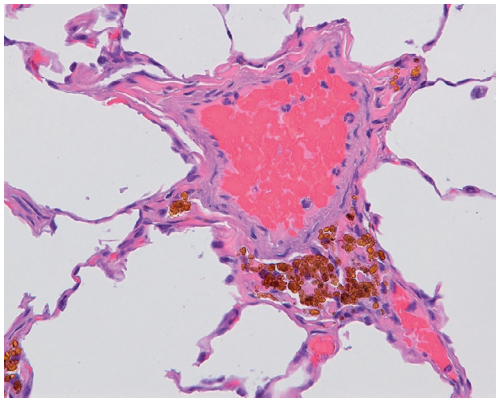


FIGURE 12.11 Cynomolgus monkey, lung, hemosiderin pigment, original magnification 400×. The yellow-brown, iron-positive pigment is contained within macrophages and is usually perivascular.

VetBooks.ir

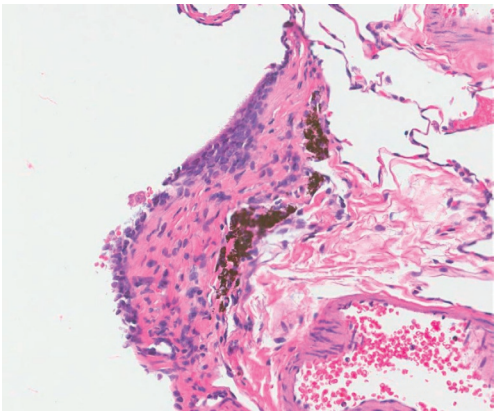


FIGURE 12.12 Cynomolgus monkey, lung, anthracosis, original magnification 200×. The fine black pigment (carbon particles) is usually located within the interstitium around bronchi.

used in nonclinical research today. Yellow-brown mite pigment can usually be differentiated by its association with arthropods and/or granulomatous bronchiolitis and peribronchitis and its refractile nature. The small granulomas within the lungs usually contain foreign material such as inhaled plant material or, if the animal was part of a study with an intravenous route of administration, small fragments of keratin or hair introduced into the vasculature during administration. Small fibrin emboli can also be observed in the lungs of these animals, particularly if there is a thrombus formed at the intravenous injection or infusion site.

Renal Lesions

Interstitial inflammation of the kidneys (also known as interstitial nephritis or tubulointerstitial nephritis) consists primarily of lymphocytes, plasma cells, and small numbers of neutrophils and can be associated with tubular regeneration, interstitial fibrosis, intraepithelial pigment, and mineralization [15,17]. Kidney glomerular lesions can include focal membranous and proliferative glomerulonephritis and glomerulosclerosis [15,21] (Figure 12.13). There are usually no gross pathological findings or systemic disease associated with these glomerular or interstitial changes [17].

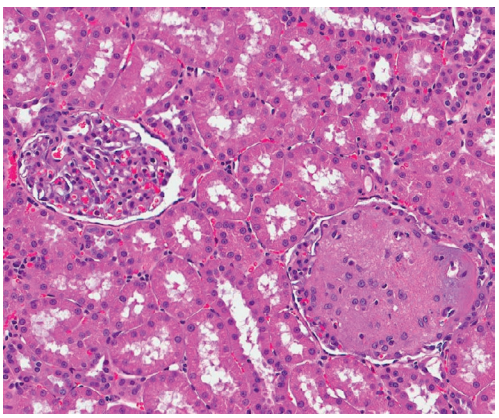


FIGURE 12.13 Cynomolgus monkey, kidney, increased glomerular matrix (focal global membranous glomerulonephritis) with normal glomerulus on the left, original magnification 180×.

VetBooks.ir

Skeletal Muscle

Background lesions in skeletal muscle are rare but important to recognize because they need to be separated from drug-induced skeletal muscle injury. Biomarkers of muscle injury include ALT, AST, creatine kinase, and/or lactate dehydrogenase. Some of the more common non-drug-related background lesions in cynomolgus macaques include focal accidental trauma, vaccination-related inflammation (granulomas), and infection with *Sarcocystis* spp. coccidian parasites [17]. Sarcocystosis is usually not associated with other lesions; however, cases occasionally exhibit inflammation characterized by infiltrates of lymphocytes, plasma cells, and eosinophils, as well as myofiber degeneration and fibrosis.

Reproductive Lesions

The greatest challenge regarding the evaluation of the reproductive tract of either male or female NHPs is the lack of sexual maturity. The study pathologist must identify maturity-related changes in the various reproductive organs and determine how best to record them, making sure not to misinterpret these changes as related to the test article when they are merely age related. Males become sexually mature around 4-5 years of age and females at 2.5-4 years of age; however, most nonclinical studies use animals that are around 2 years of age, and since studies are often <6 months in duration, the animals are often sexually immature at the terminal necropsy [31]. Regarding testicular test article-related effects, detection requires that the test animal be sexually mature and spermatogenesis be active; without active spermatogenesis, germ cell degeneration cannot be detected or compromised by peripubertal germ cell degeneration. It is also important to recognize that in "maturing" (peripubescent) and even "fully mature" males there is a spectrum of findings that can resemble pathologic changes. Other than the difficulties with sexual immaturity, background changes in reproductive organs include mononuclear cell infiltrates and small foci of inflammation, fibrous hypoplasia of the testes (Figure 12.14), and ovarian cysts [17].

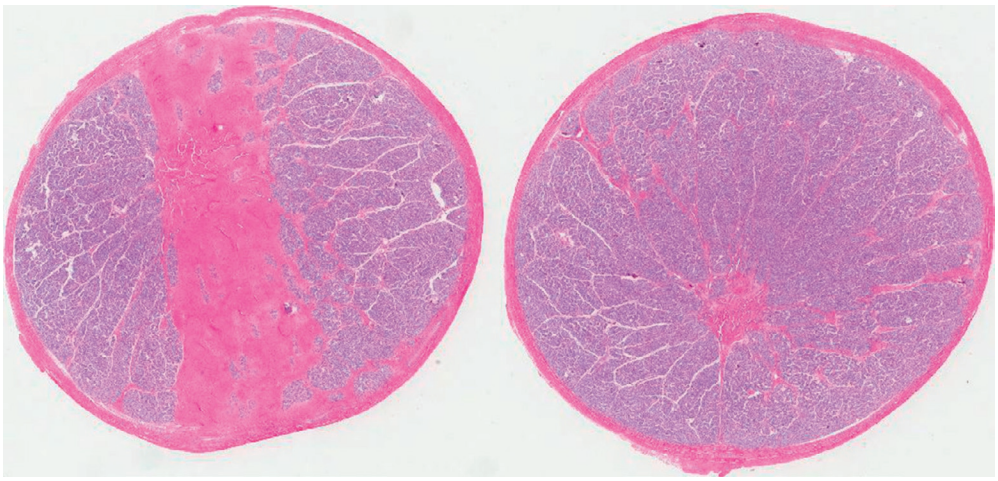


FIGURE 12.14 Cynomolgus monkey, testes, fibrous hypoplasia, unilateral (left), immature normal (right), original magnification 6×.

VetBooks.ir

Lymphoid Lesions

Because of the recent interest in immunomodulatory compounds and biopharmaceuticals, which can elicit immune responses, a thorough understanding of the spontaneous lymphoid changes in NHPs is critical. Observing lymphoid hyperplasia or prominent lymphoid follicles in various organs, including the spleen, lymph nodes, bone marrow, salivary glands, stomach, lungs, and urinary bladder, is not uncommon (Figures 12.15 and 12.16). These findings have been associated with subclinical type D retroviral infection [32,33], but since most of the NHPs that are used in nonclinical research are reared in relatively disease-free environments and known to be negative for type D simian retroviruses, these hyperplastic lymphoid follicles are considered to most likely represent a heightened nonspecific immunosurveillance. Other common findings include hyalinization (most likely antigen-antibody complex material), fibrosis, and macrophages within the germinal centers of splenic lymphoid follicles; lymphoid depletion (atrophy), mineralization, and cystic tubules within the thymus; or inflammatory cell infiltrates and pigment within the lymph nodes [17]. Since NHPs originate from areas where malaria is endemic, a significant but variable proportion of the monkeys have had previous exposure to *Plasmodium* spp. This can be confirmed by the presence of

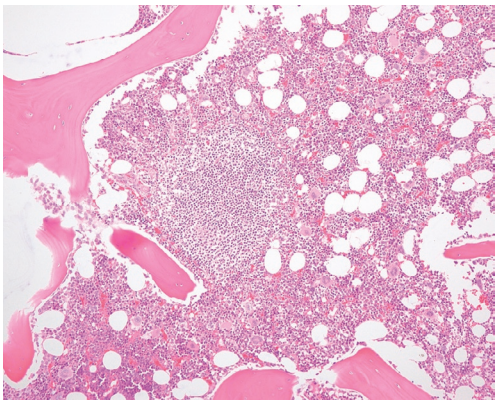


FIGURE 12.15 Cynomolgus monkey, spleen, lymphoid hyperplasia, nodular, original magnification 20×.

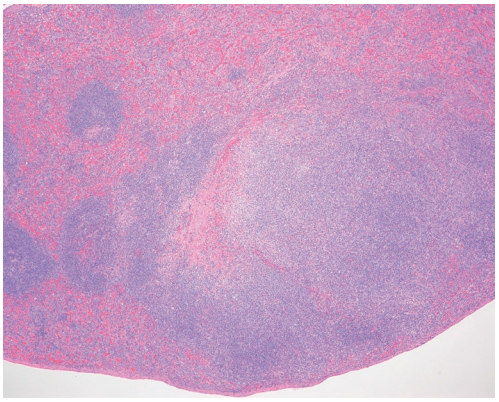


FIGURE 12.16 Cynomolgus monkey, bone marrow, secondary lymphoid follicle, original magnification 100×.

VetBooks.ir

malarial pigment, or hemozoin, in splenic macrophages. This golden-brown granular pigment is birefringent and can be easily identified under polarized light [34]. Thymic lymphoid depletion can also be caused by stress (discussed previously) or as part of normal age-related involution. The thymus normally begins to undergo involution (physiological atrophy) at 4-5 years of age [15].

Marmosets (C. *jacchus*)

Many of the incidental changes in Old World macaques discussed above, such as mononuclear cell infiltrates of various organs, chronic colitis, myocarditis, cardiomyopathy, vasculocentric inflammation, amyloidosis, glomerulonephritis/glomerulonephropathy, and lymphoid hyperplasia, can also be observed in marmosets [19,25,35,36]. Mononuclear cell infiltrates are most commonly observed in the kidneys (79%), liver (36%), adrenals (28%), thyroid (25%), heart (14%), lung (13%), and sciatic nerve (9%) [35]. One of the most common background findings in marmosets is extramedullary hematopoiesis (EMH) in a wide variety of organs, including the adrenal glands, liver, kidney, and spleen. This finding often correlates with the amount and frequency of blood sampling, and care must be taken by the pathologist to differentiate it from inflammatory cell infiltrates. EMH in macaques is rare and typically restricted to lymph nodes [17]. Other common background findings in marmosets that are less common in macaques include focal random necrosis of the liver, inflammation of the small intestine (enteritis), dilated Brunner glands in the duodenum, mineralization (primarily of the vasculature, atrial myocardium, lungs, kidneys, and adrenals), hemosiderosis and inflammation in the liver, C-cell hyperplasia of the thyroid gland, mineralization and pigmentation of the kidneys, lipid vacuolation and glycogen storage/accumulation in the liver, luteal hyperplasia of the ovary, and fibrous osteodystrophy of the bones [16,19,33,35,37,38]. In macaques soft-tissue mineralization is rare and almost always dystrophic in nature, whereas in marmosets the mineralization is usually representative of metastatic calcification and is usually due to excessive vitamin D in the diet (hypervitaminosis D) [16,38].

CONCLUSION

Toxicologic pathology plays a key role in the preclinical safety assessment of new drugs in NHPs. Crucial factors in a successful assessment are a well-designed study, a detailed and systematic histopathologic examination, and a comprehensive pathology report integrated with other applicable study end points. The histopathologic assessment presents many challenges, including accurate identification and nomenclature of microscopic changes, interpretation of findings within the context of the study design, and differentiation of test article-related from background findings. Toxicologic pathologists rely heavily on their training and experience, and the ultimate decision on what observations to record and the overall interpretation involves a systematic thought process using a weight-of-evidence approach. The references included in this chapter provide a solid foundational overview of toxicologic pathology and the many spontaneous findings in NHPs. However, there is no single atlas, list, table, or publication that can easily detail or substitute for the overall art and practice used in the safety assessment of NHPs. We hope this chapter provides a useful introductory

overview this practice, facilitating a greater understanding for both nonpathologists as well as pathologists who are early in their journey as toxicologic pathologists.

VetBooks.ir

References

[1] Crissman JW, Goodman DG, Hildebrandt PK, Maronpot RR, Prater DA, Riley JH, et al. Best practices guideline: toxicologic histopathology. Toxicol Pathol 2004;32:126–31.

[2] Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, et al. Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. Toxicol Pathol 2007;35:751–5.

[3] Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, et al. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicol Pathol 2007;35:742–50.

[4] Editorial. Society of Toxicologic Pathologists' position paper on blinded slide reading. Toxicol Pathol 1986;14(4):493–4.

[5] Goodman DG. Factors affecting histopathologic interpretation of toxicity-carcinogenicity studies. In: Carcinogenicity: the design, analysis, and interpretation of long-term animal studies. ILSI monographs. New York: Springer-Verlag; 1988.

[6] House DE, Berman E, Seely JC, Simmons JE. Comparison of open and blind histopathologic evaluation of hepatic lesions. Toxicol Lett 1992;63:127–33.

[7] Iatropoulos MJ. Editorial. Toxicol Pathol 1984;12(4):305–6.

[8] Neef N, Nikula KJ, Francke-Carroll S, Boone L. Regulatory forum opinion piece: blind reading of histopathology slides in general toxicology studies. Toxicol Pathol 2012;40:697–9.

[9] Newberne PM, de la Iglesia FA. Editorial: philosophy of blind study reading. Toxicol Pathol 1985;13(4):255.

[10] Prasse K, Hildebrandt P, Dodd D. Letter to the editor. Vet Pathol 1986;23:540–1.

[11] Mann PC, Vahle J, Keenan CM, Baker JF, Bradley AE, Goodman DG, et al. International harmonization of toxicologic pathology nomenclature: an overview and review of basic principles. Toxicol Pathol 2012;40:7S–13S.

[12] Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, et al. Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. Toxicol Pathol 2013;41:560–614.

[13] Deschl U, Kittel B, Rittinghausen S, Morawietz G, Kohler M, Mohr U, et al. The value of historical control data-scientific advantages for pathologists, industry and agencies. Toxicol Pathol 2002;30(1):80–7.

[14] Morton D, Sellers RS, Barale-Thomas E, Bolon B, George C, Hardisty JF, et al. Recommendations for pathology peer review. Toxicol Pathol 2010;38:1118–27.

[15] Sato J, Doi T, Kanno T, Wako Y, Tsuchitani M, Narama I. Histopathology of incidental findings in cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. J Toxicol Pathol 2012;25:63–101.

[16] Chamanza R. Non-human primates: cynomolgus (*Macaca fascicularis)* and rhesus (*Macaca mulatta*) macaques and the common marmoset (*Callithrix jacchus*). In: McInnes EF, editor. Background lesions in laboratory animals: a color atlas. New York, NY: Elsevier; 2012. p. 1–15.

[17] Chamanza R, Marxfeld HA, Blanco AI, Naylor SW, Bradley AE. Incidences and range of spontaneous findings in control cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. Toxicol Pathol 2010;38:642–57.

[18] Kaspareit J, Friderichs-Gromoll S, Buse E, Habermann G. Background pathology of the common marmoset (*Callithrix jacchus*) in toxicological studies. Exp Toxicol Pathol 2006;57(5–6 (July)):405–10.

[19] David JM, Dick EJ, Hubbard GB. Spontaneous pathology of the common marmoset (*Callithrix jacchus*) and tamarins (*Saguinus oedipus*, *Saguinus mystax*). J Med Primatol 2009;38(5 (October)):347–59.

[20] Dunn ME, Coluccio D, Zabka TS, Gopalakrishnan G, Hirkaler G, Geng W, et al. Myocardial mononuclear cell infiltrates are not associated with increased serum cardiac troponin I in cynomolgus monkeys. Toxicol Pathol 2012;40:647–50.

[21] Drevon-Gaillot E, Perron-Lepage M, Clement C, Burnett R. A review of background findings in cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins. Exp Toxicol Pathol 2006;58:77–88.

[22] Sinha DP, Cartwright ME, Johnson RC. Incidental mononuclear cell infiltrate in the uvea of cynomolgus monkeys. Toxicol Pathol 2006;34:148–51.

[23] Brady AG, Carville AAL. Digestive system diseases of nonhuman primates. In: Abee CR, Mansfield K, Tardif SD, Morris T, editors. Nonhuman primates in biomedical research. 2nd ed. Diseases (American College of Laboratory Animal Medicine), vol. II. New York: Academic Press; 2012. p. 589–628.

[24] Zabka TS, Irwin M, Albassam MA. Spontaneous cardiomyopathy in cynomolgus monkeys (*Macaca fascicularis*). Toxicol Pathol 2009;37:814–8.
[25] Chamanza R, Parry NM, Rogerson P, Nicol JR, Bradley AE. Spontaneous lesions of the cardiovascular system in purpose-bred laboratory nonhuman primates. Toxicol Pathol 2006;34:357–63.
[26] Keenan CM, Vidal JD. Standard morphologic evaluation of the heart in the laboratory dog and monkey. Toxicol Pathol 2006;34:67–74.
[27] Vidal JD, Drobatz LS, Holliday DF, Geiger LE, Thomas HC. Spontaneous findings in the heart of Mauritian-origin cynomolgus macaques (*Macaca fascicularis*). Toxicol Pathol 2010;38:297–302.
[28] Albassam MA, Lillie LE, Smith GS. Asymptomatic polyarteritis in a cynomolgus monkey. Lab Anim Sci 1993;43:628–9.
[29] Porter BF, Frost P, Hubbard GB. Polyarteritis nodosa in a cynomolgus macaque (*Macaca fascicularis*). Vet Pathol 2003;40:570–3.
[30] Rojko JL, Evans MG, Price SA, et al. Formation, clearance, deposition, pathogenicity, and identification of biopharmaceutical-related immune complexes: review and case studies. Toxicol Pathol 2014;42:725–64.
[31] Creasy D. Reproduction of the rat, mouse, dog, non-human primate and minipig. In: McInnes EF, editor. Background lesions in laboratory animals: a color atlas. New York, NY: Elsevier; 2012. p. 108–22.
[32] Guzman RE, Kerlin RL, Zimmerman TE. Histologic lesions in cynomolgus monkeys (Macaca fascicularis) naturally infected with simian retrovirus type D: comparison of seropositive, virus-positive, and uninfected animals. Toxicol Pathol 1999;27:672–7.
[33] Lowenstine LJ. A primer of primate pathology: lesions and nonlesions. Toxicol Pathol 2003;31(Suppl. (January–February)):92–102.
[34] Hanscheid T, Egan TJ, Grobusch MP. Haemozoin: from melatonin pigment to drug target, diagnostic tool, and immune modulator. Lancet Infect Dis 2007;7:675–85.
[35] Kaspareit J, Friderichs-Gromoll S, Buse E, Habermann G. Background pathology of the common marmoset (*Callithrix jacchus*) in toxicological studies. Exp Toxicol Pathol 2006;57(5–6 (July)):405–10.
[36] Yamada N, Sato J, Kanno T, Wako Y, Tsuchitani M. Morphological study of progressive glomerulonephropathy in common marmosets (*Callithrix jacchus*). Toxicol Pathol 2013;41(8 (February)):1106–15.
[37] Foster JR. Spontaneous and drug-induced hepatic pathology of the laboratory beagle dog, the cynomolgus macaque and the marmoset. Toxicol Pathol 2005;33(1):63–74.
[38] Zühlke U, Weinbauer G. The common marmoset (*Callithrix jacchus*) as a model in toxicology. Toxicol Pathol 2003;31(Suppl. (January–February)):123–7.

VetBooks.ir

CHAPTER

13

Nonhuman Primate Models of Human Disease

Jennifer A. Cann
MedImmune, Gaithersburg, MD, USA

OUTLINE

Introduction 257
Type 2 Diabetes Mellitus, Cardiovascular Disease, and Metabolic Syndrome 259
Diseases of Aging 262
Female Reproductive Conditions 263
Menopause and the Perimenopausal Transition 264
Endometriosis 265
Immunosuppressive Conditions 266
Malaria 268
References 269

INTRODUCTION

Animal models of human disease are commonly used in exploratory and efficacy studies, but their use in toxicity testing is less common and largely limited to tier II and III studies designed to understand the significance or mechanism of an adverse effect identified in conventional preclinical toxicology studies [1]. In some cases, however, it may be more appropriate to use an animal model of disease, rather than a healthy animal, for toxicity and safety assessment studies. Specifically, in diseases where the pharmacodynamic effect of the drug differs in healthy individuals compared with diseased individuals, toxicity testing in diseased individuals may more accurately predict risks and benefits in the target population. For example, drugs designed to decrease blood glucose falsely predict risk (hypoglycemia) if tested in a healthy animal model, yet accurately predict benefit (normoglycemia) if tested in a diabetic

http://dx.doi.org/10.1016/B978-0-12-417144-2.00013-5
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

animal model. Drugs designed to treat complex disease conditions, such as metabolic and age-related conditions, may also benefit from disease model testing. These conditions develop with a host of comorbidities that may predispose diseased individuals to adverse drug effects that would not be expected to occur in healthy individuals. This poses a significant challenge for toxicity and safety testing, and a model system that accurately recapitulates all of the comorbidities is more likely to reveal unintended effects. In addition, some of these complex conditions, such as female reproductive diseases, cannot be accurately modeled in a nonprimate animal model. This chapter reviews some common disease conditions for which toxicity testing using nonhuman primate models of disease may be scientifically justified. The commonly used nonhuman primate models are summarized in Table 13.1.

TABLE 13.1 Commonly Used Nonhuman Primate Models of Human Diseases

Disease/condition	Commonly used nonhuman primate models	References
Type 2 diabetes mellitus	Cynomolgus macaque	[3–5]
	Rhesus monkey	[6,7]
	African green monkey	[8,9]
Atherosclerosis	Cynomolgus macaque	[3,16–19]
Metabolic disease	Cynomolgus macaque	[20,21]
	Rhesus monkey	[22–28]
	Pigtail macaque	[24]
Aging	Rhesus monkey	[29–32]
Sarcopenia	Rhesus monkey	[33,34]
Osteoporosis	Rhesus monkey	[35–38]
	Cynomolgus macaque	[38,39]
Cognitive decline	Rhesus monkey	[40–44]
	Cynomolgus macaque	[45]
Menopause/perimenopause	Rhesus monkey	[46–48]
	Cynomolgus monkey	[47,49–52]
Endometriosis	Rhesus monkey	[53,54]
	Cynomolgus monkey	[53]
Simian immunodeficiency virus	Rhesus monkey	[55–61]
	Cynomolgus monkey	[56,58,59,61,62]
	Pigtail macaque	[55,59,61,63]
Radiation	Rhesus monkey	[64–69]
Malaria	Rhesus monkey	[70–81]
	Cynomolgus macaque	[70]
	Japanese macaque	[70,82,83]

VetBooks.ir

TYPE 2 DIABETES MELLITUS, CARDIOVASCULAR DISEASE, AND METABOLIC SYNDROME

Deranged glucose metabolism is a feature of type 2 diabetes mellitus (T2DM) that is not present in healthy individuals; thus to accurately assess the safety of drugs targeting glucose metabolic pathways, an animal model of T2DM must be used. Likewise, cardiovascular disease, including atherosclerosis, results in multiorgan vascular pathology to the extent that drugs targeting this indication may have effects in diseased individuals that do not occur in healthy individuals. Further, these conditions often occur simultaneously and manifest other features, collectively known as metabolic syndrome. Metabolic syndrome is defined as having at least three of five of the following risk factors common to both cardiovascular disease and T2DM: hypertension, dyslipidemia (elevated triglycerides and reduced high-density lipoprotein [HDL] cholesterol), elevated fasting glucose, and obesity [2]. These chronic, complex comorbidities are inexorably intertwined to the extent that disease modulation targeted to one area may have unintended effects in another. This poses a challenge for toxicologic and safety assessment studies, which, to be most predictive of adverse effects, must be conducted in a model system that recapitulates the key characteristics of all the comorbidities.

Spontaneous T2DM occurs in macaques [3–7], African green monkeys [8,9], chimpanzees [10,11], baboons [12,13], mandrills [14], and marmosets [15]. Among these, cynomolgus and rhesus macaques are the most commonly used and well-characterized experimental models. When fed a high-fat/high-carbohydrate diet they progressively develop obesity and insulin resistance that escalates to impaired glucose tolerance followed by T2DM [4]. Pathophysiologically, the process by which insulin secretion is abolished mimics that of the human condition. Initially, pancreatic islets become hypercellular as beta cells produce increasing amounts of insulin in response to hyperglycemia. As islet amyloid polypeptide (cosecreted with insulin) accumulates over time, it effaces the islets and eliminates all insulin production [5]. These changes are accompanied by increases in total plasma cholesterol and triglycerides and decreases in HDL cholesterol [5]. Thus, the macaque model fulfills the criteria of having metabolic syndrome [84]. The model is further strengthened by the finding that prolonged consumption of a high-fat diet also results in the development of atherosclerosis [3].

Macaque models of atherosclerosis are well described and have been used to elucidate many mechanisms and features of the disease [16]. The pathogenesis, lesion distribution and composition, and response to treatment resemble the human condition, making them a scientifically sound model for many types of investigations, including preclinical efficacy testing, toxicology, and safety assessment. Lipid metabolism in macaques has been studied extensively, and, as in humans, plasma lipid concentrations change with dietary lipid intake; the particles predominantly circulate as low-density lipoprotein (LDL) cholesterol rather than HDL cholesterol [17]. Atheroma distribution also resembles that of humans, with plaques forming at the branchpoints of medium and large arteries such as the coronaries and carotids [18,19]. Histopathologically, the fibrofatty lesions contain abundant foamy macrophages and lymphocytes, reflecting the inflammatory nature of the disease. Limitations of this model include the lack of a strong clinical end point, such as myocardial infarction. Although myocardial infarction has been reported to occur at a rate similar to that in humans, is not common enough to be useful in experimental settings [16].

VetBooks.ir

A clear mechanistic link between the development of T2DM and the development of atherosclerosis has been a scientific focus for many years, and increasing evidence points to the role of white adipose tissue as a key common factor in the context of obesity [85]. Although classically thought of as an energy-storing organ, it is now recognized that white adipose secretes numerous pro- and anti-inflammatory mediators and, as such, acts as an important endocrine organ [86,87]. To date the majority of known adipokines, including leptin, resistin, lipocalin-2, tumor necrosis factor, angiopoietin-like protein-2, monocyte chemoattractant protein-1, interleukin (IL)-6, IL-18, retinal binding protein-4 (RBP-4), and C-X-C chemokine ligand-5 (CXCL5), have been shown to have proinflammatory effects, and many have been found to be positively associated with markers of insulin resistance in humans [88–95]. Tumor necrosis factor and RBP-4 have been shown to play a direct role in the development of T2DM by inhibiting phosphorylation of the insulin receptor, and RBP-4 also was found to correlate with hypertension, dyslipidemia, and increased body mass index in humans [96–98]. Plasminogen activator inhibitor-1, a classic mediator of fibrinolysis, also has been shown to be strongly upregulated in visceral adipose stores, suggesting a mechanistic link between obesity and thrombotic vascular disease [99]. Likewise, IL-18 is thought to play a role in atherogenesis, specifically destabilization of plaque [100]. Leptin, well known for its inverse effect on food consumption, promotes type 1 immune responses by activating the Janus kinase (JAK)/signal transducer and activator of transcription and 5′ adenosine monophosphate (AMP)-activated protein kinase pathways, as well as inhibiting cytokine signaling 3 receptors [101–106]. At the level of the atheroma, type 1 immune responses promote intraplaque inflammation, destabilization, and rupture; circulating leptin concentrations have been associated with an increased risk of myocardial and cerebral infarction independent of obesity and other cardiovascular disease risk factors [107]. Resistin is also directly linked to cardiovascular disease via its induction of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, both of which are key players in the establishment and maintenance of intraplaque inflammation [108]. Clinically, circulating resistin concentrations have been positively correlated with unstable angina and myocardial infarction [109]. In contrast to most other adipokines, adiponectin and secreted frizzled-related protein have anti-inflammatory properties and are downregulated in obese individuals [110,111]. Several observational studies suggested an inverse association between serum adiponectin concentrations and risk of T2DM and cardiovascular disease [112–114]. In macaques, the biology of adipokines and their association with T2DM and cardiovascular disease is less well characterized; however, adiponectin, leptin, and resistin have been investigated to varying degrees [22–28]. In cynomolgus and rhesus macaques, as in humans, serum leptin concentrations positively correlate with body weight, percentage of body fat, and serum insulin concentrations [20,21,27]. In rhesus monkeys, circulating adiponectin concentrations are inversely proportional to body weight and decrease in parallel with reduced insulin sensitivity, findings that also mimic the human condition [25,26,112].

Many pharmacological options are available to treat and manage T2DM and cardiovascular disease, and many more are in preclinical development. Current T2DM treatment options include sulfonylureas, metformin, glinides, alpha-glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 analogs, sodium glucose cotransporter 2 inhibitors, and insulin. By acting on various targets including pancreatic beta cells, hepatocytes, renal tubular epithelia, and skeletal muscle, their primary effect is to lower blood

VetBooks.ir

glucose concentrations. As expected, use in nondiabetic subjects results in hypoglycemia, making healthy animals a poor choice for toxicologic and safety assessment studies. In addition, some agents, such as the peroxisome proliferator activated receptor agonists, have effects on other related metabolic pathways such as adipocyte differentiation and lipid metabolism [115]. Likewise, other drug classes, such as lipid modulators, obesity/weight loss drugs, next-generation anti-inflammatories, and antihypertensives, can be expected to have multisystemic effects and to behave differently in healthy individuals compared with those with T2DM, cardiovascular disease, and metabolic syndrome. Since their introduction in the 1980s as lipid-lowering agents, HMG-CoA reductase inhibitors (statins) have been shown to have pleiotropic effects on many fundamental pathologic processes including inflammation, coagulation, and fibrinolysis [116]. The clinical effects have been beneficial for various noncardiovascular diseases including nonalcoholic fatty liver disease, inflammatory bowel disease, experimental autoimmune encephalitis, and various neoplasias, yet two independent meta-analyses concluded that their use may increase the risk of developing diabetes [116–122]. Predicting the risk or benefit of a specific drug for specific comorbidities can be extremely difficult, and use of an animal model of disease may help to elucidate the mechanisms at play and more accurately predict the outcome. An obese rhesus macaque model was recently used to investigate unexpected anti-inflammatory and insulin-sensitizing effects of an anti-oxidized LDL monoclonal antibody that was intended to reduce LDL-cholesterol concentrations [123]. Nonsteroidal anti-inflammatory drugs also are known to have disparate effects in individuals with cardiovascular disease compared with healthy individuals. In 2004 the selective cyclooxygenase-2 (COX-2) inhibitor rofecoxib was withdrawn from the U.S. market when its use was associated with higher rates of myocardial infarction and stroke [124]. Two additional, structurally distinct COX-2 inhibitors also increased the risk of myocardial infarction and stroke in multiple placebo-controlled clinical trials; the mechanistic basis for this outcome was recently confirmed using a mouse model of vascular COX-2 deficiency [124,125]. As the worldwide obesity epidemic grows and the search for more effective treatments broadens, it is likely that similar unintended effects—both beneficial and therapeutic—will occur, and those effects are more likely to be identified at the preclinical toxicology and safety assessment stage of drug development if an animal model of obesity and metabolic syndrome is used.

Numerous clinical and investigative assays are available for monitoring and characterizing T2DM, cardiovascular disease, and metabolic syndrome. Diabetes can be diagnosed and monitored using commonly described end points such as fasting blood glucose concentrations; oral and intravenous glucose tolerance tests; and plasma insulin, glycated hemoglobin, fructosamine, leptin, and adiponectin concentrations. For at-risk individuals who have not yet progressed to T2DM, the hyperinsulinemic-euglycemic clamp is the gold standard for assessing insulin resistance [126]. Immunohistochemistry is helpful for assessing islet insulin and glucagon production, and Congo red stain is useful for identifying islet amyloidosis. Cardiovascular disease risk is commonly assessed using serum lipid profiles (total plasma cholesterol, HDL, LDL, very LDL, triglycerides), arterial systolic and diastolic blood pressure, and angiography. Atherosclerosis burden can be directly assessed in postmortem tissues via histomorphometric measurement of intimal area, intimal thickness, intraplaque inflammatory cell density, necrotic core area, and fibrous cap thickness. Obesity can be characterized by measuring the body weight-to-height ratio, abdominal circumference, and skinfold thickness,

as well as by using advanced imaging techniques such as dual energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), and computed tomography.

VetBooks.ir

DISEASES OF AGING

Aging is associated with the onset of numerous disease conditions and pathologies, some of which, such as Alzheimer disease, are exclusive to the elderly. With advancing age, reductions in important homeostatic parameters such as basal metabolic rate and core body temperature; loss of sensory functions, most notably eyesight and hearing; and significant cognitive decline are common [127–129]. Immune function is gradually impaired with age and results in increased susceptibility to infectious diseases. On a molecular level, the deranged immune response is attributed to reductions in lymphocyte proliferation and alterations in cytokine production. Type 1 cytokines, including IL-2 and interferon-γ, are reduced, and type 2 cytokines, including IL-4, IL-10, IL-1, and IL-8, are increased [130]. Pathologies such as sarcopenia and osteoporosis are also common and result in frailty and increased risk for fractures.

Sarcopenia refers to the loss of skeletal muscle mass and is accompanied by loss of strength, increased fat mass, and disability [131]. The prevalence of sarcopenia in both men and women increases with age, and nearly 50% of all people are affected by 80 years of age [132]. Type II fast fibers are almost exclusively affected, leaving the type I fatigue-resistant fibers intact [133]. Several age-related factors have been implicated in the development of sarcopenia, including neuromuscular junction degeneration, changes in steroid hormone production and sensitivity, and proinflammatory shifts in cytokine and adipokine production [134–136]. This proinflammatory shift, as well as increases in white adipose mass, contributes to the development of metabolic syndrome and cardiovascular disease. In addition, aged skeletal muscle fibers are less insulin sensitive, and the loss of skeletal muscle mass equates to loss of the body's primary insulin-responsive tissue [132,137]. This manifests clinically as a strong correlation between loss of muscle mass and the development of T2DM [138]. Adding to the complexity is the recognition that sarcopenia is also associated with osteoporosis [139]. Bone architecture is dynamic; remodeling occurs in response to increases or decreases in force exerted on the skeleton, and muscle mass is the most powerful loading force conferred on the skeleton. Thus reduction in muscle mass is one means by which bone loss occurs [140]. In addition, as occurs with sarcopenia, age-related changes in endocrine and inflammatory mediators also directly potentiate bone loss [134]. The effects of osteoporosis are not trivial; the World Health Organization estimates that approximately 8.9 million fractures each year are due to osteoporosis, and hip fractures are associated with premature death [141]. In addition, bone loss in women, like cardiovascular disease, is accelerated at menopause and is attributed to changes in sex steroid hormone concentrations.

It is also worth noting that T2DM, cardiovascular disease, and their shared risk factors—hyperlipidemia, obesity, and hypertension—are also risk factors for age-related dementia [142,143]. There is an epidemiological correlation between hypercholesterolemia and Alzheimer disease, and statin use is associated with reduced disease incidence [144–146]. Alzheimer disease is the leading cause of dementia worldwide, and it was estimated that 35.6 million people were living with some form of dementia in 2010 [129,147]. The course of

VetBooks.ir

Alzheimer disease is progressive and irreversible, and there are few approved drugs for treatment or prevention. Cholinesterase inhibitors, such as donepezil, rivastigmine, and galantamine, as well as memantine, an *N*-methyl-D-aspartate receptor antagonist, are commonly prescribed, but functional improvements are modest [148,149]. Clinical trials using secretase inhibitors that block the amyloidogenic pathway have shown little improvement in cognitive function but significant liver, gastrointestinal, and hematologic toxicity [150,151]. Drugs against other pathophysiologic targets, including aggregated tau and defective mitochondria, also are under investigation and have shown some benefit in clinical trials [152]. Other therapies including omega-3 fatty acids, vitamins, antioxidants, and complementary/alternative medicines have produced inconsistent results [153]. The large unmet need for effective dementia therapeutics makes this an active area of preclinical investigation. Given the complex interrelatedness and possible mechanistic synergism between Alzheimer disease/dementia, metabolic syndrome, T2DM, cardiovascular disease, sarcopenia, and osteoporosis, the effects that a drug targeting one indication may have on the others are difficult to predict. Thus an animal model that includes one or more of these comorbidities can be useful for predicting effects in the target population.

Rhesus monkeys have been the subject of continuous and ongoing aging studies for more than 25 years [29]. Elderly rhesus monkeys demonstrate impaired hearing and eyesight, as well as changes in immune function, that parallel those seen in elderly humans, including reductions in the type 1 cytokine interferon-γ and increases in the type 2 cytokines IL-6 and IL-10 [30–32,127,154]. Sarcopenia and osteoporosis also occur, and age-associated changes in glucose metabolism are reported [33–37]. Impaired cognitive function is also well documented, and both aged rhesus and cynomolgus macaques have been used to model Alzheimer disease [40–45]. Macaque models of menopause and the perimenopausal transition also have been developed, making possible the study of osteoporosis, sarcopenia, cardiovascular disease, insulin resistance/diabetes, and dementia within the context of age-associated changes in hormone production and immune function [38,39,46,49]. The menopause-perimenopausal transition models are discussed in more detail below.

Many techniques and assays for monitoring and characterizing age-related conditions in both humans and macaques are available. Those specific to T2DM, cardiovascular disease, and metabolic syndrome are discussed above. Sarcopenia can be assessed using anthropometry, DEXA, bioelectrical impedance analysis, and clinical imaging (MRI and computed tomography). Bone mineral density is commonly determined using DEXA. A battery of tests can be used to assess cognitive function, including tasks designed to assess learning and memory, executive function, attention, and processing speed [42].

FEMALE REPRODUCTIVE CONDITIONS

The reproductive physiology of female anthropoid primates, including women, differs from that of other mammals. Menstrual cyclicity is exclusive to Old World primates; as such, nonhuman primates from these regions are uniquely suited for the study of human female reproductive disorders.

VetBooks.ir

Menopause and the Perimenopausal Transition

In women reproductive senescence (menopause) typically occurs in the sixth decade of life. Profound endocrine changes, such as increases in follicle-stimulating hormone and luteinizing hormone and reductions in 17β-estradiol, inhibin B, and anti-Müllerian hormone, drive the process and begin in the years leading up to menopause [155–157]. During this period, 17β-estradiol, the most biologically active estrogen in women, drops from a premenopausal range of 50-1000 to 40-150 pM in the postmenopausal years [158]. During the perimenopausal transition, pathophysiologic alterations begin to manifest in the brain, heart, breast, skeleton, skeletal muscle, pancreas, white adipose, thyroid gland, skin, and mucous membranes. Of all the organ systems affected, the central nervous system is perhaps the most significant because it is affected on many levels: neurological changes affect memory and cognition; hypothalamic vasomotor changes result in hot flashes and night sweats; psychological changes can produce affective disorders; and cardiovascular disease can lead to stroke. Cardiovascular disease also affects other medium and large arteries throughout the body, and the risk of an ischemic event, including stroke and myocardial infarction, increases sharply with menopause. Likewise, the incidence of obesity, insulin resistance, sarcopenia, and osteoporosis increases during the postmenopausal period. These complex comorbidities are further complicated by the hormonal fluctuations that occur during the perimenopausal transition, and treatments aimed at alleviating the symptoms of menopause can greatly impact the underlying conditions.

Various types of hormone replacement therapies effectively alleviate menopausal symptoms such as hot flashes, night sweats, vaginal dryness, and dyspareunia. Hormone therapy regimens typically include an estrogen, either alone or in combination with a progestin. Numerous estrogens and progestins are available, and the biological activity of each varies. Premarin, composed of conjugated equine estrogens, is the form of estrogen replacement most commonly used in the United States. Its exact composition is undefined, but it is known to contain at least 10 different biologically active estrogens, and the metabolism and physiologic effects of each vary [159,160]. In addition, other compounds without estrogenic activity are also present. In Europe, estradiol and estriol are more commonly used. Numerous progestin choices are also available. Some have strong androgenic and glucocorticoid activity, whereas others have strong antimineralocorticoid activity; thus the biological effects are significantly diverse [161]. Other pharmacologic options include tibolone and selective estrogen receptor modulators, such as bazedoxifene. The choice of drugs, as well as the dose and frequency of administration (continuous vs. sequential), are determined for each individual based on age, menopause duration, and underlying disease status. All of these patient and drug-related variables contribute to the wide variation of effects on underlying diseases. Women with osteoporosis typically benefit from hormone replacement therapy. Multiple randomized controlled trials have shown improvement in bone mineral density and reduced risk of fractures [162–166]. However, the negative effects of hormone replacement therapy on other common comorbidities limit its utility in osteoporosis management; in the absence of menopausal symptoms, other effective treatments, including calcium, vitamin D, bisphosphonates, synthetic parathyroid hormone, strontium ranelate, and denosumab, are recommended. The effect of hormone therapy on cardiovascular disease is not clear. Observational studies and prospective studies using animal models have demonstrated a cardioprotective effect; however, randomized clinical trials, such as the Heart and Estrogen/Progestin Replacement Study and the Women's Health

VetBooks.ir

Initiative, suggest either no benefit or an increased risk of a cardiovascular event for women receiving hormone therapy [167–170]. The divergent results are largely thought to be due to the individual's age and menopausal duration when hormone therapy is initiated. Beneficial cardioprotective effects are seen in younger women entering the early stages of menopause, whereas adverse cardiovascular effects are seen in older women in mid- to late menopause [171]. Hormone therapy also seems to increase a woman's risk of ischemic stroke and venous thromboembolism; however, the effect of menopausal duration and timing on these extracardiac vascular events has not been evaluated [172–174]. Last, continuous, prolonged use (>5 years) of hormone replacement therapy is associated with increased risk of developing breast cancer [175]. This wide variation in disease-specific effects makes the development and safety testing of novel hormone replacement therapies challenging and error prone. The verity of the results may be improved by using a macaque model of aging/cardiovascular disease/metabolic disease, specifically in females that are progressing through or have undergone menopause.

Nonhuman primate models of natural and surgical menopause are available. Gorillas have been reported to undergo menopause at approximately 40 years of age; chimpanzees at around 35-50 years; baboons at approximately 25 years; and macaques at about 26 years [47,48,50,176]. Naturally occurring menopause models have the advantage of encompassing the complex endocrine changes that occur during the perimenopausal transition; however, few animals that have reached this transition are available. In addition, incorporating the necessary dietary and environmental conditions to produce the other comorbidities of interest (cardiovascular disease, metabolic syndrome/T2DM, osteoporosis, cognitive decline) requires advanced planning and adds to the logistical difficulty. To aid in the selection of individuals, serum anti-Müllerian hormone concentrations can be used to identify animals with low ovarian reserve that may be in or nearing perimenopause [177]. Inhibin B and follicle-stimulating hormone concentrations can also help to further characterize each individual's reproductive status. Menopause can also be experimentally induced by surgically removing the ovaries. This model has the advantage of making large numbers of individuals of similar age and menopausal duration readily available for study; however, the perimenopausal endocrine and physiologic changes are not recapitulated, making the validity of the model more questionable. Some researchers also investigated the utility of chemically induced perimenopause models. When administered to rodents, the chemical 4-vinylcyclohexene diepoxide selectively destroys primordial and primary ovarian follicles but leaves the steroidogenic ovarian stroma intact [178]. Development of a 4-vinylcyclohexene diepoxide model of perimenopause in macaques has been met with mixed success, largely because of difficulties in administrating the compound [49,51,52].

Endometriosis

Endometriosis is a complex disease condition in which functional endometrial glands and stroma ectopically grow in extrauterine sites throughout the pelvis, abdomen, and thorax. This disease is unique to Old World primates, and the ectopic glands undergo cyclic menstrual bleeding concurrent with uterine menstruation. It is estimated that 5-10% of all reproductive-age women are affected; however, definitive diagnosis requires laparoscopy, making it likely that the incidence is underestimated [179]. The etiology and precise pathogenesis of endometriosis are unknown, but endocrine and inflammatory processes are

VetBooks.ir

known to play key mechanistic roles. In healthy women the endometrial response to ovarian progesterone production is anti-inflammatory and antiproteolytic. In contrast, in women with endometriosis both ectopic and eutopic endometria are markedly less responsive to progesterone, resulting in a chronic proinflammatory and proteolytic environment. The result is ongoing glandular proliferation, hemorrhage, inflammation, and fibrosis. Clinically, women with endometriosis experience debilitating pain and reduced fertility. Treatment options are limited to hormonal manipulation and/or surgery, neither of which is curative and both of which are wrought with side effects. The rate of recurrence after treatment is high, and the need for new effective therapies is great [180].

Among nonhuman primates endometriosis is a naturally occurring disease in gorillas, baboons, macaques, mangabeys, and African green monkeys [53]. Disease prevalence in these species is largely unknown; however, the Wisconsin National Primate Research Center reported that 31.4% of female rhesus monkeys who died over a 20-year period had endometriosis [54]. In addition, a familial pattern was identified, allowing assembly of a large, multigenerational pedigree of >1600 females, 142 of which have been diagnosed with endometriosis. Models such as these may more accurately predict the effect new drugs will have not only on the ectopic endometria but also on other sex steroid hormone-responsive tissues such as bone, skeletal muscle, breast, and the thyroid gland. Surgical models involving either the direct implantation of endometrium into the peritoneal cavity or manipulation of the reproductive tract to facilitate retrograde menstruation also have been described, but these models do not recapitulate the complex endocrine and inflammatory changes that occur in the natural disease. The primary challenge for using macaques with naturally occurring endometriosis lies in identifying affected individuals. As in humans, definitive diagnosis requires laparoscopy or laparotomy; however, diagnosis using MRI in macaques has been reported [54,181].

IMMUNOSUPPRESSIVE CONDITIONS

Immunosuppression secondary to various insults such as viral infection, radiation exposure, chemotherapy, or genetic abnormalities is another complex multisystemic disease state in which drug activity may manifest differently in affected individuals versus healthy individuals. Since the emergence of lentivirus-associated acquired immunodeficiency syndrome (AIDS) in the 1980s, macaques have been used as models to study numerous etiologic, immunologic, virologic, and pathologic aspects of the condition [55]. A detailed discussion of the host, viral, and environmental factors associated with human and simian immunodeficiency virus (HIV and SIV, respectively) infection is beyond the scope of this chapter; however, the associated pathologies and comorbidities that may affect the pharmacodynamic behavior of a drug are reviewed below.

SIV infection in nonhuman primates of African origin generally results in a benign and self-limiting disease; however, infection in Asian macaques results in progressive immunosuppression and secondary opportunistic infections similar to HIV/AIDS in humans. Historically, rhesus monkeys of Indian origin have been the most commonly used model, followed by Chinese-origin rhesus monkeys, cynomolgus macaques, and pigtail macaques [56]. CD4-expressing cells, including subsets of T lymphocytes and antigen-presenting cells, are the primary target of HIV and SIV; as such, secondary lymphoid tissues exhibit an array of histopathologic and functional changes [57,58]. Follicular and paracortical regions of

VetBooks.ir

the lymph nodes may undergo hyperplasia or involution, and in some cases a generalized lymphoproliferative syndrome occurs [59]. As in human AIDS, giant-cell inflammatory infiltrates occur in many organs including lymph nodes, spleen, heart, lung, liver, intestine, central nervous system, and peripheral ganglia [62]. The gastrointestinal tract, with its dense population of resident $CD4^+$ T cells, is a primary target, and diarrhea and wasting are common clinical signs [60]. Up to 20% of infected rhesus monkeys may also develop SIV-associated glomerulosclerosis and tubulointerstitial nephritis, a common presentation in human AIDS [61,63]. In addition, a myriad of secondary opportunistic infections, including but not limited to cytomegalovirus, mycobacteriosis, cryptosporidiosis, pneumocystosis, and entamoebiasis, may occur. These widespread multisystemic manifestations of AIDS, coupled with the diverse range and severity of changes that may occur among different individuals, makes predicting the effects a candidate drug may have on the greater HIV-positive population challenging. Well-designed studies using a simian AIDS model are more likely to reveal potential toxic effects than those using healthy macaques. Peripheral neuropathy presents a unique challenge in this arena. Primary peripheral neuropathy is the most common neurologic complication associated with HIV infection and it is caused by a direct infection of the ganglia or peripheral nerve fiber by the virus [182–185]. In human patients with AIDS, however, peripheral neuropathy also occurs secondary to antiretroviral drug therapy. In this situation, simian AIDS models of peripheral neuropathy are helpful for distinguishing treatment-related effects from virus-related effects [185].

Immunosuppression, whether primary (HIV/AIDS) or secondary (chemotherapy, radiation oncology), also poses a unique challenge for the development of novel anticancer therapies. Immunotherapy of cancer leverages immune cell stimulatory, inhibitory, or regulatory mechanisms to enhance the antitumor immune response [186]. By targeting new candidate drugs to specific receptors, individual pathways can be enhanced or antagonized to promote or inhibit pro- or anti-inflammatory mechanisms. However, the immune targets are not limited to tumor-associated leukocytes and may have broad, possibly toxic, effects in other tissues. These effects may differ in immunosuppressed individuals compared with immunocompetent individuals. Nonhuman primate models of immunosuppression can help to align the toxicology assessments with the treatment-targeted immunosuppressed population. Likewise, secondary bacterial and fungal infections are a common cause of morbidity and mortality in immunosuppressed individuals. The increased incidence of opportunistic infections, coupled with the globally increasing phenomenon of antibiotic resistance, makes the need for new, effective antimicrobial drugs high. Predicting the safety margin of these compounds in the immunosuppressed population is likely to be more accurate using animal models of immunosuppression than immunocompetent animals. Likewise, efforts to develop newer, safer vaccines specifically for use in immunosuppressed populations, such as with the development of a next-generation smallpox vaccine, are well suited for testing in macaque models of immunosuppression [187–189].

Sublethal exposure to radiation, whether therapeutic, accidental, or militant, can be severely toxic. In 2004 the U.S. government established eight Centers for Medical Countermeasures Against Radiation with the intent to develop agents for prophylaxis, mitigation, and treatment of radiation injury [190]. Determining the therapeutic and/or toxic effects of these countermeasures is hampered by the broad adverse effects of radiation exposure on hematopoietic and lymphoid tissues, gastrointestinal tract, skin, lung, kidney, and

VetBooks.ir

eye. The hematopoietic and immune effects are both acute and chronic and result in not only immune cell death but also marked derangement of activity and cytokine production among surviving immune cell populations, such as natural killer T cells [191–194]. Macaques have long been used to study the effects of irradiation on the immune system, and controlled studies have demonstrated an aberrant immune response to both simple antigens and vaccines [64–66]. In addition, extensive radiodamage to the mucosa of the gastrointestinal tract and the skin, both important barriers to environmental and opportunistic pathogens, results in localized and systemic infections, as well as alterations to the normal microbiota—all in the face of a compromised immune system [195–197]. This complex immunity-pathogen relationship makes accurate prediction of unintended effects of a new drug unlikely in a healthy host. However, rhesus models of radiation-induced hematopoietic, immune, gastrointestinal, and lung injury are described and may provide more useful data [67–69,198].

MALARIA

Nonhuman primate models of human infectious diseases are primarily used for pathogenesis and efficacy studies, but for some complex infections that pose significant treatment challenges, such as malaria, use of nonhuman primates in toxicology and safety assessment studies may be warranted.

Malaria is the most important parasitic disease of humans, killing approximately 2000 people each day, most of whom are children [199]. The epidemiology, pathogenesis, and clinical presentations are complex, and resistance to effective therapies is common. More than 100 species of the apicomplexan protozoan *Plasmodium*, the causative agent of malaria, have been identified and are known to infect numerous mammalian, avian, and reptilian hosts [70]. *Plasmodium* infection in the natural host typically results in only mild self-limiting or subclinical disease. However, infection in aberrant hosts causes a spectrum of conditions ranging from mild or subclinical disease in endemic areas to a severe, multisystemic, often life-threatening condition in seasonal areas, travelers, and youth. Five *Plasmodium* species are known to infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Clinically, *P. falciparum* and *P. vivax* are associated with the most severe forms of the disease, although increasing reports implicate *P. knowlesi* as an important cause of life-threatening illness in areas where the natural hosts, cynomolgus and pig-tail macaques, are common [200]. In human malaria, disease presentation and progression are highly variable and depend on many epidemiologic determinants including host factors, such as age and immune status; vector factors, such as density of the anopheline mosquito; social factors, such as the availability of prophylactic drugs, insecticides, and mosquito nets; transmission seasonality (i.e., stable nonseasonal transmission versus unstable seasonal transmission); and, as noted above, the virulence of the infecting *Plasmodium* species [199]. After transmission from an infected female *Anopheles* mosquito, sporozoites invade hepatocytes and undergo asexual replication to produce schizonts containing myriad daughter merozoites. Bursting of the schizonts releases the merozoites, which go on to invade erythrocytes via receptor-mediated mechanisms [199]. It is this erythrocytic stage of infection that results in fever, chills, malaise, myalgia, nausea, and vomiting; anemia and thrombocytopenia are common. As the intraerythrocytic trophozoites mature, structural alterations in the erythrocyte membrane, including expression of

VetBooks.ir

strain-specific adhesive proteins, conformational deformities, and changes in cellular rigidity, result in intravascular erythrocyte aggregation and microvascular obstruction [201–203]. In the brain this causes edema, seizures, and coma, a condition known as cerebral malaria; in the kidney microvascular obstruction results in acute tubular necrosis. Additional heme-related renal damage occurs secondary to lysis of infected erythrocytes, and with chronic infections, immune complex deposition and glomerulonephropathy further compromise renal function. Hepatic damage secondary to the initial extraerythrocytic phase of infection often adds to the complexity of the disease. Splenomegaly and splenic rupture occur with some forms of the disease. Severe malaria is also associated with pulmonary edema and acute respiratory distress syndrome, metabolic acidosis, coagulopathies, intravascular hemolysis, and death. Growing resistance to antiprotozoal drugs makes the need for novel effective treatments high, yet the complexity of disease presentations makes testing safety challenging. These studies may be enhanced by using a nonhuman primate model of malaria.

Rhesus macaques and, less commonly, Japanese macaques are used to model human malaria. Natural infections occur, and experimental infection with *P. coatneyi* or *P. knowlesi* results in a multisystemic disease that recapitulates many of the features of severe *P. falciparum* malaria in humans [71,72]. Importantly, *P. coatneyi* infection results in erythrocytic alterations and microvascular obstruction as is seen in human cerebral malaria [71,73,82,83,204]. Conversely, erythrocyte sequestration, microvascular obstruction, and cerebral edema do not occur with *P. knowlesi* infection in macaques; however, other features of severe malaria, including anemia, thrombocytopenia, coagulopathies, renal disease, metabolic acidosis, disseminated intravascular coagulation, and high fatality rates, are common [70,205]. Rhesus monkeys experimentally infected with *P. coatneyi* also have been used to model gestational malaria in humans [74–77]. It is noteworthy that a rhesus model of SIV-*P. fragile* coinfection has been developed [78,79]. Given the high prevalence of HIV infection in areas where malaria is endemic, there is concern that coinfection may enhance the virulence of one or both pathogens or alter the pharmacodynamic effect of antiviral or antimalarial drugs. Thus, the rhesus coinfection model may be useful for predicting the safety of drugs intended for use in patients with chronic immunodeficiency and malaria. The ability of rhesus monkey models to reproduce many of the complex features of severe human malaria makes them suitable for efficacy, immunogenicity, and toxicity and safety testing of new antimalarial drugs [81].

References

[1] Morgan SJ, Elangbam CS, Berens S, Janovitz E, Vitsky A, Zabka T, et al. Use of animal models of human disease for nonclinical safety assessment of novel pharmaceuticals. Toxicol Pathol 2013;41:509–18.

[2] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640–5.

[3] Clarkson TB, Koritnik DR, Weingand KW, Miller LC. Nonhuman primate models of atherosclerosis: potential for the study of diabetes mellitus and hyperinsulinemia. Metabolism 1985;24:51–9.

[4] Wagner JD, Carlson CS, O'Brien TD, Anthony MS, Bullock BC, Cefalu WT. Diabetes mellitus and islet amyloidosis in cynomolgus monkeys. Lab Anim Sci 1996;46:31–5.

[5] Wagner JD, Cline JM, Shadoan MK, Bullock BC, Rankin SE, Cefalu WT. Naturally occurring and experimental diabetes in cynomolgus monkeys: a comparison of carbohydrate and lipid metabolism and islet pathology. Toxicol Pathol 2001;29:142–8.

VetBooks.ir

[6] Hansen BC, Bodkin NL. Heterogeneity of insulin responses: phases leading to type 2 (non-insulin-dependent) diabetes mellitus in the rhesus monkey. Diabetologia 1986;29:713–9.
[7] Bremer AA, Stanhope KL, Graham JL, Cummings BP, Wang W, Saville BR, et al. Fructose-fed rhesus monkeys: a nonhuman primate model of insulin resistance, metabolic syndrome, and type 2 diabetes. Clin Transl Sci 2011;4:243–52.
[8] Kavanagh K, Fairbanks LA, Bailey JN, Jorgensen MJ, Wilson M, Zhang L, et al. Characterization and heritability of obesity and associated risk factors in vervet monkeys. Obesity (Silver Spring) 2007;15:1666–74.
[9] Cann JA, Kavanagh K, Jorgensen MJ, Mohanan S, Howard TD, Gray SB, et al. Clinicopathologic characterization of naturally occurring diabetes mellitus in vervet monkeys. Vet Pathol 2010;47:713–8.
[10] Rosenblum IY, Barbolt TA, Howard CF. Diabetes mellitus in the chimpanzee (*Pan troglodytes*). J Med Primatol 1981;10:93–101.
[11] McTighe MS, Hansen BC, Ely JJ, Lee DR. Determination of hemoglobin A1c and fasting blood glucose reference intervals in captive chimpanzees (*Pan troglodytes*). J Am Assoc Lab Anim Sci 2011;50:165–70.
[12] Stokes WS. Spontaneous diabetes mellitus in a baboon (Papio cynocephalus anubis). Lab Anim Sci 1986;36:529–33.
[13] Comuzzie AG, Cole SA, Martin L, Carey KD, Mahaney MC, Blangero J, et al. The baboon as a nonhuman primate model for the study of the genetics of obesity. Obes Res 2003;11:75–80.
[14] Pirarat N, Kesdangsakolvut S, Chotiapisitkul S, Assarasakorn S. Spontaneous diabetes mellitus in captive Mandrillus sphinx monkeys: a case report. J Med Primatol 2008;37:162–5.
[15] Tardif SD, Mansfield KG, Ratnam R, Ross CN, Ziegler TE. The marmoset as a model of aging and age-related diseases. ILAR J 2011;52:54–65.
[16] Register TC. Primate models in women's health: inflammation and atherogenesis in female cynomolgus macaques (*Macaca fascicularis*). Am J Primatol 2009;71:766–75.
[17] Rudel LL. Genetic factors influence the atherogenic response of lipoproteins to dietary fat and cholesterol in nonhuman primates. J Am Coll Nutr 1997;16:306–12.
[18] Bullock BC, Lehner ND, Clarkson TB, Feldner MA, Wagner WD, Lofland HB. Comparative primate atherosclerosis. I. Tissue cholesterol concentration and pathologic anatomy. Exp Mol Pathol 1975;22:151–75.
[19] Clarkson TB, Hamm TE, Bullock BC, Lehner ND. Atherosclerosis in old world monkeys. Primates Med 1976;9:66–89.
[20] Chen Y, Ogawa H, Narita H, Ohtoh K, Yoshida T, Yoshikawa Y. Ratio of leptin to adiponectin as an obesity index of cynomolgus monkeys (*Macaca fascicularis*). Exp Anim 2003;52:137–43.
[21] Chen Y, Ono F, Yoshida T, Yoshikawa Y. Relationship between body weight and hematological and serum biochemical parameters in female cynomolgus monkeys (*Macaca fascicularis*). Exp Anim 2002;51:125–31.
[22] Ceccarini G, Flavell RR, Butelman ER, Synan M, Willnow TE, Bar-Dagan M, et al. PET imaging of leptin biodistribution and metabolism in rodents and primates. Cell Metab 2009;10:148–59.
[23] Adams SH, Stanhope KL, Grant RW, Cummings BP, Havel PJ. Metabolic and endocrine profiles in response to systemic infusion of fructose and glucose in rhesus macaques. Endocrinology 2008;149:3002–8.
[24] Muehlenbein MP, Campbell BC, Richards RJ, Watts DP, Svec F, Falkenstein KP, et al. Leptin, adiposity, and testosterone in captive male macaques. Am J Phys Anthropol 2005;127:335–41.
[25] Shaw KA, Hennebold JD, Edelman AB. Effect of a combined estrogen and progesterone oral contraceptive on circulating adipocytokines adiponectin, resistin and DLK-1 in normal and obese female rhesus monkeys. Contraception 2013;88:177–82.
[26] Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, et al. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 2001;50:1126–33.
[27] Ramsey JJ, Laatsch JL, Kemnitz JW. Age and gender differences in body composition, energy expenditure, and glucoregulation of adult rhesus monkeys. J Med Primatol 2000;29:11–9.
[28] Wahab F, Bano R, Jabeen S, Irfan S, Shahab M. Effect of peripheral kisspeptin administration on adiponectin, leptin, and resistin secretion under fed and fasting conditions in the adult male rhesus monkey (*Macaca mulatta*). Horm Metab Res 2010;42:570–4.
[29] Roth GS, Mattison JA, Ottinger MA, Chachich ME, Lane MA, Ingram DK. Aging in rhesus monkeys: relevance to human health interventions. Science 2004;305:1423–6.
[30] Torre P, Fowler CG. Age-related changes in auditory function of rhesus monkeys (*Macaca mulatta*). Hear Res 2000;142:131–40.

VetBooks.ir

[31] Moscrip TD, Ingram DK, Lane MA, Roth GS, Weed JL. Locomotor activity in female rhesus monkeys: assessment of age and calorie restriction effects. J Gerontol A Biol Sci Med Sci 2000;55:B373–80.
[32] Mascarucci P, Taub D, Saccani S, Paloma MA, Dawson H, Roth GS, et al. Age-related changes in cytokine production by leukocytes in rhesus monkeys. Aging 2001;13:85–94.
[33] Colman RJ, McKiernan SH, Aiken JM, Weindruch R. Muscle mass loss in Rhesus monkeys: age of onset. Exp Gerontol 2005;40:573–81.
[34] Pugh TD, Conklin MW, Evans TD, Polewski MA, Barbian HJ, Pass R, et al. A shift in energy metabolism anticipates the onset of sarcopenia in rhesus monkeys. Aging Cell 2013;12:672–81.
[35] Black A, Tilmont EM, Handy AM, Scott WW, Shapses SA, Ingram DK, et al. A nonhuman primate model of age-related bone loss: a longitudinal study in male and premenopausal female rhesus monkeys. Bone 2001;28:295–302.
[36] Champ JE, Binkley N, Havighurst T, Colman RJ, Kemnitz JW, Roecker EB. The effect of advancing age on bone mineral content of female rhesus monkeys. Bone 1996;19:485–92.
[37] Tigno XT, Gerzanich G, Hansen BC. Age-related changes in metabolic parameters of nonhuman primates. J Gerontol A Biol Sci Med Sci 2004;59:1081–8.
[38] Smith SY, Jolette J, Turner CH. Skeletal health: primate model of postmenopausal osteoporosis. Am J Primatol 2009;71:752–65.
[39] Jayo MJ, Weaver DS, Adams MR, Rankin SE. Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys. Am J Obstet Gynecol 1990;163:614–8.
[40] Price DL, Martin LJ, Sisodia SS, Wagster MV, Koo EH, Walker LC, et al. Aged non-human primates: an animal model of age-associated neurodegenerative disease. Brain Pathol 1991;1:287–96.
[41] Voytko ML. Impairments in acquisition and reversals of two-choice discriminations by aged rhesus monkeys. Neurobiol Aging 1999;20:617–27.
[42] Nagahara AH, Bernot T, Tuszynski MH. Age-related cognitive deficits in rhesus monkeys mirror human deficits on an automated test battery. Neurobiol Aging 2010;31:1020–31.
[43] Hara Y, Rapp PR, Morrison JH. Neuronal and morphological bases of cognitive decline in aged rhesus monkeys. Age 2012;34:1051–73.
[44] Bailey ME, Wang AC, Hao J, Janssen WG, Hara Y, Dumitriu D, et al. Interactive effects of age and estrogen on cortical neurons: implications for cognitive aging. Neuroscience 2011;191:145–58.
[45] Darusman HS, Sajuthi D, Kalliokoski O, Jacobsen KR, Call J, Schapiro SJ, et al. Correlations between serum levels of beta amyloid, cerebrospinal levels of tau and phospho tau, and delayed response tasks in young and aged cynomolgus monkeys (*Macaca fascicularis*). J Med Primatol 2013;42:137–46.
[46] Hodgen GD, Goodman AL, O'Connor A, Johnson DK. Menopause in rhesus monkeys: model for study of disorders in the human climacteric. Am J Obstet Gynecol 1977;127:581–4.
[47] Walker ML, Herndon JG. Menopause in nonhuman primates? Biol Reprod 2008;79:398–406.
[48] Gilardi KV, Shideler SE, Valverde CR, Roberts JA, Lasley BL. Characterization of the onset of menopause in the rhesus macaque. Biol Reprod 1997;57:335–40.
[49] Appt SE, Clarkson TB, Hoyer PB, Kock ND, Goode AK, May MC, et al. Experimental induction of reduced ovarian reserve in a nonhuman primate model (*Macaca fascicularis*). Comp Med 2010;60:380–8.
[50] Kavanagh K, Koudy Williams J, Wagner JD. Naturally occurring menopause in cynomolgus monkeys: changes in hormone, lipid, and carbohydrate measures with hormonal status. J Med Priamtol 2005;34:171–7.
[51] Appt SE, Kaplan JR, Clarkson TB, Cline JM, Christian PJ, Hoyer PB. Destruction of primordial ovarian follicles in adult cynomolgus macaques after exposure to 4-vinylcyclohexene diepoxide: a nonhuman primate model of the menopausal transition. Fertil Steril 2006;86:1210–6.
[52] Appt SE. Peer review and reflection continues forever. Fertil Steril 2008;89:1034–5.
[53] Story L, Kennedy S. Animal studies in endometriosis: a review. ILAR J 2004;45:132–8.
[54] Zondervan KT, Weeks DE, Colman R, Cardon LR, Hadfield R, Schleffler J, et al. Familial aggregation of endometriosis in a large pedigree of rhesus macaques. Hum Reprod 2004;19:448–55.
[55] Gardner MB, Luciw PA. Macaque models of human infectious disease. ILAR J 2008;49:220–55.
[56] Sui Y, Gordon S, Franchini G, Berzofsky JA. Nonhuman primate models for HIV/AIDS vaccine development. Curr Protoc Immunol 2013;102:12.14:12.14.1–12.14.30.
[57] Spira AI, Marx PA, Patterson BK, Mahoney J, Koup RA, Wolinsky SM, et al. Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J Exp Med 1996;183:215–25.

[58] Estes JD. Pathobiology of HIV/SIV-associated changes in secondary lymphoid tissues. Immunol Rev 2013;254:65–77.
[59] Wachtman L, Mansfield K. Viral diseases of nonhuman primates. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. 2nd ed. Nonhuman primates in biomedical research, vol. 2. London: Academic Press; 2012. p. 68.
[60] Baskin GB, Murphey-Corb M, Watson EA, Martin LN. Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. Vet Pathol 1988;25:456–67.
[61] Alpers CE, Tsai CC, Hudkins KL, Cui Y, Kuller L, Benveniste RE, et al. Focal segmental glomerulosclerosis in primates infected with a simian immunodeficiency virus. AIDS Res Hum Retroviruses 1997;13:413–24.
[62] Li SL, Kaaya EE, Feichtinger H, Putkonen P, Parravicini C, Böttiger D, et al. Monocyte/macrophage giant cell disease in SIV-infected cynomolgus monkeys. Res Virol 1991;142:173–82.
[63] Stephens EB, Tian C, Dalton SB, Gattone VH. Simian-human immunodeficiency virus-associated nephropathy in macaques. AIDS Res Hum Retroviruses 2000;16:1295–306.
[64] Gengozian N, Moriarty C, Good RA. Functional evaluation of T helper, T suppressor, and B lymphocytes in lethally irradiated rhesus monkeys injected with autologous bone marrow. Transplantation 1992;53:1313–22.
[65] Bogden AE, Eltringham JR, Anderson DR. Effect of total body irradiation on the immune response of rhesus monkeys to a single antigenic stimulus. Radiat Res 1970;42:90–104.
[66] Hilmas DE, Spertzel RO. Response of sublethally irradiated monkeys to a replicating viral antigen. Infect Immun 1975;12:592–601.
[67] Vigneulle RM, Rao S, Fasano A, MacVittie TJ. Structural and functional alterations of the gastrointestinal tract following radiation-induced injury in the rhesus monkey. Dig Dis Sci 2002;47:1480–91.
[68] Garofalo MC, Ward AA, Farese AM, Bennett A, Taylor-Howell C, Cui W, et al. A pilot study in rhesus macaques to assess the treatment efficacy of a small molecular weight catalytic metalloporphyrin antioxidant (AEOL 10150) in mitigating radiation-induced lung damage. Health Phys 2014;106:73–83.
[69] MacVittie TJ, Bennett AW, Cohen MV, Farese AM, Higgins A, Hankey KG. Immune cell reconstitution after exposure to potentially lethal doses of radiation in the nonhuman primate. Health Phys 2014;106:84–96.
[70] Galinski MR, Barnwell JW. Nonhuman primate models for human malaria research. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. 2nd ed. Nonhuman primates in biomedical research, vol. 2. London: Academic Press; 2012. p. 299–323.
[71] Smith CD, Brown AE, Nakazawa S, Fujioka H, Aikawa M. Multi-organ erythrocyte sequestration and ligand expression in rhesus monkeys infected with *Plasmodium coatneyi* malaria. Am J Trop Med Hyg 1996;55:379–83.
[72] Moreno A, Cabrera-Mora M, Garcia A, Orkin J, Strobert E, Barnwell JW, et al. *Plasmodium coatneyi* in rhesus macaques replicates the multisystemic dysfunction of severe malaria in humans. Infect Immun 2013;81:1889–904.
[73] Nakano Y, Fujioka H, Luc KD, Rabbege JR, Todd GD, Collins WE, et al. A correlation of the sequestration rate of *Plasmodium coatneyi*-infected erythrocytes in cerebral and subcutaneous tissues of a rhesus monkey. Am J Trop Med Hyg 1996;55:311–4.
[74] Davison BB, Cogswell FB, Baskin GB, Falkenstein KP, Henson EW, Krogstad DJ. Placental changes associated with fetal outcome in the *Plasmodium coatneyi*/rhesus monkey model of malaria in pregnancy. Am J Trop Med Hyg 2000;63:158–73.
[75] Davison BB, Cogswell FB, Baskin GB, Falkenstein KP, Henson EW, Tarantal AF, et al. *Plasmodium coatneyi* in the rhesus monkey (*Macaca mulatta*) as a model of malaria in pregnancy. Am J Trop Med Hyg 1998;59:189–201.
[76] Davison BB, Kaack MB, Rogers LB, Rasmussen KK, Rasmussen T, Henson EW, et al. Alterations in the profile of blood cell types during malaria in previously unexposed primigravid monkeys. J Infect Dis 2005;191:1940–52.
[77] Davison BB, Kaack MB, Rogers LB, Rasmussen KK, Rasmussen TA, Henson EW, et al. The role of soluble tumor necrosis factor receptor types I and II and tumor necrosis factor-alpha in malaria during pregnancy. J Infect Dis 2006;194:123–32.
[78] Trott KA, Richardson A, Hudgens MA, Abel K. Immune activation and regulation in simian immunodeficiency virus-*Plasmodium fragile*-coinfected rhesus macaques. J Virol 2013;87:9523–37.
[79] Trott KA, Chau JY, Hudgens MG, Fine J, Mfalila CK, Tarara RP, et al. Evidence for an increased risk of transmission of simian immunodeficiency virus and malaria in a rhesus macaque coinfection model. J Virol 2011;85:11655–63.
[80] Coban C, Philipp MT, Purcell JE, Keister DB, Okulate M, Martin DS, et al. Induction of *Plasmodium falciparum* transmission-blocking antibodies in nonhuman primates by a combination of DNA and protein immunizations. Infect Immun 2004;72:253–9.

[81] Dutta S, Kaushal DC, Ware LA, Puri SK, Kaushal NA, Narula A, et al. Merozoite surface protein 1 of *Plasmodium vivax* induces a protective response against *Plasmodium cynomolgi* challenge in rhesus monkeys. Infect Immun 2005;73:5936–44.
[82] Kawai S, Aikawa M, Kano S, Suzuki M. A primate model for severe human malaria with cerebral involvement: *Plasmodium coatneyi*-infected *Macaca fuscata*. Am J Trop Med Hyg 1993;48:630–6.
[83] Kawai S1, Kano S, Suzuki M. Rosette formation by *Plasmodium coatneyi*-infected erythrocytes of the Japanese macaque (*Macaca fuscata*). Am J Trop Med Hyg 1995;53:295–9.
[84] Zhang X, Zhang R, Raab S, Zheng W, Wang J, Liu N, et al. Rhesus macacques develop metabolic syndrome with reversible vascular dysfunction responsive to pioglitazone. Circulation 2011;124:77–86.
[85] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–7.
[86] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85–97.
[87] Samaras K, Botelho NK, Chisholm DJ, Lord RV. Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. Obesity (Silver Spring) 2010;18:884–9.
[88] Santos-Alvarez J, Goberna R, Sanchez-Margalet V. Human leptin stimulates proliferation and activation of human circulating monocytes. Cell Immunol 1999;194:6–11.
[89] Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR. Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. Biochem Biophys Res Commun 2003;309:286–90.
[90] Zhang J, Wu Y, Zhang Y, Leroith D, Bernlohr DA, Chen X. The role of lipocalin 2 in the regulation of inflammation in adipocytes and macrophages. Mol Endocrinol 2008;22:1416–26.
[91] Tabata M, Kadomatsu T, Fukuhara S, Miyata K, Ito Y, Endo M, et al. Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. Cell Metab 2009;10:178–88.
[92] Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, Tso AW, et al. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem 2007;53:34–41.
[93] Chavey C, Lazennec G, Lagarrigue S, Clape C, Iankova I, Teyssier J, et al. CXC Ligand 5 is an adipose-tissue derived factor that links obesity to insulin resistance. Cell Metab 2009;9:339–49.
[94] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.
[95] Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327–34.
[96] Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 1994;94:1543–9.
[97] Ost A, Danielsson A, Liden M, Eriksson U, Nystrom FH, Stralfors P. Retinol-binding protein-4 attenuates insulin-induced phosphorylation of IRS1 and ERK1/2 in primary human adipocytes. FASEB J 2007;21:3696–704.
[98] Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006;354:2552–63.
[99] Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med 1996;2:800–3.
[100] Mallat Z, Corbaz A, Scoazec A, Besnard S, Lesèche G, Chvatchko Y, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation 2001;104:1598–603.
[101] Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 1998;394:897–901.
[102] Zabeau L, Lavens D, Peelman F, Eyckerman S, Vandekerckhove J, Tavernier J. The ins and outs of leptin receptor activation. FEBS Lett 2003;546:45–50.
[103] Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Müller C, Carling D, et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002;415:339–43.
[104] Ren J. Leptin and hyperleptinemia—from friend to foe for cardiovascular function. J Endocrinol 2004;181:1–10.
[105] Sahu A. Minireview: a hypothalamic role in energy balance with special emphasis on leptin. Endocrinology 2004;145:2613–20.
[106] Fruhbeck G. Intracellular signalling pathways activated by leptin. Biochem J 2006;393:7–20.
[107] Sierra-Johnson J, Romero-Corral A, Lopez-Jimenez F, Gami AS, Sert Kuniyoshi FH, Wolk R, et al. Relation of increased leptin concentrations to history of myocardial infarction and stroke in the United States population. Am J Cardiol 2007;100:234–9.

VetBooks.ir

[108] Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, et al. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. Biochem Biophys Res Commun 2004;314:415–9.
[109] Lubos E, Messow CM, Schnabel R, Rupprecht HJ, Espinola-Klein C, Bickel C, et al. Resistin, acute coronary syndrome and prognosis results from the AtheroGene study. Atherosclerosis 2007;193:121–8.
[110] Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003;107:671–4.
[111] Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, et al. Adiponectin as a biomarker of the metabolic syndrome. Circ J 2004;68:975–81.
[112] Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2009;302:179–88.
[113] Sattar N, Wannamethee G, Sarwar N, Tchernova J, Cherry L, Wallace AM, et al. Adiponectin and coronary heart disease: a prospective study and meta-analysis. Circulation 2006;114:623–9.
[114] Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004;291:1730–7.
[115] Marx N, Schönbeck U, Lazar MA, Libby P, Plutzky J. Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. Circ Res 1998;83:1097–103.
[116] Youssef S, Stüve O, Patarroyo JC, Ruiz PJ, Radosevich JL, Hur EM, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. Nature 2002;420:78–84.
[117] Nakahara T, Hyogo H, Kimura Y, Ishitobi T, Arihiro K, Aikata H, et al. Efficacy of rosuvastatin for the treatment of non-alcoholic steatohepatitis with dyslipidemia: an open-label, pilot study. Hepatol Res 2012;42:1065–72.
[118] Athyros VG, Papageorgiou AA, Mercouris BR, Athyrou VV, Symeonidis AN, Basayannis EO, et al. Treatment with atorvastatin to the National Cholesterol Educational Program goal versus 'usual' care in secondary coronary heart disease prevention. The GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study. Curr Med Res Opin 2002;18:220–8.
[119] Grip O, Janciauskiene S, Lindgren S. Circulating monocytes and plasma inflammatory biomarkers in active Crohn's disease: elevated oxidized low-density lipoprotein and the anti-inflammatory effect of atorvastatin. Inflamm Bowel Dis 2004;10:193–200.
[120] Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nat Rev Cancer 2005;5:930–42.
[121] Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, et al. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. Lancet 2010;375:735–42.
[122] Preiss D, Seshasai SR, Welsh P, Murphy SA, Ho JE, Waters DD, et al. Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis. JAMA 2011;305:2556–64.
[123] Li S, Kievit P, Robertson AK, Kolumam G, Li X, von Wachenfeldt K, et al. Targeting oxidized LDL improves insulin sensitivity and immune cell function in obese rhesus macaques. Mol Metab 2013;2:256–69.
[124] Fitzgerald GA. Coxibs and cardiovascular disease. N Engl J Med 2004;351:1709–11.
[125] Yu Y, Ricciotti E, Scalia R, Tang SY, Grant G, Yu Z, et al. Vascular COX-2 modulates blood pressure and thrombosis in mice. Sci Transl Med 2012;4:132–54.
[126] Wagner JD, Kavanagh K, Ward GM, Auerbach BJ, Harwood Jr. HJ, Kaplan JR. Old world nonhuman primate models of type 2 diabetes mellitus. ILAR J 2006;47:259–71.
[127] Lane MA, Baer DJ, Rumpler WV, Weindruch R, Ingram DK, Tilmont EM, et al. Calorie restriction lowers body temperature in rhesus monkeys, consistent with a postulated anti-aging mechanism in rodents. Proc Natl Acad Sci USA 1996;93:4159–64.
[128] Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol 2010;9:1118–27.
[129] Reitz C, Brayne C, Mayeux R. Epidemiology of Alzheimer disease. Nat Rev Neurol 2011;7:137–52.
[130] Rink L, Cakman I, Kirchner H. Altered cytokine production in the elderly. Mech Ageing Dev 1998;102:199–209.
[131] Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 1998;147:755–63.
[132] Kim TN, Choi KM. Sarcopenia: definition, epidemiology, and pathophysiology. J Bone Metab 2013;20:1–10.
[133] Evans WJ, Campbell WW. Sarcopenia and age-related changes in body composition and functional capacity. J Nutr 1993;123:465–8.

[134] Lang T, Streeper T, Cawthon P, Baldwin K, Taaffe DR, Harris TB. Sarcopenia: etiology, clinical consequences, intervention, and assessment. Osteoporos Int 2010;21:543–59.
[135] Zadik Z, Chalew SA, McCarter Jr. RJ, Meistas M, Kowarski AA. The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. J Clin Endocrinol Metab 1985;60:513–6.
[136] Schrager MA, Metter EJ, Simonsick E, Ble A, Bandinelli S, Lauretani F, et al. Sarcopenic obesity and inflammation in the InCHIANTI study. J Appl Physiol 2007;102:919–25.
[137] Rasmussen BB, Fujita S, Wolfe RR, Mittendorfer B, Roy M, Rowe VL, et al. Insulin resistance of muscle protein metabolism in aging. FASEB J 2006;20:768–9.
[138] Park SW, Goodpaster BH, Strotmeyer ES, Kuller LH, Broudeau R, Kammerer C, et al. Accelerated loss of skeletal muscle strength in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes Care 2007;30:1507–12.
[139] Ilich JZ, Kelly OJ, Inglis JE, Panton LB, Duque G, Ormsbee MJ. Interrelationship among muscle, fat, and bone: connecting the dots on cellular, hormonal, and whole body levels. Ageing Res Rev 2014;15:51–60.
[140] Lang TF. The bone-muscle relationship in men and women. J Osteoporos 2011; 702735.
[141] WHO. The World Health Report 2004: changing history, http://www.who.int/whr/2004/en/; 2004 [accessed 17.04.14].
[142] Pappolla MA, Bryant-Thomas TK, Herbert D, Pacheco J, Fabra Garcia M, Manjon M, et al. Mild hypercholesterolemia is an early risk factor for the development of Alzheimer amyloid pathology. Neurology 2003;61:199–205.
[143] Fernández Martínez M, Castro Flores J, de Las P, Heras S, Mandaluniz Lekumberri A, Gordejuela Menocal M, et al. Risk factors for dementia in the epidemiological study of Munguialde County (Basque Country—Spain). BMC Neurol 2008;8:39.
[144] Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. Arch Neurol 2000;57:1439–43.
[145] Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. Statins and the risk of dementia. Lancet 2000;356:1627–31.
[146] Chuang CS, Lin CL, Lin MC, Sung FC, Kao CH. Decreased prevalence of dementia associated with statins: a national population-based study. Eur J Neurol 2014; http://dx.doi.org/10.1111/ene.12402 [Epub ahead of print].
[147] Sosa-Ortiz AL, Acosta-Castillo I, Prince MJ. Epidemiology of dementias and Alzheimer's disease. Arch Med Res 2012;43:600–8.
[148] Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev 2006;(1):CD005593.
[149] McShane R, Areosa Sastre A, Minakaran N. Memantine for dementia. Cochrane Database Syst Rev 2006;(2):CD003154.
[150] Mikulca JA1, Nguyen V, Gajdosik DA, Teklu SG, Giunta EA, Lessa EA, et al. Potential novel targets for Alzheimer pharmacotherapy: II. Update on secretase inhibitors and related approaches. J Clin Pharm Ther 2014;39:25–37.
[151] Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. Alzheimer's Disease Cooperative Study Steering Committee, Siemers E, Sethuraman G, Mohs R; Semagacestat Study Group. A phase 3 trial of semagacestat for treatment of Alzheimer's disease. N Engl J Med 2013;369:341–50.
[152] Doody RS, Gavrilova SI, Sano M, Thomas RG, Aisen PS, Bachurin SO, et al. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. Lancet 2008;372:207–15.
[153] Fan LY, Chiu MJ. Combotherapy and current concepts as well as future strategies for the treatment of Alzheimer's disease. Neuropsychiatr Dis Treat 2014;10:439–51.
[154] Kim MJ, Aiken JM, Havighurst T, Hollander J, Ripple MO, Weindruch R. Adult-onset energy restriction of rhesus monkeys attenuates oxidative stress-induced cytokine expression by peripheral blood mononuclear cells. J Nutr 1997;127:2293–301.
[155] Sowers MR, Zheng H, McConnell D, Nan B, Harlow S, Randolph Jr. JF. Estradiol rates of change in relation to the final menstrual period in a population-based cohort of women. J Clin Endocrinol Metab 2008;93:3847–52.
[156] Sowers MR, Zheng H, McConnell D, Nan B, Harlow S, Randolph Jr. JF. Follicle stimulating hormone and its rate of change in defining menopause transition stages. J Clin Endocrinol Metab 2008;93:3958–64.
[157] Rannevik G, Jeppsson S, Johnell O, Bjerre B, Laurell-Borulf Y, Svanberg L. A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. Maturitas 1995;21:103–13.

[158] Nedergaard A, Henriksen K, Karsdal MA, Christiansen C. Menopause, estrogens and frailty. Gynecol Endocrinol 2013;29:418–23.
[159] Bhavnani BR. Pharmacokinetics and pharmacodynamics of conjugated equine estrogens: chemistry and metabolism. Proc Soc Exp Biol Med 1998;217:6–16.
[160] Notelovitz M. Clinical opinion: the biologic and pharmacologic principles of estrogen therapy for symptomatic menopause. MedGenMed 2006;8:85.
[161] Nath A, Sitruk-Ware R. Different cardiovascular effects of progestins according to structure and activity. Climacteric 2009;12:96–101.
[162] Rozenberg S, Vandromme J, Kroll M, Pastijn A, Degueldre M. Osteoporosis prevention with sex hormone replacement therapy. Int J Fertil Menopausal Stud 1994;39:262–71.
[163] Dören M, Nilsson JA, Johnell O. Effects of specific post-menopausal hormone therapies on bone mineral density in post-menopausal women: a meta-analysis. Hum Reprod 2003;18:1737–46.
[164] Wells G, Tugwell P, Shea B, Guyatt G, Peterson J, Zytaruk N, et al. Meta-analyses of therapies for postmenopausal osteoporosis. V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. Endocr Rev 2002;23:529–39.
[165] Cummings SR1, Ettinger B, Delmas PD, Kenemans P, Stathopoulos V, Verweij P, et al. The effects of tibolone in older postmenopausal women. N Engl J Med 2008;359:697–708.
[166] Nelson HD, Walker M, Zakher B, Mitchell J. Menopausal hormone therapy for the primary prevention of chronic conditions: a systematic review to update the U.S. Preventive Services Task Force recommendations. Ann Intern Med 2012;157:104–13.
[167] Barrett-Connor E, Slone S, Greendale G, Kritz-Silverstein D, Espeland M, Johnson SR, et al. The Postmenopausal Estrogen/Progestin Interventions Study: primary outcomes in adherent women. Maturitas 1997;27:261–74.
[168] Grodstein F, Manson JE, Colditz GA, Willett WC, Speizer FE, Stampfer MJ. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. Ann Intern Med 2000;133:933–41.
[169] Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/Progestin Replacement Study (HERS) Research Group. JAMA 1998;280:605–13.
[170] Grodstein F, Stampfer MJ. Estrogen for women at varying risk of coronary disease. Maturitas 1998;30:19–26.
[171] Hodis HN, Mack WJ. A "window of opportunity:" the reduction of coronary heart disease and total mortality with menopausal therapies is age- and time-dependent. Brain Res 2011;1379:244–52.
[172] Lobo RA, Clarkson TB. Different mechanisms for benefit and risk of coronary heart disease and stroke in early postmenopausal women: a hypothetical explanation. Menopause 2011;18:237–40.
[173] Bath PM, Gray LJ. Association between hormone replacement therapy and subsequent stroke: a meta-analysis. BMJ 2005;330:342.
[174] Sare GM, Gray LJ, Bath PM. Association between hormone replacement therapy and subsequent arterial and venous vascular events: a meta-analysis. Eur Heart J 2008;29:2031–41.
[175] Anderson GL, Chlebowski RT, Rossouw JE, Rodabough RJ, McTiernan A, Margolis KL, et al. Prior hormone therapy and breast cancer risk in the Women's Health Initiative randomized trial of estrogen plus progestin. Maturitas 2006;55:103–15.
[176] Van Wagenen G. Vital statistics from a breeding colony. Reproduction and pregnancy outcome in *Macaca mulatta*. J Med Primatol 1972;1:2–28.
[177] Appt SE, Clarkson TB, Chen H, Adams MR, Christian PJ, Hoyer PB, et al. Serum antimüllerian hormone predicts ovarian reserve in a monkey model. Menopause 2009;16:597–601.
[178] Muhammad FS, Goode AK, Kock ND, Arifin EA, Cline JM, Adams MR, et al. Effects of 4-vinylcyclohexene diepoxide on peripubertal and adult Sprague-Dawley rats: ovarian, clinical, and pathologic outcomes. Comp Med 2009;59:46–59.
[179] Giudice LC, Kao LC. Endometriosis. Lancet 2004;364:1789–99.
[180] Bruner-Tran KL, Herington JL, Duleba AJ, Taylor HS, Osteen KG. Medical management of endometriosis: emerging evidence linking inflammation to disease pathophysiology. Minerva Ginecol 2013;65:199–213.
[181] Waterton JC, Miller D, Morrell JS, Dukes M, West CD, Wadsworth PF. A case of endometriosis in the macaque diagnosed by nuclear magnetic resonance imaging. Lab Anim 1992;26:59–64.

[182] Ellis RJ, Rosario D, Clifford DB, McArthur JC, Simpson D, Alexander T, et al. Continued high prevalence and adverse clinical impact of human immunodeficiency virus-associated sensory neuropathy in the era of combination antiretroviral therapy: the CHARTER Study. Arch Neurol 2010;67:552–8.
[183] Ghosh S, Chandran A, Jansen JP. Epidemiology of HIV-related neuropathy: a systematic literature review. AIDS Res Hum Retroviruses 2012;28:36–48.
[184] McArthur JC, Brew BJ, Nath A. Neurological complications of HIV infection. Lancet Neurol 2005;4:543–55.
[185] Mangus LM, Dorsey JL, Laast VA, Ringkamp M, Ebenezer GJ, Hauer P, et al. Unraveling the pathogenesis of HIV peripheral neuropathy: insights from a simian immunodeficiency virus macaque model. ILAR J 2014;54:296–303.
[186] Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity 2013;39:1–10.
[187] Gordon SN, Cecchinato V, Andresen V, Heraud JM, Hryniewicz A, Parks RW, et al. Smallpox vaccine safety is dependent on T cells and not B cells. J Infect Dis 2011;203:1043–53.
[188] Chen RT, Lane JM. Myocarditis: the unexpected return of smallpox vaccine adverse events. Lancet 2003;362:1345–6.
[189] Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part II. Adverse events. Clin Infect Dis 2003;37:251–71.
[190] Williams JP, Brown SL, Georges GE, Hauer-Jensen M, Hill RP, Huser AK, et al. Animal models for medical countermeasures to radiation exposure. Radiat Res 2010;173:557–78.
[191] McBride WH, Vegesna V. The role of T-cells in radiation pneumonitis after bone marrow transplantation. Int J Radiat Biol 2000;76:517–21.
[192] Kusunoki Y, Hayashi T. Long-lasting alterations of the immune system by ionizing radiation exposure: implications for disease development among atomic bomb survivors. Int J Radiat Biol 2008;84:1–14.
[193] Park HR, Jo SK, Paik SG. The NK1.1+T cells alive in irradiated mice play an important role in a Th1/Th2 balance. Int J Radiat Biol 2006;82:161–70.
[194] Riggs JE, Lussier AM, Lee SK, Appel MC, Woodland RT. Differential radiosensitivity among B cell subpopulations. J Immunol 1988;141:1799–807.
[195] Mason KA, Withers HR, McBride WH, Davis CA, Smathers JB. Comparison of the gastrointestinal syndrome after total-body or total-abdominal irradiation. Radiat Res 1989;117:480–8.
[196] Withers HR, Elkind MM. Dose-survival characteristics of epithelial cells of mouse intestinal mucosa. Radiology 1968;91:998–1000.
[197] Potten CS, Hendry JH. Radiation and gut. Amsterdam: Elsevier Science; 1995.
[198] Augustine AD, Gondré-Lewis T, McBride W, Miller L, Pellmar TC, Rockwell S. Animal models for radiation injury, protection and therapy. Radiat Res 2005;164:100–9.
[199] White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria. Lancet 2014;383:723–35.
[200] Singh B, Daneshvar C. Human infections and detection of *Plasmodium knowlesi*. Clin Microbiol Rev 2013;26:165–84.
[201] Aikawa M, Iseki M, Barnwell JW, Taylor D, Oo MM, Howard RJ. The pathology of human cerebral malaria. Am J Trop Med Hyg 1990;43:30–7.
[202] Turner GD1, Morrison H, Jones M, Davis TM, Looareesuwan S, Buley ID, et al. An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. Am J Pathol 1994;145:1057–69.
[203] Ahlqvist J. Decreased red cell deformability and vascular obstruction in falciparum malaria illustrated by a fatal case. Scand J Haematol 1985;35:531–5.
[204] Aikawa M, Brown A, Smith CD, Tegoshi T, Howard RJ, Hasler TH, et al. A primate model for human cerebral malaria: *Plasmodium coatneyi*-infected rhesus monkeys. Am J Trop Med Hyg 1992;36:391–7.
[205] Craig AG, Grau GE, Janse C, Kazura JW, Milner D, Barnwell JW, et al. The role of animal models for research on severe malaria. PLoS Pathog 2012;8(2):e1002401.

VetBooks.ir

VetBooks.ir

PART IV

THE 3 Rs AND ANIMAL WELFARE

VetBooks.ir

VetBooks.ir

CHAPTER

14

Opportunities for Implementing the 3 Rs in Drug Development and Safety Assessment Studies Using Nonhuman Primates

Kathryn Chapman[*], *Kathryn Bayne*[†], *Jessica Couch*[‡], *Thierry Decelle*[§], *John Finch*[¶], *Lolke de Haan*[‖], *Tina Koban*[~], *Lars Fris Mikkelsen*[**], *Wolfgang Müller*[††], *Helen Palmer*[#], *Mark Prescott*[*]

[*]National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, United Kingdom

[†]AAALAC International, Frederick, MD, USA

[‡]Genentech, South San Francisco, CA, USA

[§]Sanofi Pasteur, Marcy L'Etoile, France

[¶]Charles River Laboratories, Edinburgh, United Kingdom

[‖]Medimmune, Cambridge, United Kingdom

[~]Huntingdon Life Sciences, Princeton, NJ, USA

[#]Huntingdon Life Sciences, Huntingdon, United Kingdom

[**]Independent

[††]Covance, Münster, Germany

http://dx.doi.org/10.1016/B978-0-12-417144-2.00014-7
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

OUTLINE

Introduction 282
The "3 Rs" Principles 282
The Current Global Legislative Environment 283

Study Design Considerations to Minimize Nonhuman Primate Use 283
Opportunities to Reduce Nonhuman Primate Use in Individual Studies 290
Opportunities to Reduce Nonhuman Primate Use Across the Whole Drug Development Program 292

Refinement of Husbandry and Procedures to Improve Animal Welfare and Quality of Science 293
Housing Design and Environmental Enrichment 293
Managing Social Groups 296
Study Design and Scientific Procedures 297
Staff-Animal Interactions 298

References 299

INTRODUCTION

The "3 Rs" Principles

The concepts of replacement, reduction, and refinement as guiding principles for humane science were first elaborated by Russell and Burch [1]:

- Replacement of animals with nonanimal methods
- Reduction of the number of animals used to the minimum necessary to obtain information of a given amount and precision
- Refinement of scientific procedures and husbandry to minimize pain and distress and improve animal welfare.

The principles of the "3 Rs" have since become accepted internationally and adopted in legislation on the protection and use of laboratory animals. They are increasingly viewed as an integral part of mainstream scientific practice, and there is recognition that application of the 3 Rs during the design and conduct of scientific research studies can benefit not only animal welfare but also the quality of the science and business efficiency. This has led to the 3 Rs also being included in international legislation on the development of pharmaceuticals for human use.

Effective implementation of the 3 Rs requires a team approach, with information sharing, buy-in from senior management, and collaboration within and between organizations. It is worth mentioning, however, that the 3 Rs is not a competitive part of drug development. Hence, more than 30 international pharmaceutical companies, contract research organizations, and regulators have collaborated with the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) to identify opportunities to reduce and refine the use of nonhuman primates. Most of this work has been done outside the demands of the regulatory framework and has led to, for example, opportunities to reduce by up to 64% the number of nonhuman primates used per monoclonal antibody in development.

VetBooks.ir

To fully apply the 3 Rs and maximize the benefits there is a need to ensure that as new knowledge, technologies, and approaches emerge there is timely assessment and evolution of scientific procedures and husbandry, research strategies, and study designs to meet best practices. Ideally, this would be a continuous process. The NC3Rs website (www.nc3rs.org.uk) is one source of contemporary information and advice.

The Current Global Legislative Environment

The use and care of nonhuman primates in research and testing is regulated by national and international laws and governmental regulations and policies on the use of animals for scientific purposes [2]. These are often supplemented by nongovernmental systems of oversight, such as voluntary participation by institutions in accreditation programs (e.g., the Association for Assessment and Accreditation of Laboratory Animal Care International), company global animal welfare policies, and the peer-review processes and grant terms and conditions of research funding bodies (e.g., the National Institutes of Health, European Commission, Wellcome Trust). Together, these oversight mechanisms help to provide the structures to support application of the 3 Rs to nonhuman primate use. Key elements common to many laws on the protection of animals used in science include a requirement for scientific and ethical evaluation of research projects before their authorization, appropriate veterinary care, trained and competent staff, and implementation of measures to minimize pain and distress, such as anesthesia, analgesia, and humane end points (Table 14.1).

The use of nonhuman primates in regulated scientific procedures raises ethical issues [3] and is of great concern to the public [4]. Accordingly, some laws place restrictions on nonhuman primate use, and most contain special provisions aimed at improving the health and psychological well-being of these animals, such as minimum space allowances and requirements for social housing and environmental enrichment (Table 14.2). It is worthwhile to note that while adherence to the prescriptive engineering standards recommended or mandated by law is a starting point for good animal welfare, often much more can be done to refine housing and care to deliver truly high welfare standards. Another important issue is the international supply of nonhuman primates for research, which raises logistical and animal welfare concerns [5,6]. For practical advice on refining supply and transport, see Jennings and Prescott [7] and Swallow et al. [8].

STUDY DESIGN CONSIDERATIONS TO MINIMIZE NONHUMAN PRIMATE USE

Nonclinical safety testing of new chemical and biological entities is a mandatory regulatory requirement before initiating clinical trials in humans. Nonhuman primates are increasingly being used in nonclinical safety testing of pharmaceuticals, which is in large part due to the significant increase in the number of biologics that are in development across the global pharmaceutical industry [9]. While there is a comprehensive set of International Committee of Harmonisation (ICH), US Food and Drug Administration, and Committee for Medicinal Products for Human Use guidelines that lay out the framework, end points, relative timing, and requirements for nonclinical safety testing, none of these guidelines makes

TABLE 14.1 General Legal Provisions Supporting the 3 Rs (Not Intended to be Exhaustive)

Provision	Europe	USA	China	Singapore	Thailand	India
Use of alternatives	E1	U1, U2	C1	S1, S2	T1	I1
Minimization of animal numbers	E1	U1, U2	C1	S1, S2	T1	I1
Minimization of pain, suffering, and distress	E1	U1, U2, U3	C1	S1, S2	T1	I1
Use of humane end points	E1	U2	C1	S1, S2	T1	I1
Use of anesthesia	E1	U2, U3	C1	S1, S2	T1	I1
Appropriate veterinary care	E1	U2	C1	S1, S2	T1	I1
Trained and competent staff	E1	U1, U2, U3, U4	C1	S1, S2	T1	I1
Ethical review committee or animal care and use committee, with advice on the 3 Rs	E1	U2	To be established at national and provincial levels	S1, S2	T1	I1
Project evaluation before authorization, including compliance with the 3 Rs	E1	U2, U3, U6	To be established	S1, S2	T1	I1
Retrospective review	E1	–	–	–	–	–
Harm-benefit assessment as part of the project evaluation	E1	U2	IACUC to review justification for and objectives of animal use	S1	T1	I1
National committee, with advice on the 3 Rs	E1	–	CALAS Laboratory Animal Welfare and Ethics Committee	National Advisory Committee for Laboratory Animal Research	The National Research Council of Thailand	Committee for the Purpose of the Control and Supervision of Experiments on Animals
Minimum enclosure sizes/space allocations (i.e., national animal welfare standard)	E1, E2, E3	U2, U3	C2	S2	T1: The type of animal cage depends upon the standard required for the species, weight, and number of animals in the cage	I1

Provision of environmental enrichment	E1, E2, E3	U2, U3	C1: The special husbandry demand of animals in certain pathogenic and physiological conditions, including welfare requirements, shall be satisfied and be subject to IACUC review and where possible, appropriate items or devices shall be provided to laboratory animals based on their species and intended uses	S2	T1	–

E1: European Community (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September on the Protection of Animals Used for Scientific Purposes, OJ L276/33. Brussels: European Commission. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

E2: Council of Europe (2005). European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986 - Text amended according to the provisions of the Protocol (ETS No. 170) as of its entry into force on 2 December 2005. Strasbourg: Council of Europe. http://conventions.coe.int/Treaty/en/Treaties/Html/123.htm#ANX.

E3: Council of Europe (2006). Appendix A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). Guidelines for Accommodation and Care of Animals (Article 5 of the Convention). Approved by the Multilateral Consultation. Cons 123 (2006) 3. Strasbourg: Council of Europe. http://conventions.coe.int/Treaty/EN/Treaties/PDF/123-Arev.pdf.

U1: Interagency Research Advisory Committee (1985). U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples.

U2: National Research Council (2011). Guide for the Care and Use of Laboratory Animals, Eighth Edition. Washington, D.C.: National Academies of Science Press. http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf.

U3: US Animal Welfare Act—7 US Code 2131-2157 (Agriculture) 9 CFR Parts 1, 2, and 3. http://www.gpo.gov/fdsys/pkg/CFR-2013-title9-vol1/xml/CFR-2013-title9-vol1-chapI-subchapA.xml.

U4: National Institutes of Health (1985). Health Research Extension Act of 1985, Public Law 99-158, November 20, 1985, Animals in Research. Bethesda, MD: National Institutes of Health. http://history.nih.gov/research/downloads/PL99-158.pdf.

U6: National Institutes of Health (2002). Public Health Service Policy on Humane Care and Use of Laboratory Animals. Bethesda, MD: National Institutes of Health. http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf.

C1: National Standard of the People's Republic of China, Laboratory Animal Facilities—General Requirements for Quality and Competence, Draft Standard for Approval, 12th April, 2013, Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and Standardization Administration of the People's Republic of China, 2013.

C2: National Technical Committee on Laboratory Animal of Standardization Administration of China (2010). National Standard of the People's Republic of China, Laboratory animal—Requirements of Environmental and Housing Facilities.

S1: Ministry for National Development (2004). Animals and Birds Act (Chapter 7) Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004. http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/12801/Attach24_legislation_AB_CareandUse_rules.pdf.

S2: National Advisory Committee for Laboratory Animal Research (2004). Guidelines on the Care and Use of Animals for Scientific Purposes. http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/13557/Attach3_AnimalsforScientificPurposes.PDF.

T1: National Research Council of Thailand (2006). Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes. https://www.aaalac.org/resources/Ethical_Principles_and_Guidelines_for_the_Use_of_Animals_for_Scientific_purposes.pdf.

I1: Ministry of Environment and Forestry, India (2010). Standard Operation Procedure (SOP) for Institutional Animal Ethics Committee (IAEC), Guidelines on the Regulation of Scientific Experiments on Animals, Guidelines for Laboratory Animal Facility and Breeding of and Experiments on Animals (Control and Supervision) Rules, Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). https://www.aaalac.org/resources/SOP_CPCSEA.pdf.

VetBooks.ir

TABLE 14.2 Legal Provisions on the Use of Nonhuman Primates[a]

Provision	Europe	USA	China	Singapore	Thailand	India
Requires justification of nonhuman primates over other vertebrate species	E1	U2: Guide requires justification of any species proposed for use	C1: Animals higher in the phylogenetic scale shall be replaced with lower ones C3: Nonhuman primates can only be used for experiments where they are indispensable	–	–	I1
Restrictions on the purpose of use	E1: Limited to basic research, preservation of the nonhuman primate species, or translational/applied research for the avoidance, prevention, diagnosis or treatment of disease, ill health, or other abnormality of their effects in human beings	–	–	–	–	–
Ban on the use of great apes	E1: Effective ban on great ape use, save exceptional circumstances approved by the European Commission (safeguard clause)	National Institutions of Health has ceased funding most types of studies. The CHIMP Act provides funds a sanctuary system for retired research chimpanzees	–	–	–	–
Species/genera specific provisions	E1	U2, U3	C3: Playground and access to it should be established for breeding stock and enrichment items appropriate for the species should be placed in the playground	S2	–	I1: Nonhuman primates should have a run for free ranging activities

VetBooks.ir

TABLE 14.2 Legal Provisions on the Use of Nonhuman Primates[a]—Cont'd

Provision	Europe	USA	China	Singapore	Thailand	India
Social housing	E1	U2, U3	C1: In general, where possible, social husbandry of animals shall be provided	S2	–	I1
Environmental enrichment	E1	U2, U3	C3: Playground and access to it should be established for breeding stock, and enrichment items appropriate for the species should be placed in the playground	–	–	I1: Provision should be made for animals with specialized locomotor patterns, especially when held for long periods, e.g., artificial trees, ropes, bars and perches for nonhuman primates
Inspection by competent authorities	E1: At least once per year for breeders, suppliers and users of nonhuman primates Drug agencies for Good Laboratory Practices	USDA (annual) AAALAC (triennial) FDA (for GLP studies) OLAW IACUC (local oversight)	Semiannual by the IACUC	S1, S2 (all species/ facilities): Annually by IACUC and as required by AVA	–	–
Retrospective assessment	E1: All projects involving nonhuman primates must undergo a retrospective assessment of the outcomes, harms, and implementation of the 3 Rs	–	–	S2: Retrospective reporting of all adverse events for all species	–	–
Individual marking and history file	E1: Individual marking and history file, including social information	USDA U2	C1: Methods used shall be subject to IACUC review	S1, S2	–	I1: Required for all species

(*Continued*)

TABLE 14.2 Legal Provisions on the Use of Nonhuman Primates[a]—Cont'd

Provision	Europe	USA	China	Singapore	Thailand	India
Breeding strategy/ supply	E1: Requirement to move to use of nonhuman primates of F2 or greater generation or else sourced from self-sustaining colonies; subject to feasibility study	National Institutes of Health	In general experimental animals shall be procured from qualified producers and have known sources and acceptable quality	–	–	–
Thematic reviews	E1: Thematic reviews by the European Commission of the 3 Rs and nonhuman primate use especially	–	–	–	–	–

E1: European Community (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September on the Protection of Animals Used for Scientific Purposes, OJ L276/33. Brussels: European Commission. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

U2: National Research Council (2011). Guide for the Care and Use of Laboratory Animals, Eighth Edition. Washington, D.C.: National Academies of Science Press. http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf.

U3: US Animal Welfare Act—7 US Code 2131-2157 (Agriculture) 9 CFR Parts 1, 2, and 3. http://www.gpo.gov/fdsys/pkg/CFR-2013-title9-vol1/xml/CFR-2013-title9-vol1-chapI-subchapA.xml.

C1: National Standard of the People's Republic of China, Laboratory Animal Facilities—General Requirements for Quality and Competence, Draft Standard for Approval, 12th April, 2013, Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and Standardization Administration of the People's Republic of China, 2013.

C3: Ministry of Science and Technology (2006). Instructive Guideline on Humane Care of Laboratory Animals.

S1: Ministry for National Development (2004). Animals and Birds Act (Chapter 7) Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004. http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/12801/Attach24_legislation_AB_CareandUse_rules.pdf.

S2: National Advisory Committee for Laboratory Animal Research (2004). Guidelines on the Care and Use of Animals for Scientific Purposes. http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/13557/Attach3_AnimalsforScientificPurposes.PDF.

I1: Ministry of Environment and Forestry, India (2010). Standard Operation Procedure (SOP) for Institutional Animal Ethics Committee (IAEC), Guidelines on the Regulation of Scientific Experiments on Animals, Guidelines for Laboratory Animal Facility and Breeding of and Experiments on Animals (Control and Supervision) Rules, Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). https://www.aalac.org/resources/SOP_CPCSEA.pdf.

[a]This list is not intended to be exhaustive.

clear recommendations on study design, in particular in relation to the number of animals to be used. This flexible approach to study design within the guidelines allows for scientific and evidence-based decisions depending on the specific drug candidate in development. Table 14.3 shows how and where the regulatory guidelines refer to numbers of animals in study design.

The ICH guidelines describe a stepwise process for evaluating both animal and human efficacy and safety information as a drug progresses through development. With respect to nonclinical safety study objectives, it is stated that these studies should characterize toxic effects, identify target organs, and assess the dose-toxicity relationship. Furthermore, when appropriate, the potential reversibility of adverse effects should be demonstrated.

TABLE 14.3 Regulatory Guidelines and Group Sizes for Repeat Dose Regulatory Toxicology Studies

Relevant guideline	Typical group size for nonrodent studies	Notes
ICH M3 (R2)	Main study: 3 males+3 females Recovery: 2 males+2 females	See footnote to Table 3 within the ICH M3 (R2) guideline relating to extended single dose studies
ICH S6 (R1)	No numbers given	Indicates that small group sizes may in part be mitigated by increasing the frequency and duration of monitoring of individual animals
CHMP (previously CPMP) note for guidance on repeated-dose toxicity	No numbers given	Size of treatment groups should be sufficient to allow for meaningful scientific justification of the data generated
OECD 409	At least four per sex per group	Studies of 90 days' duration for chemicals

Drug-specific and scientifically driven study design is critical to ensure that all human dose and safety predictions are accurate. There are risks associated with routinely following regulatory guidelines without a tailored case-by-case study design because specific questions related to the individual drug may not be answered and safety concerns may be missed. This is particularly important when considering the nonhuman primate as a nonclinical species; although they may be perceived as the gold standard because of their closer phylogenetic relationship to humans, this may not always be a correct assumption. Thus appropriate scientific and ethical justification is needed for the choice of species as well as the number of animals used to generate safety data, and opportunities to minimize the use of animals without affecting study outcome should be explored [10–12].

A typical design, including the number of animals per group and the number of dose groups, of a nonhuman primate toxicology study is shown in Table 14.4. Individual companies may implement alternatives to this template design during the course of drug development, all of which are acceptable within the current regulatory guidance. Some of this variation is related to unique aspects of the individual drug target, whereas some is due to company practices. Where the variation in study design is associated with company practice rather than a case-by-case approach, there are opportunities to minimize the number of nonhuman primates in the nonclinical program, which yield scientific, business, and ethical benefits. There is substantial evidence in the literature to suggest that in many cases the design of nonhuman primate nonclinical safety studies is driven largely by company practice and internal standard (or default) study designs, rather than an assessment of the scientific requirements for each molecule [13, 15–16]. For example, according to an industry-wide survey on the design of chronic toxicity studies of 54 monoclonal antibodies using nonhuman primates, many instances of variation in the numbers of animals used despite similar study objectives were reported [13]. Furthermore, there was evidence to suggest that findings from short-term toxicity studies were not leveraged to inform the design of subsequent chronic long-term studies in terms of the number of dose groups needed, the number of animals

TABLE 14.4 Alternative Study Designs for Repeat Dose Regulatory Toxicology Studies

Dose group	Low	Medium	High	Control
FREQUENTLY USED STUDY DESIGN				
Animals in main study group (n)	3M+3F	3M+3F	3M+3F	3M+3F
Recovery animals (n)	2M+2F	2M+2F	2M+2F	2M+2F
			Total per study	40
ALTERNATIVE STUDY DESIGN 1				
Animals in main study group (n)	3M+3F		3M+3F	3M+3F
Recovery animals (n)			2M+2F	2M+2F
			Total per study	26
ALTERNATIVE STUDY DESIGN 2				
Animals in main study group (n)			5M+5F	3M+3F
Recovery animals (n)			2M+2F	2M+2F
			Total per study	24

Chapman et al. [13] and Baldrick [14].
M, male; F, female.

per group, or the inclusion of recovery animals. This is perhaps related in part to the limited available (regulatory) guidance on study design. However, general risk averseness within the pharmaceutical industry is also likely to play an important role.

Opportunities to Reduce Nonhuman Primate Use in Individual Studies

A number of nonclinical safety studies are required over the course of drug development. For each of these studies, a range of scientific questions and end points, as well as a priori knowledge about the compound/class, influences the numbers of animals used. For example, is the drug a novel entity or are there similar molecules in the class? What toxicity profile might be expected based on findings in previous studies (e.g., non-Good Laboratory Practice studies or those determining dose ranges)? Was the high dose used in short-term (subchronic) toxicology studies considered the no observed adverse effect level?

There are many opportunities to reduce the number of animals in a typical study design by considering both an individual study and how an individual study integrates into the whole drug development program. When initially focusing on the design of an individual study, the key factors that should be considered are the number of animals in a group, the number of dose groups, and the number of recovery animals. The group size for nonhuman primate studies generally varies from 6 (3 males plus 3 females) to 10 (5 males plus 5 females). The goal of general toxicity studies is hazard identification, and studies are not routinely

VetBooks.ir

designed to be statistically powered. One opportunity to minimize the number of nonhuman primates is to use a group size of six unless there is specific scientific justification to increase the number. On some occasions, group size for chronic studies tends to be larger than that in short-term studies [13]. However, this may be because of a default decision based on standard company practice rather than case-specific scientific rationale. Individual group size should also be balanced with consideration of the number of dose groups. For example, if only the control and one dose group is used, increasing the number of animals in the one dose group to 10 (5 males and 5 females) may be necessary; however, in such a study the total number of animals would be fewer. There are some situations where statistical power may be required, for example, in reproductive toxicology studies. Calculations to determine group sizes that yield group sizes of six to eight infants are available in Chapter 9 and have been provided by Jarvis et al. [17] and Cappon et al. [18].

Typically, a control and three dose levels are included in a general toxicology study to determine a dose response. In some instances there may be opportunities to reduce this to one or two dose levels, for example, if the drug is a monoclonal antibody and pharmacokinetic/pharmacodynamic information is available to optimize dose selection. From a scientific perspective, if 100% saturation of the receptor has been demonstrated at the lowest dose, including an additional two doses above this may be unnecessary. In reproductive toxicology studies, the opportunity to use fewer dose groups is high because there is already information about dose response as well as the toxic effects of the drug candidate from prior nonclinical and clinical studies.

The inclusion of recovery animals can also be adapted in study design; for instance, there are opportunities to include only assessment of recovery in the control and one dose (low or high, depending on the drug candidate). Table 14.4 show examples of minimized study designs by using fewer animals in a group, fewer doses, and fewer recovery animals. These study designs are not always appropriate, but they illustrate the flexibility and incentive to carefully consider the study designs used for individual drugs on a case-by-case basis. For assessment of reproductive toxicity in nonhuman primates, an enhanced pre- and postnatal development study design can be used in place of traditional independent segment II and segment III study designs to minimize animal use while still capturing critical measurements and end points [19]. There are potential opportunities to further reduce the number of nonhuman primates in reproductive toxicology studies by reusing maternal animals treated with vehicle control and by reviewing the timing of the studies so that they are carried out later in development (phase III) on fewer compounds overall. In addition, on occasion there is the potential to reuse nonhuman primates, particularly for small molecules, if no terminal end points are required and no adverse toxicology is observed.

Inclusion of multiple end points and combining studies presents a topical and interesting approach to minimizing animal use, particularly nonhuman primates. This has been successfully implemented in the now widely used enhanced pre- and postnatal development study design but can also be expanded to include safety pharmacology or fertility end points and/or robust pharmacokinetic/pharmacodynamic measurements in general toxicology studies [20,21]. One example of this is the use of telemetry devices in short-term toxicity studies to evaluate cardiovascular end points, particularly for biologics. In addition, sexually mature nonhuman primates may be included in chronic toxicology studies for evaluation of reproductive toxicity and fertility-related end points to minimize the need for stand-alone studies.

VetBooks.ir

Opportunities to Reduce Nonhuman Primate Use Across the Whole Drug Development Program

To optimize both the 3 Rs and overall resources during the drug development process, considering how the overall toxicology program can be streamlined—from initial studies supporting first-in-human clinical trials through those required for marketing authorization—is important. Individual toxicity studies should be designed to enable the specific needs of the development program at any given stage and should take advantage of any prior information available on the compound or drug class, rather than using a generic or "default" approach.

Certain oncology indications have different regulatory requirements for safety characterization than, for example, a drug for rheumatoid arthritis. This is defined within ICH S9, which provides opportunities to reduce animal use by limiting the scope of nonclinical safety studies required to support safety in specific patient populations based on risk/benefit assessment. Using a single 12-week study in nonhuman primates to support the entire clinical development program for certain oncology indications may be possible. Similarly, whether a reproductive toxicology study is necessary is also highly dependent on the indication of the drug candidate. The considerations for waiving a reproductive toxicology study or using a species other than the nonhuman primate include if the primary drug indication is for a geriatric population or a life-threatening disease and/or if historical information about the target already exists [22,23].

Additional important considerations to streamline drug development include maximizing the use of information available from studies that have been carried out earlier in development and continued review of whether assessment of certain end points is necessary and when assessment should be carried out. Much information can be gathered from the first pivotal short-term toxicity study to support first-in-human clinical trials. In addition to the importance of these studies in informing early clinical trials, they have critical importance in informing subsequent nonclinical chronic toxicology studies. The findings gathered from short-term (subchronic) studies may provide further opportunities to reduce the number of doses in the chronic toxicology study, such as in circumstances where no toxicity has been observed and/or the no observed adverse effect level is the highest dose tested in the short-term study. In addition, in the case of biologics that are potent in multiple species, ICH S6 indicates that if the toxicological findings in short-term studies of rodents and nonrodents are similar or have been characterized based on the mechanism of action, then longer-term general toxicity studies in one species are considered sufficient—this presents a clear opportunity for reducing the use of nonhuman primates.

ICH M3 indicates that evaluation of recovery from toxicity is necessary in at least one study during the development program. However, it is not necessary to assess recovery in all studies. The study in which recovery animals are included can vary, and this can impact animal use. If recovery has been evaluated in the short-term toxicity study, scientific justification should be provided for inclusion of recovery on the long-term study. One example of this may be if more detail on the nature of recovery from an observed toxic effect is needed. In this case, however, limiting assessment of recovery to only the highest dose and the control in the long-term study may be appropriate. There are different approaches to the inclusion of recovery animals in a development program to reduce animal use. For instance, assessment of recovery during the initial short-term study may enable determination of whether a

VetBooks.ir

candidate drug should be continued/discontinued in development, reducing further animal use for a compound that will be dropped later in development. However, this may lead to the inclusion of recovery animals in more studies overall, as more short-term studies of a greater number of compounds are carried out. Alternatively, recovery groups may be excluded from the short-term study in favor of inclusion on the longer-term study once specific drug-related toxicities have been defined and a need for recovery assessment has been identified.

The number of studies carried out over the course of drug development may vary from one (e.g., 12 weeks for an oncology indication for a biological) to three (e.g., 4 weeks, 12 weeks, 6/9 months). However, for biologics (not for small molecules) repeat-dose toxicity can be evaluated with one short-term (2 weeks' to 2 months' duration) and one chronic study (6 months) to support market authorization [24]. Although additional repeat-dose toxicity studies may be required for small molecules (e.g., 1, 3, and 9 months), the entire development program should be considered at each stage to maximize information obtained from individual studies and minimize the total number of studies needed.

REFINEMENT OF HUSBANDRY AND PROCEDURES TO IMPROVE ANIMAL WELFARE AND QUALITY OF SCIENCE

Key aspects of the refinement of nonhuman primate use include spacious and complex accommodation, effective environmental enrichment, housing in stable social groups, well-trained staff, careful study design, use of best humane technical practices, and training the animals with positive reinforcement techniques. All of these important elements are linked and ultimately help to support good animal welfare and high-quality science (e.g., see the three-pillar housing concept presented by Müller [25]). For more extensive reviews of opportunities to refine nonhuman primate use and care see Refs. [2,7,26–29].

Housing Design and Environmental Enrichment

Accommodation for nonhuman primates in the laboratory should be designed to meet the biological and behavioral needs of the species and give them a degree of choice and control over their environment [30]; it has a primary impact on the welfare of the research animals [31]. General requirements are specified in Appendix A of the European Convention ETS 123 [4] and the ILAR Guide for the Care and Use of Laboratory Animals [5]. There should be sufficient space in which to provide a complex and enriched environment, so that the animals can exhibit a wide range of natural, species-specific behaviors (e.g., resting, running, climbing, leaping, foraging, grooming and other social interactions). Full use should be made of the enclosure volume (i.e., the three-dimensional space) by incorporating fixed and moveable substrates (e.g., shelves, ladders, hammocks, swings, ropes). Enclosure height is important; nonhuman primates flee upward when alarmed and prefer to rest on elevated perches; ideally enclosures should be floor-to-ceiling high, allowing the animals to perch above human eye level. Elevated verandas/balconies on the front of the enclosure are particularly attractive resting places and improve the animals' visibility of the holding room and other pens, which can help habituate them to human activity. To meet these requirements many research organizations house

VetBooks.ir

their nonhuman primates in customized rooms rather than standard, freestanding metal cages, which has resulted in less aggressive, more cooperative animals [6]. Even free-standing conventional caging can be converted into linked gang-housing by removing the partitions between adjacent and tiered units [32]. Figure 14.1 shows a flexible approach with pens for three to five macaques each and the flexibility to combine them to accommodate larger groups.

A good amount and complexity of space is also required to enable social housing of nonhuman primates, which is crucial for their psychological well-being [33]. Adequate perching at different heights, visual barriers, and multiple escape routes help hierarchical species such as macaques to manage their social interactions; subordinate animals can more easily modify their behavior to avoid conflict with dominant animals. Solid floors are preferable to grid floors because they allow foraging for fine food items scattered in suitable litter such as wood shavings; this occupies the animals for extended periods and helps promote calm behavior. Enrichment items, such as toys that the animals can manipulate, mirrors, foraging devices (e.g., puzzle feeders), and swimming pools should be provided and rotated to maintain novelty as part of a documented environmental enrichment program. No evidence of an effect of enrichment on within- or between-experiment variation has been documented thus far [34]. Therefore these elements of good nonhuman primate housing and care in the laboratory do not conflict with scientific aims and are commonly provided in regulatory toxicology and other studies by contract research organizations in Europe and increasingly elsewhere [7,35–37].

Housing design also needs to consider the operational needs of the laboratory facility and research projects. These include the ease of capturing the animals for dosing and sampling, ease of visual inspection for monitoring clinical signs and welfare, and safeguarding the health and safety of the staff. Flexible enclosure designs with moveable panels allow the pen

FIGURE 14.1 Gang housing system for macaques, showing pop holes to connect adjacent enclosures, elevated verandas, and widely-spaced horizontal bars on the enclosure fronts—the latter give better visibility down the room than do vertical bars, facilitate human-animal interaction and can be used for climbing.

VetBooks.ir

space to be divided to temporarily separate individuals or groups of animals (e.g., for capture or cleaning) or to combine adjacent enclosures to allow additional space or to house larger groups. Innovative enclosure design can help to facilitate training of the animals and to refine procedures; for example, areas within the enclosure that allow temporary individual separation and collection of urine or feces avoid the need for extra metabolism cages and hence reduce the stress induced by cage change and removal from social companions (Figures 14.2 and 14.3).

FIGURE 14.2 Multifunctional box at the rear of the enclosure which provides: (a) a high resting place; and (b) a means to temporarily separate individuals for short periods for procedures such as urine collection whilst allowing interaction with the social group.

FIGURE 14.3 Macaque temporarily housed in the multifunctional box for urine collection.

VetBooks.ir

Managing Social Groups

Attending to the social environment of captive primates is fundamental to their welfare; it helps to decrease the incidence of behavioral abnormalities, expands the behavioral repertoire, provides stimulation, and reduces the impact of stress [38,39]. Allowing cage mates to continuously and fully interact with each other is more beneficial than providing temporary or partial social contact [40]. In the wild cynomolgus and rhesus macaques live in multimale, multifemale groups with strong hierarchical social structures. While it is rarely possible to replicate these same structures in a laboratory environment, macaques used in pharmaceutical studies can be successfully housed in pairs or groups [37,41]. Careful selection of companions and ongoing monitoring and management is necessary to ensure stable and harmonious pairings/groupings and to minimize any aggression [42–44]. Staff should have a basic knowledge of the ecology and behavior of the relevant species. Housing and enrichment should be designed to minimize the likelihood and impact of aggressive encounters; for example, care should be taken so that dominant animals cannot monopolize resources such as feeders, water spouts, and enrichment items; multiple resources per cage will help limit competition and potential conflict.

Stock groups of 10-20 small macaques (2-3 kg) can be housed together. The size of study animal groups is usually determined by the study design but should generally be as large as the design permits so that a normal social hierarchy can be established. Although a pair is not a natural social configuration for macaques, housing mature males in pairs may be preferable so that the dominant/submissive roles can be established easily, reducing the likelihood of aggression and injury. Removing or adding animals to an established group often results in reestablishment of the hierarchy and should therefore be avoided as much as possible. If a new social group is to be established or a new animal is to be included in an established group, moving all of the animals into a novel enclosure simultaneously may be preferable so that the newcomer is less disadvantaged and can more easily find their own position within the social structure.

Successful establishment and maintenance of a group with a stable hierarchy is greatly facilitated by using knowledge from the breeder/supplier about the social history, behavior, and health of individual animals, previous and current caging, as well as socialization methods. Breeding centers generally hold young adults in nonfamilial, same-sex groups. Consideration should be given to selecting experimental groups from within the same holding cage so that the animals are familiar with each other before reaching the laboratory environment. If the information available from the breeder does not provide sufficient information to assign cage mates, the likely compatibility of prospective social partners can be assessed by first observing their behavior in a large group and recording incidents of affiliative and hierarchical behaviors between the animals (snapshot ethograms) [25]. These data can then be used as the basis for assigning cage mates for smaller enclosures. Comingling in the enclosures should occur under the direct observation of caretakers. The animals should be allowed adequate time to get used to the enclosure and their cage mates before the start of a study. The duration of this acclimatization period may depend on the study type; changes in caging or cage mates can influence reproductive parameters such as menstrual cyclicity for several months [45].

Some laboratories house males and females (particularly sexually mature animals) in separate rooms to limit visual, auditory, and olfactory contact between the sexes and reduce

VetBooks.ir

competition within same-sex groups. Another factor to consider in the establishment of stable hierarchies is position within the room, with particular regard to foreknowledge of impending procedures or interruption by staff. Provision of balconies, "windows," or glass doors to enable animals within all cages to have good visibility of the room and corridor and an opportunity to vocalize to inform the group of human activity may facilitate equality between the cages.

Study Design and Scientific Procedures

Refining the study design and scientific procedures is a process based on continuous reassessment of routine practice. The ultimate goal is an experiment in which the animal feels, does, and expects exactly what would occur without the experimental intervention having been imposed. Throughout the monkey's life and experience, the highest standards of handling, procedural techniques, and empathy must be used. With such a culture of care, all experimental interventions will become more tolerable to the animals. Furthermore, physiological biomarkers return to a common baseline in unstressed animals, making experiments more sensitive and reproducible [46].

Single housing of nonhuman primates can have a major negative effect on their welfare and should be avoided whenever possible. Separation not only causes stress for the affected animal but for the cage mates as well. Therefore, the need for any data requiring single housing should be critically reassessed. For example, individual food consumption data are not routinely needed in toxicity testing; for biologics (monoclonal antibodies), urinalysis is a largely irrelevant end point and has been omitted from a number of studies without comment from European regulators. When total collection of urine from individual animals is required and the normal housing does not permit this, metabolism cages may be required. The duration of time the animals spend in the metabolism cages should be the minimum possible, and the cages should be designed to minimize negative impacts on animal welfare [7]. Whenever there is a strong scientific need to separate animals from their cage mates, they should be given visual and auditory access to them so they can continue to interact socially; there should never be a need for social isolation. The positioning of freestanding cages must be mindful of dominance hierarchies in the home colony.

Instrumentation (pumps for port catheter systems, telemetry devices, etc.) are often carried in jackets. The use of such devices is often associated with single housing because of concerns about cage mates interfering with the instrumentation, causing loss of data, repeat surgery, and so on. Tailoring each monkey's jacket to its own body shape greatly improves comfort and reduces interference from cage mates, enabling significant durations of social housing. The Joint Working Group on Refinement has published guidance on the refinement of telemetry procedures [47] and husbandry [48].

Social housing enhances the accurate assessment of individual well-being because socially housed animals show behaviors that animals housed singly do not. Any deterioration in well-being shows a marked contrast against both the behaviors of cage mates and the previous behaviors of the affected animals. Furthermore, the possibility of detecting changes in locomotion, balance, coordination, and peer interaction mean that socially housed animals allow a range of end points that are not detectable in depressed individuals housed singly.

VetBooks.ir

The duration and intensity of procedures, such as wearing jackets and chair/tube restraint, should be built up stepwise over time as part of a documented training program. Most important, the procedures should be conducted in a calm, empathetic way with gentle handling. Animals showing appropriate, desired behavior should be rewarded with kind words and food treats.

Staff-Animal Interactions

The quality of interaction between staff and the nonhuman primates they work with can have a major impact on both animal welfare and science. All staff interacting with the nonhuman primates should be trained to ensure that they can carry out their work competently, safely, and with minimal stress to the animals. Competence and opportunities for continuing professional development should be assessed regularly. An important part of this is training to understand and interpret the monkeys' gestures, facial expressions, and vocalizations and to respond appropriately when performing routine husbandry and procedures; in the case of common marmosets, see www.marmosetcare.com. Even simple modifications to behavior can benefit welfare, such as knocking on the door or ringing a bell before entering a room to avoid startling the animals and increase the predictability (signaling) of potentially adverse events, allowing the animals to relax at other times [49,50].

Using positive reinforcement techniques to desensitize and habituate animals to handling and procedures, and to gain their voluntary cooperation, is a major refinement goal [51]. Such techniques help to reduce stress and give the animals greater control over their lives, which is beneficial for welfare. For example, a study comparing trained versus untrained animals resulted in a statistically significant reduction in cortisol, lower heart rates, and normal stress leukograms for trained subjects [52]. It is important to be clear about the behavior modification technique being employed:

- Desensitization: Systematically pairing positive rewards directly with an uncomfortable or aversive experience or stimulus to reduce any associated fear or anxiety response.
- Habituation: The waning of a response as a result of repeated stimulation, but not fatigue. This kind of learning is of importance in familiarizing an animal with aspects of the environment to which it is not expected to react. Ignoring nonthreatening stimuli (e.g., the sound of clippers, wearing jackets, restraint in a chair) is of value in the training of nonhuman primates.
- Training: Shaping the behavior of an animal so that it actively responds in a way that is desired by the trainer (e.g., offers a limb for injection, stands on a weighing scale, waits and allows subordinate individuals to feed uninterrupted).

While developing equal animal training skills in all animal care personnel may not be feasible, all should have a basic working knowledge of the techniques involved to ensure consistency across staff and facilities. Establishing a formal institutional behavioral management program assists with this, improving the quality of care and outcome of research procedures [53]. The most effective program for nonhuman primates would begin at the facility of origin and continue through all other institutions in which the animals are housed. The desired training goals are more likely to be achieved if these are in line with the behavioral preferences of the monkeys; for instance, areas of the enclosure for capture of the animals should be situated in an elevated position.

VetBooks.ir

Institutions may encounter challenges during the implementation of an effective behavioral management program, such as start-up and maintenance costs, resource implications, achieving buy in, and securing the long-term commitment of key personnel. These can usually be addressed by collecting quantitative data to demonstrate to technical staff, toxicologists, and senior management the effectiveness and benefits of the program for animal welfare and science. A progressive program should be subject to continual review in light of empirical data, experimental design, and the behavior and welfare of the animals.

References

[1] Russell WMS, Burch RL. The principles of humane experimental technique. Potters Bar, England: Universities Federation for Animal Welfare; 1959.

[2] Bayne K, Morris TH. Laws, regulations and policies relating to the care and use of nonhuman primates in biomedical research. In: Abee CR, Mansfield K., Tariff SD, Morris T., editors. Nonhuman primates in biomedical research: biology and management, vol. 1. London: Elsevier; 2012. p. 35–57.

[3] Prescott M. Ethics of primate use. Adv Sci Res 2010;5:11–20.

[4] Council of Europe. Appendix A of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123). Guidelines for accommodation and care of animals (Article 5 of the convention). Approved by the multilateral consultation. Cons 123 (2006) 3. Strasbourg: Council of Europe; 2006.

[5] Council NR. Guide for the care and use of laboratory animals. 8th ed. Washington, DC: National Academies of Science Press; 2011.

[6] Wolfensohn SE. Brief review of scientific studies of the welfare implications of transporting primates. Lab Anim 1997;31:303–5.

[7] Jennings M, Prescott MJ. Refinements in husbandry, care and common procedures for nonhuman primates: ninth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Lab Anim 2009;43: S1:1–S1:47.

[8] Swallow J, Anderson D, Buckwell A, Harris T, Hawkins P, Kirkwood J, et al. Guidance on the transport of laboratory animals: report of the Transport Working Group established by the Laboratory Animal Science Association (LASA). Lab Anim 2005;39:1–39.

[9] Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. Nat Rev Drug Discov 2010;9(10):767–74.

[10] Chapman K, Pullen N, Graham M, Ragan I. Preclinical safety testing of monoclonal antibodies: the significance of species relevance. Nat Rev Drug Discov 2007;6:120–6.

[11] Chapman K, Pullen N, Coney L, Dempster M, Andrews L, Bajramovic J, et al. Preclinical development of monoclonal antibodies: considerations for the use of non-human primates. MAbs 2009;1(5):505–16.

[12] Buckley LA, Chapman K, Burns-Naas LA, Todd MD, Martin PL, Lansita JA. Considerations regarding nonhuman primate use in safety assessment of biopharmaceuticals. Int J Toxicol 2011;30(5):583–90.

[13] Chapman KL, Andrews L, Bajramovic JJ, Baldrick P, Black LE, Bowman CJ, et al. The design of chronic toxicology studies of monoclonal antibodies: implications for the reduction in use of non-human primates. Regul Toxicol Pharmacol 2012;62(2):347–54.

[14] Baldrick P. Safety evaluation of biological drugs: what are toxicology studies in primates telling us? Regul Toxicol Pharmacol 2011;59:227–36.

[15] Baldrick P. Safety evaluation to support first-in-man investigations II: toxicology studies. Regul Toxicol Pharmacol 2008;51(2):237–43.

[16] Sparrow SS, Robinson S, Bolam S, Bruce C, Danks A, Everett D, et al. Opportunities to minimise animal use in pharmaceutical regulatory general toxicology: a cross-company review. Regul Toxicol Pharmacol 2010;61(2):141–260.

[17] Jarvis P, Srivastav S, Vogelwedde E, Stewart J, Mitchard T, Weinbauer GF. The cynomolgus monkey as a model for developmental toxicity studies: variability of pregnancy losses, statistical power estimates, and group size considerations. Birth Defects Res B Dev Reprod Toxicol 2010;89(3):175–87.

[18] Cappon GD, Potter D, Hurtt ME, Weinbauer GF, Luetjens CM, Bowman CJ. Sensitivity of male reproductive endpoints in nonhuman primate toxicity studies: a statistical power analysis. Reprod Toxicol 2013;41:67–72.

VetBooks.ir

[19] Weinbauer GF, Luft J, Fuchs A. The enhanced pre- and postnatal development study for monoclonal antibodies. Methods Mol Biol 2013;947:185–200.
[20] Vargas HM, Amouzadeh HR, Engwall MJ. Nonclinical strategy considerations for safety pharmacology: evaluation of biopharmaceuticals. Expert Opin Drug Saf 2013;12(1):91–102.
[21] Redfern WS, Ewart LC, Lainée P, Pinches M, Robinson S, Valentin JP. Functional assessments in repeat-dose toxicity studies: the art of the possible. Toxicol Res 2013;2:209–34.
[22] Chapman K, Pullen N, Andrews L, Ragan I. The future of non-human primate use in mAb development. Drug Discov Today 2010;15(5–6):235–42.
[23] Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicology studies in nonhuman primates. Birth Defects Res B Dev Reprod Toxicol 2009;86(6):446–62.
[24] Clarke J, Hurst C, Martin P, Vahle J, Ponce R, Mounho B, et al. Duration of chronic toxicity studies for biotechnology-derived pharmaceuticals: is 6 months still appropriate? Regul Toxicol Pharmacol 2008;50(1).
[25] Müller W. The new European housing regulations for nonhuman primates: opportunities and challenges. In: Weinbauer GF, Vogel F, editors. Critical contributions of primate models for biopharmaceutical drug development. Münster: Waxmann Verlag; 2008. p. 53–73.
[26] Rennie AE, Buchanan-Smith HM. Refinement of the use of non-human primates in scientific research, part I: the influence of humans. Anim Welf 2006;15:203–13.
[27] Rennie AE, Buchanan-Smith HM. Refinement of the use of non-human primates in scientific research, part II: housing, husbandry and acquisition. Anim Welf 2006;15:215–38.
[28] Rennie AE, Buchanan-Smith HM. Refinement of the use of non-human primates in scientific research, part III: refinement of procedures. Anim Welf 2006;15:239–61.
[29] Wolfensohn SE, Honess P. Handbook of primate husbandry and welfare. Oxford: Blackwell Publishing Ltd.; 2005.
[30] Buchanan-Smith HM, Prescott MJ, Cross NJ. What factors should determine cage sizes for primates in the laboratory? Anim Welf 2004;13:S197–201.
[31] Bayne K, Turner P. Animal environments and their impact on laboratory animal welfare. In: Bayne K, Turner P, editors. Laboratory animal welfare. New York: Elsevier; 2013. p. 77–93.
[32] Frost S. Huntingdon Life Sciences: Old World primate cage replacement. Anim Technol Welf 2004;3:185–7.
[33] Council NR. The psychological well-being of nonhuman primates. Washington, DC: National Academy Press; 1998.
[34] Bayne K, Würbel H. Environmental enrichment, outcome variability and scientific validity of laboratory animal studies. In: Mellor DJ, Bayvel ACD, editors. Animal welfare: focusing on the future. OIE Sci Tech Rev 2014;33(1).
[35] Hartner M, Hall J, Penderghest J, Clark LP. Group-housing subadult male cynomolgus macaques in a pharmaceutical environment. Lab Anim 2001;30:53–7.
[36] Andrews-Kelly G. Primate enrichment and positive reinforcement training in toxicology: challenges and possibilities. Enrichment Rec 2011;9:16–8.
[37] Couch J, Taylor H, Chapman K. Social housing for nonhuman primates: a global perspective. In: Weinbauer GF, Vogel F, editors. Challenges in nonhuman primate research in the 21st century. Münster: Waxmann Verlag; 2012. p. 47–58.
[38] Visalberghi E, Anderson JR. Reasons and risks associated with manipulating captive primates' social environments. Anim Welf 1993;2:3–15.
[39] Koban TL, Schapiro SJ, Kusznir T, Lambeth SP, Ogbin J, Garofalo E. Effects of international transit and relocation on cortisol values in cynomolgus macaques (*Macaca fascicularis*). Am J Primatol 2010;72:51.
[40] Baker KC, Bloomsmith MA, Oettinger B, Neu K, Griffis C, Schoof VA. Comparing options for pair housing rhesus macaques using behavioral welfare measures. Am J Primatol 2014;76:30–42.
[41] Turner PV, Smiler KL, Hargaden M, Koch MA. Refinements in the care and use of animals in toxicology studies: regulation, validation, and progress. Contemp Top Lab Anim Sci 2003;42:8–15.
[42] Watts E, Meder A. Introduction and socialization techniques for primates. In: Carlstead K, Kleiman DG, Allen ME, Thompson KV, Lumpkin S, Harris H, editors. Wild mammals in captivity: principles and techniques. Chicago: University of Chicago Press; 1996. p. 67–77.
[43] Watson LM. A successful program for same- and cross-age pair-housing adult and subadult male *Macaca fascicularis*. Lab Primate Newsletter 2002;41:6.
[44] Carlson J. Safe pair housing of macaques. Washington, DC: Animal Welfare Institute; 2008.

VetBooks.ir

[45] Niehoff M, Bergmann M, Müller W, Weinbauer GF. Effects of group housing on reproductive parameters in the sexually mature cynomolgus monkey. In: Weinbauer GW, Buse E, Müller W, Vogel F, editors. New developments and challenges in primate toxicology. Münster: Waxmann Verlag; 2005. p. 137–43.

[46] Tasker L. Linking welfare and quality of scientific output in cynomolgus macaques (*Macaca fascicularis*) used for regulatory toxicology. [Ph.D. thesis]. University of Stirling; 2012. http://dspace.stir.ac.uk/handle/1893/9801#.VGh3p_msUXg.

[47] Morton DB, Hawkins P, Bevan R, Heath K, Kirkwood J, Pearce P, et al. Refinements in telemetry procedures: seventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement, part A. Lab Anim 2003;37:261–99.

[48] Hawkins P, Morton DB, Bevan R, Heath K, Kirkwood J, Pearce P, et al. Husbandry refinements for rats, mice, dogs and non-human primates used in telemetry: seventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement, part B. Lab Anim 2004;38:1–10.

[49] Bassett L, Buchanan-Smith HM. Effects of predictability on the welfare of captive animals. Appl Anim Behav Sci 2007;102:223–45.

[50] Gottlieb DH, Coleman K, McCowan B. The effects of predictability in daily husbandry routines on captive macaques (*Macaca mulatta*). Appl Anim Behav Sci 2012;143:117–27.

[51] Prescott MJ, Buchanan-Smith HM. Training nonhuman primates using positive reinforcement techniques: guest editor's introduction. J Appl Anim Welf Sci 2003;6:157–61.

[52] Koban TL, Miyamoto M, Donmoyer G, Hammar A. Effects of positive reinforcement training on cortisol, hematology and cardiovascular parameters in cynomolgus macaques (*Macaca fascicularis*). Am J Primatol 2005;66:148.

[53] Prescott MJ, Bowell VA, Buchanan-Smith HM. Training laboratory-housed nonhuman primates, part 2: resources for developing and implementing training programmes. Anim Technol Welf 2005;4:133–48.

VetBooks.ir

VetBooks.ir

CHAPTER

15

Training and Selection of (Mature) Nonhuman Primates for European Housing

Wolfgang Müller[*], *Susan Henwood*[†]

[*]Covance, Münster, Germany

[†]Covance, Madison, WI, USA

OUTLINE

Introduction 303

Training of (Mature) Monkeys for European Housing 304

Selection of (Mature) Monkeys for European Housing 307

References 312

INTRODUCTION

A training program to encourage cooperation and to reduce stress during procedures can be beneficial to animals, the staff caring for the animals, and the scientific program. Stress avoidance and housing conditions in compliance with species-specific biological needs—both to the greatest possible extent—are central requirements of the European guidelines on accommodation and care of laboratory animal species [1–3]. For nonhuman primates, with their complex behavioral repertoire, the training program, accommodations, and environmental enrichment, require particularly intense efforts—even more so for mature animals with distinct hierarchical behavior, which may be the reason for incompatibilities when cage mates are assigned.

While the previous chapter covers general nonhuman primate husbandry in social groups, environmental enrichment, study procedures, and staff-animal interactions, this chapter highlights training experiences and a systematic approach for the selection of (mature) monkeys

http://dx.doi.org/10.1016/B978-0-12-417144-2.00015-9
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

for European housing. The content of this chapter is generally applicable to many nonhuman primates species, but it mainly refers to macaque monkeys, particularly the cynomolgus monkey (*Macaca fascicularis*).

TRAINING OF (MATURE) MONKEYS FOR EUROPEAN HOUSING

Training of monkeys cannot be addressed in isolation and separate from training of the involved caretaker staff and general facility and particularly animal housing conditions. An integrated training program therefore needs to be based on a review of such underlying aspects and designed accordingly. Although there is much overlap between the approaches of different facilities, there are also facility-specific characteristics that indicate a thorough review with specific adaptations. Below are listed determining factors for the establishment of a training program.

Facility- and staff-related aspects include:

- Number of animals in a study/on site to be included in the program
- Duration of scientific programs, including time for preparation (i.e., the overall duration of stay)
- Housing and animal care conditions, including the size of social groups, design of cages, extent of manual handling, and the type of animal transportation routines
- Method, frequency, and duration of test substance administration, blood sampling events, and monitoring of physiological parameters
- Qualifications of caretaker staff, who need to understand the biology of the nonhuman primates and the animals' signals (body language, facial and vocal expressions) and need to know how to behave (firmly and calmly) when approaching an animal. For the training of staff, the Federation of European Laboratory Animal Science Associations [4] recommendations should be followed.

Animal-related aspects include:

- Biological characteristics of nonhuman primates that are not domesticated, tend to avoid proximity to humans, are more on alert, show strong reaction to unknown stimuli (and therefore, at least initially, perceive being caught by a caretaker as being caught by a predator), and have a vertical flight reaction and therefore perceive most elevated locations as safest.
- Individual history of the nonhuman primates, including degree of desensitization toward proximity to humans and being touched, held, and transported.

The training of nonhuman primates must aim for increasing self-confidence and cooperation because "training non-human primates to cooperate rather than resist during handling procedures offers a simple refinement to traditional, involuntary restraint techniques" [5, and cf. 6]. We apply positive reinforcement training (PRT) with our animals [7–9] as an essential tool to improve husbandry and veterinary care, to increase the safety of the attending personnel, and to desensitize animals to uncomfortable, frightening, or stressful events [10], for example, the caretaker coming very close and even entering the pen.

Such training needs time, so determining the content and milestones for any training early enough in any study is critical. In a toxicology laboratory, such study-related preparations

VetBooks.ir

can be very challenging because they are often time critical. Establishing a facility-specific system that brings forward the basic training elements and makes them part of the general acclimation and socialization period after animal arrival or—even better—part of the working routines at the breeding facilities is therefore favorable. In this context staff must be aware that every interaction with a caretaker or other involved person is an experience for the animal and that nonhuman primates cannot distinguish between training efforts and other interactions. This is why handling procedures when animals arrive at the facility must be consistent and in line with handling approaches used later during study performance.

Each facility needs general decisions and guidance for the staff on the extent of manual handling and restraining versus the use of physical restraint devices; this also needs to be considered to define the steps of desensitization and training. Particularly for pregnant animals, we prefer manual catching, manual handling, and manual transportation over short distances.

Depending on the animals' individual histories, they may be more or less prepared for targeted PRT. If animals are unused to being manually handled, initial efforts must focus on overcoming uncertainty and intimidation (particularly shortly after arrival), on acceptance of a caretaker coming very close (even violating the individual escape distance by entering a larger pen) and, in steps, accepting treats manually given directly into the mouth. Such desensitization and habituation are essential to prepare practical PRT [11]. Before starting PRT, the desired behaviors that the monkeys should show in reaction to a certain trigger must be defined. In a toxicology facility, such desired behaviors include:

- moving to a desired location in the cage when the caretaker opens the door;
- accepting being touched;
- accepting an arm or leg being held;
- accepting being held, removed from the cage, and transported; and
- accepting being held for and exposed to a procedure.

Beyond this, PRT rewards need to be specified; they should not interfere with any of the study parameters. We usually use tasty rewards such as fruit pellets, nuts, or raisins.

On the basis of the defined training goals, a training plan that considers the facility-, staff-, and animal- related aspects, as well as any specific study-related aspects. This plan needs to include a review of each animal with efforts spent on desensitization and habituation before starting the PRT and identification of which behaviors will be trained, in which order, and within what time frame, including how many sessions per day/per week, duration and frequency to establish success, and which training milestones must be achieved before starting a study. Trainers should know the hierarchy of animals socially housed in a pen and start any training initiative with the dominant animal first.

Training will lead to the desired results faster if the PRT reinforces behaviors that the animals prefer anyway since they are in line with their normal behaviors. This is why we train the animals to move to elevated enclosures at the back wall of the pens to be captured by a caretaker. In line with their normal flight behavior, such locations are perceived by the animals as safe. Figure 15.1 shows a sequence of behaviors of a full-grown male animal (almost 14 kg body weight) after several days of PRT with the aim of easing capture. Opening the sliding door at the front of the pen (possibly combined with knocking at the grid) is the signal for the animals to move to this location in the back. It usually requires a few days of PRT until the animals show the desired behavior (moving to the back of the cage) in reaction to the signal (opening the door).

VetBooks.ir

FIGURE 15.1 A full-grown male cynomolgus monkey has been trained by PRT to collaborate in the process of being captured by moving to an elevated enclosure in the back of the pen (top) when the caretaker opens the door at the cage front and accepting the enclosure being closed (bottom left) and accepting being manually removed (bottom middle). A tasty reward is then placed directly into the animal's mouth (bottom right). During such a sequence of behaviors, particularly high-ranking animals tend to demonstrate their competitiveness by displaying their canines (top middle).

If an animal is trained to move into a lockable enclosure within the pen, this not only makes capturing safer for the staff but also it is useful for temporarily separating animals, for example, during spot cleaning of the pen or for urine or fecal sampling. Such animal behaviors can also be achieved by clicker training, perhaps in conjunction with the use of a laser pointer if a particular route should be followed by the animal.

Another helpful element of our approach is the distinction of roles between the staff involved in a study. The staff who mainly focus on animal husbandry tasks, such as feeding,

VetBooks.ir

cleaning, and handling, as well as providing most of the rewards, play the role of the "good guys" for the nonhuman primates. They work as motionless as possible so that they know their animals well and the animals know them well. These "good guys" should try to avoid performing application or diagnostic procedures, which may not always be pleasant for the animals; such tasks should be done by the "bad guys" (technicians), who are less familiar with individual animals. This principle of having caretaker staff in roles that nonhuman primates perceive as the "good guys" can positively and strongly influence training success (as in humans, who usually learn better from more favored teachers).

In bigger facilities with more staff, establishing a mechanism that ensures that the defined uniform, colony-wide handling and training routines are maintained over time, without drifting away from the original definitions, is crucial. Therefore, the training and all other staff interactions with the animals need to be constantly monitored and kept within limited variations.

A training concept that is tailored according to the facility conditions and study requirements substantially contributes to refinement, as first defined by Russell and Burch [12] in the concept of the 3 Rs (replacement, reduction, and refinement). It clearly strengthens the self-confidence of and cooperation by the monkeys, thus reducing the animals' stress. This is also in the interest of the caretakers because it makes handling the nonhuman primates easier and safer.

SELECTION OF (MATURE) MONKEYS FOR EUROPEAN HOUSING

The European guidelines [1,2] allow single housing of nonhuman primates under only exceptional circumstances and explicitly require that the normal social organization of the species should be taken into account when groups are created. Therefore, having a systematic approach for selecting compatible cage mates is a crucial element for successful social housing of nonhuman primates. Ideally, such a system can be based on existing data and requires only limited additional data collection. In addition to the usual data on the individual animal's history (e.g., age and weight), we ask breeders to provide information about special observations, incompatibilities, or preferences and as much information as possible about former social partners (cage mates and roommates) and housing conditions in a small or larger group.

If one (or more) former cage mate(s) is available from the same shipment of animals, these animals could more easily commingle. Also, the animals' health records may play an important role in preparing cage mate assignments. If, for example, an animal needed veterinary care for injuries resulting from hierarchical behaviors in a group, veterinary records may provide important information about an animal's social behavior in the former group; biting wounds to the face or on the front of the animal, as well as the upper extremities, usually reflect active fighting and competition for a higher rank. Wounds on the back or the tail usually result from escape or submissive behavior in situations when an animal of lower social rank tries to end or avoid such ranking competitions. To what extent the nonhuman primates are used to the proximity of humans—coming very close and violating their flight distance or possibly manually handling them—should also be explored. Such a data review can provide a useful and sometimes sufficient basis for assigning cage mates. Although in cynomolgus monkeys the best predictor of eventual social status is the animal's body weight immediately before the formation of the group [13], the assignment of cage mates for certain studies requires additional data, at least for some of the animals.

VetBooks.ir

More than 20 years ago we started routine social housing of (mature) macaques by combining the cages of neighboring monkeys that had shown affiliative behaviors. We observed that the best enrichment for a monkey is another monkey; a compatible cage mate—unlike inanimate toys—maintains its stimulatory effect over time, probably because of its inherent ever-changing nature [14]. Since then, we refined our approach to assess affiliative behaviors and also included the assessment of hierarchical behaviors to more representatively consider social ranking of the macaques when groups of two or more animals were created.

In 2005 we started a research project with the aim to refine our procedures for social ranking and cage mate assignment (as well as cage design features, which are not presented in this chapter). Specifics of the entire project have been published by Coenen de Roo [15].

Social ranking was examined with a group 26 captive-bred female cynomolgus monkeys 4 to 8 years old with a history of single or paired housing. These animals were brought together in a large pen (4×5×2.5 m), individually marked with dye from "A" to "Z," and observed for approximately 10 min every hour from 06:30 to 14:00 for 4 weeks to calculate a dominance matrix using the focal animal technique [16]. For these examinations, the observer stood outside the pen and recorded as many interactions between the group members as possible (frontal view scan sampling; no videotaping).

The behavioral observations focused on affiliative and ranking behavior. Affiliative (friendly or cooperative) behavior comprises playing, wrestling, grooming, close physical contact, possibly face to face, and hugging, as well as food sharing. Ranking (dominant or subordinate) behavior comprises avoidance (turning away), submissive, or agonistic behavior; nonphysical aggression (threatening) and chasing; as well as physical aggression (beating, fighting, and biting).

When evaluating hierarchical behavior we found that submissive behaviors were most reliable in establishing a dominance matrix. The group's hierarchical structure therefore was determined as the frequency of submissive behavior versus that of peers: The animal showing no submissive behaviors was considered the alpha animal; the one with only submissive behavior toward the alpha animal was considered number 2 in the hierarchy, and so forth, down to the lowest-ranking animal showing submissive behavior toward all other group members.

Six weeks later, the ranking assessment was repeated on one day only and led to the same results. This confirmed the stable social structure in the undisturbed group. Tis short focal sampling is a suitable tool with which and a reliable basis on which to assess the social ranking within a group of monkeys. This focal sampling or focal behavioral assessment can be described as a "one-time snapshot dominance matrix ethogram," which we often abbreviate as a "snapshot ethogram."

During the behavioral observations for the generation of a snapshot ethogram, the types and incidence of the behaviors are recorded. This data collection is step 1 in preparing cage mate assignments.

The lack of ranking behavior between two animals, for example, can be accepted, and a decision to assign two animals as cage mates is possible if these animals display affiliative behavior and show that they are good friends.

The results of the snapshot observation then are visualized using colors for the two behavioral categories, for example, red for ranking behavior and green for affiliative behavior. This visualization is step 2 in preparing cage mate assignments (Figure 15.2).

Sex	Tier Nr.	Geburt	13208	13228	13216	13225	13221	13212	13220	13230	13211	13222	13224	13219	13226	affiliativ
M	13208	11.09.99		–1				–1		–3			5	–2	1	13208
M	13228	02.07.01							1	2						13228
M	13216	04.12.99							1			–1				13216
M	13225	16.09.00					–1	1	1			1	2	3		13225
M	13221	06.02.00						1		–1						13221
M	13212	18.09.99				–2				–3	–1	–1	2	4		13212
M	13220	19.12.99		–1		–2				–4	–2	–2				13220
M	13230	31.05.02		–1				1					2	3		13230
M	13211	20.09.99	2	–2		–1	–1		1	–2		–1	1			13211
M	13222	14.03.00	3	–2		–3		2		–3			1	2		13222
M	13224	23.07.00		–1		–1				–1						13224
M	13219	11.12.99		–1		–5				–4	–1		3			13219
M	13226	14.10.00		–1				–1		–2		–1				13226
	sub/dom															

FIGURE 15.2 Snapshot dominance matrix ethogram originating from frontal view sampling of affiliative and ranking behaviors in a group of 13 male cynomolgus monkeys. During data collection, affiliative behaviors are marked with a "+" and ranking behaviors with a "–"; behaviors then are listed in a table and subsequently visualized by, for example, green and red.

The visualized data overview (Figure 15.2) is then rearranged according to the incidence of ranking behaviors and affiliative behaviors to ease the evaluation and the assignment of cage mates, for example, starting with the highest-ranking animal on the left. This data sorting is step 3 in preparing cage mate assignments (Figure 15.3).

In contrast to "full-scale" systematic ethograms, which often are generated with the help of video equipment to precisely describe, define, and classify the behavioral

Sex	Tier Nr.	Geburt	13228	13230	13221	13225	13222	13211	13212	13219	13208	13216	13224	13220	13226	affiliativ
M	13228	02.07.01		2										1		13228
M	13230	31.05.02	–1						1	3			2			13230
M	13221	06.02.00		–1					1							13221
M	13225	16.09.00			–1		1		1	3			2	1		13225
M	13222	14.03.00	–2	–3		–3			2	2	3		1			13222
M	13211	20.09.99	–2	–2	–1	–1	–1				2		1	1		13211
M	13212	18.09.99		–3		–2	–1	–1		4			2			13212
M	13219	11.12.99	–1	–4		–5		–1					3			13219
M	13208	11.09.99	–1	–3					–1	–2			5		1	13208
M	13216	04.12.99					–1							1		13216
M	13224	23.07.00	–1	–1		–1										13224
M	13220	19.12.99	–1	–4		–2	–2	–2								13220
M	13226	14.10.00	–1	–2			–1		–1							13226
	sub/dom															

FIGURE 15.3 Results of the snapshot dominance matrix ethogram originating from frontal view sampling, as visualized in Figure 15.2, and then rearranged according to position in the hierarchy of the group, with the highest-ranking animal (animal 13228) on the left. While animal 13230 has shown the highest incidence of dominant behaviors of the entire group, animal 13228 once displayed dominance over animal 13230, which had shown affiliative behaviors toward animal 13228 on two occasions. Hence not the highest ranking but the second ranking animal of the group had most hierarchical interaction with the other members.

Sex	Tier Nr.	Geburt	13228	13230	13221	13225	13222	13211	13212	13219	13208	13216	13224	13220	13226	affiliativ
M	13228	02.07.01		2										1		13228
M	13230	31.05.02	–1						1	3			2			13230
M	13221	06.02.00		–1					1							13221
M	13225	16.09.00			–1		1		1	3			2	1		13225
M	13222	14.03.00	–2	–3		–3			2	2	3		1			13222
M	13211	20.09.99	–2	–2	–1	–1	–1				2		1	1		13211
M	13212	18.09.99		–3		–2	–1	–1		4			2			13212
M	13219	11.12.99	–1	–4		–5		–1					3			13219
M	13208	11.09.99	–1	–3					–1	–2			5		1	13208
M	13216	04.12.99					–1							1		13216
M	13224	23.07.00	–1	–1		–1										13224
M	13220	19.12.99	–1	–4		–2	–2	–2								13220
M	13226	14.10.00	–1	–2			–1		–1							13226
	sub/dom															

FIGURE 15.4 Examples of the selection of compatible animals from a group of 13 nonhuman primates for, in this case, four cages with two animals in each and one cage with three animals.

interactions between the members of a social group [17], such snapshot ethograms are not intended to provide a complete and fully accurate overview of all hierarchical relationships between the group members; rather, they provide only a sufficient data base that allows decision making in the process of assigning cage mates. In the case of insufficient data regarding certain animals and their compatibility, another snapshot observation might be performed only for these animals until sufficient data are available. Examples of how cage mates for paired housing are selected from a group of 13 animals are presented in Figure 15.4.

Cage 1:Animals 13228 and 13230, with one clear ranking behavior and two affiliative contacts (the two highest-ranking animals that like each other and where 13230 accepts the other as dominant, as shown in Figure 15.3).
Cage 2:Animals 13225 and 13222, with one affiliative and three clear ranking behaviors (two higher-ranking animals that like each other and where 13225 is accepted as dominant).
Cage 3:Animals 13208 and 13224, with five affiliative contacts and no ranking behavior (two lower-ranking animals with no obvious hierarchical behaviors but a high preference for each other).
Cage 4:Animals 13212 and 13221, with one affiliative contact and no ranking behavior (both animals like each other and have not shown much ranking behavior during sampling and none against each other but are in the upper ranks).
Cage 5:Animals 13216, 13220, and 13226 showed one affiliative behavior and no ranking behavior (all three males are low in the hierarchy and as such are socially compatible, and at least two of them like each other).

For several years now we also have successfully applied social housing to pregnant animals. More recently we established social housing for male animals, which are used as breeders for nonhuman primate development and reproduction toxicology studies.

VetBooks.ir

It is commonly believed that adult cynomolgus males do not tolerate one another well and that research or management procedures in a biomedical research environment may render continuous full-contact pairing of macaques unfeasible; for example, refer to the article by Baker et al. [18], who examined housing conditions and behavioral benefits. However, successful social housing of adult male cynomolgus macaques is possible, but potential effects on body weight and reproductive parameters related to hierarchical position—even if transient—must be taken into account [19]. Successful pairing of such males can be achieved when using a familiarization period before full contact. Particularly for the handling of large, sexually mature, male nonhuman primates, this must be done by trained caretakers who understand the animals' behavioral signals.

Individual animal history and thorough behavioral assessment are important when attempting to establish compatible male pairings. Pair assignments are made using a three-phase approach. During phase 1, animals are assessed to determine their ranking on the dominant-subordinate spectrum. Behavioral assessments in singly housed males are performed by a trained animal behaviorist and based on initiation and/or reaction to confrontational behavior and position in the pen. A hierarchical structure ranking animals as dominant (showing no submissive behavior), submissive, or subordinate is developed. During phase 2, protected contact is established by placing Plexiglas panels in the upper and lower ports of the common wall between two pens. If the pair seems to be compatible, the process progresses to the last phase. In phase 3, full contact of animals is facilitated by removing the Plexiglas panels, allowing commingling within the two-pen space. Further observation is done to fully assess the relationship in this setting.

During active mating, each male of a breeding pair is housed individually. The female is brought to the male and the breeding pair is housed together for up to 48 h. At the completion of the mating period, the female is removed. The dividing panels are removed to allow reintroduction of the commingled males.

Even the thorough preparation of the selection of cage mates cannot guarantee the smooth interaction of the animals throughout the entire study duration; numerous factors, including test article effects, could potentially influence the hierarchical behaviors. It is therefore necessary that such groups of socially housed nonhuman primates are continuously monitored by competent staff.

To keep the animals' stress at a minimum, restricting the number of relocations for the animals is important. This applies to both relocations to other rooms and to other cages, which are often underestimated sources of stress [20]. Determining general clinical pathology data or reproduction parameters requires sampling from undisturbed and unstressed animals, and it has been shown that male and female fertility studies should not be performed in newly formed groups of animals [21]. It is therefore mandatory to restrict animal moves to a minimum; establish groups of cage mates early enough and move them to their assigned room and cage at the appropriate time point to increase animal welfare and avoid effects on studies.

Social housing of nonhuman primates can be successful only if compatible animals are assigned as cage mates and held in enclosures with adequate space and design and with staff and animals being adequately trained to increase collaboration and safety and to reduce stress. These three pillars complement each other and are equally indispensable [22].

VetBooks.ir

References

[1] European Community. Directive 2010/63/EU of the European Parliament and of the Council of 22 September on the protection of animals used for scientific purposes. Off J Eur Union 2010;L 276:33–79. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF [accessed in 2014].

[2] Council of Europe. Appendix A of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123). Guidelines for accommodation and care of animals (Article 5 of the convention). Approved by the multilateral consultation. Cons 123 (2006) 3. Strasbourg: Council of Europe; 2006. http://conventions.coe.int/Treaty/EN/Treaties/PDF/123-Arev.pdf [accessed in 2014].

[3] Bayne K, Morris TH. Laws, regulations and policies relating to the care and use of nonhuman primates in biomedical research. In: Abee C.R., Mansfield K., Tariff S.D., Morris T., editors. Nonhuman primates in biomedical research: biology and management, vol. 1. London: Elsevier; 2012. p. 35–57.

[4] FELASA. Recommendations on the education and training of persons working with laboratory animals: categories A and C and recommendations for the education and training of persons carrying out animal experiments (category B), http://www.felasa.eu/recommendations.htm; 2008 [accessed in 2014].

[5] Reinhardt V, Liss C, Stevens C. Restraint methods of laboratory non-human primates: a critical review. Anim Welf 1995;4:221–38.

[6] Reinhardt V. Training nonhuman primates to cooperate during handling procedures: a review. Anim Technol 1997;48:55–73.

[7] Andrews-Kelly G. Primate enrichment and positive reinforcement training in toxicology: challenges and possibilities. Enrichment Rec 2011;9:16–8.

[8] Prescott MJ, Buchanan-Smith HM. Training nonhuman primates using positive reinforcement techniques: guest editor's introduction. J Appl Anim Welf Sci 2003;6:157–61.

[9] Prescott MJ, Bowell VA, Buchanan-Smith HM. Training laboratory-housed nonhuman primates, part 2: resources for developing and implementing training programmes. Anim Technol Welf 2005;4:133–48.

[10] Laule GE, Bloomsmith MA, Schapiro SJ. The use of positive reinforcement training techniques to enhance the care, management, and welfare of primates in the laboratory. JAAWS 2003;6(3):163–73.

[11] Koban TL, Miyamoto M, Donmoyer G, Hammar A. Effects of positive reinforcement training on cortisol, hematology and cardiovascular parameters in cynomolgus macaques (*Macaca fascicularis*). Am J Primatol 2005;66:148.

[12] Russell WMS, Burch RL. The principles of humane experimental technique. Potters Bar, England: Universities Federation for Animal Welfare; 1959.

[13] Morgan D, Grant KA, Prioleau OA, Nader SH, Kaplan JR, Nader MA. Predictors of social status in cynomolgus monkeys (*Macaca fascicularis*) after group formation. Am J Primatol 2000;52:115–31.

[14] Reinhardt V. Arguments for single-caging of rhesus macaques: are they justified? AWIC Newsletter 1995;6(1):1–2.

[15] Coenen de Roo CJJ. Do behavioural studies contribute to the development of a new primate vivarium? [Thesis]. Utrecht: University of Utrecht, Department of Animals, Science and Society; 2007, pp. 1–28.

[16] Altmann J. Observational study of behaviour: sampling methods. Behaviour 1974;49:227–67.

[17] Xu F, Xie L, Li X, Li Q, Wang T, Ji Y, et al. Construction and validation of a systematic ethogram of *Macaca fascicularis* in a free enclosure. PLoS One 2012;7(5):e37486.

[18] Baker KC, Bloomsmith MA, Oettinger B, Neu K, Griffis C, Schoof VA. Comparing options for pair housing rhesus macaques using behavioral welfare measures. Am J Primatol 2014;76(1):30–42.

[19] Niehoff MO, Bergmann M, Weinbauer GF. Effects of social housing of sexually mature male cynomolgus monkeys during general and reproductive toxicity evaluation. Reprod Toxicol 2010;29:57–67.

[20] Crockett CM, Bowers CL, Shimonji M, Leu M, Bowden DM, Sackett GP. Behavioural responses of longtailed macaques to different cage sizes and common laboratory experiences. J Comp Psychol 1995;109:368–83.

[21] Niehoff M, Bergmann M, Müller W, Weinbauer GF. Effects of group housing on reproductive parameters in the sexually mature cynomolgus monkey. In: Weinbauer GF, Buse E, Müller W, Vogel F, editors. New developments and challenges in primate toxicology. Münster: Waxmann Verlag; 2005. p. 137–43.

[22] Müller W. The new European housing regulations for nonhuman primates: opportunities and challenges. In: Weinbauer GF, Vogel F, editors. Critical contributions of primate models for biopharmaceutical drug development. Münster: Waxmann Verlag; 2008. p. 53–74.

VetBooks.ir

PART V

THE ROLE OF NONHUMAN PRIMATES IN SAFETY ASSESSMENT/ REGULATORY TOXICOLOGY

VetBooks.ir

VetBooks.ir

CHAPTER

16

Nonclinical Toxicology Testing Strategies and Applicable International Regulatory Guidelines for Using Nonhuman Primates in the Development of Biotherapeutics

Marque Todd[*], *Michael W. Leach*[†]

[*]Pfizer, Inc., San Diego, CA, USA

[†]Pfizer, Inc., Andover, MA, USA

OUTLINE

Introduction 316

Species of NHPs Used in Nonclinical Toxicity Studies 317

Overview of Nonclinical Toxicity Testing Strategy Using NHPs and Applicable International Regulatory Guidelines 317

Species Selection 319

Tiered Approach to Species Selection 319

Interpretation and Utilization of Species Selection Data 321

When a Pharmacologically Relevant Species Cannot Be Identified 322

In Vivo Pharmacology/Toxicology Studies 323

General Considerations for In Vivo Studies 323

Early Non-GLP Exploratory Toxicity Studies 324

GLP Repeat-Dose Toxicity Studies 324

Recovery Groups in In Vivo Toxicity Studies 326

Considerations for Safety Pharmacology Assessment 326

http://dx.doi.org/10.1016/B978-0-12-417144-2.00016-0

© 2015 Elsevier Inc. All rights reserved.

Considerations for Developmental and Reproductive Toxicity Testing 327
Other Considerations for Using NHPs in Nonclinical Testing Programs 328
Immunotoxicity Testing 328
Carcinogenicity Testing 329
Immunogenicity and Antidrug Antibody Formation 330
Hypersensitivity 330
Conclusion 333
References 334

INTRODUCTION

The use of nonhuman primates (NHPs) in nonclinical toxicity testing is based on the regulatory expectation that the toxicity of new drugs should be tested in animals before testing in humans. Regulatory guidelines indicate that two animal species should generally be used for nonclinical toxicity testing supporting the development of drugs for human use: a rodent (e.g., mouse or rat) and a nonrodent (e.g., dog or NHP). Exceptions to the need for two species may occur if only one species is considered relevant to understanding the effects that might occur in humans. Thus, the decision on whether to use an NHP species in studies should be scientifically driven and based on the expected relevance to humans; when an NHP species is the most pharmacologically-relevant nonrodent species, it should be used in nonclinical toxicity studies. Inappropriate use of NHPs, such as when they are not the most relevant nonrodent species, should be avoided.

When comparing NHP use between new chemical entities (NCEs) and biotherapeutics, there are clear differences. Using NHPs for NCE development is uncommon because dogs are often an equally (or more) relevant species, but NHPs are used if there is a strong scientific rationale (primarily if NHPs are the most relevant nonrodent species). In contrast, biotherapeutics (e.g., monoclonal antibodies, recombinant proteins) often have better pharmacologic activity and lower immunogenicity in NHPs compared with other species; therefore, many biotherapeutics programs use an NHP species to fulfill the regulatory requirement to test the drug candidate in a nonrodent species. When NHPs are the only relevant species, which occurs in a number of biotherapeutics programs, the NHP is the only nonclinical species used in the toxicity studies; the use of a second, nonrelevant species is not required. Therefore, this chapter focuses on the use of NHPs in nonclinical biotherapeutic programs; however, many of the strategies described here also would be generally applicable to nonclinical NCE programs using an NHP species.

A variety of regulatory guidances are available to help direct the use of NHPs in nonclinical toxicity studies, as well as to provide suggestions on study designs to minimize NHP use. Most notable are the guidelines from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), which detail many different aspects of drug development. In addition, there are many regional and country-specific guidelines available. Because these guidelines can undergo revision, it is important for scientists to be sure they are using the most up-to-date information that is available. The goal of this chapter is to review nonclinical strategies and applicable global regulatory guidelines when using NHPs in biotherapeutic drug development programs, focusing on toxicology-related activities.

VetBooks.ir

SPECIES OF NHPs USED IN NONCLINICAL TOXICITY STUDIES

There are hundreds of NHP species. Historically, only a few of these have been used in toxicity studies, including cynomolgus (*Macaca fascicularis*) and rhesus monkeys (*Macaca mulatta*), common marmosets (*Callithrix jacchus*), and, in limited cases, African green monkeys (*Chlorocebus sabaeus*), baboons (*Papio anubis* and *Papio hamadryas*), and chimpanzees (*Pan troglodytes*). Today, the cynomolgus monkey is the most common choice for use in toxicity studies by a significant margin over other NHP species. There are several practical reasons why cynomolgus monkeys are most frequently used NHP species: they weigh less than rhesus monkeys and are therefore easier to handle and require smaller amounts of total drug for a study, they breed year-round (unlike the rhesus monkey, which is a seasonal breeder), and there are extensive historical data available. The rhesus is used infrequently. Although the common marmoset is used infrequently, its use as a nonclinical toxicity species has increased recently [1]. The use of rhesus macaques and marmosets in a toxicology program should be based on specific scientific considerations (e.g., target binding and/or pharmacological activity). African green monkeys and baboons are almost never used in toxicity testing. Chimpanzees are not used in toxicity studies at the present time for ethical reasons. Table 16.1 shows some of the characteristics relevant to using each NHP species in nonclinical toxicology programs.

OVERVIEW OF NONCLINICAL TOXICITY TESTING STRATEGY USING NHPs AND APPLICABLE INTERNATIONAL REGULATORY GUIDELINES

The nonclinical toxicity testing strategy when using NHPs differs in some aspects from strategies using other species, particularly for programs for which the only relevant species is an NHP. This is because of scientific considerations related to NHP physiology and animal husbandry, regulatory expectations, and animal welfare/use concerns. For biotherapeutics programs where both relevant rodent and nonrodent species have been identified, a nonclinical program may use mice or rats as the rodent species and dog, NHP, mini-pig, or even rabbit as the nonrodent species, depending upon a variety of factors including target binding/affinity, pharmacologic activity, pharmacokinetics (PKs), pharmacodynamics (PDs), species physiology, and/or immunogenicity. However, for biotherapeutics in which the only identified pharmacologically-relevant species is the NHP, all nonclinical toxicity studies are usually conducted using NHPs. Table 16.2 outlines studies commonly used in the nonclinical

TABLE 16.1 Relative Characteristics of NHPs Used in Toxicity Testing

Species	Size	Handling and care	Breeding	Availability of historical toxicity data
Cynomolgus monkey	Intermediate	Easy	Year-round	High
Rhesus macaque	Large	Difficult	Seasonal	Intermediate
Common marmoset	Small	Difficult	Year-round	Intermediate
African green monkey	Intermediate	Intermediate	Seasonal	Low
Baboon	Large	Intermediate	Year-round	Low

TABLE 16.2 Nonclinical Toxicity Studies/Programs Using NHPs for Biotherapeutics and Applicable International Regulatory Guidelines

Study type	Toxicology program using rodents and NHPs	Toxicology program using NHPs only	International regulatory guidelines
Exploratory (single- and repeat-dose) and GLP repeat-dose general toxicity	Shorter-term studies of rodents and NHPs; consider non-terminal exploratory studies for NHPs; terminal studies using only rodent species	Studies of NHPs; consider nonterminal exploratory studies for NHPs	ICH M3(R2) ICH S6(R1) ICH S3A
Safety pharmacology (CV, CNS, and respiratory)	Stand-alone studies of rodents; consider adding safety pharmacology end points in NHP general toxicity studies	Add safety pharmacology end points to NHP general toxicity studies; stand-alone studies of NHPs only if cause for concern	ICH S7A ICH S6(R1)
Fertility	Study of rodents (single species)	Assess male and female reproductive organs (organ weight and histopathology) in mature animals on repeat-dose toxicity studies ≥3 months in duration; add additional end points (e.g., sperm motility, reproductive hormones) if cause for concern	ICH S5(R2) ICH S6(R1)
EFD	Studies of rodents and rabbits	Study of NHPs; consider ePPND study to assess EFD and postnatal development in one study	ICH S5(R2) ICH S6(R1) ICH M3(R2)[a]
PPND	PPND study of rodents (single species)	PPND study of NHPs; consider ePPND study to assess EFD and postnatal development in one study	ICH S5(R2) ICH S6(R1) ICH M3(R2)
Carcinogenicity	Carcinogenecity studies of rodent species may be considered on a case-by-case basis (i.e., short-term transgenic mouse and 2-year rat bioassay)	Carcinogenicity studies are not conducted using NHPs; cell proliferation end points may be added to repeat-dose toxicity studies to support carcinogenicity risk assessment	ICH S1A ICH S6(R1)
Immunotoxicity	If cause for concern, additional end points can be added to rodent and NHP studies	If cause for concern, additional end points can be added NHP studies	No ICH guidelines; FDA and EU guidelines
Juvenile toxicity	If needed, study of single rodent species	If needed, study of NHPs	No ICH guidelines

CNS = central nervous system, CV = cardiovascular, EFD = embryo-fetal development, ePPND = enhanced pre- and postnatal development, EU = European Union, FDA = Food and Drug Administration, GLP = Good Laboratory Practice, ICH = International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, PPND = pre- and postnatal development.

[a]*ICH M3(R2) includes a note on the timing of NHP EFD/ePPND studies specifically for monoclonal antibodies.*

VetBooks.ir

development of biotherapeutics using NHPs and the most applicable ICH regulatory guidelines for each study type. Each of these study types is discussed in greater detail, along with regulatory and strategic considerations, in the sections below.

SPECIES SELECTION

Species selection is a key scientific activity and represents the foundation of the nonclinical toxicology program and regulatory strategy. Not only is it important to ensure an accurate nonclinical assessment of a drug's pharmacological and toxicological properties, but it is also required by worldwide health authorities as part of regulatory submissions to support the relevance of the nonclinical program to the clinical program. This concept has been particularly stressed for biotherapeutics in the literature [2–4] and in regulatory guidance related to biotherapeutics [5].

A pharmacologically-relevant species is defined in ICH S6(R1) as one in which the drug binds to the intended target (e.g., receptor, epitope) and elicits a pharmacological response similar to that expected in humans [5]. The assessment of species relevance should be based on comparative evaluations between humans and animals, which determine the similarity of drug activity and biological responses between species. All of the standard nonclinical species should be assessed during species selection screening because any can be considered for use in the nonclinical toxicology program if it is pharmacologically relevant. It should not be presumed that NHPs are the most relevant species just because they are phylogenetically closer to humans compared with dogs, rabbits, rats, or mice. If an NHP species is considered the most pharmacologically-relevant species, they should be selected. As noted above, because of the restricted species selectivity of many biotherapeutics, it is not uncommon that the only an NHP species is pharmacologically relevant, and the species most often used is the cynomolgus monkey.

Tiered Approach to Species Selection

Species relevance compared with humans may be assessed in a tiered manner. First, whether the candidate molecule has the anticipated interaction with the planned target is considered. Next, whether that species also has the same downstream pathways resulting in similar pharmacologic activity is assessed. Assays that can be conducted to support species relevance are summarized in Table 16.3.

In a tiered approach to species selection, assessment usually starts in very early development with a comprehensive literature review to determine what is known about comparative pharmacology, including information from transgenic animal models versus human genetic diseases in which the target may be under- or overexpressed. At the same time, comparison of amino acid sequence homology across standard nonclinical species and humans should be conducted; additional sequence homology comparisons can also be made for DNA and messenger RNA. If amino acid sequence homology for a species is very low compared with humans (i.e., 10% identity vs. humans), particularly at the binding epitope of the target, additional work may not need to be conducted for that species. However, there is no specific rule for when homology is too low, and even high homology (99%) is not a guarantee that target binding will be the same.

VetBooks.ir

TABLE 16.3 Tiered Approach to Nonclinical Species Selection (Evaluations Should Compare Animal and Human Data when Feasible)

Tier	Examples of approaches
Tier 1: In silico and literature	• DNA, RNA, and/or protein sequence homology • Literature assessment of target pharmacology, expression, and distribution • Literature assessment of transgenic animals (e.g., knock-in and knockout models) and/or human genetic diseases
Tier 2: Binding and target distribution	• Cell binding/affinity assays (e.g., Biacore assay or flow cytometry) • Immunohistochemistry to assess target tissue binding/distribution
Tier 3: In vitro, ex vivo, and in vivo studies to assess pharmacology	• In vitro cell-based assays using species-specific cell lines • Ex vivo assays using cells or tissues • Pharmacologically-relevant biomarkers assessed in in vivo pharmacology or toxicity studies • Pharmacologically-mediated toxicity assessed in in vivo pharmacology or toxicity studies

As a next tier, assays can be conducted to assess comparative binding/binding affinity and target distribution. The nonclinical species with the greatest similarity in relative binding affinity to humans is preferred, although a high degree of similarity may not always be possible. It is recommended that binding data be generated—even if target homology across species is believed to be 100%—because there have been many instances where homology data does not predict binding very well or homology data may be incorrect.

One type of assay that sometimes is confused with early target distribution assays is the tissue cross-reactivity (TCR) assay. TCR assays usually use immunohistochemistry methodologies, are conducted later in development, and are required by health authorities for antibody and antibody-like biotherapeutic molecules that contain a complementarity-determining region [6]. The goal of TCR assays is to use the candidate molecule to identify tissues and cells in humans where there is off-target binding and, secondarily, to identify sites of on-target binding that were not previously identified [6]. The information then is compared with the animal species that have been selected for toxicity studies based on in vitro binding, in vitro pharmacology, and in vivo pharmacology data. While this assay historically played a significant role in species selection for nonclinical toxicity studies, it is now recognized that other methods are usually more appropriate for species selection [6].

Amino acid sequence analysis and bind affinity/tissue binding data alone are not sufficient to demonstrate that a nonclinical species is pharmacologically relevant. If it is scientifically feasible, additional in vitro/in vivo PD and/or toxicity data should be collected. ICH S6(R1) highlights that functional activity data can be obtained from cell-based assays and/or in vivo pharmacology or toxicity studies. Species-specific cell-based assays should be well-characterized for target expression, target modulation, and downstream activity, and data from animals and humans should be compared using the same (or a similar) assays.

VetBooks.ir

The strongest data supporting species selection comes from using target pathway-specific PD markers in in vivo studies, if these are available. Ideally, the PD marker can be monitored in humans. However, a toxicity finding, such as a morphologic change in a tissue that can be associated with the mechanism of action of the test article, can also serve as a biomarker of pharmacologic activity, suggesting species relevance. In some cases, in vivo PD markers may not be available and in vitro data are the only information that can be used to support pharmacologic activity in the nonclinical species. In addition to selecting relevant species, the screening process is also important to justify when a species is not relevant. It is the expectation of health authorities that the nonclinical program be conducted with both a rodent and nonrodent species unless data are presented showing that a species is not pharmacologically relevant; this is particularly important when justifying a single-species (i.e., NHP only) nonclinical testing strategy. Thus, demonstration that a species is not relevant is critical to having an appropriate nonclinical testing strategy that does not conduct inappropriate studies using irrelevant species.

Interpretation and Utilization of Species Selection Data

Once the evaluations related to species selection are completed, species relevance should be determined to select the most appropriate nonclinical species to use in the nonclinical program. While ideally the comparative animal and human data would match, in reality this is rarely the case. In some cases it is clear that an animal species is not relevant, such as when there is no binding to the target. In other cases the data may suggest less relevance (i.e., lower binding affinity and less pharmacologic effect in in vitro pharmacology studies compared with human data). In still other cases, some assays may suggest differences with humans, and other data may suggest a high degree of similarity. In these cases, placing more weight on comparative in vitro pharmacology data relative to binding data, and placing the most weight on ex vivo and in vivo data, is recommended. As an example, it might be possible that limited binding to cells might still result in a full pharmacologic effect. Therefore, lower binding in animals might not impact the in vivo pharmacology.

When the overall data suggest that a species is of marginal relevance, questions as to whether it is "relevant enough" arise. For example, if the binding affinity in an animal is 50% of that in humans, is that close enough? What about 10%? ICH S6(R1) suggests that higher dose concentrations may be used to overcome these differences in binding affinity, but at some point this may no longer be feasible. There is no exact cutoff for when a species should no longer be considered relevant. We suggest that the comparative sequences, binding, and in vitro data be considered, taking into account the anticipated pharmacology and planned indication, to make a final case-by-case determination regarding whether the species should be considered "relevant enough" to warrant conducting an in vivo study. In some cases, it may require an early pharmacology or non-Good Laboratory Practice (GLP) toxicity study to assess in vivo pharmacology and/or toxicity to make the final determination of species relevance for the program.

Evidence of immunogenicity (the formation of antidrug antibodies) is sometimes used as a justification that a species is not relevant and to exclude that species from use in a nonclinical program. However, immunogenicity can be managed in a variety of ways (e.g., increasing the frequency of dosing or dose concentrations to maintain exposure and any PD effects). Increasing the number of animals per dosing group may increase the likelihood that animals

VetBooks.ir

will make it to the end of the study without developing immunogenicity that interferes with interpretation of the results in those animals. Thus, immunogenicity does not automatically exclude a species as being potentially relevant.

Pharmacologic and/or toxicologic effects in animals and humans can be related to either interactions with the anticipated target of the pharmacologic modulation or interactions with an unanticipated target (i.e., an off-target effect). The "ideal" animal species would fully replicate the combination of effects in humans; however, in many cases one animal species may only replicate some responses or may only partially replicate a response. In these cases, scientists should determine which combination of species is needed to best evaluate potential toxicities in humans.

In contrast to small molecules, biotherapeutics often do not have off-target effects because of their high target specificity, and they lack metabolites since they are degraded by catabolism into small peptides and individual amino acids [5]. Thus, these types of molecules typically produce only on-target effects related to the drug. However, there have been a few cases where biopharmaceuticals have affected unintended targets. An example is when an antibody binds unexpectedly to a protein that is not the intended target [7–9]. This may occur because the same or a related target epitope is present on more than one protein, resulting in an interaction of the drug with an unexpected target; in the case of an anti-amyloid β antibody, the cross-reactive protein was shown to be platelet factor 4 [7].

A special situation occurs when the target is present only in the disease state. In this case, it is not expected that pharmacologic effects will occur in the normal animals used in standard toxicity studies. Studies using animal models of disease may be more appropriate to determine potential on-target effects. However, unexpected pharmacological effects or off-target effects may still occur in normal animals in general toxicity studies, and health authorities currently find studies of normal animals acceptable to support clinical development.

Overall, the most relevant species are those that replicate human biology for the pathway(s) that are being affected (whether on or off target) and have the same pharmacological response to the drug. In reality, it is uncommon for any animal species to mirror perfectly what happens in humans, including NHPs. Because of this, nonclinical testing may need to include more than one species, with different species assessing different effects that may occur in humans. Regardless of which species are selected, that selection should be based on information that suggests there is relevance to humans. In cases where NHPs are not the most relevant species, their use may not be warranted.

When a Pharmacologically-Relevant Species Cannot Be Identified

In some cases for biotherapeutics, the only relevant species that can be identified is the chimpanzee. Because the chimpanzee is endangered, only animals currently held in captivity can be used for studies, and this is a very small number of animals. In addition, the use of the chimpanzee for nonclinical toxicity studies is not legal in many parts of the world. Even if legal in a given country, there are several disadvantages to selecting the chimpanzee as a nonclinical species for a drug development program. Because of the limited number of animals available, none of the animals are naïve; all have been exposed to numerous NCEs, vaccines, and/or biotherapeutics. Only nonterminal studies can be conducted with chimpanzees, and therefore end points are limited to clinical signs, clinical pathology, and PKs. The doses

VetBooks.ir

selected must be conservative and the toxicity of a drug candidate cannot be fully explored. Many companies do not allow the use of chimpanzees for these reasons, and many health authorities do not favor their use as a nonclinical species. Modern pharmaceutical scientific techniques usually make it feasible to engineer a drug candidate that is pharmacologically active in at least one standard nonclinical species.

In those cases where a pharmacologically-relevant species cannot be identified (i.e., the drug only binds to a human target), there is still strong regulatory expectation that in vivo toxicity testing will be conducted using alternative methods [5]. Alternatives may include the use of homologous proteins, transgenic animals, and/or disease models [10]. The most common alternative approach is to develop a homologous protein that binds the target in a nonclinical species and has similar pharmacological activity to the clinical candidate. Homologous proteins can be developed for any nonclinical species, including NHPs, but the most common practice is to develop a rodent homolog. Toxicity studies conducted using a homologous protein can be used for assessing hazards and understanding potential adverse effects of a biotherapeutic, but not for quantitative risk assessment. Homologous proteins need to be well characterized biochemically and biologically and show a similar mechanism of action and PD effects as the clinical candidate. A homolog also needs to be manufactured using processes, characterization, and documentation that can support its use in GLP toxicity studies. If at all feasible, health authorities prefer that the clinical candidate be assessed in a pharmacologically-relevant species rather than using a homolog (i.e., clinical candidate tested in monkeys versus homolog tested in a rodent is preferred), unless there is strong scientific rationale to use the homolog [5]. There is no expectation that a two-species nonclinical program will be conducted if it would require the development of a homologous protein to enable testing in a second species [5].

IN VIVO PHARMACOLOGY/TOXICOLOGY STUDIES

General Considerations for In Vivo Studies

As noted above, conducting in vivo studies in relevant species is critical. NHPs should be used only when they have been shown to be relevant based on data, and not on a presumption related to being closely related from a phylogenetic standpoint. The majority of in vivo pharmacology studies supporting a drug development program are usually conducted before the initiation of clinical trials because these studies often provide the fundamental supporting information that directs the indication, dosing, and regimen in the human trials. In vivo pharmacology studies are usually conducted in rodents, and less frequently in NHPs, for several reasons: (1) NHPs are more genetically diverse, which can lead to more variable results and more difficult data interpretation. (2) Fewer NHPs can be used per study, lowering the power of the studies and lessening the ability to detect true differences. (3) Models are usually more easily developed and validated in rodents. (4) Many reagents for assessing pharmacology and efficacy are more readily available for rodents. (5) NHPs are much larger and require much more test article. (6) NHP studies are typically significantly more expensive. However, there are cases when NHPs are the most relevant pharmacology model. An example is the targeting of CD20 to deplete B cells for the treatment of B-cell malignancies and various immune diseases. A number of antibodies have been developed that target CD20 [11], and these have

VetBooks.ir

activity only in primates (or in rodent models modified to have the primate CD20) [12]. Most nonclinical studies (i.e., pharmacology and toxicity studies) in anti-CD20 programs have been conducted using cynomolgus monkeys because the candidate drugs have no PD effects in nonprimate species, and conducting studies in these species would not provide relevant pharmacologic data.

When it is determined that NHPs are the most relevant large animal species for in vivo testing, scientists should use strategies to minimize overall NHP use. Because of their large size relative to rodents, a larger number of samples can potentially be collected from the same individual NHP, including more serial samples, to better follow the progress of any effects. With careful planning, conducting fewer overall studies and using fewer NHPs may be possible; this is usually acceptable, and many times preferred, by worldwide health authorities. As an example, PK and toxicity assessments may be included in pharmacology studies, as long as they do not negatively impact the necessary pharmacology assessments. Data obtained from these "combination" studies may help guide better designs of future pharmacology or toxicity studies or may eliminate the need for some additional studies.

Early Non-GLP Exploratory Toxicity Studies

As programs progress, studies in which the primary focus is toxicity are often conducted. Initially these are usually non-GLP exploratory studies that may use small numbers of animals, and there are no specific regulatory guidelines directing the design and conduct of these studies. Safety pharmacology end points may be included to provide an early assessment of potential effects on these parameters. Depending on the program status, these studies may be designed to provide information that (1) suggests whether a program has significant toxicity issues that may preclude development (i.e., allowing early program termination), (2) selects between several candidate molecules, and/or (3) allows appropriate design of GLP toxicity studies that support entry into clinical trials, including dose concentrations, regimen, and identification of additional end points that might be helpful. In the case of molecules that are expected to be relatively nontoxic, such as monoclonal antibodies against soluble cytokines, it is possible the NHP study could consist of a single-dose PKs/tolerability study, as the high dose level for the study is often set by targeting at least a 10× exposure multiple and/or maximum pharmacological effect rather than toxicity [5]. The low dose in these studies is usually based on trying to match the highest planned human exposure and/or pharmacological activity. At the end of these studies, animals may be returned to the colony for other uses, although typically not for use with other biologics because of immunogenicity concerns. A modification of this design is where an initial dose is given, toxicokinetics are followed for approximately five half-lives, and then another dose is given approximately 24-72 h before necropsy. This design allows a macroscopic and microscopic assessment of the animals at a time point when exposure is high.

GLP Repeat-Dose Toxicity Studies

Studies directly supporting human safety are usually conducted in compliance with US FDA 21 CFR Part 58, European, and/or Japanese GLP standards. GLP regulations apply to nonclinical laboratory studies supporting research or marketing applications for regulated

VetBooks.ir

products and set forth the minimum basic requirements for study conduct, personnel, facilities, equipment, written protocols, operating procedures, study reports, and a system of quality assurance oversight for each study to help ensure the safety of regulated products [13]. The design of individual toxicity studies should be based on the ICH guidelines (Table 16.2). In vivo general toxicity studies in support of the initiation of human clinical trials (i.e., clinical trial-enabling studies) often consist of shorter-term (up to 1 month's duration), medium-term (1-3 months' duration), and long-term (≥6 months' duration) studies; in some programs, such as for molecules with limited toxicity (e.g., certain monoclonal antibodies), the medium-term studies can be eliminated.

As noted above, current regulations require general toxicity studies to be conducted in one rodent species (usually mouse or rat) and one nonrodent species (typically dog or cynomolgus monkey) [5,14]. If both the dog and an NHP species are considered to be of equivalent relevance, it is encouraged that dogs be used for in vivo general toxicity studies whenever possible based on ethical considerations. However, for biotherapeutics there are general concerns (particularly for monoclonal antibodies and associated derivatives) that significant immunogenicity may occur in dogs even if they are a pharmacologically-relevant species. If dogs and NHPs have similar immunogenicity, then in vivo studies should usually be conducted in dogs. If significant immunogenicity is observed in the dog that impacts drug exposure/pharmacology or causes unmanageable hypersensitivity reactions, then studies should be conducted using NHPs if similar immunogenicity-related effects are not observed. Practical experience has shown that dogs are used only infrequently with biotherapeutics programs (mostly used with recombinant proteins); in most cases NHPs are the nonrodent species used for in vivo studies of biotherapeutics.

In many cases, the biotherapeutic drug is only active in NHPs—or activity is much lower in other species—and the NHP is the considered the only relevant nonclinical species. In a review of approximately 40 biotherapeutics that had entered clinical trials at one drug company, 40% had activity in only NHPs (Leach, unpublished data); the remainder had activity in at least one other standard nonclinical species used in general toxicity studies (mouse, rat, dog). For programs with activity only in NHPs, all in vivo general toxicity studies need to be conducted using NHPs.

When there is also activity in rodents, it is acceptable to conduct chronic studies (i.e., ≥6 months duration) in only one rodent species, provided the findings in shorter-term studies are similar or the findings are understood from the mechanism of action of the product [5]. The need for only one rodent species in these circumstances can significantly reduce the use of NHPs in a development program. Another difference between small molecule and biotherapeutics programs is the duration of the long-term (i.e., chronic) studies. While small molecules often need chronic studies of large animals of 9 months duration [14], chronic studies for biotherapeutics do not need to be longer than 6 months unless there is a scientific rationale for longer studies [5,15].

As mentioned above, in some cases target molecules may be expressed only in disease states, and testing in normal animals may not be representative of what may occur in patients. Although most disease models with biotherapeutics are conducted in rodents, it is possible that the only model might be in NHPs. In this case, toxicity studies using the NHP disease model might be considered. However, such a study might have challenges in interpretation related to differentiating toxic changes from the effects of the disease

VetBooks.ir

itself, and it is recommended that such a study be conducted only after careful consideration of and determination that other methods (e.g., use of a rodent model with a homologous product or use of genetically-modified animals such as knockout mice) would not provide sufficient overall data [10]. Seeking advice and approval from worldwide health authorities is also recommended when undertaking a nonclinical program with alternative animal models.

Recovery Groups in In Vivo Toxicity Studies

The purpose of the recovery period is to understand the reversibility of clinically relevant pharmacological and toxicological effects, not to understand potential delayed toxicity and, in the case of biotherapeutics, not to further characterize immunogenicity [5,16]. The demonstration of recovery, or of complete recovery, may not be necessary if the process of recovery from specific findings is well understood or if the findings occur at doses that are not clinically relevant [5]. An assessment of recovery may not be warranted for every program, every study, or every group within a study, particularly when a drug has shown no to minimal toxicity in previous studies and there are no significant concerns/risks for the target or mechanism of action of the drug [17]. Fewer recovery groups may be needed for drugs being developed for life-threatening indications or unmet medical need because the risk/benefit profile for the drug in these cases allows for more risk. Addition of recovery animals/groups for NHP studies should be determined on a case-by-case basis and carefully considered so that the numbers of animals used for each study and the entire nonclinical program can be minimized to only what is scientifically justified [17].

CONSIDERATIONS FOR SAFETY PHARMACOLOGY ASSESSMENT

The ICH S7B and ICH S6(R1) guidelines outline requirements for conducting safety pharmacology studies [5,18]. Cardiovascular, central nervous system (CNS), and respiratory function should be assessed before first-in-human clinical trials are conducted. If rodents are being using in the nonclinical safety program, standard stand-alone safety pharmacology studies can be conducted and additional end points/dose groups can be added to repeat-dose toxicity studies as warranted. If safety pharmacology end points need to be assessed in a nonrodent species (typically when the NHP is the only relevant species), they can be incorporated into the repeat-dose general toxicity studies. Cardiovascular assessments added to general toxicity studies usually include electrocardiograms, heart rate, and blood pressure [19]. Respiratory and CNS function data are challenging to collect from NHPs, and end points are generally limited to respiration rate and clinical observations for CNS function, although more detailed evaluations are possible in general toxicity studies [20]. This approach is accepted by health authorities and helps to reduce the number of NHPs used in the toxicology program. Stand-alone safety pharmacology studies using NHPs may be warranted if there is cause for concern based on previous study results, if there are theoretical concerns about target biology or the mechanism of action of the drug candidate; however, stand-alone studies should only be considered on a case-by-case basis.

VetBooks.ir

CONSIDERATIONS FOR DEVELOPMENTAL AND REPRODUCTIVE TOXICITY TESTING

Developmental and reproductive toxicity (DART) needs to be assessed so that women of child-bearing potential and men who may father children can be included in clinical trials. The main purpose of these nonclinical studies is to minimize the risks to the embryo and fetus and understand risk to future fertility. The studies include assessment of male and female fertility, embryo-fetal development (EFD), and pre- and postnatal development (PPND). Program strategies, study designs, and timing for DART studies are outlined in the ICH S5(R2), M3(R2), and S6(R1) guidelines [5,16,21]. If the clinical drug candidate is active in rodents/and or rabbits, then these species should be used for DART studies. When rodents are the relevant species in a biotherapeutics program, a second nonrodent species is not required for assessment of EFD, thus reducing the use of NHPs [5].

For programs in which the NHP is the only pharmacologically relevant species, the NHP can be used to assess DART [22]. NHPs have a number of characteristics in common with humans, including similar reproductive physiology and response to teratogens. However, because of low fertility rates, high rates of fetal abortion, small group sizes, and limited fetuses/offspring, the NHP should be used for DART studies only if there are no other pharmacologically-relevant species (i.e., rodents and/or rabbits). Because of these limitations, NHP DART studies are used to assess hazard (i.e., large effects) rather than qualitative risk [23,24].

For NHP-only programs, conducting separate EFD and PPND studies is acceptable. Alternatively, a generally more preferred option is to conduct one appropriately designed enhanced PPND (ePPND) study covering organogenesis to birth with some duration of the neonatal period [25,26]. The duration of the neonatal period is determined by the mechanism of action of the drug and the specific end points (e.g., immunotoxicity end points) in the study. A single study that incorporates several stages of development is considered to be a scientifically valid approach and is acceptable to worldwide health authorities. The ePPND study should not be conducted as just a combined EFD and PPND study but should take into consideration the pharmacology and mechanism of action of the clinical candidate in the study design; this design also reduces the number of animals used versus doing separate EFD and PPND studies.

Large-molecular-weight biotherapeutics (>20,000-30,000 Da) in general exhibit minimal to no transfer across the placenta of NHPs or humans [27]. However, monoclonal antibodies cross the placental barrier in these species using a specific transport mechanism (FcRn), with exposure increasing through gestation and peaking around birth [28]. In addition, immunoglobulin (including exogenously administered antibodies) is secreted only in the colostrum and not during later lactation and nursing phases in the NHP. Because of this physiology in primates, including monkeys and humans, there is limited fetal exposure to immunoglobulins during organogenesis or postnatally [29,30]. Even with limited fetal exposure early during pregnancy, a highly potent molecule affecting a sensitive target could cause effects, and DART studies can provide useful information about maternal toxicity, placental effects, and even fetal effects due to FcRn transport. For example, an EFD study of figitumumab, an anti-insulin-like growth factor-1 receptor monoclonal antibody, using cynomolgus monkeys showed pregnancy loss and fetal growth deficits consistent with the inhibition of insulin-like growth factor signaling, as well as fetal malformations secondary to altered placental

VetBooks.ir

function and/or reduced fetal growth [31]. In some cases, a well-designed ePPND study may be more useful to assess DART effects than an EFD study. Administration of denosumab (an anti-RANK ligand monoclonal antibody) showed no effects in cynomolgus monkeys in an EFD study, but there were maternal and infant effects including increased stillbirths and postnatal mortality, and effects on growth and development of bones and lymph nodes were observed in an ePPND study [32]. These types of studies not only fulfill regulatory expectations around DART testing but are used to develop language for the label of a marketed drug.

Assessing fertility and effects on the embryo through implantation in accordance with ICH S5(R2) is difficult in the NHP because fertility and mating studies as conducted in rodents and/or rabbits are not feasible in NHPs. Therefore, collecting male and female fertility end points from general repeat-dose toxicity studies that are at least 3 months in duration is acceptable [5]. To assess these end points, sexually mature animals (i.e., males that are producing viable sperm and females that are cycling) need to be used in the study [33]. Standard end points include reproductive tract histology and reproductive organ weights in males and females. If there is cause for concern, menstrual cycling, sperm motility/counts, or relevant hormone concentrations can be incorporated as warranted. If there is specific concern about conception or implantation, then a characterized homologous molecule or the evaluation of genetically-modified (knockout or knock-in) animals can be considered.

There are some informational points in the ICH guidelines related specifically to the timing and conduct of NHP DART studies. Because of the smaller numbers of animals in each dose group compared with rodent studies, NHP DART studies should be for hazard identification only, and the number of doses groups should be adjusted accordingly (i.e., one or two dose groups that cover target saturation and at least one group that provides at least 10× higher exposure compared with the clinical exposure) [5]. For biotherapeutics where the only relevant species is the NHP, the EFD and PPND studies or the ePPND study may be conducted during phase III if women of child-bearing potential are using adequate forms of birth control to prevent pregnancy [14]. Because DART study designs can be more complex for NHPs, consulting with health authorities and reaching agreement before study designs or labeling approaches are finalized may be useful.

OTHER CONSIDERATIONS FOR USING NHPs IN NONCLINICAL TESTING PROGRAMS

There are other regulatory and scientific considerations when developing a strategy for a nonclinical program using NHPs and rodents or only NHPs. These include considerations around immunotoxicity, carcinogenicity assessment, and immunogenicity and hypersensitivity reactions.

Immunotoxicity Testing

According to the guidelines for immunotoxicology evaluation, immunotoxicity parameters (beyond standard clinical pathology and immune system organ weight/histology) do not need to be added to the repeat-dose toxicity studies unless there is a specific cause for concern [34–36]. If specialized end points do need to be added to toxicity studies using NHPs, commonly used end points such as immunophenotyping and the T-cell-dependent antibody

VetBooks.ir

response assay have been extensively validated in the cynomolgus monkey [37]. These end points also have been used for common marmosets and rhesus monkeys and should be accepted by health authorities, but they are not as well validated. When planning for a more detailed immunological assessment in NHPs, one consideration is that differences in white blood cell parameters and potentially other immunologic end points do exist between animals from different regions of the world (i.e., cynomolgus monkeys of Chinese origin vs. those of Vietnamese origin) [38]. Therefore, it is recommended that NHPs of the same origin be used throughout the nonclinical program. Another consideration for molecules that might induce a cytokine storm (i.e., drugs that activate the immune system) is that the NHP is not considered a good model and responses may not correlate well with potential responses in humans. However, other species have not been found to be any more predictive of human cytokine storm.

Carcinogenicity Testing

A decision on how to evaluate the carcinogenic potential of a new drug (literature review vs. in vitro vs. in vivo) depends on its intended clinical use (i.e., duration of treatment and severity of disease) and specific concerns based on pharmacological activity and the potential for genotoxicity [39]. For biotherapeutics that are pharmacologically active in the rodent, ICH S6(R1) states that 2-year rodent bioassays "…are generally inappropriate for biotechnology-derived pharmaceuticals." This is because biotherapeutics are typically not genotoxic; however, chronic administration could potentially lead to tumor promotion, depending on the mechanism of action of the drug, and it may be the expectation of health authorities that a 2-year bioassay be conducted [40]. For drugs that are not pharmacologically active in the rodent (i.e., NHP-only programs), there is no regulatory expectation (or precedent) that carcinogenicity studies be conducted in a nonrelevant species, nor that a homologous molecule to use in these studies be developed. In vivo carcinogenicity studies using NHPs are not recommended (and have no regulatory precedent) because administering drug for a sufficient duration of their lifespan or having a large enough number of animals to reach meaningful conclusions is not practical [15]. In addition, there is no expectation to increase the duration of the chronic repeat-dose NHP toxicity studies as a means of assessing carcinogenic potential.

In all cases, however, it is a regulatory expectation that an in-depth, product-specific risk assessment of carcinogenic potential be conducted and include a review of the literature (e.g., information on transgenic knockout/-in models and human genetic diseases), the available data from existing in vivo studies, and the information on related drugs and an understanding of any potential issues with the drug class (e.g., immunomodulators and growth factors). Doing the risk assessment and developing a carcinogenicity strategy early in the development program (i.e., before the initiation of clinical trials) is advantageous so that additional studies or end points can be included in nonclinical studies, if needed. For NHP-only programs, in accordance with ICH S6(R1), adding end points to the repeat-dose toxicity studies to evaluate potential effects on cell proliferation is feasible and acceptable; these data can be incorporated into a weight-of-evidence assessment of carcinogenicity risk [40]. As with DART studies (and particularly for NHP-only programs), health authorities should be consulted on the nonclinical carcinogenicity strategy; a thorough understand of drug pharmacology and appropriate product labeling may be more important than additional nonclinical studies.

VetBooks.ir

Immunogenicity and Antidrug Antibody Formation

Immunogenicity occurs when the immune system in an animal administered a drug recognizes it as foreign and generates a humoral and/or cellular immune response. Immunogenicity can occur in NHPs following administration of a biotherapeutics. Biotherapeutics are often large proteins that can be antigenic and stimulate the immune system without any covalent binding to endogenous molecules. Although the use of humanized or fully human biotherapeutics has reduced the immunogenicity of biotherapeutics in NHPs relative to the rodent chimeric molecules that were used in the past, both humanized and human biotherapeutics can result in immunogenicity in NHPs.

Immunogenicity in NHPs frequently results in the generation of antidrug antibodies (ADAs) [41]. Most commonly, these ADAs against biotherapeutics (1) do nothing (i.e., have no discernable effect), (2) bind to the drug and clear it (thus reducing the pharmacology), or (3) bind to the drug and neutralize the activity (also reducing the pharmacology) [41,42]. Although rare, it is also possible that the ADA might enhance or prolong the activity of the test article, which can occur if an antibody response does not clear or neutralize but instead increases the time in circulation of the active molecule without blocking its activity [42]. Another rare event can be the neutralization of an endogenous molecule, which can occur following the development of an ADA to a replacement therapy. This has been reported in humans following erythropoietin therapy and resulted in aplastic anemia in a few patients [43,44]. Other toxicities associated with immunogenicity can also occur and are discussed in section "Hypersensitivity."

Regulatory guidances for immunogenicity to biotherapeutics in animals have been published [5,45]. Several regulatory guidelines directed toward humans also have been published [34,35], and many of the principles can be applied to NHPs. In accordance with ICH S6(R1), it is expected that immunogenicity will be evaluated in repeat-dose toxicity studies if the following occurs: (1) evidence of altered PD activity; (2) unexpected changes in exposure in the absence of a PD marker; or (3) evidence of immune-mediated toxicity (immune complex disease, vasculitis, anaphylaxis, etc.). Because predicting whether such analysis will be necessary before completion of the in-life phase of the study is difficult, obtaining appropriate samples during the course of the study and then analyzing them, if warranted, to aid in interpretation of the study results is often useful.

Interpretation of study data should consider the potential impact of immunogenicity on any findings. Immunogenicity may reduce or eliminate exposure and thus reduce or eliminate drug-related effects. Development of an ADA can also result in hypersensitivity, which is discussed in more detail in the next section. Correlation of exposure and ADA data with findings in individual animals may be necessary to reach correct interpretations [42]. It is important to note that the presence of high concentrations of the drug in serum or plasma usually interfere with the detection of an ADA against biotherapeutics [41]. Thus, an animal may test negative for an ADA if the biotherapeutic is still present in the serum or plasma, even if the ADA is present.

Hypersensitivity

Hypersensitivity is an undesirable reaction caused by the immune system. While hypersensitivity reactions are uncommon in toxicity studies of small molecules [46], they have

VetBooks.ir

proven to be a significant issue in toxicity studies of biotherapeutics, including those using NHPs [47]. In an internal review of approximately 40 biotherapeutics programs at Pfizer, approximately 35% of biotherapeutics programs had clinical or microscopic evidence of immunogenicity in general toxicity studies (Leach, unpublished data).

Hypersensitivity reactions were historically divided into types I (immediate, anaphylaxis), II (antibody-mediated cytotoxic reactions), III (immune complex-mediated reactions), and IV (delayed reactions) using the classification defined by Gell and Coombs [48,49]. It has been suggested more recently that this classification should be modified [50] (Table 16.4). Others have suggested that drug reactions are not easily categorized using the classic Gell and Coombs system, and instead they should be classified as (1) pseudoallergic reactions, (2) primarily antibody-mediated reactions, and (3) cell-mediated reactions [51,52]. The effector mechanisms of hypersensitivity involved in the Gell and Coombs type I, II, and III reactions are related to antibodies (although a variety of cells are also involved), whereas the mechanisms involved in type IV reactions are related primarily to cellular immune responses; all are related to immunogenicity. In contrast, pseudoallergic reactions do not involve specific recognition of the test article by the immune system [51,52]. Regardless of the classification system used, recognizing that hypersensitivity reactions involve multiple cell types interacting in concert with one another to produce an effect (such as antigen-presenting cells, $CD4^+$ T cells, and B cells interacting to produce antibodies) is important, and the reaction in any animal or group of animals may involve multiple mechanisms; for example, both type II and III reactions might occur.

When they occur in NHPs, hypersensitivity reactions can be quite variable between individual animals and between studies [47]. The genetic variability between individual NHPs may play a role in at least part of this variability. The most common types of hypersensitivity reactions in nonclinical toxicity studies of biotherapeutics, including those using NHPs, are related to development of antibody-mediated reactions, in particular type I and type III reactions [42,47]. Type I reactions may take the form of classical anaphylaxis, with effects occurring within minutes after dosing. However, effects often may not be observed for up to an hour or more after dosing. Whether such reactions are related to classic immunoglobulin E-mediated anaphylaxis, nonimmunoglobulin E-mediated mechanisms, other immune reactions, or combinations of these is not usually clear. Affected animals typically have clinical signs; if they are severe enough, elective euthanasia may be appropriate. Premedication with an antihistamine or other drug to block the immune response may reduce or prevent reactions. Data interpretation should consider the use of any drugs given to prevent these reactions. Alternative dosing regimens, such as more frequent dosing, may reduce these reactions [53]; such strategies do not confound study interpretation. In contrast, study interpretation may be confounded when attempting to control reactions using concurrent medications.

Immune complex-mediated injury related to deposition of immune complexes in tissues is the other hypersensitivity reaction most commonly observed in NHP toxicity studies [42,47]. The inciting immune complexes are typically believed to form from ADAs binding to the administered biotherapeutic. However, aggregated monoclonal antibodies with intact effector functions in the dosing solution could fix the complement upon administration and result in circulating immune complexes that could deposit in tissues [42]. Alternatively, the aggregates could lodge in tissues and activate the immune system in situ [54]. Either could result in immune complex disease, or other immunologically-mediated disease, without the need

TABLE 16.4 Classification of Hypersensitivity Reactions

	Hypersensitivity reaction type						
	Type I	**Type II**	**Type III**	**Type IVa**	**Type IVb**	**Type IVc**	**Type IVd**
Immune reactant	IgE	IgG1, IgG3, IgM	IgG	IFN-γ, TNF-α (Th1 cells)	IL-4, IL-5/IL-13 (Th2)	Perforin, Granzyme B, FasL (CTL)	CXCL8, GM-CSF (T cells)
Antigen	Soluble antigen	Cell or matrix-associated antigen	Soluble antigen	Antigen presented by APCs	Antigen presented by APCs	Antigen presented by target cells	Antigen presented by APCs
Effector cells	Mast cells	Complement, FcR^+ cells	Complement, FcR^+ cells	Macrophages	Eosinophils	$CD8^+$ T cells	Neutrophils
Examples	Anaphylaxis	Hemolytic anemia, thrombocytopenia	Vasculitis, serum sickness, Arthus reaction	Tuberculin reaction	Asthma, allergic rhinitis	Contact dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis	Acute generalized exanthematous pustulosis, Behçet disease

APC = antigen-presenting cell, CTL = cytotoxic T lymphocyte, GM-CSF = granulocyte-macrophage colony-stimulating factor, IFN = interferon, Ig = immunoglobulin, IL = interleukin, TNF = tumor necrosis factor. Adapted from Ref. [50]

VetBooks.ir

for development of ADAs. The most common findings in NHP toxicity studies related to immune complex disease are glomerular injury and vasculitis following deposition of immune complexes in these tissues [42,47]. Demonstrating the presence of immune complexes (i.e., monkey immunoglobulin and complement) in affected glomeruli or blood vessels to attempt to identify the cause of the finding(s) may be possible [47,55]. However, normal animals may also have detectable immune complexes, and a comparison with concurrent and historical controls is often useful to determine whether there is a drug-related increase present. In some cases, the deposition of immune complexes in glomeruli can result in clinical pathology findings such as proteinuria, hypoalbuminemia, and nephrotic syndrome [42,55]. In other cases, however, the glomerular injury does not result in significant clinicopathologic alterations despite the presence of morphologic changes. The drug can sometimes be detected in the microscopic findings using antibodies that detect the drug specifically, for example, using antibodies that detect the human biologic that do not cross-react with the NHP molecules (e.g., anticomplementarity-determining region antibodies, specific antihuman Fc antibodies, monkey-adsorbed antihuman immunoglobulin G) [55].

Regardless of the mechanism of hypersensitivity, distinguishing effects related to hypersensitivity from those that are related directly to the drug is critical. Because immunogenicity and hypersensitivity, at least against biotherapeutics, are not considered predictive of similar effects in humans [5,56] and typically are not used in determining risk to humans or setting dose concentrations in clinical trials. Erroneous interpretation of findings may lead to incorrect predictions of what may occur in humans. An incorrect interpretation regarding the translation of immunogenicity-related findings from NHPs to humans (i.e., believing the finding will occur in humans) could lead to inappropriate program termination. It is also the expectation of worldwide health authorities that, if a finding or set of findings in an animal is attributed to immunogenicity-related toxicity, a weight-of-evidence approach will be used to support this interpretation. Data should include, at a minimum, an attempt to correlate ADA-related effects to drug exposure and ADA measurements in the animal [41], although the presence of the drug may prevent detection of ADAs. Additional information might be collected and include correlative clinical pathology parameters (e.g., complement split products); immunohistochemistry to assess to colocalization of drugs, ADAs, and complement products in suspect lesions; and/or electron microscopy to confirm the presence of complexes detected during microscopic evaluation [41]. Experience has shown that the correlation between ADAs and findings within individual animals can be somewhat variable, and scientists should evaluate all data from all animals to attempt to reach the most accurate overall study interpretation [47,53]. It is also important to recognize that non-ADA-related mechanisms of immunologic injury can occur.

CONCLUSION

NHPs are frequently used in nonclinical toxicity testing of biotherapeutics. The decision to use NHPs should be made after an NHP species has been determined to be pharmacologically relevant and appropriate to use in the development program. A determination of species relevance should be based on information in the literature, as well as in silico, in vitro, ex vivo, and in vivo data. It should not be assumed that a NHP species is the most relevant based on phylogenetic closeness to humans; the selection should be made on a case-by-case

basis after careful consideration of all the available data. When an NHP species is considered relevant and appropriate, the cynomolgus monkey is the most widely used in nonclinical toxicity testing. There is a deep understanding of the physiology and biology of this species, as well as a robust historical database; cynomolgus monkey data have been successfully used to support clinical trials throughout the world. The common marmoset is also used occasionally in nonclinical testing, whereas the rhesus monkey is used only rarely.

There are many well-developed regional and international guidelines to help guide nonclinical development using NHPs. The most important are the ICH guidelines, which cover a variety of nonclinical topics including species selection, study design, timing of studies relative to clinical trials, and specialty studies including safety pharmacology, DART, and carcinogenicity studies. Because NHPs are most frequently used in programs testing biotherapeutics, one of the most notable ICH guidelines related to NHP use is S6(R1), which covers all aspects of the nonclinical development of biotherapeutics. ICH S6(R1) also gives advice specific to nonclinical strategies using NHPs either in conjunction with rodents or as the sole species in a nonclinical program. When using NHPs, studies can be designed to use as few animals as possible while still gathering robust nonclinical safety data to support entry into humans and the ongoing clinical development of a new drug candidate.

References

[1] Zühlke U, Weinbauer G. The common marmoset (*Callithrix jacchus*) as a model in toxicology. Toxicol Pathol 2003;31:123–7.
[2] Buckley LA, Chapman K, Burns-Naas LA, Todd MD, Martin PL, Lansita JA. Considerations regarding nonhuman primate use in safety assessment of biopharmaceuticals. Int J Toxicol 2011;30:583–90.
[3] Bussiere JL. Species selection considerations for preclinical toxicology studies for biotherapeutics. Expert Opin Drug Metab Toxicol 2008;7:871–7.
[4] Chapman K, Pullen N, Coney L, Dempster M, Andrews L, Bajramovic J, et al. Preclinical development of monoclonal antibodies: considerations for the use of non-human primates. MAbs 2009;1:505–16.
[5] ICH. ICH S6(R1). Preclinical safety evaluation of biotechnology-derived pharmaceuticals; 2011. www.ich.org/products/guidelines/safety/article/safety-guidelines.html.
[6] Leach MW, Halpern WG, Johnson CW, Rojko JL, MacLachlan TK, Chan CM, et al. Use of tissue cross-reactivity studies in the development of antibody-based biopharmaceuticals: history, experience, methodology, and future directions. Toxicol Pathol 2010;38:1138–66.
[7] Loberg LI. Case study: off target toxicity of an anti-amyloid beta antibody for Alzheimer's disease immunotherapy. In: Presented at "A future vision for nonclinical protein-based biotherapeutic development", Washington, DC, June 9-10, 2014; 2014.
[8] Rudmann DG, Page TJ, Vahle JL, Chouinard L, Haile S, Poitout F, et al. Rat-specific decreases in platelet count caused by a humanized monoclonal antibody against sclerostin. Toxicol Sci 2012;125:586–94.
[9] Santostefano MJ, Kirchner J, Vissinga C, Fort M, Lear S, Pan W-J, et al. Off-target platelet activation in macaques unique to a therapeutic monoclonal antibody. Toxicol Pathol 2012;40:899–917.
[10] Bussiere J, Martin P, Horner M, Couch J, Flaherty M, Andrews L, et al. Alternative strategies for toxicity testing of species-specific biopharmaceuticals. Int J Toxicol 2009;28:230–53.
[11] Oflazoglu E, Audoly LP. Evolution of anti-CD20 monoclonal antibody therapeutics in oncology. MAbs 2010;2:14–9.
[12] European Union (EU) European Public Assessment Report (EPAR). MabThera: scientific discussion. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Scientific_Discussion/human/000165/WC500025817.pdf; 2005.
[13] US FDA. The applicability of good laboratory practice in premarket device submissions: questions and answers—draft guidance for industry and food and drug administration staff. www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm366338.html; 2013.

[14] ICH. ICH M3(R2). Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. www.ich.org/products/guidelines/multidisciplinary/article/multidisciplinary-guidelines.html; 2009.
[15] Clarke J, Hurst C, Martin P, Vahle J, Ponc R, Mounho B, et al. Duration of chronic toxicity studies for biotechnology-derived pharmaceuticals: is 6 months still appropriate? Regul Toxicol Pharmacol 2008;50:2–22.
[16] ICH. ICH M3(R2) Q&A(R2). Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals questions and answers. www.ich.org/products/guidelines/multidisciplinary/article/multidisciplinary-guidelines.html; 2011.
[17] Pandher K, Leach MW, Burns-Naas LA. Appropriate use of recovery groups in nonclinical toxicity studies: value in a science-driven case-by-case approach. Vet Pathol 2012;49:357–61.
[18] ICH. ICH S7B. The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. www.ich.org/products/guidelines/safety/article/safety-guidelines.html; 2005.
[19] Vargas HM, Bass AS, Breidenbach A, Feldman HS, Gintnat GA, Harmer AR, et al. Scientific review and recommendations on preclinical cardiovascular safety evaluation of biologics. J Pharmacol Toxicol Methods 2008;58:72–6.
[20] Leach MW, Smith MO, Cartwright ME, Mirro E, Buono T, Kornbrust D. Use of multi-modality electrophysiological testing to monitor central nervous system function in cynomolgus monkeys (*Macaca fascicularis*). Poster presented at the 2001 Society of Toxicology Annual Meeting, San Francisco, California, March 25–29. Toxicol Sci (Suppl) 2001;60:322.
[21] ICH. ICH S5(R2). Detection of toxicity to reproduction for medicinal products and toxicity to male fertility, www.ich.org/products/guidelines/safety/article/safety-guidelines.html; 2005.
[22] Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicity studies in the nonhuman primate model. Toxicology 2003;185:221–7.
[23] Henck JW, Hilbish KG, Serabian MA, Cavagnaro JA, Hendrickz AG, Agnish ND, et al. Reproductive toxicity testing of therapeutic biotechnology agents. Teratology 1996;53:185–95.
[24] Jarvis P, Sritasta S, Vogelwedde E, Stewart J, Mitchard T, Weinbauer GF. The cynomolgus monkey as a model for developmental toxicity studies: variability of pregnancy loses, statistical power estimates, and group size considerations. Birth Defects Res (Part B) 2010;89:1750–87.
[25] Weinbauer GF, Fuchs A, Niehaus M, Luetjens CM. The enhanced pre- and postnatal study for nonhuman primates: update and perspectives. Birth Defects Res C Embryo Today 2011;93(4):324–33.
[26] Weinbauer GF, Luft J, Fuchs A. The enhanced pre- and postnatal development study for monoclonal antibodies. Methods Mol Biol 2013;947:185–200.
[27] Martin PL, Breslin W, Rocca M, Wright D, Cavagnaro J. Considerations in assessing the developmental and reproductive potential of biopharmaceuticals. Birth Defects Res (Part B) 2009;86:176–203.
[28] Simister NE. Placental transport of immunoglobulin G. Vaccine 2003;21:3365–9.
[29] Fujimoto K, Terao K, Cho F, Honjo S. The placental transfer of IgG in the cynomolgus monkey. Jpn J Med Sci Biol 1983;36:171–6.
[30] Pentsuk N, van der Laan JW. An interspecies comparison of placental antibody transfer: new insights into developmental toxicity testing of monoclonal antibodies. Birth Defects Res (Part B) 2009;86:328–44.
[31] Bowman CJ, Chmielewski G, Oneda S, Finco D, Boucher M, Todd M. Embryo-fetal developmental toxicity of figitumumab, an anti-insulin-like growth factor-1 receptor (IGF-1R) monoclonal antibody in cynomolgus monkeys. Birth Defects Res (Part B) 2010;89:326–38.
[32] Bussiere JL, Pyrah I, Boyce R, Branstetter D, Loomis M, Andrews-Cleavenger D, et al. Reproductive toxicity of denosumab in cynomolgus monkeys. Reprod Toxicol 2013;42:27–40.
[33] Smedley JV, Bailey SA, Perry RW, O'Rourke CM. Methods for predicting sexual maturity in male cynomolgus macaques on the basis of age, body weight, and histologic evaluation of the testes. Comtemp Top Lab Anim Sci 2002;41:18–20.
[34] EMA. Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins. EMA/CHMP/BMWP/14327/2006. London, UK: EMA; 2007.
[35] EMA. Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use. EMA/CHMP/BMWP/86289/2010. London, UK: EMA; 2012.
[36] US FDA. Guidance for industry: immunotoxicology evaluation of investigational new drugs. US Food and Drug Administration, Center for Drug Evaluation and Research; 2002.

VetBooks.ir

[37] Caldwell R, Guirguis M, Kornbrust E. Evaluation of various KLH dosing regimens for the cynomolgus monkey TDAR assay. In: Assay abstract 1723, 2007 SOT annual meeting, Charlotte, NC; 2007.
[38] Zuehlke U, Srivastav S, Stanley MA, Friedhelm V, Korte R, Mueller W, et al. Cynomolgus monkeys of Mauritian origin: reference data and comparison to Chinese/Vietnamese animals. In: Weinbauer GF, Vogel F, editors. Novel approaches towards primate toxicology. Munster, Germany: Waxman Munster; 2006. p. 185–201.
[39] ICH. ICH S1A. Need for carcinogenicity studies of pharmaceuticals. www.ich.org/products/guidelines/safety/article/safety-guidelines.html; 1995.
[40] Valhe JL, Finch GL, Heidel SM, Hovland Jr DN, Ivens I, Parker S, et al. Carcinogenicity assessments of biotechnologically-derived pharmaceuticals: a review of approved molecules and best practice recommendations. Toxicol Pathol 2010;38:522–53.
[41] Ponce R, Abad L, Amaravadi L, Gelzleichter T, Gore E, Green J, et al. Immunogenicity of biologically-derived therapeutics: assessment and interpretation of nonclinical safety studies. Regul Toxicol Pharmacol 2009;54:164–82.
[42] Leach MW. Differences between protein-based biologic products (biotherapeutics) and chemical entities (small molecules) of relevance to the toxicologic pathologist. Toxicol Pathol 2013;41:128–36.
[43] Randolph JF, Stokol T, Scarlett JM, MacLeod JN. Comparison of biological activity and safety of recombinant canine erythropoietin with that of recombinant human erythropoietin in clinically normal dogs. Am J Vet Res 1999;60:636–42.
[44] Verhelst D, Rossert J, Casadevall N, Krüger A, Eckardt KU, Macdougall IC. Treatment of erythropoietin-induced pure red cell aplasia: a retrospective study. Lancet 2004;363:1768–71.
[45] US FDA. Draft guidance for industry: immunogenicity assessment for therapeutic protein products. US Food and Drug Administration, Center for Drug Evaluation and Research; 2013.
[46] Kawabata TT, Piccotti JR. Non-clinical testing approaches for drug development: possibilities and limitations. In: Pichler WJ, editor. Drug hypersensitivity. Basel: Karger; 2007. p. 140–50.
[47] Leach MW, Rottman JB, Hock MB, Finco D, Rojko JL, Beyer JC. Immunogenicity/hypersensitivity of biologics. Toxicol Pathol 2014;42:293–300.
[48] Coombs RRA, Gell PGH. Classification of allergic reactions responsible for drug hypersensitivity reactions. In: Coombs RAA, Gells PGH, editors. Clinical aspects of immunology. 2nd ed. Philadelphia, PA: Davis; 1968. p. 575–96.
[49] Gell PGH, Coombs RRA. The classification of allergic reactions underlying disease. In: Gell PGH, Coombs RRA, editors. Clinical aspects of immunology. London, UK: Blackwell Science; 1963.
[50] Pichler WJ. Drug hypersensitivity reactions: classification and relationship to T-cell activation. In: Pichler WJ, editor. Drug hypersensitivity. Basel, Switzerland: Karger; 2007. p. 168–89.
[51] Descotes J, Choquet-Kastylevsky G. Gell and Coombs's classification: is it still valid? Toxicology 2001;158:43–9.
[52] Kuper CF, Ruehl-Fehlert C, Elmore SA, Parker GA. Immune system. In: 3rd ed. In: Haschek WM, Rousseaux CG, Wallig MA, Bolon B, Ochoa R, editors. Haschek and Rousseaux's handbook of toxicologic pathology, vol. 3. London, England: Elsevier; 2013. p. 1795–862.
[53] Rohde CM, Markiewicz V, Thibault S, Besteman E, Clarke DW, et al. Impact of dosing frequency on anaphylaxis in cynomolgus monkeys administered TRU-015, an anti-CD20 SMIP™ therapeutic. In: Poster presented at the 2012 society of toxicology annual meeting, San Francisco, California, March 11–15; 2012.
[54] Demeule BL, Lawrence MJ, Drake AF, Gurny R, Arvinte T. Characterization of protein aggregation: the case of a therapeutic immunoglobulin. Biochim Biophys Acta 2007;17774(1):146–53.
[55] Heyen JR, Rojko J, Evans M, Brown TP, Bobrowski WF, Vitsky A, et al. Characterization, biomarkers, and reversibility of a monoclonal antibody-induced immune complex disease in cynomolgus monkeys (*Macaca fascicularis*). Toxicol Pathol 2014;42:765–73.
[56] Bugelski PJ, Treacy G. Predictive power of preclinical studies in animals for the immunogenicity of recombinant therapeutic proteins in humans. Curr Opin Mol Ther 2004;6:10–6.

VetBooks.ir

CHAPTER

17

Alternatives to the Use of Nonhuman Primates in Regulatory Toxicology

Georg Schmitt, Paul Barrow, Markus Stephan-Gueldner

F. Hoffmann-La Roche Ltd., Basel, Switzerland

OUTLINE

Introduction 337

Conventional Nonrodent Species as Alternatives to NHPs 339
- *Minipigs/Pigs* 340
- *Dogs* 343
- *Rabbits* 345
- *Ferrets* 346
- *Sheep and Goats* 347
- *Cats* 348
- *Tree Shrews* 348

Conventional Rodent Species as Alternatives to NHPs 349

Genetically Modified Rodent or Nonrodent Species as Alternatives to NHPs 350

Specific Animal Models for Targets Only Present in Humans/Human Disease 351

Alternative Lower-Order Species 352

Omission of Regulatory Toxicity Animal Studies If No Suitable Models Exist (Alternatives to In Vivo Testing) 352

References 352

INTRODUCTION

For the development of new medicines, nonclinical safety assessment is required to identify potential risks for patients. Nonrodent animal models often are used to satisfy scientific and regulatory needs, particularly when it comes to predicting human safety outcomes (i.e., regulatory toxicology). While there is no best and universal animal model, the choice of an appropriate species is multifaceted and complex. In general, a balanced decision primarily

http://dx.doi.org/10.1016/B978-0-12-417144-2.00017-2
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

reflecting scientific, ethical, and practical aspects is needed. For regulatory toxicology in nonclinical research and the development of therapeutic molecules, the selected species should ideally show a pharmacodynamic, pharmacokinetic, and metabolic profile similar to humans. For biopharmaceuticals, adverse effects are mainly related to exaggerated pharmacology, and therefore any appropriate animal species needs to show pharmacological activity similar to humans. Potential immunogenicity is an additional key consideration in species selection. For example, humanized proteins are more likely to cause an immune reaction in lower-order species than in nonhuman primates (NHPs), potentially resulting in the formation of anti-drug antibodies (ADAs) with downstream neutralization of the molecule or ill effects. Ideally, a model species will display extensive sequence homology with the human target, allowing the assessment of pharmacodynamic and toxic effects at the same time. In that case establishing a therapeutic window within the selected species is possible. For further information see the work by Smith and Hubrecht [1] and Smith and Trennery [2].

In recent years the number of NHPs, particularly cynomolgus monkeys, used in regulatory toxicity testing has been constantly increasing [3]. Public perception, however, strongly disfavors the laboratory use of NHPs. Scientists are thus obliged to seek alternative models whenever possible. This is particularly the case in regulatory toxicology because of the large numbers of animals used. Regardless of these considerations, regulatory bodies insist that the most appropriate and feasible animal models for the detection of human hazards, and extrapolation of risks to humans, must be used.

A close phylogenetic proximity to the human represents an important aspect in the choice of an animal model species. Although NHPs by definition are the closest relative to the human among all feasible test species, this does not mean that the NHP is necessarily the best model for medical research and safety testing in all circumstances. van Meer et al. [4] concluded that the value of extensive NHP use in routine safety and efficacy studies for nonclinical assessment of monoclonal antibodies (mAbs) is scientifically questionable. Nevertheless, there are some human anatomic and physiological features shared only with other primates, ruling out the use of alternative lower-order species in some circumstances (e.g., the macula is present only in the eyes of primates, so pigs, dogs, cats, rabbits, and rodents are not appropriate for the efficacy and safety testing of many ophthalmologic medicines).

NHPs should be selected only if unequivocally necessary and once all other possible avenues of potential alternatives have been explored. Such alternatives include:

- other conventional nonrodent or rodent species
  - those that may show a pharmacologic response to the human therapeutic molecule
  - those for which a species-specific surrogate molecule may be available
  - those in which an immune response to the therapeutic molecule may be mitigated
- genetically modified nonrodent or rodent species (e.g., humanized species)
- specific animal models (e.g., of a disease) for targets present only in humans or human disease
- omission of animal studies if no suitable models exist.

In this chapter we describe options for the selection of species/alternative approaches that can replace the NHP as a model in biomedical research and development (R&D). While concentrating on the toxicology of human pharmaceuticals, similar principles and thinking may apply to food additives, chemicals, veterinary medicines, and medical devices.

CONVENTIONAL NONRODENT SPECIES AS ALTERNATIVES TO NHPs

In principle, any animal species, rodent or nonrodent, that is scientifically valid and practically feasible may be chosen for biomedical research experiments instead of NHPs. The term *nonrodent* is not a taxonomic order and simply describes mammalian species that do not belong to the biologically defined order of rodents (Rodentia). The evolutionary tree of the placental mammal is still developing and numerous key aspects of relationships remain controversial [5]. The tree shown in Figure 17.1 is an example.

Besides the NHP, the most commonly used nonrodent species in nonclinical R&D are dogs, minipigs/pigs, and rabbits. Other nonrodent species, including ferrets, sheep, goats, cats, and tree shrews, are used rarely if at all and are unlikely to play a future role in replacing the NHP as a model in regulatory safety testing. The guinea pig is not discussed here because there are differing views of whether it is actually a nonrodent, and although this species is used in some areas of research (e.g., as a model in developmental and reproductive toxicology), the guinea pig clearly plays a negligible role overall in safety testing and particularly in replacing the NHP.

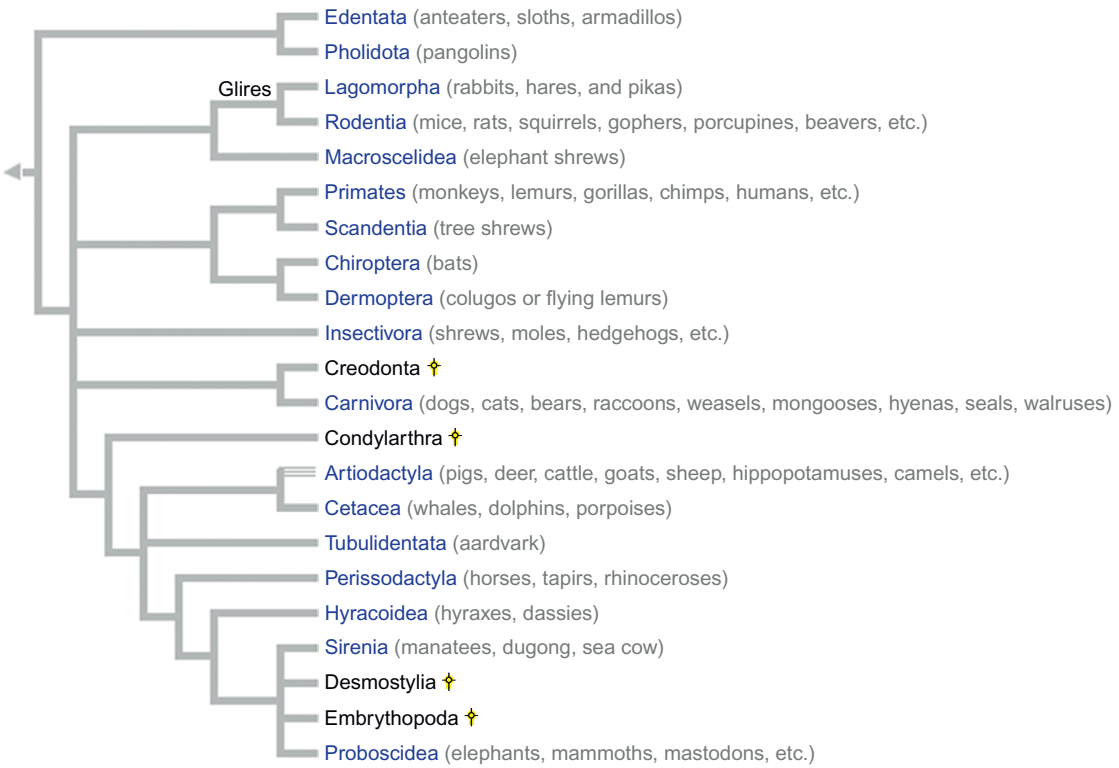


FIGURE 17.1 Example for an evolutionary tree of placental mammalians. *Source:* http://www.tolweb.org/Eutheria/15997.

VetBooks.ir

Exclusive target cross-reactivity and manageable immunogenicity are the major reasons for using the NHP rather than other species to test biopharmaceuticals. The use of species-specific surrogates has been applied to overcome the absence of target cross-reactivity. This may allow a new biopharmaceutical to be tested in species other than the NHP. However, stringent criteria need to be applied for surrogates to ensure sufficient similarity to the human therapeutic molecule. A surrogate typically recognizes the target in the selected animal species, which is analogous to the human target, and allows the selected animal species to express a pharmacologic response similar to the original molecule in the human. Careful consideration is needed whenever safety data are generated using a surrogate because they may not fully translate to the clinical candidate, for example, because of differences in quality characteristics, different production methods, and lack of dose equivalency between the human/humanized and surrogate homologous proteins. Depending on the characteristics of the biopharmaceutical, immunogenicity may be an issue and require thoughtful consideration. Because of a high degree of target sequence homology, biopharmaceuticals are often less immunogenic in NHPs than in any other animal species. Immunogenicity can sometimes also be managed in rodents or rabbits. If ADAs are produced in treated animals in response to administration of the biopharmaceutical, this may neutralize the pharmacologic activity and/or influence the pharmacokinetic characteristics of the biopharmaceutical. For human-specific proteins, this is actually a limiting aspect for all animal species, including NHPs. If the pharmacokinetic and/or pharmacodynamic impact of the ADAs can be overcome, by dosing through the immune reaction, for instance, the pertinence of the model will not be reduced.

Minipigs/Pigs

The pig (*Sus scrofa*) belongs to the order of Artiodactyla, the even-toed ungulates, and is a member of the *Suidae* family. Domesticated pigs, including landrace and miniature races, have their origin in the Eurasian wild boar. The main centers of domestication were Europe and China. In the middle of the last century, smaller miniature races, called mini- and micropigs, were developed. There are multiple miniature breeds, some of them of native (e.g., Yucatan minipig) and others of crossbred origin (e.g., Göttingen minipig). Although the term *micropig* does not necessarily reflect a body size and weight inferior to that of a minipig, some micropig breeds indeed have a smaller body size and weight than the commonly used Göttingen minipig.

Pigs (including the minipig) share many anatomic and physiological similarities with humans, making them remarkably good in vivo models. As true omnivores, pigs properly chew food and have a digestive system that is similar to that of humans. As scavenging animals, pigs are also known for their excellent olfactory sense, reflected by a large number of functional olfactory receptor genes [6], which humans exploit in many ways (e.g., for locating truffles). Because of their ready availability and similar size to humans, landrace pigs have often been used as an anatomic model and for the assessment and refinement of surgical techniques. Pigs have more recently been genetically modified to produce organs for human transplantation. They are also a major target species for veterinary drugs. The teeth of pigs have an enamel coating, like human teeth, which makes the pig a good model for dental research. The pathology of the minipig is well studied and the common background lesions are well documented. These lesions are generally minimal and do not hinder the identification

VetBooks.ir

of compound-related effects and hazard signals [7]. Although the Göttingen minipig has a whitish skin color and is sensitive to sunburn, it is not albino; melanin is present in skin and retina [8].

The sequencing of the genome of common landrace and minipig breeds has extended their potential use in R&D [9–11]. The pig genome is only about 7% smaller than the human genome, showing extensive homology [9]. By far the genomically best-characterized miniature pig breed is the Göttingen minipig, for which the genome was recently sequenced, further enhancing its usefulness as a model species. Tissue gene expression profiles of Göttingen minipigs and humans also are very similar. Recent data show that the Göttingen minipig has little genomic variability but significantly higher gene expression differences among individuals than cynomolgus monkeys (U. Certa, unpublished data). Specific microarrays for minipig gene expression and copy number variations are also available and will deepen the understanding and thus improve the translational value of this species.

The use of pigs for laboratory research is generally viewed much more favorably by the general public than the use of dogs. For this reason, the minipig is likely to increase in popularity as a laboratory species as it gradually replaces the dog. We estimate that a significant proportion of therapeutic development projects (mainly small-molecule drugs) that currently use the NHP or dog could actually be adequately conducted in the minipig instead. Public perception, however, is not logically founded; the pig deserves exactly the same ethical consideration as any other species.

Today, the Göttingen minipig is by far the porcine breed most used in safety testing, reflected by worldwide breeding capacities in Europe, the USA, and Japan to satisfy the increasing needs. Over the years, a large database of physiological and historical data has been accumulated worldwide, though mostly for small-molecule drug testing. Experience with small molecules from various chemical classes shows that the minipig often has a similar metabolism to that of the NHP and dog. It should be noted, however, that delayed gastrointestinal absorption, atypical pharmacokinetic profiles, and low bioavailability may be encountered.

The minipig may be a suitable species for the testing of biopharmaceuticals, including peptides, enzymes, hormones, therapeutic antibodies, and oligonucleotides [12]. Yet the use of minipigs in the development of biopharmaceuticals is still in its infancy. Although a number of peptides and proteins have been successfully tested in the Göttingen minipig, no complete safety programs with mAbs have been published so far. The main reasons for this are the current knowledge and technology gaps with regard to minipig immunology. While the pig immune system generally has a structure and function analogous to the human immune system, there are also species-specific features (e.g., inverted morphology of the lymph nodes). Tissue banks of the minipig are available, and tissue cross-reactivity studies are feasible. Likewise, immune function testing in minipigs (e.g., keyhole limpet hemocyanin assay) is largely established. From a pure genetic perspective, key immune genes such as immunoglobulin (Ig), T-cell receptor, major histocompatibility complex, and natural killer cell loci have been characterized in detail [6]. Yet the biological role of cytokine release and the Fc gamma receptor is currently not clear, and effector function may thus be different to that of humans and NHPs. Future research in this area will investigate the pertinence of the minipig for the safety testing of biopharmaceuticals. A recent demonstrative study of anakinra showed that the predictive value of the minipig for immunogenicity testing is comparable to that of NHPs [13].

VetBooks.ir

In addition to their biological similarity to humans, pigs—especially minipigs—offer many practical advantages over other nonrodent models, namely dogs and NHPs. Minipigs reach sexual maturity much faster than dogs or NHPs. Although initial publications refer to sexual maturity at the ages of 3-4 (male) and 4-5 months (females) [14], more recent data indicate full sexual maturity is reached at 5 and 7.5 months of age, respectively [15]. An often-used argument against the use of the minipig is the larger amounts of test material required than for the NHP or dog because of their larger body size. In fact, the overall amount of test material required for a therapeutic molecule development program is not substantially different between minipigs and dogs in the early stages of development because of the earlier sexual maturity of the minipig (i.e., at the start of sexual maturity, the dog and minipig have comparable body weights). At the later stages of pharmaceutical development, the compound needs are generally less of a problem; production methods have been scaled up to meet clinical needs and deliver a larger supply of the test material. Administration procedures in the minipig are usually more laborious and require more skilled technical staff than in dogs and even NHPs. This is particularly true for intravenous administration because of the absence of easily accessible peripheral veins and for oral capsule administration.

Probably the strongest ethical argument for the use of the pig/minipig over the dog and NHP is their easy adaptation to laboratory housing; they require smaller spaces and have less need for exercise. Existing dog facilities can generally easily be adapted to house minipigs as well. As foraging animals with highly developed exploratory behavior, the housing environment of pigs needs to be sufficiently enriched and varied to avoid stereotypies. A potential downside to the use of the pig/minipig are restrictions and regulations imposed to prevent the spread of contagious diseases among livestock (e.g., a swine flu epidemic may prevent the transport of minipigs). In addition to the highly increased use of toxicological testing in human therapeutic molecule development, the pig also has been invaluable in the development of gene therapies [16], vaccines, veterinary pharmaceuticals, food additives, and medical devices. While pigs have often been successfully used in hormonal and dermal testing, the development of porcine disease models in indication areas such as diabetes, arteriosclerosis, osteoporosis, and wound healing will likely continue to add value to the utility of this species for safety testing.

The RETHINK project (funded by the European 6th Framework Programme) evaluated the impact of toxicity testing in the minipig and came to the conclusion that "the minipig presents a favorable profile as a non-rodent toxicology model, in terms of similarity to man and also in terms of applicability to different study types," and "the minipig might be a better non-rodent model than the dog" [14]. A more recent publication concluded that "minipigs/pigs present a viable and perhaps more reliable model of non-rodent testing" [17].

Beyond general (repeat-dose) toxicity testing, the minipig also has been successfully used in developmental and juvenile toxicity testing, in drug metabolism and pharmacokinetic studies, and in safety pharmacology. Some species specificities are highlighted.

The minipig has gained regulatory acceptance for developmental toxicity testing. The anatomy of the swine placenta differs from that of the human and other primates, which may influence its susceptibility to teratogens. It is also assumed that mAbs are less readily transported across the porcine placenta to reach the conceptus than in humans and NHPs. This assumption is based on the observation that maternal antibodies (IgGs) are not transferred to the piglet before birth, as in primates, but are transmitted postnatally via milk [18]. Whether

VetBooks.ir

this assumption holds true for humanized biopharmaceuticals remains undetermined. The porcine placenta is diffuse and spread over the surface of the uterus. It is epitheliochorial in structure, with six persistent layers of tissue (three maternal and three embryonic) separating the maternal and embryonic circulations. By contrast, humans and NHPs have a hemochorial placenta with three layers of separation, the three maternal layers having disintegrated. Rodents and rabbits also have a hemochorial placenta, which develops during the later stages of gestation. This difference in placental morphology may render the pig more or less sensitive to teratogenic agents than humans. Despite the apparent differences in the thickness and permeability of the placental barrier between species, however, all have evolved to fulfill the same function, that is, ensure the nutrition of the developing embryo. In practice, pharmacokinetic investigations of embryo-fetal exposure rarely reveal any significant differences between NHPs, rodents, rabbits, or minipigs. It is possible that the surface area of exchange counterbalances the influence of the permeability of the barrier.

To date the minipig has been infrequently used for juvenile toxicity studies, though it offers many advantages over other nonrodent species [19]. Even if the general toxicology package has been performed in NHPs, the investigator has every interest in finding an alternative species for juvenile toxicity studies (i.e., the rodent or minipig). Minipigs can be of value for various types of safety pharmacology studies. In cardiovascular telemetry studies, the effect of feeding and the long postprandial phase on the heart rate is significant. The long QT interval and the specificities of minipig electrocardiography waveforms also need to be taken into account [20]. Reliable blood pressure measurements in the minipig usually require invasive methods because the tail and limb anatomy is not well suited to reliable cuff measurements. Also, pigs are prone to stress-related changes in blood pressure [21].

The recent availability of genetically modified minipig/pigs, such as transgenic and knockout models, has resulted in many new applications of this species. In addition to porcine disease models, pigs have provided donor tissues in xenotransplantation models, for example, for type 1 diabetes [22]. This approach has the potential to provide the only relevant nonrodent model for the safety assessment of some novel categories of biologics, such as bispecific human antibodies (see also below).

Overall, nonclinical R&D using the pig, and primarily the Göttingen minipig, has gained considerable importance over the past two decades. The minipig is now an established and indispensable nonrodent model, in particular for safety testing of pharmaceutical products, and is fully accepted by regulatory authorities such as European Medicines Agency and US Food and Drug Administration. The increased demand for the minipig as a model is reflected by the many minipig facilities that have been established within the pharmaceutical industry and by contract research organizations.

The following are recommended as further reading related to the minipig: McAnulty et al., *The Minipig in Biomedical Research* (Boca Raton, FL: CRC Press; 2011); Swindle, *Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques*, 2nd edition (Boca Raton, FL: CRC Press; 2007); and Bode et al. [14].

Dogs

The domestic dog (*Canis lupus familiaris*) belongs to the order of Carnivora, which means that the gastrointestinal tract is adapted to eating meat. They are members of the *Canidae*

VetBooks.ir

family. Dogs have been domesticated for at least 15,000 years. In Western cultures it is not considered acceptable to eat "man's best friend," which may explain the public's sensitivity to the use of dogs in research compared with pigs. Beagles have been moving toward the top of the animal rights agenda in the past few years.

The dog genome has been sequenced [10], and for many predicted human genes there was no convincing evidence of a corresponding dog gene [23]. Furthermore, polymerase chain reaction arrays are available to characterize the expression of key genes from various pathways to better characterize toxicity in the dog.

The beagle is the most common purpose-bred race used in research and nonclinical safety testing, with large breeding facilities worldwide to satisfy demand. A large part of modern internal medicine relies on knowledge gained from dogs. Examples of the crucial role that dogs have played in medical advances include the development of electrocardiography, heart transplant surgery, and treatments for prostate cancer. Beagles are of medium size, short-haired, sufficiently intelligent, easy to handle, even tempered, cooperative; they are well adapted to working with humans. Almost any procedure and study type can be done using dogs, including acute and repeat-dose, reproductive, juvenile, pharmacology, and safety pharmacology studies (including telemetry). Because of their ready availability, their lack of inherited health problems, the rich knowledge of their physiology, and the historical background data available, beagles have been used for decades and are still the most common nonrodent species used for the regulatory safety testing of small-molecule drugs. Beagles are also not very demanding with regard to space requirements and exercise compared with other dog breeds. Housing regulations have recently changed in Europe to require more exercise space than in other regions of the world. Finally, beagles are relatively cheap, most notably compared to the cost of NHPs.

Although there are multiple arguments in favor of the beagle as a nonrodent model, there are also some limitations to be considered. Beagles are oversensitive to some types of adverse effects. They are prone to vomiting, which can lead to insufficient exposure. Beagles may also develop lesions that are of no or little relevance to humans. For example, nonsteroidal anti-inflammatory drugs are often associated with gastrointestinal lesions in beagles at very low doses, which generally do not translate to humans at comparable levels of exposure [24]. Likewise, some vasodilators and antihypertensive drugs induce arteriopathy and cardiotoxicity at low exposures [25]. The mammary glands in the female dog are very sensitive to progestagens and have a high individual sensitivity to estrogens and antigestagens compared with other mammals, including humans [26]. Beagles also have shown dose-limiting toxicity due to pseudo-allergy following the administration of compounds causing histamine release. Thus their sensitivity to detergents, such as Cremophor and Tween, may render them unsuitable for the testing of some vehicle formulations [27]. The "beagle pain syndrome," a rare idiopathic syndrome seemingly of genetic origin and characterized by necrotizing arteritis, can be a complicating factor in the interpretation of results from toxicity studies [28]. A controversial, recently published analysis casts significant doubt on the universal applicability of the dog in predicting human toxicity and drug safety [29]. In 1992 at the International Workshop on Use and Misuse of the Dog in Experimental Toxicology in Zurich, Gerhard Zbinden criticized the historically derived tendency to automatically use the beagle as the nonrodent species in risk assessment of drugs and other chemicals.

VetBooks.ir

Although the dog is extensively used in small-molecule testing, its use in the field of large molecules is limited because of the general lack of cross-reactivity to human targets and because dogs share only low homology with the human Fc protein, making this species irrelevant for mAb testing [30]. Exceptions to the successful use of beagles in the field of novel/advanced therapies include gene therapy to successfully treat type 1 diabetes [31] and safety testing of the recombinant coagulation factor IX BeneFIX [32] and the recombinant glutamate carboxypeptidase, glucarpidase (Voraxaze) [33].

Genetically modified beagle models have only just become available [34]. A good recent overview of the classical use of the dog in biomedical research and safety testing was published by Hasiwa et al. [35].

Rabbits

The rabbit (*Oryctolagus cuniculus*) was originally classified as a rodent species, and only later was given its own family, *Leporidae*, in the taxonomic order of the Lagomorpha. Rabbits have been domesticated for centuries. Today, rabbits are bred by professional breeders as well as on a private basis for their meat and fur. Rabbits are easy and cheap to maintain and, with professional handling, a vast variety of procedures and study types using this species are technically feasible. A few particularities, however, must be mentioned. Rabbits hardly ever show obvious clinical symptoms other than changes in feces. Cases of necrosis in the stomach have been observed postmortem without any visible abnormal in vivo clinical symptoms of discomfort (G., Schmitt, personal observation). Rabbits are unable to vomit because of the restriction to striated muscle in the esophagus and stomach. They can tolerate only limited volumes of oily formulations orally (usually about 1 mL/kg body weight) because of their sensitive gastrointestinal tract. For the same reason, antibiotics interfering with the microbiological gut flora can generally not be tested in this species. Furthermore, rabbits may easily damage their spine when restrained improperly.

High-quality breeding facilities for rabbits exist worldwide, yet supply can be an issue, and regional shortages are common. The main uses of rabbits as the nonrodent species in nonclinical R&D are reproductive toxicity [36] and pyrogen testing. By far the most-used strain is the New Zealand white rabbit, but other strains such as the Himalayan and the pigmented Dutch-Belted rabbit are also used [37].

The rabbit is widely used in teratogenicity testing. The thalidomide catastrophe played a key role in establishing national and international guidelines calling for a second, nonrodent species to be used (in addition to a rodent, generally the rat). While thalidomide was first detected to be teratogenic in humans after having passed limited preclinical teratogenicity testing in rats, it was later shown to induce malformations in the New Zealand white rabbit as well. Consequently, since 1966, the standard for teratogenicity testing of small molecules in the chemical and pharmaceutical industries is a two-species paradigm (as for repeat-dose toxicity testing). For practical and economic reasons the rabbit is generally chosen as the nonrodent species for teratogenicity testing (whereas the dog, minipig, or NHP are commonly used in general toxicity studies). A large amount of historical data is available [38]. Fertility studies are rarely performed using rabbits but are feasible. In particular, sperm can be sampled manually, allowing repeated sampling for mechanistic male fertility examinations [39]. Postnatal examinations were rarely performed in the past because of the tendency

VetBooks.ir

of does to cannibalize their offspring in the laboratory. This difficulty has now been overcome and postnatal examinations of rabbits have become commonplace in the developmental testing of vaccines [40,41]. The transplacental transfer of imummoglobulins in the rabbit shows a profile similar to that of humans and other primates [42]. When testing humanized biopharmaceuticals, the dosing period in developmental toxicity studies using rabbits is much shorter than for NHPs because of a shorter gestation period, allowing less time for an immune response to develop.

Rabbits can also be used for juvenile toxicity studies and as in vivo models in local (skin) and ocular tolerance testing. Consequently, physiological and historical background data are also available for the rabbit in these fields of use. Despite these uses, rabbits have been used only rarely in general toxicity repeat-dose testing. There are a few examples of rabbits being used as a second species for general toxicity testing in the development of biopharmaceuticals (e.g., in addition to the dog for TNKase [43] and in addition to the NHP for Avastin [44]).

Another major use of the rabbit is in testing the safety of prophylactic and therapeutic vaccines. The rabbit is the species of choice for these studies because its size conveniently allows the administration of an entire human dose, without scaling for body weight, by the intramuscular or subcutaneous routes. The local, systemic, and immune effects of the vaccine are assessed following a course of vaccinations comprising the number of administrations given to the human plus one [45].

Laboratory rabbits are also commonly used for the testing of implanted materials [46] and for the production of polyclonal antibodies [47]. The cardiovascular system of rabbits shares some structural aspects with humans, making this species useful in cardiovascular research and safety pharmacology studies (e.g., Purkinje fiber tests).

The genome of the rabbit has not been completely sequenced yet (Rabbit Genome Project; [48]), but available data provide a wealth of sequence information for comparative genetics. Tools such as polymerase chain reaction arrays exist to characterize the expression of key genes from various pathways in response to toxic insult. The first transgenic rabbit was generated in 1985 [49]; since then, transgenic rabbits have been used as animal models for a variety of human diseases (e.g., atherosclerosis and cardiomyopathy) and as a source of diagnostic and therapeutic proteins (e.g., erythropoietin and insulin-like growth factor).

For further reading on the use of rabbits in research, the following references are recommended: Suckow et al., *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents*, Part II (New York: Academic Press; 2012), and Suckow and Douglas, *The Laboratory Rabbit*, a volume in the Laboratory Animal Pocket Reference Series (Boca Raton, FL: CRC Press; 1997).

Ferrets

Ferrets (*Mustela putorius furo*) are small carnivores and belong to the family *Mustelidae*. The romans used ferrets for hunting rabbits. Today, ferrets are widely accepted as pets. Handling and manual restraint of ferrets can be tricky, but most conventional methods of dosing are feasible.

Ferrets share many anatomic, metabolic, and physiological features with humans. They are used as experimental models in research involving cystic fibrosis, respiratory virological diseases such as sudden acute respiratory syndrome and influenza, lung cancer, endocrinology, and neuroscience (particularly neurological changes associated with brain and spinal cord

VetBooks.ir

injury). Ferrets' capacity to vomit—and their high sensitivity to it—makes this species the most widely used animal model in emesis research, particularly to detect the emetic potential of developmental candidates in oncology, as well as to test potential antiemetic compounds [50].

There are established ferret breeders, and the supply seems to cope with the current demand. Nevertheless, a broader use of this species would clearly require the production of virus-free animals.

The ferret genome was assembled in 2011 by the Broad Institute [51]. The first genetically engineered ferrets were described in 2008 [52] and further developed as a disease phenotype model of cystic fibrosis [53].A recent histologic investigation characterized the distribution of immune system cells in healthy ferret tissues to better understand pathological changes in this species [54].

Because of its small size and related relatively low requirement for test compounds, there has been renewed interest in the ferret as a nonrodent model in safety testing. An additional advantage of the ferret is its early sexual maturity (at about 6 months of age) compared with dogs and NHPs. Overall, the use of ferrets in toxicity testing has remained limited since its introduction a few decades ago [55,56]. Disadvantages of the ferret include a continued lack of extensive reference data, a low number of quality breeders, and limited availability. The low demand from the pharmaceutical industry is also reflected in the reluctance of contract research organizations to invest in ferret facilities [57].

Ferrets, like many carnivores, have a zonary, endotheliochorial placenta, as opposed to the discoid, hemochorial placenta in humans, NHPs, and rodents. However, like humans, the ferret does not have the inverted yolk sac placenta common to rodents and lagomorphs, making the ferret a feasible animal for teratogenicity testing [58], in particular for compounds that are teratogenic in rodents because of an action on the yolk sac [59]. The ferret is sensitive to some known human teratogens, such as ethyl alcohol, methyl mercury, thalidomide, vitamin A analogs, and alkylating anticancer agents. The ferret was used as the nonrodent species to demonstrate the teratogenicity of mechlorethamine hydrochloride [60].

To our knowledge, to date the ferret has never been used as the primary nonrodent species for the toxicity/safety evaluation of any marketed drug. This is the case despite the potential advantages of this species, in particular in indications where the ferret is a disease model (e.g., antiemesis). In such cases, its use as a toxicology model could deliver valuable translational information (i.e., a therapeutic window within the same species). Regulators are not used to seeing safety data gathered from ferrets, although the acceptance of such data should be a given, provided the species selection is justified and the study data are of high quality and meet international regulatory standards.

For further reading on use of the ferret in research, the following references are recommended: Matchett et al., *The Laboratory Ferret*, in the Laboratory Animal Pocket Reference Series (Boca Raton, FL: CRC Press; 2012), and Fox and Marini, *Biology and Diseases of the Ferret*, 3rd edition (Ames, IA: Wiley-Blackwell; 2014).

Sheep and Goats

Sheep (*Ovis aries*) and goats (*Capra aegagrus*) are closely related ruminant species belonging to the order of Artiodactyla in the family *Bovidae*. They are among the earliest animals domesticated by humans. In addition to being used for agricultural purposes (production

VetBooks.ir

of fleece, meat, milk, and cheese), sheep have been used as an exceptional model of human pregnancy [61]. Furthermore, sheep have been useful as a model of hypertension and heart failure in the development of the human drug Bosentan [62] and as a target species for the development of veterinary drugs [63]. In the USA, goats have become the main animal species used to train combat medics after the Pentagon phased out dogs for medical training in the 1980s. In 1996, Dolly became famous as the first mammal to be cloned from an adult somatic cell.

While housing and animal transport are established and comparably easy, the availability of laboratory-quality sheep and goats is virtually nonexistent. Furthermore, the lack of practical experience and background data in toxicology presents a serious drawback for the use of these species in safety testing. Consequently, sheep or goats have rarely been used as nonrodent species for the toxicity/safety evaluation of any marketed drug. One of the rare uses of the sheep in safety testing comes from studies of zinc in pregnant sheep [64], which is also referenced in the US Food and Drug Administration approval package for Galzin. Thus regulators are not accustomed to seeing safety data gathered from sheep or goats, although the acceptance of such data is probable, provided that the selection of species is justified and the study data are of high quality and meet international regulatory standards.

Cats

The cat (*Felis catus*) belongs to the *Felidae* family in the taxonomic order Carnivora. Cats are popular pets and companions. In the 20th century cats were often used as animal models based on ease of access (street catch). Today, purpose-bred cats are available for research in very limited numbers, and their use has decreased over recent decades.

Cats have been used as models to evaluate potential therapeutic treatment in various oncological areas such as sarcoma, carcinoma, lymphoma, myeloma, and leukemia. Because of multiple similarities to human breast cancer, feline mammary carcinoma is an important research model. Furthermore, cats and humans share several infectious diseases, such as toxoplasmosis and cholera, making the cat a good model for the development of effective therapies and preventive treatments. The feline acquired immunodeficiency syndrome (AIDS) virus is similar to the human immunodeficiency virus. Diabetic cats have proven to be useful models for the study of diabetes mellitus. In spite of its many advantages, however, nowadays the cat is rarely used as a model in safety testing and is unlikely to become a model in the future, largely because of public perception.

Tree Shrews

Tree shrews (*Tupaiidae* family) are widely distributed in south Asia, southeast Asia, and southwest China. They were originally described as Insectivora but nowadays belong to the order of Scandentia, which is close to primates based on the immediate phylogenic relationship and anatomic similarities (e.g., in the brain). The recently published genome of the Chinese tree shrew supports its close affinity to primates [65]. Tree shrews are small, omnivorous animals and have been used as an alternative to NHPs in experimental studies of myopia, depression, psychosocial stress, and hepatitis virus infections. Of note, their immune system shows multiple similarities to that of the human (e.g., the entire major histocompatibility

VetBooks.ir

complex region), and there is conservation of therapeutic targets and signaling pathways [65]. Overall, these characteristics suggest that the tree shrew could be a viable alternative to NHPs in biomedical research and safety testing [66]. Yet there are very few reliable sources of tree shrews for research purposes. The general lack of practical experience and of historical background information (e.g., the incidence of histopathological changes) is a clear hindrance to the selection of this species for regulatory toxicity testing. Overall, tree shrews are unusual models in safety testing and largely unknown to regulatory authorities, making any use without previous agreement a potential source of delays in drug development or even an obstacle for final market authorization.

CONVENTIONAL RODENT SPECIES AS ALTERNATIVES TO NHPs

Conventional rodent species that show the intended pharmacological response to the test item may play a role in nonclinical safety testing where, for instance, marked immunogenicity or exaggerated pharmacology of a biopharmaceutical prevents the use of an NHP and no other appropriate nonrodent species is available. The most relevant rodent species in this regard are the mouse and rat.

A conventional rodent species may be used if a species-specific surrogate molecule is available that allows human-relevant assessments (e.g., a mouse-specific antibody to test the safety of a human-specific antibody). The surrogate molecule needs to recognize the target in the selected rodent species and to induce a pharmacological response similar to that of the original molecule in the human. In the past, mAbs(e.g., Actemra, Cimzia) have been successfully evaluated for general as well as developmental and reproductive toxicity in rodents using surrogates, that is, a homologous murine molecule, thus avoiding the need to resort to the use of NHPs. These days, however, this approach is not readily accepted by regulatory authorities because of, for instance, the differences in quality characteristics, production methods, and the lack of dose equivalency between the humanized and murine homologous proteins. A surrogate approach, however, is applicable for antisense oligonucleotides in which only the nucleotide sequence has been modified to render the therapeutic molecule active in the rodent (most often the mouse). Since oligonucleotides are synthesized chemically, their chemical structure can be closely controlled to ensure that the chemical backbone is identical between the homologous proteins. Often, therapeutic antisense oligonucleotides are equally active in humans and various animal species, and can thus be tested directly in rodents and rabbits.

The rat is by far the most common species used for fertility testing, followed by the mouse [67]. NHPs are not well suited for this type of study because mating trials are not feasible [68]. Male rats produce much greater numbers of spermatozoa than humans and have a high reserve capacity. Despite this difference, the male reproductive system of the rat has been extensively studied and techniques have been developed to reliably predict effects on human male fertility [69]. The process of spermatogenesis between the rat and the human is remarkably similar. In fact, the duration of spermatogenesis in the rat (about 56 days) is closer to that of the human (64-74 days) than is that of the cynomolgus monkey (40-46 days). The female reproductive system differs significantly between primates and

VetBooks.ir

lower species. The monthly menstrual cycle, which is common to the cynomolgus monkey and the human, is the main characteristic that makes mating trials in NHPs impractical. The 4-day estrous cycle, high fertility, and rapid sexual maturity of the rat allows even multigeneration studies to be completed within a relatively short time. Because of the differences in the endocrine control of the reproductive organs, there may be no alternative to NHPs for the evaluation of some hormonal products. On the other hand, rodents are proposed as the best mammalian models to detect human endocrine-disrupting substances [70].

When pre- and postnatal studies are required for the registration of new pharmaceuticals, they are almost always performed using rodents. Certainly, if a rodent species has been successfully used for embryo-fetal development studies, there would be no reason to use a NHP model for the pre- and postnatal study. For biopharmaceuticals necessitating the use of NHPs for developmental toxicity testing, the current standard is to conduct an enhanced pre- and postnatal development study covering the objectives of both embryo-fetal and pre- and postnatal studies at the same time [71].

GENETICALLY MODIFIED RODENT OR NONRODENT SPECIES AS ALTERNATIVES TO NHPs

Transgenic models are created by genetic manipulation of a host species so that they carry exogenous genetic material or genes from another species in their genome. Knock-in and knockout animals have been genetically modified to over- or underexpress the protein coded by one or more genes. Genetically modified laboratory models have been developed to produce human proteins, model human physiology, or simulate a range of human diseases.

The mouse is the species of choice for genetic modification because its genome is well characterized and it is sufficiently similar to the human genome. Techniques that allow mouse cells and embryos to be altered comparably easily, quickly, and with high rate of success are available. Transgenic mice are therefore widely used in basic research and pharmacology and toxicology testing. The translatability to humans of the results gained using these models is in general reasonably well understood. The primary initial use of transgenic mouse models within toxicology was in the field of carcinogenicity. Transgenic mouse strains with deficient DNA repair mechanisms or overactive oncogenes are highly sensitive to carcinogens, allowing carcinogenicity tests to be performed much more rapidly than in conventional mice or rats (i.e., 6 months as opposed to 2 or 1.5 years). Transgenic animal models may also be used to reduce or replace nonrodent models, including the NHP, when the data generated allow an assessment of relevance to humans.

Transgenic immunotolerant mice have been generated and may be used to for the nonclinical safety testing of biopharmaceuticals that are immunogenic in other nonprimate models. For example, human immunoglobulin G-transgenic mice, tolerant to human immunoglobulin G, were successfully used as an animal model to assess the immunogenicity of engineered mAbs [72]. Even combination products including three humanized biopharmaceuticals can be tested using triple-transgenic mouse models [73].

Many biopharmaceuticals are designed to target protein sequences that are overexpressed in disease conditions. The target protein may nonetheless have a physiological role at lower

VetBooks.ir

levels in the healthy individual. For example, an mAb may target β-amyloid or phosphorylated tau protein for the treatment of Alzheimer disease. Neither protein is expressed at significant levels in adults of any routine laboratory species. Both proteins, however, seem to play a role—albeit at very low levels—in normal brain development [74]. Transgenic mouse models, for example, PS2APP, that express the human form of these proteins are available, offering a much more pertinent test system than NHPs. Genetically modified mice will probably be available for other research areas, such as human immunodeficiency virus, in the future.

Similarly, in situations where the therapeutic target of interest is specific to humans (i.e., absent in any conventional animal species, including NHPs) transgenic animals may offer a unique possibility to introduce the target of interest into a selected animal model. These "humanized animals" may allow efficacy as well as safety testing in an animal model. Nonrodent transgenic models, including humanized minipigs, can be produced today and may provide relevant targeted models for the safety assessment of some novel categories of biopharmaceuticals, such as bispecific human antibodies.

SPECIFIC ANIMAL MODELS FOR TARGETS ONLY PRESENT IN HUMANS/HUMAN DISEASE

Certain diseases or disease conditions (e.g., infectious diseases such as hepatitis B virus [HBV]) are specific to humans, and the use of classical animal models, including NHPs, may not be appropriate because of the absence of disease-specific pathology. Under such conditions, the use of unsuited animal models may be a waste of animals and resources, which is ethically questionable. In the case of HBV, intensive searching for alternative models led to the discovery of an HBV-like infection in the woodchuck (*Marmota monax*), a large rodent species, and its use in pharmaceutical research. The same is true for feline AIDS virus as a model in human AIDS research. Likewise, mice bearing human tumors (i.e., human tumor xenograft models) are used to test anticancer therapies for efficacy (mainly biomarker responses) and potential safety liabilities. These examples illustrate that in some circumstances unconventional animal models may be equally or more pertinent than large animal species, including NHPs.

Many biopharmaceuticals are designed to interact with cellular proteins, which are markers of disease, for example, cancer. If the target protein is absent in healthy humans and animal species, including NHPs, there is little point in using target affinity as a criterion for species selection. In this case the toxic reactions to the therapeutic molecule can be elicited only by off-target effects (i.e., secondary pharmacology). Some anti-infective proteins are targeted against the cell markers of pathogenic organisms. If the target protein sequence is not expressed by the host human tissues, there are no particular constraints on the choice of model species. In the above cases, the NHP is unlikely to be more pertinent as a model species than the rat, rabbit, mouse, dog, or minipig. In developmental toxicity testing, however, verifying that the primary target protein, although absent in the adult, does not have a role in the developing organism before being deactivated during the course of maturation (e.g., polysialiated cellular adhesion molecules) is necessary.

VetBooks.ir

ALTERNATIVE LOWER-ORDER SPECIES

Alternative lower-order species such as the zebrafish may be considered when a mammalian species is not necessary or explicitly requested (e.g., for screening purposes). Otherwise, such low-order species are unlikely to play a role in replacing the NHP in regulatory toxicology, at least not under current regulatory guidance. A deeper understanding of species differences, together with more advanced use of translational biomarkers and genetic engineering techniques, may make these low-order species adoptable for a meaningful use to replace NHPs in the future.

OMISSION OF REGULATORY TOXICITY ANIMAL STUDIES IF NO SUITABLE MODELS EXIST (ALTERNATIVES TO IN VIVO TESTING)

In rare situations, data from any animal species may not be relevant for the human situation and in vitro data, possibly supported by the assessment of existing packages of similar characteristics, are considered the only and/or most appropriate data for predicting human outcomes (e.g., the minimal anticipated biological effect level (MABEL) approach [75]). Examples of such approaches include bispecific antibodies, fusion proteins, immune system modulators, and therapeutic cancer vaccines. In these situations the omission of animal studies seems most appropriate, and replacement by careful evaluation of all other biological data and careful clinical study design with a low starting dose and careful dose escalation may be an alternative.

References

[1] Smith D, Hubrecht R. Good practice guidelines—the selection of non-rodent species for pharmaceutical toxicology, series 3/issue 1. Tamworth, UK: Laboratory Animal Science Association; 2001.
[2] Smith D, Trennery P. Non-rodent selection in pharmaceutical toxicology. A 'Points to Consider' document, developed by the ABPI in conjunction with the UK home office. London, UK: ABPI; 2002.
[3] Buckley LA, Chapman K, Burns-Naas LA, Todd MD, Martin PL, Lansita JA. Considerations regarding nonhuman primate use in safety assessment of biopharmaceuticals. Int J Toxicol 2011;30(5):583–90.
[4] van Meer PJK, Kooijman M, van der Laan JW, Moors EHM, Schellekens H. The value of non-human primates in the development of monoclonal antibodies. Nat Biotechnol 2013;31:882–3.
[5] Song S, Liu L, Edwards SV, Wu S. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proc Natl Acad Sci USA 2012;109(37):14942–7.
[6] Groenen MAM, Archibald AL, Uenishi H, Tuggle CK, Takeuchi Y, Rothschild MF, et al. Analyses of pig genomes provide insight into porcine demography and evolution. Nature 2012;491:393–8.
[7] Svendsen O. The minipig in toxicology. Exp Toxicol Pathol 2006;57:335–9.
[8] Durairaj C, Chastain JE, Kompella UB. Intraocular distribution of melanin in human, monkey, rabbit, minipig and dog eyes. Exp Eye Res 2012;98:23–7.
[9] Walters EM, Wolf E, Whyte JJ, Mao J, Renner S, Nagashima H, et al. Completion of the swine genome will simplify the production of swine as a large animal biomedical model. BMC Med Genet 2012;5(55):1–11.
[10] Vamathevan JJ, Hall MD, Hasan S, Woollard PM, Xu M, Yang Y, et al. Minipig and beagle animal model genomes aid species selection in pharmaceutical discovery and development. Toxicol Appl Pharmacol 2013;270(2):149–57.

VetBooks.ir

[11] Certa U, Ebeling M, Weiser T, Singer T. Ellegaard Newsletter 2011;36. http://minipigs.dk/fileadmin/filer/Newsletters/Newsletter_36.pdf [accessed 20.11.2014].
[12] Zheng Y, Tesar DB, Benincosa L, Birnböck H, Boswell CA, Bumbaca D, et al. Minipig as a potential translatable model for monoclonal antibody pharmacokinetics after intravenous and subcutaneous administration. MAbs 2012;4(2):243–55.
[13] van Mierlo GJD, Cnubben NHP, Kuper CF, Wolthoorn J, van Meeteren-Kreikamp AP, Nagtegaal MM, et al. The Göttingen minipig® as an alternative non-rodent species for immunogenicity testing: a demonstrator study using the IL-1 receptor antagonist anakinra. Immunotoxicology 2013;10(1):96–105.
[14] Bode G, Clausing P, Gervais F, Loegstedd J, Luft J, Nogues V, et al. The utility of the minipig as an animal model in regulatory toxicology. J Pharmacol Toxicol Methods 2010;672:196–220.
[15] Tortereau A, Howroyd P, Lorentsen H. Onset of puberty and normal histological appearances of the reproductive organs in peripubertal female Gottingen minipigs. Toxicol Pathol 2013;41(8):1116–25.
[16] Morrissey RE, Horvath C, Snyder EA, Patrick J, Collins N, Evans E, et al. Porcine toxicology studies of SCH 58500, an adenoviral vector for the p53 gene. Toxicol Sci 2002;65(2):256–65.
[17] Helke KL, Swindle MM. Animal models of toxicology testing: the role of pigs. Expert Opin 2013;9(2):127–39.
[18] DeSesso JM, Williams AL, Ahuja A, Bowman CJ, Hurtt ME. The placenta, transfer of immunoglobulins, and safety assessment of biopharmaceuticals in pregnancy. Crit Rev Toxicol 2012;42(3):185–210.
[19] Barrow PC. Use of the swine pediatric model. In: Hoberman A, editor. Pediatric non-clinical drug testing. Hoboken, NJ: Wiley; 2012. p. 213–30.
[20] Milano S. The minipig in safety pharmacology. In: McAnulty PA, Dayan AD, Ganderup NC, Hasting KL, editors. The minipig in biomedical research. Boca Raton, FL: CRC Press; 2012. p. 159–81.
[21] Baudet PS, Milano S, Boucheix O, Chalencon E, Lege P, Bory C, et al. Minimally-invasive telemetric measurement of arterial blood pressure in the freely moving minipig. Isoprenaline validation. Toxicologist 2011;120(Suppl. 2), Poster Abstract 559, SOT 2011.
[22] Klymiuk N, van Buerck L, Bähr A, Offers M, Kessler B, Wuensch A, et al. Xenografted islet cell clusters from INSLEA29Y transgenic pigs rescue diabetes and prevent immune rejection in humanized mice. Diabetes 2012;61(6):1527–32.
[23] Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 2005;438(8):803–19.
[24] Lehmann H. The minipig in general toxicology. Scand J Lab Anim Sci 1998;25(Suppl. 1):59–62.
[25] Stephan-Gueldner M, Inomata A. Coronary arterial lesions induced by endothelin antagonists. Toxiciol Lett 2000;112–113:531–5.
[26] Hart JE. Endocrine pathology of estrogens: species differences. Pharmacol Ther 1990;47(2):203–18.
[27] Lorenz W, Reimann HJ, Schmal A, Dormann P, Schwarz B, Neugebauer E, et al. Histamine release in dogs by Cremophor El and its derivatives: oxethylated oleic acid is the most effective constituent. Agents Actions 1977;7(1):63–7.
[28] Hayes TJ, Roberts GK, Halliwell WH. An idiopathic febrile necrotizing arteritis syndrome in the dog: beagle pain syndrome. Toxicol Pathol 1989;17(1,2):129–37.
[29] Bailey J, Thew M, Balls M. An analysis of the use of dogs in predicting human toxicity and drug safety. ATLA 2013;41:335–50.
[30] Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals. A science-based approach to facilitating clinical trials. USA: John Wiley & Sons, Inc.; 2008.
[31] Callejas D, Mann CJ, Ayuso E, Lage R, Grifoll I, Roca C, et al. Treatment of diabetes and long-term survival following insulin and glucokinase gene therapy. Diabetes 2013;62:1718–29.
[32] Product Monograph BeneFIX®. Pfizer Canada. 2012. http://www.pfizer.ca/en/our_products/products/monograph/224 [accessed 20.11.2014].
[33] FDA—Center for Drug Evaluation and Research. Office Director Memo Application Number: 125327Orig1s000, 2012. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/125327Orig1s000ODMemo.pdf) [accessed 20.11.2014].
[34] Kim MJ, Oh HJ, Park JE, Kim GA, Hong SG, Jang G, et al. Generation of transgenic dogs that conditionally express green fluorescent protein. Genesis 2011;49(6):472–8.
[35] Hasiwa N, Bailey J, Clausing P, Daneshian M, Eileraas M, Farkas S, et al. Critical evaluation of the use of dogs in biomedical research and testing in Europe. Altex 2012;28(4/11):326–40.
[36] Footea RH, Carney EW. The rabbit as a model for reproductive and developmental toxicity studies. Reprod Toxicol 2000;14:477–93.

[37] Spence S. The Dutch-Belted rabbit: an alternative breed for developmental toxicity testing. Birth Defects Res B Dev Reprod Toxicol 2003;68(5):439–48.
[38] Allais L, Reynaud L. Teratology studies in the rabbit. In: Barrow PC, editor. Teratogenicity testing: methods and protocols. New York: Humana Press; 2013. p. 139–56.
[39] Barrow PC. Technical procedures in reproduction toxicology. Laboratory animals handbooks. vol. 11. London: Royal Society of Medicine; 1990.
[40] Barrow PC. Developmental and reproductive toxicity testing of vaccines. J Pharmacol Toxicol Methods 2012;65:58–63.
[41] Barrow PC, Allais L. Developmental toxicity of vaccines. In: Barrow PC, editor. Teratogenicity testing: methods and protocols. New York: Humana Press; 2013. p. 81–90.
[42] Pentsuk N, van der Laan JW. An interspecies comparison of placental antibody transfer: new insights into developmental toxicity testing of monoclonal antibodies. Birth Defects Res B 2009;86:328–44.
[43] FDA. Tenecteplase product approval information—clinical pharmacology and toxicology review of tenecteplase, 99-0903. http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/UCM200877.pdf; 2009 [accessed 20.11.2014].
[44] Health Canada. Summary basis of decision (SBD): AVASTIN®. Submission Control No. 089366. http://www.hc-sc.gc.ca/dhp-mps/prodpharma/sbd-smd/drug-med/sbd_smd_2006_avastin_089366-eng.php; 2006 [accessed 20.11.2014].
[45] Forster R. Study designs for the nonclinical safety testing of new vaccine products. J Pharmacol Toxicol Methods 2012;66(1):1–7.
[46] Mapara M, Thomas BS, Bhat KM. Rabbit as an animal model for experimental research. Dent Res J 2012;9(1):111–8.
[47] Diano M, Le Bivic A, Hirn M. A method for the production of highly specific polyclonal antibodies. Anal Biochem 1987;166:224–9.
[48] Craig DB, Kannan S, Dombkowski AA. Augmented annotation and orthologue analysis for *Oryctolagus cuniculus*: better bunny. BMC Bioinf 2012;13(84):1–6.
[49] Hammer RE, Pursel VG, Rexroad Jr. CE, Wall RJ, Bolt DJ, Ebert KM, et al. Production of transgenic rabbits, sheep and pigs by microinjection. Nature 1985;315:680–3.
[50] du Sert NP, Rudd JA, Apfel CC, Andrews PLR. Cisplatin-induced emesis: systematic review and meta-analysis of the ferret model and the effects of 5-HT3 receptor antagonists. Cancer Chemother Pharmacol 2011;67(3):667–86.
[51] Broad Institute. Ferret Genome Project (MusPutFur1.0), http://www.broadinstitute.org/scientific-community/science/projects/mammals-models/ferret-genome-project; 2011 [accessed 20.11.2014].
[52] Sun X, Yan Z, Yi Y, Li Z, Lei D, Rogers CS, et al. Adeno-associated virus-targeted disruption of the CFTR gene in cloned ferrets. J Clin Invest 2008;118(4):1578–83.
[53] Sun X, Sui H, Fisher JT, Yan Z, Liu X, Cho HJ, et al. Disease phenotype of a ferret CFTR-knockout model of cystic fibrosis. J Clin Invest 2010;120(9):3149–60.
[54] Vidana B, Majo N, Perez M, Montoya M, Martorell J, Martinez J. Immune system cells in healthy ferrets: an immunohistochemical study. Vet Pathol 2013;51(4):775–86.
[55] Thornton PC, Wright PA, Sacra PJ, Goodier TE. The ferret, *Mustela putorius furo*, as a new species in toxicology. Lab Anim 1979;13(2):119–24.
[56] Beach JE. The ferret for non-rodent toxicity studies—a pathologist's view. Arch Toxicol Suppl 1982;5:279–82.
[57] Gad SC. Pigs and ferrets as models in toxicology and biological safety assessment. Int J Toxicol 2000;19:149–68.
[58] Mould GP, Curry SH, Beck F. The ferret, a useful model for teratogenic study. Naunyn Schimiedebergs Arch Pharmacol 1973;279(Suppl.):R18.
[59] Beck F. Use of the ferret in teratology. Teratology 1981;24(2):10A.
[60] Beck F, Schon H, Mould G. Comparison of the teratogenic effects of mustine hydrochloride in rats and ferrets. the value of the ferret as an experimental animal in teratology. Teratology 1976;13(2):151–60.
[61] Barry JS, Anthony RV. The pregnant sheep as a model for human pregnancy. Theriogenology 2008;69(1):55–67.
[62] Gien J, Tseng N, Seedorf G, Roe G, Abman SH. Endothelin-1 impairs angiogenesis through rho-kinase activation after chronic intrauterine pulmonary hypertension in fetal sheep. Pediatr Res 2013;73(3):252–62.
[63] Fajt VR. Drug laws and regulations for sheep and goats. Vet Clin North Am Food Anim Pract 2011;27:1–21.
[64] Campbell JK, Mills CF. The toxicity of zinc to pregnant sheep. Environ Res 1979;20:1–13.
[65] Fan Y, Huang ZY, Cao CC, Chen CS, Chen YX, Fan DD, et al. Genome of the Chinese tree shrew. Nat Commun 2013;4:1426.
[66] Cao J, Yang EB, Su JJ, Li Y, Chow P. The tree shrews: adjuncts and alternatives to primates as models for biomedical research. J Med Primatol 2003;32(3):123–30.

[67] Barrow PC. Reproductive toxicity testing for pharmaceuticals under ICH. Reprod Toxicol 2009;28:172–9.
[68] Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicology studies in nonhuman primates. Birth Defects Res B 2009;86(6):446–62.
[69] Creasy DM. Evaluation of testicular toxicity in safety evaluation studies: the appropriate use of spermatogenic staging. Toxicol Pathol 1997;25(2):119–31.
[70] EPA. Endocrine disruptor screening program. http://www.epa.gov/endo/pubs/assayvalidation/status.htm; 2011 [accessed 20.11.2014].
[71] Fuchs A, Buse E, Weinbauer GF. Embryo fetal development studies in nonhuman primates. In: Barrow PC, editor. Teratogenicity testing: methods and protocols. New York: Humana Press; 2013. p. 169–84.
[72] Kronenberg S, Baumann A, de Haan L, Hinton HJ, Moggs J, Theil FP, et al. Current challenges and opportunities in nonclinical safety testing of biologics. Drug Discov Today 2013;18(23/24):1138–43.
[73] Brenden N, Madeyski-Bengtson K, Martinsson K, Svärd R, Albery-Larsdotter S, Granath B, et al. A triple-transgenic immunotolerant mouse model. J Pharm Sci 2013;102(3):1116–24.
[74] Löffler J, Huber G. β-Amyloid precursor protein isoforms in various rat brain regions and during brain development. J Neurochem 1992;59:1316–24.
[75] EMA, Committee for medicinal products for human use (CHMP). Guideline on strategies to identify and mitigate risks for first-inhuman clinical trials with investigational medicinal products. London, 19 July 2007. Doc. EMEA/CHMP/SWP/28367/07.

VetBooks.ir

VetBooks.ir

PART VI

TRANSLATIONAL ASPECTS FOR THE USE OF NONHUMAN PRIMATES IN DRUG DEVELOPMENT

VetBooks.ir

VetBooks.ir

CHAPTER

18

Pharmacokinetics of Human Therapeutics Characterized in Nonhuman Primates

Chih-Ming L. Tseng, Raffaella Faggioni, Lorin K. Roskos
Clinical Pharmacology & DMPK, MedImmune LLC, Mountain View, CA, USA

OUTLINE

Nonhuman Primate Pharmacokinetic Study Designs 360
NHP PK Study Results 360
Determinants for PK in NHPs 367
Target Mediated Clearance 367
Interaction with Neonatal Fc Receptor for mAbs 368
Renal Elimination of Biologics and Small Molecules 369
Hepatic Metabolism 369
Nonspecific CL of Biologics 370
Other Special PK Studies Using NHPs 370
PK Analysis of Data Obtained from NHPs 370
Species Difference and Prediction for Human PK 371
References 372

Nonhuman primates (NHPs) are common animal models for pharmacokinetic (PK) studies characterizing human therapeutics. This chapter discusses PK studies using NHPs and their respective results. PK data from both biologics and small molecules are included, and these data can be used as a reference for future comparison with data for other biologics or small molecules. Key determinants such as elimination mechanisms to explain the PK of biologics and/or small molecules are summarized. An understanding of the elimination mechanisms provides the basis for evaluating human PK prediction scaled from NHP data.

http://dx.doi.org/10.1016/B978-0-12-417144-2.00018-4
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

NONHUMAN PRIMATE PHARMACOKINETIC STUDY DESIGNS

Pharmacokinetic (PK) studies for 44 molecules were selected to represent both the small-molecule and biologics classes (monoclonal antibodies [mAbs], cytokines, peptides, etc.) and are reviewed in this chapter. The designs of these PK studies using nonhuman primates (NHPs) are summarized in Table 18.1. Fourteen of these molecules (abiraterone acetate, belimumab, brentuximab vedotin, cabozantinib, carfilzomib, ipilimumab, obinutuzumab, pazopanib, pertuzumab, pomalidomide, ponatinib, tocilizumab, trastuzumab emtansine, and ziv-aflibercept) are new molecular entities recently approved by the U.S. Food and Drug Administration after 2009 [42].

The rationale to choose NHPs as animal models for safety and PK evaluation of biologics is based on the similar pharmacology of therapeutic targets between NHP and humans, which includes in vitro binding affinity and potency and target expression. For example, hepatocyte growth factor (HGF) is 98% homologous in humans and cynomolgus monkeys. The binding affinity of AMG 102 in cynomolgus monkey and human HGF are 19±15 and 41±20 pM, respectively. A tissue cross-reactivity study using fluorescent-conjugated AMG 102 showed that the pattern of tissue binding of AMG 102 in cynomolgus monkey tissues was similar to that in human tissues [4]. Therefore the PK of AMG 102, a fully human mAb that binds to HGF, was studied in cynomolgus monkeys as part of a preclinical safety and PK evaluation package. Although cynomolgus monkeys are commonly used for PK studies of biologics (Table 18.1), other NHPs such as rhesus monkeys also have been used as animal models for PK studies of biologics [27,36]. PK of c-Mpl fusion proteins were studied in both cynomolgus and rhesus monkeys [36]; a twofold difference in clearance values that is not explained by the differences in body weight was observed (Table **18.2**). Therefore data obtained from different NHP species must be interpreted with caution.

For small molecules, NHPs also are used as preclinical species for PK and safety studies; both cynomolgus and rhesus monkeys are commonly used in PK studies (Table 18.1). Dogs also are a common nonrodent species for small molecules, including recently approved molecules such as afatinib, axitinib, bosutinib, dabrafenib, enzalutamide, and trametinib [43–48].

The doses used in PK studies are usually chosen to provide coverage over the toxicology and the dose range in first-time-in-human (FTIH) studies. Three dose levels (i.e., low, medium, and high) are the most common (Table 18.1). Each dose is 3- to 10-fold higher than the previous dose. Three or four monkeys are included in each dose group. Dosing routes include intravenous (IV), subcutaneous (SC), oral administration (small molecules only), among others, depending on the proposed route for clinical use and/or study purposes. In single-dose PK studies, large enough blood samples should be collected to characterize the terminal phase of the concentration profiles. In repeat-dose PK studies, intensive blood samples for PK analysis can be collected after the first and the last dose. Additional samples maybe collected during the study.

NHP PK STUDY RESULTS

PK parameters (clearance [CL], volume of distribution [Vd], and terminal phase half-life [$t_{1/2}$]) of the molecules, as shown in Table 18.1, are categorized as therapeutic antibodies, peptides and proteins (except mAbs), and small molecules and are presented in Tables **18.2–18.4**. The majority of the data presented are from single-dose studies for the purpose of

TABLE 18.1 Nonhuman Primate pharmacokinetic Studies Reviewed in This Chapter

Study molecule	Species and other information	Dose	Duration of dosing	Reference
5c8 (humanized anti-CD154 mAb)	Female cynomolgus monkeys (n = 3 per group)	1, 5, and 20 mg/kg, IV	Single dose on day 1	[1]
Abiraterone acetate (prodrug) Abiraterone (drug, 3β-17-[pyridin-3-yl] androsta-5,16-diene)	Male cynomolgus monkeys (n = 2)	24 mg/kg, IV 250 mg/animal, oral	Single dose on day 1	[2]
Albugranin (recombinant human G-CSF fused to human albumin)	Male and female cynomolgus monkeys (n = 5/sex/group)	0.1, 0. 5, and 1 mg/kg, SC	Single dose on day 1	[3]
AMG 102 (human anti–hepatocyte growth factor IgG2)	Single dose: male cynomolgus monkeys (n = 4 per group), Repeat dose: male and female cynomolgus monkeys (n = 4/sex/group)	Single dose: 0.5, 1, 5, 10, or 50 mg/kg, IV, or 75 mg/kg, SC 4-week repeat dose: 5, 25, or 100 mg/kg 13-week repeat dose: 5, 25, or 150 mg/kg	Single dose on day 1 or once weekly for either 4 or 13 weeks	[4]
Anacetrapib ([4S,5R]-5-[3,5-bis(trifluoromethyl) phenyl]-3-{[4-fluoro-2-methoxy-5-(propan-2-yl)-4-(trifluoromethyl)[1,1-biphenyl]-2-yl] methyl}-4-methyl-1,3-oxazolidin-2-one)	Male rhesus monkeys (n = 3)	0.1 mg/kg, IV	Single dose on day 1	[5]
Anti-amyloid β Ab2 (humanized antibody against human amyloid β peptide)	Cynomolgus monkeys (n = 3 per group)	2 and 10 mg/kg, IV	Single dose on day 1	[6]
Anti-IL-21R antibodies (Ab-01 and Ab-02 with Kd values for human IL-21 R at 2 and 0.4 nM, respectively)	Cynomolgus monkeys (n = 3 per group)	10 mg/kg IV for both Ab-01 and Ab-02	Single dose on day 1	[7]
Belimumab (human anti-BLyS IgG1)	Male and female cynomolgus monkeys (n = 2/sex/dose)	5 and 50 mg/kg, IV	Single dose on day 1	[8]
Bevacizumab (chimeric anti-VEGF)	Male cynomolgus monkeys (n = 4 per group)	2, 10, 50 mg/kg,IV 10 mg/kg, SC	Single dose on day 1	[9]
Brentuximab vedotin (anti-CD30 antibody conjugated with monomethyl auristatin E)	Female cynomolgus monkeys (n = 6 per dose)	0.3, 1 mg/kg, IV	Single dose on day 1	[10]

(*Continued*)

TABLE 18.1 Nonhuman Primate pharmacokinetic Studies Reviewed in This Chapter—Cont'd

Study molecule	Species and other information	Dose	Duration of dosing	Reference
Cabozantinib (N-(4-((6,7-dimethoxyquinolin-4-yl)oxy)phenyl)-N-(4-fluorophenyl) cyclopropane-1,1-dicarboxamide)	Male cynomolgus monkeys (n = 3 per dose)	3 mg/kg, IV and oral	Single dose on day 1	[11]
Carfilzomib (2S)-N-((S)-1-((S)-4-methyl-1-((R)-2-methyloxiran2-yl)-1-oxopentan-2-ylcarbamoyl)-2-phenylethyl)-2-((S)-2-(2-morpholinoacetamido)4-phenylbutanamido)-4-methylpentanamide	Male cynomolgus monkeys (n = 3 per dose)	1, 2, or 4 mg/kg, IV	Single dose on day 1	[12]
CD4-BFFI bifunctional fusion protein containing an anti-CD4 human IgG1 and a fusion inhibitor peptide T651	Male cynomolgus monkeys (n = 3 per group)	1 or 10 mg/kg, IV	Single dose on day 1	[13]
CI-1007 (PD 143188, I) [1,2,3,6-tetrahydro-4-phenyl-1-[(3-phenyl-3-cyclohexen-1-yl) methyl]pyridine, R-(1)-enantiomer]	Male cynomolgus monkeys (n = 4)	5 mg/kg, IV	Single dose on day 1	[14]
CPT-11 (7-ethyl-10-[4-(1-piperidino)-1-piperi-dino]carbonyloxycamptothecin)	Female rhesus monkeys (n = 1-3 per group)	3, 7, 15, and 25 mg/kg, IV	Single dose on day 1	[15]
Dacliximab (humanized anti-IL-2R)	Cynomolgus monkeys (n = 4 per group)	0.05, 0.5, or 5 mg/kg	Single dose on day 1	[16]
E2074 (2-[(2R)-2-fluoro-3-{(3r)-[(3-fluorobenzyl)oxy]-8-azabicyclo[3.2.1] oct-8-yl}propyl]-4,5-dimethyl-2,4-dihydro-3H-1,2,4-triazol-3-one)	Monkeys (n = 2)	1 mg/kg, IV; 3 or 10 mg/kg, oral	Single dose on day 1	[17]
ILT-threo (di-[2S,3S]-2-amino-3-methyl-pentanoic-1,3-thiazolidine fumarate)	Bile-duct-cannulated male monkeys (n = 2 or 3)	1, 5, and 10 mg/kg, IV	Single dose on day 1	[18]
Infliximab (Chimeric anti-TNF-α)	Chimpanzees	30 mg/kg	Single dose on day 1	[19]
Ipilimumab (human anti-CTLA-4 IgG1)	Female cynomolgus monkeys (n = 4 per group)	10 mg/kg, IV	Single dose on day 1	[20]
MLN8866 (humanized anti-B7-H4 IgG1 with a maytansine cytotoxic analog)	Female cynomolgus monkeys (n = 2 per group)	5, 15, and 30 mg/kg, IV	Single dose on day 1	[21]

MNRP1685A (human anti-neuropilin-1 mAb)	Male and female cynomolgus monkeys (n = 2/sex/group for single dose; n = 6/sex/group for multiple-dose study)	Single dose: 0.5, 3, 15, or 50 mg/kg Multiple dose: 10, 30, or 100 mg/kg	Single dose on day 1 or 9 weekly doses, IV	[22]
Obinutuzumab (humanized monoclonal IgG1 antibody against CD20)	Cynomolgus monkeys (n = 2 per group)	1 and 10 mg/kg, IV	Single dose on day 1	[23]
Odanacatib (N-(1-cyanocyclopropyl)-4-fluoro-N2-[4]-L-leucinamide)	Male rhesus monkeys (n = 4)	1 mg/kg, IV and 5 mg/kg, oral	Single dose on day 1	[24]
Pazopanib (5-[[4-[(2,3-dimethyl-2H-indazol-6- yl)methylamino]-2-pyrimidinyl] amino]-2-methylbenzenesulfonamide monohydrochloride)	Male cynomolgus monkeys (n = 4)	5 mg/kg, IV, oral	Single dose on day 1	[25]
Pegylated interferon-β1a	Cynomolgus monkeys (n = 4 per group)	0.3, 1, and 3 MIU/kg, SC	Single dose on day 1	[26]
Pegylated recombinant human methionyl G-CSF (pegfilgrastim)	Male rhesus monkeys after irradiation and autologous bone marrow transplantation (n = 4 per group)	100, 300 μg/kg, IV	Single dose on day 1	[27]
Pertuzumab (humanized anti-HER2)	Male and female cynomolgus monkeys (n = 2/sex/group)	15, 50 or 150 mg/kg, IV 50 mg/kg, SC	Single dose on day 1	[28]
Pomalidomide (4-amino-2-([(3RS)-2,6-dioxopiperidin-3-yl)2Hisoindole-1,3-dione	Male cynomolgus monkeys (n = 4)	10 mg/kg, IV 100 mg/kg, oral	Single dose/crossover	[29]
Ponatinib	Cynomolgus monkeys (n = 3)	1 mg/kg, IV and oral	Single dose on day 1	[30]
Prodrug DB289 (2,5-Bis[4-(N-methoxyamidino)phenyl]furan Monomaleate)	Cynomolgus monkeys (n = 3/sex/group)	1 mg/kg, IV 1 or 5 mg/kg, oral	Single dose on day 1	[31]
Recombinant human erythropoietin	Male cynomolgus monkeys (n = 3 per group)	500, 2000, or 4000 IU/kg, IV 400, 1,000, 2,400,5,000, 20,000, or 40,000 IU/kg, SC	Single dose on day 1	[32]
Recombinant human methionyl G-CSF (Filgrastim)	Male rhesus monkeys after irradiation and autologous bone marrow transplantation (n = 4)	300 mcg/kg, IV	Single dose on day 1	[27]

(*Continued*)

TABLE 18.1 Nonhuman Primate pharmacokinetic Studies Reviewed in This Chapter—Cont'd

Study molecule	Species and other information	Dose	Duration of dosing	Reference
Recombinant IL-2	Male cynomolgus monkeys (n = 1 per group)	9.6×10^{6} or 1.18×10^{7} cpm (Note: 1 CPM = 0.125 unit of bioactivity)	Single dose on day 1	[33]
Rituximab (chimeric anti-CD20)	Male and female cynomolgus monkeys (n = 1/sex/group)	10, 30, 100 mg/kg, IV	Single dose on day 1	[34]
Rivoglitazone ((RS)-5-{4-[(6-methoxy-1-methyl-1H-benzimidazol-2-yl)methoxy] benzyl}-1,3-thiazolidine-2,4-dione monohydrochloride)	Monkeys (n = 3 or 4 per group)	0.0986, 0.493, or 0.986 mg/kg, IV and oral	Single dose on day 1	[35]
Romiplostim (a 59-kD fusion protein that consists of c-Mpl binding domain covalently linked to the N-terminus of the carrier Fc domain)	Male cynomolgus monkeys (n = 3) Male rhesus monkeys (n = 3)	0.5, 2, or 5 mg/kg, IV	Single dose on day 1	[36]
SGN-40 (humanized anti-CD40)	Male and female cynomolgus monkeys (n = 4-6 per group)	1 and 10 mg/kg, IV	Single dose on day 1	[37]
Simvastatin	Male cynomolgus monkeys (n = 4 per group)	1 mg/kg, IV 20 mg/kg, oral	Single dose on day 1	[38]
Tocilizumab (humanized anti-IL-6 receptor IgG1)	Male and female cynomolgus monkeys (n = 2/sex/dose)	0.5, 5, and 50 mg/kg, IV	Single dose on day 1	[39]
Trastuzumab emtansine, T-DM1 (humanized anti-HER2 covalently bound to DM1)	Male and female cynomolgus monkeys (n = 2/sex/group)	0.3, 3, 30 mg/kg, IV	Single dose on day 1	[40]
Ziv-aflibercept (VEGFR-1 and VEGFR-2 IgG1 Fc fusion protein)	Cynomolgus monkeys (n = 3/sex/dose)	0.75, 1.5, 5, and 15 mg/kg, SC 5 mg/kg, IV	Single dose on day 1	[41]

G-CSF, granulocyte colony-stimulating factor; Ig, immunoglobulin; IL, interleukin; IV, intravenous; Kd,; mAb, monoclonal antibody; SC, subcutaneous; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VEGF, vascular endothelial growth factor receptor.

VetBooks.ir

TABLE 18.2 Pharmacokinetic Parameters of Therapeutic Antibodies in NHP

Study molecule	Clearance (mL/h/kg)	Volume of distribution (mL/kg)	Terminal phase half-life (hours)	Others
5c8	0.18-0.24	70.9-74.6	228-324	-
Anti-amyloid β Ab2	0.837-0.941	53-58	52.6-54.7	-
Anti-interleukin-21R (Ab-01)	-	-	264	-
Anti-interleukin-21R (Ab-02)	-	-	138	-
Belimumab	0.233	85-108	268-336	-
Bevacizumab	4.76-5.78	64-73.9	210-247	F (%): 98
Brentuximab vedotin	0.771-1.15	67.4-68	43.7-64.6	-
CD4-BFFI bifunctional fusion protein	0.563-1.35	28.4-39.4	35-50	-
Dacliximab	0.221	52.5	214	-
Infliximab	6-8.6	33.6-40	140-193	-
Ipilimumab	0.19	44.1	203	-
MLN8866	1.2-1.5	60.5-63.5	-	-
Obinutuzumab	0.173-0.235	23.9-28.1	172-194	-
Pertuzumab	4.98-5.24	68.1-72.7	237-250	F (%): 81.5
Rituximab	0.450-0.706	-	61-143	-
SGN-40	0.45-2.40	87.1-96.7	27.6-162	-
Tocilizumab	0.2297-1.331	38.71-61.17	135-197	-
Trastuzumab emtansine	0.421-1.68	44.2-68.6	12-22	-

F = bioavailability.

comparison among molecules regardless of regimens or repeat-dosing schedules. However, PK properties after repeat doses may change. For example, upregulation of the elimination mechanism resulting from pharmacodynamic effects contributes to time-dependent PK for pegylated granulocyte colony-stimulating factor (G-CSF), as discussed in a later section describing target-mediated clearance. In general, CL and Vd values for small molecules are higher than those for peptides, small proteins, and mAbs; mAbs typically have the longest half-life—longer than small molecules, peptides, or small proteins. Differences in PK properties among classes of molecules can be attributed to their respective elimination mechanisms, as further explained later in the section "Determinants for PK in NHPs."

For therapeutic antibodies (except antibody drug conjugates [ADCs]), CL values range from 0.17 to 8.6 mL/h/kg (4.08-206.4 mL/day/kg); Vd values range from 28.4 to 108 mL/kg; $t_{1/2}$ values range from 27.6 to 336 hours (1.15-14 days) (Table **18.2**). The range in PK parameters for five recently approved mAbs (i.e., belimumab, ipilimumab, obinutuzumab, pertuzumab, tocilizumab), shown in Table **18.2**, are narrower; CL values range from 0.19 to 5.24 mL/h/kg

VetBooks.ir

TABLE 18.3 Pharmacokinetic Parameters of Proteins (Except Monoclonal antibodies) in Nonhuman Primates

Study molecule	Clearance (mL/h/kg)	Volume of distribution (mL/kg)	Terminal phase half-life (hours)	Others
c-Mpl fusion protein[a]	7.44-7.76	184-196	-	-
c-Mpl fusion protein[b]	17.3-20.1	110-198	-	-
G-CSF fused to human albumin[c]	7.9-27.5	90.1-591	7.73-13.3	-
Interleukin-2	20-24	246-341	8.6-10.5	-
Pegylated G-CSF	1.8-2.2	46.2-48.8	16.1-22.2	-
Pegylated interferon-β1a[c]	15-122	-	3.89-21.0	-
Recombinant G-CSF	47.9	89.5	4.7	-
Recombinant human erythropoietin	-	-	-	Vmax (IU/kg/h): 480.3 Km (IU/L): 35190 F (%): 30-100
Ziv-aflitrap (115-kDa protein)	0.50	62	98	-

[a]*Cynomolgus monkeys.*
[b]*Rhesus monkeys.*
[c]*Subcutaneous administration.*

TABLE 18.4 Pharmacokinetic Parameters of Small Molecules in Nonhuman Primates

Study molecule	Clearance (mL/h/kg)	Volume of distribution (mL/kg)	Terminal phase half-life (hours)	Others
Abiraterone	1214	-	6.6	F (%)[a] = 1.6
Anacetrapib	6	300	34	-
Carbozantinib	640	2670	3.92	F (%)[a] = 13-73
Carfilzomib	11280-17100	219-1077	0.125-0.133	-
CI-1007	1668	18000	19.4	-
CPT-11	660-996	1503-3832	-	-
DB289	1620-1800	9100-9400	11	-
E2074	1350	3980	-	-
ILT-threo	480-540	900-1300	1.6-2.5	-
Odanacatib	366	1600	18	-
Pazopanib	96	283	4.7	F (%)[a] = ~50
Pomalidomide	239.5	2283.3	6.7	F (%)[a] = 15
Ponatinib	522	2600	5.3	F (%)[a] = 20.6
Rivoglitazone	18.6-22.3	138-166	6.21-6.79	-
Simvastatin	1288	2567	-	-

[a]*Oral administration.*

VetBooks.ir

(4.56-125.8 mL/day/kg), Vd values range from 44.1 to 108 mL/kg, and $t_{1/2}$ values range from 135 to 336 hours (5.62-14 days).

PK properties for ADCs are affected not only by the antibody component but also by the stability of the payload. CL and Vd values of two approved ADCs (brentuximab vedotin and trastuzumab emtansine) are within the respective ranges for antibodies, although the $t_{1/2}$ values of these two ADCs are shorter (12-64.6 hours or 0.5-2.69 days; Table **18.2**).

Nonlinearities were observed for CD4-BFFI bifunctional protein (1-10 mg/kg dose range), rituximab (10-100 mg/kg dose range), SGN-40 (1-10 mg/kg dose range), trastuzumab emtansine (0.3-30 mg/kg dose range) and tocilizumab (0.5-50 mg/kg dose range), as shown in Table **18.2**. The dose range (0.3-100 mg/kg) for these biologics overlaps with those of mAbs observed with linear PK. In addition, CL values of these biologics range from 0.421 to 2.40 mL/h/kg and also overlap with observed CL values for the other mAbs. The magnitude of nonlinearity is influenced by a number of factors, including dose, target expression, target turnover rate, binding affinity of the drug to the target, and the presence and magnitude of parallel linear clearance pathways.

For proteins (except mAbs), as shown in Table **18.3**, CL values range from 0.5 to 122 mL/h/kg; Vd values range from 46.2 to 591 mL/kg; and $t_{1/2}$ values range from 3.89 to 22.2 hours. These reference intervals represent different classes of proteins such as cytokines, pegylated molecules, and fusion proteins. Wide ranges of parameter values of these proteins are observed than those of mAbs; the lower molecular weight molecules are cleared more rapidly by nonspecific clearance mechanisms, such as renal clearance. Nonlinearities were observed for many of the molecules in this category.

For small molecules reviewed in this chapter, almost all have high CL values (6-17100 mL/h/kg), large Vd (138-18,000 mL/kg), and relatively short $t_{1/2}$ (0.125-34 h), as summarized in Table **18.4**. Among these small molecules, abiraterone acetate, cabozantinib, carfilzomib, pazopanib, pomalidomide, and ponatinib were recently approved by the U.S. Food and Drug Administration (after 2009). Their PK properties in NHPs are quite varied: CL values range from 96 to 17100 mL/h/kg, Vd values range from 219 to 2600 mL/kg, and $t_{1/2}$ values range from 0.125 to 6.7 hours.

DETERMINANTS FOR PK IN NHPs

Target Mediated Clearance

Target or receptor-mediated clearance is one of the key determinants of the PK of therapeutic mAbs and other protein drugs. This elimination mechanism has been extensively reported in the literature. Antibody internalization upon binding to the target receptor and degradation can affect the PK of the drug. The target-mediated CL represents an additional saturable clearance mechanism. The PK can provide useful insights into saturation of the target receptor, where the half-life increases with increasing dose as the receptor is progressively blocked. The clearance of a fully human mAb, panitumumab, against epidermal growth factor receptor (EGFR) was compared in nude mice and cynomolgus monkeys [49]. Panitumumab cross-reacts with monkey EGFR but does not cross-react with murine EGFR. The PK of panitumumab was nonlinear in monkeys because of EGFR-mediated clearance.

VetBooks.ir

At high doses in monkeys, EGFR-mediated clearance is saturated. In mice, the clearance was linear and there was no saturable EGFR-mediated clearance.

The PK of two anti-interleukin-21R antibodies with binding affinity (Kd) values of 2 and 0.4 nM, respectively, were studied in cynomolgus monkeys [7]. The antibody that has stronger target binding (i.e., a smaller Kd) also has a shorter half-life (2.3 vs. 11 days).

Target-mediated clearance also was observed with recombinant pegylated G-CSF. The clearance of pegylated G-CSF after a single intravenous administration decreased with increasing dose, consistent with saturable G-CSF receptor-mediated clearance on neutrophils and neutrophil precursors [27]. Enhanced target-mediated clearance also was observed with G-CSF resulting from its pharmacodynamic effect of increasing the absolute neutrophil count over time. As reported in a 1-month repeat-dose study of cynomolgus monkeys, exposure (area under the curve [AUC]) values of G-CSF on day 22 were approximately threefold lower than the respective day 1 values [50]. This pharmacodynamically enhanced clearance was observed not only in monkeys but also in the clinic. Concentrations of G-CSF in serum from patients treated with different G-CSF therapy schedules were inversely correlated with circulating neutrophil counts [51]. Using phase 1 data of pegylated G-CSF, Roskos et al. [52] established a mathematical relationship between G-CSF receptor-mediated clearance and absolute neutrophil count to describe feedback regulation of pegylated G-CSF clearance by neutrophils.

Interaction between Neonatal Fc Receptor for mAbs

The elimination of IgG antibodies results primarily from lysosomal catabolism, which occurs after antibody internalization by a target receptor or by fluid-phase pinocytosis [49]. If the antibody target is saturated or does not contribute substantially to total clearance, the elimination occurs primarily through reticuloendothelial pinocytosis. Following pinocytosis, salvaging immunoglobulin (Ig) G from lysosomal catabolism occurs by binding IgG to the neonatal Fc receptor (FcRn), which recycles the antibody to the cell surface and prolongs its half-life [49]. Engineering the FcRn binding domain of IgG to improve affinity to FcRn at pH 6.0 (reflecting the pH of the acidified endosome) has reduced clearance and prolonged the half-life of human IgG [53]. The humanized antirespiratory syncytial virus mAb was modified with the triple mutation M252Y/S254T/T256E (YTE). There was 10-fold increase in its binding to both cynomolgus monkey and human FcRn at pH 6.0 [54]. The PK of wild-type and YTE antibodies were compared in cynomolgus monkeys and a fourfold increase in the serum half-life of YTE antibodies was observed [54]. These results demonstrate the interaction with FcRn is a key determinant of the PK of mAbs in NHP.

Pharmacokinetics of a full-length, humanized antitumor necrosis factor-α mAb IgG1 and its FcRn variants (N434H and N434A)—with a 7.6- to 23-fold increase in the binding affinity of mAbs to FcRn at pH 6.0 while keeping a low binding affinity at pH 7.4—were studied [54]. The results show that AUC exposure values increased significantly (1.27- to 1.38-fold) for variants.

Datta-Mannan et al. [55] used five humanized IgG4 wild-type and their respective V308PFC variants to compare their respective in vivo half-lives in NHPs after a single intravenous dose at 5 mg/kg. The results showed that the variants, which have lower Kd

VetBooks.ir

values (~1 vs. ~115 nM) determined by BIAcore, have improved pharmacokinetic properties such as increases in half-lives from approximately 100 to 300 hours to 300 to 650 hours in cynomolgus monkeys.

Albumin has been used as a platform to extend terminal half-lives of therapeutic proteins with a molecular mass <60 kDa [56]. Albumin can not only increase the mass of these proteins, allowing them to escape renal elimination, but it can also carry them through FcRn-mediated recycling pathways. The albumin fusion proteins glucagon-like peptide (GLP)-1, G-CSF, factor VIIa, factor IX, and interferon-α-2b elicit a significantly longer half-life (i.e., 3.1- to 5760-fold longer) than the respective original molecules in cynomolgus monkeys [56]. Take as an example GLP fused to albumin: its half-life increased from 1.5 to 2 minutes to 2 to 4 days in cynomolgus monkeys. These data further support interaction with FcRn as a key determinant for PK.

Renal Elimination of Biologics and Small Molecules

Renal elimination is usually not a key determinant for mAbs because of their size. For smaller biologics and small molecules, renal elimination can also play a role in determining PK properties. Exendin-4 is a 39-amino-acid GLP-1 analog, sharing approximately 53% sequence identity with mammalian GLP-1 [57]. Gao and Jusko [58] reported that the CL from the central compartment (monkeys, 2.39 mL/[min·kg]) was similar to the reported renal CL. The results support a key role for renal elimination in determining the PK of small molecules, peptides, and proteins (except mAbs) and are consistent with the size of the molecule (e.g., 39 amino acids). In addition, the PK model by Gao and Jusko also includes a target-mediated CL or target-mediated drug disposition (TMDD) component, and the model reasonably describes the concentration profiles of exendin-4. Therefore both renal and target-mediated clearances determine the PK of this molecule.

The distribution of sunitinib was studied with ^{14}C-labeled compound in cynomolgus monkeys. Five to 6% of radioactivity was excreted through urine [59]. Similarly, only 1% of radioactivity was excreted through urine for labeled anacetrapib [5] and 14% for CI-1007 [14]. For rivoglitazone, however, recovery of urinary radioactivity can be up to 47% in monkeys [35]; for ILT-threo it is >60% [18], and for lodenosine (2′-b-fluoro-2′,3′-dideoxyadenosine) it is ~50% [60].

Hepatic Metabolism

Hepatic metabolism by CYP450 enzymes is a key determinant of the PK of small molecules such as those cited in Table 18.1. Additional examples of hepatic metabolism are summarized here. Carbamazepine is an inducer of CYP450 enzymes and a model substrate for measuring changes in drug metabolism. Wedlund et al. [61] used two different carbamazepine dose regimens (15 mg/hour for a total of either 4 hours or 101 hours) to induce different levels of CYP450 in rhesus monkeys. Liver samples were taken using biopsy to measure cytochrome P450. In addition, serum carbamazepine concentrations also were measured to calculate CL. The data show a good correlation of total body CL or intrinsic CL of carbamazepine versus CYP450 (r^2 = ~0.6).

VetBooks.ir

Using cynomolgus monkey hepatocytes, Ballard et al. [62] showed that MK-0457 (N-[4(thio) phenyl]cyclopropanecarboxamide) [18] was biotransformed to two major metabolites, VRT07500074 and VRT171335, via CYP3A4 and CYP2C8. For ILT-threo, additional phase II metabolism–glucuronidation was also identified in monkeys [18].

Nonspecific CL of Biologics

Anti–amyloid β Ab2 is a humanized mAb against amino acids 3-6 of primate amyloid β. The pharmacokinetics of Ab2 were studied in nonclinical species [6]. In cynomolgus monkeys, the $t_{1/2}$ of Ab2 was faster (<2.5 days) compared with that of the control antibody (~13 days). The mechanism(s) contributing to this difference in PK was studied in whole-blood partitioning experiments, where the interactions of Ab2 with plasma proteins from multiple primate species (cynomolgus and rhesus monkeys, baboons, marmosets, and humans) were evaluated. The data show that similar amounts of fibrinogen were immunoprecipitated from cynomolgus and rhesus monkey plasma, slightly less immunoprecipitated from baboon plasma, and markedly less to undetectable (with some interdonor variability) from human and marmoset plasma. Therefore, the fast elimination of Ab2 in cynomolgus monkeys was linked to an off-target binding to cynomolgus monkey fibrinogen.

OTHER SPECIAL PK STUDIES USING NHPs

Azithromycin PK in maternal plasma (MP), fetal plasma, and amniotic fluid were studied in pregnant rhesus monkeys following intra-amniotic infection with *Ureaplasma* [63]. Maternal monkeys were dosed intravenously with azithromycin at 12.5 mg/kg every 12 hours for 10 days. The results show that azithromycin transfers from maternal circulation through the placenta to the fetus. The MP–to–amniotic fluid AUC ratio after multiple doses was 1.2. The median MP–to–fetal plasma AUC ratio was 3.2.

Roth et al. [60] collected cerebrospinal fluid (CSF) from a chronically indwelling subcutaneous Ommaya reservoir attached to a fourth ventricular Pudenz catheter in rhesus monkeys. They reported that the amount of CSF following lodenosine (20 mg/kg) intravenous administration is low, with a CSF-to-plasma AUC ratio of 0.068.

PK ANALYSIS OF DATA OBTAINED FROM NHPs

In addition to noncompartmental analysis, several PK models have been applied to describe concentration profiles observed in NHPs. The choice of a model depends on the concentration profiles and the elimination mechanisms. For examples, a two-compartment model adequately described concentration profiles of anti–HGF [4,36]. A Michaelis-Menten model was used to capture the nonlinear PK of a CD4-anchoring bifunctional fusion inhibitor [13]. For biologics, the TMDD model can be also used to capture PK nonlinearity and target binding [64]. The main component of TMDD includes a bimolecular interaction between the drug and the receptor target.

SPECIES DIFFERENCE AND PREDICTION FOR HUMAN PK

VetBooks.ir

For small molecules, preclinical species differences in PK have been reported: PK of lododoxorubicin and its active metabolite, iododoxorubicinol, were studied in rats, dogs, and monkeys. The mean iododoxorubicinol-to-lododoxorubicin AUC ratios were 0.02, 0.47, and 0.58 in rats, dogs, and monkeys, respectively, values considerably lower than those reported in human studies [65]. The PK and metabolic pathways of odanacatib also were studied in rats, dogs, and monkeys [24]. In monkeys, odanacatib was almost completely eliminated by metabolism; metabolism also played a major role in the CL of odanacatib in rats and dogs. However, the major metabolites are M8 and M3 for monkeys; M4, M5, and M6 for dogs; and M4, M5, M8, and M6 for rats. Therefore, odanacatib displayed species-dependent metabolism. DB289 is a prodrug of DB75. The conversions of this prodrug were compared, and almost half of an oral dose of DB289 to rats and about one-third of that to monkeys was metabolized to DB75 [31]. M1, M2, and M3 metabolites of anacetrapib (MK-0859) were detected in rat but not in monkey plasma [5]. O-demethyl-O-sulfate (M11) of rivoglitazone was observed as the major metabolite in rats but as only one of many minor metabolites in monkeys [35].

In spite of species differences in the metabolism of small molecules, PK data derived from NHPs frequently can be used to predict human PK: 103 nonpeptide xenobiotics were identified with intravenous PK data in rats, dogs, monkeys, and humans for the purpose of evaluating extrapolation of CL data from preclinical species to humans [66]. The majority (70%) of the data are compounds with low CL values (<30% liver blood flow). CL values were normalized with liver blood flow of the respective species. CL values (percentage of liver blood flow) from rats, dogs, and monkeys then were compared against human CL. The results show that monkey CL seemed to correlate with human CL better than rat and dog data, although quantitative correlation analysis was not shown.

However, Akabane et al. [67] argued that cynomolgus monkeys have lower oral bioavailability than humans. They used 13 commercial drugs (small molecules), representing different types of CYP450 isozyme selectivity, and P-glycoprotein affinity to compare PK parameters obtained experimentally in cynomolgus monkeys following both intravenous and oral administration with those for human PK parameters that have been reported. They found that monkeys have lower bioavailability than humans. Since there was a good correlation in hepatic CL between monkeys and humans, the key determining factor of the difference in monkey's versus human's oral bioavailability is intestinal metabolism. The first-pass intestinal metabolism is greater in cynomolgus monkeys than in humans. Since oral administration is a major route of delivery for small molecules, allometric scaling based on NHP data should be applied with caution.

Among the mAbs reviewed in this chapter (Tables 18.1 and **18.2**), dacliximab has relatively long $t_{1/2}$ (214 hours, or 8.91 days) in monkeys. In the clinic, dacliximab's $t_{1/2}$ is also long (480 hours, or 20 days), consistent with the expected half-life determined by allometric scaling [68]. Trastuzumab emtansine, measured as intact antibody drug conjugate, has a short $t_{1/2}$ (i.e., 12-22 hours) in monkeys; in the clinic, this molecule also has a short $t_{1/2}$ (i.e., 31-98.4 hours) [69]. A more comprehensive study to evaluate scalability of NHP data was conducted by Dong et al. [70]. They retrospectively compared the pharmacokinetics of 16 mAbs from FTIH studies to those predicted in humans based on allometric scaling of cynomolgus monkey pharmacokinetic data. Among 16 mAbs, 10 exhibited linear PK in monkeys and the

VetBooks.ir

rest exhibited nonlinear PK. Simple allometric scaling based on body weight [71] was applied to predict human CL and Vd for mAbs with linear PK. A good correlation was shown between observed and predicted PK and the ratios of predicted CL and Vd versus the respective observed values, which are in the range of 0.43 to 1.88.

For the remaining six mAbs exhibiting nonlinear PK in monkeys, the data were analyzed by a two-compartment PK model with parallel linear and nonlinear elimination (Michaelis-Menten kinetics) from the central compartment. In addition to linear parameters (CL and Vd), the additional Michaelis-Menten parameters (maximal capacity [Vmax] and the Michaelis-Menten constant [Km]) also were scaled up by body weight. For these mAbs with nonlinear PK in monkeys, predictions were concentration dependent: overprediction (up to 5.3-fold higher) occurred when the concentrations were below the predicted Km but were within 2.3-fold when the concentrations greatly exceeded the Km.

We should also mention that species differences in targets (e.g., differences in target level and binding affinity) may affect the prediction of human PK based on preclinical data from NHPs or other species. Potential PK differences and differences in target expression in disease states in patients may affect predictability too.

The NHP PK study designs and results summarized in this chapter can be used as guidance to prepare preclinical PK evaluation packages to support FTIH dosing. Prediction of human PK based on NHP data represents a key component of PK/pharmacodynamic models that are developed to translate PK and pharmacodynamics from animal models and support dose selection for FTIH studies.

References

[1] Gobburu JV, et al. Pharmacokinetics/dynamics of 5c8, a monoclonal antibody to CD154 (CD40 ligand) suppression of an immune response in monkeys. J Pharmacol Exp Ther 1998;286(2):925–30.
[2] US FDA. Drug approval package: Zytiga (Abiraterone acetate). Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202379Orig1s000PharmR.pdf.
[3] Halpern W, et al. Albugranin, a recombinant human granulocyte colony stimulating factor (G-CSF) genetically fused to recombinant human albumin induces prolonged myelopoietic effects in mice and monkeys. Pharm Res 2002;19(11):1720–9.
[4] Kakkar T, et al. Pharmacokinetics and safety of a fully human hepatocyte growth factor antibody, AMG 102, in cynomolgus monkeys. Pharm Res 2007;24(10):1910–8.
[5] Tan EY, et al. Pharmacokinetics, metabolism, and excretion of anacetrapib, a novel inhibitor of the cholesteryl ester transfer protein, in rats and rhesus monkeys. Drug Metab Dispos 2010;38(3):459–73.
[6] Vugmeyster Y, et al. Complex pharmacokinetics of a humanized antibody against human amyloid beta peptide, anti-abeta Ab2, in nonclinical species. Pharm Res 2011;28(7):1696–706.
[7] Vugmeyster Y, et al. Correlation of pharmacodynamic activity, pharmacokinetics, and anti-product antibody responses to anti-IL-21R antibody therapeutics following IV administration to cynomolgus monkeys. J Transl Med 2010;8:41.
[8] US FDA. Drug approval package: Benlysta (Belimumab) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125370Orig1s000PharmR.pdf.
[9] US FDA. Drug approval package: Avastin (Bevacizumab) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/STN-125085_Avastin_Pharmr.pdf.
[10] US FDA. Drug approval package: Adcetris (Brentuximab vedotin) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125399Orig1s000PharmR.pdf.
[11] US FDA. Drug approval package: Cometriq (cabozantinib) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203756Orig1s000PharmR.pdf.
[12] US FDA. Drug approval package: Kyprolis (carfilzomib) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202714Orig1s000PharmR.pdf.

[13] Liu X, et al. Pharmacokinetics and pharmacodynamics of CD4-anchoring bi-functional fusion inhibitor in monkeys. Pharm Res 2014;31(3):809–18.
[14] Feng MR, Loo J, Wright J. Disposition of the antipsychotic agent CI-1007 in rats, monkeys, dogs, and human cytochrome P450 2D6 extensive metabolizers. Species comparison and allometric scaling. Drug Metab Dispos 1998;26(10):982–8.
[15] Inaba M, et al. Pharmacokinetics of CPT-11 in rhesus monkeys. Cancer Chemother Pharmacol 1998;41(2):103–8.
[16] US FDA. Drug approval package: Zenapax (Dacliximab) nonclinical pharmacology and toxicology review. Available from: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm113476.pdf.
[17] Nagaya Y, et al. Species difference in the mechanism of nonlinear pharmacokinetics of E2074, a novel sodium channel inhibitor, in rats, dogs, and monkeys. Drug Metab Dispos 2013;41(5):1004–11.
[18] Beconi MG, et al. Metabolism and pharmacokinetics of a dipeptidyl peptidase IV inhibitor in rats, dogs, and monkeys with selective carbamoyl glucuronidation of the primary amine in dogs. Drug Metab Dispos 2003;31(10):1269–77.
[19] US FDA. Drug approval package: Remicade (Infliximab) pharmacology review. Available from: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm107706.pdf.
[20] US FDA. Drug approval package: Yervoy (Ipilimumab) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125377Orig1s000PharmR.pdf.
[21] Hsieh FY, et al. Toxicological protein biomarker analysis–an investigative one-week single dose intravenous infusion toxicity and toxicokinetic study in cynomolgus monkeys using an antibody-cytotoxic conjugate against ovarian cancer. Pharm Res 2008;25(6):1309–17.
[22] Xin Y, et al. Anti-neuropilin-1 (MNRP1685A): unexpected pharmacokinetic differences across species, from preclinical models to humans. Pharm Res 2012;29(9):2512–21.
[23] US FDA. Drug approval package: Gazyva (Obinutuzumab) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/125486Orig1s000PharmR.pdf.
[24] Kassahun K, et al. Pharmacokinetics and metabolism in rats, dogs, and monkeys of the cathepsin k inhibitor odanacatib: demethylation of a methylsulfonyl moiety as a major metabolic pathway. Drug Metab Dispos 2011;39(6):1079–87.
[25] US FDA. Drug approval package: Votrient (Pazopanib) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022465s000_PharmR.pdf.
[26] Mager DE, Neuteboom B, Jusko WJ. Pharmacokinetics and pharmacodynamics of PEGylated IFN-beta 1a following subcutaneous administration in monkeys. Pharm Res 2005;22(1):58–61.
[27] Farese AM, et al. Pegfilgrastim, a sustained-duration form of filgrastim, significantly improves neutrophil recovery after autologous marrow transplantation in rhesus macaques. Bone Marrow Transplant 2003;32(4):399–404.
[28] US FDA. Drug approval package: Perjeta (Pertuzumab) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/125409Orig1s000PharmR.pdf.
[29] US FDA. Drug approval package: Pomalyst (Pomalidomide) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/204026Orig1s000PharmR.pdf.
[30] US FDA. Drug approval package: Iclusig (Ponatinib) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203469Orig1s000PharmR.pdf.
[31] Midgley I, et al. Pharmacokinetics and metabolism of the prodrug DB289 (2,5-bis[4-(N-methoxyamidino)phenyl]furan monomaleate) in rat and monkey and its conversion to the antiprotozoal/antifungal drug DB75 (2,5-bis(4-guanylphenyl)furan dihydrochloride). Drug Metab Dispos 2007;35(6):955–67.
[32] Ramakrishnan R, et al. Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after intravenous and subcutaneous dose administration in cynomolgus monkeys. J Pharmacol Exp Ther 2003;306(1):324–31.
[33] Nadeau RW, et al. A comparison of mass balance, pharmacokinetics and disposition of [14C(U)]- and [125I] recombinant human interleukin-2 in cynomolgus monkeys. Drug Metab Dispos 1995;23(9):904–9.
[34] US FDA Drug approval package: Rituxan (Rituximab) preclinical and clinical pharmacology review. Available from: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm113334.pdf.

VetBooks.ir

[35] Uchiyama M, et al. Pharmacokinetics, metabolism, and disposition of rivoglitazone, a novel peroxisome proliferator-activated receptor gamma agonist, in rats and monkeys. Drug Metab Dispos 2011;39(4):653–66.
[36] Krzyzanski W, et al. Pharmacokinetic and pharmacodynamic modeling of romiplostim in animals. Pharm Res 2013;30(3):655–69.
[37] Kelley SK, et al. Preclinical pharmacokinetics, pharmacodynamics, and activity of a humanized anti-CD40 antibody (SGN-40) in rodents and non-human primates. Br J Pharmacol 2006;148(8):1116–23.
[38] Ogasawara A, et al. Effect of oral ketoconazole on oral and intravenous pharmacokinetics of simvastatin and its acid in cynomolgus monkeys. Drug Metab Dispos 2009;37(1):122–8.
[39] US FDA. Drug approval package: Actemra (Tocilizumab) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/125276s000PharmR.pdf.
[40] US FDA. Drug approval package: Kadcyla (Trastuzumab emtansine) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/125427Orig1s000PharmR.pdf.
[41] US FDA. Drug approval package: Zaltrp (Ziv-Aflibercept) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/125418Orig1s000PharmR.pdf.
[42] Drugs@FDA, http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.
[43] US FDA. Drug approval package: Gilotarif (afatinib) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/201292Orig1s000PharmR.pdf.
[44] US FDA. Drug approval package: Mekinist (trametinib) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/204114Orig1s000PharmR.pdf.
[45] US FDA. Drug approval package: Tafinlar (dabrafenib) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/202806Orig1s000PharmR.pdf.
[46] US FDA. Drug approval package: Bosulif (bosutinib) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000PharmR.pdf.
[47] US FDA. Drug approval package: Inlyta (Axitinib) pharmacolgy review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202324Orig1s000PharmR.pdf.
[48] US FDA. Drug approval package: Xtandi (enzalutamide) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203415Orig1s000PharmR.pdf.
[49] Tabrizi MA, Tseng CM, Roskos LK. Elimination mechanisms of therapeutic monoclonal antibodies. Drug Discov Today 2006;11(1–2):81–8.
[50] US FDA Drug approval package: Neulasta (Pegfilgrastim) pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/125031_000_Neulasta_pharmr.pdf.
[51] Takatani H, et al. Levels of recombinant human granulocyte colony-stimulating factor in serum are inversely correlated with circulating neutrophil counts. Antimicrob Agents Chemother 1996;40(4):988–91.
[52] Roskos LK, et al. Pharmacokinetic/pharmacodynamic modeling of pegfilgrastim in healthy subjects. J Clin Pharmacol 2006;46(7):747–57.
[53] Dall'Acqua WF, Kiener PA, Wu H. Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). J Biol Chem 2006;281(33):23514–24.
[54] Deng R, et al. Pharmacokinetics of humanized monoclonal anti-tumor necrosis factor-{alpha} antibody and its neonatal Fc receptor variants in mice and cynomolgus monkeys. Drug Metab Dispos 2010;38(4):600–5.
[55] Datta-Mannan A, et al. FcRn affinity-pharmacokinetic relationship of five human IgG4 antibodies engineered for improved in vitro FcRn binding properties in cynomolgus monkeys. Drug Metab Dispos 2012;40(8):1545–55.
[56] Sleep D, Cameron J, Evans LR. Albumin as a versatile platform for drug half-life extension. Biochim Biophys Acta 2013;1830(12):5526–34.
[57] Doyle ME, Egan JM. Mechanisms of action of glucagon-like peptide 1 in the pancreas. Pharmacol Ther 2007;113(3):546–93.
[58] Gao W, Jusko WJ. Target-mediated pharmacokinetic and pharmacodynamic model of exendin-4 in rats, monkeys, and humans. Drug Metab Dispos 2012;40(5):990–7.
[59] Speed B, et al. Pharmacokinetics, distribution, and metabolism of [14C]sunitinib in rats, monkeys, and humans. Drug Metab Dispos 2012;40(3):539–55.
[60] Roth JS, et al. 2'-beta-fluoro-2',3'-dideoxyadenosine, lodenosine, in rhesus monkeys: plasma and cerebrospinal fluid pharmacokinetics and urinary disposition. Drug Metab Dispos 1999;27(10):1128–32.
[61] Wedlund PJ, et al. Linear relationship between cytochrome P-450 and carbamazepine clearance in rhesus monkey. Drug Metab Dispos 1982;10(5):480–5.

VetBooks.ir

[62] Ballard JE, Prueksaritanont T, Tang C. Hepatic metabolism of MK-0457, a potent aurora kinase inhibitor: interspecies comparison and role of human cytochrome P450 and flavin-containing monooxygenase. Drug Metab Dispos 2007;35(9):1447–51.
[63] Acosta EP, et al. Transplacental transfer of Azithromycin and its use for eradicating intra-amniotic ureaplasma infection in a primate model. J Infect Dis 2014;209(6):898–904.
[64] Chen T, Mager DE, Kagan L. Interspecies modeling and prediction of human exenatide pharmacokinetics. Pharm Res 2013;30(3):751–60.
[65] Edwards DM, et al. Pharmacokinetics of iododoxorubicin in the rat, dog, and monkey. Drug Metab Dispos 1991;19(5):938–45.
[66] Ward KW, Smith BR. A comprehensive quantitative and qualitative evaluation of extrapolation of intravenous pharmacokinetic parameters from rat, dog, and monkey to humans. I. Clearance. Drug Metab Dispos 2004;32(6):603–11.
[67] Akabane T, et al. A comparison of pharmacokinetics between humans and monkeys. Drug Metab Dispos 2010;38(2):308–16.
[68] US FDA. Drug approval package: Zenapax (Dacliximab) clinical pharmacology review. Available from: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm113472.pdf.
[69] US FDA. Drug approval package: Kadcyla (Trastuzumab emtansine) clinical pharmacology review. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/125427Orig1s000ClinPharmR.pdf.
[70] Dong JQ, et al. Quantitative prediction of human pharmacokinetics for monoclonal antibodies: retrospective analysis of monkey as a single species for first-in-human prediction. Clin Pharmacokinet 2011;50(2):131–42.
[71] Mordenti J. Man versus beast: pharmacokinetic scaling in mammals. J Pharm Sci 1986;75(11):1028–40.

VetBooks.ir

VetBooks.ir

PART VII

NONHUMAN PRIMATE-SPECIFIC ASPECTS OF STANDARD TOXICOLOGY STUDIES

VetBooks.ir

VetBooks.ir

CHAPTER

19

Advantages and Limitations of Commonly Used Nonhuman Primate Species in Research and Development of Biopharmaceuticals

Annick J. Cauvin[*], *Christopher Peters*[*], *Frank Brennan*[†]

[*]UCB Biopharma, New Medicine, Non-Clinical Development, Braine L'Alleud, Belgium

[†]UCB Pharma, New Medicines, Non-Clinical Development, Slough, UK

OUTLINE

Introduction 379

Use of NHPs in Research and Development 382

Rhesus Macaques 382

Cynomolgus Monkeys 383

Marmosets 384

Evaluation of Reproductive and Developmental Toxicity 385

Cynomolgus Monkeys 386

Marmosets 388

Evaluation of Immune Functions 389

Conclusion 392

References 393

INTRODUCTION

Biopharmaceuticals, also known as a biologics or biologicals, differ from other pharmaceutical products, or small molecules, in that they are created using biological processes rather than being chemically synthesized. They include a wide variety of medicinal products such as vaccines, blood or blood components, somatic cells, gene therapy, tissue, recombinant

http://dx.doi.org/10.1016/B978-0-12-417144-2.00019-6

© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

therapeutic proteins, or living cells that are used as therapeutics to treat diseases. This chapter focuses on the main classes of biopharmaceuticals that are tested in nonhuman primates (NHPs), i.e., monoclonal antibodies (mAbs) and recombinant proteins/peptides.

The goal of biopharmaceutical development is to maximize therapeutic benefit while minimizing the risk of treatment-related toxicity. Animal testing is key in the prediction of toxicity; pharmacokinetics and pharmacodynamics of these drugs are a prerequisite for the drug approval process. To maximize the chances of detecting toxicity of biopharmaceuticals, including prediction of putative interpatient variability in the responsiveness to the test article, selecting a relevant species for conducting safety assessment studies is important; according the International Conference for Harmonization (ICH) S6 guidance [1], this is a species in which the test article is pharmacologically active due to the expression of the target or, in the case of antibodies, to the expression of an epitope. In addition, understanding the relative sensitivity of the species or the strain to the pharmacological effect, as well as to other effects that may be mediated by the nature of the test item, is important. Finally, the physiological similarity between humans and the test species considered for the pathway or system to be evaluated is key. Appropriate selection of the relevant species that is sensitive to the pharmacological effects of the biopharmaceutical of interest enables the identification of factors that most reproducibly affect the margin between safety and efficacy (the therapeutic index) and the selection of safe doses for human testing.

Although there is growing pressure to use lower species and to limit the use of animals in drug development, NHPs cannot be easily replaced in numerous area of safety evaluation. Their phylogenetic closeness to humans has naturally identified them as a model of choice for the safety assessment of large molecules. The high homology of sequences of many proteins between humans and NHPs enables target receptor binding or epitope recognition by a human protein or mAb therapeutic more often in NHPs than in lower species. This sequence homology for many proteins also limits immunogenicity, often allowing treatment with a human or humanized protein over long periods of time [2]. The rationale and experimental means by which the appropriate species is selected differ for small and large molecules. Nevertheless, for some specific targets, similarities with human anatomy and physiology are crucial in evaluating the effect resulting from target occupancy and modulation. For example, aging primates are used as models for geriatric diseases and osteoporosis [3]. Primates also are a species of choice for many ocular indications such as macular degeneration, retinopathy, testing of retinal implants, treatments targeting the reproductive system, or bone remodeling in various pathophysiologic conditions. NHPs are especially useful in the risk assessment for immunotoxicology of biotherapeutic proteins [3].

Given ethical considerations, the number of NHPs used in a study or across a safety assessment program is limited when compared with the use of rodents and dogs. Nevertheless there is good knowledge of the background lesions and abnormalities that can be encountered, aiding in the interpretation of any findings [4–6].

Like rodents, NHPs are not a single entity. The most commonly used species are cynomolgus monkeys, rhesus monkeys, and marmosets. These three species have characteristics and physiological differences that may render them either attractive, inappropriate, or unsuitable for a given risk assessment (Table 19.1). The presence of the target protein and the pharmacological activity of the biopharmaceutical are required, but not always sufficient, to ensure an optimum safety assessment; other factors need to be considered. For example, reproductive and developmental functions cannot easily be evaluated in the rhesus monkey because of

VetBooks.ir

TABLE 19.1 Comparison of NHP: Selected Species-Specific Differences

	Cynomolgus macaque (*Macaca fascicularis*)	**Rhesus macaque (*Macaca mulatta*)**	**Marmoset (*Callithrix jacchus*)**
Size (cm), range	44-53	47-53	18.5-18.8
Weight (kg), range	4.1-7.0	5.3-7.7	0.24-0.26
Blood volume (mL/kg)	65	55	70
Respiration rate (breaths/min), mean±SD	36±13	51±15	101±14
Tidal volume (mL)	46±6	39±11	0.98±0.1
Heart rate (beats/min)	123±30	174±27	400±45
PT (s)	12.1±1.1	14.5±0.9	6.7±0.5
Gamma GT (U/L; male)	70.5±16	76.5±16.7	5.1±4.3
ALT (U/L; male)	55.3±30.9	59±22.4	3±2.4
Reproductive physiology	Like human physiology	Like human seasonality	Different from human (males and females)
Sexual maturity	Females: 2.5-3 years Males: >4 years[a]	Females: 3 years Males: >4 years[a]	17-20 months
Menstrual cycle (days)	28±5	28±5 only 3-6 months per year	24-30 days (polyestrous, up to 4)
Gestation duration (days)	134-184	164	140-150
Litter size (*n*)	1	1	1-3 (usually twins)
Birth weight (g)	320	480-520	25-35
Interbirth interval (months)	15±5	14	6
Reproductive toxicology	Well established	Well established	Partially established
Developmental toxicology	Well established	Well established	Partially established
Palate fusion (gestational days)	45-50	45-50	75-80

[a]*Males may not be able to reproduce until 6-8 years of age.*
ALT, alkaline phosphatase; gamma GT, gamma glutamyl transferase; PT, prothrombin time.

seasonal variations. Some data can be generated from marmosets; however; their interpretation and their translatability to humans is questionable. One species may also be more predictive than another for a specific safety concern in humans. In addition, substrains of a given species from different geographical origins may also present genetic differences that may have a considerable impact on the outcome of biopharmaceutical evaluation.

Therefore selecting an NHP species for research or nonclinical development requires the careful evaluation of numerous ethical and scientific considerations. The possibility of using the clinical candidate is always an advantage. Confidence in the similarity of target distribution, density, downstream signaling, and physiology are important. Nevertheless, hazard

VetBooks.ir

identification also has to be taken into consideration, and in this respect, NHPs are not always superior to other species, perhaps because of the limited number of individuals allocated to nonclinical studies. The purpose of this chapter is to help the reader understand how to best select the right NHP species or strain and to illustrate this with examples.

USE OF NHPs IN RESEARCH AND DEVELOPMENT

Several NHP species have been used for research and drug development in many research areas because of their close evolutionary relationship to humans. Recent development of genomic resources also have boosted their value as model organisms. The Old World primates rhesus macaque (*Macaca mulatta*) and cynomolgus macaque (*Macaca fascicularis*) are by far the most commonly used species. The New World primate marmoset (*Callithrix jacchus*) is more evolutionary diverged from humans than Old World monkeys but is sometimes used when the two other species are not pharmacologically relevant for the biopharmaceutical or when specific models exist only in the marmoset. Each of these three species presents advantages and disadvantages for pharmacologic and toxicologic assessment of biopharmaceuticals, which are reviewed in this chapter.

Rhesus Macaques

Because of its relatively easy maintenance in captivity, wide availability, and anatomic and physiological proximity to humans, the rhesus macaque has been used extensively in medical and biological research on human and animal health-related topics and has given its name to the rhesus factor, one of the elements of a human blood group. The rhesus macaque is widely used as an NHP model to study infection and immunity because of the close genetic relationship with humans (93% average human-macaque sequence identity) and because of the homology between human and rhesus pathogen genomes. Rhesus macaques have been used to study fundamental aspects of immunology, including the development and maintenance of T-cell memory, immune dominance, and the aging immune system. There also have been many studies of immune responses in rhesus macaque models of human infections such as human immunodeficiency virus (HIV), influenza virus, tuberculosis, Epstein-Barr virus (EBV), cytomegalovirus, smallpox, measles, and severe acute respiratory syndrome [7]. Furthermore, rhesus macaques have been instrumental in designing and testing of vaccines against infections such as HIV and smallpox, in creating drugs to manage HIV/AIDS [7], and in understanding the female reproductive cycle and development of the embryo and the propagation of embryonic stem cells [8]. The genome of the rhesus macaque was cloned in 2007 [9]. Surprisingly, some normal gene sequences in healthy macaques cause profound disease in humans. For example, the normal sequence of phenylalanine hydroxylase in macaques is the mutated sequence responsible for phenylketonuria in humans. Some gene families are conserved or under evolutionary pressure and expansion in humans, macaques, and great apes such as chimpanzees (e.g., cholesterol pathways), whereas some are uniquely under expansion in humans, chimpanzees, or macaques (e.g., major histocompatibility genes are more abundant in macaques) [9]. The rhesus macaque was instrumental in understanding key features of reproduction. However, males and females are only fertile 4-5 months per

VetBooks.ir

year; seasonality is influenced by the geographic origin and is still observed after several years of being housed in a research colony in Western countries [10].

Since the rhesus macaque is no longer available from India, it has started to be used less in research; there is often considerable lead time in sourcing these animals. In addition, it has been progressively replaced by the cynomolgus monkey in preclinical toxicology studies, in part because of its bigger size (requiring large amounts of a drug for dosing and making them more difficult to handle) and its seasonal reproduction, which makes its use in reproductive and developmental studies more difficult. This has resulted in less toxicology background data being available for interpretation of any findings.

Cynomolgus Monkeys

The cynomolgus monkey has become the most widely used primate species in preclinical toxicology studies. It is widely available, being purpose bred for laboratory use, is of a size that represents a good compromise with regard to the amount of drug required for dosing and the amount of blood/tissue that can be sampled since it is generally smaller than the rhesus macaque (Table 19.1). Cynomolgus macaques are especially useful for reproductive and developmental toxicology when lower species are not pharmacology relevant or given their close resemblance to human. They are also good models for the development of locally administered drugs, such as those for use in specific ocular diseases or prostate disorders, and for inhalation. Nevertheless, although specific information is easily obtained from numerous breeding colonies, the cynomolgus monkey has not been as extensively characterized as the rhesus macaque in terms of genomics; however, efforts are underway to comparatively screen rhesus, cynomolgus, and other macaque species genomes.

Both rhesus and cynomolgus macaque species exhibit substantial variations dependent on origin and genetic background. As an example, Chinese-derived populations of rhesus macaques have been shown to be more resistant to simian immunodeficiency virus than Indian-derived populations. Hematology and clinical chemistry differences also have been demonstrated in these two populations. In rhesus macaques, such dependence on genetic background is understood and used to guide research. Although cynomolgus monkeys are the most commonly used in the development of biopharmaceuticals such as mAbs and recombinant proteins, there is relatively little information on their genome in public domain databases. Nevertheless, cynomolgus monkeys originate from three different populations (e.g., Indochina, insular Asia and the Philippines, and Mauritius) with own genetic features. Animals from Mauritius and the Philippines exhibit potentially lower genetic diversity, even for genes involved in immune response [11]. Differences in the gene sequences of some target proteins have been encountered within cynomolgus monkeys sourced from different geographic origins (e.g., China, the Philippines, Mauritius), raising concerns that certain genetic variants might result in a loss or gain of pharmacologic responses in the cynomolgus monkey colony used for toxicology studies. Phenotypic differences in cynomolgus monkeys of diverse geographic origin, in particular for immune system functions, are well established, as illustrated by the diverse major histocompatibility complex (MHC) genetics of cynomolgus monkey from different geographic origin [12,13]. Collectively, these observations suggest that a prospective assessment of target gene variation may be warranted for cynomolgus monkey

VetBooks.ir

(and other NHP species) pharmacology and toxicology studies. Moreover, identifying a sequence variation within a target gene requires complementary target expression/distribution, epitope/binding, and/or functional readouts.

Marmosets

C. jacchus (common marmoset) is one of the more primitive NHP species and is used widely in fundamental biology, pharmacology, and toxicology studies. Utilizing this species may provide the optimal compromise between ethical considerations, disease understanding, and drug development. Once the scientific rationale has been carefully considered and their use justified, there are several advantages to using the marmoset as a model in the nonclinical development of pharmaceutical products.

There are a number of unique physiologic differences between Old World primates such as rhesus and cynomolgus macaques and New World primates such as marmosets. For example, anatomic differences in placentation and frequent twinning lead to bone marrow chimerism of dizygotic twins. Limited diversity at both MHC class I and II loci has been identified in both tamarins and marmosets and may be responsible for differences in disease susceptibilities that are exploited in infectious disease studies. While the availability of research tools has been a limitation in the past, making marmosets less attractive than rhesus macaques as animal models, this is no longer the case in a number of key areas.

Experimental autoimmune encephalomyelitis (EAE) is a well-established animal model of multiple sclerosis (MS). The disease induced in common marmosets more closely resembles MS than that in rodent or other primate models in terms of clinical signs, lesions, and evolution. The unique particularities of marmosets in terms of full bone marrow chimerism of littermates have been key in the understanding of disease pathogenesis. The EAE model has been used in the preclinical evaluation of novel immunotherapies such as testing new anti-inflammatory agents, tolerance-based therapies, and costimulated target therapy [14]. Marmosets also have been used widely in neuroscience research programs in models of cerebral vascular disease, tardive dyskinesia, and neurodegenerative diseases and in studies of normal neurophysiology [15].

Common marmosets have been used extensively in infectious disease research in large part because of their unique sensitivity to a number of important human infectious agents, including viral, bacterial, and parasitic agents. In particular, marmosets are susceptible to a variety of herpes virus agents, including gammaherpes viruses, such as EBV and herpesvirus saimiri, herpes simplex virus, hepatitis A virus infection, Junin virus, malaria, measles, and GB virus B, a surrogate model of human hepatitis C virus. The reason marmosets are highly susceptible to infection with many of these agents is not understood, but limited diversity at MHC class I and II loci is hypothesized to play a role, as mentioned above. In particular, marmosets have been extensively used to investigate the molecular pathogenesis of viral-induced lymphoma and EBV and lymphocrypto virus (LCV) model of viral oncogenesis or viral persistence [15].

The marmoset also has been used as a nonrodent second species in drug safety assessment of new chemical entities (NCEs) and, more recently, of biopharmaceuticals based on side effects, findings of given drugs, and metabolizing enzymes or receptors found to be similar to humans [16–19]; because of the closer phylogenetic relationship to humans than other second species such as the dog, common marmosets may be more suitable for certain types of

VetBooks.ir

pharmacokinetic and toxicological screening. When the test item is a biopharmaceutical and the mechanism of action is dependent on specific receptors or epitopes, relevant species may be restricted to NHPs. The small size of common marmosets may represent an additional advantage because it reduces the quantity of compound to be synthesized and may translate into shorter development times. However, their short stature, sensitivity, and activity level may also pose unique challenges [16,17]. Nevertheless, for some development programs, as for canakinumab, the marmoset may be the only suitable species [20]. In addition, marmosets are largely free of diseases harmful to humans.

As a result, this species is being used in studies ranging from behavioral studies to reproductive and developmental protocols. However, this species poses unique challenges because their sensitivity, small size, housing conditions, and high activity level.

EVALUATION OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Many drugs in development are intended for chronic administration to infants, women of child-bearing potential (WoCBP), fertile men, and infants, requiring the evaluation of hazard on fertility and/or early development of the offspring and juvenile toxicity. With biologics, often the only relevant species with which to assess these is the NHP.

If for NCEs the reproductive strategies benefit from standard and well-validated study designs in rodents and in rabbits, for which extensive historical databases are available, some specific considerations for biopharmaceutical proteins have to be taken into account. They do not freely diffuse across membranes, including placenta, and are therefore not expected to affect early stages of development, especially embryogenesis, because the embryo/fetus is not directly exposed during early life [21].

Although mAbs and Fc fusion proteins are also large proteins, they can be actively transported from mother to fetus in mammals via the neonatal Fc receptor for immunoglobulin (Ig) G (FcRn), with variable efficacy as a function of the IgG subclass of antibodies from which they are derived, with $IgG_1 > IgG_4 > IgG_3 > IgG_2$ [21,22]. The long half-life of many therapeutic IgGs may result in prolonged pharmacological activity and effects on the developing fetus, including the immune system (developmental immunotoxicity). Transfer of antibodies occurs mainly during the second and third trimester in humans, when FcRn starts being expressed on the placenta. All antibodies or recombinant proteins with an intact IgG Fc portion can be transported across placenta, whereas antibody fragments (Fab, dAb, scFv, $F(ab)'_2$) without Fc are not [23]. Similar placental transfer of IgGs has been demonstrated in humans and NHPs [22,24,25].

Because IgG mAbs are not transferred to the fetus until later in pregnancy when organ development has ended, the evaluation of the effects of therapeutic IgG antibodies and Fc fusion proteins on embryo-fetal development is not systematically required prior the enrollment of WoCBP in phase II clinical trials (as for NCEs), according to the revised ICH M3 guideline [26]. This is because if a woman accidentally became pregnant while being administered an IgG mAb in a clinical trial, the risk of exposure to the developing offspring in the early weeks of pregnancy is very low. Potential adverse effects to the fetus in these early stages are more likely to be due to secondary effects of maternal toxicity rather than direct effects. However,

VetBooks.ir

some cases of fetal abnormalities without maternal toxicity have been reported and considered secondary to altered placental function and/or reduced fetal growth, even if the direct inhibition of target signaling in the conceptus cannot be ruled out, as reported with anti-receptor for the insulin-like growth factor 1 (IGFR) [27]. Therefore the assessment should be designed based on the risks that may be related to target modulation in the mother or in the infant. A careful review of the distribution and the role of the target in different physiological conditions is important to appreciate risks and adapt the study design as a consequence. Standard study designs for use with rodents and rabbits allow all steps of reproduction and development to be evaluated (as described in ICHS5(R2)) and are generally predictive, provided the therapeutic mAb does not modulate a pathway that is physiologically different between rodents/rabbits and humans. Conversely, study designs for use with NHPs do not cover all aspects of reproduction (e.g., direct fertility assessment through mating or egg implantation—pregnancy cannot be confirmed until day 20 of gestation) and so may not allow a full evaluation of the specific risks in humans. However, the physiology of reproduction, pregnancy, and parturition, including hormonal control, are very close between human and NHPs, allowing better identification of hazards, provided an appropriate study design is applied.

Unlike rodents, the relevance of the NHP model for developmental toxicity is well established [8]. The spontaneous incidence of congenital defects is low [28,29]. Clinical relevance and superiority over rodents has been demonstrated for thalidomide, vitamin A, and valproic acid [28].

Choosing the NHP for assessing the effect of a therapeutic protein on reproductive and developmental functions may help identify hazards that would not be identified in other species and allows, in general, an evaluation of the clinical candidate. However, there are cases where no relevant NHP species is suitable for assessing reproductive toxicity of the clinical drug product. In those cases, mAb surrogates are used for reproductive toxicity studies. Well-known examples are infliximab (anti-tumor necrosis factor alpha (TNFα)), efalizumab (anti-CD11a), and eculizumab (anti-C5), respectively.

Among others, an important consideration is the choice of the NHP species or even substrain for reproductive and developmental testing (Table 19.1). The physiology of the menstrual cycle of Old World monkeys closely resembles that of humans. For this reason, monkey species belonging to this group are preferred as experimental models in studies of human reproduction, pharmacology, and toxicology. Especially for studies of sex hormones, synthetic steroidal compounds, or compounds that interfere with the hypothalamus-pituitary-ovarian (or testicular) axis, such monkey species are predictive [30].

Cynomolgus Monkeys

Because of its lack of reproductive seasonality, convenient body size, close resemblance to human physiology, and thanks to the availability of a large historical database, the cynomolgus monkey is the species of choice for reproductive toxicity studies. The average duration of the menstrual cycle in the female is 28 days, and the endocrinology of the ovarian cycle is similar to that in women, with one predominant follicle maturing to ovulation. Markers of the menstrual cycle are also similar to those in women, and the cycle can be monitored by daily vaginal smears [31].

There are also strong similarities between the male cynomolgus monkeys and men in terms of endocrine control of testicular function (follicle stimulating hormone (FSH) is especially

VetBooks.ir

important for spermatogenesis and inhibin B is an important marker of testicular toxicity), prostate anatomy, and control of prostate function. Fertility rates are typically around 40-70% and preimplantation loss is evaluated at 25% [10].

The most relevant period in which to evaluate adverse drug effects on organogenesis takes place mainly between gestation days 20 and 50, with palate fusion occurring around gestation days 45-50. Pregnancy lasts around 160 days, with a range of 134-184 days. The maturation of different organs and systems evolves similarly to that of humans, with some noticeable differences, including earlier bone ossification, respiratory and urinary systems being mature at birth, and more rapid maturation of the digestive system [32]. Twin pregnancies are extremely rare in macaques, with an overall twin live birth incidence around 0.1%.

The low fertility rates and high abortion rates (10-30%) in this species limit the detection of infrequent risks [10]. Nevertheless, its size, ease of housing, and extensive panel of reagents make it an attractive model. Several marketed products have been tested in this species (basiliximab, adalimumab, rituximab, natalizumab, ustekinumab, alefacept, and omalizumab) for a partial or complete evaluation of embryo fetal and postnatal development.

There is a much ethical pressure to limit the use of NHPs, especially in reproductive toxicology. However, there are examples where rodents are not helpful in hazard identification, as illustrated in the two cases below.

An IgG4 directed against a soluble target for inflammatory indications was developed in the cynomolgus monkey and general toxicity studies were uneventful. The potential effects on the fetus and developing infant were addressed in a single enhanced pre and postnatal development study in the cynomolgus monkey. The decision was based on normal fertility, reproduction, and development in target knockout mice, or in mice treated with a therapeutic antibody blocking upstream signaling of the receptor, an based on an uneventful embryo-fetal development (EFD) study in cynomolgus monkeys treated with a therapeutic antibody targeting the same signaling pathway from gestation days 20 to 50 and undergoing a cesarean delivery on gestation day 100. Therefore, pregnant female macaques were administered the IgG4 weekly from gestation day 20 to approximately gestation day 160 and were allowed to deliver their offspring naturally and raise their infants. Maternal animals tolerated the antibody treatment as in general toxicity studies, without changes compared with control animals, and delivered healthy infants that developed normally with a functional immune system. However, an increased incidence of treatment-related perinatal mortalities was observed in infants. Macroscopic examination of the decedents revealed skull bone luxation; subdural, cerebellar, or superficial brain hemorrhage, all suggesting difficult delivery; and an empty gastrointestinal tract, indicating the absence of suckling after birth. Unexpectedly, treated maternal animals experienced a longer pregnancy than controls (up to a mean of 10 days) and a higher incidence of retained placenta, and prenatal maternal mortality associated in some cases with genital hemorrhages was observed. Dystocia is one of the leading causes of late pregnancy loss in cynomolgus monkeys, rhesus monkeys, and baboons, often resulting in intracranial trauma/hemorrhages in the nonviable fetus/infant [33–35]. However, difficult delivery rarely results in maternal death and is not usually associated with important genital blood losses, even in the case of placental retention. A careful review of the literature in light of the findings of the study suggested that the soluble target may indeed play a role in parturition, as indicated by increased circulating concentrations of the target during the third trimester of pregnancy and increased messenger RNA and protein concentrations in the cervix,

VetBooks.ir

myometrium, and choriodecidua during labor [36]. The target is thought to be involved in the local process of inflammation occurring at parturition, enabling recruitment of leukocytes in the cervix and uterine wall [37] to induce cervical ripening and dilation and probably resorption of the interface between the placenta and uterine wall [38,39]. It also promotes the expression of the prostaglandin synthase type-2 in the cervix, myometrium, choriodecidua, and placenta [40], enabling efficient contractions.

Although additional factors interfering with parturition cannot be ruled out, the inhibition of target signaling may explain the observations in maternal animals. Maternal mortality was considered to be a direct consequence of the long and difficult labor associated in some instances with hemorrhage caused by incomplete resorption of the placenta/uterine interface and with genitourinary infections resulting from the prolonged labor. Perinatal infant mortality was considered indirectly linked to the treatment, resulting from head injuries at parturition or lack of maternal care during the first critical hours after birth.

Such findings could not have been observed in mice, whose physiology of parturition is very different, or in monkey studies not specifically addressing parturition, but they highlight a risk for pregnant women still exposed to the mAb by the end of the pregnancy based on the similar physiology of pregnancy and parturition in NHPs and humans. This example illustrates the need to carefully evaluate target expression, the biology of the target, and its potential role in pregnancy, as well as the need to understand how close the test species and humans are for the pathway to be explored.

Another example in which the cynomolgus monkey was shown to be more sensitive than the rodent for detection of adverse effects on pregnancy is the case of the soluble receptor for interleukin-4 (IL-4R) [41]. The treatment of pregnant cynomolgus monkeys with human soluble IL-4R (0.2 and 2.0 mg/kg intravenous) during organogenesis (gestational days 20-51) resulted in increased incidence of spontaneous abortion, embryo/fetal death, and stillborn neonates. However, pregnant Crl:CD1 mice treated with an intravenously soluble mouse (surrogate) IL-4R during organogenesis presented no adverse effects up to the equivalent dose of 20 mg/kg. CD-1 mice may be Th1-biased (like C57BL/6), a possible reason why no reproductive effects were seen in this mouse study—i.e., they already have more Th1 bias, so tipping them further that way has little effect [42].

Marmosets

Although the cynomolgus monkey is the established NHP model for reproductive and developmental toxicology, there are several advantages to conducting developmental toxicity studies using marmosets. Considerations include specific cross-reactivity—particularly with biologics—and some specific teratogenic risks that are more difficult to demonstrate in species with only one offspring.

From a reproductive point of view, marmosets are unique among NHPs and differ from humans in some key respects. Females have no menstrual bleeding, each cycle results in multiple ovulations, and postpartum conception is not uncommon. Duration of the menstrual cycle varies from 24 to 30 days, with a long luteal phase, a mid-phase FSH peak, and simultaneous maturation of one to four eggs [43]. Ovarian cyclicity can be controlled by monitoring progesterone after triggering luteolysis by administering prostaglandin-F2α. As a result, controlled EFD studies are feasible. Multiple, simultaneous ovulations result in litter

VetBooks.ir

sizes ranging from one to four births; twins (30-70%) and triplets (20-25%) are most frequent. Interestingly, marmoset have a naturally high abortion rate to reduce the number of fetuses in utero, as they generally can nurse only two offspring. Compared with Old World monkeys, the father has to stay with the mother for the entire duration of the pregnancy to prevent abortion. Gestation lasts between 140 and 150 days. The interbirth interval is approximately 6 months, but conception can occur 10-50 days postpartum. In contrast to humans, twins' placental disks are supplied by blood vessels of both fetuses. Shared anastomoses in the chorion commonly occur, exhibiting a hematopoietic XX/XY chimerism in the case of twins of opposite sex. In addition, most marmosets are chimera from at least two fertilized eggs or early embryos fused together. The difference in phenotype may be subtle (hitchhiker's thumb and straight thumb, different hair growth on opposite sides of the body, etc.) or completely undetectable [43]. In marmosets, as in most New World monkey species, chorionic gonadotropin hormone plays the role of luteinizing hormone (LH). Key differences are present in males too. The control of Leydig cell function and testosterone production by LH is entirely different from that of humans. Regulating genes on the Y chromosome are different from those of male Old World monkeys and humans, and some key fertility genes (e.g., *DAZ*) are absent. As a consequence, even if the organization of the testicular germinal epithelium is similar to that of man, the correspondence with the functional and hormonal spermatogenesis in human remains unknown. The reproductive cycle is considerably shorter than in macaques, and males attain fertility at 17-20 months of ages (for a review, see refs. 10,16,44).

This species is valid and relevant for EFD studies (segment II) in the event that cynomolgus monkeys cannot be used (e.g., a biopharmaceutical that is not pharmacologically active in cynomolgus or rhesus monkeys). Effects of canakinumab on embryo-fetal development were assessed in marmosets using the drug product. The only finding associated with canakinumab treatment was a slight reduction in the number of fetuses at the high dose, which correlated with a slight trend toward decreased placental weight. There were no treatment-related effects on fetal development. Despite the confirmation of placental transfer of IgG at the time of cesarean delivery (gestational days 112-114), given that transfer of IgG across the placenta starts late during gestation, it is unlikely that the fetus was exposed to canakinumab during organogenesis [22]. There are no other published examples of the use of marmosets in reproductive and developmental toxicology. The feasibility of the marmoset model for a full range of developmental studies, including segment III pre/postnatal studies, must be more fully evaluated [10,16,44].

EVALUATION OF IMMUNE FUNCTIONS

The high species specificity of the sequence/structure of most immune receptors targeted by mAbs makes the selection of an appropriate animal model challenging. This is probably less true for small-molecule drugs. However, the NHP is the most commonly selected animal model for safety assessment of immunomodulators, given its genetic similarity to humans and the homology of its immune system to that of humans. For reasons of practicability/availability, the cynomolgus monkey is the preferred NHP species, although occasionally marmosets and rhesus macaques are used. Nevertheless, there are safety-relevant functional differences between primate and human immune systems, despite a close evolutionary relationship. These include,

VetBooks.ir

for example, differences in MHC genes, distribution and function of Fc receptors and Toll-like receptors, and sensitivity to complement activation, which all need to be considered while assessing the safety of immunomodulatory compounds and, more specifically, agonists [45].

A range of potential liabilities associated with immune agonism may be explored in NHPs [46]. The cynomolgus monkey has been used as a relevant toxicology species for immunostimulatory agents such as Toll-like receptor agonists; however, while special recognition regarding the clinical risk of systemic cytokine release for certain targets is warranted, the NHP is not always predictive of this potential toxicity in humans. An important difference between humans and other NHPs such as cynomolgus macaques is the stronger human T-cell proliferative responses upon activation via the T-cell receptor. This is the consequence of human-specific loss of T-cell Siglec expression during the later stages of evolution. CD33-related Siglecs, which are inhibitory signaling molecules expressed on most immune cells, downregulate cellular activation pathways via cytosolic immunoreceptor tyrosine-based inhibitory motifs. Concordant with this species-related difference in Siglec expression is the observation that several common human T-cell-mediated diseases, such as bronchial asthma, rheumatoid arthritis, and Type 1 diabetes, have not been reported in chimpanzees or other great apes. The lack of Siglec expression in humans may partially explain why the cytokine release syndrome (CRS) in humans cannot be accurately predicted by NHPs or rodents. The life-threatening cytokine storm effects induced by TGN1412 were a result of the unexpected TGN1412-mediated activation (proliferation and cytokine release) of CD28-expressing $CD4^{+}CD45RO^{+}$ effector memory T cells predominantly residing in tissue [47]. These effects were not observed in cynomolgus monkeys, which do not express CD28 on effector memory T cells, nor in mice, in which the rapid T regulatory cell expansion quenched the proinflammatory effects of the relatively small effector T-cell population in these animals compared with a mature human immune system [48,49]. These human-specific differences in T-cell activation need to be carefully considered during safety assessment of immunostimulatory mAbs against T-cell targets.

Immune genes are expanded in macaques. The macaque genome has 33 major histocompatibility genes, three times that of humans. This has clinical significance because the macaque is used as an experimental model of the human immune system. By contrast, marmosets exhibit limited diversity at MHC class I and II loci [50,51].

Fc receptors are plasma membrane glycoproteins that bind to the Fc region of antibodies. Crosslinking of Fc receptors by Ab-opsonized antigen complexes initiate cellular immune responses, including phagocytosis, Ab-dependent cell-mediated cytotoxicity, respiratory burst, release of cytokines and inflammatory mediators, and antigen presentation. Binding human IgG to cynomolgus monkey FcγRs is broadly similar to that observed with human FcγR [52]; however, there are some important differences. In humans, FcγRIIIA (CD16A) is expressed on monocytes, macrophages, and NK cells, whereas the FcγRIIIB (CD16B) isoform is expressed on neutrophils, eosinophils, and other cells. In NHPs there is only one CD16 gene, homologous to the human CD16A, which is restricted to NK cells and monocytes [53]. This difference necessitated the testing of an antihuman CD16 (FcγRIIIA and FcγRIIIB)-specific mAb in human CD16 transgenic mice to assess the impact on neutrophils [54]. Even greater differences in Fc receptor expression, distribution, and internalization profiles between humans and mice also are observed [55]. Anatomic differences in human and animal immune systems and their responses to immunomodulatory drugs have been reviewed [56].

VetBooks.ir

The importance of ensuring that human-relevant Fc binding and effector function activity of IgG is occurring in the toxicology species is further illustrated by the case of hu5c8, an anti-CD40L IgG1. Development was stopped in clinical phase II after it induced thromboembolism in patients [57]. No thromboembolisms were detected in preclinical studies conducted using cynomolgus monkeys. Further investigations showed that treatment in rhesus monkeys, but not in cynomolgus monkeys, resulted in thrombi formation at various locations and vasculopathy characterized by intimal hyperplasia. The full-length IgG1 induced platelet aggregation and serotonin release once bound to its target in humans and in rhesus monkeys. Other constructs based on Fab fragments or full-length antibody with aglycosyl Fc did not cause platelet aggregation or activation in vitro, nor thromboembolisms or intimal vascular hyperplasia in vivo, while binding to the target with comparable affinity. The mechanism is not fully understood, but one hypothesis is that CD40 may play a role in hemostasis and platelet biology by functioning as a primary signaling receptor for CD40L and by serving as a docking molecule for CD40L immune complexes. CD40 may drive translocation of FcγRIIa (CD32a) to the platelet surface, enabling the Fc part of antibodies bound to their target to be captured and inducing strong platelet activation [58]. Based on these observations, the nonclinical development of a new anti-CD40L with a different format preventing Fc interaction was therefore conducted in the rhesus monkey [59]. This case study also demonstrates that the rhesus monkey may for some toxicities be the most relevant NHP species.

IgG1 and IgG3 antibodies are capable of mediating complement activation (binding to C1q). By contrast, IgG2 shows little complement activation and IgG4 shows none at all. Therefore, for immunomodulatory mAbs that bind to membrane-based targets, to avoid cell depletion, the IgG2 or IgG4 format or silent IgG1 (with reduced Fc effector function) is often used because of the absence of Fc effector function. In addition to complement-dependent cytotoxicity, which is an intended pharmacological effect of certain mAbs (e.g., direct killing of tumor cells), complement activation may lead to a number of safety-relevant consequences, including cytokine release, hematological effects, and/or potentially life-threatening hemodynamic disturbances. Despites large concentrations of complement components in cynomolgus monkey serum compared with human serum, the hemolytic activity is comparable. However, mechanistic differences in terms of (cross-)species-specific IgG/Fc-mediated complement activation, particularly related to IgG aggregation status, cannot be excluded and deserve further investigation [60].

Immunophenotyping of peripheral blood cells in NHPs is readily available for a variety of populations of lymphocytes and is usually included in the safety assessment of immunomodulators. A standard panel can include total T lymphocytes, helper T lymphocytes, cytotoxic T lymphocytes, B lymphocytes, and NK lymphocytes. Populations that typically are not evaluated unless warranted to measure a specific pharmacodynamic response may include regulatory T helper lymphocytes, activated T helper lymphocytes, activated cytotoxic T lymphocytes, effector memory T helper lymphocytes, effector memory cytotoxic T lymphocytes, central memory T helper lymphocytes, central memory cytotoxic T lymphocytes, naïve T helper lymphocytes, and naïve cytotoxic T lymphocytes. The selection of such assessments should be driven by the biology of the drug target and should be considered as part of the safety assessment on a case-by-case basis [60]. Some qualitative and quantitative differences exist with respect to surface markers on lymphocytes relevant for studies of immunotoxicity. For example, the marmoset lacks natural killer cells with typical markers

found in humans ($CD2^{-}CD56^{+}$); conversely, cytotoxic killer effector $CD4^{+}$ cells ($CD4^{+}CD56^{+}$) found in marmosets are not found in humans [17].

The T-cell-dependent antigen response (TDAR) assay encompasses multiple aspects of the immune response (i.e., antigen capture and presentation, T-cell help, and antibody formation [including class switching]) or the ability to mount an immune response in one end point: antigen-specific antibody titers [61]. In toxicology regulatory guidance documents, the TDAR is considered to be one of the functional assays of choice to evaluate potential immunotoxicity of investigational new drugs. However, the interanimal variability limits the sensitivity of the assay in studies powered for general toxicology evaluations, despite the similar behavior of sexes or animals from different countries of origin. Inhibition of the TDAR can be clearly evidenced with immunosuppressive agents in NHPs, but demonstrating a lack of activity in the assay has been problematic and the definition of biologically irrelevant variations is not established. Keyhole limpet hemocyanin, (KLH), sheep red blood cells (SRBC), and tetanus toxoid (TT) are the most commonly used antigens for challenge; KLH and TT show similar interanimal variability and a trend to less variability than SRBC. Power analysis of numerous studies has demonstrated that animal group sizes of 8-12, combining males and females, could detect ≤3.1-fold differences in anti-KLH IgG responses. Assay optimization and harmonization of the TDAR in NHPs remains a priority [60]. A functional *ex vivo* assay for NK cell-mediated lysis is available for NHPs (using the K562 human myelogenous leukemia target cell line), but the development of a functional assay for CTL activity is needed and is the subject of significant effort.

If there is generally a good concordance of pharmacodynamic effects in humans and NHPs, there is often limited concordance for adverse effects when considering mAbs or fusion proteins for cell surface targets or soluble targets [62,63]. The rare but serious adverse events that are of greater concern for patients are usually not predicted in studies conducted in normal healthy animals. Part of the reason why the animal studies fail to detect these effects is that NHP studies are not powered to detect rare events. Also, serendipitous infectious challenge may not occur in a controlled laboratory environment, and animals are generally prescreened for certain infectious agents before inclusion in studies; only those animals that test negative are included in the study. Laboratory animals do not receive comedications and are healthy when enrolled in toxicology studies. Regarding cell surface targets, downstream signaling may also be different because the antibody or fusion protein tested does not bind to the same epitope.

NHPs are often the only relevant species for the development of biopharmaceuticals; however, a comprehensive assessment of immunotoxicity remains a challenge because of the interanimal variability associated with certain parameters. The stage of development of certain immune assays, the incomplete knowledge of target distribution or effector function compared with humans, and a greater understanding of the background incidence of certain pathogens in NHPs are needed for proper interpretation of infectious agent-related findings that may be misinterpreted as treatment-related findings as a result of immunomodulation.

CONCLUSION

NHPs are used for research and development and are often the only relevant species for the development of biopharmaceuticals for many different indications. Many similarities with humans generally result in a good concordance of pharmacodynamics effects in humans and

NHPs. Several examples have demonstrated the need to use NHPs, especially to identify hazards in the reproductive and developmental assessment of biopharmaceuticals. Nevertheless, some important challenges that may impact safety and risk assessment for humans remain. These include limited numbers of animals and interanimal variability associated with some parameters, the stage of development of certain assays, the incomplete knowledge of target distribution or effector function compared with humans, and differences inherent to the species used or the provenance.

References

[1] ICHS6 (R1). Preclinical safety evaluation of biotechnology-derived pharmaceuticals. Step 2 Addendum; 2009. Available at http://www.ich.org/LOB/media/MEDIA5784.pdf.

[2] Swanson SJ, Bussiere JL. Immunogenicity assessment in non-clinical studies. Curr Opin Microbiol 2012;15:337–47.

[3] Bussiere JL. Immune function evaluation strategies in the nonhuman primate. In: Weinbauer GF, Buse E, Müller W, Vogel F, editors. New developments and challenges in primate toxicology. Muenster: Waxmann; 2004. p. 23–31.

[4] Chamanza R, Maxfeld HA, Blanco AI, Naylor SW, Bradley AE. Incidences and range of spontaneous findings in control cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. Toxicol Pathol 2010;38:642–57.

[5] Kaspareit J, Friderichs-Gromoll S, Buse E, Habermann G. Spontaneous neoplasms observed in cynomolgus monkeys (*Macaca fascicularis*) during a 15-year period. Exp Toxicol Pathol 2007;59:163–9.

[6] Kaspareit J, Friderichs-Gromoll S, Buse E, Habermann G. Background pathology of the common marmoset (*Callithrix jacchus*) in toxicological studies. Exp Toxicol Pathol 2006;57:405–10.

[7] Greenaway HY, Kurniawan M, Price DA, Douek DC, Davenport MP, Venturi V. Extraction and characterization of the rhesus macaque T-cell receptor b-chain genes. Immunol Cell Biol 2009;87:546–53.

[8] Hendrickx AG, Cukierski MA. Reproductive and developmental toxicology in nonhuman primates. In: Graham CR, editor. Preclinical safety of biotechnology products intended for human use. New York: Alan R. Liss, Inc.; 1987. p. 73–88.

[9] Rhesus Macaque Genome Sequencing and Analysis Consortium, Gibbs RA, Rogers J, Katze MG, Bumgarner R, Weinstock GM, et al. Evolutionary and biomedical insights from the rhesus macaque genome. Science 2007;316(5822):222–34.

[10] Weinbauer GF, Frings W, Fuchs A, Niehaus M, Osterburg I. Reproductive/developmental toxicity assessment of biopharmaceuticals in nonhuman primates. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals. Hoboken, NJ: John Wiley & Son; 2008. p. 379–98.

[11] Stevison LS, Kohn MH. Determining genetic background in captive stocks of cynomolgus macaques (*Macaca fascicularis*). J Med Primatol 2008;37:311–7.

[12] Campbell KJ, Detmer AM, Karl JA, Wiseman RW, Blasky AJ, Hughes AL, et al. Characterization of 47 MHC class I sequences in Filipino cynomolgus macaques. Immunogenetics 2009;61:177–87.

[13] Kita YF, Hosomichi K, Kohara S, Itoh Y, Ogasawara K, Tsuchiya H, et al. MHC class I A loci polymorphism and diversity in three Southeast Asian populations of cynomolgus macaque. Immunogenetics 2009;61:635–48.

[14] 't Hart BA, Massacesi L. Clinical, pathological, and immunologic aspects of the multiple sclerosis model in common marmosets (*Callithrix jacchus*). J Neuropathol Exp Neurol 2009;68:341–55.

[15] Mansfield K, Tardif S, Eichler EE. White Paper for complete sequencing of the common marmoset (*Callithrix jacchus*) genome, http://www.genome.gov/Pages/Research/Sequencing/SeqProposals/MarmosetSeq.pdf; 2004.

[16] Zühlke U, Korte S, Niggermann B, Fuchs A, Müller W. The common marmoset (*Callithrix jacchus*) as a model in biotechnology. In: Korte R, Vogel F, Weinbauer G, editors. Primate models in pharmaceutical drug development. Münster: Waxmann; 2002. p. 119–25.

[17] Orsi A, Rees D, Andreini I, Venturella S, Cinelli S, Oberto G. Overview of the marmoset as a model in nonclinical development of pharmaceutical products. Regul Toxicol Pharmacol 2011;59:19–27.

[18] Grace J, Wong H, Sinz M, Lentz K. Comparison of the formation kinetics of acetaminophen in chimpanzee, cynomolgus monkey, marmoset, rhesus monkey and human liver microsomes. Drug Metab Rev 2009;41:58–9.

[19] Schulz TG, Thiel R, Neubert D, Brassil PJ, Schulz-Utermoehl T, Boobis AR, et al. Assessment of P450 induction in the marmoset monkey using targeted anti-peptide antibodies. Biochim Biophys Acta 2001;1546:143–55.
[20] Ilaris. Toxicology summary. 2009. Available at http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/125319s000_PharmR_P2.pdf.
[21] Pentsůk N, Van der Laan JW. An interspecies comparison of placental antibody transfer : new insight into developmental toxicity testing of monoclonal antibodies. Birth Defects Res B Dev Reprod Toxicol 2009;86:328–44.
[22] Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 2007;7:715–25.
[23] Miller RK, Mace K, Polliotti B, DeRita R, Hall W, Treacy G. Marginal transfer of ReoPro (Abciximab) compared with immunoglobulin G (F105), inulin and water in the perfused human placenta in vitro. Placenta 2003;24:727–38.
[24] Fujimoto K, Terao K, Cho F, Honlo S. The placental transfer of IgG in the cynomolgus monkey. Jpn J Med Sci Biol 1983;36:171–6.
[25] Bowman CJ, Breslin WJ, Connor AV, Martin PL, Moffat GJ, Sivaraman L, et al. Placental transfer of Fc-containing biopharmaceuticals across species, an industry survey analysis. Birth Defects Res B Dev Reprod Toxicol 2013;98:459–85.
[26] ICHM3(R2). Nonclinical safety studies for the conduct of human clinical trials with pharmaceuticals. 2009. Available at http://www.ema.europa.eu/pdfs/human/ich/028695en.pdf.
[27] Bowman CJ, Chmielewski G, Oneda S, Finco D, Boucher MA, Todd M. Embryo-fetal developmental toxicity of figitumumab, an anti-insulin-like growth factor-1 receptor (IGF-1R) monoclonal antibody, in cynomolgus monkeys. Birth Defects Res B Dev Reprod Toxicol 2010;89:326–38.
[28] Peterson PE, Short JJ, Tahara R, Valverde C, Rothgarn E, Hendrickx AG. Frequency of spontaneous congenital defects in rhesus and cynomolgus macaques. J Med Primatol 1997;26:267–75.
[29] Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicology studies in nonhuman primates. Birth Defects Res B Dev Reprod Toxicol 2009;86:446–62.
[30] Cline JM, Söderqvist G, Register TC, Williams JK, Adams MR, von Schoultz B. Assessment of hormonally active agents in the reproductive tract of female nonhuman primates. Toxicol Pathol 2001;29:84–90.
[31] Van Esch E, Cline M, Buse E, Wood CE, de Rijk EPCT, Weinbauer GF. Summary comparison of female reproductive system in human and the cynomolgus monkey (*Macaca fascicularis*). Toxicol Pathol 2008;36:171S–2S.
[32] Martin PL, Weinbauer GF. Developmental toxicity testing of biopharmaceuticals in nonhuman primates: previous experience and future directions. Int J Toxicol 2010;29:552–68.
[33] Hendrie TA, Peterson PE, Short JJ, Tarantal AF, Rothgarn E, Hendrie MI, et al. Frequency of prenatal loss in a macaque breeding colony. Am J Primatol 1996;40:41–53.
[34] Sesbuppha W, Chantip S, Dick Jr EJ, Schlabritz-Lousevitch NE, Guardado-Mendoza R, Butler SD, et al. Stillbirths in Macaca fascicularis. J Med Primatol 2008;37:169–72.
[35] Schlabritz-Loutsevitch NE, Moore CM, Lopez-Alvarenga JC, Dunn BG, Dudley D, Hubbard GB. The baboon model (*Papio hamadryas*) of fetal loss: maternal weight, age, reproductive history and pregnancy outcome. J Med Primatol 2008;37:337–45.
[36] Tattersall M, Engineer N, Khanjani S, Sooranna S, Roberts V, Grigsby P, et al. Pro-labour myometrial gene expression: are preterm labour and term labour the same? Reproduction 2008;135:569–79.
[37] Osman I, Young A, Ledingham MA, Thomson AJ, Jordan F, Greer IA, et al. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod 2003;9:41–5.
[38] Hassan SS, Romero R, Haddad R, Hendler I, Khalek N, Tromp G, et al. The transcriptome of the uterine cervix before and after spontaneous term parturition. Am J Obstet Gynecol 2006;195:778–86.
[39] Winkler M. Role of cytokines and other inflammatory mediators. BJOG 2003;110:118–23.
[40] Sooranna SR, Grigsby PL, Engineer N, Liang Z, Sun K, Myatt L, et al. Myometrial prostaglandin E2 synthetic enzyme mRNA expression: spatial and temporal variations with pregnancy and labor. Mol Hum Reprod 2006;12:625–31.
[41] Carlock LL, Cowan LA, Oneda S, Hoberman A, Wang DD, Hanna R, et al. A comparison of effects on reproduction and neonatal development in cynomolgus monkeys given human soluble IL-4R and mice given murine soluble IL-4R. Regul Toxicol Pharmacol 2009;53:226–34.
[42] Masubuchi Y, Sugiyama S, Horie T. Th1/Th2 cytokine balance as a determinant of acetaminophen-induced liver injury. Chem Biol Interact 2009;179:273–9.

[43] Ross CN, French JA, Ortí G. Germ-line chimerism and paternal care in marmosets (*Callithrix kuhlii*). Proc Natl Acad Sci U S A 2007;104:6278–82.
[44] Fuchs A, Weinbauer G. Feasibility of embryofetal development studies in the marmoset (*Callithrix jacchus*). In: Weinbauer GF, Vogel F, editors. Novel approaches towards primate toxicology. Münster: Waxmann; 2006. p. 110–22.
[45] Bussiere JL. General toxicity testing and immunotoxicity testing for biopharmaceuticals. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals. A science based approach to facilitating clinical trials. Hoboken, NJ: John Wiley & Son; 2008. p. 343–56.
[46] Gribble EJ, Sivakumar PV, Ponce RA, Hughes SD. Toxicity as a result of immunostimulation by biologics. Expert Opin Drug Metab Toxicol 2007;3:209–34.
[47] Eastwood D, Findlay L, Poole S, Bird C, Wadhwa M, Moore M, et al. Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on $CD4^+$ effector memory T-cells. Br J Pharmacol 2010;161:512–26.
[48] Gogishvili T, Langenhorst D, Lühder F, Elias F, Elflein K, Kevin M, et al. Rapid regulatory T-cell response prevents cytokine storm in CD28 superagonist treated mice. PLoS One 2009;4(2):e4643.
[49] Frigault MJ, June CH. Predicting cytokine storms: it's about density. Blood 2011;118:6724–6.
[50] Nguyen DH, Hurtado-Ziola N, Gagneux P, Varki A. Loss of Siglec expression on T lymphocytes during human evolution. Proc Natl Acad Sci U S A 2006;103:7765–70.
[51] Muller PY, Brennan FR. Safety assessment and dose selection for first-in-human clinical trials with immunomodulatory monoclonal antibodies. Clin Pharmacol Ther 2009;85:247–58.
[52] Warncke M, Calzascia T, Coulot M, Balke N, Touil R, Kolbinger F, et al. Different adaptations of IgG effector function in human and nonhuman primates and implications for therapeutic antibody treatment. J Immunol 2012;188:4405–11.
[53] Rogers KA, Scinicariello F, Attanasio R. IgG Fc receptor III homologues in nonhuman primate species: genetic characterization and ligand interactions. J Immunol 2006;177:3848–56.
[54] Flaherty MM, MacLachlan TK, Troutt M, Magee T, Tuaillon N, Johnson S, et al. Nonclinical evaluation of GMA161- an antihuman CD16 (FcγRIII) monoclonal antibody for treatment of autoimmune disorders in CD16 transgenic mice. Toxicol Sci 2012;125:299–309.
[55] Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol 2008;8:34–47.
[56] Haley PJ. Species differences in the structure and function of the immune system. Toxicology 2003;188:49–71.
[57] Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. Nat Med 2000;6:114.
[58] Langer F, Ingersoll SB, Amirkhosravi A, Meyer T, Siddiqui FA, Ahmad S, et al. The role of CD40 in CD40L- and antibody-mediated platelet activation. Thromb Haemost 2005;93:1137–46.
[59] Wakefield I, Harari O, Hutto D, Burkly L, Ferrant J, Taylor F, et al. An assessment of the thromboembolic potential of CDP7657, a monovalent Fab' PEG anti-CD40L antibody, in rhesus macaques. Arthritis Rheum 2010;62(Suppl. 10):1243.
[60] Lebrec HN. Regulatory forum opinion piece: immunotoxicology assessments in nonhuman primates—challenges and opportunities. Toxicol Pathol 2013;41:548–51.
[61] Picotti JR. T-cell-dependent antibody response tests. In: Danuta JH, Bussiere J, editors. Immunotoxicology strategies for pharmaceutical safety assessment. New York, NY: John Wiley and Sons Inc.; 2008. p. 67–76.
[62] Martin PL, Bugelski PJ. Concordance of preclinical and clinical pharmacology and toxicology of monoclonal antibodies and fusion proteins: soluble targets. Br J Pharmacol 2012;166:806–22.
[63] Bugelski PJ, Martin PL. Concordance of preclinical and clinical pharmacology and toxicology of monoclonal antibodies and fusion proteins: cell surface targets. Br J Pharmacol 2012;166:823–46.

VetBooks.ir

VetBooks.ir

CHAPTER

20

Nonhuman Primate Health Screening During Acclimation/Prestudy Conditioning

William Oliver Iverson
MedImmune, Gaithersburg, MD, USA

OUTLINE

Introduction 398
Animal Sourcing 398
Qualification of Animals at the Source 399
Quarantine of Animals in the Source Country 400
Transportation to the Test Facility 400
Quarantine in the Receiving Country 401
Acclimation 401
Study Selection 402
Socialization 402
Summary of Animal Sourcing and Acclimation 402
References 404

Abbreviations

CITES	Convention on International Trade in Endangered Species
HVAC	Heating, ventilation, and air conditioning
IATA	International Air Transport Association
PPE	personal protective equipment
TB	tuberculosis (species or strain not specified)

http://dx.doi.org/10.1016/B978-0-12-417144-2.00020-2
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

The objective of every toxicology study designed to assess the safety of a test article is to eliminate as many variables as possible except the test substance itself; this allows researchers to more easily determine which changes that occur during the course of the study may be related to the test article. Many environmental factors such as temperature, humidity, air exchange, and light cycle should be maintained within preset limits by modern heating, ventilation, and air conditioning systems. Fresh water, a standard nutritious diet, and appropriately sized cages must be provided for all animals within a given study. The source of the animals, quarantine and health testing, vaccinations and prestudy treatments, transportation, social groupings, and training and acclimation to study procedures can and should be controlled.

The factor over which the study director has the least control is the genetics of the test system (the animals). Heterogeneous rodent and nonrodent populations are still the most common test systems for general toxicology studies. Although the strain or breed of the species is controlled, the animals are purposely outbred and siblings are randomized within study groups, resulting in potentially wide genetic variability between animals. This is especially true in nonhuman primates (NHPs), in which the gestation period is long and the number of offspring produced over a given period of time is less than that of any other in vivo test system.

ANIMAL SOURCING

Although there are toxicology test facilities in some countries that breed and produce NHPs, the vast majority are exported. Contract research organizations supply animals for toxicology studies as requested by the client. These companies often have divisions that specialize in providing laboratory animals from a variety of species, including NHPs. Breeding of macaques and marmosets occurs most often in a semitropical climate in the country of origin. For *Macaca fascicularis* this is Southeast Asia, the Philippines, China, and Mauritius. *M. mulatta* are bred in China for export, and *Callithrix jacchus* in several countries in South and Central America as well as large primate centers in the European Union and North America [1].

If a company desires to conduct studies with these species in its own facilities, it is best to use an animal vendor to supply the animals. International transport of NHPs is complicated and subject to Convention on International Trade in Endangered Species and International Air Transport Association regulations. In addition, provincial and national requirements vary and permitting differs by country. Vendors who export animals maintain familiarity with these rules and regulations, know the carriers who will transport the animals and the quarantine regulations in the receiving country, and can arrange all necessary ground transportation as well.

Regardless of the company used to procure the animals, it is still the client's obligation to determine by which animal welfare standards they measure their suppliers and to ask questions about the vendor's inspections of the breeding farm. Factors such as housing, climate

VetBooks.ir

control, nutrition, and preventive and routine veterinary care at the breeding farm must be considered.

The specific health requirements for the animals in a study must be known when the animals are selected in the exporting country and before they enter quarantine. Beginning with animals that have met the protocol inclusion/exclusion requirements is always helpful. In particular, testing for agents that cannot be eliminated or for which treatment is difficult, for example, viruses and Mycobacteria species, is essential. This testing will be repeated during the acquisition process, and some animals may seroconvert and test positive. A single-dose pharmacokinetic/pharmacodynamic study may have minimal health requirements, but these animals will eventually be reused in longer, repeated-dose toxicity studies in which there will be many exposures to the test article, and the health requirements will be more critical and strict.

QUALIFICATION OF ANIMALS AT THE SOURCE

Animals are selected in the source country according to criteria defined by the vendor or the customer. These criteria should exclude the potential pathogens listed in Table 20.1 [2–5]. These agents are evaluated using a combination of serologic and microbiologic methods. For parasites, treating all animals on a routine basis with a combination of effective broad-spectrum drugs that eliminate both internal and external parasites is preferable. This regimen typically includes a drug from the avermectin class, for example, ivermectin, as well as a benzimidazole to eliminate trematodes (flukes) and cestodes (tapeworms). If the animals are intended for a study testing a known or suspected immunosuppressant and are coming from an area where malaria is enzootic, the infection must be eliminated. All animals in the group should be treated with a combination of quinines or the highly effective drug Coartem. Because treatment is equal to the cost of testing, treatment alone should be considered. Intradermal testing of macaques for tuberculosis is a standard part of quarantine procedures in both exporting and receiving countries, and it is repeated multiple times during export and importation. If measles and hepatitis A are considered a risk for the animals, vaccination

TABLE 20.1 Recommended Prescreening in the County of Origin

Agent	Acronym	Testing Method(s)
Simian retrovirus 1, 2, 3, 4, 5	SRV	Serology, PCR
Simian immunodeficiency virus	SIV	Serology
Simian T-cell leukemia virus-1	STLV	Serology
Macacine herpesvirus-1	B-Virus	Serology
Mycobaterium spp.	Tuberculosis (TB)	Intradermal skin test, in vitro test for interferon-δ release

VetBooks.ir

can be performed. After the animals that meet these criteria have been selected, they can be moved to the quarantine facility and begin the process specific to the exporting country.

QUARANTINE OF ANIMALS IN THE SOURCE COUNTRY

Quarantine procedures are specified by the governmental authorities in the country of origin. Some common procedures include isolating the quarantine animals from other animals and minimizing contact of the personnel caring for the animals in quarantine with animals in other areas of the facility. This may be done by using dedicated staff or through the rigorous use of personal protective equipment (PPE). Health testing usually includes multiple intradermal tuberculosis (TB) tests and removing from the group any animals with a positive or suspicious reaction. During quarantine, additional testing or treatment may be allowed, and consideration should be given to agents in Table 20.2 [2,3–5]. However, intradermal tuberculin and other types of microbiological tests are not required for marmosets, and *C. jacchus* is not considered a significant source of zoonotic diseases.

TRANSPORTATION TO THE TEST FACILITY

Animal welfare regulations in each country specify the size and type of caging to be used for ground transport. International transport is governed by guidelines—the Live Animal Regulations—promulgated by International Air Transport Association, which publishes standards for the air transport of all types of animals, including pets, agricultural animals, competitive sporting (e.g., equestrian events), and animals intended for special breeding programs, exhibition, or research. Caging for NHPs must be destroyed or disinfected in the

TABLE 20.2 Testing, Treatment, or Vaccination Before or During Quarantine

Agent	Common Name	Testing, Treatment, or Vaccination
Morbilivirus	Measles	Serology, vaccination
Hepatitis A	Hep A	Vaccination
Multiple	Endoparasites	Avermectin and benzimidazole class drugs
Multiple	Ectoparasites	Avermectin class drug
Plasmodium spp.	Malaria	Polymerase chain reaction or treat with at least two quinines or Coartem
For export to a rabies-free country		
Lyssavirus	Rabies	Serology, vaccination
Some countries require		
Filoviruses	Ebola/Marburg	Serology

VetBooks.ir

receiving country to eliminate the spread of any potential pathogens [6]. The caging is frequently constructed of combustible materials that can be incinerated.

QUARANTINE IN THE RECEIVING COUNTRY

Procedures for quarantine in the receiving country are determined by the public health authorities, whose focus is on preventing zoonotic disease from entering their country [7,8]. In the European Union quarantine is regulated by the health authorities within each member country and can vary from 30 days to 12 weeks in duration. In Singapore quarantine is 30 days but requires certification of the absence of a number of diseases at the farm of origin for the prior 2 years, and that facility must also be approved by the Singapore Veterinary Authority. A special concern in Singapore is testing for yellow fever, which is of special relevance in Southeast Asia [9].

In the United States quarantine is regulated and controlled by the Centers for Disease Control and Prevention in Atlanta, Georgia [7]. This second quarantine presents an opportunity for testing and treatment for additional potential pathogens [10–12]. Following any required quarantine in the receiving country, the animals must be transported to the study site. This might be movement within the same building in which the quarantine room is located or transport several hundred miles to the test facility site. This last phase of transport is usually accomplished via ground transport in environmentally controlled vehicles by approved carriers.

ACCLIMATION

Acclimation reduces stress to both the animals and the study personnel, results in more meaningful data, and is a key component of preparing for a toxicology study. Acclimation occurs at several levels: acclimation to study personnel and procedures, acclimation to other animals of the same species (socialization), acclimation to the environmental conditions of the test facility, acclimation to the study room, and acclimation to the specific animal enclosure(s) in which the NHPs will be housed during study conduct [2]. Upon arrival at the test facility, animals should be placed in the room in which the study will be conducted to begin the room and enclosure acclimation phase. Animals must be housed individually during quarantine, but if the quarantine room is in the same building as the study rooms the environmental conditions may be very similar. If the basic primate chow during final quarantine is different from that used at the test facility, a portion of feed from the same lot last fed to the animals should accompany them to their final destination, and they should be gradually transitioned to the new diet. The types of fruits and vegetables they are fed should also be changed gradually if they are significantly different. Acclimation to study procedures should begin during quarantine by training animals to present an arm or leg, even if the quarantine personnel are different from the actual study personnel [2,13,14]. The staff should wear the same PPE, including face masks, that will be worn during the study to protect them as well as the animals, and no person feeling ill is allowed to enter the room. Training is done through the use of positive reinforcement with edible treats, which may be solid or liquid and are determined by

VetBooks.ir

the type of dosing and sampling to be performed in the specific study. Common methods include inducing a desired response with hand signals, a laser pointer, or a small noise device, for example, "clicker" training. A training experience with an experienced trainer twice daily for 14 days is successful for about 90% of animals [15]. Some animals learn quickly; others never learn in the time allotted.

STUDY SELECTION

Following a 24- to 48-hour rest period at the final destination, each animal needs to have a thorough physical examination and blood sampling to screen for inclusion in the study. Animals with minor defects or anomalies may still be used but should be assigned to the control group. Results of the physical examination, electrocardiography (EKG), complete blood counts, and clinical chemistries are used to eliminate from consideration animals that are out of the acceptable range for that specific study. Remaining animals are usually randomized by body weight and assigned to the study groups.

SOCIALIZATION

The three species described in this book are social animals that live naturally in families or social groups [1,16]. It is now well established that allowing study animals to live in groups or at least pairs for the majority of the time before and during a study reduces individual stress and results in more meaningful data [17]. Although separation following dosing or during sample collection is necessary, group housing the majority of the time is extremely beneficial. Procedures for selecting animals to be housed together have been described [18] and are most critical for older, sexually mature animals. Juvenile animals weighing 2-3 kg can usually be combined in the study groups, as dictated by body weight randomization, without incident. This must be done as soon as the prestudy data have been collected and reviewed so animals selected for study can be assigned to their respective groups and begin social housing. Animals remain in these groups from that time until completion of the study. NHPs designated for a treatment-free (recovery) period should be assigned to the same social group so they can remain together until study termination.

SUMMARY OF ANIMAL SOURCING AND ACCLIMATION

- Evaluate the source of the animals in the country of origin.
- Select healthy animals according to the weight and sex specifications of the proposed protocol.
- For macaques, perform serology and/or intradermal tuberculosis testing to the group of agents described in Table 20.1, plus any others of specific concern to the study director, on the group of selected animals plus 20% more animals.

VetBooks.ir

- Administer any necessary vaccination(s) and treat with antiparasitic agents to eliminate both internal and external parasites (Table 20.2).
- Begin first quarantine (before export) on the farm or in a quarantine facility in the country of origin.
- Transport animals to the receiving country where the test facility is located.
- Second quarantine occurs in an approved facility in the receiving country according to specific government regulations.
- Begin training animals to present an arm or leg for either manual restraint or study procedures through the use of positive reinforcement.
- During the second quarantine, test or treat for undesirable potential pathogens listed in Table 20.3 (macaques only). Repeat any questionable or equivocal serology from earlier testing.
- Following quarantine, transport animals to the test facility or move to the study room if they are already on site.
- Qualified veterinary staff perform a thorough physical and ophthalmic examination of each animal; collect body fluids for hematology, clinical chemistry, and urinalysis (if desired) and perform an EKG, if required.
- Within 48 hours, select animals for study based on acceptable results, assign to groups based on body weight randomization, and begin preliminary housing in social groups.
- Sexually mature males may require an extended observation and selection process to establish pairs or social groups, but this grouping is still highly desirable.
- After 2 weeks conduct baseline body fluid and data collections from all study animals, per protocol.
- Continue training and acclimation to all study procedures, including but not limited to manual restraint, dosing, EKG, blood collection, and chair restraint (if needed to perform any of the study procedures).
- On the first day of the study, begin sample collection, dosing. and data collection, per protocol.
- Ensure all study and animal care personnel have current negative tuberculosis tests, always wear appropriate PPE, including face masks, and do not enter the study rooms if they are ill (even a mild cold may be contagious).

TABLE 20.3 Additional Testing or Treatment during Second Quarantine or During Acclimation

Agent	Test	Treatment
Shigella spp.	Culture	Antibiotics
Salmonella spp.	Culture	Antibiotics
Yersinia spp.	Culture	Antibiotics
Helicobacter spp.	Culture	Antibiotics
Mycobacteria spp.	Intradermal skin test	None (eliminate from colony)

References

[1] Smith D, Trennery P, Farningham D, Klapwijk J. The selection of marmoset monkeys (*Callithrix jacchus*) in pharmaceutical toxicology. Lab Anim 2001;35:117–30.

[2] Taylor K. Clinical veterinarian's perspective of non-human primate (NHP) use in drug safety studies. J Immunotoxicol 2010;7:114–9.

[3] Wachtman LM, Mansfield KG. Opportunistic infections in immunologically compromised nonhuman primates. ILAR J 2008;49:191–208.

[4] Sasseville VG, Mansfield KG. Overview of known non-human primate pathogens with potential to affect colonies used for toxicity testing. J Immunotoxicol 2010;7:79–92.

[5] Bailey C, Mansfield K. Emerging and reemerging infectious diseases of nonhuman primates in the laboratory setting. Vet Pathol 2010;47:462–81.

[6] U. S. Department of Health and Human Services. (2013) Requirements for importers of nonhuman primates. CFR 42, 71.53 (k) (3).

[7] Roberts JA, Andrews K. Nonhuman primate quarantine: its evolution and practice. ILAR J 2008;49:145–56.

[8] Rollin PE, Williams RJ, Bressler DS, Pearson S, Cottingham M, Pucak G, et al. Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. J Infect Dis 1999;179:S108–14.

[9] Agri-Food & Veterinary Authority of Singapore - Laboratory. Veterinary Conditions for the Importation of Laboratory Animals (3/4) - Primates (1/1). The Animals and Birds Act, Republic of Singapore, J-5/060213, 7.

[10] Reindel JF, Fitzgerald AL, Breider MA, Gough AW, Yan C, Mysore JV, et al. An epizootic of lymphoplasmacytic gastritis attributed to *Helicobacter pylori* infection in cynomolgus monkeys (*Macaca fascicularis*). Vet Pathol 1999;36:1–13.

[11] Sasseville VG, Diters RW. Impact of infections and normal flora in nonhuman primates on drug development. ILAR J 2008;49:179–90.

[12] Patton DL, Sweeney YC, Tsai CC, Hillier SL. *Macaca fascicularis* vs. *Macaca nemestrina* as a model for topical microbicide safety studies. J Med Primatol 2004;33:105–8.

[13] Abbott DH, Barnett DK, Colman RJ, Yamamoto ME, Schultz-Darken NJ. Aspects of common marmoset basic biology and life history important for biomedical research. Comp Med 2003;53:339–50.

[14] Bassett L, Buchanan-Smith HM, McKinley J, Smith TE. Effects of training on stress-related behavior of the common marmoset (*Callithrix jacchus*) in relation to coping with routine husbandry procedures. J Appl Anim Welf Sci 2003;6:221–33.

[15] Bliss-Moreau E, Theil JH, Moadab G. Efficient Cooperative Restraint Training With Rhesus Macaques. J Appl Anim Welf Sci 2013;16:98–117.

[16] Roder EL, Timmermans PJ. Housing and care of monkeys and apes in laboratories: adaptations allowing essential species-specific behaviour. Lab Anim 2002;36:221–42.

[17] Benton CG, West MW, Hall SM, Marko ST, Johnson JC. Effect of short-term pair housing of juvenile rhesus macaques (*Macaca mulatta*) on immunologic parameters. J Am Assoc Lab Anim Sci 2013;52:240–6.

[18] West AM, Carey M, Wagner W, Erwin J. Full-contact social housing of non-human primates (*Macaca mulatta* and *Macaca fascicularis*) in a biomedical research facility: initiation and implementation. Am J Primatol 2013;75:98.

VetBooks.ir

CHAPTER

21

Study Design Considerations, Technical Procedural Aspects, Scheduling, and Dosing Sampling Volumes

Werner Frings[*], *Marc Niehoff*[†], *Sven Korte*[†]

[*]Chugai Pharmaceutical Co., Ltd., Gotemba, Japan

[†]Covance Laboratories GmbH, Münster, Germany

OUTLINE

Introduction 406

Animal Welfare, Care, and Use 406

Applicable Guidelines and Regulations 406

Justification of the Test System 407

Animal Specification 407

Use of Juvenile Animals 408

Evaluation of Clinical Signs: Food and Water Consumption 409

Veterinary Considerations 409

Blood Sampling Procedures and Volumes 410

Dosing 411

Cardiovascular Investigations 411

Neurobehavioral Observations and Activity Monitoring 412

Fertility Assessment 412

Clinical Pathology 413

Immunotoxicology 413

Ophthalmology 414

Study Group Size, Randomization, and Data Evaluation 415

References 415

http://dx.doi.org/10.1016/B978-0-12-417144-2.00021-4

© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

Nonhuman primates (NHPs) are widely used in testing the toxicity of new drug candidates. Because of their close proximity to humans in the mammal pedigree, they might be the only relevant species for use in this context. For small molecules this can be because of common metabolism pathways; for large molecules the reason is often the absence of binding and/or pharmacological activity in other test species. Because NHPs are not inbred and in most cases come from outdoor breeding facilities, and because of species-specific behavioral patterns, some points need to be considered when planning and preparing a study using NHPs.

ANIMAL WELFARE, CARE, AND USE

Most test facilities conducting studies using NHPs are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and thus provide a high standard of animal welfare, with an institutional animal care and use committee (IACUC) as local internal control organ. However, all procedures in the study plan must be in compliance with the local authority's regulations and should be approved by the local IACUC before beginning the study.

In Europe, studies need to be performed in consideration of the Commission Recommendation 2007/526/EC on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (Appendix A of Convention ETS 123). This European guideline requires, for example, paired or group housing for laboratory primates and a larger cage volume, as required by other countries. Because different housing can have a varying impact on the stress of animals, and because some study parameters cannot be easily followed under group housing conditions, this surely has an impact on protocol designs.

APPLICABLE GUIDELINES AND REGULATIONS

Preclinical studies with NHPs in Europe are designed to meet the known requirements of the Council/Commission Directives, as follows:

- 65/65/EEC on the approximation of provisions laid down by law, regulation, or administrative action relating to medicinal products
- 75/318/EEC on the approximation of the laws of Member States relating to analytical, pharmacotoxicological, and clinical standards and protocols with respect to the testing of medicinal products
- 75/319/EEC on the approximation of provisions laid down by law, regulation, or administrative action relating to medicinal products
- 83/570/EEC on the approximation of provisions laid down by law, regulation, or administrative action relating to proprietary medicinal products
- 91/507/EEC modifying the Annex to Council Directive 75/318/EEC on the approximation of the laws of Member States relating to analytical, pharmacotoxicological, and clinical standards and protocols with respect to the testing of medicinal products.

VetBooks.ir

In addition, preclinical studies also should meet the requirements in the ICH guidelines:

- ICH-S3A, Toxicokinetics: A Guidance for Assessing Systemic Exposure in Toxicology Studies, issued in EMA as CPMP/ICH/384/95
- ICH-S3B, Pharmacokinetics: Repeated Dose Tissue Distribution Studies, issued by the European Medicines Agency (EMA) as CPMP/ICH/385/95
- ICH-S4, Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing), issued by the EMA as CPMP/ICH/300/95
- ICH-S6, Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, issued by the EMA as CPMP/ICH/302/95, and the first revision, issued by the EMA as CHMP/ICH/731268/1998
- ICH-S8, Immunotoxicity Studies for Human Pharmaceuticals, issued by the EMA as CHMP/167235/2004 and by the drug law "Arzneimittelgesetz" in Germany.

For safety pharmacology studies, the following guidelines could also be considered valuable:

- ICH-S7A, Safety Pharmacology Studies for Human Pharmaceuticals, issued by the EMA as CPMP/ICH/539/00
- ICH-S7B, The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals, issued by the EMA as CPMP/ICH/423/02.

Furthermore, studies should be conducted in accordance with the following regulatory framework, in addition to national regulations:

- Organisation for Economic Co-Operation and Development: Principles of Good Laboratory Practice, ENV/MC/CHEM (98) 17 (revised in 1997, issued January 1998)
- Consensus document "The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies" ENV/JM/MONO(2002)9.

Studies conducted in compliance with the preceding standards are in accordance with the Good Laboratory Practice (GLP) standards required by the US Food and Drug Administration's GLP regulations.

JUSTIFICATION OF THE TEST SYSTEM

If NHPs are selected as the relevant species for use in a study, a clear justification must be given in the study plan. Reasons can be the pharmacologic mechanism and/or metabolism of xenobiotics, previous study experience, extensive historical data available, species-specific cross-reactivity of the test item, or similarity of the target organ system (e.g., reproduction, development).

The planned study should not duplicate previous studies, and the number of monkeys to be used needs to be chosen to yield meaningful results in the context of the study. This should be obligatory for all animal work but is surely even more important when using NHPs.

Animal Specification

For most studies using NHPs, relatively young cynomolgus monkeys are used (approximately 2-3 years old). At this age the animals are normally not yet sexually mature, which

VetBooks.ir

TABLE 21.1 Postnatal Phase Definitions in Cynomolgus Monkeys Compared with Rhesus Monkeys and Humans Based on Reproductive Organ Maturation/Sexual Maturity

	Cynomolgus monkey	Rhesus monkey	Human
Newborn	24 hour postnatal	24 hour postnatal	At term
Neonate	0-4 months	0-1 month	0-1 month
Infant	Up to 8 months	1-12 months	1-24 months
Juvenile	Up to 36 months	12-24 months	Not defined
Adolescent	3-5 years	2-4 years	12-16/18 years
Young adult	5-7 years	4-8 years	16/18-20 years
Adult	>7 years	8-15 years	>20-25 years

Modified from Ref. [3].

makes group housing, as required by European legislation [1], easier compared with group housing fully mature male and female animals. On the basis of sexual maturity, age factors of 3 to 5 would apply for cynomolgus monkeys when compared to human development [2]. Mature animals (see Table 21.1 for typical NHP age ranges) require stronger consideration of hierarchy or how individuals within a small group tolerate each other [4]. Mature animals also tend to have comparable body weights, which—in addition to the social aspects—can result in some limitations for group allocation and randomization. This is of special interest when the use of mature animals is required by legislation. For small-molecule testing, this applies to fertility studies, and when testing biopharmaceuticals mature animals can be used in a long-term study (at least 13 weeks) to avoid an additional fertility study if no macroscopic or microscopic findings in reproductive organs are observed in such long-term studies [5].

Use of Juvenile Animals

To support marketing of new drugs for treatment of children, studies using juvenile animals might be required. For rodent species, the animals' smaller size might be the largest challenge. For macaques, this is normally not a major issue. Adulthood in monkeys is defined by epiphyseal closure. Hence, the terms *young adult* versus *adult* seem to be somewhat arbitrary.

Species-specific breeding properties and logistics provide the largest hurdles. Until a certain age (normally 6 months), cynomolgus monkeys should not be separated from their mothers and transferred to the test facility. Transport of cynomolgus monkeys also requires a 6-week export and another 6-week import quarantine, plus time for acclimatization, before predose assessments can be implemented. Shipment of mother and offspring together is not possible, however, because the mother animals are typically part of breeding program for several years and shipping these animals might be business risk for breeders. This therefore implies that if the first 6 to 12 months of the animals' development must be investigated, purpose-based breeding must be conducted at the test site. Because monkeys, like humans, have a small litter size with relatively long pregnancies, accessing a certain number of similarly aged animals at the same time might be difficult. Any issues that might lead to delays

in starting the study (including test item availability) can jeopardize the entire study because the animals might reach an age that exceeds the study requirements.

VetBooks.ir

Evaluation of Clinical Signs: Food and Water Consumption

Historically, food and water consumption has always been an important parameter for evaluating the general health condition of a test animal. However, in contrast to other species, NHPs often use water supplies as toys, resulting in a certain but highly variable loss of water. Consequently, reduced water intake might be easily overlooked. Food (pellet) intake can be well monitored for animals housed singly by counting or weighing the remaining food. However, some animals also tend to play with pellets, resulting in a more or less pronounced loss outside of the cage. When food supplementation for enrichment are offered, such as fruits, vegetables, or nuts, which can have a varying energy content, calculation of the consumed food in terms of energy is even more difficult.

When animals are housed under group housing conditions, an animal-by-animal assessment of food intake is almost impossible without separating them for a limited period of time during feeding. The exact amount of food eaten per animal cannot be monitored because the food will be picked at over a long period of time. In addition, the hierarchy between the animals can have a major impact on eating habits. Therefore performing only a general overall assessment of food intake per cage, and using the development of body weight as a better and more precise measure for the animals' general condition, should be considered. However, if the pharmacology of a test item is linked to a possible influence on food intake, single housing might be considered and is justifiable, or the animals could be separated, at least during feeding time, with removal afterward. Similar approaches might be justified if monitoring feces is of special interest.

Veterinary Considerations

Species most commonly used for toxicity testing are bred under highly controlled hygienic standards and are known to be free of certain pathogens (specified pathogen free) and/or inbred. Cynomolgus monkeys are typically bred in outdoor facilities in different countries in Southeast Asia, China, or Mauritius. This requires a veterinary treatment plan that might differ from that used for other species. The country of origin can have an impact on assessing the potential background incidence of different diseases. Cynomolgus monkeys from Mauritius are known to be free of macacine herpesvirus 1 (also known as the herpes B virus), measles, simian immunodeficiency virus, and some other simian retroviruses or herpes viruses. Although these viruses typically do not have a major effect on study data, an impact of these diseases cannot always be excluded. Other background diseases in monkeys include diarrhea or parasites (both ecto- and endoparasites). Diarrhea is in most cases self-limiting but can also be recurrent. Chronic enteropathies might be associated with infectious etiologies that can affect study data. Individual stress sensitivity can also affect background diseases as an immunomodulatory action of the test item. Consequently, a tiered treatment plan—starting with more frequent monitoring, supporting care (e.g., supplemental fluids or food, additional heating) as the second step, and medication as the last step—might be considered. Medication should, then, consider possible side effects and drug–test item interactions. For a

VetBooks.ir

more detailed review of a veterinarian's perspective of the use of NHPs in drug safety studies see the article by Taylor [6].

Blood Sampling Procedures and Volumes

Among the different laboratories that have extensive experience working with NHPs, blood sampling procedures and the veins or arteries used can vary. One question that is often discussed is the eventual need for disinfection of the injection site before venipuncture. When the injection site is not disinfected, there is a certain risk of exposing the blood to microorganisms that belong to the normal skin flora. On the other hand, a disinfectant might irritate skin, especially when combined with wiping, and the cold feeling on the skin can cause unease in the test animal and thus interfere with a smooth and stress-reduced procedure. This can even be increased when multiple blood samples have to be taken over a short period of time, for example, for toxicokinetics. Normally, each laboratory has established procedures to deal best with the different risks under the local conditions; therefore, this point should not be considered critical when developing a study plan. Sampling can routinely be conducted from the arm veins in cynomolgus monkeys using syringes or Vacutainers, making sure not to use the same vein used for dosing.

The question of what blood volume can be collected within a certain period of time without causing too much stress to the animal has been widely discussed, and recommendations for best practices have been published [7]. In addition to these recommendations, local legislation and test site experience also have to be considered. Nowadays, most responsible laboratories have their own IACUC to set and control reasonable limits and to assist in the development of a study plan. Table 21.2 shows one example of IACUC limits.

Sometimes not all parameters needed for an investigational or pivotal toxicity study can be measured within these limits. Because of the large body size of the most used cynomolgus monkeys, this is rarely an issue. For the much smaller marmosets (280-520 g), blood limits have to be considered much more carefully and thus provide a certain disadvantage for the

TABLE 21.2 Example of a Typical Institutional Animal Care and Use Committee Regulation for Blood Volumes to be Taken from Nonhuman Primates and Examples of Typical Body Weights of Cynomolgus and Marmoset Monkeys.

	General volume (mL/kg and % of total blood volume)	Sampling volume Cynomolgus monkey (body weight 3 kg)	Sampling volume Marmoset monkey (body weight 350 g)
Blood samplings within 24 hours and a 7 day recovery (or within 7 days)	7 mL/kg (10 %)	21 mL	2.4 mL
Maximum accepted for Marmosets within 24 hours if no alternative for volume reduction is possible	1.05 mL/kg (15%)	-	3.7
Blood sampling within 14 days	14 mL/kg (20 %)	42 mL	4.9 mL

use of this test species. In these cases whether an exception to the rule is justified needs to be discussed on a case-by-case basis. If not, the use of satellite animals for testing, for example, toxicokinetics might be an option. However, for biopharmaceutical testing with a certain likelihood of antidrug antibody development, which might require an integrated discussion of all data on a single-animal basis, the use of satellite animals also can result in a suboptimal study design. To combine good study conduct with animal welfare, the last blood sampling can be done directly before sedation for necropsy, allowing a larger volume to be collected.

Dosing

Standard dosing of test items in monkeys is performed via the oral (gavage), intravenous, and subcutaneous routes. The intraperitoneal route, which is commonly used for rodents, is not applicable for NHPs. As for blood sampling, recommendations for the maximum volume of liquids collected via the different dosing routes have been published [7]. However, details for the dosing procedures can differ among laboratories. For example, oral dosing by gavage can be performed through either the nose or the mouth. For both methods, misdosing by inserting the gavage tube into the trachea can occur, with a fatal outcome for the test animal. Given that well-trained staff are working with the animals, however, a higher risk of misdosing with either method is not recognized.

To resemble the intended dosing route in humans, unusual dosing routes are more often requested. These include, but are not limited to, different infusion techniques. [8] When short-term bolus administrations are limited to approximately 5 min, manual dosing (e.g., for volumes up to 10 mL/kg), chair-restrained infusion (e.g., up to 4 hours) can be conducted in adapted cynomolgus monkeys (up to 60 mL/kg once weekly into the leg vein using a butterfly system). If continued (24 hours/7 days) infusion is required, an implanted port catheter system, together with ambulatory or tethered infusion, is the method of choice. Infusion techniques for NHP species are also well-established for the intrathecal route; however, the use of this route is limited to macaques.

Cardiovascular Investigations

Cardiovascular end points such as electrocardiography and blood pressure are usually collected as snapshot measurements in physically restrained animals in toxicology studies [9] and can be applied for cynomolgus monkeys as well as marmosets. If performed, measurements should be taken at least once during the predose phase and at multiple time points in the early and late dosing phase and again around the expected maximum time after dosing. Although a comprehensive cardiovascular assessment after single dosing is usually done using implanted telemetry when testing the safety of pharmacology, the use of jacket external telemetry could be considered to enhance sensitivity in repeated-dose toxicity studies. This might be of special interest if the compound or class of the compound is known to possibly affect the cardiovascular system. While fully implanted cynomolgus as well as marmoset models are available, jacket external telemetry can be applied only in cynomolgus monkey [10,11]. For further reading see Chapter 22. However, for measurement of all cardiovascular end points, it must be considered that the assessment is negatively affected by stress and thus should be avoided as much as possible.

VetBooks.ir

Neurobehavioral Observations and Activity Monitoring

The use of neurobehavioral methods for determining neurotoxic potentials of a new chemical entity is recommended and implemented in the ICH guideline S7A, 2000. Compared with rodents, for which a variety of standard tests for behavioral monitoring are available, only a few methods are available for NHPs. Qualitative behavioral observations using functional observation batteries, for example, the modified Irwin test, often are used for testing cynomolgus monkeys in their home cage [12,13]. The modified Irwin is used to investigate behavioral parameters (locomotion, stereotypy, awareness, aggression, and vocalization), neurological parameters (body posture, body tone, grip strength), gait parameters (ataxia, other impaired gait), and excitation parameters (tremors, twitches, and convulsions), as well as autonomic effects (piloerection, hypothermia), of a test item. The modified Irwin test for cynomolgus monkeys is usually validated under GLP conditions and often is included as a parameter in standard toxicology studies beginning from a dosing phase duration of 4 weeks or longer. Since it is a qualitative observation, results sometimes lack sensitivity and reproducibility because of the subjective bias of the observer. Quantitative behavioral monitoring using, for example, video recording of group-housed animals and quantification of locomotor parameters such as distance moved and velocity gives a more precise analysis of behavior [14]. Other approaches used include implantable devices, ultrasonic motion detectors [15], radiotelemetry collars, and infrared motion sensors [16]. Robust tests for neurobehavioral evaluation in marmosets in the preclinical setting, however, are rare. For further reading see Chapter 9.

Fertility Assessment

Considerations for development and reproductive toxicity testing are not discussed in this chapter; this topic is discussed in detail elsewhere (see Chapter 9). As discussed earlier, fertility assessment in general toxicity studies is most relevant for biopharmaceuticals because this assessment can support clinical trials without additional fertility studies when the NHP is the only relevant species [5]. In general, different definitions of maturity are used by different laboratories. Some laboratories accept a certain age and body weight as indicators of maturity, eventually in combination with testicular volume determination and hormone concentrations. Other laboratories prefer the proof of sperm in male animals and the presence of at least two menstrual cycles for female monkeys. Although age, body weight, and testicular volume give a certain likelihood of male sexual maturity, clinically there is neither a single parameter nor a combination of parameters that allows the certain prediction of fertility. One study compared age, body weight, testis size, semen parameters, and testosterone concentrations in male cynomolgus monkeys with proven fertility and in male animals that failed to impregnate females [17]. In that study detecting clear differences in these parameters was not possible. Consequently, proof can be given only by the presence of sperm [18]. Recognition of sexual maturity is comparatively easier to achieve in female long-tailed macaques by collecting daily vaginal swabs for identification of menstrual bleeding.

The end points for biopharmaceutical development per ICH S6(R1) [5] focus on histopathology and organ weight assessment in long-term studies of at least 13 weeks. Therefore, that the development of reproductive organs has been completed before the study starts just needs to be ensured; evidence of sperm cells in male animals is sufficient for this type of

VetBooks.ir

studies because it does not have a relevant impact on histopathology. The same applies for the quality of cycle duration of female animals; a regular cycle of a certain duration is not required.

If pharmacological activity or previous findings of a biopharmaceutical raise specific concerns regarding a potential impact on reproduction, specialized assessments such as sperm count, sperm morphology, and hormone concentrations eventually need to be added to the study plan. In these cases the bar for animal selection should be higher. For male animals, the sperm count should be above a certain level (to be defined by each laboratory based on control data); for female animals a regular cycle duration is required to allow for hormone sample collection based on the cycle data from each individual animal.

Clinical Pathology

The timing for evaluation of hematology (eventually including coagulation) and clinical chemistry (serum and urine) basically does not differ between studies using NHP and other species. The relatively large body size of macaques, however, offers the advantage of eventually including additional time points for better monitoring (see Blood Sampling Procedures and Volumes, above). Because these animals are not inbred, NHPs have a large interanimal variation of many parameters (e.g., white blood cell count); for some parameters, differences between animals from Mauritius and the Asian mainland have been reported [19]. As for other species, procedure-related stress can have an impact on clinical pathology data from NHPs. Furthermore, circadian changes in at least some parameters (e.g., glucose) need to be considered. Depending on the age and the individual character of NHPs, there might be a significant difference in the effect of stress on individual animals. The social environment is a major parameter in the well-being of laboratory animals. Having stable pairs/groups for some weeks before starting the predose phase makes a longer prestudy period for planning and study setup preferable, especially for group-housed animals. Another opportunity to get a better overview of individual baselines and group mean values is to have, for example, two predose time points rather than just one, which is normally considered sufficient for standard toxicity studies. In addition, some animals show a more or less pronounced adaptation to study procedures, which can lead to individual differences between predose- and dosing-phase values. This surely also applies to other test species, but because the number of animals per group is fewer in NHP studies than, for example, rodent studies for humane reasons, the statistical impact is substantially larger for NHPs.

Immunotoxicology

Stress hormones have a relevant impact on immune system regulation. Therefore, the above-described impact of stress perhaps applies even more for the assessment of immune system function. Consequently, the study plan should be designed to reduce stress as much as possible. Because animal welfare has become more relevant for study design considerations, this emphasizes the correlation of scientific and animal welfare efforts. For each investigation the relevance of data at a given time point should thus be considered carefully. As an example, stress induced by multiple blood samplings in a short time might be perfect for monitoring the pharmacodynamic profile of a new drug. The stress on the animals, however,

might impact the quality of the data. No basic rule to overcome this discrepancy can be given. The study design in such cases must always be selected on a case-by-case basis.

It is well known that the degree of immune response can have enormous interindividual variations in NHPs, even without additional stress-related variation. Lebrec et al. [20] performed an extensive evaluation of T-cell-dependent antibody response data obtained after vaccination with keyhole limpet hemocyanin. One of the outcomes was that, as a basic rule, there is no sex difference, which allows for sex-merged data analysis, resulting in a larger number of values for a more powerful statistical evaluation. However, analysis of immunophenotyping data from multiple laboratories showed that different approaches for optimizing the study plan might be preferable [21]. Depending on the leukocyte subtype, for example, B cells, there can be statistically significant sex differences, supporting a separate statistical analysis. Or, when no other data from both sexes are needed, using only one sex might be considered to increase the statistical power but minimize animal usage. For other cell types, an additional statistical analysis of data from both sexes might be acceptable to enlarge the mathematical size of the group. Because this analysis comprises data from multiple laboratories, checking the reference data of each test facility for intersex variation before deciding on a merged or separated statistical approach might be useful. As for the T-cell-dependent antibody response assay, a high degree of interanimal variation of lymphocyte subtypes is present in NHP blood, which by far exceeds the intra-animal variation when looking at different time points in the course of a toxicity study. Consequently, the predose values of each individual animal are the better comparators to identify drug effects than a comparison between different animals or treatment groups at a selected time point.

When cynomolgus monkeys are used, they are normally bred either in the Asian mainland or in Mauritius. Animals from Asia tend to have higher absolute numbers of T and B cells and lower absolute numbers of natural killer cells [22]. These observations are in line with the information from this chapter's authors (unpublished data), with the difference of natural killer cell counts being low. However, the relative numbers (percentages within the lymphocytes) are comparable; therefore the difference seems to be related to a different number of lymphocytes in general, with no suspicion for a functional difference. Consequently, the animals' origin is likely not relevant for an investigation of the immune system by immunophenotyping. For investigative purposes, or if the lowest dose does not necessarily allow for complete target saturation in lymphocytes, these differences might be considered for dose optimization.

Ophthalmology

The morphology and physiology of the eye is not homogenous within different mammal species. The rodent eye has a larger lens for recognizing a larger portion of the surrounding environment, resulting in a smaller volume of the interior chamber. Dogs eyes, as those of other species active at night, have the *tapetum lucidum* located behind the retina to reflect incoming light and thus increase night vision. Unique in humans and NHPs is the macula lutea, a region of the retina containing concentrated visual photoreceptors for high-resolution vision. These properties can not only be relevant for selection of the best species for toxicity testing but also can have an impact on protocol development. In addition to standard ophthalmology methods, subjective loss of visibility could theoretically be tested using purpose-trained NHPs. Practically, however, this is not feasible in routine toxicity studies.

VetBooks.ir

Alternatively, methods such as electroretinographic investigation can be included. Standard electroretinography is considered a useful tool to screen for general toxicity of the entire retina, and for a more detailed investigation focusing on the area of the macula lutea, multifocal electroretinography might be the method of choice [23].

Study Group Size, Randomization, and Data Evaluation

For animal welfare reasons, the number of animals per study should be as small as possible but large enough to allow for a good data interpretation. This is normally more critical for large animals than for rodents, and within the large animal species even more so than for dogs. Together with the high interanimal variation, which is normal for animals that have never been inbred, this view can lead to significant challenges for an optimal study design. In any case defining one or a few of the most critical study parameters is unavoidable. One option to overcome some challenges is, then, to screen potential study animals in advance and select only those that fulfill certain criteria. A minimum of three animals (either of one or of each sex) should be considered for each treated group, allowing for statistical analysis. Disadvantages are the time and eventually availability of study animals. Sometimes screening animals while they are still at the breeder or just screening a few animals more than required at the test facility and excluding outliers only might be preferential. In addition, predose serum and plasma backup samples allow later screening in the case of unexpected findings. Furthermore, a tendency for prestudy virus screening seems to be conducted occasionally, with scientific rationale determined in the forefront of the study.

Another option is optimizing groups using modified randomization procedures. For standard toxicity studies, body weight normally serves as the randomization parameter. However, for studies that focus on parameters that have a high interanimal but low intra-animal variability, such as immunophenotyping, the cell population of interest can easily be used as the (primary) randomization parameter. This allows for better comparability between groups and avoids incidental statistically relevant differences from baseline time points on.

Data evaluation normally is done by comparing data collected under dosing conditions, with data from the control group collected during the same study interval, and with predose data. Depending on the variability, however, the focus can be different for single parameters, or the most relevant data evaluation might be on the basis of a single animal. In addition, running statistical analysis with merged data from both sexes to increase the statistical power might be beneficial. In this case, sufficient data should be available to show that there occurred no relevant sex-specific test item changes that might interfere with the parameter of interest. However, if study design and randomization optimization still leave concerns about obtaining relevant data, increasing the number of animals used should be considered on a case-by-case basis.

References

[1] ETS 123. European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Appendix A. Official Journal of the European Communities K. 2007, 2525.

[2] Morford LL, et al. Preclinical safety evaluations supporting pediatric drug development with biopharmaceuticals: strategy, challenges, current practices. Birth Defects Res, Part B 2011;92:359–3803.

VetBooks.ir

[3] Morford LL, Bowman CJ, Blanset DL, Bogh IB, Chellman GJ, Halpern WG, et al. Preclinical safety evaluations supporting pediatric drug development with biopharmaceuticals: strategy, challenges, current practices. Birth Defects Res, Part B 2011;92(4):359–80.
[4] Niehoff MO, Bergmann M, Weinbauer GF. Effects of social housing of sexually mature male cynomolgus monkeys during general and reproductive toxicity evaluation. Reprod Toxicol 2010;29:57–67.
[5] ICH. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ICH harmonised tripartite guideline Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals S6 (R1). 2011.
[6] Taylor S. Clinical veterinarian's perspective of non-human primate (NHP) use in drug safety studies. J Immunotoxicol 2010;7(2):114–9.
[7] Diehl K-H, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 2001;21:15–23.
[8] Green OP, Healing G. Non-clinical vascular infusion technology. The techniques. Boca Raton, FL: CRC Press, Taylor & Franchis Group; 2014, Volume II.
[9] Schmelting B, Niehoff M, Egner B, Korte S-H, Weinbauer GF. High definition oscillometry: A novel technique for non-invasive blood pressure monitoring in the cynomolgus monkey (*M. fascicularis*). J Med Primatol 2009;10:1–9.
[10] Tabo M, Hara T, Sone S, Shishido N, Kuramoto S, Nakano K, et al. Prediction of drug induced QT interval prolongation in telemetered common marmodets. J Toxicol Sci 2008;33(3):315–25.
[11] McMahon C, Mitchell AZ, Klein JL, Jenkins AC, Sarazan RD. Evaluation of blood pressure measurement using a miniature blood pressure transmitter with jacketed external telemetry in cynomolgus monkeys. J Pharmacol Toxicol Methods 2010; 62(2):127–35.
[12] Gauvin DV, Baird TJ. A functional observational battery in non-human primates for regulatory-required neurobehavioral assessments. J Pharmacol Toxicol Methods 2008;58:88–93.
[13] Korte S, Fuchs A, Weinbauer GF, Moscardo E, Giarola A. Modified Irwin test as diagnostic tool to monitor neurobehavioural changes in monkeys. J Pharmacol Toxicol Methods 2007;56:47.
[14] Rose C, de Heer RC, Korte S, van der Harst JE, Weinbauer GF, Spruijt BM. Quantified tracking and monitoring of diazepam treated socially housed cynomolgus monkeys. Regul Toxicol Pharmacol 2012;62:292–301.
[15] Hauty GT, Yellin AM. Activity cycles of the monkey (*Macaca mulatta*) under different environmental conditions. J Interdiscrip Cyclic Res 1970;1:181–91.
[16] Walton A, Branham A, Gash DM, Grondin R. Automated video analysis of age-related motor deficits in monkeys using EthoVison. Neurobiol Aging 2006;10:1477–83.
[17] Meyer JK, Fitzsimmons D, Hastings TF, Chellman GJ. Methods for the prediction of breeding success in male cynomolgus monkeys (Macaca fascicularis) used for reproductive toxicology studies. J Am Assoc Lab Anim Sci 2006;45:31–6.
[18] Luetjens CM, Weinbauer GF. Functional assessment of sexual maturity in male macaques (cynomolgus monkeys). Regul Toxicol Pharmacol 2012;63:391–400.
[19] Zühlke U, Srivastav S, Stanley MA, Vogel F, Korte R, Müller W, et al. Cynomolgus monkeys of Mauritian origin: Reference data and comparison to Chinese/Vietnamese animals. In: Weinbauer G, Vogel F, editors. Novel Approaches towards Primate Toxicology. Münster: Waxmann Verlag GmbH; 2006. p. 185–201.
[20] Lebrec H, Cowan L, Lagrou M, Krejsa C, Neradile KMB, Polissar NL, et al. An inter-laboratory retrospective analysis of immunotoxicological endpoints in non-human primates: T-cell-dependent antibody responses. J Immunotoxicol 2011;8(3):238–50.
[21] Krejsa CM, Neradilek MB, Polissar NL, Cox N, Clark D, Cowan L, et al. An inter-laboratory retrospective analysis of immunotoxicological endpoints in non-human primates: flow cytometry immunophenotyping. J Immunotoxicol 2013;10(4):361–72.
[22] Baker DL, Finco-Kent DL, Reagan WJ, Conklyn MJ, Kawabata TT. Optimization and validation of a flow cytometric method for immunophenotyping peripheral blood lymphocytes from cynomolgus monkeys (*Macaca fascicularis*). Vet Clin Pathol 2008;37(1):42–8.
[23] Barker F, Niggemann B. The multi-focal Electro-retinogram (mERG) in Primate Toxikology. In: Weinbauer G, Vogel F, editors. Novel Approaches Towards Primate Toxicology. Münster: Waxmann Verlag GmbH; 2006. p. 145–57.

VetBooks.ir

CHAPTER

22

Functional Assessments in Nonhuman Primate Toxicology Studies to Support Drug Development

Jean-Pierre Valentin[*], *Annie Delaunois*[*], *Pierre Lainée*[†], *Matthew Skinner*[‡], *Hugo M. Vargas*[§]

[*]UCB Biopharma SPRL, Braine-l'Alleud, Belgium

[†]Animal Welfare and Research, Sanofi, Montpellier, France

[‡]AstraZeneca R&D Alderley Park, Macclesfield, Cheshire, England

[§]Department of Toxicology Sciences, Amgen Inc., Thousand Oaks, CA, USA

OUTLINE

Introduction 419
Why Measure Functional End Points in Toxicology Studies? 419
Principles of Repeat Toxicology Studies in Drug Development 419
Drivers for Inclusion of Functional End Points in NHP Toxicology Studies 420

Application to Specific Organ Functions 422
Cardiovascular System 422
Telemetric Approaches for Hemodynamics and ECG 422
Imaging of Cardiac Structure and Function 423
Limitations of Cardiovascular Assessments in Toxicology Studies 424
Respiratory System 425
Quantitative Evaluation of Ventilation and Lung Resistance Parameters 425

http://dx.doi.org/10.1016/B978-0-12-417144-2.00022-6
© 2015 Elsevier Inc. All rights reserved.

Nervous System 426
Global Neurobehavioral Assessment 427
Automated Activity Analysis 427
Body/Core Temperature 428
Renal System 429
Hepatobiliary System 430
Gastrointestinal System 430
Conclusion 431
Acknowledgements 431
References 432

Abbreviations

ABP	arterial blood pressure
C_{max}	maximal drug concentration
CNS	central nervous system
DIKI	drug-induced kidney injury
DRF	dose range finding
ECG	electrocardiography
EPA	US Environmental Protection Agency
FDA	Food and Drug Administration
FOB	functional observational battery
GFR	glomerular filtration rate
GI	gastrointestinal
GLP	Good Laboratory Practice
HDO	high definition oscillometry
HR	heart rate
ICH	International Conference on Harmonisation
IND	investigational new drug
KIM-1	kidney injury molecule-1
LV	left ventricle
LVP	left ventricular pressure
MTD	maximal tolerated dose
NBE	new biological entity
NCE	new chemical entity
NHP	nonhuman primate
NOAEL	no observed adverse effect level
NRC	National Research Council
QTc	heart rate corrected QT interval
RFID	radio frequency identification
RIP	respiratory inductive plethysmography
TFF-3	trefoil factor-3
WHO	World Health Organization

INTRODUCTION

VetBooks.ir

Why Measure Functional End Points in Toxicology Studies?

This chapter aims to provide evidence and illustrations of how the value of nonhuman primate (NHP) in vivo toxicology studies can be enhanced by including the measurement of functional end points. By "functional end points" we mean behavioral, physiological, and biochemical measurements of function as opposed to pathological changes in morphology or biomarkers thereof, including blood-borne biomarkers.

Principles of Repeat Toxicology Studies in Drug Development

Toxicology studies are conducted at any point from early drug discovery (to assess adverse effects related to the intended molecular target and/or chemical series) through to late-stage development (i.e., regulatory studies to support clinical trials, approval, and marketing). The primary aim of regulatory repeat-dose toxicity studies in animals is to identify the potential hazards of new chemical or biological entities (NCEs or NBEs, respectively) or new modalities (e.g., gene therapy products). This is achieved by evaluating target organ toxicities and the exposure levels at which these occur, thereby enabling detection of potential safety hazards and a safety risk assessment before human exposure.

The standard duration of regulatory toxicology studies run before human exposure is 28 days of dosing (termed *1-month studies*), which permits dosing of the same duration in humans [1–4]; they are required in a rodent and a nonrodent species and are conducted according to the principles of Good Laboratory Practice [3]. These 1-month studies are preceded by maximal tolerated dose (MTD)/dose range-finding (DRF) studies. The MTD phase involves escalating and adjusting the dose until the maximal tolerated dose for a single administration is established. The DRF phase involves administering the MTD (along with lower doses and a vehicle control) for usually 7 or 14 days. MTD/DRF studies provide a degree of confidence that a 1-month study can proceed safely and successfully using these doses throughout [2,5,6]. For chronic use products, repeat-dose toxicity studies of 6 months' duration using rodents or nonrodents are considered sufficient, providing the high dose is appropriately selected; studies of longer duration have not generally provided useful information that changed the clinical course of development.

Longer-term toxicology studies (longer than 1 month in duration) are conducted in parallel to early clinical trials to enable the dosing of patients over longer periods [1–4]. In addition to these general toxicology studies, specialized repeat-dose toxicity studies are also required and include rodent carcinogenicity, reproductive toxicology, and juvenile toxicity studies [3,7–13].

The primary end points of general toxicology studies are pathological ones: histopathological examination of a standard set of tissues postmortem. In addition, during the in-life phase of the study, blood samples are taken to assess effects of the test article on hematology and plasma chemistry and to measure plasma exposure to the test article (toxicokinetics), urine is collected for urinalysis, and ophthalmoscopy is performed. The only routine physiological assessments are those of body weight, food consumption (per cage, in the case of group-housed animals), urine biochemistry, and, in non-rodent species,

VetBooks.ir

electrocardiography (ECG). A key outcome of the studies is the establishment of a "no observed adverse effect level" [14].

Regulatory general toxicology studies are usually run according to well-defined study designs, standard operating procedures, and Good Laboratory Practice. In such a highly regulated environment, changing the status quo is difficult, leading to a relatively conservative mind-set within the pharmaceutical toxicology community. As a result, the basic design of the 1-month rodent and nonrodent toxicology studies have remained largely unchanged since the 1970s. The default nonrodent species of choice is usually the dog; however, there are a number of instances and circumstances where the NHP is used preferentially, as illustrated below and elsewhere in this book.

Drivers for Inclusion of Functional End Points in NHP Toxicology Studies

Clinical and nonclinical safety liabilities remain a major cause of adverse drug reactions, candidate drug attrition, delays during development, labeling restrictions, nonapproval, and product withdrawal [15]. Many of the toxicities are functional in nature and/or in origin. Whereas pharmacological responses tend to have fairly rapid onset and are therefore detectable after a single dose, some diminish upon repeated dosing; others increase in magnitude and therefore can be missed or underestimated in single-dose safety pharmacology studies [13].

As described above, general toxicology studies have, in the past, been fairly prescriptive and performed using standard designs, with the primary end point being the histopathological assessment of a standard set of postmortem tissues. There have been calls, however, for better assessment of physiological functions within standard toxicology studies for many years [16], and it has been suggested that integration of functional end points within these studies could help to address safety-related drug attrition by improving the concordance of the toxicity of pharmaceuticals in humans with that which occurs in animals [17].

Agencies regulating the safety testing of food additives and chemicals have led the way in this regard, producing guidelines that recommend inclusion of quantitative tests to assess neurological disorders, behavioral abnormalities, physiological dysfunctions, and any other signs of nervous system toxicity into standard toxicity testing protocols [18,19]. Regulatory drivers now also exist in the pharmaceutical industry; several current guidelines describe circumstances where inclusion of functional assessments in toxicity studies is acceptable. One example is the preclinical safety evaluation of biopharmaceuticals described in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidance document S6(R1) and confirmed in S7A [20–22]. Biopharmaceuticals, such as humanized monoclonal antibodies and antibody fragments, usually have a high specificity for the molecular target and therefore a lower risk of "off-target" activity; they also have high tissue/or species specificity, which often results in the NHP being the most, or only, relevant species for use. Safety pharmacology studies to measure the functional effect of biopharmaceuticals can, according to the guidelines, be incorporated into the regulatory toxicology study *instead of* performing stand-alone studies, as is the usual case with NCEs. According to a recent industry survey, 74% of respondents reported using this integrated approach in the development of NBEs, whereas 26% of respondents reported that the inclusion of functional end points on toxicity studies is performed

VetBooks.ir

in addition to stand-alone safety pharmacology studies [23]. The latter group may represent biopharmaceuticals from a novel class or molecules that have less receptor-specific targeting, or where the target itself has potential safety liabilities and the risk warrants dedicated safety pharmacology studies.

In a bid to expedite the development of new anticancer pharmaceuticals or biopharmaceuticals intended for life-threatening malignancies, the ICH guidance document S9 recommends that, unless specific risks have been identified, assessment of vital organ function can be incorporated into general toxicology studies instead of performed in separate safety pharmacology studies [24]. Similar recommendations have been made in the US Food and Drug Administration guidance document for preclinical assessments to support exploratory investigational new drug studies in which trial patients are exposed to limited doses without risk of toxic effects.

Clearly, there are circumstances where stand-alone safety pharmacology studies can be replaced with a combination toxicology study; however, the survey by Authier et al. [23] revealed that inclusion of functional end points in toxicology studies was conducted *in addition* to stand-alone safety pharmacology studies for both NCEs (reported by 41% of respondents) and NBEs (reported by 26% of respondents). This situation is warranted if there is prior knowledge of the target's potential safety liabilities or if adverse clinical signs or functional effects have been observed in any efficacy or safety study performed earlier in the development program. In this regard, the most frequent motivation for adding functional assessment to toxicity studies is to generate *additional* data for the integrated risk assessment, thereby enhancing confidence in the safety assessment [23].

An obvious benefit of incorporating functional end points into toxicology studies is that it allows assessment of repeat dosing and long-term exposure on vital organ function, in contrast to stand-alone safety pharmacology studies, which typically assess the effects of single-dose administration [13,25]. After multiple doses, functional effects with delayed onset may occur (e.g., following accumulation of the test article in a particular organ or inhibition of ion channel trafficking) or tolerance of initial effects may develop (e.g., due to receptor internalization). The effects on physiological function can be monitored at regular intervals throughout the time course of the enhanced toxicology study to characterize these phenomena and, with the usual inclusion of recovery groups in toxicology studies, the reversibility of any sustained functional effect may also be easily investigated.

The ability to assess functional end points, toxicokinetics, clinical pathology, and histopathology in the same animals is considered to be a great advantage. It may aid in the interpretation of histopathological findings, some of which can occur as a result of functional changes (e.g., vascular toxicity related to changes in blood pressure and heart rate [HR]). Conversely, specific organ toxicity may first manifest as a functional change, either at an earlier time point or at lower exposures compared with those at which histopathological changes are detected. In this situation, the functional effect may be used as a biomarker in clinical development to ensure safe dose escalation in the phase I clinical trials.

There is increasing public scrutiny of the use of NHPs in scientific research, while at the same time demand for the NHP as a test species is increasing because of the growing proportion of NBEs in drug development pipelines within the pharmaceutical industry. Performing an enhanced toxicology study instead of stand-alone safety pharmacology studies has clear ethical benefits, resulting in a reduction in the total number of animals used. Even when

VetBooks.ir

performed in addition to stand-alone studies, reductions in animal usage may occur because this may obviate the need for investigational follow-up repeat-dose studies and reduce the number of inherently unsafe compounds progressing to later-stage animal studies.

One should be mindful of the logistical challenges that exist in incorporating functional end points into general toxicology studies and of some of the possible disadvantages. The most prominent concern is that this may result in a lack of sensitivity of detection due to the interference of safety pharmacology measurement by multiple toxicology procedures that occur in the animal room, particularly on the busy day 1 of the study [23]. The use of parallel group designs rather than the more statistically powerful crossover designs (used in some stand-alone studies) and the use of less sensitive techniques (e.g., jacketed telemetry vs. implantable telemetry) may also affect sensitivity of detection. These challenges can, however, be overcome if some flexibility is allowed, such as making functional measurements in day 2 rather than day 1 and the addition of extra animals to key dose groups to ensure sufficient sensitivity is achieved [13,25].

APPLICATION TO SPECIFIC ORGAN FUNCTIONS

Cardiovascular System

Based on historical practice, the prototypical assessment of cardiovascular function in an NHP toxicology study includes a noninvasive evaluation of the ECG, HR determination, and a cuff-based arterial blood pressure (ABP) measurement in restrained animals [13,26]. Although these methods are practical to implement, the cardiovascular safety information is sparse (i.e., few time points) and lack scientific robustness (i.e., low quality) because of the restraint process used to immobilize NHPs for monitoring (e.g., manual, chair or tube restraint, light anesthesia or sedation) [27]. Physical restraint is problematic because, unlike dogs, NHPs have an intense physical reaction to restraint, including tachycardia and elevated ABP, which confounds signal quality (e.g., ECG) and can lead to misinterpretation of findings [27–29]. A potential additional confounding factor is the use of light anesthesia or sedation to minimize the stress response. The common practice has been to limit cardiovascular monitoring to a "data snapshot" (e.g., <1–2 min of data) taken at one time point after dosing; this restricted sampling raises the probability that peak effects will be missed and does not allow for the onset, duration, and reversibility of the effect to be determined. Thus the opportunity to identify functional indices of cardiotoxicity associated with repeated drug exposure is lost or diminished, which is a current gap in the risk assessment of new drug candidates [13,15].

Telemetric Approaches for Hemodynamics and ECG

Cardiovascular safety assessment in unrestrained NHPs has been facilitated by the use of telemetry for monitoring ABP, HR, cardiac contractility, and ECG [27,30]. In addition, telemetry studies using cynomolgus monkeys, rhesus macaques, and marmosets have demonstrated high translational value for the determination of HR-corrected QT interview (QTc) prolongation risk in humans [31–33]. Surgically implanted telemetry devices are an essential component of cardiovascular safety pharmacology assays, but the need for invasive surgery and a long recovery time (e.g., weeks), coupled with the terminal outcome of the toxicity

VetBooks.ir

study animals, limit the practical and routine use of implanted cardiovascular telemetry in general toxicology studies. That said, it may still be an alternative solution for some unique and challenging drug development programs [34]

This resource investment barrier has been overcome with the development of jacket-based telemetry systems that enable the collection of high-quality cardiovascular signals from unstressed NHPs, especially cynomolgus monkeys, the most common NHP species used in NCE and NBE safety assessment [25,27,32]. For example, a thorough test of jacket-based ECG telemetry demonstrated that this method was equivalent to implant telemetry in its ability to capture high-quality cardiac interval and HR data in awake cynomolgus monkeys [35]. In this validation study, which reflected the design of a 28-day toxicology study, jacketed monkeys (1) had baseline HR and QTc values consistent with those measured in the unstressed (unjacketed) state; (2) demonstrated normal diurnal HR variation over multiple 24-hour sessions; (3) exhibited reproducible HR and cardiac interval values on ECG with vehicle treatment over 4 weeks of observation; and (4) had cardiovascular parameters that were highly correlated with implant-derived values obtained in the same animals [35]. An important additional finding was that sotalol-induced QTc prolongation and bradycardia were detected with equivalent accuracy and sensitivity compared with implant telemetry. A critical factor for optimal performance of jacket telemetry in NHPs is complete acclimation to the processes involved with ECG lead placement, jacketing (on and off), and dosing [35]. Jacket-based ECG monitoring (e.g., Holter system) of QTc prolongation risk also has been conducted in conscious rhesus monkeys, which further supports the use of this approach in the toxicology setting [36]. There are no reports, however, of jacket-based telemetry in marmosets, possibly because of the small size of these animals relative to external equipment requirements. The utility of jacket-based telemetry in NHP toxicology studies has gained traction and has animal welfare benefits (3 Rs—replacement, reduction, and refinement) (see the previous section Telemetric Approaches for Hemodynamics and ECG) [23,25].

Jacket-based telemetry systems have evolved to enable the direct measurement of ABP in large animals [26,34]. Miniature telemetry devices for ABP, coupled with jacket ECG telemetry, have been developed for the cynomolgus monkey [37]. This alternative method requires minor surgery to introduce a pressure transducer into an accessible artery (e.g., femoral) and has no impact on organ histopathology, body weight, food consumption, or clinical pathology end points, which makes including jacket-based ECG and ABP measurements in repeat dose toxicity studies feasible [34]. Telemetry-based ABP recording (with jacket-based or fully implanted devices) is more robust and reliable for hemodynamic evaluation (e.g., a best practice) than oscillometric cuff-based ABP methods. Cuff methods are subject to the same data quality and interpretation issues as "snapshot" ECG collection in restrained or sedated monkeys (vide supra). In general, cuff-based ABP methods are inferior and are not recommended for safety assessment evaluations of new drugs, especially in primates [25,27,38]. A new cuff technology called high-definition oscillometry (HDO) may offer improved accuracy and sensitivity for the noninvasive detection of arterial pressure in cynomolgus monkeys, but it still requires the manual restraint of the monkey, which is a significant issue [39,40].

Imaging of Cardiac Structure and Function

In the NHP, cardiac function can be evaluated directly using two methods: echocardiographic imaging or left ventricular pressure (LVP) measurement and its various indices of

VetBooks.ir

inotropy [26,27]. Of these two approaches, echocardiography is advantageous for inclusion in toxicology studies because it is a noninvasive method, and imaging-based findings can be used to track the onset (and reversal) of drug-induced structural changes in the myocardium (e.g., organ size, chamber size, wall thickness, valve lesions) and make functional correlations to pathology-based end points at final necropsy. In contrast, LVP evaluation requires surgical implantation of a pressure transducer through the apex of the left ventricle and thus is more amenable to dedicated cardiovascular telemetry studies [27]. Echocardiography can be quite useful in the toxicology study setting because functional and structural parameters can be evaluated in the same animals, which allows for direct comparison of end points and direct translation of findings to humans, given the clinical use of this modality. For example, the technique was used to characterize cardiac pump function, for example, increased systolic ejection time and cardiac output, for a novel NCE that had did not alter LVP-based contractility measures (e.g., LV dP/dt_{max}) [41]. Echocardiography imaging procedures for cynomolgus monkeys, rhesus macaques, and marmosets have been reported, including response to drug treatment [27,42–45]. Unlike continuous monitoring of left ventricular function by telemetry, echocardiography is a "snapshot" measurement (vide supra), which requires chemical restraint to ensure proper and stable positioning of the NHP for the duration of image collection, which can take several minutes (5–20 min) depending on the end points of interest. This is an important factor to consider when integrating cardiac imaging into a toxicology study—that is, determining an appropriate echocardiogram examination time relative to the initiation of dosing—since it can impact risk assessment.

Limitations of Cardiovascular Assessments in Toxicology Studies

It should be mentioned that jacket-based telemetry systems with ECG and ABP capabilities are a new technology, with only a few reports of use in NHPs available in the literature, so there is little shared knowledge in the public domain at this moment; the same can be said for NHP echocardiographic analysis. The scientist studying nonclinical safety should be aware that every method and study design has its limitations, and functional assessments in toxicology have a common downside: the standard parallel-dose group design introduces greater interanimal variability and relies on comparisons between separate vehicle and test article groups, which can reduce signal detection sensitivity compared with crossover designs [25,26]. In addition, toxicology study activities (pharmacokinetic/toxicokinetic bleeds, clinical observations, etc.) can disturb or excite animals, so that functional effects are masked. The loss of sensitivity can be mitigated in part by including additional animals in some dose groups or conducting functional tests on study days devoid of toxicity-related procedures [13,25]. Despite the limitations imposed by toxicology study designs, the use of jacket technologies for cardiovascular hazard identification is an improvement over snapshot evaluations of ABP, HR, and ECG because (1) they allow for continuous monitoring over 24 hours, which is ideal for characterizing functional effects that occur at maximal exposure and those that emerge afterward (due to tissue distribution, pathway modulation, metabolites, etc.); (2) the time to peak effect may differ in individual animals; and (3) drug-induced cardiovascular effects, for example, arrhythmia incidence, could be influenced by circadian changes in autonomic tone [46]. In addition, imaging technologies (e.g., echocardiography) are not readily available because of the cost of the equipment and the training and specific expertise required to adequately collect, measure, and interpret the end points. In general, noninvasive

VetBooks.ir

cardiovascular assessments can identify hazards in early stage safety studies, for example, MTD or DRF, which can inform the design of subsequent investigational new drug–enabling safety studies and optimize the cardiovascular safety pharmacology strategy.

Respiratory System

The ability of a new drug to alter respiratory ventilation is an essential safety pharmacology evaluation to be conducted before human clinical trials because severe respiratory depression can have adverse consequences (ICHS7A) [20]. The respiratory system is physiologically composed of a pumping apparatus, which moves air between the lung and environment, and a gas exchange unit, which facilitates oxygen and carbon dioxide movement between the airways and blood [47,48]. A variety of noninvasive and invasive methods have been developed to study respiratory ventilation and mechanics in the rat because this is the most common species used in respiratory safety pharmacology studies of small-molecule therapeutics [13,47–49]. However, there are fewer options available for noninvasive investigation in the NHP. This can create a problem for compliance with ICHS7A guidelines, especially when the NHP may be the only valid animal species for nonclinical safety assessment (e.g., NBEs) [25].

In the NHP toxicology study setting, some qualitative respiratory assessment options are cage-side clinical observations and arterial blood gas evaluation [47,48,50,51]. Clinical observations can provide valuable information on adverse changes in lung ventilation, such as dyspnea and labored breathing, which can be assessed by veterinary examination. The clinical evaluation, in conjunction with pulmonary histopathology evaluation, could be used to grossly inform a respiratory risk assessment in the NHP, which might be adequate for agents with low inherent risk for pulmonary dysfunction (e.g., NBEs) [25]. There are important issues with this approach: the subjective nature of cage-side observations (e.g., dependent on the experience/skill of the assessor) and the restricted examination times (per protocol) make this approach useful only for detection of overt respiratory symptoms [50,51]. With regard to the utility of arterial blood gases (partial pressure of oxygen, partial pressure of carbon dioxide) and hemoglobin oxygen saturation (measured by pulse oximetry), a recent review by Murphy [47,48] concluded that these end points "are not a sensitive measure of drug-induced ventilatory change in conscious, healthy animals used in safety pharmacology studies." Thus, while these end points may be easy to acquire, their low value in respiratory risk assessments negates their usefulness in toxicology studies.

Quantitative Evaluation of Ventilation and Lung Resistance Parameters

In addition to clinical observation, it is possible to use unbiased quantitative measures of respiratory function with methods recently developed or adapted for use in the NHP: jacket-based respiratory inductive plethysmography (RIP) [52], whole-body plethysmography [53], and airwave oscillometry [54]. The jacket-RIP technology utilizes inductions bands, placed on the chest and abdomen, to record inspiration and expiration continuously for long-periods of time (e.g., 24 hours) from unrestrained animals. This noninvasive method is suited for incorporation into an NHP toxicity study to record acute and chronic changes in breathing rate, tidal volume, and minute volume, which are sensitive indicators of altered ventilation [47,48]. The RIP technology can also be an "add-on" feature to a cardiovascular

VetBooks.ir

telemetry study to perform an integrated cardiorespiratory safety evaluation [52]. The RIP method may also allow for measuring airway obstruction (based on phase angle shifts), which could be indicative of increased airway resistance, including bronchoconstriction [47,48]. Whole-body plethysmography can also be used to capture ventilation end points in the NHP, but this method requires chair restraint and acclimation to a biased airflow helmet fitted over the animal's head to measure respiration. Given the restraint condition and the limited duration of data collection (e.g., a few hours), this method may be less amenable to application in a toxicology study. Last, airwave oscillometry has been shown to be a novel approach for the simultaneous measurement of drug-induced changes in respiratory ventilation and total pulmonary resistance [54]. A validation study using conscious cynomolgus monkeys demonstrated that methacholine infusion increased pulmonary resistance, as expected, but had little effect on breathing rate and no effect on tidal and minute volumes. These findings clearly indicate that increased pulmonary resistance can occur in the absence of ventilation changes in the NHP, a finding that stresses the need to routinely evaluate ventilation *and* resistance end points [47,48]. This novel technology has great potential to improve respiratory safety assessment because it is noninvasive and recording periods are very brief (e.g., 16 sec). The downsides of this method are the same as other snapshot techniques: need for animal restraint, acclimation as a requirement for successful implementation, and limited data collection.

Of the methods outlined above, including routine qualitative evaluation and quantitative assessments of respiratory function in NHP toxicity studies when necessary is reasonable. The respiratory assessment strategy in the NHP model may need to be flexible and scientifically based on risks (e.g., ventilation or mechanical effects) associated with the target, off-target pharmacology concerns, therapeutic modality (NCEs or NBEs), known toxicity concerns, and the clinical indication. The new quantitative methods—jacket-based RIP and airwave oscillometry—have promise as valuable additions to the safety assessment toolbox, but their utility in toxicology studies and the creation of respiratory risk assessments will depend on their availability, especially to toxicology service providers that specialize in NHP studies.

Nervous System

The evaluation of the neurotoxic potential of NCEs emerged as a cause of concern far before the safety pharmacology guidelines were finalized, and several expert committees (National Research Council [55], World Health Organization [56], and the US Environmental Protection Agency [57]) have recommended the use of expanded clinical observations or functional observational battery (FOB) for assessing neurotoxicity. As for the two other organ functions defined as the core battery needs in the ICH S7A guideline, central nervous system (CNS) effects can also be investigated during toxicity studies. This is often considered as part of rodent studies, embedding FOBs at different times through subchronic or chronic toxicity studies. However, rodents are not always the most relevant species, and NHPs have become more relevant as the contributions of NBEs have progressed in pharmaceutical portfolios. Although most biopharmaceuticals are unlikely to penetrate the blood–brain barrier because of their size [25], engineering efforts to improve CNS penetration [58–60] and therefore expand therapeutic domains toward neurodegenerative diseases or primary or metastatic cancers are being made. Because NBEs developed in those indications are prone to benefit from

VetBooks.ir

options offered by the ICH S6 or S9 guidelines, identifying options to include CNS safety pharmacology end points in NHP toxicology studies is necessary. Finally, stand-alone studies are not adequate to identify cumulative and long-term effects or tolerance and habituation to initial adverse effects. In such cases, the inclusion of specific tests or the enhancement of the daily clinical examinations already in place in toxicology studies may be beneficial for CNS safety assessment.

Global Neurobehavioral Assessment

Drug-induced CNS effects are likely to be detected during daily clinical observations performed in toxicology studies. This is especially true during MTD and DRF studies, in which repeated administrations of high dose concentrations occur and special attention is paid to clinical signs. Animal technicians therefore have the relevant professional background to implement dedicated testing strategies. Starting from the extended list of neurobehavioral signs included in the rodent FOBs defined over the past fifty years [19,61–63], the most relevant tests have been streamlined to be implemented in rodent toxicology studies [13] and in nonrodent studies [64–66].

Keeping the basic principles, two examination phases are separated, with observation at the home cage (postural, gait, and activity changes) and specific behavioral tests outside the cage (sensorimotor reactions and reflexes). The influence of housing conditions and handling procedures have led to adjustments depending on species. In NHPs, housing conditions have moved from single to group housing over the past decade, with a significant impact on clinical observation methodologies. In single-housed conditions, observations often consisted of stereotypies, hypo- or hyperactivity, or excitement and abnormal postures or vocalization. Group housing improved the detection of abnormal behaviors (grooming, dominance, isolation, and other game interactions) and postural changes (gait, coordination, limb synchronization, etc.). Specific scoring of all changes have been adapted and published [65–68] and is considered for inclusion in stand-alone studies with as few as four animals per group [68]. With regard to sensitivity and overall predictivity, NHPs present a particular challenge because they naturally hide most of their weaknesses, especially when housed in groups (to keep their group status). However, the different FOB patterns have been tested with reference compounds and effects detected through an integrated analysis of the different observations after administration of D-amphetamine [66,68,69], diazepam [68], ketamine [66,69], and chlorpromazine [70]. As with other additional functional end points, these methods require quiet conditions and additional time to acquire (7 to 10 min/animal, depending on the facility), which need to be considered when planning activities. Therefore, initiating these investigations in the very first toxicology studies to take advantage of the small scale (e.g., fewer animals) before progressing to larger animal numbers along the path of preclinical development is recommended.

Automated Activity Analysis

Historically, locomotor activity was available by means of dedicated cages with infrared beam detectors, mainly used for stand-alone studies, depending on project strategies. The time necessary for large toxicology studies and group-housing requirements have made this solution inappropriate. Animal activity can first be scored during FOB home-cage observations (see the previous paragraph), but other electronic systems have been developed as well.

VetBooks.ir

Different remote systems using radiofrequency identification accelerometers placed in collars can be used for marmosets [71] or macaques [72] to quantify long-term periods. Actimetry can also be obtained from jacketed telemetry devices (e.g., EMKA Technologies; VivoMetrics Life-Shirt; DSI JET) used for cardiovascular recordings (see the previous section), with only minimal investment to extract additional relevant data available for both adverse effects detection and ECG interpretation [68]. Those solutions are valuable tools when CNS effects are expected; they can also contribute to the evaluation of sleep disorders, an area of growing concern for indications in elderly populations, but are still underestimated in preclinical development. As follow-up evolutions of video systems used previously for efficacy models [73], recent options include video-tracking capabilities [74]; however, this has the major problem of needing either to isolate animals and induce a behavioral impact or to find an optimized solution to identify individuals within a group, such as the use of colored jackets [75]. In the context of toxicology studies, these video systems are more relevant for detecting or confirming adverse effects such as convulsions over long periods when they are suspected, rather than trying to quantify activity per se.

Body/Core Temperature

Body temperature is often measured as part of the FOB but can also be used as an isolated marker of toxicity. While it is often inferred by clinical observations such as "cold extremities" or "cold to the touch," direct measurements are preferable and can lead to better comparisons between groups and study phases—and variations in both directions are informative. In NHPs, nycthemeral temperature cycle variations can be as high as 1.5 °C above and below the normal value (often 37 °C or 37.5 °C, depending on the species), and hyperactivity can also increase body temperature by 1 °C, so that relevant variations should be considered only when they exceed 1.5 °C. Hypothermia can potentially be correlated to direct toxicity, exaggerated pharmacology, alteration of physiological responses (e.g., cutaneous vasodilation, hypoglycemia, hypoxia), or central thermoregulatory control or metabolic heat production. Hyperthermia can reveal infection, skeletal muscle toxicity, or defects in immunological responses. Bearing in mind the impact of stress on body temperature in NHPs, the methods have to be well selected to ensure data quality. While rectal probes are still used in many laboratories, technical improvements have been made; different remote systems are available for recording temperature, starting with radiofrequency identification transponders available for animals as small as mice [76], and therefore suitable for all monkeys including marmosets or squirrel monkeys. Telemetry implants (TSE, Remo, DSI) also include temperature sensors and deliver high-quality data as long as the implantation site is appropriate and reproducible from one animal to another. Temperature is routinely extracted in stand-alone NHP safety pharmacology studies [30] but could also be considered in early toxicology studies to provide a quantified, reliable, limiting toxicity end point. Whatever the method selected, the time of measurement needs to remain constant across time points and animals, with a preference for an early assessment if manual handling is necessary (i.e., during the FOB for example). The room temperature can also have an impact and should be considered as a reference throughout the study.

In toxicology studies, a significant amount of time is dedicated to clinical observations. Therefore, adjustments of study designs to better identify and characterize neurotoxicity does not necessarily require huge efforts. Animal technicians are usually properly trained to

perform FOB assessment, and the input of safety pharmacology expertise and techniques can allow neurobehavioral assessment and CNS function to be investigated during NHP toxicology studies as well.

VetBooks.ir

Renal System

The kidney is a complex excretory organ and plays a crucial role in various physiological processes such as fluid and electrolyte balance, control of blood pressure, removal of waste products, and drug disposition. Drug-induced kidney injury remains a significant cause of attrition during drug development, and the underlying mechanisms are still poorly understood. Because renal function is not part of the ICH S7A core battery, stand-alone safety pharmacology studies are not routinely conducted by pharmaceutical companies, in particular in NHPs. The most common approach to evaluating renal function in many animal species consists of integrating renal/urinary functional end points in repeat-dose toxicity studies through analyses of urine and/or blood samples [77]. Urine collection in NHPs is not as easy as in dogs or rodents. For a quantitative urinalysis, determination of the urine volume is necessary. Small species such as marmosets can be placed in rat metabolism cages for 24 hours, after preliminary acclimation to the cage to limit stress. Some investigators were able to train marmosets to urinate in a special box after a light is turned on for a food reward [78]. Various methods also have been tested in macaques, from a simple metallic plate and vat placed under the home cage to an adapted diaper containing a sponge to absorb the urine [79]. Urinary bladder catheterization requires anesthesia and is difficult to integrate in a repeat-dose study design.

While tubular function can be addressed by fractional excretion of electrolytes or by determining urinary concentrations of glucose, protein, enzymes, or other newer biomarkers, glomerular filtration rate (GFR) is usually considered the best quantitative measurement of overall renal function because it is directly related to the functional renal mass. This parameter is therefore a good indicator of glomerular function and of the progress of a renal injury. GFR is usually estimated by measuring the plasma clearance of an inert marker, which is ideally exclusively excreted by the kidneys without reabsorption or secretion. Various exogenous substances such as inulin, iodixanol, or cystatin C have been used in dogs and rats, some of which also are applicable to NHPs [80]. The use of radiolabeled markers (^{125}I-iothalamate or ^{99}mTc-DTPA) also has been reported [81]. Alternatively, GFR can be simply estimated by the creatinine clearance rate, although it might be biased by extrarenal factors such as age and muscle mass. It represents the volume of plasma cleared of creatinine per minute. The general excretory function of the kidney can be estimated by some plasma markers such as urea or creatinine, which depend on the renal perfusion and the functional renal mass, whereas urinalysis allows the identification of the functional status of particular nephron segments.

In general toxicology studies, routine urine analysis usually includes visual assessment of urine (color, clarity), volume, pH, gravity or osmolarity, and semiquantitative (dipsticks) or quantitative measurements of glucose, protein, and electrolytes. In addition to these standard parameters and to histopathological examination of the kidneys, novel promising urinary biomarkers have emerged over the past decade, offering greater sensitivity and specificity than traditional renal parameters. Seven biomarkers (KIM-1, clusterin, TFF-3, β2-microglobulin, cystatin C, albumin, and total protein) have been "qualified" by regulatory agencies for rat

VetBooks.ir

studies because of their translatability in clinical trials. Beside these qualified seven, novel exploratory biomarkers are being investigated for potential use in preclinical studies, such as specific microRNAs indicative of proximal or distal tubular injury. Although these qualified or emerging biomarkers have been primarily developed for rodent studies and remain less studied in NHPs, some investigators demonstrated the utility of urinary KIM-1 and lipocalin for early detection of acute injury in proximal tubules [82] and of clusterin and calbindin D28 to monitor the progression of distal nephron drug-induced kidney injury [83].

Hepatobiliary System

Like the kidneys, the liver is involved in a large number of functions, including synthesis of proteins, hormones, and coagulation factors; production of bile; and metabolism of carbohydrates, lipids, and many drugs and other exogenous substances before their excretion. Assessment of liver function is routinely integrated in DRF and 1-month toxicity studies. Various parameters are measured in blood samples collected at different time points throughout the study duration. They include at least bilirubin, urea, cholesterol, triglycerides, glucose, albumin, albumin-to-globulin ratio, total protein, and liver enzymes. However, except bilirubin, none of these parameters are primary markers of liver dysfunction because they can be influenced by many extrahepatic factors. An interesting and easy end point for liver function assessment is bile acid profiling. Primary bile acids (such as cholic acid) are produced from cholesterol in hepatocytes, stored in the gall bladder, and released in bile into the duodenum. There they are transformed into secondary bile acids (such as deoxycholic acid) and mostly (90%) reabsorbed into the portal circulation or excreted in feces. In healthy animals, the concentrations of bile acids in the systemic circulation are very low. Bile acids can be measured in serum, plasma, or feces. Assays for total bile acids and for identification of the different types of bile acids (primary or secondary) are available. An increase in bile acid concentrations in plasma can indicate decreased functional liver mass, reduced enterohepatic cycle, or cholestasis. Increased bile acid concentrations in feces, usually associated with diarrhea in monkeys, is due to malabsorption or disturbed portal circulation. In humans, a noninvasive tool called the Entero-Test is used to analyze intestinal bile secretions and has been recently applied in dogs [84]. The device consists of a gelatine capsule containing a highly absorbent nylon string, which is swallowed by the dog; the proximal end of the string is taped to the animal's face. The capsule dissolves in the stomach and the string passes into the duodenum. After a few hours, the string with any adsorbed gastrointestinal (GI) fluid is withdrawn through the mouth and analyzed. Although not yet reported, the use of this tool in NHPs could be investigated.

Gastrointestinal System

Because the oral route is the most widely used method of drug administration, the GI tract is particularly exposed to high concentrations of test articles. GI side effects may account for about 9% of project failures because of safety concerns in clinical phases but only 3% in preclinical development [85]. This discrepancy could indicate that GI signs are not monitored carefully enough during preclinical studies or that animal species used for safety purposes are not sensitive enough to GI effects. Dogs are known to be very sensitive to nausea and emesis [86], and NHPs are prone to soft feces and diarrhea, in particular when they are stressed by transportation, handling, or single-housing or group composition change, or

VetBooks.ir

if they are treated with drugs inducing immunosuppressive effects. Indeed, the GI tract of NHPs usually carries a large number of opportunistic agents (bacteria, protozoans, parasites) that are not associated with any symptoms in normal conditions but may become symptomatic in the case of stress or immunomodulation. Then it becomes difficult to distinguish direct drug-induced GI effects from indirect effects caused by infectious agents.

Integrating GI end points in toxicology studies can first consist of a simple observation of feces for consistency, color, and odor and a check for the presence of vomit in the home cage. However, these observations may be complicated by group housing, which is now mandatory for all NHPs in Europe, and the use of bedding or foraging material on the cage floor. In case of doubt, an individual fecal sampling through rectal swab might be useful for parasitological examination or bacterial culture. GI transit is rarely evaluated in monkey safety studies. Orally dosed telemetric capsules, such as SmartPill or Bravo, measuring pH, temperature, and/or pressure in the GI tract, have been used in dogs to evaluate the adverse effects of drugs on GI transit [87,88], but their use in NHPs has not been reported so far, probably because of the large size of the device (marketed for humans). Other approaches such as imaging (ultrasonography, radiography using radio-opaque material, or scintigraphy) or more invasive methods (aspiration of stomach liquid for pH measurement) also remain rarely used in NHPs.

CONCLUSION

Clinical and nonclinical safety liabilities remain a major cause of drug attrition; many of the toxicities are functional in nature and/or in origin and can manifest after single-dose or chronic administration. Over recent years there has been growing use of NHPs in safety assessment testing for NCEs or NBEs as well as novel modalities; the drivers of this are scientific, technological, regulatory (e.g., ICH S6, S7, S9), ethical, societal, and economic. Functional measurements (i.e., behavioral, physiological, and biochemical measurements of function) can be incorporated into repeat-dose toxicity studies using NHPs, either routinely or on an ad hoc basis, to enhance the value of the toxicology studies. There are inherent challenges in achieving this: the availability of suitable technical and scientific expertise in the test facility; unsuitable laboratory conditions; use of simultaneous (as opposed to staggered) dosing; requirement for toxicokinetic sampling; unsuitability of certain techniques (e.g., use of anesthesia, surgical implantation, food restriction); availability of equipment in close proximity; and the sensitivity of the methods to detect small, clinically relevant changes. Nonetheless, "fit-for-purpose" data can still be acquired without requiring additional animals. Examples include assessment of cardiovascular (e.g., ECG, arterial blood pressure, cardiac function); nervous system (e.g., behavior, general activity, body temperature); respiratory; GI; renal; and hepatic functions. This is entirely achievable if functional measurements are relatively unobtrusive, both with respect to the animals and to the toxicology study itself. Carefully validating the pharmacology of any methods used, and establishing their detection sensitivity, is vital to ensuring the credibility of generated data.

Acknowledgements

The authors thank colleagues within the safety pharmacology and toxicology groups within their respective organizations.

VetBooks.ir

References

[1] Keller KA, Banks C. Multidose general toxicology studies. In: Keller KA, Jacobson-Kram D, editors. Toxicological Testing Handbook: Principles, Applications and Data Interpretation. 2nd edn. Taylor & Francis Ltd.: London; 2006. p. 149–84.

[2] Baldrick P. Safety evaluation to support first-in-man investigations II: toxicology studies. Regul Toxicol Pharmacol 2008;51:237–43.

[3] Anon. ICH M3(R2): Guidance on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals; 2009. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2_Guideline.pdf.

[4] Sparrow SS, Robinson S, Bolam S, Bruce C, Danks A, Everett D, et al. Opportunities to minimise animal use in pharmaceutical regulatory general toxicology: a cross-company review. Regul Toxicol Pharmacol 2011;61:222–9.

[5] Herlich JA, Taggart P, Proctor J, Stahle P, Colis R, Hall L, et al. The non-GLP toleration/dose range finding study: design and methodology used in an early toxicology screening program. Proc West Pharmacol Soc 2009;52:94–8.

[6] Roberts RA, Kavanagh SL, Mellor HR, Pollard CE, Robinson S, Platz SJ. Reducing attrition in drug development: smart loading preclinical safety assessment. Drug Discov Today 2014;19:341–7.

[7] Hew KW. Juvenile animal toxicology studies. In: Keller KA, Jacobson-Kram D, editors. Toxicological Testing Handbook: Principles, Applications and Data Interpretation. 2nd ed. Taylor & Francis Ltd.: London; 2006. p. 265–304.

[8] Anon. ICH S5(R2): Detection of toxicity to reproduction for medicinal products and toxicity to male fertility; 2005. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S5_R2/Step4/S5_R2__Guideline.pdf.

[9] Anon. FDA Guidance for Industry: Nonclinical Safety Evaluation of Pediatric Drug Products; 2006. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079247.pdf.

[10] Committee for Human Medicinal Products (CHMP). Guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications; 2008. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003305.pdf EMEA/CHMP/SWP/169215/2005.

[11] Organisation for Economic Co-operation and Development. Guidance document on mammalian reproductive toxicity testing and assessment, http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2008)16&doclanguage=en, ENV/JM/MONO(2008) 16.

[12] Baldrick P. Juvenile animal testing in drug development – is it useful? Regul Toxicol Pharmacol 2010;57:291–9.

[13] Redfern WS, Ewart LC, Lainee P, Pinches M, Robinson S, Valentin J-P. Functional assessments in toxicology studies: the art of the possible. J Toxicol Res 2013;2:209–34.

[14] Dorato MA, Engelhart JA. The no-observed adverse- effect-level in drug safety evaluations: use, issues and definition(s). Regul Toxicol Pharmacol 2005;42:265–74.

[15] Laverty H, Benson C, Cartwright E, Cross M, Garland C, Hammond T, et al. How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? Br J Pharmacol 2011;163:675–93.

[16] Zbinden G. Neglect of function and obsession with structure in toxicity testing. In: Proceedings of 9th International Congress of Pharmacology, vol. 1. New York, NY: Macmillan Press; 1984. p. 43–9.

[17] Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 2000;32:56–67.

[18] Toxicological Principles for the Safety Assessment of Food Ingredients. Redbook 2000. 2000, Chapter IV.C.10. Neurotoxicity Studies.

[19] Moser VC, Becking GC, MacPhail RC, Kulig BM. The IPCS collaborative study on neurobehavioral screening methods. Fundam Appl Toxicol 1997;35:143–51.

[20] Anon. ICHS7A: Safety pharmacology studies for human pharmaceutical. 2000. CPMP/ICH/539/00, London, November 16, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002831.pdf.

[21] Anon. ICHS6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; 1997. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S6_R1/Step4/S6_Guideline.pdf.

[22] Anon. ICH S6(R1) Guidance for Industry S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; 2012. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM194490.pdf.

[23] Authier S, Vargas HM, Curtis MJ, Holbrook M, Pugsley MK. Safety pharmacology investigations in toxicology studies: an industry survey. J Pharmacol Toxicol Methods 2013;68:44–51.
[24] Anon. Nonclinical evaluation for anticancer pharmaceuticals; 2009. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S9/Step4/S9_Step4_Guideline.pdf.
[25] Vargas HM, Amouzadeh HR, Engwall MJ. Nonclinical strategy considerations for safety pharmacology: evaluation of biopharmaceuticals. Expert Opin Drug Saf 2013;12:91–102.
[26] Sarazan RD, Mittelstadt S, Guth B, Koerner J, Zhang J, Pettit S. Cardiovascular function in nonclinical drug safety assessment: current issues and opportunities. Int J Toxicol 2011;30:272–86.
[27] Heyen JR, Vargas HM. The use of nonhuman primates in cardiovascular safety evaluation. In: Bluemel J, editor. The Nonhuman Primate in Drug Development and Safety Assessment. Elsevier, Inc.: Waltham, MA; 2014, Chapter 31.
[28] Guth BD, Bass AS, Briscoe R, Chivers S, Markert M, Siegl PK, et al. Comparison of electrocardiographic analysis for risk of QT interval prolongation using safety pharmacology and toxicological studies. J Pharmacol Toxicol Methods 2009;60:107–16.
[29] Bass AS, Hanson LA, Jackson TA. Measuring the risk of torsades de pointes: electrocardiographic evaluation of PNU-142093 in conscious cynomolgus non-human primates using restraint and non-restraint procedures. J Pharmacol Toxicol Methods 2009;60:51–7.
[30] Gauvin DV, Tilley LP, Smith Jr. FW, Baird TJ. Electrocardiogram, hemodynamics, and core body temperatures of the normal freely moving cynomolgus monkey by remote radiotelemetry. J Pharmacol Toxicol Methods 2006;53:140–51.
[31] Chaves AA, Keller WJ, O'Sullivan S, Williams MA, Fitzgerald LE, McPherson HE, et al. Cardiovascular monkey telemetry: sensitivity to detect QT interval prolongation. J Pharmacol Toxicol Methods 2006;54:150–8.
[32] Caruso A, Frances N, Meille C, Greiter-Wilke A, Hillebrecht A, Lavé T. Translational PK/PD modeling for cardiovascular safety assessment of drug candidates: Methods and examples in drug development. J Pharmacol Toxicol Methods 2014;70:73–85.
[33] Holzgrefe H, Ferber G, Champeroux P, Gill M, Honda M, Greiter-Wilke A, et al. Preclinical QT safety assessment: cross-species comparisons and human translation from an industry consortium. J Pharmacol Toxicol Methods 2014;69:61–101.
[34] Baird TJ, Bailie M, Patrick DJ, Moddrelle D, Yoder J, Gauvin DV, et al. Influence of surgically implantable telemetry solutions on in-life and post-mortem toxicology endpoints. J Pharmacol Toxicol Methods 2013;67:148–61.
[35] Derakhchan K, Chui RW, Stevens D, Gu W, Vargas HM. Detection of QTc Interval Prolongation Using Jacket Telemetry in Conscious Non-Human Primates: Comparison with Implant Telemetry. Br J Pharmacol 2014;171:509–22.
[36] Hassimoto M, Harada T, Kaga N, Murano H, Obata M. Accurate evaluation of QT interval in conscious rhesus monkeys (*Macaca mulatta*) by use of Holter ECG. J Electrocardiol 2002;35:333–42.
[37] McMahon C, Mitchell AZ, Klein JL, Jenkins AC, Sarazan RD. Evaluation of blood pressure measurement using a miniature blood pressure transmitter with jacketed external telemetry in cynomolgus monkeys. J Pharmacol Toxicol Methods 2010;62:127–35.
[38] Kurtz TW, Griffin KA, Bidani AK, Davisson RL, Hall JE. Recommendations for blood pressure measurement in humans and experimental animals. Hypertension 2005;45:299–310.
[39] Mitchell AZ, McMahon C, Beck TW, Sarazan RD. Sensitivity of two noninvasive blood pressure measurement techniques compared to telemetry in cynomolgus monkeys and beagle dogs. J Pharmacol Toxicol Methods 2010;62:54–62.
[40] Schmelting B, Neihoff M, Egner B, Korte SH, Weinbauer GF. High-definition oscillometry: a novel technique for non-invasive blood pressure monitoring in the cynomolgus monkey. J Med Primatol 2009;38:293–301.
[41] Shen YT, Malik FI, Zhao X, Depre C, Dhar SK, Abarzúa P, et al. Improvement of cardiac function by a cardiac Myosin activator in conscious dogs with systolic heart failure. Circ Heart Fail 2010;3:522–7.
[42] Korcarz CE, Padrid PA, Shroff SG, Weinert L, Lang RM. Doppler echocardiographic reference values for healthy rhesus monkeys under ketamine hydrochloride sedation. J Med Primatol 1997;26:287–98.
[43] Tsusaki H, Yonamine H, Tamai A, Shimomoto M, Iwao H, Nagata R, et al. Evaluation of cardiac function in primates using real-time three-dimensional echocardiography as applications to safety assessment. J Pharmacol Toxicol Methods 2005;52:182–7.
[44] Hanton G, Sobry C, Daguès N, Rochefort GY, Bonnet P, Eder V. Cardiovascular toxicity of minoxidil in the marmoset. Toxicol Lett 2008;180:157–65.

VetBooks.ir

[45] Hanton G, Eder V, Rochefort G, Bonnet P, Hyvelin JM. Echocardiography, a non-invasive method for the assessment of cardiac function and morphology in preclinical drug toxicology and safety pharmacology. Expert Opin Drug Metab Toxicol 2008;4:681–96.
[46] Chui RW, Derakhchan K, Vargas HM. Comprehensive analysis of cardiac arrhythmias in telemetered cynomolgus monkeys over a 6 month period. J Pharmacol Toxicol Methods 2012;66:84–91.
[47] Murphy DJ. Optimizing the use of methods and measurement endpoints in respiratory safety pharmacology. J Pharmacol Toxicol Methods. 2014;70(3):204–9.
[48] Murphy DJ. Respiratory safety pharmacology - current practice and future directions. Regul Toxicol Pharmacol 2014;69:135–40.
[49] Hoymann HG. Lung function measurements in rodents in safety pharmacology studies. Front Pharmacol 2012;3:156.
[50] Lawler JV, Endy TP, Hensley LE, Garrison A, Fritz EA, Lesar M, et al. Cynomolgus macaque as an animal model for severe acute respiratory syndrome. PLoS Med 2006;3:e149.
[51] Nalca A, Livingston VA, Garza NL, Zumbrun EE, Frick OM, Chapman JL, et al. Experimental infection of cynomolgus macaques (*Macaca fascicularis*) with aerosolized monkeypox virus. PLoS One 2010;5(e1):2880.
[52] Ingram-Ross JL, Curran AK, Miyamoto M, Sheehan J, Thomas G, Verbeeck J, et al. Cardiorespiratory safety evaluation in non-human primates. J Pharmacol Toxicol Methods 2012;66:114–24.
[53] Iizuka H, Sasaki K, Odagiri N, Obo M, Imaizumi M, Atai H. Measurement of respiratory function using whole-body plethysmography in unanesthetized and unrestrained non-human primates. J Toxicol Sci 2010;35:863–70.
[54] Bassett L, Troncy E, Robichaud A, Schuessler TF, Pouliot M, Ascah A, et al. Non-invasive measure of respiratory mechanics and conventional respiratory parameters in conscious large animals by high frequency Airwave Oscillometry. J Pharmacol Toxicol Methods 2014;70:62–5.
[55] Anon. NR—Toxicity testing: Strategies to determine needs and priorities. Washington, DC: National Academy Press; 1984.
[56] Anon. WH—Environmental Health Criteria 60: Principles and methods for the assessment of neurotoxicity associated with exposures to chemicals. Geneva: World Health Organization; 1986.
[57] Anon. EP—Guidelines for neurotoxicity risk assessment. Fed Regist 1998;63:26926–54.
[58] Gabathuler R. Approaches to transport therapeutic drugs across the blood brain barrier to treat brain diseases. Neurobiol Dis 2010;37:48–57.
[59] Pardridge WM. Biopharmaceutical drug targeting to the brain. J Drug Target 2010;18:1157–67.
[60] Yu YJ, Zhang Y, Kenrick M, Hoyte K, Luk W, Lu Y, et al. Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. Sci Transl Med 2011;3:1–8.
[61] Moser VC. Screening approaches to neurotoxicity: a functional observational battery. J Am Coll Toxicol 1989;8:85–93.
[62] Catalano PJ, McDaniel KL, Moser VC. The IPCS collaborative study on neurobehavioral screening methods: VI. Agreement and reliability of the data. Neurotoxicology 1997;18:1057–64.
[63] Redfern WS, Strang I, Storey S, Heys C, Barnard C, Lawton K, et al. Spectrum of effects detected in the rat functional observational battery following administration on non-CNS targeted compounds. J Pharmacol Toxicol Methods 2005;52:77–82.
[64] Tontodonati M, Fasdelli N, Moscardo H, Giarola A, Dorigatti R. A canine model used to simultaneously assess potential neurobehavioural and cardiovascular effects of candidate drugs. J Pharmacol Toxicol Methods 2007;56:265–75.
[65] Korte S, Fuchs A, Weinbauer GF, Moscard E, Giarola A. Modified Irwin test as diagnostic tool to monitor behavioural changes in monkeys. J Pharmacol Toxicol Methods 2007;56:e47.
[66] Gauvin DV, Baird TJ. A functional observational battery in non-human primates for regulatory-required neurobehavioral assessments. J Pharmacol Toxicol Methods 2008;58:88–93.
[67] Froget G, Cruz G, Hubert I, Virley D, Lemaire M, Duxon M, et al. The development of an Irwin-like test in the non-human primate for CNS safety assessment. J Pharmacol Toxicol Methods 2012;66:164.
[68] Moscardo E, McPhie G, Fasdelli N, Giarola A, Tontodonati M, Dorigatti R, et al. An integrated cardiovascular and neurobehavioural functions assessment in the conscious telemetered cynomolgus monkey. J Pharmacol Toxicol Methods 2010;62:95–106.
[69] Li Y, Niui N, Cai Q. Method validation of functional observational battery assessement in rodents and nonhuman primates. J Pharmacol Toxicol Methods 2013;68:e5.

VetBooks.ir

[70] Monroe PJ, Hawk MA, Richie GD, Hanjora KR, Hassler CR. Characterization of a functional observational battery in rodents and non-human primates. J Pharmacol Toxicol Methods 2011;64:e20.
[71] Mann TM, Williams KE, Pearce PC, Scott EA. A novel method for activity monitoring in small non-human primates. Lab Anim 2005;39:169–77.
[72] Papailiou A, Sullivan E, Cameron JL. Behaviors in rhesus monkeys (*Macaca mulatta*) associated with activity counts measured by accelerometer. Am J Primatol 2008;70:185–90.
[73] Chassain C, Eschalier A, Durif F. Assessment of motor behavior using a video stsem and a clinical rating rating scalme in parkinsonian monkeys lesioned by MPTP. J Neurosci Methods 2001;111:9–16.
[74] Bassett L, Troncy E, Pouliot M, Paquette D, Ascah A, Authier S. Telemetry video-electroencephalography (EEG) in rats, dogs and non-human primates: Methods in follow-up safety pharmacology seizure liability assessments. J Pharmacol Toxicol Methods. 2014;70(3):230–40.
[75] Rose C, De Heer RC, Horte S, Van der Harst JE, Weinbauer GF, Spruijt BM. Quantified tracking and monitoring of diazepam treated socially housed cynomolgus monkeys. Regul Toxicol Pharmacol 2012;62:292–301.
[76] Prior H, Motyer V, Cobey D, Marks L, Ewart L, Redfern WS, et al. Inclusion of microchip transponder body temperature measurements in safety pharmacology and toxicology studies. J Pharmacol Toxicol Methods 2012;66:192.
[77] Hart SGE. Renal System in Safety Pharmacology. In: Vogel HG, et al., editors. Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays. Berlin, Heidelberg: Springer-Verlag; 2013.
[78] Anzenberger G, Gossweiler H. How to obtain individual urine samples from undisturbed marmoset families. Am J Primatol 1993;31:223–30.
[79] Kurien BT, Everds NE, Scofield RH. Experimental animal urine collection: a review. Lab Anim 2004;38:333–61.
[80] Timchalk C, Finco DR, Quast JF. Evaluation of renal function in rhesus monkeys and comparison to beagle dogs following oral administration of the organic acid triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid). Fundam Appl Toxicol 1997;36:47–53.
[81] Rabito C, Van Tongeren S, Zavorskas P, Stricker-Krongrad A, Robb J, Haupert Jr. G. Fast, effortless and accurate measurement of glomerular filtration rate in conscious monkeys. J Nucl Med 2008;49(Suppl. 1):162P.
[82] Burt D, Crowell SJ, Ackley DC, Magee TV, Aubrecht J. Application of emerging biomarkers of acute kidney injury in development of kidney-sparing polypeptide-based antibiotics. Drug Chem Toxicol 2014;37:204–12.
[83] Guha M, Heier A, Price S, Bielenstein M, Caccese RG, Heathcote DI, et al. Assessment of biomarkers of drug-induced kidney injury in cynomolgus monkeys treated with a triple reuptake inhibitor. Toxicol Sci 2011;120:269–83.
[84] Guiney WJ, Beaumont C, Thomas SR. Use of the Entero-test, a novel approach for the noninvasive capture of biliary metabolites in dogs. Drug Metab Dispos 2010;38:851–6.
[85] Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite E, et al. Lessons learned from the fate of Astra Zeneca's drug pipeline: a five-dimensional framework. Nat Rev Drug Discov 2014;13:419–31.
[86] Holmes AM, Rudd JA, Tattersall FD, Aziz Q, Andrews PLR. Opportunities for the replacement of animals in the study of nausea and vomiting. Br J Pharmacol 2009;157:865–80.
[87] Penton H, Norton K. The SmartPill™ for gastrointestinal monitoring in dogs. J Pharmacol Toxicol Methods 2009;60:213.
[88] Sagawa K, Li F, Liese R, Sutton SC. Fed and fasted gastric pH and gastric residence time in conscious beagle dogs. J Pharm Sci 2009;98:2494–500.

CHAPTER

23

Considering the Use of *Callithrix jacchus*

C. Marc Luetjens[*], *Joachim Kaspareit*[*], *Sven Korte*[*], *Joachim Wistuba*[†]

[*]Covance Laboratories GmbH, Münster, Germany

[†]Institute of Reproductive and Regenerative Biology, Centre of Reproductive Medicine and Andrology, University of Münster, Münster, Germany

OUTLINE

Introduction 438

Evolutionary Origin of the Common Marmoset 439

Consequences of Phylogenetic Distance 439

Reproductive Biology 441

Evidence of Age Effects in Captivity 442

Use of Marmosets in Reproductive and Regenerative Research 442

Marmosets in Reproductive and Stem Cell Research 442

Contraception 443

Cycling Control and Oocyte Harvest, Maturation, and Offspring 444

Practical Considerations 445

Keeping: Housing and Husbandry 445

Breeding Specifics in Captivity: Triplet and Quadruplet Management 447

Use of Marmosets in Toxicological and Pharmacological Studies 447

Experimental Handling and Sampling of Specimens from Experimental Animals 447

Monitoring the Experimental Animal During the Study 448

Drug Administration 449

Dosing Techniques and Dosing Design 449

Pathology/Histology 450

Selected Examples of Special Histopathology of Marmosets 450

References 453

http://dx.doi.org/10.1016/B978-0-12-417144-2.00023-8

© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

Among primates, the common marmoset, *Callithrix jacchus*, phylogenetically belongs to the subfamiliy *Callithrichinae* within the infraorder of the *Platyrrhini*, inhabiting South and Central America. New World monkeys diverged from the Old World monkey (Catarrhini) lineage when South America broke off from the forming African continent. Thus the platyrrhine monkeys have a long-standing evolutionary history developing independent from the Catarhinii. Therefore it is not surprising that New World monkeys exhibit a couple of specific differences concerning reproduction and development. Interestingly, and of striking importance for its use as a nonhuman primate (NHP) model, there is evidence that in marmosets the hypothalamic-pituitary-gonadal (HPG) axis, regulating endocrine control of reproduction, has adapted to the action of chorionic gonadotropin (CG) alone in both sexes and that, as a consequence of this shift, luteinizing hormone (LH) is no longer expressed in this primate group at all. This altered system was likely driven by an LH receptor, the LHR type II, which was altered in parallel and has a higher affinity to CG because of the depletion of one exon compared with Old World monkeys. Based on these and other differences, it was speculated that androgens are more important for social (ranking) behavior in these monkeys but less involved in direct control of reproduction on a cellular basis. In contrast, although it evolved convergently, spermatogenesis in marmosets exhibits remarkable similarities to that in great apes, including humans, that is, the organization of the seminiferous epithelium as well as early male germ-line development are highly comparable. Conclusively, for research on reproduction, the marmoset has to be considered as a somehow limited model in terms of endocrine regulation; however, these monkeys can serve as the best NHP model available when early germ-cell differentiation and cellular spermatogenic processes are addressed.

Marmoset reproduction is peculiar among primates in another way: regularly giving birth to multiple offspring is normal. This phenomenon allows for comparative studies of reproduction to be performed with adequate numbers and, because of the relatively short gestation, in a reasonable time. Again, differing from Old World monkeys, reproduction in marmosets by far depends more on the social status of an individual. Within the family group, only the dominant couple reproduces, and preliminary data suggest that the social environment might interfere with individual pubertal development. In addition, the dominant female regulates the ovarian cycles of cohabitating females, demonstrating a natural method of "social" contraception per se. Platyrrhines reach sexual maturation after a relatively short postnatal period and have a typical life expectancy of less than a decade. Age-related changes in many toxicokinetically relevant parameters render the marmoset a suitable model for preclinical studies of age-related diseases. Marmosets are an adequate NHP model for studies of female contraception because ovarian cycles can be experimentally regulated by luteolysis and are used as model in regenerative medicine, including stem cell-related studies, showing great potential for preclinical studies.

Although standard administration techniques for toxicology safety assessment are well established for marmosets, some other specifics have to be considered when using this NHP model. Unlike Old World monkeys, marmosets require a very specialized feeding regime and, in general, several environmental factors are highly important for adequate keeping conditions, for example, their extreme territorial behavior. Furthermore, when assessing clinical pathology parameters, one has to be aware that several enzyme levels, as well as a small number of other parameters, differ significantly between cynomolgus monkeys and marmosets.

VetBooks.ir

In this chapter we review the data available on the use of marmosets as a valuable NHP model and report on histopathological specificities in a variety of organs. Based on the differences described, the marmoset model is discussed in the context of toxicological safety assessments as well as a model for general research on the pathophysiology of human diseases.

EVOLUTIONARY ORIGIN OF THE COMMON MARMOSET

According to the recent phylogenetic system, the common marmoset, *C. jacchus*, is a member of the subfamiliy *Callithrichinae*. The previous traditional classification included the genus *Callithrix* in a separate family, the Callithrichidae; however, the most recent phylogenetic analyses revised this view and situated the species in the *Cebidae* family. Within the order Primates, the anthropoid primates (Haplorrhini, Simiiformes) of the neotropics, and the *Cebidae*, together with the two other families—the *Atelidae* and the *Pitheciidae*—which very likely are of monophyletic origin [1,2], form the infraorder of the Platyrrhini, the New World monkeys [3]. There has been a debate whether owl monkeys, by separation from the Cebidae, should be assigned into a fourth family, the *Aotidae*, but this is still undecided and seems not to be supported by molecular data [3–5]. Within the order Primates, the taxon Platyrrhini, the New World monkeys, which inhabit South and Central America, diverged from the Old World monkeys (Catarrhini) when South America was detached by continental drift and the ancestors of the modern Platyrrhini were finally isolated from the Old World lineage during the Eocene era, that is, approximately 40-80 million years ago [6]. The earliest platyrrhine fossil is estimated to be 26 million years old, and from these ancestors all modern New World monkey species developed [1]. Thus, the neotropical monkeys represent a branch of the anthropoid primates with a long evolutionary history independent from the Catarhinii group. Therefore, in experimental use of *C. jacchus* for research, one always has to keep in mind that the marmoset has evolved for long time independent from the Old World lineages, which has resulted in certain adaptive features rendering these monkeys different from the Old World lineage and could limit the transfer of results obtained in this NHP model. When the marmoset is used as an NHP model for toxicology or other research purposes, the specific aspects of the studies have to be cross-checked against the physiological specifics of *C. jacchus* before conclusions are drawn.

CONSEQUENCES OF PHYLOGENETIC DISTANCE

New World monkeys exhibit a couple of specificities that differ from other primate taxa, especially concerning reproduction and development. Of particular interest and of importance for any kind of regulatory research using marmosets is the different organization of the HPG axis, which regulates reproductive endocrinology [7–10].

The first evidence that the gonadotropin system works differently in marmosets derived from the observation that the LH receptor gene, which contains 11 exons, is spliced differently in marmosets and that exon 10 of the receptor is lacking, a difference that is also observed in some patients with mutations of this gene. However, while this LH receptor type II only occurs accidentally in humans, it is the regular isoform in marmosets. Interestingly, when treating patients lacking exon 10, this alternative receptor type is far more sensitive to CG than to LH [8,11,12]. Therefore, in marmosets and other New World monkeys, the concept that a neofunctionalization

VetBooks.ir

of the receptor might be linked to different endocrine roles of LH and CG was suggested [12]. Indeed, the LHR type II was confirmed to be present in the entire Platyrrhine lineage, and later studies showed LH to be lacking completely in the New World monkeys [13].

The endocrine regulation of reproductive pathways normally found in mammals is mainly concerted between gonadotropin-releasing hormone, follicle-stimulating hormone (FSH), LH, and the sex steroids, so the complete lack of LH expression was surprising. While LH in males was functionally completely replaced by CG. It was not clear how females enabled hormonal control of their reproductive function with only CG; in other primates CG is deeply involved in pregnancy and separated from gonadal regulation, which is controlled by LH. This issue was solved when the expression of CG was analyzed on the genomic level and a novel two-promoter/one-gene system was identified for the *CGß* gene [14]. This genomic organization, which underlies epigenetic modification by methylation, allows tissue-specific expression and enables CG to control both functions: the regulation of gonadal cycling as well as placental function during pregnancy. When using marmosets for studies of the control of reproduction, these remarkable differences have to be taken into account because they might result in unexpected findings. For example, in a recent study using a marmoset model for fertility testing under long-term administration of a male contraceptive regimen, combining testosterone undecanoate with norethisterone enanthate, surprising results were obtained [15]. While this combined treatment was proven to suppress male fertility reliably in macaques and men by interfering with the endocrine regulation along the HPG axis by decreasing intratesticular testosterone to an extent that arrests spermatogenesis on the premeiotic stage [16] all male marmosets remained fertile independent of treatment duration and although intratesticular testosterone as well as pituitary CG concentrations were suppressed, as expected. What the biological relevance of these differences in regulation of spermatogenesis might be is yet not clear, but it could be related to the unique family structure of marmoset reproduction, which is extremely dependent on the social status of the individual animal [17]. Therefore, it can be speculated that androgens are more important for the social role but less involved in reproduction in this species, and thus downregulation affects spermatogenesis less. This aspect in particular is interesting because marmosets and other New World monkeys generally exhibit a strong resistance to steroids [18]. Therefore it might be speculated whether FSH—which is normally expressed (in contrast to LH, which was replaced by CG) and has a similar function in other anthropoid monkeys [19,20]—has an even stronger and perhaps more independent impact on spermatogenesis in this species [15,21].

In contrast, although marmosets evolved independent from the remaining anthropoid lineage, spermatogenesis in marmosets exhibits striking similarities to those of great apes, including humans [22,23], rendering it an excellent model for both testicular organization as well as germ-line differentiation [7,24–26,32]. These features obviously developed convergently in the Callitrichid taxa. Interestingly, among the various types of spermatogenic organization present in primates, marmosets closely resemble patterns otherwise only seen in great apes, including humans. While in prosimians as well as in Old World monkeys a so-called single-stage arrangement of the seminiferous epithelium (i.e., only one spermatogenic stage per tubular cross section) is present, in New World monkeys and great apes a multistage arrangement is found (i.e., more than one spermatogenic stage per tubular cross section) [7,10,24]. It had been suggested that the different types of spatial organization reflect "better" or "worse" efficiency of sperm production [22,27]. However, this hypothesis has

VetBooks.ir

been challenged; meiotic and spermatogenic efficiencies were shown to be similar among all primate taxa, independent of the type of spermatogenic organization [7]. To date, the most likely explanation for the underlying reason of this phenomenon is that synchronicity and size of the individual stem cell clones cause the different appearance [28,29]. The similar staging is, however, consistent with testicular development, which is also comparable between New World monkeys and great apes [25,32] and therefore renders the marmoset an excellent model in terms of testicular cell biology.

Reproductive Biology

Marmoset reproduction is peculiar among primates in another way: twinning seems to be the normal way of birthing offspring. This observation was first made and anatomically described almost 90 years ago [30]. Further studies approached this unique phenomenon in the following decades, and today it is generally accepted that twinning is the regular result of some sort of intrauterine selection, as obviously always more than two embryos are implanted, of which two survive to term in the wild. It has been discussed as a strategy to adapt reproduction to the environmental conditions; however, when bred in captivity, this fact causes some problems in colony management, as described in detail below. Interestingly, the twins share the same placenta. This has unique consequences: First, the siblings can be considered as sort of natural clones and are therefore extremely worthy for studies following up developmental changes under treatment [31]. Second, because the twins can be of different sexes, this might have provoked another marmoset-specific feature: these animals are born with gonads arrested in a late fetal state, rendering them an important model for research on gonadal development when taking the similarities to human gonadal development into account [25,32].

Puberty, defined as the onset of sexual maturity, was related to age, hormonal concentrations, and ovarian cycle or testicular size in marmosets, and several publications approached the ontogeny of pubertal onset [33–39]. Nevertheless, the pubertal dynamics of the marmoset are not yet fully understood; the findings often focus on single aspects and either males or females. In addition, the specific endocrine conditions in the marmosets, that is, the functional replacement of LH by CG [13,40] in the concerted regulatory processes along the HPG axis, and the unique social behavior of these monkeys render insights into the complex phase of puberty difficult. The latter is particularly interesting because reproduction in marmosets depends far more on the social status of an individual than in other primate models [17]. Within a family group, in general, only the dominant couple reproduces, and almost all pups are fathered by the dominant male [41]. In females, a subordinate rank efficiently suppresses fertility; however, as soon as those females gain alpha position or are artificially mated to a male partner, they start to cycle immediately [42]. In contrast, spermatogenesis in male marmosets seems to be less influenced by testosterone, that is, it is driven on by androgens at a much lower threshold, an adaptation that might be discussed as one to avoid social conflict by reducing aggressive behavior but maintaining full reproductive capacity [15,43,44]. Against this marmoset-specific background, it is not surprising that the social environment might interfere with individual pubertal development. However, compared with Old World monkeys, platyrrhines reach sexual maturation after a relatively short postnatal period and produce larger numbers of offspring per pregnancy [45,46]. Therefore, among NHPs (when taking the mentioned species-specific features into account) they are an interesting and suitable model

VetBooks.ir

to study pubertal changes in primates because larger numbers of animals become available in a reasonable time for comparative studies. Such larger group sizes would allow analysis of more than only single parameters and could also be an approach to implement the impact of social conditions into an individual development, beyond cross-sectional studies but allowing for longitudinal observations.

These findings also point to the fact that the regulatory role of CG and testosterone on marmoset spermatogenesis is limited and quite different from that observed in Old World monkeys and humans. These differences might result in unexpected findings in preclinical studies addressing endocrine issues. The regulation of male reproductive endocrinology, at least, should not be using marmosets as NHP model.

Evidence of Age Effects in Captivity

As recent research on aging came more into focus, it is not surprising that the marmoset as an NHP model was explored with regard to its suitability for studies of age-related diseases. The marmoset lifespan is, on average, between 5 and 7 years, but the animals can reach an age of 15 years and older. Thus, among anthropoid primates, they have short lifespans [47]. Ross et al. [48] analyzed in detail aging phenotypes of marmosets in colonies kept in captivity and defined these monkeys as elderly with an onset at approximately 6 years of age. Marmosets are generally considered to be elderly when they are 8 years or older. In the colonies of CLEA Inc. in Japan, Nishijima and colleagues [49] found gender-specific differences in life spans of the monkeys, with, on average, a significantly longer life expectancy in males (148.5±6.1 months) than in females (111.7±6 months). Mortality also occurred at a later age in males. Nevertheless, the maximum age reached was higher in females. In summary, the life span curves were comparable to those of rodent colonies as well as to human populations [49]. Age-related changes in body composition, hematology, inflammatory parameters, as well as bone metabolism have been reported. Interestingly, in marmosets the risk for cardiac and kidney disorders also increased with age (similar to humans). These findings provide a basis for further research and suggest the marmoset is a suitable model for preclinical studies of age-related metabolic changes and disease [48].

USE OF MARMOSETS IN REPRODUCTIVE AND REGENERATIVE RESEARCH

Marmosets in Reproductive and Stem Cell Research

Human regenerative medicine, including transplantation of various functional cells differentiated from embryonic stem cells (ESCs) or induced pluripotent stem cells, is considered to have great potential for treating several incurable diseases and has thus attracted much public attention. However, preclinical studies using animal disease models are required to evaluate the efficacy and safety of ESC/induced pluripotent stem cell-derived cells before their clinical application. The common marmoset has been recognized as a useful NHP model for such studies because of its small size, large reproductive capacity, and genetic similarity to humans.

Marmoset ESCs were established in 1996 as the first stem cell type derived from NHPs and stimulated a promising new scientific field of research on primate cells [50–52,89].

VetBooks.ir

However, growth factors and downstream pathways for self-renewal of marmoset ESCs still remain to be demonstrated. For stem cells derived from other species, for example, mice, leukemia inhibitory factor promotes the growth of cell cultures. In contrast, marmoset ESC growth is obviously driven by basic fibroblast growth factor via the activation of the phosphatidylinositol-3-kinase protein kinase B (AKT) pathway on mouse embryonic fibroblast feeders [52]. Furthermore, alternative growth factors have been tested to encourage self-renewal of marmoset ESCs, but which conditions are the most effective have not been determined. As an example, Kanda et al. [84] recently characterized marmoset mesenchymal stem cells after harvesting bone marrow-derived cells obtained from femurs of newborn male infants. They demonstrated that the morphology of the cells was similar to marmoset fibroblasts, and extracellular matrix components such as gelatin and fibronectin effectively supported their proliferation. They managed to provoke differentiation of these cells into adipocytes, osteocytes, and chondrocytes expressing several mesenchymal markers. Thus, the most appropriate conditions, that is, growth factors and the downstream pathways, for maintaining marmoset ESCs have not been completely identified and will be addressed in future studies. However, the findings so far clearly show that marmoset ESCs share phenotypical similarities with human ESCs and have putatively great potential for preclinical testing. Therefore, marmoset ESCs can facilitate the development of valuable preclinical experimental systems to test new therapeutic modalities for human diseases.

Contraception

Marmosets of both sexes seem to be an ideal model for contraceptive studies. An advantage of the females is the relatively short gestation period (138-148 days) compared with Old World monkeys and for males the similarities in its testicular development and the organization of the seminiferous epithelium [15]. Furthermore, the marmoset's high fertility rate (approximately 90%) [53] and early sexual maturity (13-15 months) enable larger numbers of animal to be used in experimental studies, allowing for the generation of unambiguous data [22]. The marmoset model provides two further advantages: the possibility of fertility studies (long-term efficacy) and use of groups of animals at regular intervals. However, as mentioned above, the marmoset's endocrine regulation differs from that of macaques and humans. Marmosets exhibit endocrine peculiarities; for example, hormones involved in reproductive regulation, such as gonadotrophins and steroids, are specifically different from those of other primates [7,18].

One central approach of achieving a reliable male hormonal contraceptive is the reduction of sperm numbers in the ejaculate. Therefore, the endocrine HPG axis has to be downregulated by testosterone and/or a gestagen or a gonadotropin-releasing hormone antagonist [16]. A good measure of male contraception in macaques and men is the decrease of free testosterone concentrations in peripheral blood. In marmosets, the approach to downregulate the HPG axis reduces testicular size but not necessarily the testosterone concentrations. This effect maybe due to the low binding affinity of plasma steroid-binding protein, an assumption that is supported by high concentrations of free testosterone observed in the serum of marmosets [54]. In addition, a possible steroid resistance has been suggested for New World monkeys [55]. The permanent presence of enhanced concentrations of testosterone could result in a certain resistance in the target organs [18], and an accelerated clearance of exogenous androgens was reported [56]. However, exogenous hormone treatment showed obvious and

VetBooks.ir

expected endocrine effects; spermatogenic disruption was weak, although testicular size decreased [15]. The seminiferous epithelium seems to be fully functional at very low concentrations of intratesticular testosterone, suggesting that the mechanisms of the proposed steroid resistance must be highly unusual. An alternative explanation could be found in the effects of FSH. This hormone is less affected by the exogenous treatment and plays an important role in primate spermatogenesis [21]—and very likely in the regulation of spermatogenesis in the marmoset, although to date its role in marmoset spermatogenesis remains speculative.

Achieving contraception in females is much easier because the ovarian cycles are susceptible to induced luteolysis (see the next section) by prostaglandin F2α (PGF2α). This is a normal phenomenon in female marmosets because marmosets live in family groups that are lead by one dominant animal, which is the only fertile female within the group of sisters and daughters. This dominant female regulates the ovarian cycles of her cohabitants by a still unknown mechanism; nevertheless, it demonstrates a natural way of "social" contraception per se. Other experimental contraception methods have been demonstrated successfully in different ways, such as inhibiting FSH [57], inhibiting vascular endothelial growth factors during the postovulatory period [53], and downstream immunocontraception methods targeting the zona pellucida proteins of oocytes [58].

In conclusion, the female marmoset is an ideal model for exploring contraceptive test article. By contrast, in males, endocrine differences from Old World monkeys and humans are still not sufficiently understood for developing test article-based contraceptives.

Cycling Control and Oocyte Harvest, Maturation, and Offspring

Unlike Old World monkeys, marmosets do not bleed during menstruation, so the menstrual cycle can only be monitored by measuring hormone concentrations. The ovarian cycle of marmosets can be followed by measuring serum progesterone values, and exogenous control of the cycle is possible by inducing luteolysis by administering PGF2α 11-14 days after ovulation [19,59]. The day of ovulation is the last day before the serum progesterone concentration exceeds 10 ng/mL or, for animals with basal progesterone values >10 ng/mL, the last day before a marked rise in serum progesterone concentration is observed.

Marmosets are the only NHPs that enable simultaneous use of multiple oocytes; therefore, they are the NHP model best suited for studying related features of reproductive biology. Great efforts have been made to create a reliable protocol for receiving mature marmoset oocytes ready for fertilization. On one side there is the approach to hyperstimulate ovaries in vivo to increase the number of mature oocytes per ovulation. After starting a new ovarian cycle by PGF2α injection, animals were treated with different regimens of recombinant human FSH for up to 7 days and were subsequently administered with human CG once, before follicular aspiration of the matured oocytes. Preovulatory follicles were aspirated with ultrasound-guided needles [60,87], and the oocytes and the surrounding cumulus cells were transferred into cell culture medium supplemented with heparin and maintained at 37 °C. In another recent successful approach oocytes were obtained before maturation and were maturated in vitro [60,61]. Although Tomioka et al. succeeded in maturing oocytes in vitro, optimization of this method is still under debate. Viable offspring derived from oocytes matured in defined medium provided evidence of stable culture conditions for marmoset oocytes and opened a future option for reliable analysis of novel factors without potential confounders from animal-derived components.

VetBooks.ir

To fertilize the oocytes, either in vivo or in vitro mature semen has to be collected from male marmosets. After sampling, semen is washed in a medium and placed in a carbon dioxide incubator for 30 min before incubation with mature oocytes. The rate of developing embryos is still highly variable, but those developing well were transferred to foster mothers after their ovarian cycles were synchronized. One to three embryos were successfully transferable into the uterine lumen. After embryo transfer, recipient animals were monitored for pregnancy by measuring plasma progesterone concentrations weekly until pregnancy could be monitored by transabdominal palpation and uterine ultrasonography [62]. Successful transplantation of embryos to gain viable marmoset offspring demonstrated that this model provides an alternative to Old World monkeys to scientifically explore reproductive aspects of human early development.

PRACTICAL CONSIDERATIONS

Keeping: Housing and Husbandry

Marmosets require a very specialized feeding regime, which often is also laboratory specific. Body weight can vary daily by ±30 g, for example, when the daily routine is altered or during experimental procedures or frequent blood sampling. In addition to dry pellets as a commercial standard diet, the main part of the food is a morning mash with over 20 ingredients freshly prepared, with a special focus on vitamin A3, vitamin D, and gummi arabicum. Alternatively, a standard feeding regime also used in many colonies is a high-calorie vitamin porridge in the morning, followed by fruits, vegetables, and a protein source (e.g., insects) in the afternoon. Feeding intervals should be 4.5-6.5 h. Extras such as cooked rice, noodles, potatoes, rusks, hardboiled eggs, cottage cheese, boiled chicken, cat food, raisins, sunflower seeds, dried figs or dates, peanuts, carob, grasshoppers, and meal- and waxworms, offered once a week in daily variation, are also offered.

Rensing and Oerke [63] list the basic requirements for breeding and maintaining a marmoset colony in great detail: like other primates, *Callitrichinae* should be housed in social groups, either as pairs, family, or peer groups. If animals have to be housed singly for experimental reasons, they should have at least visual and olfactory contact with compatible animals. In general, cages should be built and placed in such a way that monkeys can go up or escape above human eye level. The cages should use the full measure of available height rather than width (Table 23.1). The cage ground should be solid and covered with sawdust, woodchips, or hay. Animals frequently use the ground to forage for food and to encounter others in social play. A nestbox is mandatory. The minimum equipment of a cage for callitrichids consists of a nest- or sleeping box, a sitting shelf, various branches of different sizes, and, on different levels, a water bottle and a feeding bowl. Nest boxes can be made of either metal or wood. While metal is better for regular cleaning, it needs holes for ventilation. Nestboxes made out of wood are advisable because they absorb moisture better. The nestbox should be placed in the top of the cage (comparable to natural sleeping sites) and should be sufficiently sized to allow for all group members to find a place to sleep. If animals need to be caught regularly, it is worth using nestboxes that can be closed by a sliding door to catch them inside (Figure 23.1).

In general, three factors are very important for correct keeping conditions for these neotropical monkeys: temperature of 25-29 °C, 60% humidity, and a 12-h light/dark cycle. In addition, other important factors for well-being are smell, noise, and view. Since marmosets communicate through

VetBooks.ir

TABLE 23.1 Dimensions of Marmoset Cages as Specified by the New European Guideline Requirements for Housing of Nonhuman Primates

	Minimum ETS 123 Guideline Requirements[a]
Floor space (m^2)	0.5
Height (m)	1.5[b]
Volume (m^3)	0.75[c]

[a]*For one or two animals plus offspring up to 5 months old (plus 0.2 m^3 per additional animal older than 5 months).*
[b]*Top of enclosure at least 1.8 m above the ground.*
[c]*Calculated, not indicated in the guideline.*
ETS 123 [83].

odor, cleaning should not be too intensive to keep the family's smell in the home cage. Marmosets vocalize often, and it is comforting for them to hear other animals. The monkeys are usually interested in the voices of the personnel and might accept music at a low volume. Marmosets are curious animals and therefore always try to see as much as they can. It was believed that visual contact has to be avoided, for example, through separation by foliage, or otherwise the animals would suffer from permanent stress induced by their extreme territorial behavior. In the wild, the monkeys defend their home range and would, being caged, face the situation that this behavior would not have any effect in solving conflict. This could result in life-threatening stress. However, newer work shows that this is not a necessity as long as all other mentioned parameters are met.

FIGURE 23.1 Marmoset room. Each cage has an attached balcony.

VetBooks.ir

Breeding Specifics in Captivity: Triplet and Quadruplet Management

In captivity, triplet and quadruplet births are a problem often faced in marmoset colonies and seem to be becoming more and more the rule, being reported from many primate centers maintaining marmoset breeding colonies [64]. Figures on this increasing tendency of triplet and quadruplet births from the Centre of Reproductive Medicine and Andrology (Münster, Germany) have been confirmed by researchers from other institutions. The distribution of offspring per pregnancy in this colony revealed, on average, the following data: singleton births, 5.9% (range 2-10%); twins, 44.2% (43-52%); triplets, 48.5% (41-57%); quadruplets, 1.4% (0-4%). Smuncy et al. [64] summarized the average data of offspring produced from five institutions in which colonies were analyzed, and the average number of offspring per pregnancy was 2.22. Windle et al. [65] reported an average number of 2.47 offspring per pregnancy from the colony of the MRC in Cambridge, UK.

No definitive explanation for this phenomenon can be found in the literature. However, there is evidence that in marmosets generally more than two eggs are ovulated and usually more than two embryos develop; this also occurs in natural conditions among monkeys living in the wild. In the majority of cases, however, these fetuses are absorbed *in utero*, and thus triplets and quadruplets are born only rarely in the wild. It is assumed that this serves as a selective mechanism, that is, it reflects a kind of reproductive flexibility in terms of rapid adaptation to the respective prevailing ambient conditions. When an adequate, permanent food supply is provided (especially with a sufficient amount of protein) and no predator and parasite pressure is present—as in captivity—these "optimal" conditions apparently provoke an increased number of embryos to develop to term, and multiple births result [65].

The management of triplets and quadruplets is a challenge when breeding marmosets in captivity. Approaching this natural feature of marmoset reproduction, which is an adaptation to environmental conditions that do not exist in captivity, through special diets was suggested. Therefore, in the colony at the Centre of Reproductive Medicine we tested a change in diet to test this hypothesis. However, when reducing protein content, we observed no significant changes in multiple births, and this finding was confirmed by other primate centers. Thus, it is likely that there are also other factors involved that provoke increased multiple births in captivity. Unfortunately, that the adults cannot care for more than two pups is a general problem, one that often results in the loss of all offspring, which are insufficiently provided for by the parents. Unfortunately, hand-reared marmosets do not show normal behavior and can neither be integrated into existing family groups nor combined into new social entities. This is because most of marmosets' social behavior is learned and social relationships are a dominant component in normal marmoset behavior.

USE OF MARMOSETS IN TOXICOLOGICAL AND PHARMACOLOGICAL STUDIES

Experimental Handling and Sampling of Specimens from Experimental Animals

Fluid collection in marmosets is limited due to their small size. Table 23.2 details the maximum blood sample volume for marmosets adopted from the data published by Diehl et al. [66]. For clinical pathology, the maximum volume-to-body weight ratio can be increased up to 10 mL/kg (~15% of body weight).

VetBooks.ir

TABLE 23.2 Maximum Blood Volume to be Taken from Marmosets within 24 h, Followed by a 7-Day Recovery Period

Body Weight (g)	Maximum Blood Volume (mL) (10% of the Body Weight; ~0.7 mL/100 g)
200	1.4
250	1.8
300	2.1
350	2.4
400	2.8
450	3.2
500	3.5
550	3.8

Modified from Diehl (2001).

Monitoring the Experimental Animal During the Study

Korte compared standard diagnostic parameters between marmosets and cynomolgus monkeys (Korte, personal communication). Among the clinical pathology parameters, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase, creatine kinase, creatinine, and triglycerides differ significantly between cynomolgus monkeys and marmosets (Korte, personal communication). In the marmoset, mean values of alkaline phosphatase and γ-glutamyl transferase are approximately 4- to 5-fold and 13- to 17-fold lower, respectively, in comparison with values in macaques. Data reported by Clarke [91] for aspartate aminotransferase and alanine aminotransferase in the nonfasted marmoset were significantly higher than in fasted marmosets. The evaluation of marmoset hematological data is characterized by reticulocyte values approximately 11- to 17-fold higher than those of macaques. Other standard parameters show similar results for the two species (Korte, personal communication).

Ophthalmoscopic examinations of 488 marmosets resulted in only 1.2% background findings (pigment conus, medullated nerve fiber on the margin of papilla being judged as a congenital finding, bright areas on the macula, slight drusen and choroid atrophy at peripapillary conus), all of which also occurred in the macaque (Korte, personal communication). Of 1829 cynomolgus monkeys examined, a total of 134 (7.3%) spontaneous ocular findings were found [67]. These included optic nerve head variations, hyper- and hypopigmentation of the retina, drusen, macula pigment change, altered translucence, vascular variability, microaneurisms, and punctiform hemorrhages.

Electrocardiography evaluations in *C. jacchus* revealed an average of almost doubled heart beat frequency compared with macaques (405 vs. a mean of 233 beats/min) (Korte, personal communication). The average respiratory rate for male and female marmosets was 101 and 96 breaths/min, respectively; for rhesus monkeys a mean range of 51 and 44 breaths/min

VetBooks.ir

in males and females, respectively, was reported by Zühlke et al. [92]. Testosterone ranges between 0.93 and 178.06 nmol/L in adult males, with a mean of 33.61 nmol/L (Korte, personal communication). Adult male cynomolgus monkeys showed a mean testosterone level of 25.57 nmol/L (range, 1.52-122.74 nmol/L). On average, testicular volume of adult marmosets was 0.53 mL (range, 0.26-0.88 mL, volume in both testes). Spontaneous mortality in the marmoset was 2-3% (Korte, personal communication). The presumed causes of death were digestive problems inducing weight loss and acute infections. Complex studies (e.g., continuous intravenous infusion studies with a port catheter system) showed slightly increased mortality. In comparison, spontaneous mortality in macaques not related to the test article was <1% (Korte, personal communication). Conclusively, the marmoset is an established laboratory species for preclinical studies, with many physiological similarities to the macaque. Only a few significant differences have been described in marmosets, such as low alkaline phosphastase (AP), gamma glutamyl transferase (GGT), and elevated reticulocytes. Heart and respiratory rate are increased in the marmoset because of their markedly smaller body size.

Drug Administration

Standard administration techniques (such as oral, subcutaneous, intravenous) in toxicology safety assessment are well established for marmosets. Because many test articles need to be administered intravenously, in some cases prolonged or continuous infusion is required. Because marmosets have a much lower body weight and smaller body size in comparison to other NHP species (such as macaques), continuous intravenous dosing of test articles has to be adapted accordingly. Ambulatory pump systems for larger primates, consisting of a pump, backpack system, batteries, and the fluid that is to be delivered, can easily reach a weight of 300-400 g and would therefore be heavier than the experimental animals themselves. Thus, the maximum weight feasible for a free-ranging delivery system in marmosets has to be smaller and much lighter in weight (≤10% of body weight—approximately 30 g). Extensive infusion studies have been conducted using restrained marmosets, with a limited dosing time of 1.5 h (or twice daily for 1 h). This differs to macaques, where limited restrained infusion can last up to 4 h. If port surgeries are required [90], the device is commonly placed subcutaneously in macaques and extracutaneously (above the cutis, sutured to the cutis) in the marmoset. New technology using a restraint device or a mini-pump system in a backpack enables daily or even weekly continuous intravenous infusion in the marmoset [68].

Dosing Techniques and Dosing Design

Just as diagnostic techniques are adapted from use with larger NHPs to fit the purpose of applications in marmosets, other equipment, such as oral gavage tubing and needles, have to be smaller when used for marmosets. For example, a thin, vertical, metal bar supports the opening of the mouth during oral gavage. When planning daily oral dose administrations (up to 10 mL/kg/day) in starved animals, the complex and variable (multiple times daily) food intake has to be considered. When the animals have a stomach filled by the administered fluid, food intake is limited. This might provoke weight loss as a consequence of diarrhea.

Pathology/Histology

Selected Examples of Special Histopathology of Marmosets

For the interpretation of histopathology of toxicological studies using primates, precise knowledge of the background pathology of the species is necessary to differentiate test article-induced lesions from spontaneous changes. Information on the background pathology of marmosets, however, is rare, and only three larger studies of laboratory-bred marmosets have been published. Okazaki et al. [69] examined 61 marmosets for spontaneous lesions. However, only 21 of them were killed after finishing toxicological studies. The remaining were animals from the colony that died or had to be killed while moribund, a fact that may have markedly influenced the incidence and spectrum of findings (e.g., a high incidence of lymphatic organ atrophy). Tucker [70] examined 567 marmosets, including control and treated animals, but results were neither separated by treated and control animals nor by sex. Finally, Kaspareit et al. [71] examined a total of 200 control marmosets from toxicological studies to evaluate the incidence of spontaneous findings in this New World primate. Based on these studies, a number of histopathological lesions can be considered to be a special feature of common marmosets because they are not seen in other laboratory primate species, such as the cynomolgus (*Macaca fascicularis*) and rhesus monkey (*Macaca mulatta*). These histopathological changes include extramedullary haematopoiesis in a variety of organs, C-cell hyperplasia in the thyroid, hepatic hemosiderosis, mineralization and/or osseous metaplasia in different organs, enteritis, and an immune complex nephropathy.

One of the most common findings unique to marmosets is spontaneous extramedullary hematopoiesis [72]. While in rodents the spleen is the most important site of extramedullary hematopoiesis [93], in marmosets a spectrum of organs, including the adrenal gland, liver, kidney, pulmonary alveolar walls, and the choroid plexus in the brain, are involved (Table 23.3). Hematopoiesis is represented by small accumulations of erythroblastic cells (Figure 23.2a) or cells belonging to the myelopoietic cell line at different stages of maturation. Megacaryocytes can be found in scattered distribution among these nests of hematopoietic cells (Figure 23.2b). However, megacaryocytes also are often noted as single cells situated in hepatic or adrenal sinusoids or within alveolar walls. Especially in the adrenal glands of marmosets, severe extramedullary haematopoiesis may occur in a way that normal tissue

TABLE 23.3 Extramedullary Hematopoiesis in Marmosets ($n = 200$)

Organ	Males, *n*	Females, *n*	Total, *n* (%)
Adrenal gland	63	45	108 (54.0)
Brain, choroid plexus	5	5	10 (5.0)
Kidney	6	11	49 (24.5)
Liver	47	46	93 (46.5)
Spleen	24	33	57 (28.5)

From Kaspareit et al. [71].

VetBooks.ir

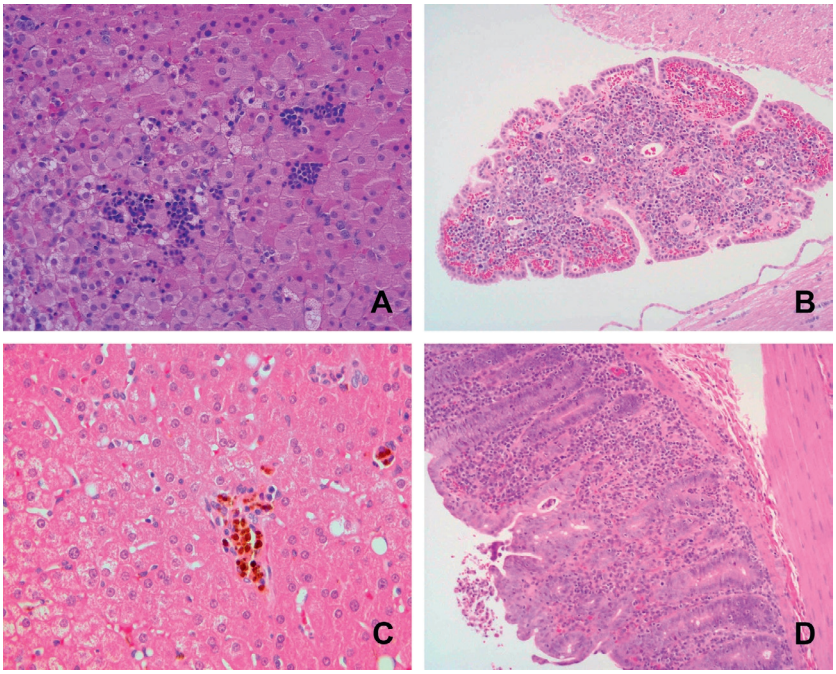


FIGURE 23.2 (a) Clusters of hematopoietic cells in the liver of a marmoset. (b) Hematopoietic cells in the choroid plexus of the brain. (c) Hemosiderin deposits in the liver of a marmoset. (d) Inflammatory cell infiltrates in the colon of a marmoset.

architecture is obscured by hematopoietic cells. Incidence of extramedullary hematopoiesis in different marmoset organs is summarized in Table 23.3. According to Tucker [70], repeated bleeding in toxicological studies can increase the degree of hematopoiesis.

In the thyroid gland of marmosets, C-cell hyperplasia is a very frequent finding. Unknown in other NHP models [72,73], it is well known in several rat strains [74,88]. While the incidence of C-cell hyperplasia is much higher in female than in male rats, equal incidences of this lesion have been observed in marmosets [71]. From rats it is known that C-cell hyperplasia frequently is associated with increased incidence of C-cell adenoma or carcinoma. This has not yet been reported in marmosets. C-cell hyperplasia may occur as one or more nodules of concentrated C-cell areas, mostly in the middle of an organ, or an increased number of C-cells may be diffusely distributed throughout an organ, causing follicles to appear smaller and fewer in number and giving the organ a more solid appearance.

Hepatic hemosiderosis is another lesion that can be variably present in captive marmosets (Figure 23.2c). In their colonies, Chalmers et al. [75] and Miller at al. [76] reported incidences of this lesion of 55% and 100%, respectively. Okazaki et al. [69] observed hepatic and Kupffer cell pigment (probably representing hemosiderin) in about 8% of their animals, whereas it was not observed in the studies by Tucker [70] and Kaspareit et al. [71]. In a feeding study of diets with different iron content, Miller et al. [76] demonstrated that

VetBooks.ir

the occurrence of hepatic hemosiderosis is strongly dependent on the hemosiderin content of the diet. High dietary hemosiderin resulted in a high frequency of hemosiderosis in the liver. Another factor discussed is the presence of chronic inflammatory processes. In the case of chronic inflammatory diseases, such as those associated with wasting marmoset syndrome (WMS), hemosiderosis is thought to develop as a sequence of the sequestration of iron and poor transfer from siderophages to erythrocytes in the anemia caused by this chronic disease [77].

Vascular or parenchymal mineralization commonly occurs in control animals. This occurs as part of metastatic mineralization in this species and may involve the aorta, kidneys, lung, heart (frequently the atria), gastric mucosa, and adrenal gland. Excessive calcium absorption as a result of hypervitaminosis D, or high calcium concentrations in the diet, are believed to be the main causes of metastatic calcification in marmosets [71]. In the lung osseous metaplasia occurs infrequently in the form of small, plaquelike ossified tissue within the pulmonary parenchyma or appears as small, elongated osseous deposits under the pulmonary surface.

Another common, but poorly understood, condition of captive-bred marmosets is enteritis of large intestine. Tucker [70] and Okazaki et al. [69] observed enteritis in 15% and 26% of animals, respectively. Kaspareit et al. [71] reported this lesion was present in about 20% of the animals examined, localized in the cecum and colon. This alteration also contributes to the so-called WMS, where it occurs in association with hemolytic anemia, severe weight loss, muscle atrophy, and hair loss [78]. However, histopathological enteritis occurs with or without intermittent clinical diarrhea. On the other hand, clinical diarrhea can occur without any histopathological correlate. The histopathological presentation of chronic enteritis is generally similar to that seen in macaques; crypt microabscesses, herniation of glands, microulceration, atrophy and loss of crypts, goblet cell depletion, and different degrees of lymphohistiocytic inflammatory cell infiltration into the lamina propria are the most common findings (Figure 23.2d). Only in a few severe cases the serosa is involved or the ulcer completely penetrates the intestinal wall. The reasons for enteritic changes in marmosets are unclear. In their natural habitat wild callitrichids have a highly variable diet, including insects, fruits, flowers, shoots, small vertebrates, eggs, and gums or saps sucked from holes dug with the teeth in the bark of trees [79]. Since a commercial diet supplemented by fruits and vegetables can hardly completely meet the demand of this species, it seems likely that marmosets in captivity are prone to disturbances of intestinal microflora, leading to malnutrition and enteritis that might easily be enhanced by stress or infection.

Marmosets also commonly suffer from a spontaneous glomerulonephropathy, which might even cause death. The most common form of glomerulonephropathy in marmosets is a diffuse, mesangioproliferative glomerulonephritis, which has been linked to the deposition of immune complexes directed against parasitic, bacterial, and dietary antigens [77]. This form is considered to be mediated by either immunoglobulin M or A or both [80,81] in marmosets and other New World NHPs. Diffuse mesangioproliferative glomerulonephritis in marmosets is characterized by enlargement of glomeruli due to proliferation of mesangium (cellular and matrix proliferation), slight thickening of the Bowman capsule accompanied by inflammation and fibrosis of the interstitium, as well as tubular damage with accumulation of tubular hyaline casts and tubular regeneration [82].

VetBooks.ir

References

[1] Opazo JC, Wildman DE, Prychitko T, Johnson RM, Goodman M. Phylogenetic relationships and divergence times among New World monkeys (Platyrrhini, Primates). Mol Phylogenet Evol 2006;40:274–80.

[2] Von Dornum M, Ruvolo M. Phylogenetic relationships of the New World monkeys (Primates, platyrrhini) based on nuclear G6PD DNA sequences. Mol Phylogenet Evol 1999;11:459–76.

[3] Wildman DE, Jameson NM, Opazo JC, Yi SV. A fully resolved genus level phylogeny of neotropical primates (Platyrrhini). Mol Phylogenet Evol 2009;53:694–702.

[4] Goodman M, Czelusniak J, Page S, Meireles CM. Where DNA sequences place *Homo sapiens* in a phylogenetic classification of primates. In: Tobias PV, Raath MA, Moggi-Cecchi J, Doyle GA, editors. Humanity from African naissance to coming millennia. Colloquia in human biology and paleoanthropology. Firenze: Firenze University Press; 2001. p. 274–89.

[5] Grooves C. Order primates. In: Wilson DE, editor. Mammal species of the world. Baltimore, MD: John Hopkins University Press; 2005. p. 111–84.

[6] Flynn JJ, Wyss AR. Recent advances in South American mammalian palaeontology. Trends Ecol Evol 1998;13:449–54.

[7] Luetjens CM, Weinbauer GF, Wistuba J. Primate spermatogenesis: comparative evidence and new insights into testis organisation, spermatogenic efficiency and endocrine control. Biol Rev Camb Philos Soc 2005;80:475–88.

[8] Maston GA, Ruvolo M. Chorionic gonadotropin has a recent origin within primates and an evolutionary history of selection. Mol Biol Evol 2002;19:320–35.

[9] Troppmann B, Kleinau G, Krause G, Gromoll J. Structural and functional plasticity of the luteinizing hormone/choriogonadotrophin receptor. Hum Reprod Update 2013;19:583–602.

[10] Wistuba J, Luetjens CM, Weinbauer GF, Gromoll J. Comparative analysis of the primate testis: size, endocrine regulation and sperm competition. In: Weinbauer GF, Buse E, Müller W, Vogel F, editors. New developments and challenges in primate toxicology. Münster/New York/München/Berlin: Waxmann Verlag; 2005. p. 73–85.

[11] Gromoll J, Eiholzer U, Nieschlag E, Simoni M. Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. J Clin Endocrinol Metab 2000;85:2281–6.

[12] Gromoll J, Wistuba J, Terwort N, Godmann M, Müller T, Simoni M. A new subclass of the luteinizing hormone/chorionic gonadotropin receptor lacking exon 10 messenger RNA in the New World monkey (Platyrrhini) lineage. Biol Reprod 2003;69:75–80.

[13] Müller T, Simoni M, Pekel E, Luetjens CM, Chandolia R, Amato F, et al. Chorionic gonadotrophin beta subunit mRNA but not luteinising hormone beta subunit mRNA is expressed in the pituitary of the common marmoset (*Callithrix jacchus*). J Mol Endocrinol 2004;32:115–28.

[14] Adams C, Henke A, Gromoll J. A novel two-promoter-one gene system of the chrionic gonadotropin ß gene enables tissue-specific expression. J Mol Endocrinol 2011;47:285–98.

[15] Wistuba J, Luetjens CM, Ehmcke J, Redmann K, Damm OS, Sandhowe-Klaverkamp R, et al. Experimental endocrine manipulation by contraceptive regimen in the male marmoset (*Callithrix jacchus*). Reproduction 2013;145:439–51.

[16] Nieschlag E. Clinical trials in male hormonal contraception. Contraception 2010;82:457–70.

[17] Saltzman W, Digby LJ, Abbott DH. Reproductive skew in female common marmosets: what can proximate mechanisms tell us about ultimate causes? Proc R Soc B Biol Sci 2009;276:389–99.

[18] Li LH, Donald JM, Golub MS. Review on testicular development, structure, function, and regulation in common marmoset. Birth Defects Res B Dev Reprod Toxicol 2005;74:450–69.

[19] Luetjens CM, Wesselmann R, Kuhlmann M. GnRH-antagonist mediated down-regulation of the estrous cycle in marmosets. J Med Primatol 2006;35:361–8.

[20] Müller T, Hupfeld T, Roessler J, Simoni M, Gromoll J, Behr R. Molecular cloning and functional characterization of endogenous recombinant common marmoset monkey (*Callithrix jacchus*) follicle-stimulating hormone. J Med Primatol 2011;40:111–9.

[21] Weinbauer GF, Schlatt S, Walter V, Nieschlag E. Testosterone induced inhibition of spermatogenesis is more closely related to suppression of FSH than to testicular androgen levels in the cynomolgus monkey model (*Macaca fascicularis*). J Endocrinol 2001;168:25–38.

[22] Millar MR, Sharpe RM, Weinbauer GF, Fraser HM, Saunders PT. Marmoset spermatogenesis: organizational similarities to the human. Int J Androl 2000;23:266–77.

[23] Weinbauer GF, Aslam H, Krishnamurthy H, Brinkworth MH, Einspanier A, Hodges JK. Quantitative analysis of spermatogenesis and apoptosis in the common marmoset (*Callithrix jacchus*) reveals high rates of spermatogonial turnover and high spermatogenic efficiency. Biol Reprod 2001;64:120–6.
[24] Wistuba J, Schrod A, Greve B, Hodges JK, Aslam H, Weinbauer GF, et al. Organisation of the seminiferous epithelium in primates: relationship to spermatogenic efficiency, phylogeny and mating system. Biol Reprod 2003;69:582–91.
[25] Albert S, Ehmcke J, Wistuba J, Eildermann K, Behr R, Schlatt S, et al. Germ cell dynamics in the testis of the postnatal common marmoset monkey testis (*Callithrix jacchus*). Reproduction 2010;140:733–42.
[26] McKinnell C, Mitchell RT, Morris K, Anderson RA, Kelnar CJ, Wallace WH, et al. Perinatal germ cell development and differentiation in the male marmoset (*Callithrix jacchus*): similarities with the human and differences from the rat. Hum Reprod 2013;28:886–96.
[27] Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neill JD, et al., editors. The physiology of reproduction. New York: Raven Press; 1994. p. 1363–434.
[28] Ehmcke J, Wistuba J, Schlatt S. Spermatogonia: physiology, pathology and clinical relevance. Invited review, Hum Reprod Update 2006;12:275–82.
[29] Wistuba J, Stukenborg JB, Luetjens CM. Mammalian spermatogenesis. Funct Dev Embryol 2007;1:99–116.
[30] Wislocki GB. Observations on twinning in marmosets. Am J Anat 1939;64:445–83.
[31] Sharpe RM, Martin B, Morris K, Greig I, McKinnell C, McNeilly AS, et al. Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity. Hum Reprod 2002;17:1692–703.
[32] Mitchell RT, Cowan G, Morris KD, Anderson RA, Fraser HM, Mckenzie KJ, et al. Germ cell differentiation in the marmoset (*Callithrix jacchus*) during fetal and neonatal life closely parallels that in the human. Hum Reprod 2008;23:2755–65.
[33] Abbott DH, Hearn JP. Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. J Reprod Fertil 1978;53:155–66.
[34] Dixson AF. Plasma testosterone concentrations during postnatal development in the male common marmoset. Folia Primatol (Basel) 1986;47:166–70.
[35] Jackson MR, Edmunds JG. Morphological assessment of testicular maturity in marmosets (*Callithrix jacchus*). Lab Anim 1984;18:173–8.
[36] Lunn SF, Cowen GM, Fraser HM. Blockade of the neonatal increase in testosterone by a GnRH antagonist: the free androgen index, reproductive capacity and postmortem findings in the male marmoset monkey. J Endocrinol 1997;154:125–31.
[37] Lunn SF, Recio R, Morris K, Fraser HM. Blockade of the neonatal rise in testosterone by a gonadotrophin-releasing hormone antagonist: effects on timing of puberty and sexual behaviour in the male marmoset monkey. J Endocrinol 1994;141:439–47.
[38] Rune GM, deSouza P, Krowke R, Merker HJ, Neubert D. Morphological and histochemical effects of 2,3,7,8-tetrachlorodibenzo-*p* dioxin (TCDD) on marmoset (*Callithrix jacchus*) testes. Arch Androl 1991;26:143–54.
[39] Sharpe RM, Fraser HM, Brougham MF, McKinnell C, Morris KD, Kelnar CJ, et al. Role of the neonatal period of pituitary-testicular activity in germ cell proliferation and differentiation in the primate testis. Hum Reprod 2003;18:2110–7.
[40] Chandolia RK, Luetjens CM, Wistuba J, Yeung CH, Nieschlag E, Simoni M. Changes in endocrine profile and reproductive organs during the onset of puberty in the marmoset monkey (*Callithrix jacchus*). Reproduction 2006;132:359–67.
[41] Nievergelt CM, Digby LJ, Ramakrishnan U, Woodruff DS. Genetic analysis of group composition and breeding system in a wild common marmoset (*Callithrix jacchus*) population. Int J Primatol 2000;21:1–20.
[42] Abbott DH, Hodges JK, George LM. Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). J Endocrinol 1988;117:329–39.
[43] Batty KA, Herbert J, Keverne EB, Vellucci SV. Differences in blood levels of androgens in female talapoin monkeys related to their social status. Neuroendocrinology 1986;44:347–54.
[44] Ross CN, French JA, Patera KJ. Intensity of aggressive interactions modulates testosterone in male marmosets. Physiol Behav 2004;83:437–45.
[45] Plant TM, Witchel SF. Puberty in nonhuman primates and humans. In: Knobil E, Neill JD, editors. 3rd ed. The physiology of reproduction, vol. 2. New York: Raven Press; 2006. p. 2177–230.

VetBooks.ir

[46] Tardif SD, Smucny DA, Abbott DH, Mansfield K, Schultz-Darken N, Yamamoto ME. Reproduction in captive common marmosets (*Callithrix jacchus*). Comp Med 2003;53:364–8.
[47] Tardif SD, Mansfield KG, Ratnam R, Ross CN, Ziegler TE. The marmoset as a model of aging and age-related diseases. ILAR J 2011;52:54–65.
[48] Ross CN, Davis K, Dobek G, Tardif SD. Aging phenotypes of common marmosets (*Callithrix jacchus*). J Aging Res 2012;2012:567143.
[49] Nishijima K, Saitoh R, Tanaka S, Ohsato-Suzuki M, Ohno T, Kitajima S. Life span of common marmoset (*Callithrix jacchus*) at CLEA Japan breeding colony. Biogerontology 2012;13:439–43.
[50] Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP. Pluripotent cell lines derived from common marmoset (*Callithrix jacchus*) blastocysts. Biol Reprod 1996;55:254–9.
[51] Müller T, Fleischmann G, Eildermann K, Mätz-Rensing K, Horn PA, Sasaki E, et al. A novel embryonic stem cell line derived from the common marmoset monkey (*Callithrix jacchus*) exhibiting germ cell-like characteristics. Hum Reprod 2009;24:1359–72.
[52] Nii T, Marumoto T, Kawano H, Yamaguchi S, Liao J, Okada M, et al. Analysis of essential pathways for self-renewal in common marmoset embryonic stem cells. FEBS Open Bio 2014;21:213–9.
[53] Fraser HM, Morris KD, Wiegand SJ, Wilson H. Inhibition of vascular endothelial growth factor during the postovulatory period prevents pregnancy in the marmoset. Contraception 2010;82:572–8.
[54] Pugeat M, Rocle B, Chrousos GP, Dunn JF, Lipsett MB, Nisula BC. Plasma testosterone transport in primates. J Steroid Biochem 1984;20:473–8.
[55] Pryce CR, Palme R, Feldon J. Development of pituitary–adrenal endocrine function in the marmoset monkey: infant hypocortisolism is the norm. J Clin Endocrinol Metab 2002;87:691–9.
[56] Mohle U, Heistermann M, Palme R, Hodges JK. Characterization of urinary and fecal metabolites of testosterone and their measurement for assessing gonadal endocrine function in male nonhuman primates. Gen Comp Endocrinol 2002;129:135–45.
[57] Wadia P, Kholkute S, Nandedkar T. Antifertility effect of an octapeptide, a fragment of FSH binding inhibitor, in the common marmoset (*Callithrix jacchus*). Contraception 2003;67:151–60.
[58] Paterson M, Jennings ZA, Wilson MR, Aitken RJ. The contraceptive potential of ZP3 and ZP3 peptides in a primate model. J Reprod Immunol 2002;53:99–107.
[59] Summers PM, Wennink CJ, Hodges JK. Cloprostenol-induced luteolysis in the marmoset monkey (*Callithrix jacchus*). J Reprod Fertil 1985;73:133–8.
[60] Gilchrist RB, Nayandu PL, Hodges JK. Maturation, fertilization, and development of marmoset monkey oocytes in vitro. Biol Reprod 1997;56:238–46.
[61] Tomioka I, Takahashi T, Shimada A, Yoshioka K, Sasaki E. Birth of common marmoset (*Callithrix jacchus*) offspring derived from in vitro-matured oocytes in chemically defined medium. Theriogenology 2012;78:1487–93.
[62] Ishibashi H, Motohashi HH, Kumon M, Yamamoto K, Okada H, Okada T, et al. Efficient embryo transfer in the common marmoset monkey (*Callithrix jacchus*) with a reduced transfer volume: a non-surgical approach with cryopreserved late-stage embryos. Biol Reprod 2013;88:115.
[63] Rensing S, Oerke AK. Husbandry and management of new world species. In: Wolfe-Coote S, editor. The laboratory primate, part 2: primate management. New York: Elsevier; 2005. p. 145–62.
[64] Smucny DA, Abbott DH, Mansfield KG, Schultz-Darken NJ, Yamamoto ME, Alencar AI, et al. Reproductive output, maternal age, and survivorship in captive common marmoset females (*Callithrix jacchus*). Am J Primatol 2004;64:107–21.
[65] Windle CP, Baker HF, Ridley RM, Oerke AK, Martin RD. Unrearable litters and prenatal reduction of litter size in the common marmoset (*Callithrix jacchus*). J Med Primatol 1999;28:73–83.
[66] Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 2001;21:15–23.
[67] Niggemann B, Korte U, Weinbauer GF, Vogel F. The cynomolgus monkey as a model for ocular toxicity testing: incidence and characterization of spontaneous lesions in the ocular fundus. Toxicologist 2003;72(S-1):312.
[68] Zühlke U, Korte S, Niggemann B, Fuchs A, Müller W. Feasibility of long-term continuous intravenous infusion in unrestrained marmoset monkeys. Toxicologist 2003;72:S-1.
[69] Okazaki Y, Kurata Y, Makinodan F, Kidachi F, Yokoyama M, Wako Y, et al. Spontaneous lesions detected in the common cotton-eared marmosets (*Callithrix jacchus*). J Vet Med Sci 1996;58:181–90.

[70] Tucker MJ. A survey of the pathology of marmosets (*Callithrix jacchus*) under experiment. Lab Anim 1984;18:351–8.
[71] Kaspareit J, Friderichs-Gromoll S, Buse E, Habermann G. Background pathology of the common marmoset (*Callithrix jacchus*) in toxicological studies. Exp Toxicol Pathol 2006;57:405–10.
[72] Ito T, Chatani F, Sasaki S, Ando T, Miyajima H. Spontaneous lesions in cynomolgus monkeys used in toxicity studies. Exp Anim 1992;41:455–9.
[73] Chamanza R, Marxfeld HA, Blanco AI, Naylor SW, Bradley AE. Incidences and range of spontaneous findings in control cynomolgus monkeys (*Macaca fascicularis*) used in toxicitiy studies. Toxicol Pathol 2010;38:642–57.
[74] Rao-Rupanagudi S, Heywood R, Gopinath C. Age-related changes in thyroid structure and function in Sprague-Dawley rats. Vet Pathol 1992;29:278–87.
[75] Chalmers DT, Murgatroyd LB, Wadsworth PF. A survey of the pathology of marmosets (*Callithrix jacchus*) derived from a marmoset breeding colony. Lab Anim 1983;17:270–9.
[76] Miller GF, Barnhard DE, Woodsward RA, Flynn BM, Bulte JW. Hepatic hemosiderosis in common marmosets, *Callithrix jacchus*: effect of diet on incidence and severity. Lab Anim Sci 1997;47:138–42.
[77] Lowenstine LJ. A primer of primate pathology: lesions and nonlesions. Toxicol Pathol 2003;31:91–102.
[78] Pritzker KPH, Kessler MJ. Diseases of the musculoskeletal system. In: Bennet BT, Abee CR, Henrickson R, editors. Nonhuman primates in biomedical research. Diseases. San Diego: Academic Press; 1988. p. 415–59.
[79] Layne DG, Power RA. Husbandry, handling and nutrition of marmosets. Comp Med 2003;53:351–9.
[80] Borda JT, Pauley DR, Mackey JJ, Alvarez X, Simon MA, Klump SA. Immunoglobulin-A nephropathy with crescentic glomerulonephritis in a pigtailed macaque (*Macaca nemestrina*). Vet Pathol 2004;41:44–9.
[81] Brack M, Schroeder C, Fooke M, Schlumberger W. IgM/IgA nephropathy in callitrichids: antigen studies. Nephron 1999;82:221–31.
[82] Isobe K, Adachi K, Hayashi S, Ito T, Miyoshi A, Kato A, et al. Sontaneous glomerular and tubulointerstitial lesions in common marmosets (*Callithrix jacchus*). Lab Anim 2013;49:839–45.
[83] ETS 123. European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Appendix A, OJEC K 2007;2525.
[84] Kanda A, Sotomaru Y, Nobukiyo A, Yamaoka E, Hiyama E. Characterization of common marmoset (*Callithrix jacchus*) bone marrow-derived mesenchymal stem cells. Folia Histochem Cytobiol 2013;51:292–9.
[85] Korte S, Nowak P, Luft J, Niggemann B. Marmoset. In: Green OP, Healing G, editors. Non-clinical vascular infusion technology. The techniques, vol. II. Boca Raton, London, New York: CRC Press, Taylor and Francis Group; 2000. p. 285–306.
[86] Korte S, Zühlke U, Kaspareit J, Friderichs-Gromoll S, Müller W, Vogel F, et al. Reference control data for the common marmoset (*Callithrix jacchus*): a comparison with macaques. Toxicologist 2004;78:205.
[87] Luetjens CM, Wesselmann R. The fate of paternal mitochondria in marmoset pre-implantation embryos. J Med Primatol 2008;37:128–40.
[88] Martin-Lacave L, Rojas F, Bernabe R, Utrilla JC, Fernandez-Santos JM, Conde MME. Comparative immunohistochemical study on normal, hyperplastic and neoplastic C cells of the rat thyroid. Cell Tissue Res 2002;309:361–8.
[89] Sasaki E. Establishment of novel embryonic stem cell lines derived from the common marmoset (*Callithrix jacchus*). Stem Cells 2005;23:1304–13.
[90] Schnell CR. Surgical preparation and multidose infusion toxicity studies in the marmoset. In: Healing G, Smith D, editors. Handbook of pre-clinical continuous intravenous infusion. London and New York: Taylor and Francis; 2000. p. 210–22.
[91] Clarke JM. The common marmoset (*Callithrix jacchus*). Anzccart News 1994;7:1–8.
[92] Zühlke U, Niehoff M, Nowak P, Weinbauer G. Primate cardiovascular research - Münster telemetry update. In: Weinbauer GF, Buse E, Müller W, Vogel F, editors. New developments and challenges in primate toxicology. Münster/New York/München/Berlin: Waxmann Verlag; 2004. p. 103–12.
[93] Andrews MC. The hemapoetic system. In: Turton J, Hooson J, editors. Target organ pathology. London (Philadelphia, PA): Taylor and Francis; 1998. p. 177–205.

VetBooks.ir

CHAPTER

24

Considerations on Immunogenicity

Thomas Gelzleichter, Eric Wakshull
Genentech, South San Francisco, CA, USA

OUTLINE

Introduction 457
Effect on PK and PD 458
Impact on Safety 459
Type I Hypersensitivity 459
Type III Hypersensitivity 460
Immune-Mediated Hematologic Disorders 460
Toxicities Due to Reduced Clearance 461
Neutralizing Endogenous Proteins 461
Altered Pharmacology 461
Strategies to Minimize Effect 462
Methods to Measure Antibodies Against Protein Therapeutics 462
Assay Formats and Technologies 463
Assay Thresholds and Sampling 465
Conclusion 465
References 466

INTRODUCTION

With the rapid rise in the development of biotechnology-derived drugs, which often have relatively limited species specificity, nonhuman primates (NHPs) are increasingly used for evaluation of pharmacology, pharmacokinetics (PK), and the safety of candidate drugs before advancement into clinical settings. An important consideration for study interpretation is that the administration of pharmaceutical drugs can be accompanied by an unwanted immune response resulting in the generation of antidrug antibodies (ADAs). This has been particularly challenging for biotechnology-derived therapeutics in which immune responses are commonly encountered. In many cases, there may be little or no impact of ADAs on study outcome, but significant generation of ADAs may also lead to changes in PK (clearing or sustaining ADAs), altered pharmacology, or adverse safety findings such as hypersensitivity reactions. Although less of an issue than it is for other species used in

http://dx.doi.org/10.1016/B978-0-12-417144-2.00024-X
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

drug development (e.g., rodents), NHPs can still have significant differences in amino acid composition between a biotechnology-derived therapeutic and its endogenous counterpart in the NHP test species, resulting in increased potential for the therapeutic to be recognized as foreign by the NHP immune system. In general, ADA responses to biotechnology-derived therapeutics in NHPs occur earlier, are more sustained, and are of a greater magnitude than those that are typically encountered in clinical settings. Given these differences and the lack of historical concordance with clinical immunogenicity-associated adverse events, the outcome of immune responses in NHP studies is not generally considered predictive of the outcome in clinical settings [1–4]. However, measurement of ADAs in NHP studies is an important study component in cases where unexplained changes in PK or pharmacodynamics (PD) are encountered and in cases where there is evidence of an immune-mediated reaction [3,4]. Although evaluating ADAs in all NHP studies of biologics may not be essential, collecting and saving samples in the event that ADA analyses are needed for appropriate study interpretation and for studies of long-term duration where ADAs are likely and where ADA data may prove essential for study interpretation may be advisable [4].

EFFECT ON PK AND PD

Low-affinity, low titer, or transient ADA responses are often not associated with significant adverse sequelae, and the effect on study interpretation may be minimal [1,2]. However, after B-cell expansion and affinity maturation, the formation of stable drug-ADA immune complexes (ICs) can become sufficient to effect drug PK and PD. In NHPs there are several proposed clearance mechanisms for drug-associated ICs. For ICs that are opsonized with C3b following complement activation, binding to complement receptor 1-expressing erythrocytes can lead to clearance via the mononuclear phagocyte system. This is in contrast to clearance in nonprimate species in which platelets play a more prominent role in complement-mediated removal of ICs [5,6]. Alternative complement-independent pathways involving cellular uptake and lysosomal destruction by FcR-expressing Kupffer cells and liver sinusoidal endothelial cells are also thought to play a prominent role in IC clearance in NHPs [7,8].

Formation of ICs can either increase or decrease drug half-life (sustaining and clearing antibodies, respectively) depending on the rate of clearance for drug-ADA complexes relative to the free drug and depending on IC-mediated changes in biodistribution. For slow clearing drugs such as monoclonal antibodies (mAbs) and fusion proteins, efficient removal of ICs typically enhance drug clearance and often loss of pharmacologic activity is the result. This rarely impacts short-term studies of 1-week duration or less, but maintenance of PK and PD can become a significant issue for longer studies, particularly for lower-dose groups in which loss of PK/PD may be more prominent. For faster clearing drugs such as short lived cytokines, ICs can result in prolonged half-life and, in some cases, unexpected toxicity because of increased exposure. Changes in drug clearance due to ADAs can be both pronounced and rapid necessitating careful consideration of PK sampling time points to ensure adequate understanding of drug disposition.

Following generation of an immune response, loss of PD is most commonly secondary to IC-mediated enhancement of drug clearance. Loss of PD can also result from the formation of neutralizing antibodies (NAbs) (i.e., those capable of directly inhibiting drug activity). NAbs often bind on or near the site of target engagement, thereby inhibiting drug activity by

VetBooks.ir

steric hindrance. ADAs may also impact PD by altering tissue distribution, for example, by reducing the apparent volume of distribution. Having a sensitive PD marker with a sufficient dynamic range is useful to gauge the impact of ADAs on drug activity as well as to understand drug dose-response relationships.

IMPACT ON SAFETY

Hypersensitivity reactions are frequently encountered in NHP studies involving biotechnology-derived therapeutics, and an important consideration for study interpretation is to differentiate ADA-mediated hypersensitivity reactions from other potential causes that may reflect direct target-mediated toxicity of the pharmaceutical candidate. Thus, a sound understanding of the biological pathways targeted by the drug is extremely useful in this regard. Historically, ADA-mediated hypersensitivity in monkeys has not been predictive of ADA-mediated hypersensitivity in clinical trials [1,2]. Typically, the amino acid sequence for biotherapeutics are optimized to replicate human sequences (e.g., replacement therapies, mAbs, fusion proteins) but can differ significantly in sequence from the NHP homolog, resulting in an ADA response to a heterologous protein that is not reflective of anticipated human responses. For example, clinical immunogenicity to humanized or fully human antibodies tends to involve anti-idiotype epitopes (i.e., targeted to the complementarity determining region), whereas in monkeys anti-Fab epitopes predominate [9]. In addition, there are significant differences between humans and NHPs in terms of Fc effector functions, complement pathways, and T-cell activation [10,11]. For most biologics, type I and type III hypersensitivity reactions are the most often encountered preclinically [12]. In cases where de novo induction of an immune response occurs, adverse events usually do not manifest until 10-14 days after initiation of dosing and may not manifest for several months. In rare cases, preexisting antibodies may impact safety after a single dose and evaluation of baseline titers may provide useful information in this event.

Type I Hypersensitivity

As an immune response progresses, isotype switching can lead to production of antidrug IgE antibodies, which have the ability to arm tissue mast cells and blood basophils by Fc-mediated binding to Fcε receptors. Once armed in this fashion, subsequent exposure to antigen (drug) can lead to an immediate type of hypersensitivity reaction involving effector cell degranulation and the release of potent inflammatory mediators such as histamine, leukotrienes, endopeptidases, prostaglandins, and other biologically active constituents. In NHPs, the "early phase" reaction is typified by hypotension, bronchospasm, and vascular leak, which sometimes can result in anaphylactic shock. Because these clinical signs may also be associated with other potential drug-related toxicities (e.g., cytokine release syndrome), it is important to distinguish true IgE-mediated hypersensitivity reactions from other potential causes. In NHPs, type I hypersensitivity reactions to human proteins are usually not manifest for the first few weeks of drug administration, with the rare exception of preexisting antibodies, but it can become acutely manifest (e.g., within 1 h) with subsequent drug administrations. Often both PK abnormalities and ADAs are detectable at the time of onset,

VetBooks.ir

and these data can be used as supporting evidence when IgE-mediated hypersensitivity is suspected. Antigen (drug)-specific IgE and delta tryptase assays have also been developed for cynomolgus monkeys and can be used as supporting evidence that the observed hypersensitivity reaction is downstream of IgE-mediated immunity [13]. However, substantial titers of antigen-specific IgE may be present in the absence of clinical signs of hypersensitivity and should not be considered definitive evidence of type I hypersensitivity. In practice, measuring antigen-specific IgE responses is rarely completed in NHP studies (or clinical studies) because the technical hurdles (discussed later in this chapter) often outweigh the informational value. Total IgE, complement, and circulating ICs have also been utilized as markers of hypersensitivity, although a lack of a strong correlation with clinically confirmed hypersensitivity may limit the utility of these as confirmatory markers. In monkeys, histological abnormalities associated with type I hypersensitivity reactions are commonly lacking but may include perivascular cuffing at injection sites and lymphoid hyperplasia. When anaphylaxis results in acute death, hemorrhage, and edema in and around the respiratory and digestive tract may be prominent [12].

Type III Hypersensitivity

ICs are normally efficiently cleared by the mononuclear phagocyte system and liver; however, under certain conditions, ICs can deposit in tissues, leading to IC-mediated disease (type III hypersensitivity). The size, type, and relative abundance of ICs can greatly influence the propensity to deposit in tissues and thus influence pathologic potential. In general, mild antigen excess relative to ADAs is thought to favor the formation of pathogenic ICs that can deposit in tissues. Renal glomeruli, vascular walls, and serosal tissues are common sites of pathogenesis; however, pathology can occur in other sites. With subcutaneous or intradermal injections, local vasculitis at the injection site (Arthus reaction) can also occur. Once deposited in tissues, ICs are capable of fixing complement with subsequent recruitment of inflammatory cells that can contribute to damage. At localized sites of inflammation, increased immunohistochemical staining for immunoglobulins, complement products, and drug products can be used as evidence of drug-associated IC-mediated disease, and, in cases of renal glomerular involvement, electron microscopy can aid in the confirmation of IC deposition [13].

Immune-Mediated Hematologic Disorders

Drug-induced, immune-mediated thrombocytopenia has been observed with numerous low-molecular-weight drugs, and immune-mediated thrombocytopenia has been associated with drug-dependent antibodies that may involve recognition of epitopes on the drug-target interface, as is hypothesized to be the case with some antagonists targeting glycoprotein IIb/IIIa [14]. This type of reaction can be considered a form of type II (cytotoxic) hypersensitivity. Typically, these responses occur after repeated dosing, but there have been documented cases of preexisting drug-dependent antibodies to platelet glycoprotein IIb/IIIa in rhesus monkeys and chimpanzees that can induce thrombocytopenia with administration of a single dose of a glycoprotein IIb/IIIa inhibitor [15]. Although relatively rarer for biotechnology-derived therapeutics, induction of ADA to abciximab, a large-molecule chimeric FAb fragment

VetBooks.ir

glycoprotein IIb/IIIa inhibitor, has also been associated with development of immune-mediated thrombocytopenia. Similarly, drug-induced immune hemolytic anemia can result from immune responses to drugs that bind red blood cells through antibody-mediated complement activation or through antibody-mediated phagocytosis [16]. When immune-mediated destruction of hematologic cells is suspected, follow-up in vitro studies can help to confirm ADA-associated binding to target cells [17].

Toxicities Due to Reduced Clearance

For very small antigens or in cases where the antigen provides limited binding sites for ADAs, small complexes containing few ADA binding sites may prolong drug half-life, thereby increasing potential for drug-induced toxicity. This has been reported to be the case for insulin ADAs for which high titers against insulin result in not only increased half-life but increased volume of distribution and a greater duration of postdose hypoglycemia [18]. Antibodies against rapidly clearing cytokines such as IL-2, IL-3, IL-4, IL-6, and IL-7 have been shown to increase cytokine half-life and duration of pharmacologic/toxicologic effects [19].

Neutralizing Endogenous Proteins

In cases where therapeutic drugs are designed to replace or augment endogenous proteins, ADA-mediated cross-neutralization of their endogenous counterpart can lead to severe toxicities. For many replacement therapies (e.g., erythropoietin, thrombopoietin, rhFVIII) there is the potential for neutralizing ADAs to not only reduce activity of the exogenously administered therapeutic but to also induce a breakdown of immune tolerance to the endogenous counterpart because of shared antigenic epitopes. During development of recombinant human thrombopoietin (rhTPO), monkeys given rhTPO developed ADA-associated thrombocytopenia that was thought to be due to loss of immune tolerance to endogenous thrombopoietin [20]. As similar ADA-associated thrombocytopenia was later manifest in a clinical setting with rhTPO, adverse events associated with loss of immune tolerance to endogenous proteins in the NHP may be a useful indicator of potential clinical outcomes following generation of NAbs [3,21], informing a clinical safety monitoring program.

Altered Pharmacology

For pharmaceutical proteins that target cellular receptors or signaling proteins that bind receptors, ADAs may have the potential to alter drug pharmacology by impacting receptor crosslinking. For example, the ADA against recombinant human Apo2L/TRAIL in cynomolgus monkeys was shown to amplify ligand-mediated signaling resulting in hepatoxicity. In this case, hepatoxicity was highly correlated with ADAs and in vitro studies were used to identify amplified receptor signaling by Apo2L/TRAIL in the presence of ADAs. In subsequent in vivo studies with a monkey homolog Apo2L/TRAIL (differed from human sequence by four amino acids), the homolog version was shown to be well tolerated [22].

VetBooks.ir

STRATEGIES TO MINIMIZE EFFECT

Evaluation of safety, PK, or pharmacology can be confounded in cases where neutralizing or clearing antibodies reduce or eliminate pharmacologic activity. If significant ADA responses to a pharmaceutical candidate are anticipated, there are study design considerations that may minimize effect. ADA-associated changes in PK and PD can often be minimized by including relatively large doses that can overcome ADA responses either by maintaining an excess of drug relative to ADAs or by inducing drug tolerance. This is often referred to as "dosing through" an ADA response [1].

In repeat-dose experiments, biotechnology-derived therapeutics sometimes can induce hypersensitivity reactions that are potentially life-threatening in NHP species [12]. When anaphylactic responses are encountered or suspected in repeat-dose studies (e.g., hypotension, vomiting, or unconsciousness) and there is a real risk of anaphylaxis with subsequent doses, premedication with antihistamines such as diphenhydramine may ameliorate subsequent hypersensitivity responses; however, this strategy is not always successful. Careful consideration should also be given to the potential for confounding effects, in particular for safety studies in which the concomitant medications may mask adverse reactions that are directly related to drug. In some research settings, immunosuppressive agents such as cyclosporine A have been successfully used to inhibit ADA formation in long-term studies [23]. For preclinical safety studies, however, prophylactic use of immunosuppressive agents to inhibit immunogenic responses is generally discouraged as their use may confound interpretation of safety findings.

In research settings, if ADA responses preclude the conduct of long-term studies, surrogate molecules (i.e., amino acid sequence modified to match the NHP sequence) can be used to study pharmacology in long-term studies. The use of surrogate molecules, however, is generally discouraged for conduct of nonclinical safety studies used for regulatory filings given the inherent difficulties in proving that the biology of the surrogate accurately reflects the properties of the clinical candidate [4]. There have been cases where surrogates have proven useful and predictive in NHP safety studies. Following ADA immune responses, both recombinant human and monkey versions of thrombopoietin were capable of inducing thrombocytopenia in normal monkeys, providing evidence that significant immunogenicity with homologous versions could neutralize endogenous thrombopoietin, and this was subsequently demonstrated in a clinical setting [24].

METHODS TO MEASURE ANTIBODIES AGAINST PROTEIN THERAPEUTICS

Immunogenic responses in NHP studies are not predictive of human responses [2]. Therefore the assessment of immunogenic responses to protein therapeutics in nonclinical studies serves two general purposes: First, its purpose is to provide a potential mechanism for any PK anomalies that may be observed, as previously described in this chapter. Second, when immune-related toxicities are noted, a correlation with ADA presence and titers may provide a mechanistic explanation for the observations. The latter is important because ADA-related toxicities in NHP studies do not necessarily translate to and thus preclude entry into human trials.

VetBooks.ir

It is important to use appropriate methods that will generate reliable data to draw conclusions regarding exposure and safety in NHP studies and that will support recommendations to proceed to clinical trials with a rationale for first-in-human doses based on exposure/safety considerations. An overview of methods, technologies, and general principles of ADA assays is described next.

There are a number of different assays that can be used to detect and characterize an ADA response. Before embarking on a discussion of which assay technologies and formats are available, first understanding which strategy is needed for assessing ADA responses is informative. The general strategy typically used involves a tiered approach to assessing the presence of ADA [25]. This entails the use of a screening assay for detecting the presence of ADAs, a confirmatory (competitive binding) assay to assess the specificity of the response, a titration assay to evaluate the relative quantities of ADA, and neutralizing bioassays to characterize the ability of the ADAs to directly neutralize drug activity. Confirmatory assays are used to eliminate "false positives" designed into the screening assay cut point (see discussion in the following section) and provide evidence of a specific ADA response. But for the purposes of NHP studies, which typically have small numbers of animals, there may not be sufficient value to justify their incorporation into the testing scheme. In addition, if a PD biomarker assay is available, neutralizing assays also may not be necessary as the former can provide evidence of drug neutralization. Further characterization assays such as epitope mapping and isotyping also might be used if a more detailed understanding of the immune response is desired. The needs of the molecule development program determine whether to limit the assay choice to simply detecting (screening assay only) and titering, eschewing more refined characterization. Because of the limited purpose of immunogenicity assessment in nonclinical studies, as noted previously, the use of neutralizing, epitope mapping, or isotyping assays in NHP studies are typically done less often than in clinical studies.

Assay Formats and Technologies

Immunoassays are the most common method available for detecting ADAs, and there are a variety of formats and technologies available within this genre (see Refs. [26,27] for more details). These methods share the same premise: capture of the ADA from serum matrix using some form of the drug as bait, followed by detection using a secondary labeled molecule that binds to the ADA and can be measured by one of a variety of technologies. Two formats are generally used in these assays. The first is a sandwich immunoassay in which the bait (again, the drug) is immobilized on a solid surface, samples potentially containing ADA are incubated with the bait to allow capture, nonspecific proteins (particularly irrelevant immunoglobulins) are washed away, and then captured ADA are incubated with a detection reagent such as an antispecies antibody conjugated to a detectable probe. The second format often used for ADA detection, particularly for mAb therapeutics, is the bridging immunoassay [28], in which the drug serves as both capture and detection reagent. The drug is conjugated to biotin or a detectable probe, and these two conjugates are then combined in a 1:1 ratio and incubated with samples potentially containing ADA. Trimolecular complexes comprised of biotin-drug/ADA/probe-drug are then captured and detected.

The detection technologies used are linked to the nature of the probe conjugated to detection reagent. The enzyme-linked immunosorbent assays (ELISA) most often used is horseradish

VetBooks.ir

peroxidase, and its substrate yield is a colorimetric (or chemiluminesence) signal that can be measured with basic spectrophotometric instrumentation, although alkaline phosphatase or beta-galactosidase have also been used. Electrochemiluminescent probes such as Tris(bipyridine)ruthenium(II) dichloride ($[Ru(bpy)_3]Cl_2$) emit light under an applied voltage and are measured using a mesoscale discovery instrument [29].

Another immunoassay format used for ADA detection is radioimmunoprecipitation (RIP), in which a radiolabeled drug (for proteins this is usually ^{125}I) is incubated with sample potentially containing ADAs, and the complex is then precipitated with beads conjugated with an immunoglobulin-binding moiety (e.g., protein A/G, anti-Ig, etc.). Historically, RIP assays have had sensitivity advantages over other detection methods because of the high specific activities obtained with ^{125}I labeling. However, the improved sensitivity with non-radioisotopic methods, such as those described previously, has diminished this advantage for RIP, and laboratories are increasingly avoiding the use of radioisotopes for these bioanalytical purposes.

Optical biosensors have also been used to detect and characterize ADAs; the two most common are surface plasmon resonance [30,31] and bilayer interferometry [32]. For these methods, ADA binding to the drug immobilized on the reacting surface is detected as a change in optical wavelength proportional to the mass. Although these methods are touted as "label free," the drug is in fact immobilized to an optically reactive surface that in essence acts as the label. True label-free methods, for example, light scattering or calorimetry, are not suitable for detecting interacting molecules in complex matrices such as serum.

All these various methods have their strengths and weaknesses, and the reader is directed to some of the references cited previously for more information. Technical performance in terms of sensitivity to the spectrum of ADA affinities encountered in such polyclonal responses and susceptibility to interference from unlabeled drug contained within samples (for NHP studies this is a particular issue because of the high dose concentrations often used in toxicology studies) are key parameters to consider. Having said that, it is also the case that these assay parameters (sensitivity, drug tolerance), regardless of the format or technology used, are usually evaluated with high-affinity surrogate positive control ADA derived from either drug-vaccinated animal sera or a murine mAbs. The parameters so derived may not reflect the performance of the assay using actual study samples that may contain ADAs of very different, often much lower, binding affinities. Sandwich immunoassays can be very sensitive to high-affinity ADAs and much less sensitive to low-affinity ADAs because of multiple wash steps and incubations; drug tolerance tends to be low relative to the amount of unlabeled drug one might find in a study sample. Bridging immunoassays, especially when complexes are allowed to form in solution as opposed to the solid phase, are very sensitive to high-affinity ADAs and have improved sensitivity to low-affinity ADAs relative to sandwich ELISAs, are much more tolerant to unlabeled drug than sandwich ELISAs, can detect all immunoglobulin isotypes (but also bivalent target molecules leading to false positives), and the same assay can be used with essentially any species. Enzyme-conjugated reagents benefit from being commonly available from multiple vendors and use instrumentation available from multiple vendors. This is not true for electrochemiluminescence-based assays or biosensors that have a single vendor source for instrumentation.

Optical biosensors have modest sensitivity to high-affinity ADAs relative to immunoassays, but they have excellent sensitivity to low-affinity ADAs. They are modestly drug tolerant and require more technical expertise for proper data interpretation relative

VetBooks.ir

to immunoassays. A singular advantage that biosensors have over routine immunoassays is the ability to do various characterizations of ADAs (on/off rates and overall affinity; isotyping). For NHP studies, these advantages may not be valuable since ADA responses tend to be more robust and manifest higher affinities than typically seen in clinical studies.

Assay Thresholds and Sampling

One of the key features of an immunogenicity assay is determination of the assay cut point. This is the decision threshold that determines when a sample-derived assay signal is considered positive or negative for an ADA. The screening assay cut point is statistically determined such that 5% of individual drug-naïve NHP tested using the assay will be considered positive (i.e., the cut point is placed at the 95th percentile of the distribution). This is done to ensure that the assay is sufficiently sensitive and will minimize the number of false-negative results [33,34]. If a confirmatory assay is employed a similar methodology is used to determine its cut point, which often targets a ≤1% false-positive rate [35].

With the method for the assay and cut point determination established, consideration must be given to when samples are taken. For ADA sampling, PK samples should also be taken concurrently so as to provide interpretive context for each result. Baseline samples should always be obtained. Since most NHP studies involve high dose concentrations that can cause interference in ADA detection, it is recommended that sampling occurs before dosing (at PK trough time points), at terminal necropsy, and at the end of the recovery phase. The actual number of ADA samples need not equal the number of doses administered, but it depends on study design, duration, and the immunogenicity risk assessment [25]. Serial sampling of NHPs can be a challenge, and sometimes study designs include satellite cohorts for this purpose. However, this is not optimal because an immunogenic response can vary by individual NHP and therefore interpretation of exposure/toxicity relationships using ADA data from a satellite cohort might be misleading.

There are a couple of technical points worth reiterating so that ADA data are properly interpreted and reported. First, in the absence of drug in a sample, a negative ADA result merely indicates that ADA could not be measured—every assay has its sensitivity limit—not that it is "truly negative." With drug in the sample, this phenomenon is exacerbated, since unlabeled drug will directly interfere in ADA detection. Related to this is the assessment of an assay's sensitivity and drug tolerance. As discussed earlier, both of these assessments are completed with a surrogate positive control and the values obtained from these assessments are dependent upon the affinity of ADA for the drug. Therefore, a negative ADA result measured in the presence of drug must be viewed with caution no matter what the sample drug levels are relative to the validated assay drug tolerance values.

CONCLUSION

Although ADA generation in NHPs is generally not considered predictive of human responses, administration of biopharmaceuticals to NHPs can induce ADAs with a range of effects from no effect to effects on PK, PD, or safety. A number of methods for measuring ADAs

VetBooks.ir

have been developed using various formats and technologies, each with their own strengths and weaknesses, advantages and disadvantages. The choice of method depends on many factors such as the molecular nature of the protein therapeutic, the needs of the molecule development program, as well as more pragmatic factors such as available expertise, time, resources, and cost.

References

[1] Ponce R, Abad L, Amaravadi L, Gelzleichter T, Gore E, Green J, et al. Immunogenicity of biotechnology-derived therapeutics: assessment and interpretation of nonclinical safety studies. Regul Toxicol Pharmacol 2009;54:164–82.
[2] Bugelski PJ, Treacy G. Predictive power of preclinical studies in animals for the immunogenicity of recombinant therapeutic proteins in humans. Curr Opin Mol Ther 2004;6:10–6.
[3] European Medicines Agency (EMEA). Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins. EMEA/CHMP/BMWP/14327/2006, 2007.
[4] International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH): S6 (R1) preclinical safety evaluation of biotechnology-derived pharmaceuticals, 2011.
[5] Male D, Brostoff J, Roth DB, Roitt IM. Immunology. 8th ed. New York: Elsevier, Ltd; 2013, ISBN: 978-0-323-08058-3, p. 405–17 [chapter 25].
[6] Davies KA, Hird V, Stewart S, Sivolapenko GB, Jose P, Epenetos AA, et al. A study of in vivo complex formation and clearance in man. J Immunol 1990;144(12):4613–20.
[7] Rojas JR, Taylor RP, Cunningham MR, Rutkoski TJ, Vennarini J, Jang H, et al. Formation, distribution, and elimination of infliximab and anti-infliximab immune complexes in cynomologus monkeys. J Pharmacol Exp Therap 2005;313(2):578–85.
[8] Jenne CN, Kubes P. Immune surveillance by the liver. Nat Immunol 2013;14(10):996–1006.
[9] Van Meer PJ, Kooijman M, Brinks V, Gispen-deWied CC, Silva-Lima B, Moors EH, et al. Immunogenicity of mAbs in non-human primates during nonclinical safety assessment. MAbs 2013;5(5):810–6.
[10] Warnche M, Calzascia T, Coulot M, Balke N, Touil R, Kolbinger F, et al. Different adaptations of IgG effector function in human and nonhuman primates and implications for therapeutic antibody treatment. J Immunol 2012;188:4405–11.
[11] Kronenberg S. Current challenges and opportunities in nonclinical safety testing of biologics. Drug Discov Today 2013;18(23/24):1138–43.
[12] Leach MW, Rottman JB, Hock B, Finco D, Rojko JL, Beyer JC. Immunogenicity/hypersensitivity of biologics. Toxicol Pathol 2014;42:293–300.
[13] Clark D, Shiota F, Forte C, Narayanan P, Mytych DT, Hock MB. Biomarkers for non-human primate Type-1 hypersensitivity: antigen-specific immunoglobulin E assays. J Immunol Methods 2013;392:29–37.
[14] Cines DB. Glycoprotein GP IIb/IIIa antagonists. Potential induction and detection of drug-dependent antiplatelet antibodies. Am Heart J 1998;135:S152–9.
[15] Bednar B, Cook JJ, Holahan MA, Cunningham ME, Jumes PA, Bednar RA, et al. Fibrinogen receptor antagonist-induced thrombocytopenia in chimpanzee and rhesus monkey associated with preexisting drug-dependent antibodies to platelet glycoprotein IIb/IIIa. Blood 1999;94(2):587–99.
[16] Salama A. Drug-induced immune hemolytic anemia. Expert Opin Drug Saf 2009;8(1):73–9.
[17] Curtis BR, Swyers J, Divgi A, McFarland JG, Aster RH. Thrombocytopenia after second exposure to abciximab is caused by antibodies that recognize abciximab-coated platelets. Blood 2002;99(6):2054–9.
[18] Van Haeften TW, Bolli BB, Dimitriadis GD, Gotesman IS, Horwitz DL, Gerich JE. Effect of insulin antibodies and their kinetic characteristics on plasma free insulin dynamics in patients with diabetes mellitus. Metabolism 1986;35:649–56.
[19] Rehlaender BN, Cho MJ. Antibodies as carrier proteins. Pharmaceut Res 1986;15(11):1652–6.
[20] Hardy L, Rogers B, Thomas D, Ryan A, Peterson M, Koren E. Toxicologist 1997;36:277.
[21] Li J, Yang C, Xia Y, Bertino A, Glaspy J, Roberts M, et al. Thrombocytopenia caused by the development of antibodies to thrombopoietin. Blood 2001;98:3241–8.

[22] de Zafra CL, Ashkenazi A, Darbonne W, Cheu M, Mounho-Zamora BJ, Totpal K, et al. Immune-mediated hepatotoxicity in monkeys following administration of a highly conserved biotherapeutic protein. Toxicologist 2014; (accepted).
[23] Ageyama N, Hanazono Y, Shibata H, Ono F, Nagashima T, Ueda Y, et al. Prevention of immune responses to human erythropoietin in cynomolgus monkeys (*Macaca fascicularis*). J Vet Med Sci 2006;68(5):507–10.
[24] Koren E, Zuckerman LA, Mire-Sluis AR. Immune responses to therapeutic proteins in humans—clinical significance, assessment and prediction. Curr Pharm Biotechnol 2002;3(4):349–60.
[25] Koren E, Smith HW, Shores E, Shankar G, Finco-Kent D, Rup B, et al. Recommendations on risk-based strategies for detection and characterization of antibodies against biotechnology products. J Immunol Methods 2008;333:1–9.
[26] Swanson SJ, Busierre J. Immunogenicity assessment in non-clinical studies. Curr Opin Microbiol 2012;15:337–47.
[27] Mendez A. Strategic selection and development of immunogenicity binding methods. Bioanalysis 2012;4:1491–508.
[28] Qiu JZ, Ying Y, Fox M, Pen K, Lewin-Koh S-C, Coleman D, et al. A novel homogeneous biotin-digoxigenin base assay for the detection of human anti-therapeutic antibodies in autoimmune serum. J Immunol Methods 2010;362:101–11.
[29] Swanson SJ, Jacobs SJ, Mytych D, Shah C, Indelicato SR, Bordens RW. Applications for the new electrochemiluminescent (ECL) and biosensor technologies. Dev Biol Stand 1999;97:135–47.
[30] Jonsson U, Fagerstam L, Ivarsson B, Johnsson B, Karlsson R, Lundh K, et al. Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology. Biotechniques 1991;11:620–7.
[31] Ritter G, Cohen LS, Williams Jr. C, Richards EC, Old LJ, Welt S. Serological analysis of human anti-human antibody responses in colon cancer patients treated with repeated doses of humanized monoclonal antibody A33. Cancer Res 2001;61:6851–9.
[32] Li J, Schantz A, Schwegler M, Shankar G. Detection of low-affinity anti-drug antobodies and improved drug tolerance in immunogenicity testing by Octet® biolayer interferometry. J Pharm Biomed Anal 2011;54:286–94.
[33] Mire-Sluis AR, Barrett YC, Devanarayan V, Koren E, Liu H, Maia M, et al. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. J Immunol Methods 2004;289:1–16.
[34] Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher R, Finco-Kent D, et al. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal 2008;48:1267–81.
[35] Wakshull E, Coleman D. Confirmatory immunogenicity assays. In: Tovey MG, editor. Detection and quantification of antibodies to biopharmaceuticals. Hoboken, NJ: Wiley; 2011.

VetBooks.ir

VetBooks.ir

PART VIII

SPECIAL TOXICITY TESTING WITH NONHUMAN PRIMATES

VetBooks.ir

VetBooks.ir

CHAPTER

25

Developmental and Reproductive Toxicity Testing

Gerhard F. Weinbauer[*], *Christopher J. Bowman*[†], *Wendy G. Halpern*[‡], *Gary J. Chellman*[§]

[*]Covance Laboratories GmbH, Münster, Germany

[†]Pfizer Inc, Groton, CT, USA

[‡]Genentech, South San Francisco, CA, USA

[§]Charles River Laboratories, Reno, NV, USA

OUTLINE

Introduction 472

Which NHP Model? 473

Fertility and Early Embryonic Development Study 474
Study Design Options 475
Sexual Maturity Determination 478

Embryo-Fetal Development 479
Study Design Options 479

Prenatal and Postnatal Development 482
Study Design Options 482

Group Size and "3 R" Considerations 486
Reproductive Toxicity Studies 487
Implications of Social Housing for Evaluation of the Reproductive System 488
Developmental Toxicity Studies 490

Relevance of ADAs 491

Placenta: Target Organ and Transport 491

Data Interpretation Challenges Associated with Background Changes in Pregnant NHPs 493

Conclusion 493

References 496

http://dx.doi.org/10.1016/B978-0-12-417144-2.00025-1
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

Developmental and reproductive toxicity (DART) testing is commonly done using rodents (mice or rats) and rabbits. Nonhuman primate (NHP) models are needed for DART testing when they are the only pharmacologically relevant species or offer critical features not found in other species. For biopharmaceuticals with highly targeted activity, pharmacological relevance is a requirement (ICH S6(R1)) [1]. DART evaluations generally use standardized experimental designs, described in detail in the ICH S5(R2) guideline [2], and comprised of three study types:

- Fertility and early embryonic development (4.1.1)
- Prenatal and postnatal development (PPND) (4.1.2)
- Embryo-fetal development (EFD) (4.1.3)

The segmented approach for DART testing is still in place for pharmaceuticals, whereas this approach has undergone substantial modification for biopharmaceuticals if NHPs are the only relevant species. In that case, a specific EFD study can be omitted, as per ICH M3(R2) [3] and ICH S6(R1), since the placental transfer of large molecules is assumed to be low during first trimester (organogenesis) and evaluation of potential external, visceral, and skeletal developmental abnormalities can be incorporated into the design of the PPND study as a postnatal assessment; this integrated design is known as an enhanced PPND (ePPND) study [4,5] and is described in detail in ICH S6(R1). ICH S6(R1) also acknowledges that functional fertility studies using NHPs are not routine if the NHP is the only pharmacologically relevant species and that reproductive parameters incorporated into chronic repeat-dose toxicity studies using sexually mature animals are appropriate in most instances.

The concept of species selection in general and in the specific context of DART evaluation have been the subject of comprehensive reviews [6,7] and are described in Chaps. 16,34 in this book. Biopharmaceutical development frequently requires the use of NHP models, necessitated by the species specificity of the test article properties [8–12]. Importantly, species selection should be based on the pharmacological relevance of the chosen species rather than a default approach (ICH S6(R1)), and it is not uncommon that NHPs represent the only relevant animal model. As a consequence of more biopharmaceuticals being developed, conducting DART studies using the NHP model is now more common, and NHPs are recognized as an important model for DART evaluation of monoclonal antibodies (mAbs) [13]. For example, in a review of the European Medicines Agency database through 2010, NHPs were used for 23 of 30 mAbs evaluated preclinically in DART studies [14].

In addition to species considerations dictated by test article-specific properties, NHPs generally offer several advantages over rodents and rabbits with regard to DART because of similarity to humans, for example, the endocrinology of testicular and ovarian function, the endocrinology of early pregnancy, placental morphology and physiology, timing of implantation and rates of embryonic development, and similar responses to known human teratogens such as thalidomide, valproic acid, and vitamin A [15,16]. On the other hand, the use of NHPs poses ethical and practical limitations. Default litter size of macaques is 1 compared with 12 and 6 in rodents and rabbits, respectively. Fertility rates in macaques are low (typically around 60% per animal), and preimplantation loss in macaques is high. Therefore, ultrasound-confirmed pregnancy is the preferred approach before starting a developmental toxicity study, with dosing rarely initiated before gestational day (GD) 20 in macaques.

VetBooks.ir

However, implantation occurs at approximately GD 9-10, and therefore the period immediately following implantation is not typically evaluated as it is in rodents and rabbits. Also, study durations of NHP EFD studies overall are three to five times longer than rodent or rabbit EFD studies and are associated with a commensurately high cost factor. For practical reasons, group sizes and number of offspring evaluated in NHP DART studies are substantially smaller compared with rodent/rabbit models [9].

Another point to note is that pregnant NHPs are not available from commercial breeders, so a mating program needs to be in place at the test site. Hence, several weeks to months may be required until all animals are enrolled in the study. Timed matings are achieved by vaginal swab-based monitoring of the ovarian cycle and pairing around the suspected time of ovulation followed by ultrasound examination for pregnancy. Since ovarian cycles cannot be synchronized [17,18], every female animal—once pregnant—is on its own timeline during the remainder of the study, and this adds significant complexity compared to a chronic study (see Figure 25.1 for details).

WHICH NHP MODEL?

Currently, *Macaca fascicularis* (long-tailed macaque, cynomolgus monkey, crab-eating macaque) is the predominant NHP model used for general and reproductive/developmental toxicity studies. In a few cases, only *Macaca mulatta* (rhesus monkey) or *Callithrix jacchus* (common marmoset) were found to be the most relevant animal model [19]. Reproductive physiology and endocrinology of Old World monkeys such as macaques is comparable to humans in many aspects. The cynomolgus monkey is generally sexually active and fertile throughout the entire year, and this species is not considered to express distinct and significant reproductive seasonality. The reproductive physiology and endocrinology of macaques have been extensively studied, and numerous publications demonstrate the relevance of the cynomolgus monkey and rhesus monkey as preclinical models of human reproduction and development. Also, a variety of immunotoxicity parameters have been developed for macaques and are available to incorporate as needed into DART study designs [20,21]. A major challenge with the rhesus monkey is that male and female reproduction is distinctly seasonal to an extent that reproductive functions are entirely shut off or severely diminished for approximately half of the year. It is crucial to consider that the annual rhythmicity of reproductive cycles can persist for years in captivity, despite conditions of controlled, indoor, artificial light patterns.

New World monkeys such as the marmoset (*C. jacchus*) are now well studied in terms of reproductive physiology and endocrinology [22,23]. However, the reproductive physiology and endocrinology of the marmoset is substantially different from that of human and Old World monkeys; among these differences are lack of menstrual bleeding, multiple ovulation (two to four ova per cycle), twin pregnancies as default, common chorion/anastomoses leading to hematopoietic XX/XY chimerism, and absence of luteinizing hormone (LH). The latter seems to be a common feature of New World monkeys [24]. In essence, the pituitary produces and releases chorionic gonadotropin (CG) instead of LH, and CG stimulates gonadal steroid hormone production. Marmosets and tamarins seem to be the only anthropoid primate species that regularly exhibit multiple ovulations per cycle [25]. Because of these substantial differences, the clinical relevance of the marmoset for reproductive toxicity

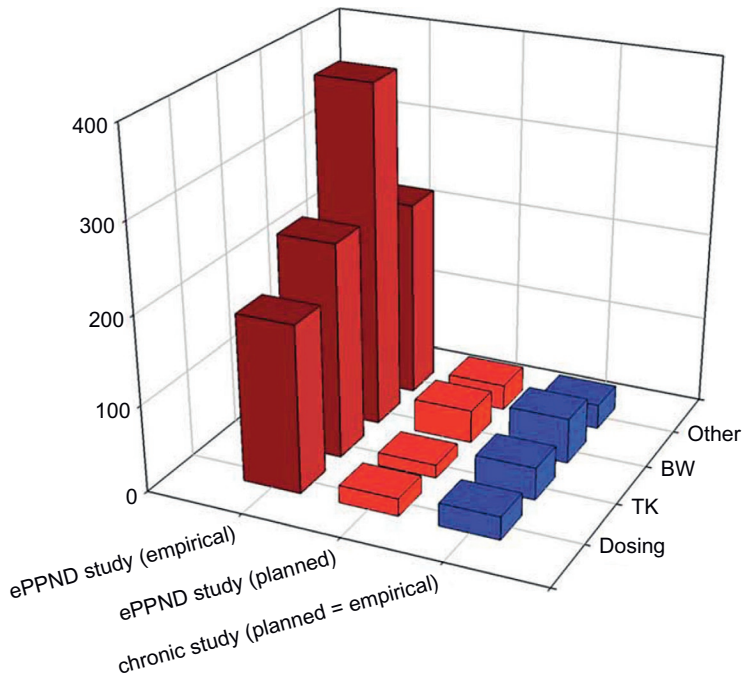


FIGURE 25.1 Graphic representation of the complexity of a nonhuman primate (NHP) developmental toxicity study, exemplified by an enhanced pre- and postnatal development (ePPND) toxicity study, compared with a chronic NHP toxicity study. "Planned" indicates the number of activities based on the study plan, and "empirical" denotes the actual numbers of activities encountered. Because pregnant NHPs are not commercially available, animals must be mated on site to generate pregnant NHPs. Hence, the study plan-based day-by-day activities in a developmental toxicity study depend on the accrual rate of pregnant animals. Empirical versus planned means the number of day-based activities according to the study plan (all animals pregnant) versus the actual entry of pregnant animals into study. In a chronic toxicity study, planned typically matches empirical. The ePPND study comprised 60 pregnant animals with weekly dosing until birth and 45 infants raised for 6 months. The chronic toxicity study comprised 40 animals dosed for 26 weeks and 10 animals followed for a 26-week treatment-free period. TK, toxicokinetic samples; BW, body weight collection; other, any other study parameter. *Reproduced from Weinbauer et al. [5] with permission.*

assessment has been questioned, and unless there is no other choice, using this species for DART evaluation is not recommended [26]. If there is no other choice, however, there is precedence and limited historical data available for all three experimental designs of the standard DART study types in the marmoset.

FERTILITY AND EARLY EMBRYONIC DEVELOPMENT STUDY

As described under 4.1.1. of ICH S5(R2), fertility and early embryonic development studies are designed to identify toxic effects resulting from exposure to the test article starting from before mating (including the development and maturation of both male and female gametes) and continuing through conception and implantation. These studies also

VetBooks.ir

assess potential changes in female cyclicity and possible functional effects (e.g., mating behavior) that may not have been detected by standard histological examinations of the reproductive organs in general toxicity testing. Per ICH M3(R2), evaluation of fertility is typically required before initiating phase III trials and/or conducted in parallel with phase II trials.

Whereas the guidelines recommend physical mating for evaluation of fertility, this is not being done routinely in NHPs for two main reasons: (1) Typically, fertility rates in macaques are clearly <100% (typically 25-45% per cycle and approximately 60% per animal, preimplantation losses are around 25%) [27], and prenatal losses can be as high as 40% [9,28] and (2) While evaluation of implantation sites is a hallmark of rodent fertility studies, this normal fertility end point is not meaningful in macaques, in which there is normally a single implantation site that is then often lost shortly after implantation. Twin pregnancies/births are extremely rare in macaques, with an overall incidence of twin live births around 0.1% [29,30]. Consistent with low fertility rates in NHPs, it is also stated in ICH S5(R2) that mating is an insensitive means to detect effects on spermatogenesis. Previous requests for physical mating in NHP fertility studies may be related to differential interpretation of ICH guidelines [31]. In general, testing fertility in NHPs via physical mating is considered impractical and rather insensitive and is therefore not recommended; however, there may be situations, such as when the test article is expected to specifically affect mating behavior or implantation, when inclusion of a mating trial may be appropriate.

Study Design Options (Table 25.1)

Since functional fertility is rarely evaluated using NHPs, a comprehensive evaluation of potential effects on the male and female NHP reproductive systems can use surrogate clinical end points used by gynecologists and andrologists for diagnosis of infertility, in addition to macroscopic and microscopic pathology. A comprehensive description of the reproductive system of the female cynomolgus monkey was reported earlier [32]. In the absence of a mating end point, fertility can be assessed in a targeted male or female reproduction study, or specific reproductive end points can be incorporated into the design of a subchronic or chronic toxicity study. When NHPs are the only relevant species and thus a rodent fertility study is inappropriate, the potential for effects on male or female reproduction can be assessed in repeat-dose toxicity studies of at least 3 months' duration, provided that sexually mature animals are used (see Section "Sexual Maturity Determination" for details). In such cases, analysis of reproductive organ weights and histopathological evaluation are considered sufficient and sensitive for the detection of reproductive toxicity (ICH S6(R1)). If there is a specific cause of concern about reproductive toxicity (e.g., findings in previous repeat-dose toxicity studies, target biology), more detailed investigation using more sophisticated parameters (e.g., testicular volume determination, semen analysis, and spermatogenesis staging, female cycle monitoring, and/or reproductive hormone evaluation) may be warranted. A weight-of-evidence approach including all available data is recommended, but the judgement of whether there is a true cause for concern can be challenging also.

With regard to the duration of targeted studies assessing the male reproductive system, periods of 9-13 weeks have been used. In cynomolgus monkeys, approximately 40 days are required to convert early spermatogonia into fully developed testicular spermatozoa, and the

TABLE 25.1 Study Design Elements for Assessment of Nonhuman Primate Reproductive Toxicity

Parameter	Detail	Note
Important elements[a]		
Age (years)	Males: ≥4 Females: ≥3	Confirmed sexual maturity: males, presence of sperm; females, cycling (2 menstrual bleedings of at least 2 days' duration and at least 20 days apart)
Animals per group and sex (*n*)	3-5	Based on necropsy of three animals per sex at the end of the dosing phase and allowing for two animals per group for assessment of reversibility of any effects
Groups (*n*)	3 or 4	Standard design (e.g., small molecule) is control group and three dose groups (treated with the test article) For biopharmaceuticals, two dose groups can be sufficient Dose selection is usually based on data from general toxicity (nonpregnant) studies
Duration of dosing (months)	≥3 months	Per ICH S6(R1)
Clinical signs	Twice daily	
Body weight	Weekly	
Food consumption	Daily	Qualitative in social housing (noting when less than approximately half of the rations are consumed)
Clinical pathology	—	Hematology, serum chemistry, coagulation parameters, and/or urinalysis
TK/ADA	First and last dose (minimum)	Samples for TK are primarily collected and analyzed to confirm expected exposures. For biologics, blood also usually collected for ADAs to help determine whether the test article is immunogenic and to consider potential impact on TK
Necropsy	At end of dosing and recovery phases	Should include gross observations and organ weights of reproductive and endocrine tissues
Histopathology	Necropsy	Standard tissue list, including reproductive tissues (male and female); end points should be included that ensure confirmation of sexual maturity
Targeted study design[b]		
Optional endpoints for males	Semen evaluation	Semen can be collected via electro stimulation at baseline and periodically during the study for repeated assessments from individual animals; alternatively, a sperm sample can be collected from the cauda epididymis at necropsy. End points for evaluation include semen volume (if ejaculate), sperm count, motility, and/or morphology

TABLE 25.1 Study Design Elements for Assessment of Nonhuman Primate Reproductive Toxicity—Cont'd

Parameter	Detail	Note
	Testis volume or size	May be measured serially using ultrasound/calipers/orchidometer
	Serum hormone concentrations	Testosterone, FSH, LH, and/or inhibin B
	Histopathology	Staging of spermatogenesis to identify spermatogenic phases (e.g., maturation, release, elongation, and meiosis) and assess cellularity, cell associations, and cell-specific changes
Optional end points for females	Menstrual cycling	Vaginal swabs can be used to monitor menstrual bleeding and determine cycle duration before the study and during the dosing and recovery phases
	Ultrasound	Changes in uterine size and relative endometrial thickness may be visualized to track cycle progression
	Serum hormone concentrations	Cyclic fluctuations in estradiol and progesterone can be tracked via blood sample collections two to three times per week for 5-6 weeks to cover a full cycle (before the study, end of dosing, end of recovery). Sampling every other day is required to detect hormone peaks. Allows confirmation of ovulation, in conjunction with cycling data, but may be difficult to incorporate into a standard toxicity study because of the frequency of sampling
	Histopathology	Detailed evaluation of reproductive tissues beyond standard histopathology may be added (e.g., concerted evaluation of all reproductive and endocrine axis tissues or step sectioning of ovaries for formal staging)

ADA, antidrug antibody; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TK, toxicokinetics.

[a]*These elements are used in a standard chronic toxicity study with male and female reproductive assessment (per ICH S6(R1), the default reproductive evaluation is based on organ weights and histopathology.*

[b]*These end points are options if cause for reproductive concern triggers a stand-alone study for more detailed assessment of male and/or female reproduction, in addition to the end points listed above; most of these end points may also be incorporated into a chronic toxicity study in mature animals, as applicable.*

ultimate duration of a male fertility study should be related to that time period plus approximately 5 days to account for sperm transport time through the rest of the male reproductive tract [33,34]. For evaluation of the female reproductive system, either two or three ovarian cycles are typically followed during the dosing phase. Importantly, in case of compounds with pronounced antigonadotropic activity, a 4-week study duration can be sufficient to detect effects on ovarian cyclicity and endocrine profiles in females, and for compounds with substantial effects on the testis a shorter follow-up study can be used to better understand the mechanism of toxicity in males.

VetBooks.ir

Another critical component in the design of male and female reproductive toxicity studies is the duration of the recovery phase to assess the reversibility of any effects identified during the treatment phase. For males, a minimum of two full spermatogenic cycles is recommended to assess reversibility, and the clearance time of the test article must also be considered, so the recovery phase may end up being several months in duration. Likewise, once regular cycling has been disrupted in females, it may take several cycles, translating to several months, for the animals to return to normal cycling. Therefore, when conducting such a study, or when effects on reproductive tissues are expected, planning for a recovery phase of adequate duration to assess reversibility is important.

Sexual Maturity Determination

Conducting studies with reproductive end points requires the use of sexually mature NHPs. Moreover, the ICH S6(R1) guideline encourages the use of sexually mature NHPs under certain circumstances during general safety assessment studies. Therefore, based on the increase in biopharmaceuticals that are pharmacologically active in only NHPs, it is not surprising that there has been an increase in the use of sexually mature NHPs. Unlike rodents, primates undergo a prolonged postnatal period of reproductive axis quiescence followed by the resumption of activity of the brain-gonadal axis [35], triggering puberty and ultimately resulting in sexual maturity and fertility. Unfortunately, in primates the sexual maturation process shows enormous individual variability both in terms of the initiation age as well as duration over months and years. Sexual maturity defines a point of maturation where gametogenesis is complete and the animal is potentially fertile. This point in development must not be confused with adulthood, which is determined by epiphyseal closure, and typically the long bones (e.g., tibia, femur) stop growing.

The long duration and enormous interanimal variability of the sexual maturation process necessitate firm and reliable criteria to assess in vivo the sexual maturity of animals to be assigned to safety assessment and toxicological studies in which there is to be any functional evaluation of the reproductive system.

Recognition of sexual maturity, indicated by regular cycling, is fairly easily achieved in female macaques by collecting (daily) vaginal swabs for identification of menstrual bleeding. Ample literature on this approach is available [9,17,18]; in practice, one common approach is to document at least 2 days of bleeding on at least two occasions that are at least 20 days apart. In contrast, assessment of sexual maturity in male animals can be less obvious, and the literature here is even conflicting. To ensure that an animal has a 90% probability of sexual maturity, body weight should be at least 5.3 kg and age at least 5 years, 5 months according to Smedley et al. [36]. According to Lawrence and Saladino [37], a 90% probability is associated with body weights of 4.52 kg or heavier. Testicular volumes of 10-20 mL were compatible with sexual maturity [38], whereas in other studies determining testis size did not prove useful for predicting sexual maturity [36,39]. The presence of sperm in a semen sample was more recently indicative of complete spermatogenesis and, therefore, is considered proof of sexual maturity [40], independent of the imperfect criteria based on age, body weight, or testis volume. Hence the use of sperm presence can be considered a reliable functional parameter of testicular maturity in primates. Spermarche is also now considered a hallmark of pubertal maturation in boys [41].

VetBooks.ir

In addition to in vivo determination of sexual maturity before or during a study, the gold standard is still microscopic evaluation. Historically, documenting maturity only by exclusion has been common practice; in other words, only immaturity was recorded and reported, and often only in males, with the implication that all other animals were mature. In males, the histology is relatively straightforward and can be readily categorized as no spermatogenesis (immature), incomplete spermatogenesis (peripubertal), or complete spermatogenesis (sexually mature). Histologic confirmation of sexual maturity in females is best achieved by evaluating several reproductive tissues, including at a minimum mammary gland, ovaries, and uterus/endometrium in the context of known age and weight. Actively cycling females should have well-lobulated mammary glands and often have evidence of multiple corpora lutea, some involution, and/or evidence of progesterone influence on the endometrium. However, in the earliest follicular/proliferative stage of the cycle, mature females cannot always be differentiated from pubertal or irregularly cycling females. Finally, macaques typically do not cycle synchronously even when cohoused. Thus if all females in a dose group or study are in the same phase of their cycle at the time of necropsy, it may represent a test article effect on cycle progression. With the increase in biopharmaceuticals that are active only in NHPs, microscopically confirming the maturity status of these animals has become even more important to provide better confidence in the assessment of potential toxicities in male and female reproductive tract tissues.

EMBRYO-FETAL DEVELOPMENT

EFD studies are conducted to detect adverse effects on the pregnant female and the development of the embryo and fetus. Basically, the critical period of organogenesis, between implantation and closure of the hard palate, is being investigated to reveal the potential for developmental toxicity (e.g., effects on embryo-fetal survival, growth, and morphology). In NHP models, however, pregnancy is not diagnosed until GDs 18-20. Thus a somewhat shortened period between implantation (approximately GD 9 or 10) and closure of the hard palate (close to GD 50 in macaques) is covered. Animals are then dosed throughout the remainder of organogenesis (GDs 20-50) and the fetus is removed by cesarean delivery for examination on GD 100±1 to obtain fetuses of practical size for examination. Ultrasound diagnosis of pregnancy can result in false-positive signals; animals will be enrolled into studies but—because they are not pregnant—an abortion will subsequently be diagnosed. Collecting a blood sample on GD 20 for analysis of monkey chorionic gonadotropin (mCG) concentrations is advantageous. This endocrine verification of pregnancy at the time of initiation of dosing helps eliminate both false-positive pregnancy diagnoses and misdiagnosed abortions.

Study Design Options (Table 25.2)

Although the test article is normally administered during GDs 20-50 followed by cesarean delivery on GD 100±1, this is occasionally extended to GD 140 or GD 150 if specific analysis of larger fetuses is needed or if an organ system of particular interest is still developing later in gestation (e.g., the immune system). Parameters to be studied include maternal clinical signs, body weight, food consumption, ultrasounds, and toxicokinetic/antidrug antibody

TABLE 25.2 Sample Design of a Nonhuman Primate Embryo-Fetal Developmental Toxicity Study

Parameter	Detail	Note
Maternal		
Age	≥3 years	Females: sexual maturity (menstrual cyclicity) reached at 3-6 years of age Males: sexual maturity (presence of sperm) reached at 4-7 years of age (used for mating only)
Animals per group	12-14 pregnancies	Based on typical abortion rate of 15-20% over the entire duration of gestation, assignment of 12-14 pregnancies per group should be adequate in most cases to obtain at least 10 viable fetuses per group at cesarean delivery; statistical estimates of pre- and postnatal survival are available [28]
Number of groups	3 or 4	Standard design (e.g., small molecule) is control group and three dose groups (treated with the test article) For biopharmaceuticals two dose groups can be sufficient Dose selection is usually based on data from general toxicity (nonpregnant) studies, but a dose-ranging study of pregnant females may be conducted (e.g., $n = 5$ per group)
Duration of dosing	GD 20-50 or later	The standard protocol has been to dose from GD 20 to 50 to encompass major organogenesis and perform cesarean delivery on GD 100 for fetal evaluation. GD 140 or GD 150 is suggested as the maximum practical time point for termination of dosing and cesarean delivery to avoid increasing probability (and confounding factor) of premature births and stillbirths after this interval
Clinical signs	Twice daily	
Body weight	Weekly	
Food consumption	Daily	Qualitative in social housing (noting when less than approximately half of the rations are consumed)
Vaginal swabs	Daily	Can be used to assist in detecting implantation bleeding and/or abortion
Ultrasounds	Every 2 weeks	Conduct throughout gestation until scheduled cesarean delivery to confirm fetal viability
TK/ADA	First and last dose (minimum)	Maternal TK allows calculation of maternal-to-fetal ratios of exposure and may help determine whether TK in pregnant females is different from that determined in nonpregnant animals in general toxicity studies For biologics, blood also usually collected for ADAs to help determine whether the test article is immunogenic and to consider potential impact on TK
Cesarean delivery	GD 100 or later	GD 140 or GD 150 suggested as maximum practical for termination of dosing and cesarean delivery to avoid increasing probability (and confounding factor) of premature births and stillbirths Evaluations immediately after cesarean delivery include evaluations of amniotic fluid (volume, clarity); placenta (weight, number of disks, disk(s) diameter, appearance of maternal and fetal surfaces, location of cord insertion); and the umbilical cord (length)

TABLE 25.2 Sample Design of a Nonhuman Primate Embryo-Fetal Developmental Toxicity Study—Cont'd

Parameter	Detail	Note
Optional end points	Fetal measures	Detailed fetal measurements can be taken at ultrasound evaluations to assess fetal growth/development
	mCG	Blood sample taken on GDs 20-22 can be collected and used to confirm pregnancy in the event of apparent abortions
	Immunology	Maternal immunologic evaluations, e.g., immunoglobulin concentrations, flow cytometry (immunophenotyping)
	Clinical pathology	Clinical pathology can include hematology, serum chemistry, coagulation parameters and/or urinalysis (e.g., documentation of expected maternal toxicity, confirmation of expected PD effect)
Fetal		
External		External examination of fetus includes fetal weight and morphometric measurements (e.g., crown-to-rump length, head circumference, long-bone length, anogenital distance)
Visceral		Visceral examination of fetus for internal organ morphology can also include weighing selected tissues/organs. Selected fetal tissues may also be evaluated by histopathology and/or immunohistopathology
Skeletal		Between GD 100 and 140/150 fetuses can be processed and stained with Alizarin red for skeletal evaluation (or evaluated by radiography for late-gestation fetuses)
TK/ADA		Umbilical cord blood collected for TK evaluations. For biologics, blood also usually collected for ADA. Concentrations of the test article in amniotic fluid can also be determined
Optional end points	Histopathology	Since the number of NHP fetuses available for gross visceral examinations is relatively small, histopathology of major organs or target organs can also be included Evidence of histologic effects may be of help in the consideration of a mechanism of abnormal development of some organs or tissues
	Immunology	Evaluations of the fetal immune system can include flow cytometry (limited by blood volume), lymphoid organ weights (mainly spleen because of the small size of fetuses), histopathology, and/or immunohistopathology
	Clinical pathology	Fetal blood can be collected by cardiac puncture but this is not encouraged

ADA, antidrug antibody; GD, gestation day; mCG, monkey chorionic gonadotropin; NHP, nonhuman primate; PD, pharmacodynamic; TK, toxicokinetics.

(ADA) samples. Studies determining dose ranges are typically not conducted using NHP models, which rely on dose information from nonpregnant animal studies. This is in contrast to rats and rabbits, for which a range-finder study often precedes the main EFD study.

Fetal evaluation comprises external, visceral, and skeletal examination [42]. Regular ultrasound inspection is used to monitor and confirm ongoing pregnancies. Ultrasound analysis

VetBooks.ir

is sometimes also used for fetal morphometry and fetal growth evaluation. At the time of cesarean delivery, maternal and fetal blood are typically collected for toxicokinetic/ADA analysis and comparison of the fetal-to-maternal test article concentration ratio. It is important to recognize that the transfer of large molecules (e.g., immunoglobulins [Igs]) across the placenta and relative to gestational duration seems to be comparable between humans and cynomolgus monkeys based on available data and occurs mainly during the second and third trimesters of pregnancy [43–46]. This has significant implications for this developmental toxicity study design; in the case of IgG-based mAbs, dosing only throughout the period of organogenesis (GDs 20-50) may not result in significant embryo-fetal exposure. This has previously been taken into account by extending the dosing phase from GD 20 until GD 120 or later. Since ICH S6(R1) has been released, however, an ePPND study is now being conducted in most cases.

PRENATAL AND POSTNATAL DEVELOPMENT

PPND studies are designed to detect adverse effects on the conceptus and the offspring following exposure of the female from implantation through weaning. In the offspring, functional parameters such as behavior, maturation throughout puberty, and fertility are also tested. For the latter, the F1 generation is mated to produce the F2 generation. Different from rodents and for feasibility considerations, dosing is not initiated until confirmation of pregnancy (as described earlier), the F1 infants are not observed throughout puberty and are not mated for production of F2 in the NHP models. If F1 and F2 generations of the cynomolgus monkey would be produced according to the prevailing guidelines, such studies would require an estimated 5-8 year duration and hence are obviously not practical. Also different from rodents is that dosing of NHPs during lactation is generally not recommended (ICH S6(R1)).

Study Design Options (Table 25.3)

Selection and enrollment of animals follow the same procedures used in an EFD study and dosing commences on GD 20 but then is extended either until termination of the pregnancy/delivery or (much less frequently or rarely) is terminated close to delivery to yield a comparable exposure duration among all animals. Duration of pregnancy in cynomolgus monkeys is around 160 days. For pharmaceuticals, maternal dosing may be continued after delivery to evaluate the transfer of the test article to the newborn via breast milk. For therapeutic antibodies, however, transfer into milk is very low and any potential contribution to systemic exposure of the infant is limited due to intestinal proteases in the infant and is not considered predictive of human infant exposure [12,47]. In fact, ICH S6(R1) does not recommend maternal dosing of biopharmaceuticals after birth.

With certain biopharmaceuticals, for example, mAbs, an EFD study can be omitted and instead an ePPND study design can be adopted. This approach is supported by ICH M3(R2) and recommended by ICH S6(R1). An ePPND study is basically a PPND study that is enhanced by incorporating "embryo-fetal" parameters into infant evaluation. This study essentially combines all the components of the EFD and PPND studies into a single study with the exception that fetal examinations are replaced by an external evaluation of infants at birth, a radiographic/computed tomographic evaluation of the infant skeleton, and a visceral

VetBooks.ir

TABLE 25.3 Example Design of a Nonhuman Primate (Enhanced) Pre- and Postnatal Developmental Toxicity (ePPND) Study

Parameter	Detail	Note
Maternal		
Age	≥3 years	Females: sexual maturity (menstrual cyclicity) reached at 3-6 years of age Males: sexual maturity (presence of sperm) reached at 4-7 years of age (used for mating only)
Animals per group	16-20 pregnancies	Based on typical combined prenatal and perinatal loss rates (includes stillbirths and infant mortality) to obtain approximately 10-16 viable infants per group. The current ICH S6 revision considers a minimum of six to eight live infants at postnatal day 7; statistical estimates of pre- and postnatal survival are available [28]
Number of groups	3-4	Standard design is control group and two to three dose groups (treated with the test article) For biopharmaceuticals, two dose groups can be sufficient
Duration of dosing	GD 20-140 or until term	The standard protocol has been to dose from GD 20 to term To have equal duration of test article administration for all pregnant females the dosing can end at the same day of gestation (e.g., GD 140)
Clinical signs	Twice daily	
Body weight	Weekly	During gestation, at birth, and weekly or monthly thereafter during postpartum period
Food consumption	Daily until delivery	Qualitative in social housing (noting when less than approximately half of the rations are consumed)
Vaginal swabs	Daily	Can be used to assist in detecting implantation bleeding and/or abortion
Ultrasounds	Approx. every 2 weeks	Conduct throughout gestation to confirm fetal viability
TK/ADA	First and last dose (minimum)	Maternal TK allows calculation of maternal-to-infant ratios of exposure and may help determine whether TK in pregnant females is different from that determined in nonpregnant animals in general toxicity studies For biologics, blood also usually collected for ADAs to help determine whether the test article is immunogenic and to consider potential impact on TK
Optional end points	Milk collection	Month 1 (e.g., day 28) postpartum and/or later. Milk samples can be collected up to approximately 1 mL volume

(*Continued*)

VetBooks.ir

TABLE 25.3 Example Design of a Nonhuman Primate (Enhanced) Pre- and Postnatal Developmental Toxicity (ePPND) Study—Cont'd

Parameter	Detail	Note
	Clinical pathology	One or more intervals during gestation and postpartum
	Immunology	Immunophenotyping (lymphocyte subsets), TDAR, NK cell activity, lymphocyte proliferation, immunoglobulin concentrations
	Fetal measures	Detailed fetal measurements can be taken at ultrasound evaluations to assess fetal growth/development
	Placental collection	As available or in a cohort of females; may include histopathology
	Necropsy	Not standard; may possibly be warranted by unique circumstances and include standard pathology end points (e.g., organ weights, histopathology)
Infant		
Gender determination	Day 1 postpartum	
Duration of postnatal observation period	Variable, up to 12 months postpartum	Duration of the infant observation period is variable, but can range from 7 to >700 days postpartum (6-12 months is most common)
Clinical signs	Twice daily	
Body weight	Weekly or monthly	
Food consumption	Not feasible	Infant consumes milk
Morphometry	At birth and every 1-3 months thereafter	External examination includes morphometric measurements (e.g., crown-to-rump length, head circumference, long-bone length, anogenital distance)
Neurobehavioral test battery	Days 1 and 7 postpartum	A comprehensive test battery for behavioral and functional development is based on the Brazleton-based human neonatal behavioral assessment scale
External evaluation	At birth	Similar to procedures for fetuses in EFD design
Visceral evaluation	At necropsy	Similar to procedures for fetuses in EFD design
Skeletal evaluation	Month 1 postpartum and/or later	Skeletal evaluation done by digital radiography (≥28 days postpartum). Later evaluations (≥3-4 months postpartum) can be used to assess ossification
TK/ADA	Monthly	Blood collected for TK evaluations. For biologics, blood also usually collected for ADA

VetBooks.ir

TABLE 25.3 Example Design of a Nonhuman Primate (Enhanced) Pre- and Postnatal Developmental Toxicity (ePPND) Study—Cont'd

Parameter	Detail	Note
Optional end points	Ophthalmology	Ophthalmologic evaluation can be done at about 3 months of age and comprises macroscopy, direct and indirect ophthalmoscopy, and slit lamp examinations
	Cardiovascular	Cardiovascular evaluation including ECGs can be initiated at 5-6 weeks of age
	Bone development	For monitoring of bone development, radiography, and DEXA can be used to determine bone age and length
	Body composition	DEXA evaluation can provide determination of whole body mass and the body composition separated into bone, muscle, and fat mass
	Bone evaluations	Quantitative evaluation of bone density and/or mineral content by DEXA and/or pQCT
	Grip strength	Grip strength evaluates the ability of the infant to hold on to a horizontal bar for at least 30 seconds (e.g., day 28 postpartum)
	Clinical pathology	At necropsy and/or intervals postpartum
	Learning and memory ability	A learning ability test based on the double choice object discrimination tasks using the Wisconsin General Testing Apparatus (6 weeks training needed). Typically conducted 6-9 months postpartum to accommodate training required for the animals
	Mother-infant interaction	Mother-infant interaction can be assessed in a qualitative or quantitative manner (e.g., the latter via a video recording, which is evaluated quantitatively using a special computer program, e.g., at 3 months postpartum)
	Immunology	Evaluations of the immune system can include flow cytometry (lymphocyte subsets), TDAR, NK cell activity, lymphocyte proliferation, immunoglobulin concentrations, and, at necropsy, lymphoid organ weights, histopathology, and/or immunohistochemistry
	Histopathology	Histopathology of major organs or target organs can also be included Evidence of histologic effects may be of help in the consideration of a mechanism of abnormal development of some organs or tissues

ADA, antidrug antibody; DEXA, dual energy X-ray absorptiometry; ECG, electrocardiography; EFD, embryo-fetal development; GD, gestational day; NK, natural killer cells; pQCT, peripheral quantitative computed tomography; TDAR, T-cell-dependent antibody response; TK, toxicokinetic.

VetBooks.ir

examination at infant necropsy (Table 25.3). The only potential information that could be lost is if there is a developmental delay during pregnancy that is not evident at birth or a direct effect on the placental functions. Slight developmental delays that occur *in utero* or altered placental functions that do not translate to abnormalities in the infants are unlikely to be of clinical concern. Therefore, there is no strong rationale that fetal examinations are critical for NHP developmental toxicity safety assessment. Fetal viability can be monitored throughout gestation by ultrasound examination. The ultrasound examination can also measure fetal growth and heart beat but, according to ICH S6(R1), fetal measurements are not required because the primary study end points are the newborn and its development. On the other hand, in our experience, fetal measurements continue to be included in some ePPND studies.

A large variety of parameters for the evaluation of infant development is available [5,9]. Recommended end points for an ePPND study are offspring viability and survival, neurobehavioral test battery, external malformations, skeletal effects (e.g., by radiography), and, ultimately, visceral morphology at necropsy. Histopathology can be included in NHP developmental studies to further understand potential effects on development. In addition, other end points in the offspring can be evaluated if they are relevant for the pharmacological activity (e.g., immune function or detailed bone evaluation). The duration of the postnatal phase is dependent on the additional end points considered relevant for the pharmacological activity and the clinical indication. In general, a large array of parameters is available for postnatal infant evaluation comprising both general and specific parameters.

For evaluation of immune system development, a postnatal period of 6 months is most commonly used to enable appropriate evaluation, for example, T-cell-dependent antibody response (TDAR), natural killer cell activity test, lymphocyte proliferation test, and/or immunophenotyping. In the case of TDAR, a response can be elicited from the age of 1-3 months. In some studies TDAR has been conducted as early as 1 month postpartum with acceptable results; however, far less historical data are available compared with testing at ≥3 months of age. ICH S6(R1) provides specific recommendations for the duration of the postnatal period relative to the development of the target organ system. For learning and memory testing (e.g., double-object discrimination test), a postnatal observation period of 9-12 months seems essential since these tests can only by applied from the age of 6 months onward. According to ICH S6(R1), a postnatal observation period of 6 months is generally sufficient, thus eliminating the need for this learning and memory test. This test is considered to be highly variable and associated with poor statistical power in rats and NHPs [48], but contrary views also have been expressed [49]. In our experience, the learning and memory test is occasionally still required by authorities and, in this case, the postnatal period needs to be extended to 9-12 months. A comprehensive test battery for behavioral and functional development is also available for the cynomolgus monkey infant and widely applied. Many of these tests are based on the Brazleton-based human neonatal behavioral assessment scale, have been modified and adopted for use in NHPs, and are usually applied within the first few days after birth [5,9].

GROUP SIZE AND "3 R" CONSIDERATIONS

The principles governing the number of animals in DART studies are described in international guidelines. According to the ICH S5(R2) guideline "Detection of Toxicity to

VetBooks.ir

Reproduction for Medicinal Products & Toxicity to Male Fertility," the number of animals per sex per group should be sufficient to allow meaningful interpretation of the data. Note 13 includes the following: "Ideally, at least the same group size should be required for all species and there is a case for using larger group sizes for less frequently used species such as primates" and "For all but the rarest events, evaluation of between 16 to 20 litters for rodents and rabbits tend to provide a degree of consistency between studies." Per ICH S6(R1), developmental toxicity studies using NHPs should aim at having a minimum of six to eight live infants on postnatal day 7 to provide a sufficient number to assess postnatal development and provide for specialty evaluation(s) (e.g., the immune system) if necessary.

Reproductive Toxicity Studies

With regard to targeted reproductive toxicity evaluation in mature NHPs, use of five to eight animals per group (with groups of eight including three animals for the recovery groups) has been recommended [9]. Since these targeted studies use only one sex, this group size is somewhat comparable to a combined group size using three or four animals per sex and group plus recovery group animals in a general toxicity study. Compared with groups in rodent fertility studies, these group sizes are comparatively low and may present a challenge with regard to the statistical power of comparisons between vehicle-exposed and test article-exposed groups. Unlike rodents, however, baseline, in-life, and recovery data are available for certain parameters (e.g., hormones, testis size, semen parameters in males and menstrual cyclicity in females), and comparisons within the same animals and between the groups aid in the interpretation of data. There have been recent attempts to address the statistical power in NHP studies targeting reproductive parameters. Conventionally, a statistical power of 0.80 (beta level) is used as the cutoff and at a significance level of $\alpha = 0.05$. One study investigated menstrual cycle duration as reproductive end point in a 6-month toxicity evaluation in cynomolgus monkeys [18]. In that study, one-sided statistical analysis was used because the potential treatment effect was assumed to induce a prolongation of the cycle duration. It was reported that at a cycle duration coefficient of variation of 20-25%, group sizes of 6, 8, and 12 would render 1.5-, 1.4-, and 1.3-fold differences, respectively, as being statistically significant. It is clear that ovarian cycle variability is a key factor in such evaluations since cycle irregularities can occur in individual animals at any time; however, these observations are encouraging in that group sizes of six to eight permit the detection of effects on a selected female reproductive toxicity end point.

Another study addressed the sensitivity of male reproductive toxicity end points in sexually mature cynomolgus monkeys [50]. The power to detect a 50% change from control was assessed at $\beta = 0.80$ and $\alpha = 0.05$ using a two-sided test. Overall, statistical power (target 80%) was rather low, with 13-30% for male reproductive organ weights, approximately 30% for testicular volume, 6-66% for seminal analyses, and 10-78% for reproductive hormones for a group size of three animals. At group sizes of six to eight animals, statistical power was achieved for testis weight and volume (Figure 25.2), sperm morphology and motility, and follicle-stimulating hormone. The addition of nonroutine end points in cases where there is reproductive cause for concern is unlikely to increase sensitivity to detect adverse effects. An important point that to our knowledge has not been addressed so far is the potential for increased statistical power using repeated measures of a single parameter and/or combining

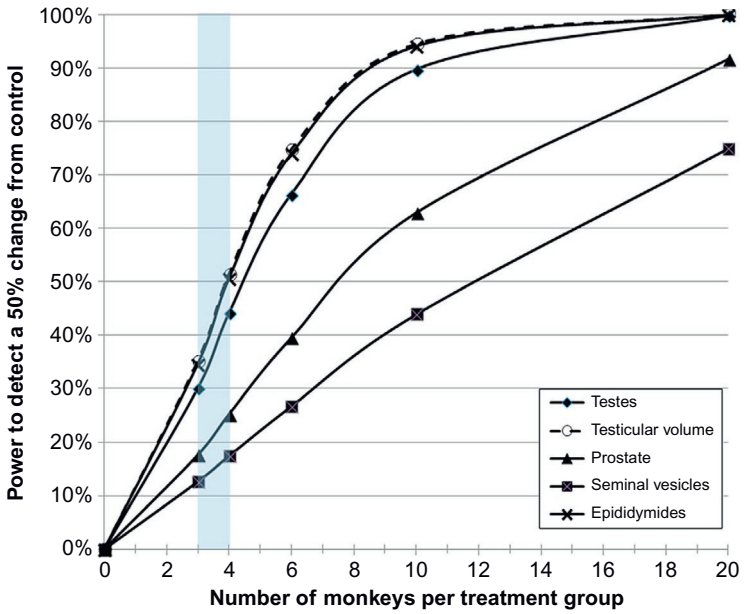


FIGURE 25.2 Power to statistically detect ($\alpha = 0.05$; $\beta = 0.80$) a 50% change from control for testicular volume and male reproductive organ weights relative to group size using a two-sided, two-sample t test. The shaded area represents the group sizes most commonly used in general toxicology studies. *Reproduced from Cappon et al. [50] with permission.*

two or more parameters. Overall, it should be remembered that while even substantial changes in some reproductive end points may not achieve statistical significance, the interpretation of results from such studies using NHPs is usually based on a weight-of-evidence interpretation of the changes that are considered biologically meaningful (rather than on statistical outcomes).

Implications of Social Housing for Evaluation of the Reproductive System

Recent developments in regulations governing the use of primates demand social housing (either in pairs or groups) whenever possible [13]. Although it is evident that NHPs generally benefit from social housing [51] (refer also to Chapters 14 and 15 in this book), systematic studies on the effects of social housing of cynomolgus monkeys in the context of reproductive toxicity evaluation are limited. Changing from individual to social housing of mature animals raises questions about the evaluation of reproductive toxicity end points. By nature, macaque societies are hierarchical, and that social rank can affect reproduction and other aspects is well documented [52].

For female cynomolgus monkeys, group housing can provoke marked alterations of reproductive hormone concentrations and other ovarian activities as judged from vaginal swab analysis [17,18]. Ovarian cycle duration can be prolonged or ovarian activity can cease for several months but subsequently returns to baseline duration within 3-6 months. Figure 25.3

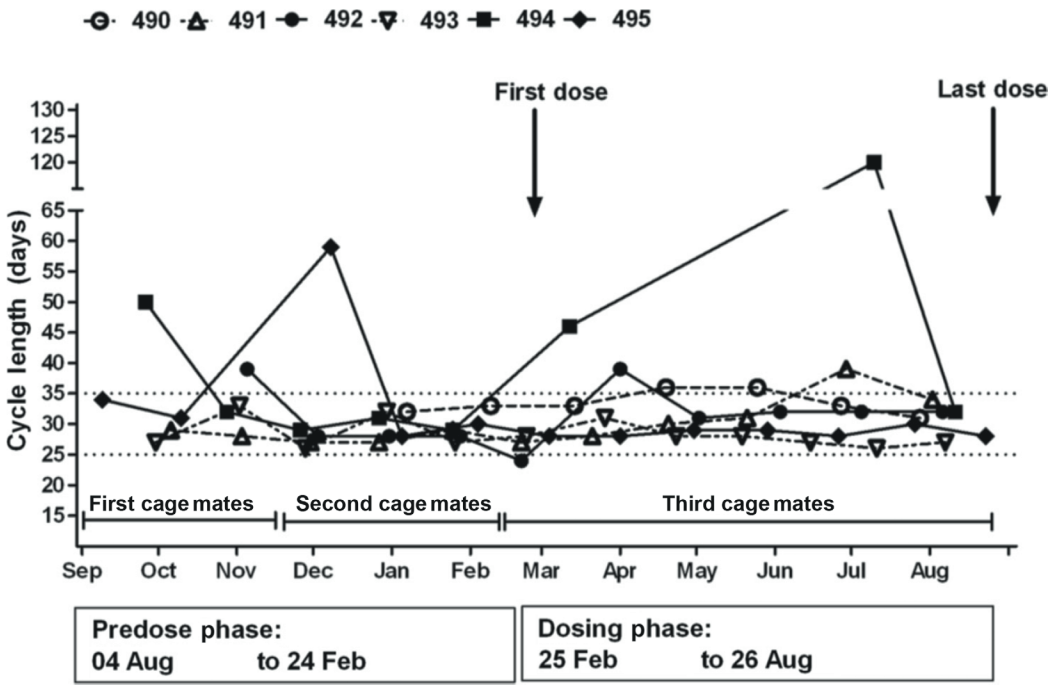


FIGURE 25.3 Effects of change of cage mates on ovarian cyclicity in cynomolgus monkeys. Animal 494 presented four consecutive cycles in the normal range from October through January. When assigned two new cage mates on February 11, this animal presented a 46-day cycle followed by three missed cycles. Animal 492 similarly had four consecutive normal cycles, but when reassigned two new cage mates on February 11, this animal presented a 24-day cycle followed by a 39-day cycle. The animal was separated in February because of cage mate fighting, which coincided with irregular cycles in March and April. These socialization issues support the conclusion that females should be socialized at least 3 months before initiating the dosing phase [17]. Dotted lines, upper and lower limits of normal cycle length (25-35 days). *Figure reproduced from Bussiere et al. [18] with permission.*

presents examples of how the change of cage mates can interfere with ovarian cyclicity. Interestingly, these types of effects on ovarian cyclicity can be apparent even among pairs of animals. Prolonged menstrual cycle duration often corresponds to an absent/decreased preovulatory estradiol peak and loss of the normal luteal progesterone increase following several normal hormone patterns concomitant with normal ovarian cycles [17]. For male cynomolgus monkeys, group housing of three to seven animals transiently decreased body weight gain compared with single housing [53]. This body weight loss is believed to result from physical activity around establishing and maintaining social rank. Testicular volumes decreased to approximately 45% of baseline within 13 weeks in low-ranking animals, followed by return to baseline during weeks 21-26, but was not accompanied by corresponding alterations of seminal and endocrine parameters. These circumstances need to be considered during social housing of mature animals in (reproductive) toxicity studies, and given the antireproductive effects in female animals, a period of at least 3 months for animals to get acquainted with each other should be included in study planning and scheduling.

VetBooks.ir

Developmental Toxicity Studies

It is important to be aware that background pregnancy failure is high, for example, postimplantation loss through postnatal loss, in normal primate populations. Hence, for developmental toxicity studies, the number of successful pregnancies and fetuses/infants available for evaluation is consistently lower than the number of pregnant animals entering the study. In macaque breeding colonies, the annual prenatal loss rates varied between 13% and 23% in the rhesus monkey (seasonal breeder), 10% and 50% in the bonnet monkey (seasonal breeder), and 8.6% and 28% in the cynomolgus monkey (nonseasonal breeder) [29]. An earlier review of the cynomolgus monkey reported abortion rates between 11.7% and 30.2% [54], which seem comparable to more recent observations of 8.8-20.3% cumulative prenatal loss rates [9]. In the context of developmental toxicity evaluation, however, it is important to recognize that—unlike cumulative loss rates—prenatal loss rates among vehicle groups in single studies can be highly variable and range from zero to >40% [28]. Early losses can be difficult to interpret, in part because the GD 18-20 pregnancy is typically diagnosed by the presence of a gestational sac, yet a fetal heartbeat cannot be visualized until 1-2 weeks later. Thus some of these may be nonviable pregnancies even before dose administration, which is typically initiated between GDs 19 and 22 in cynomolgus monkeys. Although not currently routinely available for GLP studies, assessment of mCG concentrations before administration of the first dose can be a useful way to exclude a test article relationship from nonviable pregnancies resulting in early losses.

Equally important, NHPs also experience significant infant loss at term (stillbirths) and thereafter (neonatal and infant losses). The overall stillbirth rate is 12-22% in cynomolgus monkeys [55,56]. Infant losses in the first month after delivery range from 12% to 22%, with the possibility of a similar amount of additional infant loss during the first year postpartum [54,56]. A detailed analysis of >700 control pregnancies identified the period until GD 50 (first trimester) and the birth event as the most critical periods for losses in cynomolgus monkeys [28]. A specific analysis performed to explore the influence of initial group size on the potential for data interpretation in macaque studies using the end points of embryo/fetal survival and infant survival until postnatal day 7 indicated that average loss rate until postnatal day 7 was 35% [28]. EFD studies with initial control group sizes of 16 and 20 have an 80% probability of having 13-16 ongoing pregnancies at GD 100, respectively. For ePPND studies with initial control group sizes of 16 and 20, there is an 80% likelihood of having 9-11 live infants at postnatal day 7, respectively. The pre- and postnatal loss data described above are derived from pregnant animals under single-housing conditions. There is more recent evidence that pair or group housing of pregnant animals before and (together with their infants) after delivery is associated with a 5-10% reduction in total losses [5] and a faster infant body weight gain compared with single housing (unpublished data). Interestingly, the route of administration does not seem to influence pre- or postnatal losses. Even intravenous infusions requiring chair restraint and/or wearing a jacket infusion packet have been tolerated well by pregnant animals without an apparent increase of fetal losses.

The ICH S6(R1) guideline also encourages the reuse of control animals in developmental toxicity studies for subsequent studies. This has not been implemented frequently because the concept has only recently been incorporated into the guideline. When it has been implemented, the available data indicate that while the abortion rate for female NHPs with

VetBooks.ir

successful outcomes in a previous study(s) may be similar to that of primigravid females, the postpartum success rate and infant outcome may be improved, presumably as a result of previous experience with delivery and postpartum care. It is therefore important to ensure that when reusing females from a previous NHP developmental toxicity study(s), they are randomly assigned across groups so that any bias for pregnancy success/outcome is not introduced into the study design.

RELEVANCE OF ADAs

The development of ADAs has implications for safety, including increased potential for infusion or injection reactions, neutralization of the target efficacy, altered pharmacokinetics, and potential cross-reactivity with endogenous proteins. For biopharmaceuticals, the test article may be more immunogenic in NHPs, as a xenoprotein, than in humans. In general, ADA responses are idiosyncratic and unpredictable in both humans and NHPs, and the confirmation of ADA does not always or often translate into adversity. The detection of ADAs during pregnancy may be difficult because of the low sensitivity of ADA assays when there are high concentrations of test article present. Therefore, the dam and offspring should be monitored for ADAs for several months postpartum because a positive signal may not become apparent until the serum concentration of the drug is low. Although uncommonly reported, ADAs do have the potential to contribute to adverse effects on the pregnancy via mechanisms listed above. When adverse events are observed and ADAs are also confirmed, determining the specific contributions of the drug itself versus the ADA response may be challenging. Conversely, a neutralizing ADA response may also limit the ability to interpret safety end points. Importantly, evaluation of an ADA can help to understand differences in expected exposure and/or unexpected toxicities on the overall study interpretation, but for ADA-related toxicities there is little value regarding the translation of these effects to humans because immunogenicity in NHPs is not predictive of immunogenicity in humans.

PLACENTA: TARGET ORGAN AND TRANSPORT

The placenta is a dynamic organ in its form and function, appearing as a transient and critical tissue in pregnancy. It is composed of maternal and zygotic cellular layers whose major function is as a conduit between mother and fetus, focused on the growth and viability of the next generation while preserving maternal well-being. A series of recent papers has focused on the placenta in toxicology [57–60]. In addition to providing essential nutrients and removing waste, the placenta generally permits transfer of small molecules by passive diffusion and generally limits transfer of large molecules with exceptions for specific types of molecules, including IgGs such as antibody biopharmaceuticals. Placental transfer of mAbs seems to occur by receptor-mediated transcytosis via FcRn in the syncytiotrophoblast of the chorioallantoic placenta of NHPs and humans. This process seems to transfer small amounts early in pregnancy but increases throughout pregnancy, culminating in fetal IgG concentrations comparable to, or exceeding, those of the dam near the time of parturition [43–46] (Figure 25.4). The expected low placental transport of antibodies during organogenesis has been given as

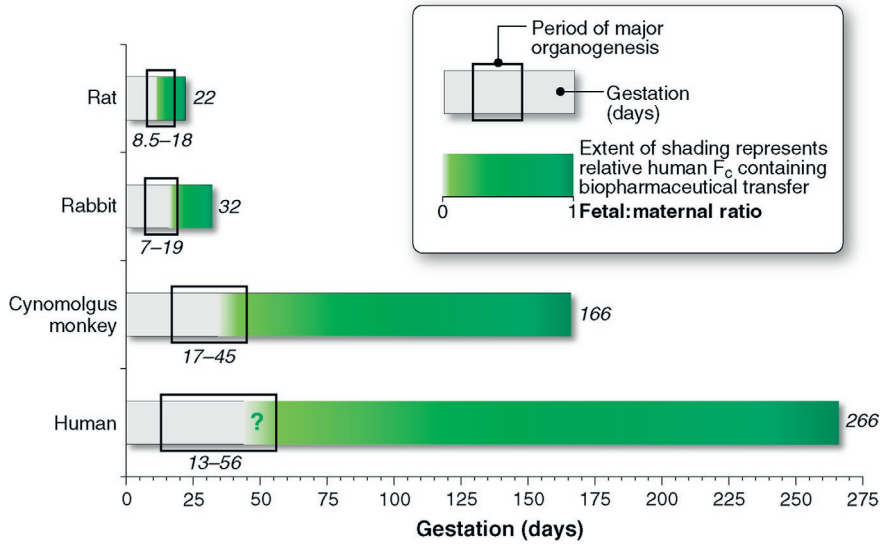


FIGURE 25.4 Comparison of the timing and extent of placental transfer of Fc-containing biopharmaceuticals in various species including humans. This diagram is a conceptual illustration of various characteristics of the gestational periods of rats, rabbits, monkeys (cynomolgus), and humans (*Courtesy of Dr. DeSesso; modified and updated from DeSesso et al. [44] based on Moffat et al. [46]*). The durations of the gestational periods (in days) are represented by the length of the horizontal bars. Gestation in the rat and rabbit is considerable shorter than in the monkeys and humans. The large rectangular outlines denote the approximate periods of organogenesis for each species. The shading within the horizontal bars denotes the approximate time of onset and increasing extent of placental transfer of Fc-containing biopharmaceuticals. Note that the transfer of these molecules is thought to initiate during the period of organogenesis in nonclinical species including monkeys. Data are very limited in humans but there is evidence of IgGs present in coelemic fluid in human embryos during organogenesis.

a reason not to conduct standard EFD studies with mAbs. However, such studies can identify developmental toxicity and inform the drug label as exemplified by pertuzumab, which is a monoclonal antibody specific for the human erbB-2 receptor tyrosine kinase, HER2. As summarized in the pertuzumab label (www.perjeta.com, most recently modified 09/13), administration of pertuzumab to pregnant NHPs from GDs 19 through 50 (the period of organogenesis) resulted in dose-dependent increases in embryo-fetal death (up to 85%) between GDs 25 and 70. At cesarean delivery on GD 100, oligohydramnios, decreased relative lung and kidney weights, and microscopic evidence of renal hypoplasia consistent with delayed renal development were identified in all pertuzumab dose groups. Pertuzumab exposure was reported in offspring from all treated groups at concentrations 29-40% of maternal serum concentrations at GD 100.

The unique physiology of biodistribution and metabolism of the placenta also renders it sensitive as a target of toxicity. Another example is figitumumab, an mAb specific for the insulin-like growth factor (IGF)-1 receptor; there was clear evidence of developmental toxicity in an NHP EFD study [61]. Pregnant NHPs dosed from GDs 20 through 48 resulted in fetal growth retardation evidenced by reduced fetal body weights and concomitant developmental delays and structural malformations observed at the fetal morphology evaluation on GD 100. The embryo-fetal effects of pregnancy loss and fetal growth deficits were considered

VetBooks.ir

consistent with inhibition of IGF signaling, but these effects and the fetal malformations were considered secondary to placental toxicity [61]; however, direct inhibition of IGF signaling in the conceptus could not be ruled out.

Therefore, there needs to be special consideration for molecules that are expected to have effects on placental structure and function. In the ePPND study design, the placenta is not routinely collected or evaluated, so placental effects may only be indirectly evident as fetal growth restriction, decreased gestational duration, decreased birthweight, developmental delays, and increased reproductive losses. If a direct effect on the placenta is expected, the study design may need to be modified to allow examination of the placenta for at least a subset of animals. Finally, direct effects on the placental trophoblasts could theoretically affect the transfer of Igs to the fetus, and fetal exposures to antibody products may be lower than expected. Alternatively, if the normal placental barrier is compromised, it may permit increased exposure to otherwise restricted molecules.

DATA INTERPRETATION CHALLENGES ASSOCIATED WITH BACKGROUND CHANGES IN PREGNANT NHPs

Similar to humans, major physiological, anatomic, metabolic, and hormonal changes occur in cynomolgus monkeys during pregnancy. Pregnancy-related changes in hormone concentrations (including estradiol and progesterone), clinical pathology parameters, lymphocyte subpopulations, and bone biomarkers have been reported. Some of these changes associated with normal pregnancy may mimic the mode of action of the test article being administered and, therefore, pose challenges to interpreting the study data. For example, serum cholesterol concentrations are significantly decreased (up to 50%) during gestation in cynomolgus monkeys [62–64] (Figure 25.5); therefore the interpretation of pharmacodynamic effects of cholesterol-lowering drugs needs to account for this. Circulating B cells, as measured by immunophenotyping (flow cytometry), also decrease up to 40% in pregnant cynomolgus monkeys [65] (Figure 25.5). This is consistent with pregnancy-related steroids being potential negative regulators of B-cell lymphopoiesis [66]. The interpretation of pharmacodynamic effects of immunomodulatory pharmaceuticals/biopharmaceuticals that target B cells needs to account for this normal decrease during pregnancy. Other typical changes during pregnancy in animals and humans include decreases in glucose, total protein, and albumin, as well as potential hematologic changes [67]. Not all changes observed in pregnant NHPs match those that occur during human pregnancy. For example, serum cholesterol concentrations decrease in various species of monkeys during pregnancy but increase during pregnancy in humans [68].

CONCLUSION

Developmental (and reproductive) toxicity evaluation in the NHP model has become more common in recent years. This is demonstrated by the substantial number of publications related to the use of NHPs in developmental toxicology testing that have appeared in the peer-reviewed literature in the past 10 years (Table 25.4). As has been the case for developmental toxicity testing in traditional species (rat/mouse/rabbit) over many years,

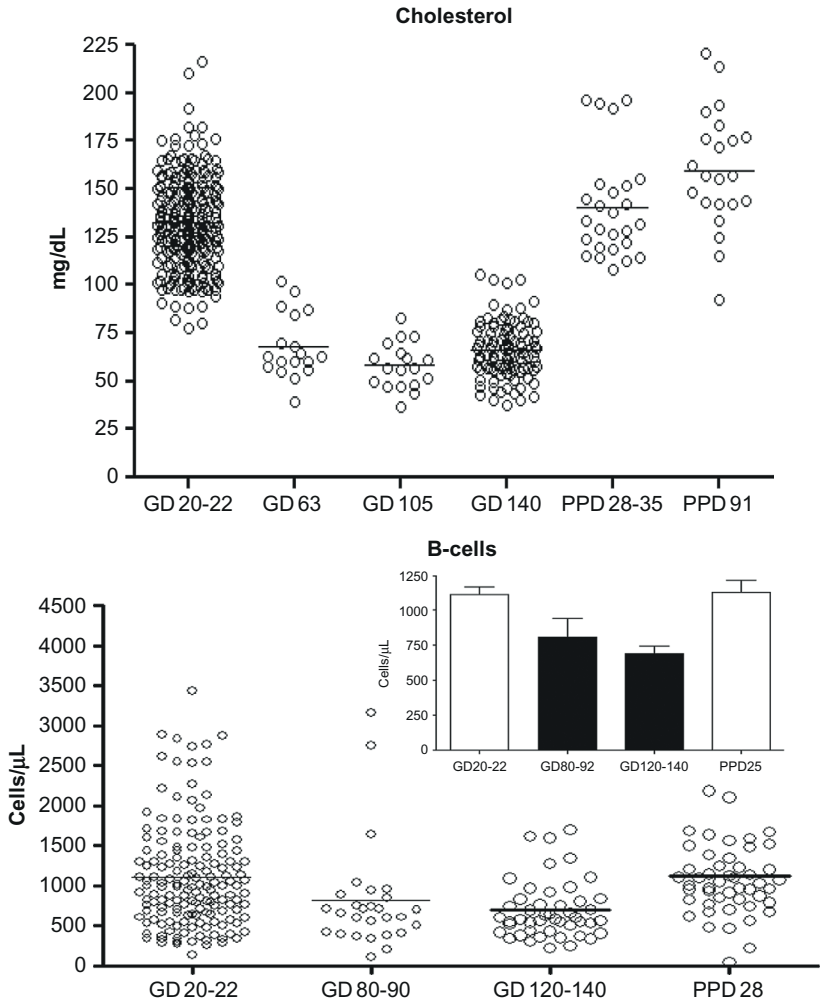


FIGURE 25.5 A number of physiologic changes occur during pregnancy in cynomolgus monkeys, including substantial decreases in serum cholesterol concentrations (top) and B-cell counts (bottom), as shown here. Some of the changes are similar to those in humans, whereas others are species specific. Awareness of such background changes is important for interpretation of potential maternal effects from test article administration during embryo-fetal development and pre- and postnatal development (PPND)/enhanced PPND studies. GD, gestational day; PPD, postpartum day.

these studies in NHPs reflect a variety of outcomes. The results range from no developmental toxicity and expression of only expected pharmacology in dosed pregnant females and/or their offspring (fetuses or infants) to studies in which the pharmacology of the test article (either intended or unintended) led to developmental effects such as altered gestational duration, fetal/infant survival, fetal/infant structural and morphological development, and offspring growth and development. Considerable operational and scientific progress has been achieved over the past 5-6 years, yielding much-improved study designs. Social housing can

TABLE 25.4 Synopsis of Published Developmental Toxicity Studies with Pharmaceuticals in NHPs During 2003-2014

NHP DART Publications (2003-2014)	Study Type	Test Article	Main Findings
Bussiere et al., Reprod Toxicol 2013;42:27–40	EFD+ePPND	Denosumab (anti-RANKL)	EFD: no maternal toxicity, fetal harm, or teratogenicity ePPND: observed effects (maternal and infant) consistent with pharmacologic action of denosumab; increased stillbirths and postnatal mortality and, in infants, effects on growth and development, bones, and lymph nodes
Bowman et al., Reprod Toxicol 2014; in press; DOI: 10.1016/j.reprotox.2014.10.004	ePPND	Tanezumab (anti-NGF)	Test article-related increased stillbirths, neuropathology and decreased postnatal survival, growth and immune function
Enright et al., Birth Defects Res B Dev Reprod Toxicol 2012;95:431–43	EFD+PPND	ABT-874 (anti-IL-12/23p400	No maternal toxicity or adverse effects on fetuses or infants
Vaidyanathan et al., Toxicol Sci 2011;119:116–25	EFD+PPND	Rituximab (anti-CD20)	B-cell depletion in fetal lymphoid tissues, with repletion in infants. Transient functional consequence on infant TDAR response
Bowman et al., Birth Defects Res B Dev Reprod Toxicol 2010;89:326–38	EFD	Figitumumab (anti-IGF-1R)	Pregnancy loss and fetal growth deficits, consistent with inhibition of IGF signaling; fetal malformations secondary to altered placental function and/or reduced fetal growth
Martin et al., Birth Defects Res B Dev Reprod Toxicol 2010;89:351–63	EFD+PPND	Ustekinumab (anti-IL-12/23p40)	No maternal or fetal effects, including infant TDAR and DTH responses
Wehner et al., Birth Defects Res B Dev Reprod Toxicol 2009;86:144–56	PPND	Natalizumab (anti-α4 integrin)	Elevated lymphocyte and nucleated red blood cell counts in infants, consistent with pharmacologic effect
Wehner et al., Birth Defects Res B Dev Reprod Toxicol 2009;86:117–30	EFD	Natalizumab (anti-α4 integrin)	No abortifacient or teratogenic effects, but changes in fetal hematopoiesis and leukocyte trafficking
Carlock et al., Regul Toxicol Pharmacol 2009;53:226–34	EFD+PPND	Soluble IL-4 receptor	Increased abortion/embryo-fetal death
Auyeung-Kim et al., Reprod Toxicol 2009;28:443–55	EFD+PPND	Belimumab (anti-BLys)	Effects limited to expected pharmacology: decreased B-lymphocytes in peripheral blood of mothers and infants and in fetal lymphoid tissues; recovery in infants upon cessation of exposure

(*Continued*)

TABLE 25.4 Synopsis of Published Developmental Toxicity Studies with Pharmaceuticals in NHPs During 2003-2014—Cont'd

NHP DART Publications (2003-2014)	Study Type	Test Article	Main Findings
Clark et al., Birth Defects Res B Dev Reprod Toxicol 2008;83:418–34	EFD	Artesunate (antimalarial)	Embryolethal, dependent on dose duration
Rasmussen et al., Reprod Toxicol 2007;23:471–9	PPND	Barusiban (oxytocin antagonist)	No test article-related effects on mothers and no adverse findings in infants
Martin et al., Am J Reprod Immunol 2007;58:138–49	PPND	Golimumab (anti-TNF-α)	No effects on development and maturation of the immune system in the offspring

EFD, embryo-fetal development; ePPND, enhanced pre- and postnatal development; DTH, delayed type hypersensitivity; IGF, insulin-like growth factor; IL, interleukin; PPND, pre- and postnatal development; TDAR, T-cell-dependent antibody response; TNF, tumor necrosis factor.

be challenging for female fertility evaluation but provides distinct advantages for developmental toxicity testing compared with single housing. Statistical power estimation and group size considerations are now coming into play. At present, NHPs have developed into a more regularly accepted model for DART testing, when appropriate, rather than representing an exotic alternative. The conduct of developmental toxicity studies using NHPs is therefore contributing to hazard identification and/or characterization of potential test article-related effects as part of the nonclinical safety assessment for drugs (especially biopharmaceuticals) being developed.

References

[1] ICH S6(R1): addendum to ICH S6: preclinical safety evaluation of biotechnology-derived pharmaceuticals; 2011. www.ich.org.
[2] ICH S5 (R2): guidance on reproductive toxicology and male fertility: detection of toxicity to reproduction for medicinal products; 2005. www.ich.org.
[3] ICH M3 (R2): guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization of pharmaceuticals; 2009. www.ich.org.
[4] Stewart J. Developmental toxicity testing of monoclonal antibodies: an enhanced pre- and postnatal study design option. Reprod Toxicol 2009;28:220–5.
[5] Weinbauer GF, Fuchs A, Niehaus M, Luetjens CM. The enhanced pre- and postnatal study for nonhuman primates: update and perspectives. Birth Defects Res (Part C) 2011;93:324–33.
[6] Bussiere JL, Martin P, Horner M, Couch J, Flaherty M, Andrews L, et al. Alternative strategies for toxicity testing of species-specific biopharmaceuticals. Int J Toxicol 2009;28:230–53.
[7] Martin PL, Breslin W, Rocca M, Wright D, Cavagnaro J. Considerations in assessing the developmental and reproductive toxicity potential of biopharmaceuticals. Birth Defects Res B Dev Reprod Toxicol 2009;86:176–203.
[8] Cavagnaro JA. The principles of ICH S6 and the case-by-case approach. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2008. p. 45–65.
[9] Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicology studies in nonhuman primates. Birth Defects Res (Part B) 2009;86:446–62.
[10] Chapman KL, Pullen N, Andrews L, Ragan I. The future of non-human primate use in mAb development. Drug Discov Today 2010;15:235–42.

[11] Baldrick P. Safety evaluation of biological drugs: what are toxicology studies in primates telling us? Regul Toxicol Pharmacol 2011;59:227–36.
[12] Bowman CJ, Martin DP. Developmental and reproductive safety assessment of biopharmaceuticals. In: Hood RD, editor. Developmental and reproductive toxicology: a practical approach. London: Informa Healthcare; 2012. p. 479–90.
[13] Chapman KL, Andrews L, Bajramovic JJ, Baldrick P, Black LE, Bowman CJ, et al. The design of chronic toxicology studies of monoclonal antibodies: implications for the reduction in use of non-human primates. Regul Toxicol Pharmacol 2012;62:347–54.
[14] van der Laan JW, Pentsuk N. ICH S6 regulatory reproduction studies to support the use of monoclonal antibodies. In: Weinbauer GF, Vogel F, editors. Future trends in primate toxicology and biotechnology. Münster: Waxmann Verlag GmbH; 2011. p. 143–61.
[15] Henck JW, Hilbish KG, Serabian MA, Cavagnaro JA, Hendrickx AG, Agnish ND, et al. Reproductive toxicity testing of therapeutic biotechnology agents. Teratology 1996;53:185–95.
[16] Weinbauer GF, Frings W, Fuchs ANM, Osterburg I. Reproductive/developmental toxicity assessment of biopharmaceuticals in nonhuman primates. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals. A science-based approach to facilitating clinical trials. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2008. p. 379–97.
[17] Weinbauer GF, Niehoff M, Niehaus M, Srivastav S, Fuchs A, Van Esch E, et al. Physiology and endocrinology of the ovarian cycle in macaques. Toxicol Pathol 2008;36:7S–23S.
[18] Bussiere JL, Moffat G, Zhou L, Tarlo KS. Assessment of menstrual cycle length in cynomolgus monkeys as a female fertility endpoint of a biopharmaceutical in a 6 month toxicity study. Regul Toxicol Pharmacol 2013;66:269–78.
[19] Martin PL, Weinbauer GF. Developmental toxicity testing of biopharmaceuticals in nonhuman primates: previous experience and future directions. Int J Toxicol 2010;29:552–68.
[20] Collinge M, Burns-Naas LA, Chellman GJ, Kawabata TT, Komocsar WJ, Piccotti JR, et al. Developmental immunotoxicity (DIT) testing of pharmaceuticals: current practices, state of the science, knowledge gaps, and recommendations. J Immunotoxicol 2012;9(2):210–30.
[21] Buse E. Development of the immune system in the cynomolgus monkey: the appropriate model in human targeted toxicology? J Immunotoxicol 2005;2:211–6.
[22] Luetjens CM, Weinbauer GF, Wistuba J. Primate spermatogenesis: new insights into comparative testicular organisation, spermatogenic efficiency and endocrine control. Biol Rev Camb Philos Soc 2005;80:475–88.
[23] Abbott DH, Barnett DK, Colman RJ, Yamamoto ME, Schultz-Darken NJ. Aspects of common marmoset basic biology and life history important for biomedical research. Comp Med 2003;53:339–50.
[24] Muller T, Gromoll J, Simula AP, Norman R, Sandhowe-Klaverkamp R, Simoni M. The carboxyterminal peptide of chorionic gonadotropin facilitates activation of the marmoset LH receptor. Exp Clin Endocrinol Diabetes 2004;112:574–9.
[25] Tardif SD, Smucny DA, Abbott DH, Mansfield K, Schultz-Darken N, Yamamoto ME. Reproduction in captive common marmosets (*Callithrix jacchus*). Comp Med 2003;53:364–8.
[26] Wistuba J, Luetjens CM, Ehmcke J, Redmann K, Damm OS, Steinhoff A, et al. Experimental endocrine manipulation by contraceptive regimen in the male marmoset (*Callithrix jacchus*). Reproduction 2013;145:439–51.
[27] Binkerd PE, Tarantal AF, Hendrickx AG. Embryonic/fetal loss and spontaneous malformations in nonhuman primates. In: Neubert D, Merker H-J, Hendrickx AG, editors. Non-human primates: developmental biology and toxicology. Berlin: Ueberreuter Wissenschaft; 1988. p. 115–27.
[28] Jarvis P, Srivastav S, Vogelwedde E, Stewart J, Mitchard T, Weinbauer GF. The cynomolgus monkey as a model for developmental toxicity studies: variability of pregnancy losses, statistical power estimates, and group size considerations. Birth Defects Res B Dev Reprod Toxicol 2010;89:175–87.
[29] Hendrie TA, Peterson PE, Short JJ, Tarantal AF, Rothgarn E, Hendrie MI, et al. Frequency of prenatal loss in a macaque breeding colony. Am J Primatol 1996;40:41–53.
[30] Van Esch E, Cline M, Buse E, Wood CE, de Rijk EPCT, Weinbauer GF. Summary comparison of female reproductive system in human and the cynomolgus monkey (*Macaca fascicularis*). Toxicol Pathol 2008;36:171S–2S.
[31] Mineshima H, Endo Y, Ogasawara H, Nishigaki K, Numa T, Hirano F, et al. Impact of globalization under the ICH guidelines on the conduct of reproductive toxicity studies – report on current status in Japan, Europe and the U.S. by questionnaire survey. J Toxicol Sci 2004;29:201–15.

[32] Buse E, Cline JM, de Rijk EPCT, Lanning LL, Van Esch E, Vidal JD, et al. A monograph on female reproductive pathophysiology in macaques. Toxicol Pathol 2008;36:5S–6S.
[33] Amann RP, Johnson L, Thompson Jr. DL, Pickett BW. Daily spermatozoal production, epididymal spermatozoal reserves and transit time of spermatozoa through the epididymis of the rhesus monkey. Biol Reprod 1976;15:586–92.
[34] Wen X-H, Yang Z-W. Quantitative (stereological) study on the spermatozoal storage capacity of epididymis in rats and monkeys. Asian J Androl 2000;2:73–7.
[35] Plant TM, Ramaswamy S. Kisspeptin and the regulation of the hypothalamic-pituitary-gonadal axis in the rhesus monkey (*Macaca mulatta*). Peptides 2009;30:67–75.
[36] Smedley JV, Bailey SA, Perry RW, O Rourke CM. Methods for predicting sexual maturity in male cynomolgus macaques on the basis of age, body weight, and histologic evaluation of the testes. Contemp Top Lab Anim Sci 2002;41:18–20.
[37] Lawrence WB, Saladino BH. Correlation of age and body weight, and testicular weight with degree of sexual maturity in male cynomolgus macaques with emphasis on peripubertal animals. Vet Pathol 2009;46:P235.
[38] Ku WW, Pagliusi F, Foley G, Roesler A, Zimmerman T. A simple orchidometric method for the preliminary assessment of maturity status in male cynomolgus monkeys (*Macaca fascicularis*) used for nonclinical safety studies. J Pharmacol Toxicol Methods 2010;61:32–7.
[39] Vogel F. How to design male fertility investigations in the cynomolgus monkey. In: Korte R, Weinbauer G, editors. Towards new horizons in primate toxicology – perspectives for the new millenium. Münster: Waxmann Verlag GmbH; 2000. p. 43–51.
[40] Luetjens CM, Weinbauer GF. Functional assessment of sexual maturity in male macaques (*Macaca fascicularis*). Regul Toxicol Pharmacol 2012;63:391–400.
[41] Laron Z. Age at first ejaculation (spermarche) – the overlooked milestone in male development. Pediatr Endocrinol Rev 2010;7:256–7.
[42] Fuchs A, Buse E, Weinbauer GF. Embryo fetal development studies in nonhuman primates. Methods Mol Biol 2013;947:169–83.
[43] Pentšuk N, van der Laan JW. An interspecies comparison of placental antibody transfer: new insights into developmental toxicity testing of monoclonal antibodies. Birth Defects Res B Dev Reprod Toxicol 2009;86:328–44.
[44] DeSesso JM, Williams AL, Ahuja A, Bowman CJ, Hurtt ME. The placenta, transfer of immunoglobulins, and safety assessment of biopharmaceuticals in pregnancy. Crit Rev Toxicol 2012;42:185–210.
[45] Bowman CJ, Breslin WJ, Connor AV, Martin PL, Moffat G, Sivaraman L, et al. Placental transfer of Fc-containing biopharmaceuticals across species, an industry survey analysis. Birth Defects Res (Part B) 2013;98:459–85.
[46] Moffat GJ, Retter MW, Kwon G, Loomis M, Hock MB, Hall C, et al. Placental transfer of a fully human IgG2 monoclonal antibody in the cynomolgus monkey, rat and rabbit from organogenesis onwards a comparative assessment. Birth Defects Res (Part B) 2014;101(2):178–88.
[47] Martin PL, Sachs C, Imai N, Tsusaki H, Oneda S, Jiao Q, et al. Development in the cynomolgus macaque following administration of ustekinumab, a human anti-IL-12/23p40 monoclonal antibody, during pregnancy and lactation. Birth Defects Res B Dev Reprod Toxicol 2010;89:351–63.
[48] Cappon GD, Bowman CJ, Hurtt ME, Grantham LE. Object discrimination reversal as a method to assess cognitive impairment in nonhuman primate enhanced pre- and postnatal developmental (ePPND) studies: statistical power analysis. Birth Defects Res B Dev Reprod Toxicol 2012;95(5):354–62.
[49] Makori N, Watson RE, Hogrefe CE, Lalayeva N, Oneda S. Object discrimination and reversal learning in infant and juvenile non-human primates in a non-clinical laboratory. J Med Primatol 2013;42:147–57.
[50] Cappon GD, Potter D, Hurtt ME, Weinbauer GF, Luetjens CM, Bowman CJ. Sensitivity of male reproductive endpoints in nonhuman primate toxicity studies: a statistical power analysis. Reprod Toxicol 2013;41:67–72.
[51] Capitanio JP, Kyes RC, Fairbanks LA. Considerations in the selection and conditioning of Old World monkeys for laboratory research: animals from domestic sources. ILAR J 2006;47:294–306.
[52] Czoty PW, Gould RW, Nader MA. Relationship between social rank and cortisol and testosterone concentrations in male cynomolgus monkeys (*Macaca fascicularis*). J Neuroendocrinol 2009;21:68–76.
[53] Niehoff MO, Bergmann M, Weinbauer GF. Effects of social housing of sexually mature male cynomolgus monkeys during general and reproductive toxicity evaluation. Reprod Toxicol 2010;29:57–67.
[54] Small M. Reproductive failure in macaques. J Med Primatol 1982;2:137–47.
[55] Sesbuppha W, Chantip S, Dick Jr. EJ, Schlabritz-Loutsevitch NE, Guardado-Mendoza R, Butler SD, et al. Stillbirths in *Macaca fascicularis*. J Med Primatol 2008;37:169–72.

[56] Gardin JF, Jerome CP, Jayo MJ, Weaver DS. Maternal factors affecting reproduction in a breeding colony of cynomolgus macaques (*Macaca fascicularis*). Lab Anim Sci 1989;39:205–12.
[57] Buse E, Haeger JD, Svensson-Arvelund J, Markert UR, Faas MM, Ernerudh J, et al. The placenta in toxicology. Part I: animal models in toxicology: placental morphology and tolerance molecules in the cynomolgus monkey (*Macaca fascicularis*). Toxicol Pathol 2014;42:314–26.
[58] Svensson-Arvelund J, Ernerudh J, Buse E, Cline JM, Haeger JD, Dixon D, et al. The placenta in toxicology. Part II: systemic and local immune adaptations in pregnancy. Toxicol Pathol 2014;42:327–38.
[59] Cline JM, Dixon D, Ernerudh J, Faas MM, Gohner C, Hager JD, et al. The placenta in toxicology. Part III: pathologic assessment of the placenta. Toxicol Pathol 2014;42:339–44.
[60] Gohner C, Svensson-Arvelund J, Pfarrer C, Hager JD, Faas M, Ernerudh J, et al. The placenta in toxicology. Part IV: battery of toxicological test systems based on human placenta. Toxicol Pathol 2014;42:345–51.
[61] Bowman CJ, Chmielewski G, Oneda S, Finco D, Boucher MA, Todd M. Embryo-fetal developmental toxicity of figitumumab, an anti-insulin-like growth factor-1 receptor (IGF-1R) monoclonal antibody, in cynomolgus monkeys. Birth Defects Res B Dev Reprod Toxicol 2010;89:326–38.
[62] Koritnik DR, Wood LL, Shandilya LN, Rudel LL. Lipids, lipoproteins, and endocrine profiles during pregnancy in the African green monkey (*Cercopithecus aethiops*). Metabolism 1984;33:840–4.
[63] Adams MR, Kaplan JR, Koritnik DR, Clarkson TB. Pregnancy-associated inhibition of coronary artery atherosclerosis in monkeys. Evidence of a relationship with endogenous estrogen. Arteriosclerosis 1987;7:378–84.
[64] Gilbert SG, Rice DC. Effects of chronic caffeine consumption in pregnant monkeys (*Macaca fascicularis*) on blood and urine clinical chemistry parameters. Fundam Appl Toxicol 1991;16:299–308.
[65] Auyeung-Kim DJ, Devalaraja MN, Migone TS, Cai W, Chellman GJ. Developmental and peri-postnatal study in cynomolgus monkeys with belimumab, a monoclonal antibody directed against B-lymphocyte stimulator. Reprod Toxicol 2009;28:443–55.
[66] Medina KL, Kincade PW. Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. Proc Natl Acad Sci U S A 1994;91:5382–6.
[67] Switzer JW, Valerio DA, Martin DP, Valerio MG, Rininger BF. Hematologic changes associated with pregnancy and parturition in *Macaca mulatta*. Lab Anim Care 1970;20:930–9.
[68] Emet T, Ustuner I, Guven SG, Balik G, Ural UM, Tekin YB, et al. Plasma lipids and lipoproteins during pregnancy and related pregnancy outcomes. Arch Gynecol Obstet 2013;288:49–55.

VetBooks.ir

VetBooks.ir

CHAPTER

26

Juvenile Animal Studies and Experience

Pauline L. Martin, Timothy P. Coogan

Janssen Research and Development, LLC, Spring House, PA, USA

OUTLINE

Introduction and Regulatory Considerations 502

The Use of Nonhuman Primates for Juvenile Toxicity Testing 502

Organ Systems that Are Well Developed in Macaques at Birth or by 6 Months of Age 504
Respiratory Tract 504
Kidney 504
Gastrointestinal Tract 505
Heart 505
Other 505

Organ Systems that Show Significant Development Beyond 6 Months of Age 506
Bone 506
Brain 506
Sexual Maturity 508
Adrenal Maturation 508
Males 508
Females 509
Immune System 510

Rationale for Not Conducting a Nonclinical Study Using NHPs 511

Rationale for Conducting a Nonclinical Study in NHPs 511

Conclusion 512

References 512

http://dx.doi.org/10.1016/B978-0-12-417144-2.00026-3
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION AND REGULATORY CONSIDERATIONS

For many years, the development of new medicines to include pediatric use was lacking. However, many new medicinal products are being developed to treat diseases in children. In addition, regulatory health authorities are requiring sponsors to consider pediatric indications for therapeutics in development. To support the use of pharmaceuticals in children, clinical trials of pediatric subjects are encouraged by many regulatory health authorities. In the European Union, plans for the evaluation of a new pharmaceutical in pediatric patients, or a waiver for not conducting such studies, is expected early during the clinical development program (usually by the end of Phase I) and is part of the pediatric investigational plan. In the USA, the plans for development in pediatric populations is expected by the end of Phase II. Regardless of region/health authority, the pediatric investigational plan should describe an assessment of the potential pediatric risks and benefits and should incorporate a preclinical discussion. The preclinical discussion includes a summary of completed and planned studies and a justification for why additional studies of juvenile animals may or may not be required. Regulatory guidance document state that juvenile animal toxicity studies should be considered only when existing animal and human data are insufficient to support pediatric studies. If a study is warranted, one relevant species (preferably a rodent) is generally considered adequate. A study using a nonrodent species can be appropriate when scientifically justified [1].

THE USE OF NONHUMAN PRIMATES FOR JUVENILE TOXICITY TESTING

Nonhuman primates (NHPs) are not routinely used for juvenile toxicity testing. Most traditional pharmaceutical drugs are tested for general toxicity in young adult rats and dogs. Only if these studies do not provide sufficient information to support the use of the pharmaceutical in a pediatric population would specifically designed juvenile toxicity studies in one species, usually the rat, be considered. An important consideration for the design of a juvenile toxicity study is whether there are major developmental events that could occur during treatment of a pediatric patient that have not been examined in other nonclinical toxicology studies and whether a nonclinical study can be designed to address these concerns. For many pharmaceutical drugs, an assessment of pre- and postnatal development in rats is conducted during the course of compound development. These studies generally involve dosing pregnant rats from implantation (gestation day 6) through lactation (postnatal day 20) [2]. During the course of these studies, the embryo/fetus/pup may be indirectly exposed to the drugs either in utero via transfer across the yolk sac or postnatally in milk. Therefore the effects of drug exposure during critical periods of development may already have been evaluated, thereby obviating the need for juvenile toxicity studies. However, it should be emphasized that rodents are born with most organs at an immature stage of development relative to humans, and some developmental events occur postnatally in the rat that would occur prenatally in humans. Therefore, if there is a gap between exposure in the pre- and postnatal development and in the general toxicity study and a critical developmental event occurs during this gap, then a juvenile toxicity study in rodents may be warranted [3].

VetBooks.ir

A nonrodent species may be used for juvenile toxicity testing, in addition to the rodent or in place of the rodent, if rodent studies alone are deemed insufficient to address patient safety. The dog is the nonrodent species of choice for small-molecule pharmaceuticals. The NHP is generally selected as the nonrodent species for nonclinical testing of pharmaceutical drugs only if the dog is not a physiologically, toxicologically, or metabolically relevant species. There are no standard study designs for NHP juvenile toxicity studies, but there are a number of end points that can be measured [4–6]. Each study needs to be designed based on the specific concern being addressed.

For biopharmaceuticals, the NHP may be the only pharmacologically relevant species for all of the nonclinical safety testing. Biopharmaceuticals mostly consist of human proteins and monoclonal antibodies that are pharmacologically active in humans and NHPs but generally not in dogs and rodents. For these molecules the nonclinical safety program may be limited to general toxicity studies in young/mature monkeys, usually cynomolgus macaques, and a developmental toxicity study. For monoclonal antibody biopharmaceuticals intended to treat chronic disease indications, the preferred developmental study design is an enhanced pre- and postnatal study in which pregnant monkeys are dosed from the beginning of organogenesis through parturition [4,7,8]. Numerous macaque studies have shown that with this study design there is extensive fetal exposure to biopharmaceuticals containing the Fc portion of IgG beginning at the end of organogenesis and that the therapeutic may persist in the infant serum for a number of months after birth [9,10]. The fetal and neonatal exposure of Fc containing biopharmaceuticals (and, to some extent, albumin) is a consequence of specific active transport via the placental FcRn receptor [11] and would not be expected to occur for other types of biopharmaceutical proteins. The fetal period in humans and NHPs is a period of extensive growth and maturation of the organ systems that were formed during the embryonic period. For some of the organ systems this maturation process continues during the postnatal period but at a progressively decreasing rate. At the time of birth most organ systems in macaques are either as developed or more developed than in humans. As a consequence, some of the events that occur postnatally in humans have already occurred prenatally in macaques and have therefore already been evaluated in the NHP developmental toxicity study. Also, for those organs that continue to develop after birth, the most rapid maturation period is during the first 3-6 months in macaques; this corresponds developmentally to the first 2-8 years in humans.

In addition, the general toxicity studies conducted using young adult animals may provide information that is relevant to the pediatric patient population. Macaques used for general toxicity testing are frequently within the 2-3-year age range. For females this represents a peripubertal age and for males it is a prepubertal age. Studies of these juvenile (adolescent) animals may be able to detect effects in the growing animal that cannot be detected in fully mature animals, for example, effects on overall growth rate. However, "filling the gap" between exposure in a pre- and postnatal developmental study and a general toxicity study of NHPs is not feasible because it would require dosing for 2-3 years if adolescent animals were used and ≥5 years if sexually mature animals were used for the general toxicity testing. The only study that could practically be done is one using a small window of exposure during a period corresponding to a comparable developmental age in the intended patient population. This study would be most valuable for evaluating differences in the pharmacokinetics and pharmacodynamics of immature animals relative to mature animals that may necessitate dose adjustments in the clinic.

VetBooks.ir

The following sections provide a brief overview of postnatal development in NHPs compared to humans and the relevance of the timing of these events for juvenile toxicity testing. This discussion is focused on macaques because these are the NHP species most frequently used for the nonclinical safety testing of pharmaceutical drugs and biopharmaceutical agents.

ORGAN SYSTEMS THAT ARE WELL DEVELOPED IN MACAQUES AT BIRTH OR BY 6 MONTHS OF AGE

Respiratory Tract

At birth the macaque lung seems to be more developmentally advanced than the human lung [12]. In humans the alveolar stage of lung development is not complete at birth but continues until about 8 years of age [13]. By contrast, the number of respiratory bronchioli and alveolar ducts in macaques is similar at birth and in the adult [14], and most of the differentiation of the tracheal epithelium occurs prenatally [15,16]. After birth the macaque lung mostly develops by growth of existing structures and increases in size with increase in body size. Despite this growth, there is no significant change in the average epithelial thickness [17]. Some of the events that do occur postnatally in macaques include mucus cell and gland development [16], development of the lamina reticularis of the basal membrane zone of the trachea [18,19], and expression of Clara cell secretory protein, a protective protein secreted from nonciliated bronchiolar epithelial cells [20]. These events mostly occur within the first 3 months after birth.

Because of the advanced stage of development of the macaque lung relative to the human lung, it has been proposed that the macaque is not an appropriate species for juvenile toxicity testing of products in which the lung may be a potential target organ for toxicity [12]. However, if the agent is intended to be administered systemically and a pre- and postnatal development study has been conducted in which macaques were exposed during the fetal and early postnatal period, then this would likely cover many of the critical events that occur postnatally in human pediatric patients and no additional testing in juvenile animals may be necessary. The exception would be local effects from agents administered by the inhalation route of administration. Overall, exposure of monkeys during the fetal and early postnatal period (first 3 months) of development covers many of the lung growth and maturation events that occur in pediatric patients up to 8 years of age.

Kidney

The pre- and postnatal development of the macaque kidney is similar to that of humans [21–25]. In both species the kidneys are well developed at birth. The definitive metanephric kidney is formed during the embryonic period (first trimester). Nephrogenesis occurs during the fetal period and is complete before birth in both macaques and humans [22–25]. During fetal development the number of glomeruli per gram of tissue triples between gestation day 50 and 100 but remains relatively constant from gestation day 100 through term and through adult [24]. Immunohistochemical assessment of the expression patterns of several proteins critical to the water balance function of the kidney have been observed as early as the late

VetBooks.ir

first trimester, similar to that of humans [23]. Overall, exposure of monkeys during the fetal period covers most of the major events in kidney development relevant to pediatric patients.

Gastrointestinal Tract

Postnatal development of the stomach involves thickening of the glandular region and the maturation of the pepsin-producing chief cells [26]. It is generally considered that at birth the stomach of the human newborn has low concentrations of acid and pepsin, and these increase with age, but the age at which full maturation occurs varies somewhat between studies. Some investigators have suggested that in humans the concentrations of acid and pepsin do not reach adult concentrations until about 2 years of age [27], whereas other investigators have suggested that adult concentrations are attained within 3 months [28,29]. In macaques (*Macaca fuscata*) the pepsin concentrations in the gastric mucosa of the stomach increase from birth through 3 years of age [30]. The macaque studies further showed that the fetus-specific pepsinogens that facilitate the absorption of milk-derived immunoglobulins (Igs) in lower order species are not present in the monkey [30]. This supports the contention that antibodies secreted in the milk are not absorbed across the primate neonatal gut [9].

The intestines grow in length and diameter during the postnatal period and mature functionally. By late infancy, most gastrointestinal functions are comparable to that of adults; in general, species with a long gestational duration exhibit postnatal development patterns more similar to humans [26]. The human small intestine is well developed at the time of birth [31]. The permeability to macromolecules may be greater during the first 3 months after birth [31].

Overall, the gastrointestinal system seems to be well developed at birth or soon after birth in both macaques and humans. Therefore, for systemically administered agents, sufficient coverage may already have been obtained during the course of pre- and postnatal development studies and additional juvenile toxicity may not be needed. The exception may be local effects from orally administered agents; the absorption of orally administered agents may be greater during the early postnatal period.

Heart

Pre- and postnatal development of the heart seems to be similar in humans and macaques [32]. In both species the heart is well developed at birth. Postnatal development is part of a continuum of changes that begins in the embryonic stages. In general, the period is characterized by the progressive addition and orientation of cellular components. Overall, exposure of monkeys during the fetal period covers most of the major events in heart development relevant to pediatric patients.

Other

The process of retinal vascularization is similar in macaques and in humans but is completed earlier in macaques. The completion of retinal vascularization occurs before birth in monkeys and after birth in humans [33]. Consequently, prenatal exposure of macaques may cover the critical events that occur during retinal vascularization in the newborn human, and a juvenile toxicity study to evaluate retinal neovascularization may not provide added value.

VetBooks.ir

ORGAN SYSTEMS THAT SHOW SIGNIFICANT DEVELOPMENT BEYOND 6 MONTHS OF AGE

Although the organs described above are well developed at the time of birth or within the first 3 months after birth, some organs systems continue to show sustained postnatal development to adulthood. These include the skeletal system, the immune system, certain parts of the central nervous system, and the reproductive system.

Bone

Bone development is generally more advanced at birth in monkeys than in humans [34]. The bones begin to form during the embryonic period, with the primary ossification centers appearing during the embryonic and early fetal periods. In macaques secondary ossification centers are either present at birth or appear within the first 6 postnatal months. Bone ossification in the macaque at birth is equivalent to that of a 5- to 6-year-old human [35]. During the postnatal period, long bones increase in length at the epiphyseal growth plate. The bones continue to increase in length until the growth plate becomes fused in adulthood. Increases in bone diameter occur by the deposition of new bone on the periosteal surface. The cessation of long bone growth with epiphyseal fusion occurs between about 2-6 years of age in macaques [34,36,37]. The long bones of macaques lengthen at a relatively steady rate throughout the postnatal period, with the exception of an initial growth spurt during the first year and a second spurt during puberty [38]. Postnatal growth and development of the macaque jaw bone occurs to the greatest extent during the first year [39].

Skeletal growth and maturation is a continuous process that occurs during the pre- and postnatal periods to adulthood. Therefore disruptions in this process at any time during development could potentially affect growth. However, disruptions in the prenatal and early postnatal periods are likely to have the most notable effects because this is when the skeletal structures are being formed and the ossification centers appear. Therefore, if a pre- and postnatal development study is conducted in macaques and fetal and neonatal exposure occurs without adversely affecting development, then it is unlikely that exposure later in development will reveal new toxicities. Any later toxicity is likely to be similar to those observed at earlier time points but to a lesser extent. A good example of this is the class of angiogenesis inhibitors that produce marked skeletal abnormalities due to a disruption of the blood supply to the developing bones when exposure occurs during the prenatal period. However, when exposure occurs in juvenile animals, an interruption in growth of the long bones can occur due to inhibition of angiogenesis in the bone growth plates, but this effect is less dramatic than that observed in fetuses and may be reversible upon cessation of treatment [40,41]. Overall, exposure of monkeys during the fetal period covers a sensitive period in bone maturation that is equivalent to events that occur in pediatric humans up to 6 years of age.

Brain

The postnatal morphological development of the monkey and human brains has been extensively reviewed by Watson et al. [42], and the functional development has been reviewed by Wood et al. [43]. The monkey brain develops in a similar pattern to the human brain, but

VetBooks.ir

there are some differences in the relative timing of the individual events. In the macaque the most rapid growth in both total brain volume and white matter is from birth to approximately 4 months and is consistent with the emergence of cognitive abilities [44]. In both humans and macaques neurogenesis begins early during embryonic development and continues during the fetal period [45]. At birth the majority of neurogenesis is complete with the exception of a few structures, including the cerebellar cortex and the dentate gyrus of the hippocampus, in which neurogenesis continues for some time after birth.

In both humans and monkeys there are considerable postnatal changes in hippocampal structure and function [46]. At birth humans have 70-80% of the adult number of dentate gyrus cells and the rhesus macaque has 50-60% [47]. In macaques the majority of the postnatal neurogenesis in the dentate gyrus occurs within the first 3 months, but gradual increases continue to occur through about 1 year of age [48]. A similar pattern occurs in humans but extends through at least the first 5 postnatal years [49–51]. A small amount of neurogenesis in the dentate gyrus may also continue throughout life [42]. Cell production in the cerebellum is completed after 2-3 postnatal months in the macaque but extends to the first postnatal year in human infants [42,52].

Synaptogenesis begins prenatally, increases through to infants (2 months in macaques, 2 years in humans), and then decreases to adulthood such that adults have approximately 50% of the synapses that are present in infants [52,53]. The process of reduction and refining of synapses from infancy to adulthood is referred to as pruning.

The functional abilities of the central nervous system develop postnatally in all species [43]. NHPs are more advanced at birth, or develop more rapidly, for many functional central nervous system end points relative to humans. In monkeys, the tactile, auditory, and visual systems are more advanced at birth than in humans, and locomotion and dexterity develop faster in macaques than in humans. Cognitive development occurs postnatally but cannot be directly compared with that in humans.

Although the developing brain may be more susceptible to potential neurotoxicants than the adult brain, an important determinant of the potential risk depends on whether the molecule can first gain access to the brain and then interfere with cellular processes. In the adult the blood-brain barrier prevents many chemicals and macromolecules from accessing the brain. However, the timing of the development of the human or NHP blood-brain barrier has not been well established. Few studies of blood-brain barrier development and function have been conducted in humans and no studies have been conducted in monkeys. Studies of mice, guinea pigs, rats, cats, chicks, opossums, wallabies, and sheep have been conducted and have produced conflicting results [42,54–56]. Some studies have shown the blood-brain barrier to be not completely mature at birth, whereas other studies have shown the opposite (a mature blood-brain barrier at birth). The only studies of humans consist of histology studies that show the presence of tight junctions of the blood-brain barrier as early as 7 weeks of gestation [55] and a study in which fresh human fetuses (16-27 weeks' gestation) were injected with dye and showed no penetration of dye into the brain [57]. However, human fetuses and newborns have been shown to have higher concentrations of plasma proteins in the cerebrospinal fluid than adults, leading to the presumption that the blood-brain barrier is immature in humans at birth and does not reach maturity until about 6 months of age [54,58,59]. Since the evidence for an immature blood-brain barrier in humans is indirect and sparse and there is no information on NHPs, a better understanding of blood-brain barrier development in primates is clearly needed before any meaningful extrapolations to humans can be made.

VetBooks.ir

Overall, because a considerable amount of postnatal development of the central nervous system occurs in macaques and humans, a juvenile toxicity study may be needed to evaluate potential effects on brain development for those agents that can cross the blood-brain barrier and for which the brain may be a potential target organ for toxicity.

Sexual Maturity

Many NHP studies exploring reproductive physiology have used the rhesus macaque. However, in recent years the cynomolgus macaque has been more frequently used because this is the only pharmacologically relevant species for the developmental and reproductive toxicity testing for biopharmaceuticals that have primate-restricted pharmacology [8,60].

Adrenal Maturation

Maturation of the zona reticularis of the adrenal cortex is essentially complete by 3 months of age in the rhesus macaque. An increase in the secretion of the adrenal androgen dehydroepiandrosterone and its sulfate marks the maturation of the adrenal cortex [61]. In rhesus macaques these androgens peak at about 6-8 weeks of age in both males and females, whereas in humans the concentrations increase throughout the first two decades of life.

Males

A cross-species comparison of the postnatal development of the male reproductive tract was reviewed by Marty et al. [62]. In humans the testes descend before birth. However, in macaques, although the testes are descended at birth they regress postnatally and descend again at approximately 3 years of age [63,64]. At 1-3 years of age the macaque testes are small and weigh approximately 0.04% of body weight. Testis size increases rapidly during puberty and comprise about 0.09% of body weight by 3.5 years of age and 0.24% of body weight by 4 years of age [65]. The immature monkey testis is characterized by the presence of only Sertoli cells and undifferentiated spermatogonia [66]. Until the prepubertal period there is a slow increase in testicular weight, tubular diameter, and the number of seminiferous cords [65].

A number of methods have been used to determine the timing of sexual maturation in the cynomolgus macaque, including age, weight [67], histopathology [68,69], orchidometry [70], and the presence of sperm in the ejaculate [71]. Based on histopathological examination of the testes, sexual maturation can be divided into six grades (1, immature; 2, prepuberty; 3, onset of puberty; 4, puberty; 5, early adult; 6, adult) [68,69]. Overall, the onset of sexual maturity in male cynomolgus macaques occurs at approximately 4-5.5 years. Interestingly, using the method of counting sperm in the ejaculate, sexual maturation was shown to occur approximately 2 years earlier in cynomolgus macaques of Mauritian origin compared with animals from the Asian mainland [71].

The control of puberty in male rhesus macaques consists of two major postnatal phases of gonadotropin secretion. The first occurs during the first 6 months of age, peaking at about 3 months, and the second begins at about 3 years of age, increasing through adulthood. The second surge marks the beginning of puberty [72]. The gonadotropin surges are associated with high rates of Sertoli cell proliferation [73–76], whereas in the period between these surges, Sertoli cell proliferation occurs but at a reduced rate [65,73,76]. The proliferation of undifferentiated spermatogonia is less dependent on gonadotropin stimulation than that of Sertoli cells.

VetBooks.ir

The steroid hormone-producing cells of the testes, Leydig cells, are prominent during fetal life in macaques. During the first year, Leydig cells decrease in number and dedifferentiate, entering a period of suspended development. By the end of the third year, the Leydig cells redifferentiate and begin to produce the primary steroid hormone testosterone [64]. The testosterone concentrations increase from approximately 30 to 250 ng/100 mL in immature male macaques to approximately 230 to 1211 ng/100 mL in mature macaques [77]. Spermatogonia in the testes become more numerous by the end of the first year, but the earliest appearance of spermatozoa is approximately 3 years of age.

Overall, for males the period between 3 months and 3 years of age is relatively quiescent with regard to testicular development [78]. Changes in the seminiferous tubule are minimal and are limited to proliferation of Sertoli cells and undifferentiated spermatogonia. Therefore, the most sensitive periods for potential disruption of normal testicular development would be during the first 3 months of life and during puberty.

Females

A cross-species comparison of the postnatal development of the female reproductive tract was reviewed by Beckman and Feuston [79]. In humans and monkeys there is an early postnatal increase in gonadotropin secretion, followed by a reduction lasting from late infancy to the end of the prepubertal period [80,81]. Adrenal androgen concentrations seem to be fairly constant throughout development in female macaques and are similar to those in adults [82]. In rhesus macaques menarche occurs at approximately 2.5 years of age [83]. Following menarche, there is a period of short luteal phases and anovulation before the monkeys develop normal menstrual cycles [83,84]. Because of this there is a lapse of about 12-18 months between the time of menarche and fertility [83,84]. A postmenarchal period of anovulation may also occur in humans [85].

Studies of the rhesus monkey [81,86–88] suggest that γ-aminobutyric acid inhibits the gonadotropin-releasing hormone (GnRH) pulse during childhood. The onset of puberty is associated with a decrease in γ-aminobutyric acid and an increase in excitatory amino acids and the reactivation of the GnRH pulse generator [89,90]. Estradiol concentrations increase in the initial stages of puberty: at 2.5-3 years in NHPs [86,91] and at 8-10 years in humans [92]. In recent years kisspeptin has been shown to be a critical regulator of the onset of puberty. Hypothalamic kisspeptin directly activates GnRH neurons to stimulate the reproductive axis; this occurs independent of the pubertal increase in circulating estradiol [93,94]. Leptin and a leptin signals also are required to achieve puberty and to maintain cyclicity and reproductive function in NHPs and humans [95].

Although puberty is a postnatal event that could be affected by perturbations during childhood, the evaluation of sexual maturation and puberty in macaques is not feasible. Sexual maturation cannot be evaluated in pre- and postnatal development studies of macaques because the time between birth and sexual maturity is about 3 years for females and 5 years for males. Clearly, it is not practical to dose NHPs for this prolonged period of time. Also, the exact timing of sexual maturation varies between animals, so designing a study to capture the transition from immaturity to fecundity would be difficult. In addition, reproductive potential cannot be accurately evaluated in NHPs because of their low natural fertility rate and high spontaneous abortion rate [4,96]. Hormones can be evaluated in immature and mature animals, and menstrual cycles and semen analysis can be conducted in mature animals.

VetBooks.ir

Immune System

The prenatal development of the macaque immune system is very similar to the human immune system [97,98]. At birth both macaques and humans have a well-developed immune system with regard to the cellular components and have the potential to generate immune responses [99]. All of the major cell types—T, B, and natural killer cells—are present before birth, and demarcation of the splenic architecture occurs during the fetal period. Immune competence is established postnatally.

Postnatal development of the immune system has been studied using various approaches such as blood immunophenotyping, T-cell-dependent antibody response, analysis of lymphocyte proliferation and natural killer cell activity, and immunocytochemical localization of immune cell populations [98,100–103]. These data collectively demonstrate general functionality of the cynomolgus monkey immune system in early postnatal life and striking similarities to human immune system development.

Serum Ig concentrations can be used as a marker for age-related humoral immune function in the cynomolgus monkeys [98,104]. At birth serum IgG concentrations are high and serum IgA and IgM concentrations are low. The IgG in the serum at birth is believed to be maternally derived because IgG, but not IgM or IgA, can cross the primate placenta during gestation. The IgG concentrations decrease during the first 6 months of life due to clearance of the maternal antibodies from the infant's serum; they increase thereafter when the monkeys start to generate their own IgG [98,104]. IgA and IgM concentrations increase from birth to adulthood. The Ig concentrations in the serum reach adult concentrations by about 5-9 years of age, suggesting that the immune system continues to mature during this time period [104]. Analysis of lymphocytes for cell surface markers have shown that the relative proportion of lymphocyte subsets with resting phenotype decreased with increasing age, whereas the subpopulations associated with activation gradually increased with age, reaching adult levels by about 5-10 years of age [104].

Although the immune system matures from birth to adulthood, the first 3-6 months is a critical period in the acquisition of immune competence. A number of macaque studies evaluating the ability of neonatal and infants to mount an immune response to vaccination have been conducted. These studies show that the ability of the neonate to mount an immune response is highly dependent on the nature of the immunogen administered and the age at which it is immunized. Immunization of newborn macaques with vaccinia virus vectors expressing measles virus proteins produced neutralizing antibody titers and cytotoxic T-lymphocyte responses that protected the neonate from viremia and rash [105], whereas vaccination with recombinant bacillus Calmette-Guérin-expressing measles virus nucleoprotein provided only partial protection [106]. Newborn macaques responded well to immunization with a live attenuated measles vaccine with both antibody and cytotoxic T-lymphocyte responses but responded poorly to DNA vaccines expressing measles virus proteins [107]. Immunization with virus replicon particles that express the measles virus proteins resulted in similar sustained levels of high-titer neutralizing antibodies and interferon-γ producing memory T cells in both infant (4 months) and juvenile macaques (1-2 years) [108]. Inoculation of 4- to 6-month-old rhesus monkeys with cytomegalovirus showed that by this age they were able to mount humoral immune responses sufficient to prevent disease [109]. At 3-6 months of age macaques showed robust T-cell-dependent antibody responses to immunization with keyhole limpet hemocyanin and tetanus toxoid [110].

VetBooks.ir

Overall, the prenatal development of the immune system is similar in macaques and humans and is well developed at birth. Immune competence is established during the first 3-6 months after birth. Therefore, exposure during the fetal period and the neonatal period in macaques covers sensitive periods in immune system development that are relevant to the pediatric population.

RATIONALE FOR NOT CONDUCTING A NONCLINICAL STUDY USING NHPs

In most organ systems the events that occur postnatally are part of a continuum of events that was initiated during the embryonic stage of development. The growth and maturation of the organ systems occurs at the greatest rate during the fetal period and the early postnatal period. For macaques the most rapid period of postnatal development occurs during the first 3 months, with the exception of the reproductive system, which matures at puberty. The period from 3 months to 3 years in macaques represents a protracted period of development in which a slow, steady progression in the maturation of the organ systems occurs. Therefore, for those organ systems that are relatively well developed at birth or soon after birth and have been evaluated for effects on embryo/fetal development in a pre- and postnatal development study, conducting additional juvenile toxicity studies may not provide sufficient additional information to justify conducting the study.

RATIONALE FOR CONDUCTING A NONCLINICAL STUDY IN NHPs

When the therapeutic agent is intended to be administered only to pediatric patients, then a justification could be made for conducting all of the general toxicology studies in juvenile animals at a developmentally equivalent age. In this case a two-species assessment (rodent and nonrodent) would likely be required unless there is a specific rationale for using a single species, for example, species-restricted pharmacology. The species most commonly used for small-molecule pharmaceutical drugs are rats and dogs. NHPs may be used in place of dogs if the dog is not a relevant species based on metabolic, pharmacological, or physiological differences between dogs and humans. For biopharmaceuticals, it using only a single species, the cynomolgus macaque, is most common because most biopharmaceuticals are pharmacologically active only in primates.

It is, however, infrequently the case that pharmaceuticals are developed only for pediatric indications. A more common scenario is that the agent is developed for an adult population first and then moves into a progressively younger patient population as information about safety in human adults becomes available. In this case the general toxicology program normally consists of toxicology studies using young adult animals. As described above, the toxicology data obtained from young adult animals and from developmental toxicity studies may be sufficient to justify the use of the therapeutic in pediatric patients without needing to conduct toxicity studies in juvenile animals. However, if developmental toxicity studies have not been conducted, or if embryo/fetal or neonatal exposure to the therapeutic did not

VetBooks.ir

occur or would not be expected, then a study evaluating the effect of treatment in juvenile animals may be warranted. This study would ordinarily be conducted in rodents unless there was a specific rationale for using a nonrodent species. Phylogenetic similarity alone is not a sufficient justification to select an NHP over a rodent species. Although rodents are born at a developmental immature age relative to NHPs, in many cases studies can be designed to evaluate the critical windows that are relevant to pediatric patients even when the relative timing between species differs.

Since the central nervous system is actively developing in children, agents that target the central nervous system and can cross the blood-brain barrier may be candidates for testing in a juvenile toxicity study. This would apply mostly to small-molecule drugs because large-molecule biopharmaceuticals are generally too large to cross the blood-brain barrier and therefore would be unlikely to have central nervous system effects, even if the target was present in the brain. Therefore, for biopharmaceuticals an NHP study to evaluate the effect on cognitive function would be unlikely to provide value. It should also be noted that the tests available for learning and memory in monkeys are relatively insensitive [111] and may not provide much information, even for those drugs that do cross the blood-brain barrier.

Maturation of the immune system and growth and maturation of the skeletal system continues from birth through maturity. Therefore, if the development of these systems has not been evaluated in other studies and these systems may be targets for the therapeutic agent, then a juvenile study may be warranted. This would apply to both small-molecule drugs and biopharmaceuticals and for biopharmaceuticals for which the NHP may be the only pharmacologically relevant species.

CONCLUSION

The potential risk to postnatal and juvenile development needs to be considered and assessed before the inclusion of children in clinical studies. If the existing nonclinical data are insufficient to justify the use in the intended patient population, then a juvenile toxicity study may be warranted. The NHP is not the recommended species for juvenile toxicity testing but may be used when scientifically justified.

References

[1] ICH (International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use) M3 (R2). Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals; 2009. www.ICH.org

[2] Bailey GP, Wise LD, Buschmann J, Hurtt M, Fisher JE. Pre- and postnatal developmental toxicity study design for pharmaceuticals. Birth Defects Res (Part B) 2009;86:437–45.

[3] Cappon GD, Bailey GP, Buschmann J, Feuston MH, Fisher JE, Hew KW, et al. Juvenile animal toxicity study designs to support pediatric drug development. Birth Defects Res (Part B) 2009;86:463–9.

[4] Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicology studies in nonhuman primates. Birth Defects Res (Part B) 2009;86:446–62.

[5] Morford LL, Bowman CJ, Blanset DL, Bogh IB, Chellman GJ, Halpern WG, et al. Preclinical safety evaluations supporting pediatric drug development with biopharmaceuticals: strategy, challenges, current practices. Birth Defects Res (Part B) 2011;92:359–80.

[6] Makori N, Watson RE, Hogrefe CE, Lalayeva N, Oneda S. Object discrimination and reversal learning in infant and juvenile non-human primates in a non-clinical laboratory. J Med Primatol 2013;42:147–57.
[7] Stewart J. Developmental toxicity testing of monoclonal antibodies: an enhanced pre- and postnatal study design option. Reprod Toxicol 2009;28:220–5.
[8] Weinbauer GF, Luft J, Fuchs A. The enhanced pre- and postnatal development study for monoclonal antibodies. Methods Mol Biol 2013;947:185–200.
[9] Martin PL, Weinbauer GF. Developmental toxicity testing of biopharmaceuticals in nonhuman primates: previous experience and future directions. Int J Toxicol 2010;29:552–68.
[10] Bowman CJ, Breslin WJ, Connor AV, Martin PL, Moffat GJ, Sivaraman L, et al. Placental transfer of Fc-containing biopharmaceuticals across species, an industry survey analysis. Birth Defects Res (Part B) 2013;98:459–85.
[11] Kuo TT, Baker K, Yoshida M, Qiao S, Aveson VG, Lencer WI, et al. Neonatal Fc receptor: from immunity to therapeutics. J Clin Immunol 2010;30:777–89.
[12] Zoetis T, Hurtt ME. Species comparison of lung development. Birth Defects Res (Part B) 2003;68:121–4.
[13] Thurlbeck WM. Postnatal growth and development of the lung. Am Rev Respir Dis 1975;111:803–44.
[14] Hislop A, Howard S, Fairweather DV. Morphometric studies on the structural development of the lung in *Macaca fascicularis* during fetal and postnatal life. J Anat 1984;138:95–112.
[15] Plopper CG, Alley JL, Weir AJ. Differentiation of tracheal epithelium during fetal lung maturation in the rhesus monkey *Macaca mulatta*. Am J Anat 1986;175:59–71.
[16] Plopper CG, Weir AJ, Nishio SJ, Cranz DL, St George JA. Tracheal submucosal gland development in the rhesus monkey, *Macaca mulatta*: ultrastructure and histochemistry. Anat Embryol 1986;174:167–78.
[17] Van Winkle LS, Fanucchi MV, Miller LA, Baker GL, Gershwin LJ, Schelegle ES, et al. Epithelial cell distribution and abundance in rhesus monkey airways during postnatal lung growth and development. J Appl Physiol 2004;97:2355–63.
[18] Evans MJ, Fanucchi MV, Van Winkle LS, Baker GL, Murphy AE, Nishio SJ, et al. Fibroblast growth factor-2 during postnatal development of the tracheal basement membrane zone. Am J Physiol Lung Cell Mol Physiol 2002;283:L1263–70.
[19] Evans MJ, Fanucchi MV, Plopper CG, Hyde DM. Postnatal development of the lamina reticularis in primate airways. Anat Rec 2010;293:947–54.
[20] Coppens JT, Plopper CG, Murphy SR, Van Winkle LS. Postnatal lung development of rhesus monkey airways: cellular expression of Clara cell secretory protein. Dev Dyn 2009;238:3016–24.
[21] Zoetis T, Hurtt ME. Species comparison of anatomical and functional renal development. Birth Defects Res (Part B) 2003;68:111–20.
[22] Batchelder CA, Lee CC, Matsell DG, Yoder MC, Tarantal AF. Renal ontogeny in the rhesus monkey (*Macaca mulatta*) and directed differentiation of human embryonic stem cells towards kidney precursors. Differentiation 2009;78:45–56.
[23] Batchelder CA, Lee CC, Martinez ML, Tarantal AF. Ontogeny of the kidney and renal developmental markers in the rhesus monkey (*Macaca mulatta*). Anat Rec 2010;293:1971–83.
[24] Batchelder CA, Keyser JL, Lee CC, Tarantal AF. Characterization of growth, glomerular number, and tubular proteins in the developing rhesus monkey kidney. Anat Rec 2013;296:1747–57.
[25] Tarantal AF, Han VK, Cochrum KC, Mok A, daSilva M, Matsell DG. Fetal rhesus monkey model of obstructive renal dysplasia. Kidney Int 2001;59:446–56.
[26] Walthall K, Cappon GD, Hurtt ME, Zoetis T. Postnatal development of the gastrointestinal system: a species comparison. Birth Defects Res (Part B) 2005;74:132–56.
[27] Deren JS. Development of structure and function in the fetal and newborn stomach. Am J Clin Nutr 1971;24:144–59.
[28] Hyman PE, Feldman EJ, Ament ME, Byrne WJ, Euler AR. Effect of enteral feeding on the maintenance of gastric acid secretory function. Gastroenterology 1983;84:341–5.
[29] DiPalma J, Kirk CL, Hamosh M, Colon AR, Benjamin SB, Hamosh P. Lipase and pepsin activity in the gastric mucosa of infants, children, and adults. Gastroenterology 1991;101:116–21.
[30] Kageyama T, Tanabe K, Koiwa O. Development-dependent expression of isozymogens of monkey pepsinogens and structural differences between them. Eur J Biochem 1991;202:205–15.
[31] de Zwart LL, Haenen HE, Versantvoort CH, Wolterink G, van Engelen JG, Sips AJ. Role of biokinetics in risk assessment of drugs and chemicals in children. Regul Toxicol Pharmacol 2004;39:282–309.

VetBooks.ir

[32] Hew KW, Keller KA. Postnatal anatomical and functional development of the heart: a species comparison. Birth Defects Res (Part B) 2003;68:309–20.
[33] Gariano RF, Luisa Iruela-Arispe M, Hendrickson AE. Vascular development in primate retina: comparison of laminar plexus formation in monkey and human. Invest Ophthalmol Vis Sci 1994;35:3442–55.
[34] Zoetis T, Tassinari MS, Bagi C, Walthall K, Hurtt ME. Species comparison of postnatal bone growth and development. Birth Defects Res (Part B) 2003;68:86–110.
[35] Michejda M. Growth standards in the skeletal age of rhesus monkey (*M. mulatta*) chimpanzee (*Pan troglodytes*) and man. Dev Biol Stand 1980;45:45–50.
[36] Fukuda S, Cho F, Honjo S. Bone growth and development of secondary ossification centers of extremities in the cynomolgus monkey (*Macaca fascicularis*). Exp Anim 1978;27:387–897.
[37] Fukuda S, Matsuoka O. Comparative studies on maturation process of secondary ossification centers of long bones in the mouse, rat, dog and monkey. Exp Anim 1980;29:317–26.
[38] Watts ES, Gavan JA. Postnatal growth of nonhuman primates: the problem of the adolescent spurt. Hum Biol 1982;54:53–70.
[39] McNamara JA, Graber LW. Manibular growth in the rhesus monkey (*Macaca mulatta*). Am J Phys Anthropol 1975;42:15–24.
[40] Ryan AM, Eppler DB, Hagler KE, Bruner RH, Thomford PJ, Hall RL, et al. Preclinical safety evaluation of rhuMAbVEGF, an antiangiogenic humanized monoclonal antibody. Toxicol Pathol 1999;27:78–86.
[41] Patyna S, Arrigoni C, Terron A, Kim TW, Heward JK, Vonderfecht SL, et al. Nonclinical safety evaluation of sunitinib: a potent inhibitor of VEGF, PDGF, KIT, FLT3, and RET receptors. Toxicol Pathol 2008;36:905–16.
[42] Watson RE, Desesso JM, Hurtt ME, Cappon GD. Postnatal growth and morphological development of the brain: a species comparison. Birth Defects Res (Part B) 2006;77:471–84.
[43] Wood SL, Beyer BK, Cappon GD. Species comparison of postnatal CNS development: functional measures. Birth Defects Res (Part B) 2003;68:391–407.
[44] Malkova L, Heuer E, Saunders RC. Longitudinal magnetic resonance imaging study of rhesus monkey brain development. Eur J Neurosci 2006;24:3204–12.
[45] Difiglia M, Pasik P, Pasik T. Early postnatal development of the monkey neostriatum: a golgi and ultrastructural study. J Comp Neurol 1980;190:303–31.
[46] Paule MG, Green L, Myerson J, Alvarado M, Bachevalier J, Schneider JS, et al. Behavioral toxicology of cognition: extrapolation from experimental animal models to humans: behavioral toxicology symposium overview. Neurotoxicol Teratol 2012;34:263–73.
[47] Coe CL, Kramer M, Czéh B, Gould E, Reeves AJ, Kirschbaum C, et al. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. Biol Psychiatry 2003;54:1025–34.
[48] Jabès A, Lavenex PB, Amaral DG, Lavenex P. Quantitative analysis of postnatal neurogenesis and neuron number in the macaque monkey dentate gyrus. Eur J Neurosci 2010;31:273–85.
[49] Seress L, Ribak CE. Postnatal development of CA3 pyramidal neurons and their afferents in the Ammon's horn of rhesus monkeys. Hippocampus 1995;5:217–31.
[50] Seress L, Abraham H, Tornoczky T, Kosztolanyi G. Cell formation in the human hippocampal formation from mid-gestation to the late postnatal period. Neuroscience 2001;105:831–43.
[51] Seress L. Comparative anatomy of the hippocampal dentate gyrus in adult and developing rodents, non-human primates and humans. Prog Brain Res 2007;163:23–41.
[52] Rakic P, Bourgeois JP, Goldman-Rakic PS. Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. Prog Brain Res 1994;102:227–43.
[53] Levitt P. Structural and functional maturation of the developing primate brain. J Pediatr 2003;143:S35–45.
[54] Adinolfi M. The development of the human blood–CSF–brain barrier. Dev Med Child Neurol 1985;27:532–7.
[55] Mollgard K, Saunders NR. The development of the human blood–brain and blood–CSF barriers. Neuropathol Appl Neurobiol 1986;12:337–58.
[56] Ek CJ, Dziegielewska KM, Habgood MD, Saunders NR. Barriers in the developing brain and neurotoxicology. Neurotoxicology 2012;33:586–604.
[57] Grontoft O. Intracranial haemorrhage and blood–brain barrier problems in the new-born; a pathologico-anatomical and experimental investigation. Acta Pathol Microbiol Scand Suppl 1954;100:8–109.
[58] Rodier PM. Developing brain as a target of toxicity. Environ Health Perspect 1995;103(Suppl. 6):73–6.
[59] Costa LG, Aschner M, Vitalone A, Syversen T, Soldin OP. Developmental neuropathology of environmental agents. Annu Rev Pharmacol Toxicol 2004;44:87–110.

[60] Fuchs A, Buse E, Weinbauer GF. Embryo fetal development studies in nonhuman primates. Methods Mol Biol 2013;947:169–83.
[61] Conley AJ, Moeller BC, Nguyen AD, Stanley SD, Plant TM, Abbott DH. Defining adrenarche in the rhesus macaque (*Macaca mulatta*), a non-human primate model for adrenal androgen secretion. Mol Cell Endocrinol 2011;336:110–6.
[62] Marty MS, Chapin RE, Parks LG, Thorsrud BA. Development and maturation of the male reproductive system. Birth Defects Res (Part B) 2003;68:125–36.
[63] Wislocki GB. Observations on the descent of the testes in the macaque and in the chimpanzee. Anat Rec 1933;57:133–48.
[64] Catchpole HR, Van Wagenen G. Reproduction in the rhesus monkey, *Macaca mulatta*. In: Bourne G.H, editor. The rhesus monkey, vol. II. New York: Academic Press; 1975. p. 132–8.
[65] Simorangkir DR, Ramaswamy S, Marshall GR, Roslund R, Plant TM. Sertoli cell differentiation in rhesus monkey (*Macaca mulatta*) is an early event in puberty and precedes attainment of the adult complement of undifferentiated spermatogonia. Reproduction 2012;143:513–22.
[66] Dreef HC, Van Esch E, De Rijk EP. Spermatogenesis in the cynomolgus monkey (*Macaca fascicularis*): a practical guide for routine morphological staging. Toxicol Pathol 2007;35:395–404.
[67] Smedley JV, Bailey SA, Perry RW, O'Rourke CM. Methods for predicting sexual maturity in male cynomolgus macaques on the basis of age, body weight, and histologic evaluation of the testes. Contemp Top Lab Anim Sci 2001;41:18–20.
[68] Haruyama E, Ayukawa Y, Kamura K, Mizutamari M, Ooshima Y, Tanimoto A. Morphometric examination for development of reproductive organs in male cynomolgus monkeys. Toxicol Pathol 2012;40:918–25.
[69] Haruyama E, Suda M, Ayukawa Y, Kamura K, Mizutamari M, Ooshima Y, et al. Testicular development in cynomolgus monkeys. Toxicol Pathol 2012;40:935–42.
[70] Ku WW, Pagliusi F, Foley G, Roesler A, Zimmerman T. A simple orchidometric method for the preliminary assessment of maturity status in male cynomolgus monkeys (*Macaca fascicularis*) used for nonclinical safety studies. J Pharmacol Toxicol Methods 2010;61:32–7.
[71] Luetjens CM, Weinbauer GF. Functional assessment of sexual maturity in male macaques (*Macaca fascicularis*). Regul Toxicol Pharmacol 2012;63:391–400.
[72] Mann DR, Plant TM. The role and potential sites of action of thyroid hormone in timing the onset of puberty in male primates. Brain Res 2010;1364:175–85.
[73] Marshall GR, Plant TM. Puberty occurring either spontaneously or induced precociously in rhesus monkey (*Macaca mulatta*) is associated with a marked proliferation of Sertoli cells. Biol Reprod 1996;54:1192–999.
[74] Sharpe RM, McKinnell C, Kivlin C, Fisher JS. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. Reproduction 2003;125:769–84.
[75] Plant TM, Ramaswamy S, Simorangkir DR, Marshall GR. Postnatal and pubertal development of the rhesus monkey (*Macaca mulatta*) testis. Ann N Y Acad Sci 2005;1061:149–62.
[76] Simorangkir DR, Marshall GR, Plant TM. Sertoli cell proliferation during prepubertal development in the rhesus monkey (*Macaca mulatta*) is maximal during infancy when gonadotropin secretion is robust. J Clin Endocrinol Metab 2003;88:4984–9.
[77] Bennett WI, Dufau ML, Catt KJ, Tullner WW. Effect of human menopausal gonadotropin upon spermatogenesis and testosterone production in juvenile rhesus monkeys. Endocrinology 1973;92:813–21.
[78] Plant TM. Undifferentiated primate spermatogonia and their endocrine control. Trends Endocrinol Metab 2010;21:488–95.
[79] Beckman DA, Feuston M. Landmarks in the development of the female reproductive system. Birth Defects Res (Part B) 2003;68:137–43.
[80] Dierschke DJ, Weiss G, Knobil E. Sexual maturation in the female rhesus monkey and the development of estrogen-induced gonadotropic hormone release. Endocrinology 1974;94:198–206.
[81] Terasawa E, Fernandez DL. Neurological mechanisms of the onset of puberty in primates. Endocr Rev 2001;22:111–51.
[82] Meusy-Dessolle N, Dang DC. Plasma concentrations of testosterone, dihydrotestosterone, delta 4-androstenedione, dehydroepiandrosterone and oestradiol-17 beta in the crab-eating monkey (*Macaca fascicularis*) from birth to adulthood. J Reprod Fertil 1985;74:347–59.
[83] Resko JA, Goy RW, Robinson JA, Norman RL. The pubescent rhesus monkey: some characteristics of the menstrual cycle. Biol Reprod 1982;27:354–61.

VetBooks.ir

[84] Foster DL. Luteinizing hormone and progesterone secretion during sexual maturation of the rhesus monkey: short luteal phases during the initial menstrual cycles. Biol Reprod 1977;17:584–90.
[85] Rosenfield RL. Clinical review: adolescent anovulation: maturational mechanisms and implications. J Clin Endocrinol Metab 2013;98:3572–83.
[86] Terasawa E, Nass TE, Yeoman RR, Loose MD, Shultz NJ. Hypothalamic control of puberty in the female rhesus macaque. In: Norman RL, editor. Neuroendocrine aspects of reproduction. New York: Academic Press; 1983. p. 149–82.
[87] Kasuya E, Nyberg CL, Mogi K, Terasawa E. A role of γ-amino butyric acid (GABA) and glutamate in control of puberty in female rhesus monkeys: effect of an antisense oligodeoxynucleotide for GAD67 messenger ribonucleic acid and MK801 on luteinizing hormone-releasing hormone release. Endocrinology 1999;140:705–12.
[88] Plant TM. Neurological bases underlying the control of the onset of puberty in the rhesus monkey: a representative higher primate. Front Neuroendocrinol 2001;22:107–39.
[89] Ma YJ, Ojeda SR. Neuroendocrine control of female puberty: glial and neuronal interactions. J Investig Dermatol Symp Proc 1997;2:19–22.
[90] Grumbach MM. The neuroendocrinology of human puberty revisited. Horm Res 2002;57:2–14.
[91] Wilson ME, Gordon TP, Collins DC. Ontogeny of luteinizing hormone secretion and first ovulation in seasonal breeding rhesus monkeys. Endocrinology 1986;118:293–301.
[92] Sizonenko PC. Endocrinology in preadolescents and adolescents. I. Hormonal changes during normal puberty. Am J Dis Child 1978;132:704–12.
[93] Guerriero KA, Keen KL, Millar RP, Terasawa E. Developmental changes in GnRH release in response to kisspeptin agonist and antagonist in female rhesus monkeys (*Macaca mulatta*): implication for the mechanism of puberty. Endocrinology 2012;153:825–36.
[94] Guerriero KA, Keen KL, Terasawa E. Developmental increase in kisspeptin-54 release in vivo is independent of the pubertal increase in estradiol in female rhesus monkeys (*Macaca mulatta*). Endocrinology 2012;153:1887–97.
[95] Chan JL, Mantzoros CS. Leptin and the hypothalamic–pituitary regulation of the gonadotropin–gonadal axis. Pituitary 2001;4:87–92.
[96] Martin PL, Breslin W, Rocca M, Wright D, Cavagnaro J. Considerations in assessing the developmental and reproductive toxicity potential of biopharmaceuticals. Birth Defects Res (Part B) 2009;86:176–203.
[97] Makori N, Tarantal AF, Lu FX, Rourke T, Marthas ML, McChesney MB, et al. Functional and morphological development of lymphoid tissues and immune regulatory and effector function in rhesus monkeys: cytokine-secreting cells, immunoglobulin-secreting cells, and CD5 B-1 cells appear early in fetal development. Clin Diagn Lab Immunol 2003;10:140–53.
[98] Buse E. Development of the immune system in the cynomolgus monkey: the appropriate model in human targeted toxicology. J Immunotoxicol 2005;2:211–6.
[99] Holsapple MP, West LJ, Landreth KS. Species comparison of anatomical and functional immune system development. Birth Defects Res (Part B) 2003;68:321–34.
[100] Hendrickx AG, Makori N, Peterson P. The nonhuman primate as a model of developmental immunotoxicity. Human Exp Toxicol 2002;21:537–42.
[101] Buse E, Habermann G, Osterburg I, Korte R, Weinbauer GF. Reproductive/developmental toxicity and immunotoxicity assessments in the nonhuman primate model. Toxicology 2003;185:221–7.
[102] Burns-Naas LA, Hastings KL, Ladics GS, Makris SL, Parker GA, Holsapple MP. What's so special about the developing immune system? Int J Toxicol 2008;27:223–54.
[103] Weinbauer GF, Frings W, Fuchs A, Niehaus M, Osterburg I. Reproductive/developmental toxicity assessment of biopharmaceuticals in nonhuman primates. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals. New Jersey: Wiley; 2008. p. 379–98.
[104] Terao K. Essentials for starting a pediatric clinical study (3): dynamic changes in early development of immune system in macaque monkeys—the significance from standpoint of preclinical toxicity test using nonhuman primates. J Toxicol Sci 2009;34:SP321–5.
[105] Zhu YD, Rota P, Wyatt L, Tamin A, Rozenblatt S, Lerche N, et al. Evaluation of recombinant vaccinia virus–measles vaccines in infant rhesus macaques with preexisting measles antibody. Virology 2000;276:202–13.
[106] Zhu YD, Fennelly G, Miller C, Tarara R, Saxe I, Bloom B, et al. Recombinant bacille Calmette–Guérin expressing the measles virus nucleoprotein protects infant rhesus macaques from measles virus pneumonia. J Infect Dis 1997;176:1445–53.

[107] Polack FP, Lydy SL, Lee SH, Rota PA, Bellini WJ, Adams RJ, et al. Poor immune responses of newborn rhesus macaques to measles virus DNA vaccines expressing the hemagglutinin and fusion glycoproteins. Clin Vaccine Immunol 2013;20:205–10.
[108] Pan CH, Valsamakis A, Colella T, Nair N, Adams RJ, Polack FP, et al. Modulation of disease, T cell responses, and measles virus clearance in monkeys vaccinated with H-encoding alphavirus replicon particles. Proc Natl Acad Sci U S A 2005;102:11581–8.
[109] Lockridge KM, Sequar G, Zhou SS, Yue Y, Mandell CP, Barry PA. Pathogenesis of experimental rhesus cytomegalovirus infection. J Virol 1999;73:9576–83.
[110] Martin PL, Buse E. Developmental immunotoxicity in nonhuman primates. In: Herzyk DJ, Bussiere JL, editors. Immunotoxicology strategies for pharmaceutical safety assessments. New York: Wiley; 2008. p. 299–317.
[111] Cappon GG, Bowman CJ, Hurtt ME, Grantham LE. Object discrimination reversal as a method to assess cognitive impairment in nonhuman primate enhanced pre- and postnatal developmental (ePPND) studies: statistical power analysis. Birth Defects Res (Part B) 2012;95:354–62.

VetBooks.ir

CHAPTER

27

Immunotoxicology Testing of Monoclonal Antibodies in *Macaca fascicularis*: From Manufacturing to Preclinical Studies

Marie-Soleil Piché[*], *Barbara Mounho-Zamora*[†], *Lynne LeSauteur*[‡], *Leigh Ann Burns-Naas*[§]

[*]Charles River Laboratories, Senneville, QC, Canada

[†]ToxStrategies, Inc., Bend, OR, USA

[‡]Human Health Therapeutics, National Research Council, Montreal, QC, Canada

§Gilead Sciences, Inc., Foster City, CA, USA

OUTLINE

Immunotoxicology Regulatory Considerations 520

Product- and Process-Related Factors and Immunogenicity 523

What Is the Effect of Antidrug Antibodies in NHP Toxicology Studies? 523

Lack of Predictivity Between NHP and Human Immunogenic Response to mAbs 523

What Are the mAb Product and Process Factors that May Impact upon ADAs in NHP Toxicology Studies? 524

Use of In Silico, In Vitro, and In Vivo Studies to Characterize Product- and Process-Related Factors that May Impact upon Immunogenicity 525

http://dx.doi.org/10.1016/B978-0-12-417144-2.00027-5

© 2015 Elsevier Inc. All rights reserved.

State-of-the-Art Immunotoxicology Testing Preclinical Study Designs 525
Study Designs for Preclinical Immunotoxicology Testing in the Cynomolgus NHP 525
Assays Most Commonly Included in NHP Preclinical Studies 527
T-Cell-Dependent Antibody Response 527
Flow Cytometry Assays 528
Cell-Based Assays 529
Biomarkers 529
Immunogenicity 532
Challenges 532
Developmental Immunotoxicology Testing 533
Study Design 533
Points to Consider 537
Study Designs 537
Methodology 538
Clinical Translation 539
Information Gaps 539
References 540

IMMUNOTOXICOLOGY REGULATORY CONSIDERATIONS

Many therapeutic monoclonal antibodies (mAbs) are indicated for the treatment of autoimmune disease (e.g., rheumatoid arthritis, psoriasis, Crohn's disease) or cancer and therefore have a mechanism of action that directly or indirectly involves interacting with one or more components of the immune system. The mechanism by which an mAb interacts with the immune system can be target-mediated and bind directly to the target expressed on immune cells and/or Fc-mediated such as antibody-dependent cell cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). This interaction ultimately results in either the activation or inhibition of the immune system. While the immunomodulatory effects of many mAbs is the intended (desired) pharmacology for clinical efficacy (e.g., immunosuppression to prevent transplant rejection), exaggerated or prolonged pharmacological activity may result in adverse immunomodulation, leading to unpredictable and unintended outcomes (immunotoxic effects), such as cytokine storm, hypersensitivity, infection, and cancer [1].

Various regulatory documents that address different aspects of nonclinical evaluation of immunotoxicity exist. Although these guidelines do not specifically address immunotoxicity testing for mAbs or assessment of immunotoxicity in cynomolgus monkeys, these guidelines can provide important direction on the scientific strategy and regulatory expectation for the immunotoxicity evaluation of mAbs.

In 2000 the Committee for Proprietary Medicinal Products within the European Medicines Evaluation Agency (EMEA; now the European Medicines Agency or EMA) issued the first immunotoxicity guideline for therapeutic products (the *Note for Guidance* first established in 1983 was updated with *Guidance on Immunotoxicity* in 2000) [2]. This guideline is primarily directed toward small-molecule products (vs. biological products like mAbs) and recommends a tiered approach to immunotoxicity testing. It recommends that the initial screening phase for immunotoxicity (tier 1) include standard nonfunctional end points such as changes in hematology, histopathology findings in lymphoid tissue (e.g., spleen, lymph nodes, thymus), as well as functional assays such as changes in lymphocyte subpopulations or in natural killer (NK)

VetBooks.ir

cell activity. If lymphocyte subpopulations or NK cell activity cannot be evaluated in the initial screening phase, then the guideline recommends including the T-cell-dependent antibody response (TDAR) assay. If the data from the tier 1 studies indicate immunotoxicity, then additional functional assays should be performed based on the type of immunotoxic effects observed in tier 1. The EMEA guideline does not specify the type of studies that should be performed in the tier 2 testing; instead, it recommends using a weight-of-evidence approach and that the types of immunotoxicity studies conducted should be based on the nature of the immunotoxic effects observed in tier 1.

In 2002, the US Food and Drug Administration (FDA) issued an immunotoxicity guideline titled "Guidance for Immunotoxicology Evaluation of Investigational New Drugs." Although this FDA guideline is directed toward small-molecule pharmaceuticals and not biological products, the scientific principles, approaches, and models/methodologies presented can be applied to biological products [3]. Similar to the EMEA guideline (2000), the FDA guidance document recommends tiered testing and applying a weight-of-evidence approach in the assessment of immunotoxicity. In addition, the FDA guideline provides a decision tree for the types of studies and assays that should be applied in the tiered immunotoxicity testing approach. In contrast to the EMEA guideline, the FDA guidance document does not recommend measuring NK cell activity in the tier 1 testing. Unlike the EMEA guideline, which does not specify the type of immunotoxicity tests to conduct, the FDA guideline provides a list of additional specialized assays to evaluate the immunotoxicity of small-molecule products, such as the guinea pig maximization test and murine local lymph node assay for topical medical products.

In 2005 harmonization on immunotoxicology testing was achieved, and the International Council on Harmonization (ICH) issued a guideline on the "Immunotoxicity Studies for Human Pharmaceuticals" (ICH S8, 2005). ICH S8 provides guidance on approaches that can be applied in nonclinical testing to identify the potential immunotoxicity of pharmaceuticals [4]. Similar to the EMEA and FDA guidance documents, ICH S8 recommends using standard toxicity studies (STSs) as the initial screening for potential immunotoxic effects of pharmaceuticals and that additional immunotoxicity studies should be based on a weight-of-evidence approach and the nature of the immunotoxic findings observed in the STS. ICH S8 defines immunotoxicity as either undesirable immunosuppression or immunoenhancement, which is the focus of this guideline. Other aspects of immunotoxicity, such as allergenicity or drug-specific autoimmunity, are not addressed in the scope of this document. ICH S8 provides a decision tree for the immunotoxicity testing strategies, and it outlines the type of data that should be evaluated in the STS (tier 1 screening), such as hematology changes, changes in organ weights, histology of immune system organs, and increased incidence of infections and/or occurrence of tumors. If changes reflective of immunosuppression or enhanced activation of the immune system are observed in the tier 1 testing, then further immunotoxicity testing to verify the immunotoxic potential of the pharmaceutical should be performed. In addition to recommending applying a weight-of-evidence approach, ICH S8 also reviews other factors that should be considered in determining the need for more immunotoxicity studies, such as the pharmacological action of the drug, patient population, the drug's structural similarity to compounds known to be immunotoxic, high disposition of the drug in immune cells, and clinical data indicative of immunotoxicity in patients.

VetBooks.ir

Overall, ICH S8 is primarily directed toward small-molecule pharmaceuticals, and because many mAbs are designed to alter immune function, ICH S8 generally does not apply to biological products such as mAbs. For drugs not covered by ICH S8, such as mAbs, the evaluation of potential immunotoxicity of the product will generally be conducted as an integral component of the pharmacological action of the molecule rather than separate studies driven by a "cause for concern" [5]. Many of the principles and studies to evaluate immunotoxicity reviewed in ICH S8, however, can apply to mAbs. For example, the parameters to evaluate adverse effects produced by small-molecule products on immune function incorporated in tier 1 testing are common end points in STSs of mAbs. Likewise, specific assays used to evaluate the immunotoxicity of small molecules, such as the TDAR, measuring NK cell activity, or immunophenotyping, can be used for mAbs. Although the guidance provided in ICH S8 does not specifically focus on evaluating potential immunotoxic liabilities of pharmaceuticals in nonhuman primates (NHPs), many of the parameters used to evaluate the effects on the immune system included in STSs, as well as certain immunotoxicity studies/assays described in ICH S8, can be applied to NHPs.

As described earlier, because many mAbs are intended to alter the immune system, evaluation of the potential immunotoxicity is likely to be a component of the overall pharmacology rather than the specific immunotoxicity studies driven by a cause for concern. This is the primary reason that ICH S8 is not directed to mAbs and actually refers the reader to ICH S6(R1), "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" (1997; addendum incorporated 2011) [6]. Although there is little guidance on immunotoxicity testing provided in ICH S6(R1), it does recommend that the strategies for immunotoxicity testing may require screening studies followed by mechanistic studies to clarify any potential findings or cause for concern. Notably, ICH S6 does explain that routine tiered testing approaches (such as those described for small-molecule products in ICH S8) are not recommended for biological products. Therefore, because many mAbs are designed to stimulate or suppress the immune system, the approach to immunotoxicity testing and the species for in vitro studies used in immunotoxicology studies (if any) should be based on the mechanism of action of the product and important factors such as the intended patient population, duration of use, and unexpected immunotoxicity occurring in patients. The mechanism of this immunotoxicity also needs to be characterized. Although S6(R1) has little guidance for immunotoxicity testing, this guideline does emphasize that the toxicology studies should be conducted in a pharmacologically relevant animal species. Thus, it is critical that any immunotoxicity tests (in vitro or in vivo) conducted for a mAbs should be performed in a pharmacologically relevant animal species (or cells from that animal.

The EMA guideline on biosimilar mAbs (*Guideline on Similar Biological Medicinal Products Containing Monoclonal Antibodies – Nonclinical and Clinical Issues*) came into effect in December 2012 [7]. This guideline addresses the nonclinical and clinical requirements for the demonstration of similarity for the approval of biosimilar mAbs, and it emphasizes that the nonclinical and clinical studies should be designed to detect any potential differences between the proposed biosimilar and reference product and determine the relevance of such differences. It explains that when demonstrating a proposed biosimilar is similar to the reference product, a step-wise approach should be applied to the nonclinical comparative studies. Using this stepwise approach, the guideline recommends that in vitro studies be conducted first, and, based on the data from the in vitro studies, a decision then be made if in vivo studies are deemed

VetBooks.ir

necessary as part of the biosimilarity exercise. Although the scope of this guideline does not address immunotoxicity testing, it does recommend that Fc-mediated effects, such as ADCC or CDC, which may be part of the antibody's mechanism of action, be fully characterized using a relevant assay. The purpose of these comparative assays is to demonstrate that the Fc-mediated effects (which can influence the immunotoxic potential of a mAbs) are similar between the proposed biosimilar and the reference product.

While immunotoxicity testing of mAbs is not specifically addressed in the described regulatory guidelines, several of the scientific principles and strategies can be applied to develop a strategic approach in evaluating the immunotoxicity of mAbs. In addition, many of the immunotoxicity studies and assays described in these guidelines can be used for mAbs as well as performed in cynomolgus monkeys.

CMC characterization of mAbs to reduce potential immunogenicity in NHPs is addressed in subsequent sections of this chapter. Then, preclinical study designs in adult and juvenile NHPs in accordance with the regulatory guidelines are described. Advantages and limitations in the most standard assays included in NHP studies also are described.

PRODUCT- AND PROCESS-RELATED FACTORS AND IMMUNOGENICITY

What Is the Effect of Antidrug Antibodies in NHP Toxicology Studies?

Because of the specificity of mAbs for their targets and the homology of these targets between NHPs and humans, the majority of investigational new drug-enabling preclinical toxicology studies of mAbs are conducted in NHPs. MAbs can be recognized as foreign in NHPs for various reasons, including product- and process-related factors, the route of administration, and species-specific factors, and they therefore generate an immune response. The immune response mounted against the mAbs is usually monitored by measuring serum antidrug antibody (ADA) responses using immunoassays (although cell-mediated responses can also occur). The need to monitor the impact of ADA in preclinical toxicology studies is highlighted in various regulatory guidance documents [3,8,9]. The impact of the ADAs in NHP toxicology studies can vary, ranging from having no effect to having an impact upon mAbs pharmacokinetics (PK) (exposure), pharmacodynamics (PD), safety, and/or efficacy. Thus, the development of ADAs in toxicology studies can potentially affect the interpretation of the study and determination of a safe starting dose for the first-in-human (FIH) studies.

Lack of Predictivity between NHP and Human Immunogenic Response to mAbs

Further complicating the issue is the lack of predictivity of the NHP immunogenicity for the human immunogenicity response (because of fundamental differences in the immune response). Thus, because animal studies, including those conducted in NHPs, are generally not predictive of clinical immunogenicity, ADA responses are measured in clinical studies regardless of whether or not ADAs are observed in the NHP. A recent retrospective analysis of all mAbs on the market in Europe through 2010 [10] highlights the issue of predictivity in ADA responses between humans and NHPs; the incidence of formation of ADA in NHPs and humans was comparable in only 59% of the cases. In addition, in the case where ADA

VetBooks.ir

responses were observed in NHPs and humans, the type of ADA response was different in 59% of those cases. This study highlights the need to first reduce immunogenic responses, as the effect on drug development can be substantial. Second, a better understanding of the factors that play a role in the lack of predictivity in ADA responses between NHPs and humans is necessary. Third, there is a need to develop tools (in vitro/in vivo) that are more predictive of human immunogenicity. Although the immunogenic response between NHPs and humans is not absolutely predictive, as highlighted above, the understanding of the immunogenic response in the context of the toxicology study interpretation is critical.

The reasons for the lack of predictivity between NHPs and humans include differences in the immune system and may also include factors related to consistency of product and process during biomanufacturing of the mAbs for preclinical and clinical studies. Although the relative contribution of these factors in terms of overall predictivity between NHPs and humans is unknown, the increased understanding of these attributes in drug development will help in selecting less immunogenic mAbs. Therefore, drug developers are working on further understanding and controlling the critical quality attributes of mAb products and mAb biomanufacturing processes that may have an impact on immunogenicity by development of in silico, in vitro (using human immune cells) and in vivo tools that aid in immunogenicity prediction and ultimately risk mitigation.

What Are the mAb Product and Process Factors that May Impact upon ADAs in NHP Toxicology Studies?

The requirements for an investigational new drug-enabling toxicology study with regard to test article characterization include appropriate data for identity, activity/potency, structure, purity, stability, and bioavailability [5,11]. Guidelines for biotechnology-derived products, including mAbs, and requirements for biophysical characterization, are outlined in ICHQ6B [12]. Ideally, the mAbs product that is tested in the NHP toxicology studies would be identical to those evaluated in human clinical trials. However, the mAb product tested in NHP toxicology studies is often from a good manufacturing process (GMP)-like production and not the final GMP product and process. If there are critical differences between the mAb product and process used in the good laboratory practice (GLP) toxicology studies and the GMP product used in clinical studies, a toxicology-bridging study may be required to ensure that there is no impact upon mAb product activity, efficacy, and safety (including immunogenicity) in the FIH trial [13].

For companies developing mAbs, it is beneficial for the drug developer to have defined critical quality attributes (CQAs) for the product and critical process parameters (CPPs) that affect immunogenicity as early as possible within development, thus mitigating risk and aiding in the selection of bridging assays. CQAs and CPP definitions are described in ICHQ8 [14]; CQAs are physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality. A CPP is a parameter whose variability has an impact upon a CQA and should be monitored or controlled to ensure the process produces the desired quality. However, a major challenge is that the CQAs and CPPs that may have an impact upon immunogenicity are not well defined, are product and process specific, and need predictive analytical methods to measure and assess effect of any changes. Common product- and process-related factors that may impact upon immunogenicity of mAbs in NHPs are outlined in Table 27.1.

VetBooks.ir

TABLE 27.1 Product- and Process-Related Factors that May Impact upon Immunogenicity of Monoclonal Antibodies in Nonhuman Primates

	Factors Potentially Impacting Immunogenicity
Product	Protein structure: sequence and chemical modifications, glycosylation, charge variants, T- and B-cell epitopes, glycoforms, size variants, deamidation, isomerization, oxidation, isoforms, degradants, aggregates, particulates, stability Formulation: excipients, container, closure
Process	Host cell proteins, DNA, chromatography ligands used for purification (e.g., protein A), endotoxin; cell line selection, upstream processing (e.g., media, selection agents), downstream processing (e.g., salts, viral inactivators)

Use of In Silico, In Vitro, and In Vivo Studies to Characterize Product- and Process-Related Factors that May Impact upon Immunogenicity

Increased understanding of mAb product- and process-related factors that may impact upon immunogenicity (both humoral and cell mediated components) and the underlying mechanisms affecting immunogenicity of mAbs create the potential to reduce immunogenicity, and will help mitigate risk. Currently, numerous laboratories are working on in silico identification of T-cell and B-cell epitopes, tools to assess propensity to aggregate, in vitro human T-cell- and antigen-presenting cell-binding and proliferation assays, cytokine release assays, and in vivo models [15–17] and identification of process and product related factors that may impact upon immunogenicity [18,19]. Ultimately, a combination of these tools and the use of these tools in lead candidate selection and monitoring of biomanufacturing processes will likely increase predictivity, help select less immunogenic mAb drug candidates, and control manufacturing changes. Additional future challenges for identification of product and process factors effecting immunogenicity will lie in the new mAb products in development, including biospecifics, fragments, antibody drug conjugates, and retrospective analysis of the effect of predictive assays on drug development and reduction of immunogenic response.

STATE-OF-THE-ART IMMUNOTOXICOLOGY TESTING PRECLINICAL STUDY DESIGNS

Study Designs for Preclinical Immunotoxicology Testing in the Cynomolgus NHP

NHPs are often considered the most relevant species to perform preclinical studies for the testing of biotechnology-derived investigational new drugs, including mAbs. The close homology of primate amino acid sequences to human molecules and receptor targets enables cross-reactivity, biological activity, and toxicology assessments of the mAbs in NHPs. Moreover, many reagents cross-react with NHP molecules and can be used for preclinical evaluations. However, when compared with rodent immunotoxicity studies, standardized procedures, assays, and protocols are generally less available for NHPs.

VetBooks.ir

Standard immunotoxicology study designs for mAbs are usually incorporated and performed within 28-day or 3-, 6-, or 9-month toxicology studies, depending on the dosing and recovery periods needed. However, 6-month studies are typically considered as being of sufficient duration, unless there are specific concerns, such as carcinogenicity, that need to be addressed. Longer study durations generally do not provide additional predictive value to a nonclinical data package [20]. Anywhere from one to all of the end points described later in this section can be included in such studies. However, blood volume requirements and collection time points may become a limiting factor when several assessments need to be conducted in a short amount of time. To overcome this barrier, several options can be considered, such as microsampling, the addition of satellite groups (although this is not routinely done for NHP studies), the reduction of time points for some of the assessments, and increasing the size of the animals used in a study. Usually, three animals per sex per group are included for the main study and two animals per sex per group for the recovery period. In a standard toxicology study for which immunotoxicology end points are added, four dose groups are routinely included: one control group and three treated (low, mid, and high dose) mAb groups. A table listing the most standard assays included in NHP studies, along with the suggested sample collection time points and considerations, is included at the end of this section as a reference (Table 27.2).

TABLE 27.2 Review of Nonhuman Primate Immunology Assessments

Assessments	Timing	Comments
Immunophenotyping	• Twice before treatment • End of dosing/recovery • In-study time points to be added depending on the length of the study or the nature of the compound	• Because of the high interanimal variability observed in the NHP, at least two predose occasions are recommended to determine whether modulations observed during the study are caused by normal variability or test article-related effects • Interpretation is done on individual animal profiles as well as on the overall predose and control group range
TDAR	• Once before KLH administration • For IgM: 5-10 days post KLH administration • For IgG: 10-21 days post KLH administration[a]	• Because of the high interanimal variability, individual animal profiles need to be taken into account in interpretation • Statistical analysis as well as qualitative interpretation is recommended
PD markers	• Twice before treatment • In-study time points to be added depending on the length of the study and on the pharmacology of the drug	• Comparison to predose is important • Whenever possible, data should be normalized with appropriate controls • Stimulation index/percentage of inhibition may be useful
Functional cell-based assays	• Twice before treatment • End of dosing/recovery • Additional time points may be added depending on the study length or pharmacology of the drug	• Assay prone to high variability because of experimental limitations • Validation or qualification of the assays is recommended to understand the limitations/performance of the assay

VetBooks.ir

TABLE 27.2 Review of Nonhuman Primate Immunology Assessments—Cont'd

Assessments	Timing	Comments
ELIspot	• End of dosing • Based on the pharmacology of the drug, predose and in-study time points may be required	• Rarely used in mAb testing • The methodology design is driven by the analyte of interest (e.g., incubation duration, matrix)
Complement	• Samples collected before and shortly after dosing (15-30 min and 24 h after dosing)	• Most commonly quantitated: CH50, Bb fragment, C3a, and C5a • Adding a time point at 24 h after dosing to assess the reversibility of the findings may be useful
Cytokines	• Samples collected before and shortly after dosing (15-30 min, 3-6 and 24 h after dosing)	• Panel selected based on the intended purpose of the measurement • Each cytokine has its own profile • Timing is critical • Adding a time point 24 h after dosing to assess the reversibility of the findings may be useful
Immunogenicity	• Before dosing • End of dosing • Additional time points may be added	• Not predictive of immunogenicity in humans • Helps in the interpretation for the toxicology findings (PK, PD, hypersensitivity), safety, and efficacy

Ig, immunoglobulin; KLH, keyhole limpet hemocyanin; PD, pharmacodynamics; PK, pharmacokinetics; TDAR, T-cell-dependent antibody response.

[a]*Following a second administration to assess the secondary immune response, antibodies can be monitored as soon as 7 days after KLH administration.*

Assays Most Commonly included in NHP Preclinical Studies

Multiple assays can be included in a NHP study depending on the anticipated mode of action of a mAb. These assays are often an adaptation of standard immunology methods, many of which can be evaluated by general toxicology and rely on the skills of pathologists examining tissues. In some cases, however, changes in the immune function may not be reflected in the morphology of lymphoid organs or hematology changes, and this is when additional specific immunology tests may be required. As many of these tests can assess immunosuppression and/or immunostimulation, they have the potential to be used for the assessment of efficacy and/or safety of mAbs.

T-Cell-Dependent Antibody Response

The TDAR assay is endorsed by regulatory agencies as a useful immune function test for evaluating the immunotoxic potential of a drug candidate as it involves antigen-presenting cells, B cells, and T cells. It measures the ability of the host species to mount a specific antibody response to an antigen. Briefly, animals are injected with an immunogenic antigen before blood collection for the measurement of specific antibodies against this antigen. In the NHP, different antigens have been used to immunize animals, including keyhole limpet

VetBooks.ir

hemocyanin (KLH). This immunogen is often used for the TDAR assessment in preclinical toxicology studies, and primary and secondary anti-KLH IgM and IgG responses are measured if required. Detailed study designs for the TDAR assays in NHP have been described by Lebrec et al. [21].

Recent advances in the development of immunostimulatory therapeutics, primarily T-cell-stimulating biologics with cross-reactivity limited to the cynomolgus monkey, have presented a need for PD assays capable of measuring increased T-cell activation in monkeys. Therefore, TDAR has recently been evaluated as a potential assessment of pharmacologically mediated immunostimulation in cynomolgus monkeys. A suboptimal dose of KLH was used for this assessment and augmentation of the TDAR response after drug administration was monitored [22]. It was determined that immunostimulation, defined as an increased magnitude of the TDAR, was more clearly demonstrated in monkeys dosed with 0.1 mg of KLH compared with monkeys dosed with 1 and 10 mg of KLH (Figure 27.1). The immunogen dose to administer should, therefore, be selected based on the intended purpose of the study (measurement of immunostimulation or immunosuppression).

Flow Cytometry Assays

Flow cytometry is commonly used in studies to assess immunomodulation as changes in relative frequency and number of lymphoid and myeloid cells in peripheral whole blood,

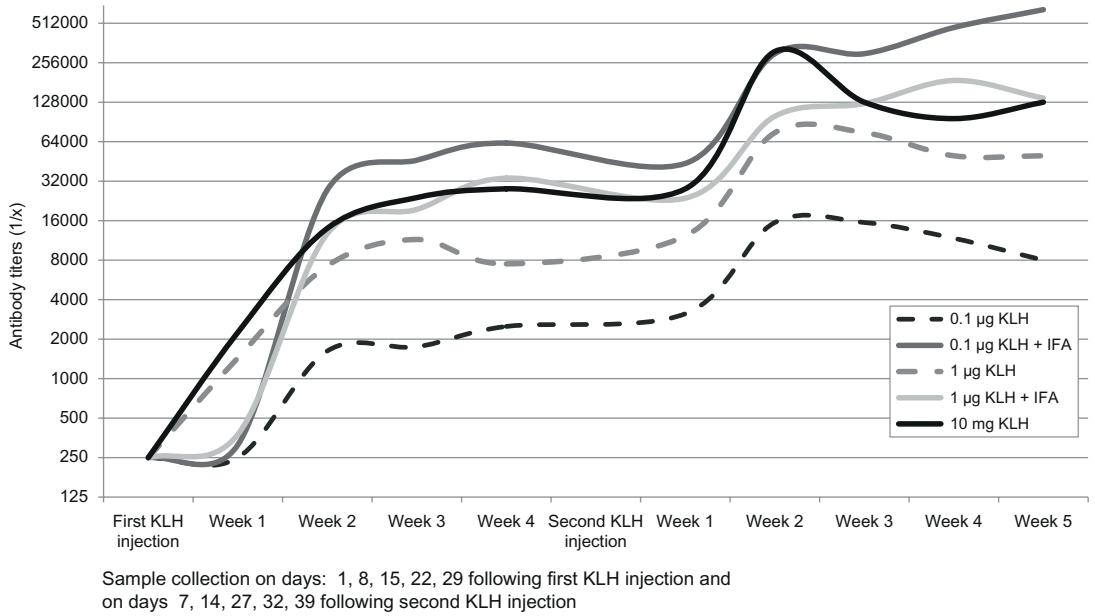


FIGURE 27.1 Primary and secondary immunoglobulin G (IgG) antibody responses to keyhole limpet hemocyanin (KLH) in adult cynomolgus monkeys. Nonhuman primates (NHPs) were challenged by subcutaneous injection with different doses of KLH with or without adjuvant. Measurement of anti-KLH antibodies was performed by enzyme-linked immunosorbent assay of serum collected on the indicated days. Each line represents the average titer from four animals.

VetBooks.ir

spleen, lymph nodes, and/or bone marrow. In NHP studies, flow cytometry is routinely used for assessing the immunotoxicology of a mAb by evaluating the immunophenotype of various cell populations in whole blood, tissue, or other matrices. In addition, receptor occupancy is included in preclinical programs to evaluate the binding of the mAb to target cells or to assess PD markers of interest for further elucidation of the on- or off-target effects of a particular drug [23].

A large panel of antibodies and assays are available to help characterize the impact of a mAb on immune cells. For example, T, B, NK, and monocyte cell subsets in blood and lymphoid tissues, as well as regulatory T cells and adhesion molecules, are often analyzed in NHP studies. Many of these molecules are associated with lineage and can be used to identify subsets as well as altered expression following exposure to drugs. There is also increasing interest in the analysis of intracellular protein expression in lymphoid cells to assess the pharmacologic effects of drugs on defined cell populations. Examples of assays that can be performed in lymphoid subsets include quantitation of various cell signaling molecules such as specific phosphoproteins and intracellular cytokines.

Customization of flow cytometry assays to address specific questions such as activation status, cell death, or functionality of specific cell types can also be used. Several publications describe technical aspects of flow cytometry in NHPs and can be reviewed for further information [24–28]. A recent publication by Krejsa et al. [29] also provides information on the interpretation of immunophenotyping data in NHPs. Some of the key considerations in the interpretation of flow cytometry data concern the animal variability. Because of a larger between-animal than within-animal variability, each animal should serve as its own control to gain power to detect treatment effect. Therefore, the inclusion of at least two pretreatment time points is essential when planning NHP studies including flow cytometry end points.

Cell-Based Assays

Various cell types of the immune system can be assessed using cell-based assays. When a drug targets cellular immunity, regulatory agencies may expect assays of antigen-specific T- or B-lymphocyte proliferation, assessment of various markers of cell activation, or cytokine expression. For example, T-lymphocytes are involved in cell-mediated immunity and are essential for the development and regulation of antibody responses. Their activity can be monitored by various ex vivo assays, including cell proliferation, cell activation (e.g., upregulation/downregulation of specific molecules), cytokine release, or functional assays. Among these, the ELIspot is a useful assay format that can detect specific cytokines (such as interferon-γ) at the single-cell level, whereas the NK cell and the cytotoxic T-lymphocyte (CTL) assays measure the killing activity of NK cells and CTL, respectively. Detailed procedures for these assays may be found in different publications [24,30,31]. Other functional assays may be included depending on the expected mode of the mAb. Basophile function assays are more widely used to monitor hypersensitivity reactions, phagocytosis/oxidative burst assays are assessed when phagocyte function may be targeted, and platelet activation experiments are included when there are specific hematology/coagulation concerns.

Biomarkers

The evaluation of biomarkers, including acute phase proteins, complement split products, cytokines, chemokines, and antibodies (such as total IgG, IgM, and IgE), are part of the

VetBooks.ir

nonfunctional assays performed to evaluate the effects of a drug on the immune system as different drugs have the potential to increase or decrease biomarker concentrations in the body. The analysis of biomarkers may, for example, detect the effect of a drug in the regulation of many processes such as inflammation. The incorporation of these biomarker assessments to monitor toxicity and efficacy within preclinical NHP studies is increasing, although challenges exist regarding the translatability of biomarkers and the biomarker concentrations associated with toxicity [32].

CYTOKINES

The panel of biomarkers to be included in a preclinical toxicology study should be selected based on the intended purpose of their measurement. If cytokines are evaluated, for example, as efficacy end points, time points should be set based on the expected PD action of the mAb. If they are measured to monitor an infusion reaction or a cytokine release syndrome, inflammatory (IL-1β, IL-2, IL-6, IL-8, tumor necrosis factor-α, and interferon-γ)/anti-inflammatory (IL-10, IL-1RA) cytokines should be selected and samples should be collected shortly after dosing (predose, 15-30 min, 3-6 and 24 h after the dose). Understanding the time course of responses is critical because these mediators may only be transiently present following stimulation/activation, which implies that they may be missed if the appropriate time points are not well selected. The transient expression of cytokines is well highlighted in Figure 27.2, where cytokine data from an NHP study is presented. The animals in this study received an anti-CD3 mAb.

Multiple features of cytokines need to be taken into consideration when interpreting data in preclinical studies. They have a short half-life, a transient activity, and may be secreted at low concentrations. Therefore, their biological activity at the cellular level in a local environment may prevent their detection in the systemic circulation. Other considerations concerning cytokine measurements include their biological variability and redundancy. Because a number of cytokines may cause similar responses, they can be interpreted collectively as markers of inflammation. On the other hand, each cytokine may have multiple actions and thus lack specificity for one outcome alone. Finally, cytokine genes are not equally conserved across species and the cytokine release may not be equivalent in all species because of different toxicity susceptibilities. To date, susceptibility of NHPs to cytokine release is not fully understood nor well described, which is important when discussing the translatability of cytokine data obtained in preclinical studies to humans. A detailed review of cytokine measurement, methodologies, limitations, and data interpretation can be found in Tarrant [32].

COMPLEMENT

Three biochemical pathways activate the complement system, all of which converge at the C3 protein level, leading to the formation of the terminal complement complex (C5b-9) [33]. Activation of the complement system leads to cytolysis, chemotaxis, opsonization, immune clearance, and inflammation. Spontaneous or continual activation of the complement system as a result of therapeutic exposure can damage healthy host tissues by producing an anaphylactoid reaction. A series of immunoassays are available to monitor various complement split products in NHPs. Historically, assays to measure complement activity and functions have been hampered by the lack of standardization, making it difficult to compare results between different laboratories [34].

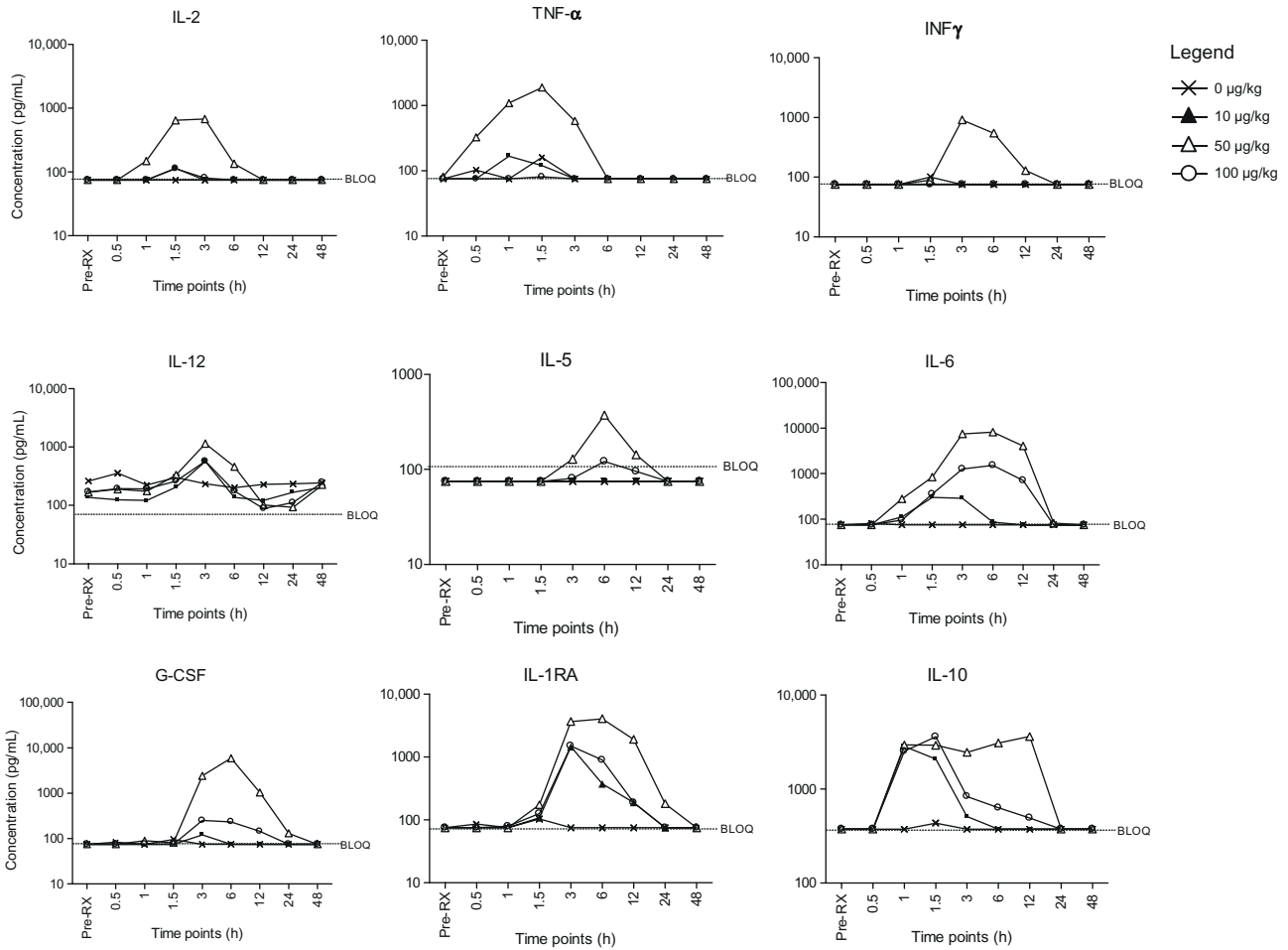


FIGURE 27.2 Cytokine and chemokine responses obtained in cynomolgus monkeys dosed with an anti-CD3. Measurement of cytokines and chemokines was performed using Luminex. Each line represents the mean cytokine concentration obtained in two animals. G-CSF, granulocyte colony-stimulating factor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

VetBooks.ir

The following complement immunoassays are most commonly incorporated into NHP studies: CH50, Bb fragment (a marker of the alternative complement pathway), C3a (a marker of total complement hemolytic activity), C5a, and C5b-9. As indicated for cytokines, samples should be collected before and shortly following dosing. Typical complement split products following oligonucleotide dosing are usually seen between 15 and 30 min after dosing. However, depending on the nature of the compound tested, this profile may change. Including time points up to 24 h after dosing is useful to evaluate the reversibility of complement activation.

Immunogenicity

Immunogenicity refers to the ability of a drug to induce an immune response and is a concern for peptide and protein therapeutics. As mentioned previously, preclinical immunogenicity is not predictive of immunogenicity in humans [35], but it helps to understand the findings in a toxicology study and therefore is an important factor in study interpretation. The preclinical consequences of antibody formation varies with the type and quantity of antibody present, but it may effect drug PK, PD, efficacy, and safety, through the alteration of endogenous molecule functions or through the induction of drug allergenicity reactions (type I and type III) [36].

Assays for ADA detection are grouped into two categories: binding assays and neutralizing antibody assays. Binding assays measure the ability of the antibodies present in the sera to bind to the antigen. They detect antibodies that are directed towards the molecule, thus detecting both neutralizing and nonneutralizing antibodies. The second assay measures the ability of the sera to neutralize the biological activity of the drug (or antigen); in other words, it detects neutralizing antibodies. In general, ADA binding assays consist of the detection of antidrug IgG and IgM antibodies. Other classes of ADAs such as IgE and IgA may be investigated on a case-by-case basis. If ADAs are detected, further characterization may be conducted including a drug competition test (confirmatory assay), titration, and isotyping. To evaluate the need and extent of immunogenicity testing in preclinical studies, one should consider the risks and whether PK and PD data are sufficient [37].

Challenges

Although more tests are now available to assess the effect of a compound on the immune system, there still remain assays that are not well characterized, developed, or cross-validated across laboratories. For some of these assays, it is critical to understand the time course of responses, because mediators may only be transiently present following stimulation/activation. The collection time points in such cases need to be carefully selected before the initiation of a study. Moreover, the quantity of test material required to perform studies in cynomolgus monkeys is much higher than for rodent studies. Therefore, if the amount of the test article is limited, smaller NHP species can be considered.

For ethical reasons and cost considerations, the group sizes in NHP studies are usually kept relatively small. This, combined with the fact that no inbred strains of NHP are available, can impair statistical power for detection of test article-related effects. Interindividual variation in the parameters of interest can be higher than for inbred rodents. Inclusion of individual comparison to baseline concentrations (predose) is recommended to appropriately

VetBooks.ir

interpret the data obtained. Moreover, assays included in NHP studies are often conducted with a variety of different methods between laboratories. For these reasons, comparing data across laboratories and compounds can be difficult [38]. Improvement in the standardized performance of some of these assays could be gained by laboratories sharing their methods and data and combining their expertise to allow further optimization [38].

DEVELOPMENTAL IMMUNOTOXICOLOGY TESTING

The evaluation of the impact of new molecular entities on developing systems is an integral part of routine safety evaluation. Typically, this has focused on embryo-fetal, reproductive, and peri- and postnatal growth and development. In the past decade, however, increased attention has turned to development of the major organ systems with an emphasis on the nervous, respiratory, and immune systems [39,40]. Although the rat has been the recommended model of choice for developmental immunotoxicology (DIT) testing, the NHP is the most likely candidate for biotherapeutics where species-specific cross-reactivity is often limited.

Study Design

A few DIT evaluations of biotherapeutics have been published [41–48] and provide insight into potential means to evaluate DIT in NHPs. The use of NHPs in developmental and reproductive toxicology (DART) testing is described in detail elsewhere [49–52]. To maximize the value of existing studies and minimize animal use, has been recommended that DIT end points be added to existing DART protocols whenever feasible. To date, most published DIT studies in NHPs have utilized one of two primary designs: the expanded embryo-fetal development (eEFD) study (Figure 27.3) or the peri- and postnatal development study (PPND) (Figure 27.4). These designs are in keeping with current practice with ICH S6(R1) (2011) [6] and ICH S5(R2) (2005) [6] and are considered in greater detail by Burns-Naas [53].

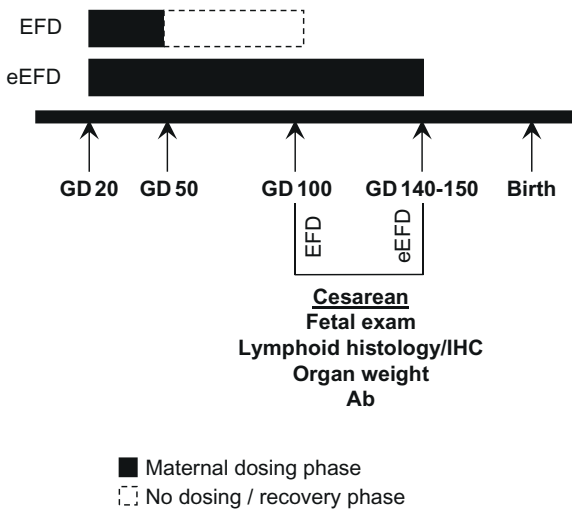


FIGURE 27.3 General study design for incorporation of developmental immunotoxicology end points into existing nonhuman primate embryo-fetal development (EFD) studies. Ab, antibody; eEFD, expanded embryo-fetal development; GD, gestational day. *Source: Adapted from Burns-Naas [54].*

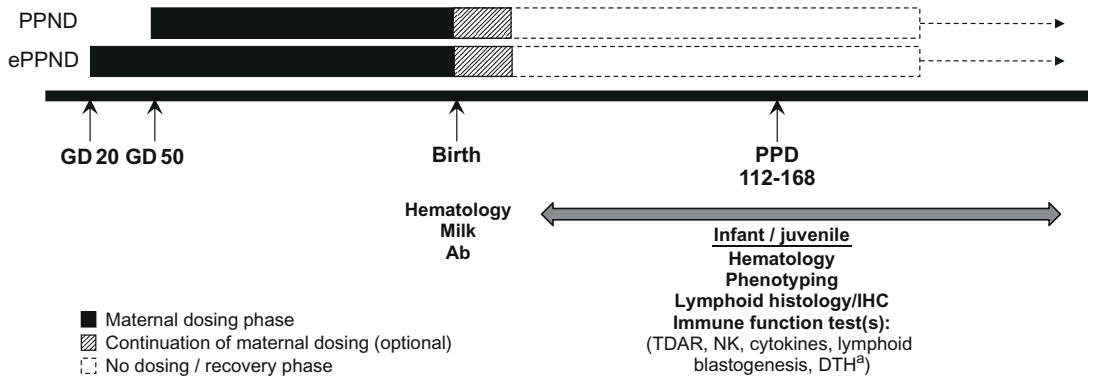


FIGURE 27.4 General study design for incorporation of developmental immunotoxicology end points into existing nonhuman primate (NHP) peri- and postnatal development (PPND) studies. [a]Assessment of the delayed hypersensitivity (DTH) response in the NHP can be problematic and difficult to reproduce consistently. AB, antibody; ePPND, enhanced peri- and postnatal development; GD, gestational day; IHC, immunohistochemistry; NK, natural killer; PPD, postpartum dat; TDAR, T-cell-dependent antibody response. *Source: Adapted from Burns-Naas [53].*

In the eEFD, dosing of the pregnant NHP usually begins on gestation day (GD) 20 following confirmation of pregnancy by ultrasound or serum chorionic gonadotropin [54] and continues through GD 140-150. This assures the possibility of exposure not only during the period of organogenesis but also during fetal growth and development. In the PPND, dosing is initiated at GD 50 and continues until birth. There is the option for continuation of maternal dosing (typically this has been for 1 month) to allow continuation of exposure through milk transfer. In addition, in an effort to reduce NHP use in drug development, an enhanced PPND (ePPND) study design, combining the EFD and PPND studies into a single study, has also been used. In it, dosing would begin at the confirmation of pregnancy and continue until birth or shortly thereafter.

In either the EFD (which incorporates a nondosing phase) or the eEFD, immunotoxicological evaluation occurs at the time of cesarean delivery, which is typically GD 100 or between GDs 140 and 150. In either case, the primary means of evaluation is by pathology (organ weights and histology and/or immunohistochemistry) of lymphoid tissues. In addition, antibody concentrations can be quantified in both maternal and fetal serum, and in some cases hematology and serum chemistry parameters may also be available. At this point in time during immune development, no immune function tests are yet available.

In either of the PPND designs, multiple end points can be assessed after birth and include not only the standard pathology evaluations (hematology, serum chemistry, lymphoid organ weights, anatomic pathology, and/or immunohistochemistry) but also immunophenotyping and functional evaluation of the immune system. Unlike rats, both NHPs and humans are born with a competent immune system. Although immunologic maturity continues after birth, the ability to mount innate and acquired cellular and humoral responses at the time of birth is measurable in both humans and NHPs. Infants can be retained for up to a year or more, which provides the opportunity for serial immune assessments in the same animals within a study. This is important because many biopharmaceuticals have extended half-lives

VetBooks.ir

such that infant exposure can continue for days or weeks after birth until the mAb is cleared. Understanding toxicokinetics in infants is, therefore, a critical piece when interpreting DIT within the context of the study.

While structural (pathology) evaluation and organ weights are currently the only means to evaluate fetal NHP immune status, the addition of functional evaluation of the immune system is considered by many to be more sensitive than relying solely on anatomic pathology evaluation of lymphoid tissue. Because of its sensitivity and predictivity, the TDAR is the single most widely accepted method for evaluation of immune function in animal models, primarily because it represents a measure of multiple key components that drive the human immune response. Although the TDAR is considered validated for use in both rodents and NHPs, many fewer functional assays are considered validated for use in the NHP and even fewer still for the developing immune system. As such, the most robust evaluation of the developing immune system includes an evaluation of both humoral (TDAR) and cell-mediated immune function. The latter may include the delayed-type hypersensitivity (DTH) response, CTL response, or the NK assay. Although cytokine measurements via multiplex evaluation and mitogen or receptor-mediated (e.g., T or B cell) proliferation assays are feasible, they are considered adjunctive at present, and they are not a replacement for more holistic immune function tests.

Drug-induced alterations in any one immune compartment can cause significant changes in the TDAR. Isotype skewing is at least as prevalent a perinatal immunotoxicity outcome as are elevated or reduced concentrations of antibodies. As a consequence, TDARs limited to measuring only one isotype (e.g., IgM) may miss detection of some T-cell-dependent class shifts in Ig production. Therefore, consideration should be given to measuring both the primary and secondary responses. In the NHP, this is typically performed using KLH and tetanus toxoid (TTX) as the antigens for immunization. Both the primary (IgM or IgG) and secondary (IgG) immune responses can be evaluated. In NHPs both are often evaluated in the same study by immunizing at different times during the study with two separate antigens. This allows for the evaluation of a primary and secondary response to the initial antigen and a primary response to a second antigen, and it can be used to evaluate temporal changes in the humoral immune response to a test article. Representative examples adapted from figures in the primary literature are provided in Figures 27.5 and 27.6.

Martin et al. evaluated ustekinumab, a human anti-IL-12/23p40 IgG1 mAb, in both an EFD and ePPND study [55]. The TDAR was evaluated in the ePPND study. Infant macaques were born to mothers treated twice weekly by subcutaneous injection with either the control or ustekinumab from GD 20 through postpartum day (PPD) 33. Infants were challenged intramuscularly with KLH on PPD 140, and the primary IgM and primary IgG responses were measured once weekly for 4 weeks afterward. Anti-KLH primary IgM titers were measureable 1 week after immunization, as expected, whereas anti-KLH primary IgG titers were maximal after 2 weeks (Figure 27.5). Treatment with ustekinumab had no effect on the TDAR to KLH. A similar lack of effect was noted for the primary IgM and IgG response to TTX (data not shown).

In a study by Vaidyanathan et al., rituximab, a chimeric murine/human IgG1 anti-CD20 mAb, was evaluated in both EFD and ePPND studies [48]. In the ePPND, infants were immunized on PPD 90 with KLH and the primary anti-KLH IgG response measured once weekly for 5 weeks thereafter. The same infants were again immunized with KLH on PPD 180 and

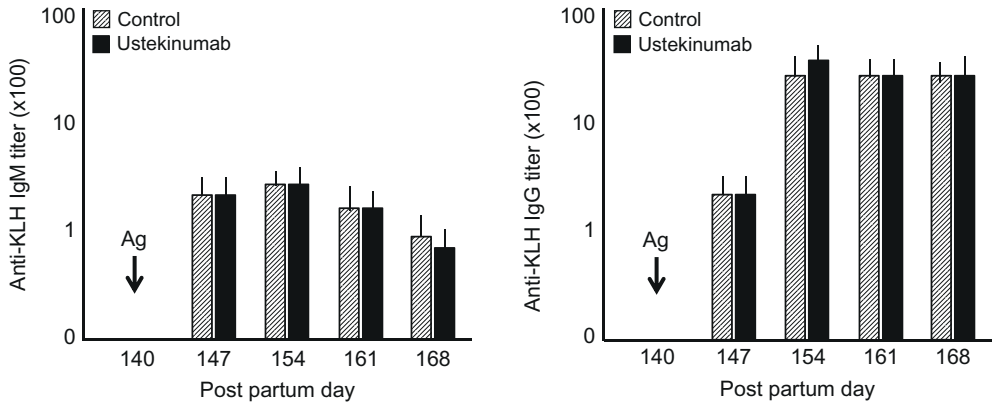


FIGURE 27.5 Primary IgM and IgG antibody responses to keyhole limpet hemocyanin (KLH) in infant macaques from mothers treated twice weekly by subcutaneous injection with 0 (control) or 45 mg/kg ustekinumab from gestational day (GD) 20 through postpartum day (PPD) 33. Infants were challenged by intramuscular injection of 2 mg/kg KLH on PPD 140. Measurement of anti-KLH antibodies was performed by enzyme-linked immunosorbent assay of serum collected on the indicated days. Each bar represents the approximate mean and standard deviation of data from 13 or 14 infants. Ag, antigen. *Source: Adapted from Martin et al. [55].*

the secondary anti-KLH IgG response was evaluated once weekly for 5 weeks. As noted with ustekinumab, the primary IgG response was not observed until the second week after immunization (Figure 27.6). The response did not reach maximal levels until 5 weeks later, which was a distinct difference from the study by Martin et al. and showed greater variability [55]. The possible reasons for this are considered in the section "Study Designs." As expected, the secondary IgG response was robust on PPDs 187 through 215. Although Figure 27.6 appears to show an effect of rituximab on the developing immune system, the authors concluded that there was no statistically significant difference in the TDAR response between the control and treated infants. Based on other end points in the evaluation, they concluded that the apparent trend toward a lower TDAR in rituximab-treated animals may have reflected a suboptimal primary response as a result of a residual lower B-cell number at the time of the initial immunization that then led to a reduced secondary response. Inhibition of CD20 would be expected to diminish the B-cell activity in the TDAR. Rituximab was detected in infant serum on PPD 90, but it was below the assay limits on PPD 180.

In the rituximab TDAR evaluation (Figure 27.6), variability of the response was much more significant than in the ustekinumab TDAR evaluation (Figure 27.5). This could have reflected the end point (titer vs. units), methodological differences in the conduct of the TDAR (e.g., use of Freund's incomplete adjuvant), the number of infants in each group (13-14 vs. 4-7), and/or the pharmacology of the target in normal animals (IL-12/23 vs. CD20). These data highlight the need for carefully planned study designs and a keen understanding of the properties of the biotherapeutic. Notably, both studies performed extensive developmental evaluations, obtaining the maximal amount of information from a single study.

As noted previously, despite being the best single functional test available to date, it is still considered best to combine the TDAR with other immunologic evaluations to provide the most robust assessment of DIT. In addition to the evaluation of the primary TDAR compared

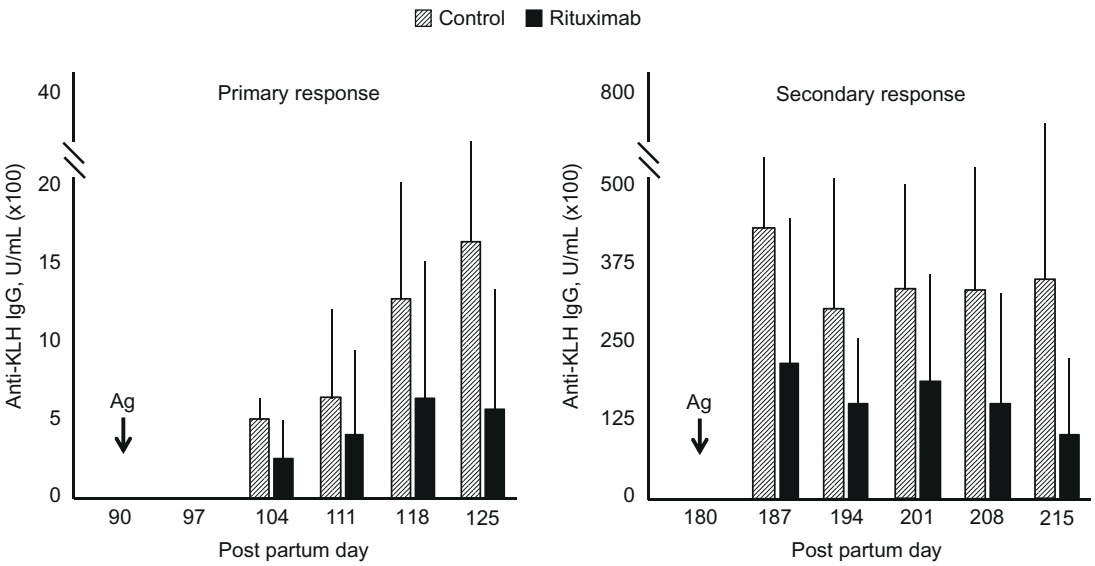


FIGURE 27.6 Primary and secondary immunoglobulin G (IgG) antibody responses to keyhole limpet hemocyanin (KLH) in infant macaques from mothers treated weekly by intravenous injection with 0 (control) or 100 mg/kg rituximab from gestational day (GD) 20 through postpartum day (PPD) 28. Infants were challenged on days 90 or 180 with 0.1 mg KLH in 0.2 mL of a 1:1 aqueous emulsion of Freund's incomplete adjuvant. Measurement of anti-KLH antibodies was performed by enzyme-linked immunosorbent assay of serum collected on the indicated days. Each bar represents the approximate mean and standard deviation of data from four to seven infants. Ag, antigen. *Source: Adapted from Vaidyanathan et al. [48].*

with both KLH and TTX, ustekinumab was also evaluated in a DTH assay, and representative data are presented in Figure 27.7. Infants were injected intracutaneously on PPD 140 and then challenged 4 weeks later (PPD 168) with TTX. Skin reactions were evaluated as the mean size of the edema (major axis × minor axis) 24 and 48 h after the challenge. An expected dose-related increase in response was observed with no effect of ustekinumab on the response. It is important to note that the DTH was successfully performed in this study. The DTH response in the NHP has generally been considered to be unreliable because it is difficult to replicate in this species. Additional data are needed to determine whether the lack of reproducibility is a methodological phenomenon or something else. To supplement the functional assays, immunophenotyping of peripheral blood and histology of lymphoid organs was also evaluated following administration of ustekinumab. The authors concluded that there was no effect on immune function and confirmed that infants were being exposed on GD 100 through PPD 120.

Points to Consider

Study Designs

Schematic designs presented in Figures 27.3 and 27.4 are intended to guide the reader in the general design of these types of studies. As with any drug development program, the design for each program should be tailored to the needs of the project and the attributes of

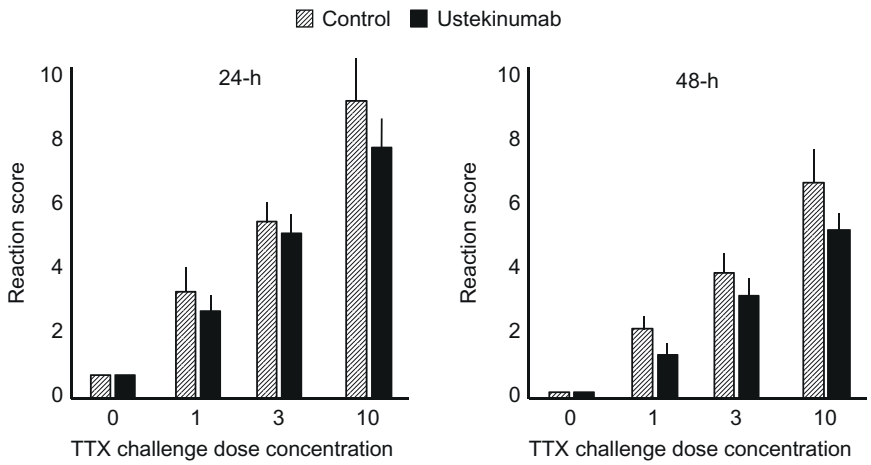


FIGURE 27.7 Delayed-type hypersensitivity (DTH) reactions (dermal edema scores) were measured in response to intracutaneous injection of tetanus toxoid (TTX) in infant macaques from mothers treated twice weekly by subcutaneous injection with 0 (control) or 45 mg/kg ustekinumab from gestational day (GD) 20 through postpartum day (PPD) 33. Infants were sensitized with 12 intracutaneous injections (50 µL of 10 Lf/mL) in the dorsal region on PPD 140 followed by 12 intracutaneous challenge injections (10 µL) of TTX (0, 1, 3, or 10 Lf/mL, three injection sites for each concentration) in the ventral region on PPD 168. Dermal edema scores were assessed 24 or 48 h after the TTX challenge. Each bar represents the approximate mean and standard deviation of data from 13 or 14 infants. *Source: Adapted from Martin et al. [55].*

the drug. For example, there may be times when the mAb is very immunogenic in the NHP. If ADA develops rapidly, a staggered dosing paradigm can be used to cover the full developmental window of interest or a limited design may be used to evaluate only the critical window of exposure. Similarly, direct dosing of the infant may be needed if children are the intended patient population, when there is cause for concern demonstrated in standard toxicology studies or in ongoing clinical trials in adults, or because of what is known about the target, the drug, and the potential to effect certain aspects of the developing immune system. In this situation, dosing could begin *in utero* (via the mothers) or approximately 1 month after birth (sooner if necessary), extend to 6-12 months, and the same end points would be utilized. Care should be taken not to jeopardize the maternal bond with the infant, particularly when starting dosing very shortly after birth. Also, the need to allow for treatment-free periods that would allow recovery of putative immune insult should be factored into the study design and interpretation. Finally, in an effort to reduce the number of animals used in safety evaluation, sponsors can consider a two-dose level (rather than three) design.

Methodology

Some specific methodological considerations are evident from the two studies described above [48,55]. In the rituximab TDAR evaluation (Figure 27.6), variability of the response was much more significant than in the ustekinumab TDAR evaluation (Figure 27.5) and, as noted in previous sections, the time course to the maximal response was different between the investigations. This could have reflected differences in the calculated end point (titer vs. units/milliliter), the number of infants in each group (13-14 vs. 4-7), and/or the pharmacology

VetBooks.ir

of the target in normal animals (IL-12/23 vs. CD20). The latter is an important consideration because the residual presence of the drug in the infant may impact the DIT assessment. There were also methodological differences in the conduct of the TDAR, including the use of Freund's incomplete adjuvant in the rituximab study and differences in the concentration of KLH utilized (2 mg/kg in the ustekinumab study vs. 100 µg in the study of rituximab). Another possible difference is the route of administration of the immunogen. KLH was injected subcutaneously in the ustekinumab study. The route utilized in the rituximab evaluation was not provided but may have been intramuscular. These data highlight the fact that there are not standardized protocols for functional immunotoxicity evaluation in the NHP, the need for carefully planned study designs, and a keen understanding of the properties of the biotherapeutic. Another study design point is the lack of a positive control in either study. While rodent TDAR studies typically use known positive controls such as cyclophosphamide, NHP studies typically do not. Notably, even though not presented here, the studies by Martin et al. [55] and Vaidyanathan et al. [48] performed extensive developmental evaluations, obtaining the maximal amount of information from a single study.

Clinical Translation

Early reports and more recent simulations based on the data suggested that little to no transfer of exogenously administered mAb would likely cross the placenta during the period of organogenesis in the NHP and that the majority of placental transfer occurred during the third trimester [51,56,57]. More recently, Moffat et al. confirmed that intravenous dosing of a fully human IgG2 mAb showed placental transfer rates similar to those previously published for human endogenous IgG [58]. Specifically, fetal concentrations were approximately 0.5% of the maternal plasma concentration during organogenesis, increased approximately 10-fold during the early stages of the second trimester (GD 50-70), and remained unchanged after that (through GD 135) at approximately 5% of the maternal plasma concentration. The data published by Moffat and colleagues are consistent with the conclusions from an analysis of a broader dataset of Fc-containing biotherapeutics [59] and together provide evidence that at high maternal exposures that are generally achieved at dose concentrations typically used in animal studies, embryonic exposure during organogenesis is likely to reach pharmacologically relevant concentrations; however, this is unlikely to occur at the lower therapeutic dose concentrations used in humans. Although a recent report indicated that data may be interpretable because of the likelihood of pharmacologically relevant exposure during critical windows of development [58], we continue to lack a solid understanding of how to translate the significance of DIT data in the NHP to potential human hazards [53]. Similarly, it is not clear what exactly constitutes true adversity in the child, even though evidence is increasing that early life insult can, in fact, have functional consequences later in life. Barriers remain to the development of clinically translatable biomarkers of DIT in both animals and humans, including gaps in our understanding of DIT in mammals as well as limitations in existing assays.

Information Gaps

As noted by others [53,60–62], even though the state of the science of DIT has moved forward over the past decade, it is still an emerging science, and there are still some gaps in

VetBooks.ir

our knowledge and limitations or challenges in the data. Relatively little is known regarding major milestones in the developing immune system in humans, and even less is known about the NHP. This is especially true concerning the structure and function of the primary and secondary lymphoid organs and the responses of the innate and acquired immune system. More knowledge regarding methodologies to reproducibly assess cell-mediated immunity in the NHP in general is needed. Some end points that can and are being measured, particularly in NHPs and humans—including immunophenotyping and evaluation of total serum immunoglobulin concentrations—have been shown to lack sensitivity in adults of either species and would, therefore, likely lack sensitivity in assessing DIT. Methods accepted by regulatory bodies to date are largely confined to those known to be sensitive and predictive in adult animals. Immunopathology forms the cornerstone of DIT assessments to date, but it is recognized that there may still be limitations in this area relative to our understanding of the time-dependent changes in structure and function.

References

[1] House RV, Hastings K. Multidimensional immunomodulation. J Immunotoxicol 2004;1:123–9.
[2] EMEA. Note for guidance on repeated dose toxicity; 2000.
[3] FDA. Guidance for industry: immunotoxicology evaluation of investigational new drugs. Available from http://www.fda.gov/cder/guidance/index.htm.
[4] ICH (International Conference on Harmonization). Guidance for industry. S8 immunotoxicity studies for human pharmaceuticals; 2006. Available from, http://www.fda.gov/RegulatoryInformation/Guidances/ucm129118.htm.
[5] Hastings K. Current regulatory expectations for immunotoxicology evaluation of pharmaceuticals. In: Herzyk D, Bussiere J, editors. Immunotoxicology strategies or pharmaceutical safety assessment. Hoboken, NJ: John Wiley & Sons; 2008. p. 3–11.
[6] International Conference on Harmonization (ICH). Harmonized tripartite guideline. ICH S6(R1). Preclinical safety evaluation of biotechnology-derived pharmaceuticals. 2011. Available from, http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html.
[7] EMA. Guideline on similar biological medicinal products containing monoclonal antibodies – nonclinical and clinical issues. EMA website, 2012.
[8] EMEA. Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins. EMA website, 2007.
[9] FDA. Guidance for industry: assay development for immunogenicity testing of therapeutic proteins. FDA website, 2009.
[10] K van Meer PJ, Kooijman M, Brinks V, Gispen-de Wied CC, Silva-Lima B, Moors EH, et al. Immunogenicity of mAbs in non-human primates during nonclinical safety assessment. MAbs 2013;5:810–6.
[11] Good laboratory practice handbook (GLP). OECD website. 2nd ed. WHO.
[12] ICHQ6B. Specification: test procedures and acceptance criteria for biotechnological–biological products. ICH website, 1999.
[13] ICHQ5E. Comparability of biotechnological–biological products subject to changes in their manufacturing process. ICH website, 2004.
[14] ICHQ8. Pharmaceutical development. ICH website, 2009.
[15] Beck A, Wurch T, Bailly C, Corvaia N. Strategies and challenges for the next generation of therapeutic antibodies. Nat Rev Immunol 2010;10:345–52.
[16] Satish JG, Sethu S, Bielsky M-C, de Hann L, French NS, Govindappa K, et al. Challenges and approaches for the development of safer immunomodulatory biologics. Nat Rev Drug Discov 2013;12:306–24.
[17] Jawa V, Cousens LP, Awwad M, Waksull E, Kropshofer H, De Groot AS. T-cell dependent immunogenicity of protein therapeutics preclinical assessments and mitigation. Clin Immunol 2013;149:534–55.
[18] Singh SK. Impact of product-related factors on immunogenicity of biotherapeutics. J Pharm Sci 2011;100:354–87.

VetBooks.ir

[19] van Beer MMC, Bardor M. Minimizing immunogenicity of biopharmaceuticals by controlling critical quality attributes of proteins. Biotechnol J 2012;7:1473–84.
[20] Bluemel J. Considerations for the use of non-human primates in non-clinical safety assessment. In: Weinbauer GF, Vogel F, editors. Challenges in nonhuman primate research in the 21st century. New York: Waxmann Publishing; 2013. p. 59–70.
[21] Lebrec H, Molinier B, Boverhof D, Collinge M, Freebern W, Henson K, et al. The T-cell-dependent antibody response assay in nonclinical studies of pharmaceuticals and chemicals: study design, data analysis, interpretation. Reg Toxicol Pharmacol 2014;69:7–21.
[22] Piché M-S, Shenton J, Dumont C, Bourgeois R, Brochu I, Grenier A-J, et al. Use of the T-cell-dependent antibody response to evaluate immunostimulation. In: SOT (San Antonio), poster 2063; 2013.
[23] Bleavins MR, Brott DA, Alvey JD, de la Iglesia FA. Flow cytometric characterization of lymphocyte subpopulations in the cynomolgus monkey. Vet Immunol Immunopathol 1993;37:1–13.
[24] Grote-Wessels S, Frings W, Sminth CA, Weinbaur GF. Immunotoxicity testing in the non-human primates. Methods Mol Biol 2010;598:341–59.
[25] Baker DL, Finco-Kent DL, Reagan WJ. Optimization and validation of a flow cytometric method for immunophenotyping peripheral blood lymphocytes from cynomolgus monkeys. Vet Clin Pathol 2008;37:42–8.
[26] Cunliffe J, Derbyshire N, Keeler S, Coldwell R. An approach to the validation of flow cytometry methods. Pharm Res 2009;26:2551–7.
[27] Verdier F, Ajoulat M, Condevaux F, Descotes J. Determination of lymphocyte subsets and cytokines levels in cynomolgus monkeys. Toxicology 1995;105:81–90.
[28] Yoshireo N, Ami Y, Terao K, Tashiro F, Honda M. Upgrading of flow cytometric analysis for absolute counts, cytokines and other antigenic molecules of cynomolgus monkeys by using anti-human cross-reactive antibodies. Exp Anim 2000;49:97–110.
[29] Krejsa CM, Neradilek MB, Polissar NL, Cox N, Clark D, Cowan L, et al. An inter-laboratory retrospective analysis of immunotoxicological endpoints in non-human primates: flow cytometry immunophenotyping. J Immunotoxicol 2013;10:361–72.
[30] Condevaux F, Guichard J, Forichon A, Aujoulat M, Descotes J. Compared effects of morphine and nickel chloride on NK cell activity in vitro in rats and monkeys. J Appl Toxicol 2001;21:431–4.
[31] Giavedoni LD, Velasquilio MC, Parodi LM, Hubbard GB, Hodara VL. Cytokine expression, natural killer cell activation and phenotypic changes in lymphoid cells from Rhesus macaques during acute infection with pathogenic simian immunodeficiency virus. J Virol 2000;74:1648–57.
[32] Tarrant JM. Blood cytokines as biomarkers of in vivo toxicity in preclinical safety assessment: considerations for their use. Toxicol Sci 2010;117:4–16.
[33] Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. Nat Rev 2009;9:729–40.
[34] Bergseth G, Ludviksen J, Kirschfink M, Giclas PC, Nilsson B, Mollnes TE. An international serum standard for application in assays to detect human complement activation products. Mol Immunol 2013;56:232–9.
[35] Bugelski PJ, Treacy G. Predictive power of preclinical studies in animals for the immunogenicity of recombinant therapeutic proteins in humans. Curr Opin Mol Ther 2004;6:10–6.
[36] Leach MW, Rottman JB, Hock MB, Finco D, Rojko JL, Beyer J. Immunogenicity/hypersensitivity of biologics. Toxicol Pathol 2014;42:293–300.
[37] Ponce R. Adverse consequences of immunostimulation. J Immunotoxicol 2008;5:33–41.
[38] Hagerty HG. Immunotoxicity testing in non-rodent species. J Immunotoxicol 2007;4:165–9.
[39] Adams J, Barone S, LaMantia A, Philen R, Rice DC, Speak L, et al. Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. Environ Health Perspect 2000;108(Suppl. 3):535–44.
[40] Dietert RR, Etzel RA, Chen D, Halonen M, Holladay SD, Jarabek AM, et al. Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary. Environ Health Perspect 2000;108(Suppl. 3):483–90.
[41] Pasparakis M, Alexopoulou L, Episkopou V, Kollias G. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. J Exp Med 1996;184:1397–411.
[42] Martin PL, Oneda S, Treacy G. Effects of an anti-TNF-alpha monoclonal antibody, administered throughout pregnancy and lactation, on the development of the macaque immune system. Am J Reprod Immunol 2007;58:138–49.

VetBooks.ir

[43] Rasmussen AD, Nelson JK, Chellman GJ, Golub M, McAnulty PA. Use of barusiban in a novel study design for evaluation of tocolytic agents in pregnant and neonatal monkeys, including behavioural and immunological endpoints. Reprod Toxicol 2007;23:471–9.
[44] Martin PL, Cornacoff JB, Treacy G, Eirikas E, Marini J, While Jr. KL, et al. Effects of administration of a monoclonal antibody against mouse tumor necrosis factor alpha during pregnancy and lactation on the pre- and postnatal development of the mouse immune system. Int J Toxicol 2008;27:341–7.
[45] Carlock LL, Cowan LA, Oneda S, Hoberman A, Wang DD, Hanna R, et al. A comparison of effects on reproduction and neonatal development in cynomolgus monkeys given human soluble IL-4R and mice given murine soluble IL-4R. Regul Toxicol Pharmacol 2009;53:226–34.
[46] Wehner NG, Shopp G, Oneda S, Clarke J. Embryo/fetal development in cynomolgus monkeys exposed to natalizumab, an alpha4 integrin inhibitor. Birth Defects Res B Dev Reprod Toxicol 2009;86:117–30.
[47] Wehner NG, Shopp G, Osterburg I, Fuchs A, Buse E, Clarke J. Postnatal development in cynomolgus monkeys following prenatal exposure to natalizumab, an alpha4 integrin inhibitor. Birth Defects Res B Dev Reprod Toxicol 2009;86:144–56.
[48] Vaidyanathan A, McKeever K, Anand B, Eppler S, Weinbauer GF, Beyer JC. Developmental immunotoxicology assessment of rituximab in cynomolgus monkeys. Toxicol Sci 2011;119:116–25.
[49] Weinbauer GF, Frings W, Fuchs ANM, Osterburg I. Reproductive/developmental toxicity assessment of biopharmaceuticals in nonhuman primates. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals: a science-based approach to facilitate clinical trials. Hoboken, NJ: John Wiley & Sons; 2008. p. 379–97.
[50] Chellman GJ, Bussiere JL, Makori N, Martin PL, Ooshima Y, Weinbauer GF. Developmental and reproductive toxicology studies in nonhuman primates. Birth Defects Res (Part B) 2009;86:446–62.
[51] Martin PL, Weinbauer GF. Developmental toxicity testing of biopharmaceuticals in non-human primates: previous experience and future directions. Int J Toxicol 2011;29:552–68.
[52] Weinbauer GF, Fuchs A, Luetjens CM, Niehaus M, Niehoff MO, Vogelwedde E. Nonhuman primates as preclinical models for developmental and reproductive toxicity evaluation. In: Hood RD, editor. Reproductive and developmental toxicity testing: a practical approach. 3rd ed. London: Informa Healthcare; 2012. p. 464–78.
[53] Burns-Naas LA. Developmental immunotoxicity testing. In: Hood RD, editor. Reproductive and developmental toxicity testing: a practical approach. 3rd ed. London: Informa Healthcare; 2012. p. 491–508.
[54] Tarantal AF, Laughlin LS, Dieter J, Tieu J, Hendrickx AG, Overstreet JW, et al. Pregnancy detection by ultrasound and chorionic gonadotropin during the periimplantation period in the macaque (*Macaca fascicularis*). Early Pregnancy 1997;3:281–90.
[55] Martin PL, Sachs C, Imai N, Tsusaki H, Oneda S, Jiao Q, et al. Development in the cynomolgus macaque following administration of ustekinumab, a human anti-IL-12/23p40 monoclonal antibody, during pregnancy and lactation. Birth Defects Res (Part B) 2010;89:351–63.
[56] Coe CL, Kennitz JW, Schneider MI. Vulnerability of placenta antibody transfer and fetal complement synthesis to disturbance of the pregnant monkey. J Med Primatol 1983;22:294–300.
[57] Fujimoto K, Terao K, Cho F, Honjo S. The placental transfer of IgG in the cynomolgus monkey. Jpn J Med Sci Biol 1983;36:171–6.
[58] Moffat GJ, Retter MW, Kwon G, Loomis M, Hock MB, Hall C, et al. Placental transfer of a fully human IgG2 monoclonal antibody in the cynomolgus monkey, rat, and rabbit: a comparative assessment from during organogenesis to late gestation. Birth Defects Res (Part B) 2014;101:178–88.
[59] Bowman CJ, Breslin WJ, Connor AV, Martin PL, Moffat GJ, Sivaraman L, et al. Placental transfer of Fc-containing biopharmaceuticals across species, an industry survey analysis. Birth Defects Res B 2013;98:459–85.
[60] Burns-Naas LA, Hastings KL, Ladics GS, Makris SL, Parker GA, Holsapple MP. What's so special about the developing immune system? Int J Toxicol 2008;27:223–54.
[61] Collinge M, Burns-Naas LA, Kawabata TT. Developmental immunotoxicology. In: Kapp RW, Tyl RW, editors. Reproductive toxicology. 3rd ed. New York: Taylor & Francis; 2010. p. 134–56.
[62] Burns-Naas LA, Pallardy MJ. Immunotoxicology. In: Brock WJ, Hastings KL, McGown KM, editors. Nonclinical safety assessment: a guide to international pharmaceutical regulations. West Sussex: J. Wiley and Sons; 2013. p. 313–46.

VetBooks.ir

CHAPTER

28

Ophthalmological Investigations in Toxicity Studies

Birgit Niggemann

Covance Laboratories GmbH, Münster, Germany

OUTLINE

Introduction 543

General Considerations 544
Standard Investigations 544
Specific Investigations 544
Fundus Photography and Fluorescein Angiography 544
Laser Scanning Ophthalmoscope 546
Optical Coherence Tomography 546
Intraocular Pressure 546
Pachymetry 547
Specular Microscopy 547
Scheimpflug Photography 547
Electroretinography 547

References 550

INTRODUCTION

Humans and nonhuman primates (NHPs) share many ocular similarities, including retinal cell anatomy, the vascular system, and the presence of a macula with fovea centralis. The macula is an area with only cones and without retinal vessels. Because of this, the macula is sensitive to changes such as the transport of metabolism products. This area is not present in other species typically used in toxicity studies, such as mice, rats, rabbits, or dogs. Because of the similarity to humans, the NHP is an excellent and often the only appropriate model of human ocular function when conducting toxicity studies. The spectrum of ophthalmoscopic techniques and equipment used for evaluation of the eye is comparable to those in humans. An exception to this are end points requiring subjective responses from the subject, such as visual acuity evaluation, which is significantly more difficult to

http://dx.doi.org/10.1016/B978-0-12-417144-2.00028-7
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

establish in monkeys [1]. The NHP, especially the cynomolgus monkey, has proven to be a highly suitable and well-established animal model for examining the ocular toxicity of drugs in preclinical research studies because of these similarities in anatomy, function, and examination technique. The investigations mentioned below are the most common methods for drug toxicity testing using NHPs. Additional methods are available and are used in specific cases.

GENERAL CONSIDERATIONS

Before the start of ophthalmological examinations, the order should be carefully considered if they are performed on the same occasion. Some investigations can have an influence on others. For most of the investigations, the animals are sedated and a mydriatic agent is used.

Standard Investigations

In standard toxicology studies, direct and indirect ophthalmoscopy as well as slit-lamp biomicroscopy examinations are used routinely. In the case of NHPs, the animals should be sedated for this examination (e.g., with ketamine). Tropicamide (0.5-1%) is commonly used to dilate the pupil.

Direct and/or indirect ophthalmoscopy is used to examine the fundus including macula, papilla, ocular vessels, and retina. By means of direct ophthalmoscopy (16-fold magnification) details of the fundus are detectable, but the evaluation of the periphery is difficult. Furthermore, it should be taken into consideration that the examiner's face needs to be very close to the monkey. Therefore, it is mainly used in the event that findings occur. Using indirect ophthalmoscopy, a collecting lens that allows only a magnification of two to sixfold is used; however, this technique enables the examiner to view more of the retina [2]. Indirect ophthalmoscopes are available as head ophthalmoscopes which provides the advantage that the ophthalmologist needs only one hand for the lens and may use the other hand to more easily position the animal for examination.

Slit-lamp examinations are performed to examine the anterior segment of the eye, including conjunctiva, cornea, iris, lens, and anterior vitreous body. Fluorescein, applied to the corneal surface, is usually used to stain the cornea to demonstrate erosions or other lesions. A scoring system can be used to assess findings of the anterior part of the eye. Digital slit-lamp camera systems are also available to document observations.

Specific Investigations

Fundus Photography and Fluorescein Angiography

Retina cameras are often used in toxicology studies to capture fundus images for detailed evaluation as well as to document clinical observations. The same equipment used for humans can also be used for NHPs (Figure 28.1). Imaging systems allow the examiner to document changes and to share these with authorities or other experts. Fluorescence angiographies can be performed with the same equipment. Fluorescence angiography is used to

VetBooks.ir

FIGURE 28.1 Topcon fundus camera and table to place the animal in front of the camera.

evaluate vascular flow and abnormalities of the vascular trees within the fundus, such as microaneurysm, capillary permeability, vascular obstructions, or defects in the retinal pigment epithelium [1]. Fluorescein sodium is injected intravenously and a series of photographs are taken as the dye courses through the ocular circulation. Fluorescein is excited by blue light and then emits yellow-green light. Due to a yellow filter, the blue light is absorbed, and only light from the fluorescein is visible on the picture (Figure 28.2).

To protect animal welfare, fluorescein angiography should be performed in both eyes on the same occasion. However, because the early phases of fluorescein circulation occur rapidly (e.g., choroidal filling), angiography of the early phases is typically feasible in only one eye. After taking the first pictures from one eye, the examiner can change to the second eye to take pictures. Indocyanine green (ICG) is the second type of dye used in ophthalmic angiography. It is a tricarbocyanine dye used to image primarily the choroidal vasculature of the eye. ICG does not pass easily from the choriocapillaris or the retinal vessels because of the high molecular weight and the high binding to proteins. It remains in larger blood vessels longer and does not easily diffuse into smaller vessels or interstitial space, particularly in the

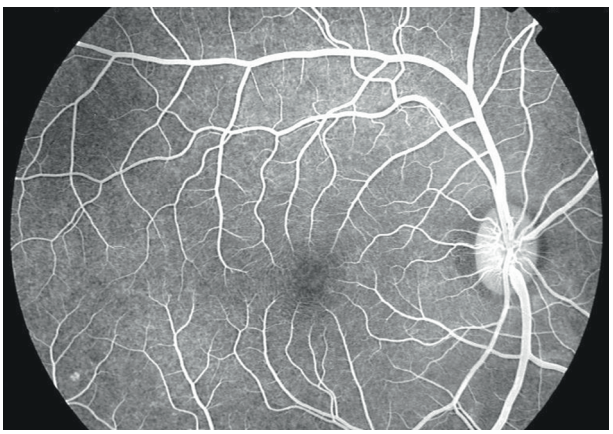


FIGURE 28.2 Fluorescence angiography in the cynomolgus monkey.

VetBooks.ir

choriocapillaris, which allows for good delineation of the choroidal vessels, in marked contrast to fluorescein [3]. In addition, the peak absorption and emission wavelengths of ICG are in the near infrared range (790-800 nm absorption and 830-840 nm emission). Infrared light penetrates deeper into tissue than visible light, which enables excitation and reflected imaging through the retinal pigment epithelium.

Laser Scanning Ophthalmoscope

In 1980, Webb et al. [4] reported a method for imaging the retina using a laser. The laser scanning ophthalmoscope uses horizontal and vertical scanning mirrors to scan a specific region of the retina point by point and to create raster images that are viewable in real time on a monitor. It can perform optical slicing through weakly scattering media, which makes it ideal for imaging the retina [5]. Furthermore, the equipment allows a three-dimensional reconstruction of the retina.

This technique is noninvasive and easy to perform if the eye is fixed and the eyelids remain open. It also allows for the exploration of retinal microvascularization and could be used for clinical, pharmacologic, and toxicological investigations as well [6].

Optical Coherence Tomography

Optical coherence tomography (OCT) is an emerging technology for performing high-resolution cross-sectional imaging. Instruments designed to image the retina and the anterior segment of the eye are available. OCT is analogous to ultrasound imaging, except that it uses light instead of sound. It allows detailed structures to be differentiated based on differences in the optical properties of the various cell layers [7].

OCT is increasingly used in ocular toxicity studies because it is a fantastic method to demonstrate the morphological changes during the in-life phase of a study. OCT measurements can be used to document the process of changes during the study or as a complement to histology at the end of the study.

Standard sedation and pupil dilatation are sufficient for these measurements. However, eye movement might be a limiting factor when it comes to duration and accuracy. Details regarding the retina profile of cynomolgus monkeys using laser scanning images and OCT measurements compared to histology have recently just published [8].

Intraocular Pressure

In NHPs, the intraocular pressure (IOP) can be determined in trained nonsedated animals or in untrained sedated animals. Sedation as well as each manipulation of the head can have a significant influence on the IOP. In toxicity studies the IOP is usually measured in sedated animals and, as such, may be useful only for detecting relatively large changes. For efficacy studies, trained nonsedated monkeys might be used to exclude the influence of general anesthesia. These trained animals can then be reused to test other drugs.

An easy method to measure the IOP is the rebound tonometer (TonoVet). Since the tip of this instrument can be exchanged after each animal, there is no risk of cross-contamination. With most applanation tonometers, the tip of the instrument needs to be cleaned between the measurements and cross-contamination can occur. Furthermore, a local anaesthesia that may also influence IOP readings is not required for rebound tonometers. The TonoPen is an alternative applanation tonometer instrument. However, local anesthesia is still required.

VetBooks.ir

Since manipulation of the head can also influence IOP, the measurements should be performed before other eye investigations and at the same time of day since IOP has a circadian rhythm. Furthermore, IOP values may be affected by the presence of a mydriatic agent. Thus, if IOP measurements are combined with other examinations that require pupil dilatation, procedure order should be considered (IOP before or after the mydriatic agent), and researchers should strive for consistent conditions for serial measures.

Pachymetry

Pachymetry is used to measure the thickness of the cornea with ultrasound. It captures an ultrahigh-definition echogram of the cornea and determines the status of the corneal endothelial cell layer. For this investigation, the animals are slightly sedated and a local ophthalmic anesthetic is instilled before the start of the measurements. Pachymetry is often used in toxicity studies with topical administration.

Specular Microscopy

Specular microscopy is a noninvasive photographic technique that allows visualization and analysis of the corneal endothelium. Using computer-assisted morphometry, modern specular microscopes analyze the size, shape, and population of the endothelial cells. The instrument projects light onto the cornea and captures the image that is reflected from the optical interface between the corneal endothelium and the aqueous humor. The reflected image is analyzed by the instrument and displayed as a specular photomicrograph [9].

Scheimpflug Photography

Scheimpflug photography is a precise technique for light scattering measurement and biometry in the anterior segment of the eye. This technique can be included in toxicological studies, not to replace slit-lamp examination and not to provide only photographic documentation, but rather to obtain density and biometry data. Scheimpflug photography is mainly used to investigate the cornea and the lens. The animals need to be sedated and the pupils should be dilated. As with most of the specific investigations, having baseline measurements and at least a final examination at the end of the dosing period is helpful [10].

Electroretinography

Electroretinography (ERG) is used to assess the function of retinal tissue. For example, ERG may be used to assess photoreceptors, neuronal cells (horizontal, bipolar, amacrine cells), and glia cells and their interaction [11]. For preclinical studies in NHPs, full-field ERG measurements should be performed in accordance to the international standards for clinical ERGs [12] recommended by the International Society for Clinical Electrophysiology of Vision (Figure 28.3). A minimum of five responses should be recorded:

1. Dark-adapted 0.01 $cd \cdot s \cdot m^{-2}$ ERG (formerly "rod response")
2. Dark-adapted 3.0 $cd \cdot s \cdot m^{-2}$ ERG (formerly "maximal or standard combined rod-cone response")
3. Dark-adapted 3.0 $cd \cdot s \cdot m^{-2}$ oscillatory potentials (formerly "oscillatory potentials")
4. Light-adapted 3.0 $cd \cdot s \cdot m^{-2}$ ERG (formerly "single-flash cone response")
5. Light-adapted 3.0 $cd \cdot s \cdot m^{-2}$ flicker ERG (formerly "30-Hz flicker").

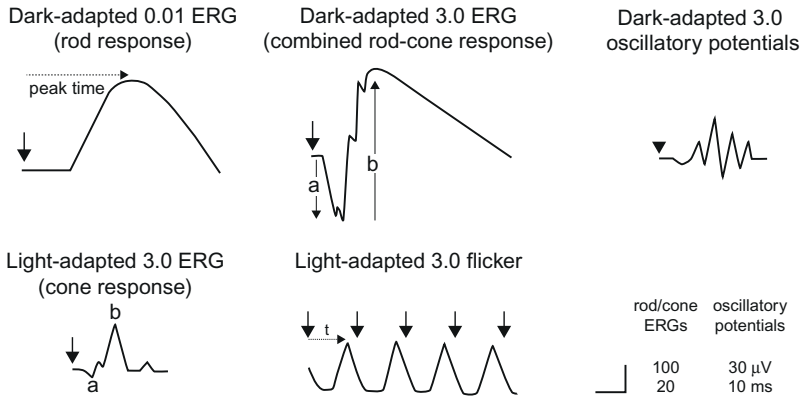


FIGURE 28.3 Diagram of the five basic electroretinography (ERG) responses [12]. Waveforms are exemplary only; short arrows indicate the stimulus flash. Dotted arrows exemplify how to measure time to peak (*t*, implicit time), a-wave amplitude, and b-wave amplitude.

The animals should be adapted to darkness for at least 30 min before the measurements start. The pupil of the eyes needs to be fully dilated to measure the response of the entire retina. Therefore, phenylephrine or atropine is used in addition to tropicamide to make sure that the pupil is fully dilated during the entire duration of measurement. Figure 28.4 shows a stimulator and a chair to place the monkey in front of the light stimulus.

FIGURE 28.4 Full-field stimulator.

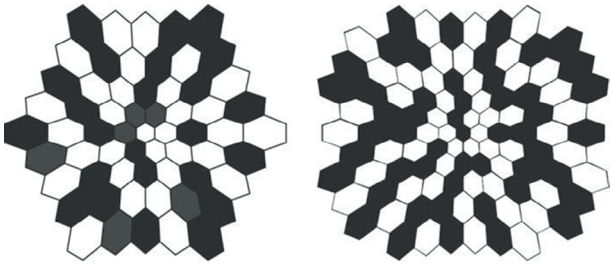


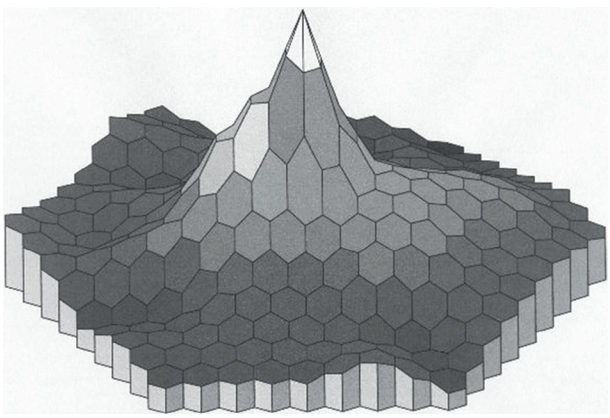
FIGURE 28.5 (a) Representative hexagonal multifocal electroretinographic stimulus array with 61 elements scaled with eccentricity. Roughly half of the elements are illuminated at any one time. (b) Same as in panel (a) for an array with 103 elements. *From Hood et al. [14].*

Since the full-field procedure captures electrical responses from the entire retina, only extensive damage of the function may be detected. Small focal changes of the retina can usually not be detected with the full-field ERG.

Multifocal ERG is a procedure that enables evaluation of focal areas of the retina. In 1992, Sutter and Tran [13] described this method as using a pattern consisting of a defined number of small black and white hexagons that flicker in a quasi-randomized sequence (Figure 28.5).

By using a special correlation technique, the local ERG that stems from the individual hexagons can be resolved. Therefore, an electrical map of retinal function is achieved [15]. Figure 28.6 shows the response from a cynomolgus monkey with the central macular peak. In NHP toxicity studies, the eye movements can make these measurements difficult; usually no additional local muscle relaxant is injected. For humans, the flickering grid is presented by a video screen. With this video screen, the investigator is not able to see which area is measured. Using a scanning laser ophthalmoscope, the flickering grid can be projected directly onto the retina and the scanning laser ophthalmoscope presents real-time video image of the macula and surrounding retina. The flickering grid is visible on the same screen and the investigator is able to see the relationship to the area of the retina [16].

FIGURE 28.6 Topography of multifocal electroretinographic P1 values from a cynomolgus monkey, showing central macular peak.



VetBooks.ir

References

[1] Niggemann B, Korte SH, Srivastav S. Ocular toxicity testing in nonhuman primates. In: Weinbauer GF, Vogel F, editors. Novel approaches towards primate toxicology. Münster: Waxmann Verlag GmbH; 2006. p. 202–17.

[2] Nasemann J. Netzhaut (retina). In: Sachsenweger M, Burggraf H, Klauß V, Nasemann J, editors. Augenheilkunde. Stuttgart: Hippokrates Verlag; 1994. p. 292–345.

[3] Clark TM, Clark TM. Retinal photography and angiography via film and digital images. In: Arevalo JV, editor. Retinal angiography and optical coherence tomography. Berlin: Springer Verlag GmbH; 2009. p. 3–25.

[4] Webb RH, Hughes GW, Pomerantzeff O. Flying spot TV ophthalmoscope. Appl Opt 1980;19:2991–7.

[5] Webb RH, Hughes GW, Delori FC. Confocal scanning laser ophthalmoscope. Appl Opt 1987;26:1492–9.

[6] Rosolen SG, Saint-Macary G, Gautier V, LeGargasson JF. Ocular fundus images with confocal scanning laser ophthalmoscopy in the dog, monkey and minipig. Vet Ophthalmol 2001;4(1):41–5.

[7] Fujimoto J, Pitris C, Boppart SA, Brezinski ME. Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy. Neoplasia 2000;2(1–2):9–25.

[8] Garcia Garrido M, Beck SC, Mühlfriedel R, Julien S, Schraermeyer U, Seeliger MW. Towards a quantitative OCT image analysis. PLoS ONE 2014;9(6):e100080.

[9] Craig T, Craig T. Use specular microscopy to diagnose corneal disease. Rev Optom 2009;146(06):69.

[10] Wegener A, Laser-Junga H. Photography of the anterior eye segment according to Scheimpflug's principle: options and limitations – a review. Clin Exp Ophthalmol 2009;37:144–54.

[11] Bee WH, Korte R, Vogel F. Electroretinography in the non-human primate as a standardized method in toxicology. In: Weisse I, Hockwin O, Green K, Tripathiy RC, editors. Ocular toxicology. New York, NY: Plenum Press; 1995. p. 53–61.

[12] Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M. Standard for clinical electroretinography (2008 update). Doc Ophthalmol 2009;118:69–77.

[13] Sutter EE, Tran D. The field topography of ERG components in man I. The photopic luminance response. Vis Res 1992;32:433–46.

[14] Hood DC, Bach M, Brigell M, Keating D, Kondo M, Lyons JS, et al. ISCEV Standard for clinical multifocal electroretinography (2011 edition). Doc Ophthalmol 2012;124:1–13.

[15] Zrenner E. Objective functional evaluation: electroretinography (ERG), electrooculography (EOG), visually evoked cortical potentials (VEP), and multifocal ERG. In: Weinbauer G, Vogel F, editors. Towards new horizons in primate toxicology. Münster: Waxmann Verlag GmbH; 2000. p. 175–88.

[16] Barker F, Niggemann B, Barker F, Niggemann B. The multi-focal electro-retinogram (mERG). In primate toxikology. In: Weinbauer G, Vogel F, editors. Novel approaches towards primate toxicology. Münster: Waxmann Verlag GmbH; 2006. p. 145–57.

VetBooks.ir

CHAPTER

29

The Use of Nonhuman Primates in Cardiovascular Safety Assessment

Jonathan R. Heyen[*], *Hugo M. Vargas*[†]

[*]Pfizer La Jolla, San Diego, CA, USA

[†]Department of Toxicology Sciences, Amgen Inc., Thousand Oaks, CA, USA

OUTLINE

Introduction 552

Basic Cardiovascular Physiology 553
- *Heart* 553
- *Vascular System* 554
- *Control of the Circulatory System* 554

Cardiovascular Evaluation Approaches and Methods 555
- *Electrocardiogram Evaluation and Cardiac Arrhythmia Assessment* 555
  - Conscious Models 555
- *Hemodynamic Assessment* 558
- *Cardiac Contractility Assessment* 560
  - Left Ventricular Pressure and dP/dt_{max} End Points 560
- *Cardiac Imaging by Echocardiography* 561
- *Cardiovascular Evaluation in Anesthetized Primates* 563
- *Cardiovascular End Points in Toxicology Studies* 567
- *Specialty Cardiovascular Assays* 568
- *In Vitro and Ex Vivo Cardiovascular Assessment* 569
- *Congenital and Background Cardiovascular Pathology in NHPs* 569
- *Cardiovascular Disease Models in NHPs* 570

Conclusion 571

Acknowledgements 571

References 572

http://dx.doi.org/10.1016/B978-0-12-417144-2.00029-9
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

The nonhuman primate (NHP) is an important animal model in the evaluation of new drug candidates in both the drug discovery and safety assessment areas. Given their significant physiological, metabolic, and genetic similarity to humans, NHP models have been used to study disease pathways and identify new drug targets for pain management [1], arthritis [2], Parkinson's disease [3], heart failure [4], and cardiovascular disease [5]. In the arena of drug safety assessment, NHPs have been used routinely in the evaluation of traditional chemical molecules and biopharmaceuticals for their value in hazard identification and risk assessment of novel drugs destined for human clinical trials [6,7]. With the increase in development of biopharmaceuticals, NHPs have become particularly important in nonclinical drug development. Because of the cross-reactivity attributes of novel biopharmaceuticals, NHPs may be the only species that is biologically and pharmacologically relevant to humans; thus, their primary use in regulatory toxicology and safety pharmacology studies [8,9]. Other scientific drivers for the role of NHPs in nonclinical development and safety assessment include their phylogenic relationship to humans, pharmacokinetic properties of drugs, target homology, and target expression.

A key driver in the utilization of NHPs in cardiovascular safety assessment has been the need to evaluate new drugs for their propensity to cause delayed repolarization of ventricular myocytes (i.e., QTc prolongation, which is a surrogate marker for the potentially fatal arrhythmia torsade de pointes [TdP]) [10,11]. Since the withdrawal of noncardiovascular drugs for high risk of TdP, nonclinical regulatory guidance documents have emerged to guide cardiovascular evaluation of new drug candidates, with an emphasis on ventricular repolarization (QTc interval). The two guidance documents endorsed by the International Conference on Harmonisation of Technical Requirements for Registrations of Pharmaceuticals for Human Use (ICH) are S7A and S7B [12,13]. ICH S7A provides guidance on the general evaluation of the cardiovascular system with a core assessment of blood pressure, heart rate, and the electrocardiogram; in vitro and/or ex vivo assays, including methods for repolarization and conduction abnormalities, can also be considered. Armed with an understanding of the effect of a novel drug on cardiovascular physiology, an accurate assessment of potential human risk can be evaluated. ICH S7B has a specific focus on the aforementioned property of a new drug to cause QTc interval prolongation in vivo, along with an in vitro study of hERG channel function. The data are used to derive an integrated QTc risk assessment before the initiation of phase I clinical trials. Although the conscious dog has been used as a primary species, the cynomolgus monkey has been used to fulfill the in vivo cardiovascular assessments described in S7A and S7B and provide a comprehensive understanding of QTc prolongation risk to humans [14–16]. The use of the NHP model in cardiovascular safety assessment of novel drug candidates is driven by several scientific factors, including the pharmacokinetic attributes of the drug candidate (similarity of drug metabolism to human), cardiovascular safety concerns associated with the primary (target-based) and secondary (off-target) pharmacology of the drug candidate, and the modality of the test article (e.g., a biopharmaceutical).

To this end, the use of monkeys has warranted an increased understanding of their cardiovascular system and the utility of this model in cardiovascular safety assessment. The peer-reviewed literature contains several examples of cardiovascular evaluations with approved drugs and experimental drug candidates that highlight the value of the NHP model to cardiovascular risk assessment [14,17–21]. This chapter reviews basic information about the NHP cardiovascular system, with reference to normal cardiovascular structure and function,

VetBooks.ir

as well as methodological approaches for cardiovascular evaluations in safety pharmacology and toxicology study settings. The focus of discussion includes methods commonly used in Old World monkeys, namely the cynomolgus monkey (*Macaca fascicularis*), as they represent the species most widely used in pharmaceutical research [22,23]. When appropriate, information on cardiovascular assessment in other primates species (e.g., rhesus monkeys [*M. mulatta*] and marmosets [*Callithrix jacchus*]), is mentioned. The reader should be aware that cynomolgus monkeys can be sourced from different regions of the globe, which may be a factor to consider when evaluating or comparing cardiovascular functional data or safety-related findings with new drug candidates [24,25]. An additional point is that experimental designs should be considered that optimize the collection of key cardiovascular data from the minimal number of animals, to reduce the overall number of NHP used during nonclinical drug development [26] (also see Chap. 14. Opportunities for Implementing the 3Rs in Drug Development and Safety Assessment Studies Using Nonhuman Primates).

BASIC CARDIOVASCULAR PHYSIOLOGY

Heart

The anatomy and structure of the NHP heart has all of the familiar characteristics of the mammalian cardiovascular system. It is a four-chambered heart consisting of two atria and two ventricles with associated atrioventricular and semilunar valves. The left side of the heart receives oxygenated blood from the pulmonary system (see Chap. 30, "Respiratory Function Measurements in Nonhuman Primates," for details) into the left atria and on to the left ventricle (LV) during diastole (relaxation) before it is pumped out via the aorta and associated artery system to the body during contraction (systole). The heart spends approximately one-third of a cycle in systole and the remaining two thirds filling with blood during diastole. The left side of the heart is more muscular, and the wall thickness is approximately three times thicker compared to the right ventricle. The interwoven longitudinal and circumferential fibers when contracted during systole (contraction) result in shortening in length but also in diameter of the LV [27]. Following this path through the arterioles into the capillaries, the oxygenated blood reaches target tissues. The capillaries also provide deoxygenated blood for return to the heart through the venous return system. Deoxygenated blood reaches the right atria via the inferior and superior vena cava as well as the coronary system. Right-ventricular systole is similar in mechanics and principal to the left side; however, the driving pressure for pulmonary artery flow into the lungs is much less. The heart is also surrounded by a thin fibrous sac (epicardium) consisting of two layers providing structural support and protection. Mechanical function of the heart can become compromised by disease of the pericardium. In addition to muscle, the heart contains an electrical conduction system that coordinates the sequential contraction of the atrial and ventricular chambers to drive blood flow (cardiac output) into the systemic circulation. The primary pacemaker is sinoatrial node, which is distributed primarily in the right atria. A second node located at the atrioventricular (AV) junction is the AV node, which spreads depolarizing electrical signals to special fibers (His bundle) before entering the left and right bundle branches that coordinate ventricular contraction. The underlying channels responsible for electrical conduction are well understood and covered later in this chapter. For a complete review of the basic cardiovascular physiology of the mammalian heart, please refer to *The Heart: Physiology, from Cell to Circulation* by Opie [28].

VetBooks.ir

Vascular System

The anatomy of the vascular system of the NHP is similar to all mammalian species and thoroughly described in the cited resources [28,29]. Briefly, the vascular system can be split into two circuits or beds, including systemic circulation from the LV and pulmonary circulation provided by the right ventricle. Following ejection from the LV, the blood enters the aorta and into smaller arteries and arterioles, finally reaching the capillary system or microcirculation. The arteriole system plays a key role in controlling the blood flow distribution into the various organs. Collectively, the arteriole system constitutes peripheral or systemic vascular resistance, and the LV pressure generated with each cardiac cycle overcomes this resistance to create aortic blood flow into the peripheral vascular system (cardiac output). Regulation of the peripheral system is extremely complex and is regulated by nervous, hormonal, and metabolic control mechanisms. Within the capillary system, oxygen and carbon dioxide are exchanged, and nutrients such as glucose and fatty acids leave the blood and enter surrounding tissues to provide energy while metabolic waste is picked up for removal. The deoxygenated blood leaves the capillary system and enters a large-volume and low-pressure venous system, returning to the heart. Changes in venous return can impact heart contractility and functionality via the Frank-Starling mechanism [28,30].

Control of the Circulatory System

The physiological mechanisms that control overall circulatory function are extremely complex and involve nervous, hormonal, and metabolic systems. The well-described autonomic pathways of the sympathetic (adrenergic) or parasympathetic (cholinergic) nervous systems are key regulators of the cardiovascular system, and there is much similarity between primate and human in the anatomic morphology of the autonomic nervous system [31]. Neurotransmitter release at neurovascular and neurocardiac synapses activates receptors located on vascular and cardiac myocytes to alter cardiovascular function. Sympathetic stimulation causes norepinephrine (nerve terminals) or epinephrine (adrenal medulla) release, which stimulates adrenergic receptors (α and β subtypes) located throughout the cardiovascular system to cause vascular contraction, tachycardia, and elevation of arterial pressure. In contrast, the parasympathetic activation, through acetylcholine-mediated activation of muscarinic and nicotinic receptors, slows the cardiovascular system and opposes sympathetic tone; cardiac parasympathetic tone has a primary role in heart rate regulation. The balance of parasympathetic and sympathetic activity has been studied in the cynomolgus monkey, and this species tends to have higher sympathetic relative to parasympathetic nerve activity [32]. Conscious cynomolgus monkeys do have cardiac vagal tone and demonstrate tachycardia following atropine administration, but the degree of vagal tone is lower relative to other species such as the beagle [33,34]. A higher degree of sympathetic function exists during the day cycle of cynomolgus monkeys, as indicated by power spectral analysis of heart rate [33]. In addition to autonomic control, many well-defined pathways, including centrally mediated baroreceptor reflexes, local vasodilatory responses (nitric oxide), the renin-angiotensin-aldosterone system, and calcium channel expression, are described for NHPs, which all play a role in the homeostasis and responsiveness of the cardiovascular system [28,35].

CARDIOVASCULAR EVALUATION APPROACHES AND METHODS

VetBooks.ir

NHPs are a vital part of cardiovascular research and are used in a variety of efficacy and safety studies. The following sections review major approaches and technical methods that have been used to study drug effects on various aspects of the primate cardiovascular system.

Electrocardiogram Evaluation and Cardiac Arrhythmia Assessment

Conscious Models

The basic cardiac electrical activity (atrial, nodal, and ventricular regions) of the cynomolgus monkey closely resembles the conduction and repolarization patterns of the human heart, making this species very useful for examining potential drug effects on cardiac electrical activity. The standard electrocardiogram (ECG), and derived cardiac intervals, are routinely captured using implant telemetry devices to analyze cardiac electrophysiology end points in awake and unrestrained animals under optimal conditions (i.e., unstressed). The biopotential leads can be placed in the subcutaneous layers of the thorax, intravascular region (superior vena cava), or directly upon the epicardium to record cardiac electrical waveforms [36–39]. The latter options provide better ECG signal resolution compared to subcutaneous leads. Telemetry-implanted cynomolgus monkeys have demonstrated sensitivity to detect QTc prolongation associated with hERG channel blockade [14,17–19], sinoatrial and ventricular conduction delays [21,40], QTc interval changes associated with the day-night cycle [41,42], spontaneous cardiac-arrhythmia patterns [39,43], as well as drug-induced ventricular tachyarrhythmia, including TdP [19,21].

Implanted telemetry devices are the preferred and optimal method for cardiovascular safety assessment of ECG changes in the NHP, with standard collection periods of approximately 24 hours following administration of small molecules [44,45]. For a small-molecule drug candidate, the typical cardiovascular telemetry study is designed as a four-way crossover (vehicle and three doses of the test article; randomly assigned dosing; N = 4-8), which enables each monkey to serve as its own control, thereby maximizing the detection sensitivity of the study [46]. Other experimental designs, such as parallel dose groups (separate vehicle and test article groups), may be used for agents with long pharmacokinetic half-lives (days or weeks). Regardless of study design, telemetry implanted NHPs are considered a very valuable resource and as such implanted animals are typically used in multiple studies. In fact, most facilities maintain a "colony" of implanted NHPs that cycle on and off study following appropriate time with no treatment. Colony animals also undergo advanced health monitoring, typically in the form of clinical chemistry and behavioral observations, to ensure quality study data during treatment. The telemetry method enables the stable collection of ECG parameters from the same animals over long time periods so that the method can also be used to assess chronic effects associated with repeated drug administration in the monkey [47]. Given the high density of data from telemetry studies, statistical approaches using superinterval analysis, coupled with computer-based analysis of ECG waveforms, have evolved to facilitate data analysis [48]. Reference cardiac interval and heart rate (HR) values for a variety of NHPs (unrestrained or retrained/anesthetized) are summarized in Table 29.1.

VetBooks.ir

TABLE 29.1 Basic Cardiac Intervals and Heart Rate Values for Monkeys Measured by Telemetry and During Restraint: Reference Values for Cynomolgus, Rhesus, and Marmoset Strains

Species	Method	N (number of monkeys)	Heart rate (bpm)	Pulse rate (msec)	QRS (msec)	QT (msec)	QTc (msec)	Reference
Cynomolgus	Telemetry	8	114±11	76±4	32±1	270±13	347±9	A1 (B)
	Telemetry	8	113±5	80±4	40±2	261±10	273±7	A2 (I)
	Telemetry	4	159±15	103±8	36±8	206±48	308±26	64 (B)
	Telemetry	6	115±2	74±1	42±1	246±2	337±2	79 (B)
	Telemetry	8	168±21	—	—	204±15	290±10	89 (F)
	Restrained	4	242±17	—	—	154±8	245±18	89 (F)
Rhesus	Telemetry	4	148±8	89±2	—	222±7	298±8	B1 (F)
	Telemetry	24	159±2	96±1	32±1	202±12	325±1	C1 (B)
	Holter	5	135±11	83±3	47±1	246±2	387±11	Hassimoto (B)[a]
	Restrained	12	140±6	85±3	49±1	261±8	400±19	Yang (B)[b,c]
Marmoset	Telemetry	4	134-173	50	27	126-152	*210-230*	51 (B)
	Telemetry	6	*200*	—	—	—	138±3	53 (I)
	Telemetry	4	*140-150*	—	—	*150*	*130-150*	54 (I)

All hemodynamic values (mean±standard error of the mean) are baseline data (pretreatment; day cycle) from the vehicle treatment group. Some data were reported as range values only.
Internal reference data: A1, A2 (Amgen Inc.); B1 (Boehringer-Ingelheim); C1 (CiToxLab). QT correction: B: Bazett; F: Fridericia; I: Individual.
[a]*Hassimoto and Harada [153].*
[b]*Yang et al. [154].*
[c]*Values derived under ketamine/midazolam sedation.*

As QT interval prolongation serves as a surrogate marker for torsadogenic risk, the analysis and interpretation of drug-induced changes in repolarization requires attention. A nonclinical cardiovascular safety assessment workshop recently outlined best practices to incorporate when designing and executing NHP telemetry studies to optimize study power, signaling detection sensitivity, generating high quality data, optimizing animal use, and improving the overall interpretation of QTc interval data [46]. In the NHP there is a well-established inverse relationship between HR and the QT interval (e.g., when HR increases, the QT interval decreases). This normal physiological phenomena requires the calculation of an HR-corrected QT interval (QTc) as part of standard practice when evaluating a drug for effects on cardiac repolarization given the dynamic range of HR and beat-to-beat HR variability that occurs in the conscious NHP [42,46]. Whenever possible, an individual animal correction factor should be implemented as it offers a significant advantage over generic QTc formulas; however, this approach requires long periods (24 hours) of ECG collection in each cynomolgus monkey (e.g., predose or vehicle treatment) to assess the physiological ranges for HR and QT and to quantify the HR/QT slope relationship over a day-night cycle [49,50]. A recent analysis of individual QT/HR slope relationships demonstrated a difference in steepness between

VetBooks.ir

Chinese- and Mauritian-sourced cynomolgus monkeys, which suggests a subtle difference in cardiac repolarization electrophysiology between the strains, but both strains were valid for drug-induced QTc prolongation detection [50]. In experimental settings where individual QTc is not possible, standard QTc correction methods (Bazett, Fridericia, and the Van Der Water formula) have been compared in the cynomolgus monkey. Of these, Bazett's correction has been shown to be an appropriate QTc correction formula for the cynomolgus monkey [42,49].

Many studies have examined the translation of test articles known to prolong the QTc interval in humans (moxifloxacin, dofetilide, terfenadine, amiodarone, and sotalol) and have consistently demonstrated that the cynomolgus monkey has predictive value for the detection of delayed cardiac repolarization [18,50–54]. A cross-company consortium effort sponsored by the Japanese Pharmaceutical Manufacturers Association evaluated 16 compounds (10 positives; 6 negatives) in cynomolgus macaques for QT prolongation and also found good concordance at appropriate exposure of the QTc prolonger in vivo [14,54].

Along with the importance of correcting the QT interval for changes in heart rate, several factors should be considered when examining QTc data and other potential changes in ECG data. These factors are collectively referred to as "indirect" effects that can modulate cardiac conduction and repolarization (e.g., alter the QTc interval). Gastrointestinal effects of novel compounds may alter the cardiovascular secondarily and alter QTc intervals because of changes in autonomic tone. For example, emesis (centrally or gastrically mediated) can alter HR, blood pressure, and QTc intervals, which can confound interpretation of drug effects on cardiovascular function [55]. Other gastrointestinal effects, such as loose stool or severe diarrhea, may disrupt electrolyte balance and energy state and impact ECG observations [55,56]. The observation and collection of clinical signs and symptoms from NHPs receiving treatment can provide valuable insight to aid the interpretation of cardiovascular effects and offer solutions to confounding data. As cage-side observations are often limited (twice daily) during cardiovascular telemetry studies, closed-circuit video monitoring can be used to observe animal behavior remotely. Locomotor activity and body temperature are standard end points collected as part of telemetry data acquisition and can provide valuable clues to observed changes in ECG or hemodynamic parameters. Changes in locomotor activity (e.g., hyperactivity) can alter HR (e.g., tachycardia); thus unanticipated behavioral effects may offer a potential hypothesis for the observed cardiovascular changes. Body temperature changes can also have a profound effect on hemodynamic and ECG parameters [56]. Lower core body temperature prolongs the QT interval, whereas increased body temperature shortens cardiac repolarization [57]. This interaction requires the use of a body temperature correction of the QT interval to account for this indirect effect on cardiac repolarization, and it may need to be considered if unexplained QTc prolongation occurs in the NHP (e.g., not related to hERG channel blockade) [57]. Altered blood glucose (and insulin) concentrations can affect cardiovascular function (i.e., hypoglycemia) and can prolong the QTc interval through indirect metabolic effects, which could also account for QTc prolongation not associated with direct hERG blockade [58]. Last, the NHP has a robust diurnal pattern, which can be observed when monitoring cardiovascular parameters over a 24-hour period; agents that modulate or target the central nervous system may disrupt this diurnal rhythm and indirectly alter cardiovascular function.

In addition to the QTc interval, other components of the ECG waveform can inform about cardiac conduction abnormalities and arrhythmias. The PR (or PQ) interval is examined to

VetBooks.ir

investigate AV conduction; a prolongation of this interval signifies AV block due to changes in cardiac sympathetic tone or sodium/calcium channel blockade [18,59]. The QRS interval is also examined because it may signify ventricular conduction delays related to sodium channel inhibition or other mechanisms [59]. Gauvin et al. [41] published the incidence rates of key background arrhythmias in research-bred cynomolgus monkeys and reported right bundle branch block and premature ventricular complexes with the most frequent occurrence, and this should be considered when examining experimental data. Additional studies recently reported similar prevalence data on atrial, nodal, and ventricular arrhythmia that occur spontaneously in healthy cynomolgus macaques or are triggered by transmural ventricular catheterization [39,43]. Supraventricular premature contraction, escape beats, sinus node pause, and ventricular premature beats were reported to have the highest incidence [43]. Some cardiac arrhythmias had a greater incidence during the night cycle, which suggests a diurnal pattern in cynomolgus monkey attributed to circadian alterations in sympathetic and vagal tone. To better understand and interpret ECG data, best practice suggests that multiple prestudy collections in individual animals are needed to establish the background arrhythmia incidence rate for individual animals for data interpretation of arrhythmias observed during drug treatment [38,43,48]. Although ECG evaluation is focused on cardiac conduction and repolarization intervals, the ECG waveform can also provide valuable information on other aspects of cardiac function including chamber size, mass, and ischemic events.

Other primate species (e.g., the marmoset and rhesus monkey) have been used in cardiovascular safety pharmacology studies to assess QTc prolongation risk. Telemetry-implanted marmosets exhibit a diurnal pattern in the QT interval, as well as HR-dependent changes in repolarization, similar to the cynomolgus monkey [50,60,61]. The circadian alterations in HR and QTc intervals reflect normal physiological alterations in autonomic tone and body temperature that occur in this species. A recent comparison of QT correction methods indicated that an individual correction approach is optimal for assessing drug-induced changes in cardiac repolarization in the marmoset, but the Bazett correction was also valid [50,62,63]. Pharmacological validation studies with agents known to prolong the QTc interval in humans (moxifloxacin, astemizole, and sotalol) also delay cardiac repolarization in the marmoset, and they confirm its predictive use as an in vivo QTc prolongation assay. Some marmosets treated with astemizole exhibit TdP, as seen in cynomolgus monkeys and humans [51,60]. Given the small body size of the primate, this animal could be quite useful for exploratory de-risking studies during early stage lead optimization and candidate selection when compound availability is limited. The rhesus monkey has also been used for QTc evaluation using standard telemetry approaches, but pharmacological validation reports are limited [64,65]. The available information also indicates that this primate model is sensitive to drug-induced QTc prolongation.

Hemodynamic Assessment

In addition to ECG data acquisition, cardiovascular telemetry studies enable the collection of other key important parameters, such as arterial blood pressure (systolic, diastolic, mean), HR, and cardiac contractility (derived from left ventricular pressure; refer to the next section). Alterations in arterial pressure in humans, in particular the onset of sustained drug-induced hypertension, can lead to cardiac hypertrophy, heart failure, and stroke; therefore chronic elevations in arterial pressure can be a significant clinical safety issue [66,67]. Likewise, acute drug-induced

VetBooks.ir

reductions in arterial blood pressure can lead to orthostatic hypotension, dizziness, and syncope [68,69]. With regard to cardiac rhythm, HR elevation is another safety risk because it is correlated to negative outcomes and an increased likelihood of a major cardiovascular event (e.g., myocardial ischemia) [70,71]. Thus, drug-induced alterations in systemic hemodynamics are an essential component of nonclinical cardiovascular risk assessment in the NHP model.

Standard telemetry methods measure blood pressure from an intravascular pressure transducer implanted into the femoral artery or another arterial segment, and HR is derived from the pulse wave interval or from the ECG (RR interval). Arterial blood pressure and HR, similar to ECG parameters, display a diurnal pattern in the cynomolgus monkey, with noted decreases in both parameters during the night photoperiod [41,46]. Numerous validation studies in the conscious cynomolgus monkey have evaluated agents known to elevate or reduce arterial pressure and modulate HR, and they have demonstrated that this species has pharmacological sensitivity to detect changes in these end points and responds accurately (i.e., as in humans) [18,34,72–74]. Because hemodynamic end points are captured continuously over long time periods as part of cardiovascular telemetry studies in the NHP, the same best practice recommendations, experimental designs, and data analysis approaches used for ECG evaluation apply to arterial pressure and HR [46].

Along with these cardiovascular end points, telemetric monitoring can also capture information of general activity of the primate and body temperature. Conscious primates exhibit normal variation in behavior (excitement, social activity, sleep) and body temperature during the course of a cardiovascular telemetry evaluation, and these phenomena can have a direct impact on functional findings. For example, pronounced elevations in arterial pressure, HR, and respiration in the NHP can occur in response to human interaction activities or procedures such as room entry or physical restraint (for chairing, dosing, and collecting samples) because of excessive emotional arousal and concomitant sympathetic activation [75–77]. When such activities occur during a telemetry study, whether scheduled or unscheduled, the cardiovascular data are compromised and need to be omitted from analysis. Table 29.2 summarizes hemodynamic and LV contractility values in conscious unrestrained or restrained/anesthetized monkeys.

A potential gap in the telemetric evaluation of cardiovascular function in conscious animals, including monkeys, is the inability to monitor other important physiological components that regulate arterial pressure, namely cardiac output and vascular resistance [45,74]. The direct measurement of cardiac output has been performed in conscious cynomolgus monkeys, but it requires placement of a flow probe around the base of the aorta to measure beat-to-beat blood flow; vascular resistance is calculated indirectly [77]. This method is surgically intensive and involves the externalization of electrical wires (for connection to instrumentation and power supply), which limits the use of the method to nonroutine or mechanistic cardiovascular studies. Another technical aspect is the demanding technical skill required to implant and maintain the aortic flow cuff for long periods of time (e.g., weeks). At the current time, cardiac output and vascular resistance (total or regional) determinations are not standard end points captured in conscious NHP telemetry studies, but one report described an external backpack platform for the conscious dog, which could be used in primates [78,79]. If a mechanistic understanding of drug action on hemodynamics is needed, measuring cardiac output in anesthetized models or through the use of cardiac imaging technology is possible (discussed later in this chapter).

VetBooks.ir

TABLE 29.2 Basic Arterial Pressure and Heart Rate Values for Monkeys Measured by Telemetry and During Restraint: Reference Values for Cynomolgus, Rhesus, and Marmoset Strains

Species	Method	N (numbers of monkeys)	Heart rate (bpm)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Mean blood pressure (mmHg)	Left ventricular dP/dt_{max} (mmHg/s)	Reference
Cynomolgus	Telemetry	8	114±11	99±3	67±4	83±3	—	A1
	Telemetry	8	113±5	102±6	66±5	84±6	3067±117	A2
	Telemetry	4	159±15	130±18	90±13	111±16	—	64
	Telemetry	8	147±13	84±2	53±4	—	—	65
	Telemetry	8	90-130	115±4	80±9	—	1809-2824	69
	Restrained	5	256±10	128±4	73±2	93±2	—	90[a]
Rhesus	Telemetry	4	148±8	199±5	85±6	—	3059±175	B1
	Telemetry	24	159±2	122±1	77±1	99±1	—	C1
	Telemetry	4	90-130	—	—	—	2168-2827	69
	Restrained	7	155±12	121±11	—	—	—	85[b]
Marmoset	Telemetry	4	134-173	92-103	63-71	—	—	51
	Telemetry	—	230±26	—	—	95±9	—	86
	Restrained	—	348±51	—	—	107±16	—	86

All hemodynamic values (mean±standard error of the mean) are baseline data (pretreatment; day cycle) from the vehicle treatment group. Some data were reported as range values only.
Internal reference data: A1, A2 (Amgen Inc.); B1 (Boehringer Ingelheim); C1 (CiToxLab). B: Bazett correction; F: Fridericia; I: Individual correction.
[a]*High-definition oscillometry performed in awake, tube-restrained animals.*
[b]*Values derived by cuff method under ketamine sedation.*

Cardiac Contractility Assessment

Left Ventricular Pressure and dP/dt_{max} End Points

In addition to alterations in cardiac electrophysiology and hemodynamics, drugs can have intended or unanticipated effects on cardiac pump function because of alterations in cardiac contractility, or inotropy [80,81]. A common way to assess cardiac contractility invasively is to place a pressure sensing catheter in through the apex of the LV and record left ventricular pressure (LVP) continuously by telemetry in the NHP model [80,81]. Cardiac contractility evaluation is not a core end point in S7A; however, LVP and derived contractility indices are recommended for monitoring in NHP cardiovascular telemetry studies to identify contractility hazards. A few reports have assessed basal and drug-induced LVP and dP/dt_{max} changes in conscious cynomolgus and rhesus monkeys, but there are no published reports on LV contractility in the marmoset [82–84] (Table 29.2).

The addition of LVP provides information in the overall mechanical performance of the heart, and it can give valuable insight on drug-induced changes in systolic and diastolic function [81]. The most common parameters reported include: LV end systolic and end diastolic pressure (LVEDP), LV dP/dt_{max}, and $dP/_{dtmin.}$ The parameter LV end systolic

VetBooks.ir

pressure is an important determinant of systemic perfusion (e.g., cardiac output); thus increases or decreases may be indicative of augmented or depressed cardiac contractility or elevated afterload [81]. LVEDP reflects the compliance of the left ventricle and is influenced directly by the volume of blood received from the left atrium during diastole (e.g., an indicator of preload). When there is an increase in LVEDP, ventricular compliance may be decreased or preload may be elevated. Correlations can be made between LVEDP and other cardiac pressures. For example, during normal mitral valve function, LVEDP should be roughly equivalent to left atrial pressure. Moreover, in the cases of normal mitral valve and lung function, LVEDP may be a surrogate for the pressure in the pulmonary-capillary bed (e.g., pulmonary capillary wedge pressure). The peak positive and negative derivatives of the LVP waveform also provides information on maximum and minimum rate of rise or fall in developed LV pressure, respectively (e.g., dP/dt_{max} or dP/dt_{min}). LV dP/dt_{max} is a well-accepted index of cardiac contractility (a surrogate) and the end point mostly commonly used to evaluate drug-induced changes in LV contractility [80,82].

It needs to be stressed that careful attention must be given to the interpretation of dP/dtmax findings because this end point can be modulated directly by alterations in preload (e.g., LVEDP), afterload (e.g., aortic pressure), and heart rate [81–83]. Several other methods of analyzing and considering contractility have been brought forth and would be relevant to any NHP analysis as well [83]. Indeed, dP/dt_{max} can be normalized for HR changes, such as the QT interval, to define the normal the H-force frequency relationship (Bowditch or Treppe effect) and serve as a baseline to determine primary drug effects on myocardial contraction in vivo [82].

Cardiac Imaging by Echocardiography

Echocardiography is a noninvasive method that can provide functional and structural information on the heart and vasculature, and it has application in a variety of animal species, including the NHP. This imaging modality can be utilized in either acute or long-term cardiac safety assessment assays on new drug candidates for the detection of chamber size, wall thickness, hypertrophy, valve lesions, and flow abnormalities [84,85]. In contrast with LVP measurements, which do not have a clinical correlate, echocardiography can be used to translate cardiac effects from animal species to humans as well as the time course of onset. The common echocardiography assessment in NHPs consists of light anesthesia with either inhaled gas (isoflurane; 1 to 2%) or chemical restraint (low-dose ketamine) to induce sedation, which enables optimal probe placement on the thorax of the NHP and the capture of images (for subsequent offline analysis). The potential direct pharmacological effect of the sedative/anesthetic on myocardial contractility is a pitfall of this approach and needs to be considered when analyzing and interpreting echocardiographic data. Sleeper and colleagues [86] completed a large study that examined the effects of different sedation protocols on echocardiography parameters. Several studies reporting a normal range of echocardiographic values for standard parameters in cynomolgus macaques have been published [86,87]. Echocardiographic values in healthy, young, and aged rhesus monkeys, as well as animals with myocardial infarcts, have also been reported [88–90]. To date, there is only one report on a cardiovascular toxicity evaluation of minoxidil in marmosets that included echocardiography [91].

VetBooks.ir

The basic echocardiography examination typically investigates the left side of the heart using clinical ultrasound systems equipped with a probe using 3-5 MHz to produce the echocardiographic images. Hanton and colleagues [84] provide an excellent overview of the basic echocardiographic exam. Briefly, two- or three-dimensional images are collected that allow for the calculation or measurement of three-dimensional volumes of the LV chamber. The volumetric data is used to calculate standard parameters such as ejection fraction (EF), stroke volume (SV), and cardiac output (CO). The use of m-mode images can allow for the measurement of wall thickness of both the septal and posterior walls facilitating the calculation of fractional shortening (FS), LV mass, and relative wall thickness. Doppler technology allows for the measurement of blood flow velocities in the heart and vessels. The measurement of flow through the mitral valve provides important information around the diastolic functioning of the heart. The typical transmitral Doppler pattern consists of two peaks; the first, or early velocity, represents the opening of the mitral valve, whereas the second peak represents the velocity from atrial contraction. Although the typical exam focuses on left ventricular structure and function in systole and diastole, echocardiography techniques may be customized or adapted from existing clinical practice to examine additional cardiovascular parameters of interest, including right ventricular structure and function, ejection times, pulmonary or aortic Doppler velocities, atrial volume, and investigation into peripheral vasculature (Tables 29.3 and 29.4).

Pharmacological and inotropic responses to standard cardiovascular agents have been evaluated in cynomolgus macaques using key echocardiographic readouts. Following treatment with beta blockers (propranolol, metoprolol) a reduction in cardiac output was observed, as expected, coupled with decreased ejection fraction and increased end-systolic volume [92]. The calcium channel blocker, verapamil, also decreased cardiac output and ejection fraction while increasing both end systolic and end diastolic volume [92]. The beta-agonist dobutamine was used to demonstrate a positive inotropic response and treatment that resulted in an increased ejection fraction, cardiac output, and stroke volume but a decreasing end-systolic and diastolic volume [87,92]. Echocardiographic techniques have also been used to track and characterize primates with disease, such as *Trypanosoma cruzi* infection [93].

Advancements and new technologies developed within the field of echocardiography may offer more sensitive measures of cardiovascular structure and function and may offer ways to detect and track subclinical cardiovascular dysfunction. Tissue Doppler techniques may offer a more sensitive method of examining diastolic function [94], whereas strain imaging, also known as speckle tracking or cardiac deformation, may offer a more sensitive measure of cardiovascular function [94,95]. Strain imaging has demonstrated some success in the clinical setting and its potential applications to preclinical research are currently being characterized and potentially validated. In human clinical studies, strain techniques have proven particularly useful at detecting subclinical cardiovascular dysfunction in patients treated with anticancer agents [96].

There are several considerations when implementing ultrasound techniques into safety studies. As with other aspects of measuring cardiac function, echocardiography techniques are also dependent upon loading conditions of the heart; therefore, a holistic approach to the interpretation of findings is important and should include an understanding of the complete hemodynamic profile of the test article. Before initiating a study, it is critical that the sonographer be fully qualified to collect and analyze echocardiographic images to ensure high quality data. It is generally recommended that a single sonographer perform the analysis of the

VetBooks.ir

TABLE 29.3 Female Reference Ranges for Basic Echocardiographic Parameters in Sedated Cynomolgus Monkeys

Parameter	N[a] (number of individual monkeys)	Mean[a]	Reference range[a]	N[b] (number of individual monkeys)	Mean[b]	Reference Range[b]
BW (kg)	118	2.56	1.47-3.67	32	3.1	2.6-4.1
HR (bpm)	117	161	86-236	32	141	81-170
AO (cm)	107	0.79	0.52-1.07	32	0.75	0.53-1.01
AV_{max} (m/s)	54	0.82	0.49-1.15	ND	ND	ND
LVET (s)	118	0.19	0.12-0.27	ND	ND	ND
LVPEP (s)	64	0.03	0.00-0.05	ND	ND	ND
LVPEP/ET	64	0.14	0.02-0.06	ND	ND	ND
EPSS (cm)	118	0.01	–0.18-0.21	ND	ND	ND
LA (cm)	107	0.89	0.51-1.28	32	.82	0.49-1.18
LA:AO	107	1.14	0.62-1.66	32	1.09	0.60-1.54
IVSd (cm)	107	0.37	0.21-0.53	32	0.35	0.20-0.49
LVPWd (cm)	107	0.36	0.22-0.50	32	0.38	0.21-0.48
LVIDd (cm)	118	1.35	0.92-1.77	32	1.23	0.87-1.74
LVIDs (cm)	118	0.81	0.43-1.19	32	0.79	0.40-1.20
FS (%)	118	40	19-60	32	38	22-58
EF (%)	ND	ND	ND	32	71	56-82
ESV (mL)	ND	ND	ND	32	1.1	0.35-2.1
EDV (mL)	ND	ND	ND	32	3.8	1.7-4.9
SV (mL)	ND	ND	ND	32	2.7	1.3-2.8

BW, body weight; HR, heart rate; ND, not determined; AO, aorta diameter; AVmax, max flow velocity of aorta; LVET, left ventricular ejection time; LVPEP, left ventricular preejection period; EPSS, E-point septal separation; LA, left atrial diameter; IVSd, intraventicular septal wall thickness in diastole; LVPWd, left ventricular posterior wall thickness diastole; LVIDd, left ventricular internal dimension diastole; LVIDs, left ventricular internal dimension systole; FS, fractional shortening; EF, ejection fraction; ESV, end-systolic volume; EDV, end-diastolic volume; SV, stroke volume. All data are mean values taken from cynomolgus monkeys sedated with ketamine (10 mg/kg) or telazol-atropine (4.0 mg/kg:0.015 mg/kg).

[a]*Data taken from Sleeper et al. [84].*

[b]*Unpublished data from Pfizer consisting of 32 vehicle-treated or untreated Mauritius cynomolgus monkeys across multiple toxicology studies.*

echocardiographic images to eliminate any potential interobserver error. In addition, clinical guidelines supplied by the American Society of Echocardiography spanning collection and analysis of a variety of echocardiographic data can be easily applied to the NHP.

Cardiovascular Evaluation in Anesthetized Primates

The ICH S7A guideline states a preference for conscious, unrestrained nonrodents for the core cardiovascular safety evaluation assay, which infers that implant telemetry is the best method to characterize the cardiovascular activity of a candidate drug. Although the use of

VetBooks.ir

TABLE 29.4 Male Reference Ranges for Basic Echocardiographic Parameters in Sedated Cynomolgus Monkeys

Parameter	N[a] (number of individual animals)	Mean[a]	Reference range[a]	N[b] (number of individual monkeys)	Mean[b]	Reference Range[b]
BW (kg)	119	3.49	1.63-5.36	32	5.4	3.9-9.2
HR (bpm)	119	152	77-227	32	145	72-189
AO (cm)	108	0.84	0.58-1.11	32	0.9	0.61-1.23
AV_{max} (m/s)	54	0.79	0.39-1.19	ND	ND	ND
LVET (s)	119	0.20	0.12-0.28	ND	ND	ND
LVPEP (s)	65	0.03	0.00-0.06	ND	ND	ND
LVPEP/ET	65	0.15	0.02-0.27	ND	ND	ND
EPSS (cm)	119	0.02	–0.36-0.41	ND	ND	ND
LA (cm)	108	0.99	0.55-1.42	32	1.04	0.61-1.51
LA:AO	108	1.18	0.69-1.66	32	1.15	0.70-1.77
IVSd (cm)	108	0.40	0.22-0.58	32	0.51	0.31-0.69
LVPWd (cm)	108	0.39	0.21-0.57	32	0.48	0.22-0.71
LVIDd (cm)	119	1.50	1.05-1.96	32	1.72	1.10-2.35
LVIDs (cm)	119	0.92	0.52-1.31	32	1.31	0.67-1.79
FS (%)	119	39	23-55	32	35	21-59
EF (%)	ND	ND	ND	32	66	51-80
ESV (mL)	ND	ND	ND	32	1.5	0.45-2.7
EDV (mL)	ND	ND	ND	32	4.3	2.7-5.9
SV (mL)	ND	ND	ND	32	2.8	1.2-3.2

BW, body weight; HR, heart rate; ND, not determined; AO, aorta diameter; AV_{max}, max flow velocity of aorta; LVET, left-ventricular ejection time; LVPEP, left-ventricular preejection period; EPSS, E-point septal separation; LA, left-atrial diameter; IVSd, intraventicular septal wall thickness in diastole; LVPWd, left-ventricular posterior wall thickness diastole; LVIDd, left-ventricular internal dimension diastole; LVIDs, Left-ventricular internal dimension systole; FS, fractional shortening; EF, ejection fraction; ESV, end-systolic volume; EDV, end-diastolic volume; SV, stroke volume. All data are mean values taken from cynomolgus monkeys sedated with ketamine (10 mg/kg) or telazol-atropine (4.0 mg/kg:0.015 mg/kg).

[a]*Data taken from Sleeper et al. [84].*

[b]*Data from Pfizer consisting of 32 vehicle-treated or untreated Mauritius cynomolgus monkeys across multiple toxicology studies.*

conscious animals in safety pharmacology is considered ideal, the downside of using conscious animals, especially primates, is that arousal, spontaneous changes in behavior, locomotion, feeding/drinking activity, body temperature, and circadian fluctuations in autonomic tone are significant sources of variability that directly alter basic cardiovascular end points [44,97]. Primates are excitable, and they typically demonstrate sympathetically driven HR elevation in response to environment cues (e.g., onset of day cycle or ambient noise and stress such as room entry or manual restraint) (Tables 29.1 and 29.2). These behavioral impacts on HR, and other cardiovascular parameters, are highly reproducible over time and do not acclimate in the conscious cynomolgus monkey (see Figure 29.1 in ref. [98]). Thus, the evaluation of drug-induced

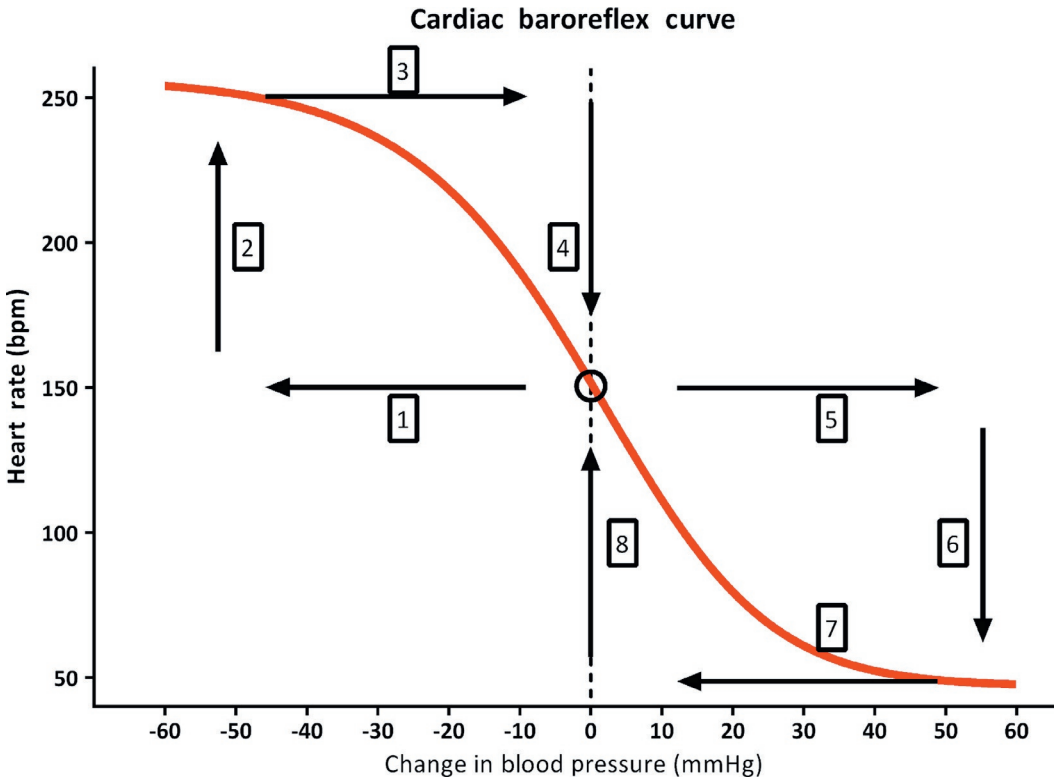


FIGURE 29.1 Cardiac baroreflex curve. Under conscious or anesthetized conditions, a peripherally mediated change in blood pressure (BP) is met by an autonomic reflex response to restore BP (i.e., cardiac baroreflex). A monkey is challenged with a drug, typically sodium nitroprusside, which reduces BP (1). The reduction in BP elicits a physiological response of increased heart rate (HR). As the drug is removed from the system, BP is returned to baseline (3), quickly followed by HR (4). Following a second baseline period with a short duration, the animal is then challenged with a pressor agent, such as phenylephrine, which results in an elevation of BP (5). A physiological reflexive decrease in HR is observed (6). The pressor effect is removed and BP decreases (7), allowing the HR to return to baseline values (8). Baroreceptor curves can be generated under conditions of vehicle and drug treatments, allowing for comparison and potential identification of drug-induced changes of the baroreflex. *The graph was adapted with permission from Zahner, M.R. and Heyen, J.R. from Autonomic regulation of blood pressure in rat; a safety pharmacology approach to assess autonomic control of blood pressure using the baroreceptor reflex (submitted).*

cardiovascular parameters in conscious monkeys requires an essential knowledge of all sources of cardiovascular variability, especially those that influence HR, and the establishment of baseline effects [46,74]. High HR variability in conscious animals can reduce the overall sensitivity to detect QTc prolongation, which can be overcome by increasing group size [46,97].

An alternative and scientifically valid approach to assess drug-induced alteration in hemodynamics, and QTc prolongation, is to conduct the study in anesthetized monkeys [65,86,98–102]. The advantage of the anesthetized monkey is the stability of cardiovascular end points, especially HR and the QT interval, over the time course of the study (i.e., is the animal is unable to respond to environmental factors or stressors). An added benefit is that each animal serves as their own control, so drug effects can be compared directly to stable baseline values

VetBooks.ir

(predrug) in the same animal. Low HR variability means that the QT interval will also be relatively stable and more sensitive for the detection of QTc prolongation [44]. For example, the QTc prolonging effect of sotalol was compared in unrestrained cynomolgus monkeys or under anesthesia with halothane [101]. When equivalent plasma sotalol concentrations were compared, anesthetized monkeys demonstrated a larger QTc interval increase relative to awake animals. The enhanced QTc sensitivity of the anesthetized preparation was attributed to the ability of halothane to reduce cardiac repolarization reserve, which may be a property of some anesthetics on myocardial electrophysiology [97]. In addition to QTc sensitivity, a separate study found that drug-induced changes in arterial pressure and HR were also greater in isoflurane-anesthetized cynomolgus monkeys compared with conscious animals based on responses to several different pharmacological challenges [65]. These investigators also found that significant functional and statistical effects could be demonstrated at doses in anesthetized cynomolgus (and rhesus) monkeys, which were not considered active in the conscious animal models. This comparison clearly suggests that the anesthetized preparation is a sensitive method for identifying hemodynamic effects. In contrast with the cynomolgus and rhesus monkey reports above, there is a paucity of information on the use of anesthetized marmosets in cardiovascular studies.

The collective findings indicate that anesthetized primates (cynomolgus and rhesus) are valid alternative models for the cardiovascular profiling of new drug candidates, and they are a valuable addition to the cardiovascular safety evaluation toolbox. It should be acknowledged, however, that only a few compounds have been evaluated in this model to assess hemodynamic and QTc changes, and there is little information on how the anesthetized primate model responds to cardiac conduction blockers or inotropic drugs, in contrast with the conscious monkey telemetry model [55,81]. So, there is a need for more pharmacological validation of other cardiovascular end points in the anesthetized monkey model, including detailed evaluation of cardiac output (with echocardiography or thermodilution methods) and vascular resistance [74]. As an evaluation tool, the anesthetized cynomolgus monkey assay could be used as follow-up or supplemental assay, especially for drug candidates that might have profound neurobehavioral or other pharmacological effects that interfere with or prevent the accurate interpretation of findings in the conscious state [98]. The anesthetized model may also be more suitable for agents with expected general toxicity risk (e.g., oncolytics) to avoid unnecessary distress or morbidity in conscious telemetered primates or for exploratory de-risking studies [65].

Some challenges to consider when using anesthesia is the need to fully characterize the impact of the anesthetic (injectable or gas) on the cardiovascular end points of interest, and the possibility that the anesthesia may modulate or blunt the cardiovascular activity of the test drug. In the previous examples, gas anesthetics were used for surgical procedures to stabilize cardiovascular parameters (after instrumentation) and then maintained an optimal degree of anesthesia over the timeframe (e.g., hours) of the experiment. Different anesthesia regimens may have differing effects on arterial pressure, HR, autonomic tone, cardiac electrical activity, and the depth of anesthesia, which are all factors that can influence basic cardiovascular parameters and drug response. Light anesthesia, or sedation (for chemical restraint), is another practice that is used to facilitate physical exams and conduct acute evaluations of the cardiovascular system. For example, ketamine is a common sedative used in clinical veterinary evaluation of primates, which can increase HR but alter blood pressure variably,

VetBooks.ir

with both hyper- and hypotensive responses reported in cynomolgus and rhesus monkeys as well as marmosets [102–105]. Regardless of the anesthesia regimen used, the establishment of stable and reproducible values at baseline (predrug) and over the experimental treatment period needs to be established to interpret drug-induced findings accurately. The interpretation of cardiovascular observations made in the presence of a short-acting sedative may be problematic because of the inability to reliably achieve a stable cardiovascular baseline, the potential for variable onset of sedation between monkeys, and the influence of the sedative on the primate cardiovascular function.

Cardiovascular End Points in Toxicology Studies

Cardiovascular evaluations in chronic toxicology studies use noninvasive methods, such as ECG monitoring, pulse rate determination, and cuff-based blood pressure, in conscious animals that are manually or chemically restrained, a clear divergence from the best practice of radiotelemetry in unstressed animals [45,98]. Historically, such methods were used to collect small samples of basic cardiovascular parameters, or "snapshots of data," from a large number of toxicology study animals. Although the application is simple and practical, the cardiovascular data collected in restrained animals is generally low quality, especially ECG waveforms, and the limited data captured can be misleading or uninterpretable. Given that conscious primates have an intense behavioral and sympathetic response to restraint stress, the interpretation of findings can be confounded by excessive tachycardia, ECG lead movement, muscle movement noise, and the evaluation of a single time point after dosing [44,45,74,106,107]. As a result, snapshot assessments of ECG and arterial pressure have lower sensitivity for detecting QTc prolongation and hypertension, respectively, and have a higher potential for false-negative findings [108,109].

A major improvement is the development of jacket ECG and blood pressure telemetry systems, which enable the collection of high quality cardiovascular signals from unstressed conscious animals [74]. Jacket technologies can be directly incorporated into toxicology studies in monkeys to inform chronic drug hazards. Derakhchan et al. [98] demonstrated that jacket-based telemetry was equivalent to implant telemetry for its ability to capture high quality ECG and HR data capture in conscious cynomolgus monkeys. In a validation study designed to mimic a 28-day toxicology study, HR and QTc values were stable over multiple 24-hour collection periods and reproducible in the vehicle treatment group over 4 weeks. The jacket method detected drug-induced QTc prolongation and bradycardia in a sensitive manner. Basal HR values were consistently low (unstressed) as well and showed normal diurnal variation like unjacketed monkeys. It is important to note that when designing experiments using jacket technologies, cynomolgus monkeys must undergo a training and acclimation period (2 or 3 days before the study) to the jacketing and dosing procedures to ensure quality data [98]. Jacket-based ECG (Holter) monitoring has also been reported in the rhesus monkey but not the marmoset (Table 29.1). The utility of jacket-based telemetry in primate toxicology studies has gained traction, and it has "3R's" (replace, reduce, refine) implications according to a recent pharmaceutical industry survey of functional investigations in toxicology studies [107].

Jacketed technologies have also been developed to monitor arterial pressure in addition to ECG [74]. Miniature telemetry devices for arterial pressure, coupled with jacket ECG telemetry,

VetBooks.ir

have been developed for the monkey [110]. This method requires minor surgery to introduce the vascular catheter, which has no impact on tissue pathology end points. The approach facilitates collection of high-fidelity blood pressure data simultaneously with ECG in chronic toxicology studies from unrestrained conscious monkeys. Current methodologies for arterial pressure evaluation in toxicology studies are oscillometric cuff-based methods. Cuff methods are subject to the same caveats as ECG collection in restrained or sedated monkeys (see "Cardiovascular Evaluation in Anesthetized Primates"), and thus they are inferior for blood pressure evaluation [111]. A new cuff method, called high-definition oscillometry, may offer improved sensitivity for the noninvasive detection of drug-induced changes in arterial pressure in cynomolgus monkeys [109,112]. High-definition oscillometry may be more accurate than prior cuff devices, but it still requires the manual restraint of the monkey, which is problematic. For additional discussion on functional evaluations in toxicology studies, see Chap. 22, "Functional Assessments in Nonhuman Primate Toxicology Studies to Support Drug Development."

Specialty Cardiovascular Assays

Therapeutic interventions that effect cardiovascular function may do so indirectly by modulating the autonomic nervous system. The autonomic nervous system is a key regulator of normal function of the heart, and vasculature several models have been developed to examine the function of key pathways suggestive of changes in autonomic tone that may allow insight into the hemodynamic effects of therapies that may be potentially neurogenic in nature. The monkey tilt model is an example of using the basic tenants of cardiovascular physiology to examine alterations in the autonomic nervous system. Orthostatic hypotension is defined as a decrease in blood pressure of 20 mmHg upon standing and is typically tested in the clinical system by having patients stand up and monitoring HR and blood pressure at 1 and 3 minutes after standing [113]. However, in animal models it is more practical to adapt a clinical model that uses a tilt table to recapitulate the alterations in blood volume that occur when changing positions from sitting to standing [114,115]. This model has been validated in cynomolgus macaques and demonstrated to be reliable with standard blood pressure lowering agents with demonstrated orthostatic hypotension. It is also technically possible to use lower body negative pressure to alter blood volume distribution and thus mimicking orthostatic hypotension [116]; this model has been successfully implemented for use in NHPs. The model has mechanistic value in providing an understanding of neurogenic hypotension and the potential role of the peripheral or central nervous system modulation in mediating the cardiovascular response.

Models that can attempt to dissect the potential causes of drug induced hemodynamic changes by taking advantage of the baroreceptor response have also been developed. It is well known that arterial pressure is maintained around a fixed value by using various aortic pressure-sensing receptors as a buffering system [117]. The baroreceptor is the primary neural control of blood pressure in response to transient stress and/or exercise [118]. Novel therapies that have unanticipated effects on cardiovascular hemodynamics may alter autonomic tone and thus alter the baroreceptor response. These effects on hemodynamics can be tested in animal models, including the NHP, by recapitulating a clinical model [117–120]. The baroreceptor response can be examined with and without treatment with the novel therapy of interest. To accomplish the evaluation of the baroreceptor response, a short intravenous

VetBooks.ir

infusion of a vasopressor, typically phenylephrine, followed by a short infusion of a vasodilator, often sodium nitroprusside, is completed [117]. The doses of the respective vasoactive compounds are targeted to change blood pressure approximately 30 mmHg in both directions. HR and blood pressure changes are plotted over the course of the infusions, and the results are a sigmoid curve that demonstrates the baroreceptor response over the physiological range of blood pressure (Figure 29.1). Vehicle-treated NHPs can be compared with drug-treated animals to compare responses and examine potential drug-related changes in the baroreceptor reflex.

In Vitro and Ex Vivo Cardiovascular Assessment

Similar to other research species, many in vitro or ex vivo methodologies have been developed to analyze cardiovascular parameters that can be theoretically applied to NHP-derived tissues. The isolation of cardiomyocytes from adult hearts is similar to other mammalian species; however, the use of isolated primary cardiomyocytes from NHPs is seldom practiced. As such, very little is known about the characteristics of isolated primate cardiomyocytes in culture. Alternative models such as stem cell derived cardiomyocytes—typically of human embryonic or induced pluripotent origin—are often used in lieu of NHP cardiomyocytes and represent a model that may be more relevant to human risk assessment [121–123]. Adult and neonatal cardiomyocyte isolation and experimentation is much more common in rodent species [124].

Techniques are available to functionally evaluate isolated tissues from NHPs. Isolated perfused vascular preparations can be completed using a variety of arteries or veins [125]. In addition, the isolated perfused (Langendorff) NHP heart has been demonstrated to be a stable preparation for the experimentation of the potential effects of compounds on inotropy or chronotropy or basic heart function. However, similar to in vitro techniques, there is a dearth of data around vascular and heart ex vivo preparations—again, likely related to the scarcity of NHP tissue available for experimentation [125,126]. Indeed, vascular and heart tissue from human donor sources are available and used more commonly instead of NHP tissue [127].

Congenital and Background Cardiovascular Pathology in NHPs

Hendrickx and Binkerd [128] thoroughly review congenital heart defects in NHPs. Briefly, spontaneous ventricular septal defects are the most commonly identified finding that occurs in both the rhesus and cynomolgus macaques [128]. Additional publications have reported similar interventricular septal defects in rhesus and cynomolgus monkeys [129,130]. Jerome [131] also published a survey of congenital malformations in breeding colonies for rhesus, cynomolgus, and stumptail macaques. Commonly identified were aortic coarctation associated with patent ductus arteriosus and atrial septal defects. Cardiac malformations were present to a greater degree compared to other organ systems [131].

Spontaneous cardiovascular lesions of research-bred NHPs have been identified and should be considered when interpreting data related to drug testing. Commonly identified pathologies include inflammatory cell infiltrate, perivasculitis/vasculitis, and focal myocarditis that were observed in 13%, 6%, and 5%, respectively, of cynomolgus macaques that were examined [132]. Other lesions identified in the same cohort of cynomolgus macaques

VetBooks.ir

observed in <1% of animals included mineralization, endocarditis, pericarditis, myocardial fibrosis, hematopoiesis, and squamous cysts [132,133]. It is important to note that there may be differences in the incidence rate of specific lesions between male and female NHPs. Vidal and colleagues [25] also reported a higher incidence rate of inflammatory cell infiltrate observed in almost 40% of Mauritian cynomolgus macaques in a much smaller cohort of animals. Dunn and colleagues [134] examined the use of troponin I as a potential biomarker for screening cynomolgus monkeys that may present with the lesion, concluding that troponin I concentrations did not differ in animals with or without lesions. Sato and colleagues [135] recently published a comprehensive review of common lesions identified in a cohort of 660 cynomolgus macaques of varying origin. Twelve common or rare lesions of the heart and artery are described, including focal inflammatory cell infiltration, focal myocardial necrosis, endocardial hemorrhage, and arteritis in coronary and kidney arteries [136].

Cardiovascular Disease Models in NHPs

Although many aspects of human disease are investigated using NHP disease models (reviewed in Chap. 13, "The Nonhuman Primate Models of Human Disease"), research in the cardiovascular field, particularly diabetes and atherosclerosis, has advanced through the use of NHP models [137–139]. Briefly, many attributes of human diabetes are present in NHPs, including dyslipidemia, obesity, insulin resistance, and pancreatic pathology [139–141]. Similar to the human disease condition in type 2 diabetes, there is increased prevalence with increase in body weight and age. Insulin resistance and associated circulating concentrations of insulin are precursors to deterioration of carbohydrate metabolism [141,142]. The progression to overt diabetes includes the transition from moderate elevations in fasting glucose to hyperglycemia and decreases insulin secretion [141]. Pancreatic pathology, including islet-associated amyloid, is present in NHPs and closely resembles the human pathology. Diabetes has been induced in NHPs by several methods with varying levels of success. Partial or total pancreatectomy, along with the use of alloxan, has been used to successfully induce a diabetic state; however, toxicity to other organs, including kidneys, adrenals, and the liver, produces side effects not typically observed in diabetes and limits the utility of the model [142]. Similar to rodents, streptozocin (STZ) has been used to successfully induce a reproducible form of diabetes. STZ is a somewhat specific β-cell toxin with limited effects to other organ systems [143,144]. STZ treatment results in hyperglycemia; however, the pancreatic islet pathology more closely resembles type 1 rather than type 2 diabetes [139,143,144]. In general, monkeys with STZ-induced diabetes are not insulin resistant unless aged or obese, and the idiosyncrasies of the model require individual laboratories to modify induction protocols to achieve success.

Cynomolgus macaques represent the most common species used in pharmaceutical research in both efficacy and toxicological models. As such, a natural occurrence of diabetes in the cynomolgus macaques is of interest for examining potential interventional therapies. Wagner and colleagues [140] and Kaplan and Wanger [141] examined the characteristics of diabetic cynomolgus macaques and found increased fasting glucose concentrations, increased glycation, and delayed glucose clearance upon challenge. As cynomolgus monkeys age, approximately 30% over the age of 15 years develop either hyperinsulinemia and/or postprandial hyperinsulinemia [140,141]. Obesity with body weight and body mass indices outside of 95% confidence intervals is associated with the diabetic cynomolgus monkey [145,146]. Cynomolgus monkeys can progress from insulin responsive to insulin resistant over time as

VetBooks.ir

the disease progresses and thus require insulin treatment. Shared aspects of human and cynomolgus monkey type 2 insulin-resistant diabetes include changes in blood lipid profiles such as increased total cholesterol, triglycerides, free fatty acids, and decreased high-density lipoprotein [139–141]. Inflammation and hemodynamic changes have also been reported such as increased C-reactive protein and blood pressure [141].

Marmosets are also used in diabetic and obesity research as they offer several advantages over other species of NHPs. Marmosets are much smaller, commonly develop obesity, and also mature faster than other species. Moreover, they are considered aged at 8 years old, allowing for the development of colonies in an accelerated timeline compared with other NHPs [147,148]. Obese marmosets display common characteristics of other diabetic/obese models, such as increases body fat, elevated glucose, triglycerides, cholesterol, and HbA_{1c} [149–151]. Wachtman and colleagues [152] developed a diet-induced model of cardiometabolic syndrome in marmosets. Marmosets were fed control, fat-, or glucose-enriched diets and tracked longitudinally to observe effects on metabolic parameters and body weight and composition and terminal pathology of the pancreas and vasculature. Obesity was induced in the glucose-enriched diet and hyperglycemia at a much earlier time point compared to controls and the animals fed a high-fat diet; the latter developed a higher severity of atherosclerosis, even with a delayed obese and hyperglycemic phenotype, that manifested after approximately 40 weeks of treatment. The glucose-enriched diet also resulted in vascular lesions of less severity, suggesting multiple mechanisms for the development of atherosclerosis [152].

CONCLUSION

As scientific research and drug development continue to drive the use of NHPs as a relevant and important model for human cardiovascular risk prediction, there will be an ongoing effort to continuously improve the current models described in this chapter to reduce the knowledge gaps identified. As NHPs continue to be a precious resource, alternative study designs, better technology, and techniques such as pharmacokinetic/pharmacodynamic modeling may allow for reduced NHP use, improved data quality, and better predictive translation to humans, the ultimate primate. More sensitive measures of structure and function through strain echocardiography or digital telemetry systems will advance cardiovascular therapeutics and safety science and improve drug development. The collection of cardiovascular end points in toxicology studies, coupled with the refinement and improvement of these techniques, will be paramount in continuing to understand long-term cardiovascular risk that may only emerge following repeat dosing. It is also important to continue to build translational cardiovascular data sets that allow proper risk assessment for human clinical trials and the appropriate context for effects observed in nonclinical primate studies. To this end, the data supporting blood pressure and contractility effects should be expanded and studied for human translation. Finally, the development and refinement of cardiovascular-specific biomarkers beyond troponin should be explored to enable early detection of cardiovascular risk.

Acknowledgements

The authors recognize and thank Kathy Derakhchan (Amgen), Simon Authier (CiToxLab) and Paul Butler (Pfizer) for providing reference data to the chapter. In addition, we thank Michael Engwall (Amgen) for constructive feedback.

VetBooks.ir

References

[1] Hama AT, Toide K, Takamatsu H. Beyond rodent models of pain: non-human primate models for evaluating noval analgesic therapeutics and elaborating pain mechanisms. CNS Neurol Disord Drug Targets 2013;12:1257–70.

[2] Vierboom M, Breedveld E, Hart BA. New drug discovery strategies for rheumatoid arthritis: a niche for non-human primate models to address systemic complications in inflammatory arthritis. Expert Opin Drug Discov 2012;7:315–25.

[3] Fox SH, Brotchie JM. The MPTP-lesioned non-human primate models of Parkinsons's disease. Past, present and future. Prog Brain Res 2010;184:133–57.

[4] Smith AH, Wolfgang EA, Flynn DM, Doe CPA, Knight DR. Tachycardia-induced primate model of heart failure in cardiovascular drug discovery. J Pharmacol Toxicol Methods 2000;43:125–31.

[5] Shen YT. Primate models for cardiovascular drug research and development. Curr Opin Investig Drugs 2010;11:1025–9.

[6] Burbacher TM, Grant KS. Non-human primates as models of toxicology research. Curr Protoc Toxicol 1999;1.1.1–9.

[7] Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 2000;32:56–67.

[8] Bussiere JL. Species selection considerations for preclinical toxicology studies for biotherapeutics. Expert Opin Drug Metab Toxicol 2008;4:871–7.

[9] Vargas HM, Amouzadeh HR, Engwall MJ. Nonclinical strategy considerations for safety pharmacology: evaluation of biopharmaceuticals. Expert Opin Drug Saf 2013;12:91–102.

[10] Shah RR. Drug-induced prolongation of the Qt interval: regulatory dilemmas and implications for approval and labelling of a new chemical entity. Fundam Clin Pharmacol 2002;16:147–56.

[11] Pollard CE, Abi Gerges N, Bridgland-Taylor MH, Easter A, Hammond TG, Valentin JP. An introduction to Qt interval prolongation and non-clinical approaches to assessing and reducing risk. Brit J Pharmacol 2010;159:12–21.

[12] ICH S7A. Safety pharmacology studies for human biopharmaceuticals, 2000. Available at ICH.org.

[13] ICH S7B. The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human biopharmaceuticals, 2005. Available at ICH.org.

[14] Ando K, Hombo T, Kanno A, Ikeda H, Imaizumi M, Shimizu N, et al. QT PRODACT: in vivo QT assay with a conscious monkey for assessment of the potential for drug-induced QT interval prolongation. J Pharmacol Sci 2005;99:487–500.

[15] Hanson LA, Bass AS, Gintant G, Mittelstadt S, Rampe D, Thomas K. ILSI-HESI cardiovascular safety subcommittee initiative: evaluation of three non-clinical models of QT prolongation. J Pharmacol Toxicol Methods 2006;54:116–29.

[16] Wallis R. Integrated risk assessment and predictive value to humans of nonclinical repolarization assays. Br J Pharmacol 2010;159:115–21.

[17] Holzgrefe HH, Cavero I, Buchanan LV, Gill MW, Durham SK. Application of a probabilistic method for the determination of drug-induced QT prolongation in telemetered cynomolgus monkeys: effects of moxifloxacin. J Pharmacol Toxicol Methods 2007;55:227–37.

[18] Lynch 3rd JJ, Wilson AW, Hernandez LE, Nelson RA, Marsh KC, Cox BF, et al. Dose–response effects of sotalol on cardiovascular function in conscious, freely moving cynomolgus monkeys. Br J Pharmacol 2008;154:1439–45.

[19] Haushalter TM, Friedrichs GS, Reynolds DL, Barecki-Roach M, Pastino G, Hayes R, et al. The cardiovascular and pharmacokinetic profile of dofetilide in conscious telemetered beagle dogs and cynomolgus monkeys. Br J Pharmacol 2008;154:1457–64.

[20] Fleury A, Lavé T, Jonsson F, Schmitt M, Hirkaler G, Polonchuk L, et al. A pharmacokinetic-pharmacodynamic model for cardiovascular safety assessment of R1551. J Pharmacol Toxicol Methods 2011;63:123–33.

[21] Misner DL, Frantz C, Guo L, Gralinski MR, Senese PB, Ly J, et al. Investigation of mechanism of drug-induced cardiac injury and torsades de pointes in cynomolgus monkeys. Br J Pharmacol 2012;165:2771–86.

[22] Mullan RJ. Nonhuman primate importation and quarantine. In: United States. Park City, Utah: Presentation at Annual Meeting of the Association of Primate Veterinarians; October 12–14; 2006.

[23] Van Meer PJK, Kooijman M, van der Laan JW, Moors EHM, Schellekens H. The value of non-human primates in the development of monoclonal antibodies. Nat Biotechnol 2013;31:882–3.

[24] Drevon-Gaillot E, Perrone-Lepage M-F, Clement C, Burnett R. A review of background findings in cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins. Exp Toxicol Pathol 2006;58:77–88.
[25] Vidal JD, Drobatz LS, Holliday DF, Geiger LE, Thomas HC. Spontaneous findings in the heart of Mauritian-origin cynomolgus macaques (*Macaca fascicularis*). Toxicol Pathol 2010;38:297–302.
[26] Chapman KL, Andrew L, Bajramovic JL, Baldrick P, Black LE, Bowman CJ, et al. The design of chronic toxicology studies of monoclonal antibodies: implications for the reduction of use of non-human primates. Reg Toxicol Pharmacol 2012;62:347–54.
[27] Pinnel J, Turner S, Howell S. Cardiac muscle physiology. Contin Educ Anes Crit Care Pain 2007;7(3):85–8.
[28] Opie LH. Heart physiology from cell to circulation-. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins Pty Ltd; 2004.
[29] Ankel-Simons F. Primate anatomy. 3rd ed. New York, NY: Academic Press; 2007.
[30] Moss RL, Fitzsimmons DP. Frank Starling mechanism: long on importance short on mechanism. Circ Res 2002;90:11–3.
[31] Kawashima T, Sasaki H. Evolutionary morphology of the autonomic cardiac nervous system in non-human primates and humans. In: Endo H, Frey R, editors. Anatomical imaging. Tokyo, Berlin, Heidelberg, New York: Springer; 2008.
[32] Hartley LH, Rodger RF. Autonomic nervous system activity in *Macaca fascicularis*. J Med Primatol 1986;15:139–42.
[33] Belloni FL, Hintze TH. Effects of adenosine on the heart rate in conscious animals: interaction between adenosine and parasympathetic mechanisms at the sinoatrial node. Prog Clin Biol Res 1987;230:361–74.
[34] Champeroux P, Martel E, Jude S, Laigot C, Lavesierre A, Weyn-Marotte A-A, et al. Power spectral analysis of heart rate variability in cynomolgus monkeys in safety pharmacology studies: comparative study with beagle dogs. J Pharmacol Toxicol Methods 2013;68:166–74.
[35] Braunwald E, Zipes DP, Libby P. Heart disease: a textbook of cardiovascular medicine. Philadelphia, PA: W.B. Saunders Co; 2001.
[36] Yao JA, Feldman HS, Illenberger A, Littell T, Schnee L, Yates D. Evaluation of electrocardiograms recorded in cynomolgus monkeys with short- and long-term intracardiac lead implantations. J Pharmacol Toxicol Methods 2009;60:185–92.
[37] Henriques TA, Beck TW, Douglas CL, Jones HM, Kremer JJ, Kruzich PJ, et al. Left thoracotomy surgical approach for chronic instrumentation in dogs and monkeys providing high-quality electrocardiogram signals. J Pharmacol Toxicol Methods 2010;62:136–42.
[38] Authier S, Moon LB, Stonerook M, Fournier S, Gervais J, Maghezzi S, et al. Evaluation of a novel ECG lead placement method in telemetered freely moving cynomolgus monkeys: assessment of an intravascular biopotential lead. J Pharmacol Toxicol Methods 2011;64:145–50.
[39] Holdsworth DL, O'Donohue KP, Yoder JD, Gesaman JE, Dalton JA, Gauvin DV, et al. Effects of telemetric epicardial leads and ventricular catheters on arrhythmia incidence in cynomolgus monkeys. J Pharmacol Toxicol Methods 2014;69:167–76.
[40] Derakhchan K, Chui RW, Vargas HM. Evaluation of cardiac conduction disturbances using jacketed external telemetry (JET) in conscious non-human primates. J Pharmacol Toxicol Methods 2011;64:e46.
[41] Gauvin DV, Tilley LP, Smith FW, Baird TJ. Electrocardiogram, hemodynamics, and core body temperatures of the normal freely moving cynomolgus monkey by remote radiotelemetry. J Pharmacol Toxicol Methods 2006;53:140–51.
[42] Soloviev MV, Hamlin RL, Barrett RM, Chengelis CP, Schaefer GJ. Different species require different correction factors for the QT interval. Cardiovasc Toxicol 2006;6:145–57.
[43] Chui RW, Derakhchan K, Vargas HM. Comprehensive analysis of cardiac arrhythmias in telemetered cynomolgus monkeys over a 6 month period. J Pharmacol Toxicol Meth 2012;66:84–91.
[44] Guth BD. Preclinical cardiovascular safety assessment in modern drug development. Toxicol Sci 2007;97:4–20.
[45] Guth BD, Bass AS, Briscoe R, Chivers S, Markert M, Siegl PK, et al. Comparison of electrocardiographic analysis for risk of QT interval prolongation using safety pharmacology and toxicological studies. J Pharmacol Toxicol Methods 2009;60:107–16.
[46] Leishman DJ, Beck TW, Dybdal N, Gallacher DJ, Guth BD, Holbrook M, et al. Best practice in the conduct of key nonclinical cardiovascular assessments in drug development: current recommendations from the Safety Pharmacology Society. J Pharmacol Toxicol Methods 2012;65:93–101.
[47] Engwall MJ, Vargas HM, Santostefano W. Cardiovascular assessment of a biotherapeutic in a multiple dose safety pharmacology study. J Pharmacol Toxicol Methods 2013;66:e31.

VetBooks.ir

[48] Sivarajah A, Collins S, Sutton MR, Regan N, West H, Holbrook M, et al. Cardiovascular safety assessments in the conscious telemetered dog: utilization of super-intervals to enhance statistical power. J Pharmacol Toxicol Methods 2010;62:12–9.
[49] Koga T, Kuwano K, Kito G, Kanefuji K. Evaluation of QT interval using a linear model in individual cynomolgus monkeys. J Pharmacol Toxicol Methods 2007;55:248–53.
[50] Holzgrefe H, Ferber G, Champeroux P, Gill M, Honda M, Greiter-Wilke A, et al. Preclinical QT safety assessment: cross-species comparisons and human translation from an industry consortium. J Pharmacol Toxicol Methods 2014;69:61–101.
[51] Haushlalter T, Friedrichs G, Reynolds D, Bareki-Roach M, Pastino G, Hayes R, et al. The cardiovascular and pharmacokinetic profile of dofetilide in conscious telemetered beagle dogs and cynomolgus monkeys. Br J Pharmacol 2008;154:1457–64.
[52] Holzgrefe HH, Cavero I, Buchanan LV, Gill MW, Durham SK. Application of probabilistic method for the determination of drug-induced QT prolongation in telemetered cynomolgus monkeys: effects of moxifloxin. J Pharmocol Toxicol Methods 2007;55:227–37.
[53] Authier S, Tanguay J, Gauvin D, Di Fruscia R, Troncy E. A cardiovascular monitoring system used in conscious cynomolgus monkeys for regulatory safety pharmacology. Part 2: Pharmacological validation. J Pharmacol Toxicol Methods 2007;56(2):122–30.
[54] Ando K, Unakami S, Obo M. QT-prolongation effect of orally administered terfenadine in conscious cynomolgus monkeys- interaction with ketoconazole. Adv Anim Cardiol 2005;38:9–20.
[55] Ishii M, Igarashi M, Patel S, Himi T, Kulecz W. Autonomic effects on R–R variations of the heart rate in the squirrel monkey: an indicator of autonomic imbalance in conflict sickness. Am J Otolaryngol 1987;8:144–8.
[56] Graybiel A, Lackner JR. Evaluation of the relationship between motion sickness symptomatology and blood pressure, heart rate, and body temperature. Aviat Space Environ Med 1980;5:211–4.
[57] Van der Linde HJ, Van Deuren B, Teisman A, Towart R, Gallacher DJ. The effect of changes in core body temperature on the QT interval in beagle dogs: a previously ignored phenomenon, with a method for correction. Br J Pharmacol 2008;154(7):1474–81.
[58] Laitinen T, Lyra-Laitinen L, Huopio H. Electrocardiographic alterations during hyperinsulinemic hypoglycemia in healthy subjects. Ann Noninvas Elect 2008;13:97–105.
[59] Erdemli G, Kim AM, Ju H, Springer C, Penland RC, Hoffmann PK. Cardiac safety implications of hNav1.5 blockade and a framework for pre-clinical evaluation. Front Pharmacol 2012;3:1–9.
[60] Horii I, Kito G, Hamada T, Jikuzono T, Kobayashi K, Hashimoto K. Development of telemetry system in the common marmoset: cardiovascular effects of astemizole and nicardipine. J Toxicol Sci 2002;27:123–30.
[61] Honda M, Komatsu R, Holzgrefe HH, Yamada Y, Isobe T, Kimura K, et al. Application of probabilistic analysis for precisely correcting the QT interval for heart rate in telemetered common marmosets. J Pharmacol Toxicol Methods 2010;61:264–70.
[62] Honda M, Komatsu R, Isobe T, Tabo M, Ishikawa T. Involvement of the autonomic nervous system in diurnal variation of corrected QT intervals in common marmosets. J Pharmacol Sci 2013;121:131–7.
[63] Tabo M, Hara T, Sone S, Shishido N, Kuramoto S, Nakano K, et al. Prediction of drug-induced QT interval prolongation in telemetered common marmosets. J Toxicol Sci 2008;33:315–25.
[64] Chaves AA, Keller WJ, O'Sullivan S, Williams MA, Fitzgerald LE, McPherson HE, et al. Cardiovascular monkey telemetry: sensitivity to detect QT interval prolongation. J Pharmacol Toxicol Methods 2006;54:150–8.
[65] Authier S, Tanguay JF, Fournier S, Gauvin D, Legaspi M, Chaurand F, et al. Conscious and anesthetized non-human primate safety pharmacology models: hemodynamic sensitivity comparison. J Pharmacol Toxicol Methods 2008;58:94–8.
[66] Laverty H, Benson C, Cartwright E, Cross M, Garland C, Hammond T, et al. How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? Br J Pharmacol 2011;163:675–93.
[67] Sager P, Heilbraun J, Turner JR, Gintant G, Geiger MJ, Kowey PR, et al. Assessment of drug-induced increases in blood pressure during drug development: report from the Cardiac Safety Research Consortium. Am Heart J 2013;165:477–88.
[68] Shibao C, Lipsitz LA, Biaggioni I. ASH position paper: evaluation and treatment of orthostatic hypotension. J Clin Hypertens 2013;15:147–53.
[69] Picard S, Goineau S, Guillaume P, Henry J, Hanouz JL, Rouet R. Supplemental studies for cardiovascular risk assessment in safety pharmacology: a critical overview. Cardiovasc Toxicol 2011;11(4):285–307.

[70] Paul L, Hastie CE, Li WS, Harrow C, Muir S, Connell JM, et al. Resting heart rate pattern during follow-up and mortality in hypertensive patients. Hypertension 2010;55:567–74.
[71] Palatini P. Heart rate and the cardiometabolic risk. Curr Hypertens Rep 2013;15:253–9.
[72] Authier S, Tanguay JF, Gauvin D, Di Fruscia R, Fournier S, Chaurand F, et al. A cardiovascular monitoring system in conscious cynomolgus monkeys for regulatory safety pharmacology. Part 1: Non-pharmacological validation. J Pharmacol Toxicol Methods 2007;56:115–21.
[73] Blasi E, Bamberger M, Knight D, Engwall M, Wolk R, Winter S, et al. Effects of CP-532,623 and torcetrapib, cholesterylester transfer protein inhibitors, on arterial blood pressure. J Cardiovasc Pharmacol 2009;53:507–16.
[74] Sarazan RD, Mittelstadt S, Guth B, Koerner J, Zhang J, Pettit S. Cardiovascular function in nonclinical drug safety assessment: current issues and opportunities. Int J Toxicol 2011;30:272–86.
[75] Bush M, Custer R, Smeller J, Bush LM. Physiologic measure of nonhuman primates during physical restraint and chemical immobilization. J Am Vet Med Assoc 1977;17:866–9.
[76] Lee JI, Hong SH, Lee SJ, Kim YS, Kim MC. Immobilization with ketamine HCl and tiletamine-zolazepam in cynomolgus monkeys. J Vet Sci 2003;4(2):187–91.
[77] Takagi G, Asai K, Vatner SF, Kudej RK, Rossi F, Peppas A, et al. Gender differences on the effects of aging on cardiac and peripheral adrenergic stimulation in old monkeys. Am J Heart Circ Physiol 2003;285:H527–34.
[78] Taylor GL, Patel B, Sullivan AT. Evaluation of blood flow parameters in addition to blood pressure and electrocardiogram in the conscious telemetered beagle dog. J Pharmacol Toxicol Methods 2007;56:212–7.
[79] McKee J, Daller J, Baumgartner B, Pettinger S. Evaluation of the sensitivity of a new fully implantable telemetry device and the importance of simultaneously measuring cardiac output and left ventricular pressure. J Pharmacol Toxicol Methods 2014;69:229–36.
[80] Markert M, Klumpp A, Trautmann T, Mayer K, Stubhan M, Guth B. The value added by measuring myocardial contractility 'in vivo' in safety pharmacological profiling of drug candidates. J Pharmacol Toxicol Methods 2007;56:203–11.
[81] Sarazan RD, Kroehle JP, Main BW. Left ventricular pressure, contractility and dP/dtmax in nonclinical safety assessment studies. J Pharmacol Toxicol Methods 2012;66:71–8.
[82] Markert M, Trautmann T, Groß M, Ege A, Mayer K, Guth B. Evaluation of a method to correct the contractility index LVdP/dt(max) for changes in heart rate. J Pharmacol Toxicol Methods 2012;66:98–105.
[83] Hamlin RL, del Rio C. dP/dt (max)- a measure of baroinotropy. J Pharmacol Toxicol Methods 2012;66(2):63–5.
[84] Hanton G, Eder V, Rochefort G, Bonnet P. Echocardiography, a non-invasive method for the assessment of cardiac function and morphology in preclinical drug toxicology and safety pharmacology. Expert Opin Drug Metab Toxicol 2008;4(6):681–96.
[85] Cheng X, Hirkaler G, Heidelberger C, Marotti J, Bartko T, Stuadner H, et al. Validation of echocardiography assessment of cardiac function in conscious beagle dogs with propranolol. J Pharmacol Toxicol Methods 2010;62(2):e9–e10.
[86] Sleeper MM, Gaughan JM, Gleason CR, Burkett DE. Echocardiographic reference ranges for sedated healthy cynomolgus monkeys (Macaca fascicularis). J Am Assoc Lab Anim Sci 2008;47(1):22–5.
[87] Tsusaki H, Yonamine H, Tamai A, Shimomoto M, Iwao H, Nagata R, et al. Evaluation of cardiac function in primates using real-time three-dimensional echocardiography as applications to safety assessment. J Pharmacol Toxicol Methods 2005;52(1):182–7.
[88] Tang HL, Wang LL, Cheng G, Wang L, Li S. Evaluation of the cardiovascular function of older adult Rhesus monkeys by ultrasonography. J Med Primatol 2008;37:101–8.
[89] Korcarz CE, Padrid PA, Shroff SG, Weinert L, Lang RM. Doppler echocardiographic reference values for healthy rhesus monkeys under ketamine hydrochloride sedation. J Med Primatol 1997;26:287–98.
[90] Cai J, Sun X, Han P, Xiao Y, Fan X, Shang Y, Kang YJ, Cai J, Sun X, Han P, Xiao Y, Fan X, Shang Y, Kang YJ. The effect of myocardial infarct size on cardiac reserve in rhesus monkeys. Cardiovasc Toxicol 2014; [epub ahead of print; March].
[91] Hanton G, Sobry C, Daguès N, Rochefort GY, Bonnet P, Eder V. Cardiovascular toxicity of minoxidil in the marmoset. Toxicol Lett 2008;180:157–65.
[92] Tsusaki H, Yonamine H, Tamai A, Shimomoto M, Kuwano K, Iwao H, et al. Left ventricular volume and function in cynomolgus monkeys using real-time three-dimensional echocardiography. J Med Primatol 2007;36(1):39–46.
[93] Zabalgoitia M, Ventura J, Anderson L, Carey K, Williams J, Vandeberg J. Morphologic and functional characterizations of the chagasic heart disease in non-human primates. Am J Med Hyg 2003;68:248–52.

VetBooks.ir

[94] Ducas R, Tsang W, Chong AA, Jassal DS, Lang RM, Leong-Poi H, et al. Echocardiography and vascular ultrasound: new developments and future directions. J Can Card 2013;29(3):304–16.
[95] Thijssen JM, de Korte CL. Cardiological ultrasound imaging. Curr Pharm Des 2014;20:1–12.
[96] Thavendiranathan P, Poulin F, Lim KD, Plana JC, Woo A, Marwick TH. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy: A systematic review. J Am Coll Cardiol 2014;63(25PA):2751–68.
[97] Guth BD, Germeyer S, Kolb W, Markert M. Developing a strategy for the nonclinical assessment of proarrhythmic risk of pharmaceuticals due to prolonged ventricular repolarization. J Pharmacol Toxicol Methods 2004;49:159–69.
[98] Derakhchan K, Chui RW, Stevens D, Gu W, Vargas HM. Detection of QTc interval prolongation using jacket telemetry in conscious non-human primates: comparison with implant telemetry. Br J Pharmacol 2014;171:509–22.
[99] Authier S, Tanguay JF, Fournier S, Gauvin D, Legaspi M, Chaurand F, et al. Conscious and anesthetized non-human primate safety pharmacology models: hemodynamic sensitivity comparison. J Pharmacol Toxicol Methods 2008;58:94–8.
[100] Ishizaka T, Yoshimatsu Y, Ozawa M, Kimotsuki T, Takasaki W, Manabe S, et al. Evaluation of drug-induced QT prolongation in a halothane-anesthetized monkey model: effects of sotalol. J Pharmacol Toxicol Methods 2009;59:86–93.
[101] Udelsman R, Bacher J, Gallucci WT, Gold PW, Morin ML, Renquist D, et al. Hemodynamic and endocrine parameters in the anesthetized cynomolgus monkey: a primate model. J Med Primatol 1984;13:327–38.
[102] Young SS, Schilling AM, Skeans S, Ritacco G. Short duration anaesthesia with medetomide and ketamine in cynomolgus monkeys. Lab Anim 1999;3:162–8.
[103] Hotchkiss CE, Wang C, Slikker Jr. W. Effect of prolonged ketamine exposure on cardiovascular physiology in pregnant and infant rhesus monkeys (*M. mulatta*). J Am Assoc Lab Anim Sci 2007;46:21–8.
[104] Lee VK, Flynt KS, Haag LM, Taylor DK. Comparison of the effects of ketamine, ketamine-medetomidine, and ketamine-midazolam on physiologic parameters and anesthesia-induced stress in rhesus (Macaca mulatta) and cynomolgus (*Macaca fascicularis*) macaques. J Am Assoc Lab Anim Sci 2010;49:57–63.
[105] Ludlage E, Mansfield K. Clinical care and diseases of the common marmoset (Callithrix jacchus). Comp Med 2003;53:369–82.
[106] Redfern WS, Ewart LC, Lainee P. Functional assessment in repeat-dose toxicity studies: the art of the possible. Toxicol Res 2013;2:209–34.
[107] Authier S, Vargas HM, Curtis MJ. Safety pharmacology investigations in toxicology studies: an industry survey. J Pharmacol Toxicol Meth 2013;68:44–51.
[108] Bass AS, Hanson LA, Jackson TA. Measuring the risk of torsades de pointes: electrocardiographic evaluation of PNU-142093 in conscious cynomolgus non-human primates using restraint and non-restraint procedures. J Pharmacol Toxicol Methods 2009;60:51–7.
[109] Mitchell AZ, McMahon C, Beck TW, Sarazan RD. Sensitivity of two noninvasive blood pressure measurement techniques compared to telemetry in cynomolgus monkeys and beagle dogs. J Pharmacol Toxicol Methods 2010;62:54–62.
[110] McMahon C, Mitchell AZ, Klein JL. Evaluation of blood pressure measurement using a miniature blood pressure transmitter with jacketed external telemetry in Cynomolgus monkeys. J Pharmacol Toxicol Meth 2010;62:127–35.
[111] Kurtz TW, Griffin KA, Bidani AK. Recommendations for blood pressure measurement in humans and experimental animals. Hypertension 2005;45:299–310.
[112] Schmelting B, Neihoff M, Egner B. High-definition oscillometry: a novel technique for non-invasive blood pressure monitoring in the Cynomolgus monkey. J Med Primatol 2009;38:293–301.
[113] Fessel J, Robertson D. Orthostatic hypotension: when pressor reflexes overcompensate. Nat Clin Pract Nephrol 2006;2:424–31.
[114] Bedette D, Santos RAS, Fontes MAP. Cardiovascular reactivity after blockade of angiotensin AT1 receptors in the experimental model of tilting test in conscious rats. Br J Pharmacol 2007;153(5):966–71.
[115] Verwaerde P, Senard JM, Mazerolles M, Tran MA, Damase-Michel C, Montastruct JL, et al. Spectral analysis of blood pressure and heart rate, catecholamine and neuropeptide y plasma levels in a new model of neuorgenic orthostatic hypotension in the dog. Clin Auton Res 1996;6(2):75–82.

[116] Murphy DJ, Renninger JP, Ju H. A model of orthostatic hypotension in the conscious monkey using lower negative pressure. J Pharmacol Toxicol Methods 2006;54(2):106–15.
[117] Berdeaux A, Guidicelli JF. Antihypertensive drugs and baroreceptor reflex control of heart rate and blood pressure. Fundam Clin Pharmacol 1987;1:257–82.
[118] Zahner MR, Schramm LP. Spinal regions involved in baroreflex control of renal sympathetic nerve activity in the rat. Am J Physiol Integr Comp Physiol 2011;300(4):R910–6.
[119] Cornish KG, Barazanji MW, Yong T, Gilmore JP. Volume expansion attenuates baroreflex sensitivity in the conscious nonhuman primate. Am J Physiol Integr Comp Physiol 1989;257:R595–8.
[120] Barazanji MW, Cornish KG. Vasopressin potentiates ventricular and arterial reflexes in the conscious nonhuman primate. Am J Physiol Heart Circ Physiol 1989;256:H1546–52.
[121] Leyton-Mange JS, Milan DJ. Pluripotent stem cells as a platform for cardiac arrhythmia drug screening. Curr Treat Options Cardiovasc Med 2014;16(9):334–7.
[122] Sharma A, Wu JC, Wu SM. Induced pluripotent stem cell-derived cardiomyocytes for cardiovascular disease modeling and drug screening. Stem Cell Res Ther 2013;4(6):150–5.
[123] Puppala D, Collis LP, Sun SZ, Bonato V, Chen X, Anson B, et al. Comparative gene expression profiling in human-induced pluripotent stem cell–derived cardiocytes and human and cynomolgus heart tissue. Toxicol Sci 2013;131(1):292–301.
[124] Louch WE, Sheehan KA, Wolska BM. Methods in cardiomyocyte isolation, culture and gene transfer. J Mol Cell Cardiol 2011;51(3):288–98.
[125] Chorro FJ, Such-Belenguer L, Lopez-Merino V. Animals models of cardiovascular disease. Rev Esp Cardiol 2009;61(1):69–84.
[126] Bell RM, Mocanu MM, Yellon DM. Retrograde heart perfusion: the Langendorff technique of isolated heart perfusion. J Mol Cell Cardiol 2011;50(6):940–50.
[127] Hillier C, Bunton D. Functional human tissue assays. Drug Discov Today 2007;12(9–10):382–8.
[128] Hendrickx AG, Binkerd PE. Congenital malformations in nonhuman primates. In: Jones TC, Mohr U, Hunt RD, editors. Nonhuman primates I. Berlin: Springer-Verlag; 1993. p. 170–80.
[129] Koie H, Ageyama N, Ono F, Kanayama K, Sakai T, Sankai T. Echocardiographic diagnosis of muscular ventricular septal defect in a cynomolgus monkey (*Macaca fascicularis*). Contemp Top Lab Anim Sci 2005;44:26–8.
[130] Swindle MM, Kan JS, Adams RJ, Starr FL, Samphilipo MA, Porter WP. Ventricular septal defect in a rhesus monkey. Lab Anim Sci 1986;36(6):693–5.
[131] Jerome CP. Congenital malformations and twinning in a breeding colony of old world monkeys. Lab Anim Sci 1987;37(5):624–30.
[132] Chamanza R, Parry NM, Rogerson P, Nicol JR, Bradley AE. Spontaneous lesion of the cardiovascular system in purpose-bred laboratory nonhuman primates. Toxicol Pathol 2006;34:357–63.
[133] Chamanza R, Marxfeld HA, Blanco AL, Naylor SW, Bradley AE. Incidences and range of spontaneous findings control cynomolgus monkeys (*Macaca fascicularis*) used in toxicology studies. Toxicol Pathol 2010;38(4):642–57.
[134] Sato J, Doi T, Kanno T, Wako Y, Tsuchitani M, Narama I. Histopathology of incidental findings in cynomolgus monkeys (*Macaca fascicularis*) used in toxicology studies. Toxicol Pathol 2012;25(1):63–101.
[135] Dunn ME, Coluccio D, Zabka TS, Gopalakrishnan G, Hirkaler G, Geng W, et al. Myocardial mononuclear cell infiltrates are not associated with increase serum cardiac troponin I in cynomolgus monkeys. Toxicol Pathol 2012;40(4):647–50.
[136] Hubbard GB, Soike KF, Butler TM, Carey KD, Butcher WI, Gauntt CJ. An encephalomyocarditis virus epizootic in a baboon colony. Lab Anim Sci 1992;42(3):233–9.
[137] Getz GS, Reardon CA. Animal models of atherosclerosis. Arterioscler Thromb Vasc Biol 2012;32(5):1104–15.
[138] Lane MA. Nonhuman primate models in biogerontology. Exp Gerontol 2000;35(5):533–41.
[139] Harwood HJ, Listrani P, Wagner JD. Nonhuman primates and other animal models in diabetes research. J Diabetes Sci Technol 2012;6(3):503–14.
[140] Wagner JD, Cline JM, Shadoan MK, Bullock BC, Rankin SE, Cefalu WT. Naturally occurring and experimental diabetes in cynomolgus monkeys: a comparison of carbohydrate and lipid metabolism and islet pathology. Toxicol Pathol 2001;29(1):142–8.
[141] Kaplan JR, Wagner JD. Type 2 diabetes an introduction to the development and use of animal models. ILAR J 2006;47(3):181–5.
[142] Howard Jr. CF. Nonhuman primates as models for the study of human diabetes mellitus. Diabetes 1982;31:37–42.

[143] Rood PP, Bottino R, Balamurugan AN, Smetanka C, Ezzelarab M, Busch J, et al. Induction of diabetes in cynomolgus monkeys with high-dose streptozocin: adverse effects and early responses. Pancreas 2006;33(3):287–92.
[144] Contreras JL, Smyth CA, Curiel DT, Eckhoff DE. Nonhuman primate models in type 1 diabetes research. ILAR J 2004;45(3):334–42.
[145] O'Brien TD, Wagner JD, Litnak KN, Carlson CS, Cefalu WT, Jordan K, et al. Islet amyloid and islet amyloid polypeptide in cynomolgus macaques (*Macaca fascicularis*): an animal model of human non-insulin-dependent diabetes mellitus. Vet Pathol 1996;33(5):479–85.
[146] Wagner JD, Carlson CS, O'Brien TD, Anthony MS, Bullock BC, Cefalu WT. Diabetes mellitus and islet amyloidosis in cynomolgus monkeys. Lab Anim Sci 1996;46(1):36–41.
[147] Austad SN, Fischer KE. The development of small primate models for aging research. ILAR J 2011;52(1):78–88.
[148] Tardif SD, Mansfield KG, Ratnam R, Ross CN, Ziegler TE. The marmoset as a model of aging and age-related diseases. ILAR J 2011;51(1):54–65.
[149] Ross CN, Davis K, Dobek G, Tardif SD. Aging phenotypes of common marmosets (*Callithrix jacchus*). J Aging Res 2012;56417:1–6.
[150] Power ML, Ross CN, Schulkin J, Tardif SD. The development of obesity begins at an early age in captive common marmosets (*Callithrix jacchus*). Am J Primatol 2012;74(3):261–9.
[151] Power ML, Ross CN, Schulkin J, Ziegler TE, Tardif SD. Metabolic consequences of the early onset of obesity in the common marmoset monkeys. Obesity 2013;21(12):E592–8.
[152] Wachtman LM, Kramer JA, Miller AD, Hachey AM, Curran EH, Mansfield KG. Differential contribution of dietary fat and monosaccharide to metabolic syndrome in the common marmoset (*Callithrix jacchus*). Obesity 2011;19(6):1145–56.
[153] Hassimoto M, Harada T. Accurate evaluation of QT interval in conscious rhesus monkeys (Macaca mulatta) by use of Holter ECG. Electrophysiology 2002;35:333–42.
[154] Yang P, Han P, Hou J, Zhang L, Song H, Xie S, et al. Electrocardiographic characterization of rhesus monkey model of ischemic myocardiacl infaction induced by left anterior descending ligation. Cardiovasc Toxicol 2011;11:365–72.

VetBooks.ir

CHAPTER

30

Respiratory Function Measurements in Nonhuman Primates

Dennis J. Murphy

GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, USA

OUTLINE

Introduction 579

Strategy for Respiratory Function Assessment 580

Methodologies for Assessing Respiratory Functions 582

Anesthetized Models 582

Conscious Restrained Models 585

Conscious Nonrestrained Models 585

References 588

INTRODUCTION

The nonhuman primate (NHP) is a well-established model for assessing the potential adverse effects of drugs on respiratory function [1] and also has been used as a disease model for evaluating the efficacy of drugs for treating lung diseases such as asthma and hyperreactive airways [2]. Advantages of using NHPs over nonprimate animal models include structural similarities to human airways, including both the upper airways [3] and the terminal and respiratory bronchioles [4], and to human lungs, including alveolar septal structure (alveolar diameter and radius of curvature), interstitial collagen and elastin content [44], types of mucosal cells in the bronchi, and intraepithelial distribution of nerves [5]. NHPs are also similar to humans both in ventilatory responses to known respiratory stimulants and depressants [6,7,43] and in airway responses to known bronchoconstrictors and dilators [8,9]. The species of NHPs that seem to be most commonly used for evaluating drug-induced effects on respiratory function are the cynomolgus monkey (*Macaca fascicularis*) and the rhesus

http://dx.doi.org/10.1016/B978-0-12-417144-2.00030-5
© 2015 Elsevier Inc. All rights reserved.

monkey (*M. mulatta*). The pigtailed monkey (*M. nemestrina*) also has been used to evaluate respiratory effects in neonatal and juvenile studies [10,11].

STRATEGY FOR RESPIRATORY FUNCTION ASSESSMENT

Developing a strategy for assessing the effects of drugs on respiratory function needs to consider the functional components of the respiratory system. The respiratory system consists basically of two functional components: the pumping apparatus and the gas exchange unit (Figure 30.1). The function of the pumping apparatus is to regulate gas exchange between the environment and the airways to help ensure that sufficient oxygen is supplied to the circulation to meet changing metabolic demands and to remove excess carbon dioxide and other metabolic products. The components of the pump include the respiratory muscles and the nerves, chemoreceptors, and mechanoreceptors that regulate the depth and frequency of

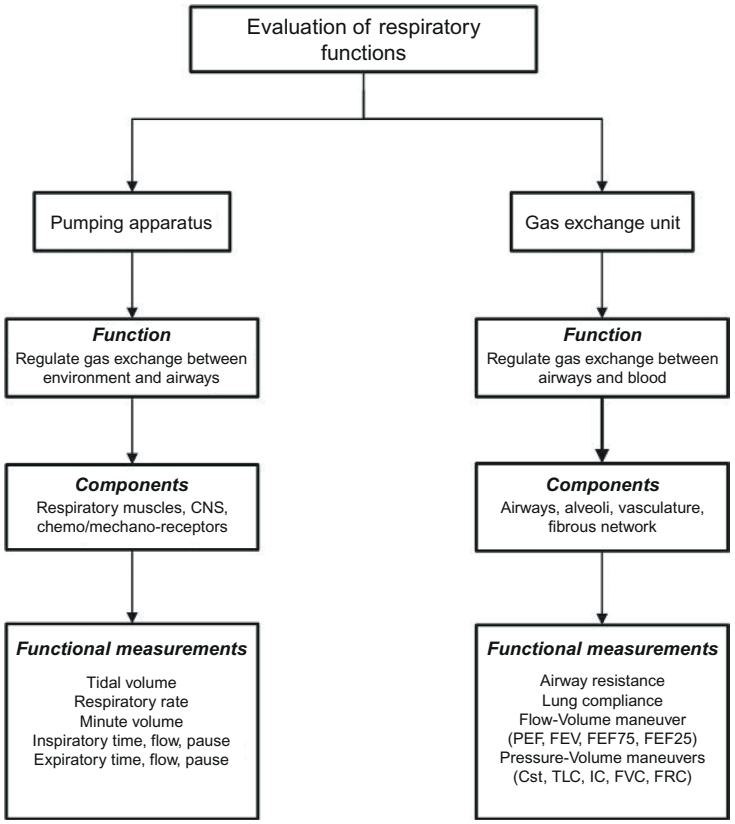


FIGURE 30.1 Flow diagram showing the functional and structural components of the respiratory system and the associated functional measurement end points. C_{st}, static lung compliance; FEF, forced expiratory flow; FEV, forced expiratory volume; FRC, functional reserve capacity; FVC, forced vital capacity; IC, inspiratory capacity; PEF, peak expiratory flow; TLC, total lung capacity.

VetBooks.ir

the pump. The other functional component is the gas exchange unit, or lung. The function of the lung is to ensure that the gas that enters the airways is appropriately exchanged with pulmonary arterial blood. To do this, the lung must have patent airways to ensure movement of gases to the alveoli during inspiration and elastic recoil to ensure the removal of gases during expiration. The components of the gas exchange unit include the airways, alveoli, pulmonary vasculature, and elastic fibrous network. Thus, to evaluate the effect of drugs on respiratory function, measurement end points that evaluate both the pumping apparatus and the gas exchange unit (lung) must be considered.

Changes in the functional status of the pumping apparatus are determined by measuring ventilatory patterns, which basically include the end points of respiratory rate (frequency) and tidal volume (depth) (Figure 30.1). By monitoring the frequency and depth of the pumping apparatus, the effects of drugs on minute volume or total pulmonary ventilation can be established. Minute volume (the product of respiratory rate and tidal volume) is an essential parameter for determining whether a treatment causes respiratory stimulation or depression. Although measures of respiratory rate and tidal volume are essential for detecting changes in pulmonary ventilation, the inclusion of additional end points such as inspiratory time and flow, expiratory time and flow, and the presence of end-inspiratory and end-expiratory pauses provide added value by helping to better characterize breathing abnormalities and/or to provide mechanistic insight. For example, a selective increase in inspiratory time or decrease in mean inspiratory flow (tidal volume/inspiratory time) is indicative of a decrease in respiratory drive [12], whereas a selective prolongation of expiratory time and/or decrease in expiratory flow can be indicative of airway obstruction [13]. In addition, the presence of an end-inspiratory or end-expiratory pause has been shown to be indicative of upper airway sensory and lower airway irritant receptor activation, respectively [14]. Such end points would be of value for detecting irritation associated with inhaled drugs. Apneic time (measure of end-expiratory pause) is the time between breaths and is used for assessing breathing instability, which is especially important during the sleep state, where it is used in clinical studies to identify and grade sleep-disordered breathing [15]. Apneic time also has been used to help grade sleep-disordered breathing in NHPs [3].

Changes in the functional status of the gas exchange unit are determined by evaluating changes in the mechanical properties of the lung (Figure 30.1). Effects on the mechanical properties of the lung are characterized by measuring changes in airway resistance and lung compliance, which are used to assess effects on airway patency and elastic recoil, respectively. An increase in airway resistance can result from obstruction of the airways caused by the constriction of airway smooth muscle (bronchoconstriction), hypertrophy or hyperplasia of cells lining the airways, or hypersecretion of airway mucus. Airway resistance is an important end point for assessing drug effects on lung function because airway obstruction can be an acute, life-threatening event [16]. Airway resistance is quantified by measuring the transpulmonary pressure (mouth pressure minus pleural pressure) required to achieve a given lung airflow. Methodologies for measuring airway resistance either during spontaneous breathing (dynamic resistance) or using a forced maneuver procedure that involves the controlled inflation and rapid deflation of the lung to evaluate forced expiratory flows are currently available [17,18].

A measure of lung compliance should also be considered when there is a need to detect functional changes associated with drug-induced injury to the lower respiratory tract. Such injury includes damage to alveoli or the interstitial components of the lung. Because these

VetBooks.ir

effects generally cause a decrease in lung elasticity [19], they can be detected by measuring lung compliance. Measuring lung compliance to detect damage to the lower respiratory tract is generally not considered in nonclinical safety studies because such changes can be detected by histopathological evaluation. However, inclusion of lung compliance as an end point should be considered when there is concern for acute lung injury following an inhaled dose of drug or when effects that may not be detected by histopathological evaluation, such as interstitial edema or alterations in alveolar surfactant production, are anticipated. In repeat-dose studies, lung compliance could be used as an in vivo biomarker for the onset and progression of drug-induced lung injury. Effects on the lower respiratory tract are characterized histopathologically as diffuse alveolar damage (with edema or hemorrhage), nonspecific interstitial pneumonia (with or without fibrosis), and bronchiolitis obliterans [20,21]. These types of changes are associated with the pathological syndromes of acute lung injury and acute respiratory distress syndrome [22]. Lung compliance is quantified by measuring the amount of transpulmonary pressure (mouth pressure minus pleural pressure) required to achieve a given lung volume. Methodology for measuring lung compliance either during spontaneous breathing (dynamic lung compliance) or using a forced maneuver procedure that involves a controlled inflation and deflation of the lung and evaluating the slope of the expiratory phase of a pressure-volume loop is currently available [17,18].

METHODOLOGIES FOR ASSESSING RESPIRATORY FUNCTIONS

Anesthetized Models

Anesthetized models offer the advantage of reducing the variability of respiratory measurements often observed in conscious models, in which movement or changing emotional states can produce artifacts or changes in respiratory parameters not directly related to drug treatment. The limitations of anesthetized models include the depressant effect most anesthetics and analgesics have on respiratory drive and the potential blunting of the response of airways to bronchoconstrictive agents [23,24]. Furthermore, the duration of measurement is generally limited to several hours, and dosing generally requires a parenteral (nonoral) route. Thus, when using an anesthetized model, the influence of anesthetics and analgesics on ventilatory parameters and airway resistance, an understanding of the duration of the test measurement time required to address the drug effect, and the feasibility of providing the drug as a solution for parenteral administration at the concentrations needed must be considered.

Methodologies for measuring respiratory parameters generally involve placing the anesthetized NHP in a dorsal recumbent position or upright and restrained in a chair, inserting a cuffed tube attached to a pneumotachometer into the trachea to monitor lung airflow, and inserting a pressure-sensitive catheter down the esophagus to the appropriate level within the thoracic cavity or directly through the rib cage into the pleural space to monitor pleural pressure. Alternative methods for obtaining lung airflow include placing the animal into a head-out body plethysmograph and obtaining lung airflow by monitoring pressure changes in the chamber or by placing a face mask attached to a pneumotachometer over the animal's snout. Obtaining an airtight fit over the snout of an NHP is difficult and requires a customized face mask [25]. Ventilatory parameters and measures of total pulmonary resistance

VetBooks.ir

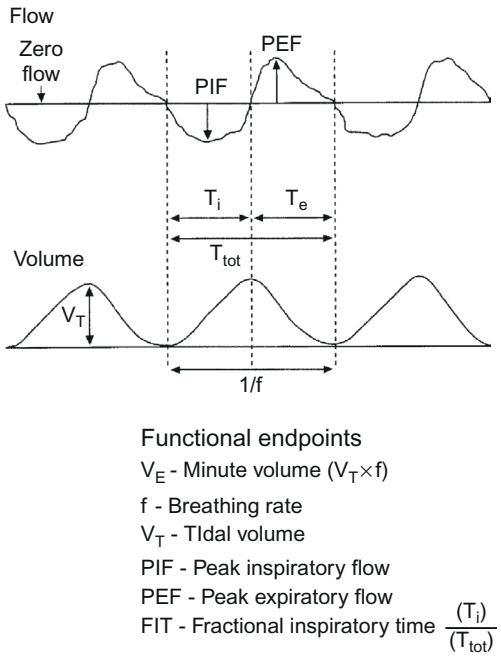


FIGURE 30.2 Flow and volume waveform obtained during spontaneous breathing. Markings demonstrate how ventilatory end points are measured. Functional end points include breathing rate (f), fractional inspiratory time (FIT; T_i/T_{tot}), peak expiratory flow (PEF), peak inspiratory flow (PIF), minute volume (V_E; $V_T \times f$), and tidal volume (V_T).

and lung compliance can be obtained in spontaneously breathing animals or ventilated animals by analyzing the flow and pleural (transpulmonary) pressure signals that are obtained (Figures 30.2 and 30.3). A variety of software applications for automated analysis of the waveforms are available. Specific details on the set-up and waveform analysis for these types of studies in NHPs can be obtained in the following publications [2,26–28].

The anesthetized model is also used to conduct forced maneuver procedures. To conduct these procedures, breathing has to be interrupted either by hyperinflating the lung to produce apnea or by administering a paralytic agent. A pressure panel that controls lung inflation and deflation is also required. One maneuver is a flow-volume maneuver that involves inflating the lung to inspiratory capacity (approximately 25 cm H_2O airway pressure) and then maximally deflating the lung by opening the tracheal tube to a negative pressure reservoir (approximately –50 cm H_2O). This maneuver provides measures of airflow resistance, including forced expiratory volumes at specific times, peak expiratory flow, and forced expiratory flows (FEF) at defined lung volumes (FEF25, FEF75). A second maneuver is the pressure-volume maneuver that involves inflating the lung to inspiratory capacity and a slow deflation to allow for the measure of pressure-volume relationships during the deflation phase. This allows for the measure of static or quasistatic lung compliance (C_{st}). These procedures can also be used to obtain lung capacities such as total lung capacity, inspiratory capacity, forced vital capacity, and functional reserve capacity. These measurements are generally used to complement the dynamic measures of airway resistance and lung compliance by (1) determining whether airway obstruction occurred in central (peak expiratory flow, FEF75) or peripheral (FEF25) airways, (2) evaluating the effect of lung mechanical changes on lung performance by detecting changes in lung capacities (inspiratory capacity, forced vital capacity, total lung

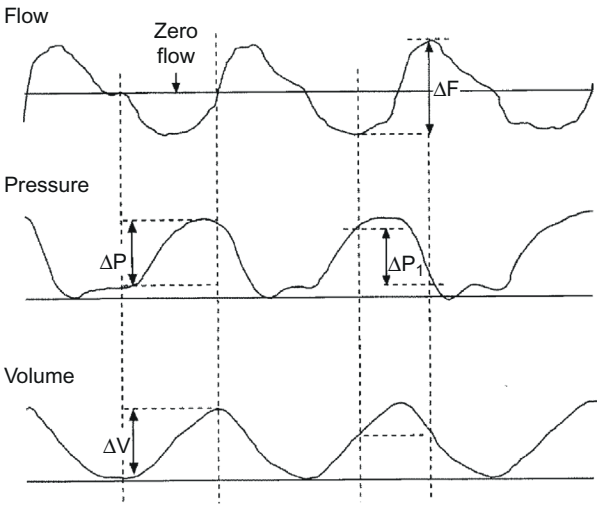


FIGURE 30.3 Flow (F), volume (V), and transpulmonary pressure (P) waveform obtained during spontaneous breathing. Markings demonstrate how lung mechanical end points are measured. Functional end points include dynamic compliance ($\Delta V/\Delta P$ at 0 flow points); dynamic resistance ($\Delta P_1/\Delta F$ at isovolume points); and dynamic conductance ($\Delta F/\Delta P_1$ at isovolume points).

Functional endpoints

$$\text{Dynamic compliance} = \frac{\Delta V}{\Delta P} \text{ (At zero flow points)}$$

$$\text{Dynamic resistance} = \frac{\Delta P_1}{\Delta F} \text{ (At isovolume points)}$$

$$\text{Dynamic conductance} = \frac{\Delta F}{\Delta P_1} \text{ (At isovolume points)}$$

capacity) and the presence of gas trapping (functional residual capacity), and (3) confirming the presence of a change in lung compliance (C_{st}) since dynamic lung compliance can be influenced by small airway obstruction. Specific details on the set-up and waveform analysis for these types of studies in NHPs can be obtained in the following publications [29,30].

Respiratory function measurements can also be obtained in anesthetized NHPs using a forced oscillation technique. This procedure generally involves placing the animal in an upright, sitting position using chair restraints and placing a cuffed tube into the trachea. Attached to the tube is a pneumotachometer for measuring tube airflow and a pressure transducer for measuring tube pressure changes. Attached to the distal opening of the tube is a loudspeaker that can generate sinusoidal pressure waves in a frequency range of 0.2-30 Hz. By comparing the induced pressure and flow oscillations for magnitude and phasing at a variety of frequencies, measures of lung compliance and airway resistance can be obtained. The impedance of the respiratory system needs to be measured to obtain these measurements. Spectral analysis of the induced waveforms using fast Fourier transform is the analysis method used [31]. A commercial device (tremoFlo C-100; Thorasys Thoracic Medical Systems Inc., Montreal, Quebec, Canada) has been developed for use in humans and has been adapted for use in NHPs to both produce and analyze forced oscillations [45]. It is important to note that these measurements are dependent on lung volume, so a measure of functional residual capacity (FRC) should be obtained and analysis should be conducted on recordings that are obtained at FRC [8]. FRC occurs at the end of a spontaneous breath. To increase the time at FRC, the lung

VetBooks.ir

can be hyperinflated to produce an apneic period. Examples of the use of forced oscillations to obtain measures of airway resistance and compliance (elastance) in NHPs can be obtained in the following publications [8,30,32].

Conscious Restrained Models

Conscious models offer the advantage of not having the respiratory complications associated with anesthetics or analgesics and reduced movement artifact relative to conscious nonrestrained models. However, the measurement of time remains limited (generally up to 6 hours) because of restraint, and, if not properly trained, emotional changes (e.g., excitement, fear) can have a significant effect on the variability of respiratory measurement end points. Restraint of NHPs generally involves the use of chair restraint. Chair restraint maintains the thorax in an upright position and does not impede movement of the thorax. Lung airflow is generally measured by placing a helmet over the animal's head. The helmet is sealed airtight around the neck, has a constant bias flow to ensure adequate ventilation, and has an opening with a pneumotachometer attached to measure the airflow in and out of the chamber (Figure 30.4). Pleural pressure is measured using a telemetry implant with a pressure-sensitive catheter inserted either directly into the pleural space or under the serosal layer of the esophagus within the thoracic cavity. Ventilatory parameters and dynamic measures of total pulmonary resistance and lung compliance can be obtained from the flow and pressure signals, as previously described in this chapter for the anesthetized model (Figures 30.2 and 30.3). Specific details on the set-up and waveform analysis for these types of studies in NHPs can be obtained in the following publications [7,33–35].

Conscious Nonrestrained Models

Conscious nonrestrained models offer the advantages of continuous, long-term monitoring of respiratory parameters, elimination of the stress associated with the conscious restraint model, as well as the effect of anesthetics and analgesics associated with the anesthetized model. The advantages of continuous monitoring include the ability to identify peak effects, time to peak effects, time for reversibility, and, by monitoring for at least 24 hours, the ability to evaluate diurnal and photoperiod effects and to monitor during both the awake and sleep states. Some of the limitations include the presence of artifact caused by animal movements and changes in emotional status as well as the influence of feeding, grooming, and other maintenance behaviors on the respiratory recordings. Methodologies for nonrestraint monitoring in NHPs currently include the use of whole-body plethysmography and telemetry for measuring thoracic and abdominal movements. Whole-body plethysmography uses a chamber that has a constant bias flow for ensuring appropriate ventilation and an attached pneumotachometer to monitor airflow in and out of the chamber [36]. This system is limited because it should only be used to measure ventilatory parameters [37], it restricts the movement of the animal (chamber dimensions for a 3- to 6-kg NHP is approximately 9 cubic feet), and tidal volume is measured indirectly. In a whole-body chamber, pressure changes in the chamber during breathing result from the difference in volume of the inhaled gas as it is humidified and heated in the lung. Tidal volume is estimated from this expansion of inhaled gas, with a calculation that is dependent on the temperature and humidity of the chamber, the animal's body temperature, and the rate of breathing since the fraction of heated/humidified

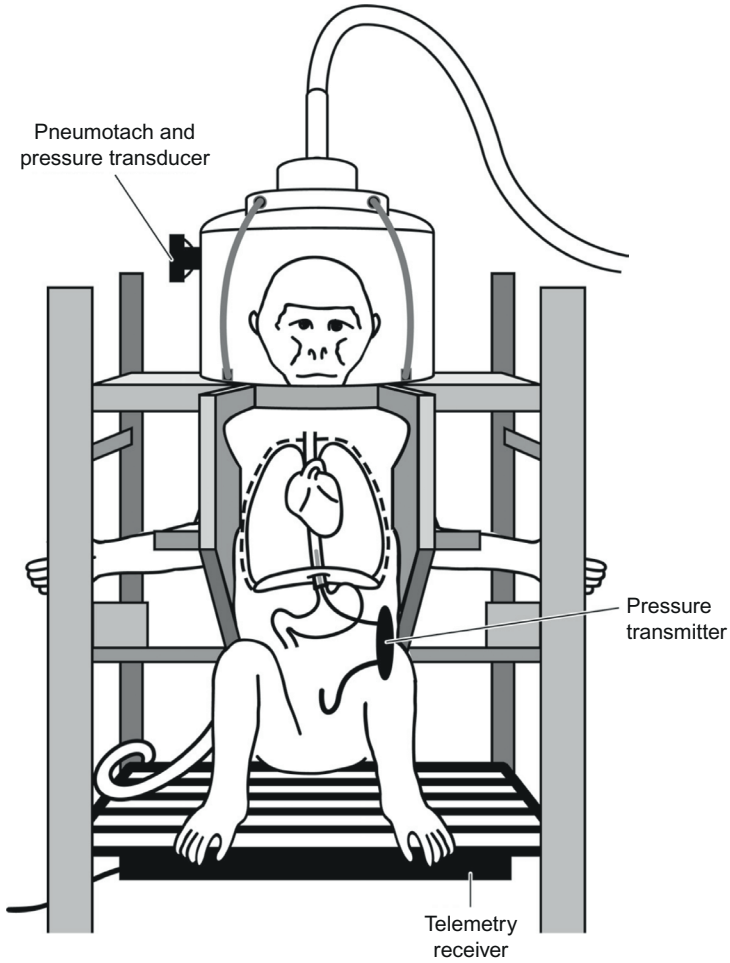


FIGURE 30.4 Illustration of a conscious monkey in a restraint chair with a helmet plethysmograph system for measuring lung airflow and a telemetry implant for measuring pleural pressure. The helmet is sealed around the neck and has an attached pneumotachometer for measuring pressure (flow) changes. Also attached to the helmet is a vacuum line and inlet ports to provide adequate ventilation.

exhaled air that is re-inhaled is dependent on breathing rate [38]. Thus, to use this procedure, the temperature of the animal and chamber and the humidity of the chamber should be monitored and used in the calculation of tidal volume. Furthermore, the effect of a change in breathing rate on tidal volume needs to be considered.

The telemetry methodologies for measuring respiratory function include the use of bioimpedance and inductive bands. The bioimpedance method involves the surgical implantation of a telemetry device with two biopotential leads (stimulation and sensing leads) that are placed subcutaneously approximately 3 cm apart in lateral intercostal spaces. The impedance changes (in Ohms) are obtained using appropriate frequency filters to obtain a combined measure of thoracic and abdominal movement, which can be converted to a volume by calibrating

VetBooks.ir

the signal using the head dome or face mask with pneumotachometer techniques [39]. A limitation of this procedure is that it can measure only ventilatory parameters and requires surgical implantation of the telemetry device. Surgical implantation may not be desired if the respiratory measurements are obtained in a toxicology study where surgical implantation could possibly confound pathology findings. The other telemetry methodology is referred to as respiratory inductive plethysmography (RIP). RIP uses straps containing inductive coils placed around the thorax and abdomen to measure lung volume changes resulting from movements of the rib cage and diaphragm, respectively. A continuous low-voltage current is passed through the inductive coils, and current changes that occur are proportional to the changes in the length of the inductive straps produced by the expansion and contraction of the thorax and abdomen during breathing. Changes in the inductance current are used to continuously monitor the changes in the circumference of the thorax and abdomen, and analysis software is used to calculate tidal volume, flow changes, and breathing rate. RIP calibration requires converting the current changes to flow using the head dome or face mask with pneumotachometry techniques (e.g., [40]) and establishing a correction factor to account for the different sensitivities of the thoracic and abdominal bands by carefully measuring the thoracic and abdominal movements during controlled spontaneous breathing before treatment [41]. One advantage of using the RIP system is that it is noninvasive; the straps are placed on the surface of the animal and protected with a customized jacket that also holds the telemetry transmitter. Another advantage is that this system also gives a measure of airway resistance by measuring shifts in the timing or phasing of the thoracic and abdominal volume waveforms (Figure 30.5). Increases in the delay of the thoracic waveform relative to the abdominal waveform are indicative of increases in airway resistance. This change in the timing of the waveforms can be visualized by

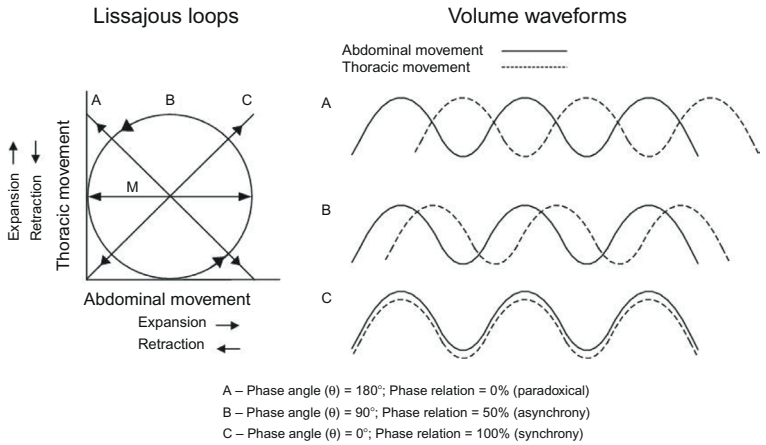


FIGURE 30.5 Patterns of thoracic and abdominal movements that can occur during spontaneous breathing. The volume waveforms are obtained using the respiratory inductive plethysmography methodology. Pattern A waveform represents a paradoxical movement in which the abdominal and thoracic movements are out of phase and in opposite directions during each breath. Pattern B represents an asynchronous pattern in which the thoracic movement lags behind the abdominal movement. Pattern C represents normal breathing in which the thoracic and abdominal movement are in phase or synchronous and moving in the same direction. The Lissajous loops are a plot of the abdominal and thoracic movements for each breath and are used to visualize and calculate the degree of asynchrony (see chapter text for further detail). The A, B, and C of the Lissajous loops (left) correspond to volume waveforms A, B and C (right).

VetBooks.ir

plotting the thoracic (y-axis) versus the abdominal (x-axis) movements for individual breaths (Figure 30.5). These plots are referred to as Lissajous loops and can be analyzed to quantify the phase shift by calculating a phase angle (θ) using the Konno-Mead method [42]. Phase angle is calculated by the equation $\theta = \sin^{-1} m/s$, where θ is the phase angle, m is the line parallel to the abscissa of the ribcage-abdominal plot set at one half the distance between the maximal ribcage excursion and the loop origin, and s is the maximal abdominal excursion measured as the perpendicular distance from the loop origin (Figure 30.5). When there is no shift in the waveforms, θ = 0° and there is no evidence for an increase in airway resistance, whereas a θ value of 90° indicates a 50% shift in the phases and an increase in airway resistance. Phase shift can also be quantified by calculating phase relation, which is the percentage of time the derivative of the thoracic and abdominal volume waveforms (flow) are moving in the same direction for a given breath. The lower the percentage the greater the increase in airway resistance (Figure 30.5). RIP methodology has been successfully used to monitor ventilatory parameters in conscious, nonrestrained NHPs [1] and dogs [40].

References

[1] Ingram-Ross JL, Curran AK, Miyamoto M, Sheehan J, Thomas G, Verbeeck J, et al. Cardiorespiratory safety evaluation in non-human primates. J Pharmacol Toxicol Methods 2012;66:114–24.

[2] Van Scott MR, Aycock D, Cozzi E, Salleng K, Stallings III HW. Separation of bronchoconstriction from increased ventilatory drive in a nonhuman primate model of chronic allergic asthma. J Appl Physiol 2005;99:2080–6.

[3] Philip P, Gross CE, Taillard J, Bioulac B, Guilleminault C. An animal model of spontaneously reversible obstructive apnea syndrome in the monkey. Neurobiol Dis 2005;20:428–31.

[4] Tyler NK, Plopper CG. Morphology of the distal conducting airways in Rhesus monkey lungs. Anat Rec 1985;211:295–303.

[5] Castleman WL, Dungworth DL, Tyler WS. Intrapulmonary airway morphology in three species of monkeys: A correlated scanning and transmission electron microscopic study. Am J Anatomy 1975;142:107–22.

[6] Authier S, Legaspi M, Gauvin D, Chaurand F, Fournier S, Troncy E. Validation of respiratory safety pharmacology models: Conscious and anesthetized beagle dogs. J Pharmacol Toxicol Methods 2008;57:52–60.

[7] Howell LL, Landrum AM. Attenuation of hypoxia-induced increase in ventilation by adenosine antagonists in Rhesus monkeys. Life Sci 1995;57(8):773–83.

[8] Chapman RW, Skeans S, Lamca J, House A, Hey JA, Celly C. Effect of histamine, albuterol and deep inspiration on airway and lung tissue mechanics in cynomolgus monkeys. Pulm Pharmacol Ther 2005;18:243–349.

[9] Seehase S, Schleputz M, Switalla S, Matz-Rensing K, Kaup FJ, Zoller M, et al. Bronchoconstriction in nonhuman primates: a species comparison. J Appl Physiol 2011;111:791–8.

[10] Guthrie RD, Standaert TA, Hodson WA, Woodrum DE. Development of CO_2 sensitivity: effects of gestational age, postnatal age, and sleep state. J Appl Physiol Respir Environ Exerc Physiol 1981;50(5):956–61.

[11] Watchko JF, Standaert TA, Mayock DE, Twiggs G, Woodrum DE. Ventilatory failure during loaded breathing: the role of central neural drive. J Appl Physiol 1988;65(1):249–55.

[12] Remmers JE. Analysis of ventilatory response. Chest 1976;70S:134–7.

[13] Glaab T, Hoymann HG, Hohlfeld JM, Korolewitz R, Hecht M, Alarie Y, et al. Noninvasive measurement of midexpiratory flow indicates bronchoconstriction in allergic rats. J Appl Physiol 2002;93:1208–14.

[14] Ferguson JS, Schaper M, Stock MF, Weyel DA, Alarie Y. Sensory and pulmonary irritation with exposure to methyl isocyanate. Toxicol Appl Pharmacol 1986;82:329–35.

[15] American Academy of Sleep Medicine Task Force. Sleep-Related breathing disorders in adults: Recommendations for syndrome definition and measurement techniques in clinical research. Sleep 1999;22(5):667–89.

[16] Hunt LW, Rosenow EC. Asthma-producing drugs. Ann Allergy 1992;68(6):453–62.

[17] Costa DL. Interpretation of new techniques used in the determination of pulmonary function in rodents. Fundam Appl Toxicol 1985;5:423–34.

[18] Diamond L, O'Donnell M. Pulmonary mechanics in normal rats. J Appl Physiol Respir Environ Exerc Physiol 1977;43(6):942–8.

VetBooks.ir

[19] Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, et al. An official American Thoracic Society workshop report: Features and measurements of experimental lung injury in animals. Am J Respir Cell Mol Biol 2011;44:725–38.
[20] Erasmus JJ, McAdams HP, Rossi SE. Drug-Induced lung injury. Semin Roentgenol 2002;37(1):72–81.
[21] Flieder DB, Travis WD. Pathologic characteristics of drug-induced lung disease. Clin Chest Med 2004;25:37–45.
[22] Myers JL, Limper AH, Swensen SJ. Drug-Induced lung disease. Semin Respir Crit Care Med 2003;24(4):445–53.
[23] Hirschman CA, Bergman NA. Halothane and enflurane protect against bronchospasm in an asthma dog model. Anesth Analg 1978;57:629–33.
[24] Nussmeier NA, Benthuysen JL, Steffey EP, Anderson JH, Carstens EE, Eisele JH, et al. Cardiovascular, respiratory, and alalgesic effects of fentanyl in unesthetized Rhesus monkeys. Anesthet Anal 1991;72:221–6.
[25] Alarie Y, Ulrich CE, Krumm AA, Haddock RH, Jennings HJ. Mechanical properties of the lung in Cynomolgus monkeys. Arch Environ Health 1971;22:643–54.
[26] O'Neil RM, Ashack RJ, Goodman FR. A comparative study of the respiratory responses to bronchoactive agents in Rhesus and Cynomolgus monkeys. J Pharmacol Methods 1981;5:267–73.
[27] Pare PD, Nicholls I. Bronchial response to histamine after inhaled propranolol and atropine in monkeys. J Allergy Clin Immunol 1982;69:213–20.
[28] Young SS, Skeans SM, Austin T, Chapman RW. The effects of body fat on pulmonary function and gas exchange in cynomolgus monkeys. Pulm Pharmacol Ther 2003;16:313–9.
[29] Pare PD, Boucher R, Michoud MC, Hogg JC. Static Lung mechanics of intact and excised rhesus monkey lungs and lobes. J Appl Physiol Respir Environ Exerc Physiol 1978;44(4):547–52.
[30] Wegner CD, Jackson AC, Berry JD, Gillespie JR. Dynamic respiratory mechanics in monkeys measured by forced oscillations. Respir Physiol 1984;55:47–61.
[31] Michaelson ED, Grassman ED, Peters WR. Pulmonary mechanics by spectral analysis and forced random noise. J Clin Invest 1975;56:1210–30.
[32] Black KR, Suki B, Madwed JB, Jackson AC. Airway resistance and tissue elastance from input or transfer impedance in bronchoconstricted monkeys. J Appl Physiol 2001;90:571–8.
[33] Allen DL, Pollard KR, Hughes BL, Dorato MA, Wolff RK. Use of a head dome system to compare i.v. methacholine-induced bronchoconstriction in conscious vs anesthetized Rhesus monkeys. J Appl Toxicol 1995;15(1):13–7.
[34] Gerak LR, Estupinan LE, France CP. Ventilatory effects of negative $GABA_A$ modulators in Rhesus monkeys. Pharmacol Biochem Behav 1998;61(4):375–80.
[35] Murphy DJ, Renninger JP, Coatney RW. A novel method for chronic measurement of respiratory function in the conscious monkey. J Pharmacol Toxicol Methods 2001;46:13–20.
[36] Lizuka H, Sasaki K, Odagiri N, Obo M, Imaizumi M, Atai H. Measurement of respiratory function using whole-body plethysmography in unanesthetized and unrestrained nonhuman primates. J Toxicol Sci 2010;35(6):863–70.
[37] Lundblad LKA, Irvin CG, Adler A, Bates JHT. A reevaluation of the validity of unrestrained plethysmography in mice. J Appl Physiol 2002;93:1198–207.
[38] Jacky JP. Barometric measurement of tidal volume: effects of pattern and nasal temperature. J Appl Physiol 1980;49:319–25.
[39] Kearney K, Metea M, Gleason T, Edwards T, Atterson P. Evaluation of respiratory function in freely moving Beagle dogs using implanted impedance technology. J Pharmacol Toxicol Methods 2010;62:119–26.
[40] Murphy DJ, Renninger JP, Schramek D. Respiratory inductive plethysmography as a method for measuring ventilatory parameters in conscious, non-restrained dogs. J Pharmacol Toxicol Methods 2010;62:47–53.
[41] Sackner MA, Watson H, Belsito AE, Feinerman D, Suarez M, Gonzalez G, et al. Calibration of respiratory inductive plethysmograph during natural breathing. J Appl Physiol 1989;66(1):410–20.
[42] Warren RH, Horan SM, Robertson PK. Chest wall motion in preterm infants using respiratory inductive plethysmography. Eur Respirat J 1997;10:2295–300.
[43] Howell LL. Effects of adenosine agonists on ventilation during hypercapnia, hypoxia and hyperoxia in Rhesus monkeys. J Pharmacol Exp Ther 1993;265(2):971–8.
[44] Mercer RR, Russell ML, Crapo JD. Alveolar septal structure in different species. J Appl Physiol 1994; 77(3):1060–6.
[45] Bassett L, Troncy E, Robichaud A, Schuessler TF, Pouliot M, Ascah A, et al. Non-invasive measure of respiratory mechanics and conventional respiratory parameters in conscious large animals by high frequency airwave oscillometry. J Pharmacol Toxicol Methods 2014;70:62–5.

VetBooks.ir

VetBooks.ir

CHAPTER

31

Central Nervous System Assessment in Nonhuman Primates: Behavioral Assessment, Neurological (Electroencephalography, Nerve Conduction), Abuse Liability, and Cognitive Assessment

Mary Jeanne Kallman
Covance Laboratories, Greenfield, IN, USA

OUTLINE

Observation/General Behavior	592	Abuse Liability Potential	601
Electrophysiological Assessment	593	Conclusion	602
Cognitive Assessment	599	References	603

Primates have become important in drug development efficacy and safety assessment because of their translatability to humans. Primates have a sophisticated central nervous system (CNS) and behavioral capabilities. Their sophistication makes them the ideal model for issue resolution work and characterization of the activity of biologics or other therapeutic compounds that are not active in rodents.

The value of CNS evaluation of NHPs in issue resolution and drug development has continued to grow in spite of increased awareness of the "3 Rs" (replacement, reduction,

http://dx.doi.org/10.1016/B978-0-12-417144-2.00031-7
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

and refinement). There are several reasons for the transition from rodents to primates as the subject of choice in drug development. First, the dog, the alternative large animal choice in drug development, has never been a preferred CNS model because of limited functional and brain dissimilarities with humans and a more limited scientific database for understanding CNS effects. The second reason is the current therapeutic focus of drug development. The current focus of drug development projects is on first-in-class drugs rather than high financial risk compounds that are not first-in-class [1]. The primary therapeutic focus is on drugs to treat all types of cancer where there currently is unmet therapeutic need. Many of the potential drugs in development for cancer indications have serious unwanted CNS side effects of convulsive profiles and peripheral neuropathies that require investigational and clinical dosing strategies for therapeutic success. Management of the side effects may be more important than elimination of the side effect because the side effect may be on target and linked to the primary mechanism of action. Drug development molecules for the treatment of CNS indications has decreased significantly in the past 10 years [1] due, in part, to the competitive off-patent drugs in this therapeutic space and the difficulties in identifying strong markers of clinical efficacy. The one caveat to the status of current CNS drug development is the search for effective treatments for cognitive deficits due to schizophrenia and Alzheimer's disease. An efficacious treatment that would reverse or slow the progression of cognitive decline would be both a financial and humanitarian breakthrough. The resolution of CNS side effects such as convulsion and peripheral neuropathies is best resolved in the primate where the measures acquired are directly translatable to the clinic for monitoring and the best understanding of general toxicology and pharmacokinetics is available in nonrodent species, which are often the primate of choice for oncology compounds. The final reason for CNS dependence on the primate for testing is the need to identify better preclinical models to predict clinical efficacy. This unmet need for model development is primarily for the development of models to assess cognitive functioning analogous to human assessment procedures. The primate may offer greater predictive success when compared to traditional rodent cognitive assessment models leading to breakthrough new treatments.

The best primate CNS assessment is conducted when primates are relaxed, familiar with the test situation, and able to anticipate the testing routine. If the monkey is distracted from the assessment procedure and excited, then performance will not be representative of the best performance. A critical issue for CNS primate assessment is that the monkey is adapted to the restraint or the assessment situation.

OBSERVATION/GENERAL BEHAVIOR

Both cynomolgus and rhesus macaques have highly developed functional capabilities that are similar to humans. Because the cynomolgus monkey is used most extensively in toxicology work as a large animal species, it is most often the primate of choice for CNS studies rather than the rhesus macaque. The availability of pharmacokinetic, pharmacodynamic, and toxicological data for test compounds clarifies the design features of CNS investigations. A rapid home cage walk-by technique can provide only limited information that may not uncover information about the details of behavioral functioning. For instance, efforts to

VetBooks.ir

evaluate motor functioning cannot be accomplished if the animal is sitting in the back of the home cage and not ambulating. A better approach would be to require the monkey to walk across the room to detect more subtle changes in motor gait and ambulatory integration. Basic assessment for multiple applications includes general behavioral observation and reflex assessment. Although behavioral assessment techniques can be specialized to evaluate specific behaviors such as gait or tremor, some general observational batteries have been developed. Examples of batteries for documentation of primate behaviors for application to a more inclusive situation are available in the literature [2–7]. A general observational battery is usually used in conjunction with an interactive assessment of cranial nerve function when trying to characterize an unknown molecule. Reflex assessment in the primate is analogous to human cranial nerve assessment that is conducted as part of an initial human neurological exam for patient assessment that is focused on response to various sensory stimuli including touch, visual, and auditory. In addition, eye coordination and hand–eye coordination are assessed. Primates must be removed from the home cage and restrained in a chair to perform a neurological exam, but the exam is short and focused and thus a good starting point for evaluation of more complex behaviors. Body temperature can also be readily recorded from a chaired primate. The most common approach to conducting observation of primate behavior is to monitor and record activity at specified discrete time points. More continuous observation over time would require videotaping and later review. Ethnovision is a commercially available software package that can be adapted to record defined and specific behaviors at variable time points. The Ethnovison software has the flexibility for applying observations to an infinite array of defined behaviors. A good example of the application of this software to the primate observational situation is described by Rose et al. [8]. One clear advantage of this approach is the ability to collect data on animal-to-animal interactions in a group-housed situation, whereas discrete observational procedures are focused on individual behaviors.

Sometimes observational techniques are used for documentation of specific behaviors of interest. The most common applications in drug development would be to document changes in motor performance/gait, types of tremor, stereotypical responses, or levels of consciousness. Although observation is a powerful tool for quantifying motor performance, locomotor activity equipment for primate use is cumbersome and not well validated. One observational supporting approach for monitoring locomotion is a telemetric chip that can be attached to the collar of the monkey and is marketed as Actical. The chip can be removed from the collar and downloaded on a computer to plot and analyze the data. The data plot in Figure 31.1 illustrates how this procedure can be effectively used to characterize circadian activity across the light and dark phases of the day. Because the collar chip is not encumbered by a specialized platform as is used by DSI for data collection, it has the advantage that the monkey can be house in a typical home cage with other monkeys.

ELECTROPHYSIOLOGICAL ASSESSMENT

Electrophysiological assessment includes monitoring of electroencephalography (EEG) and electrical nerve conduction in response to simulation. Both of these assessment endpoints are directly translated to the clinic where they are used in monitoring humans. These end

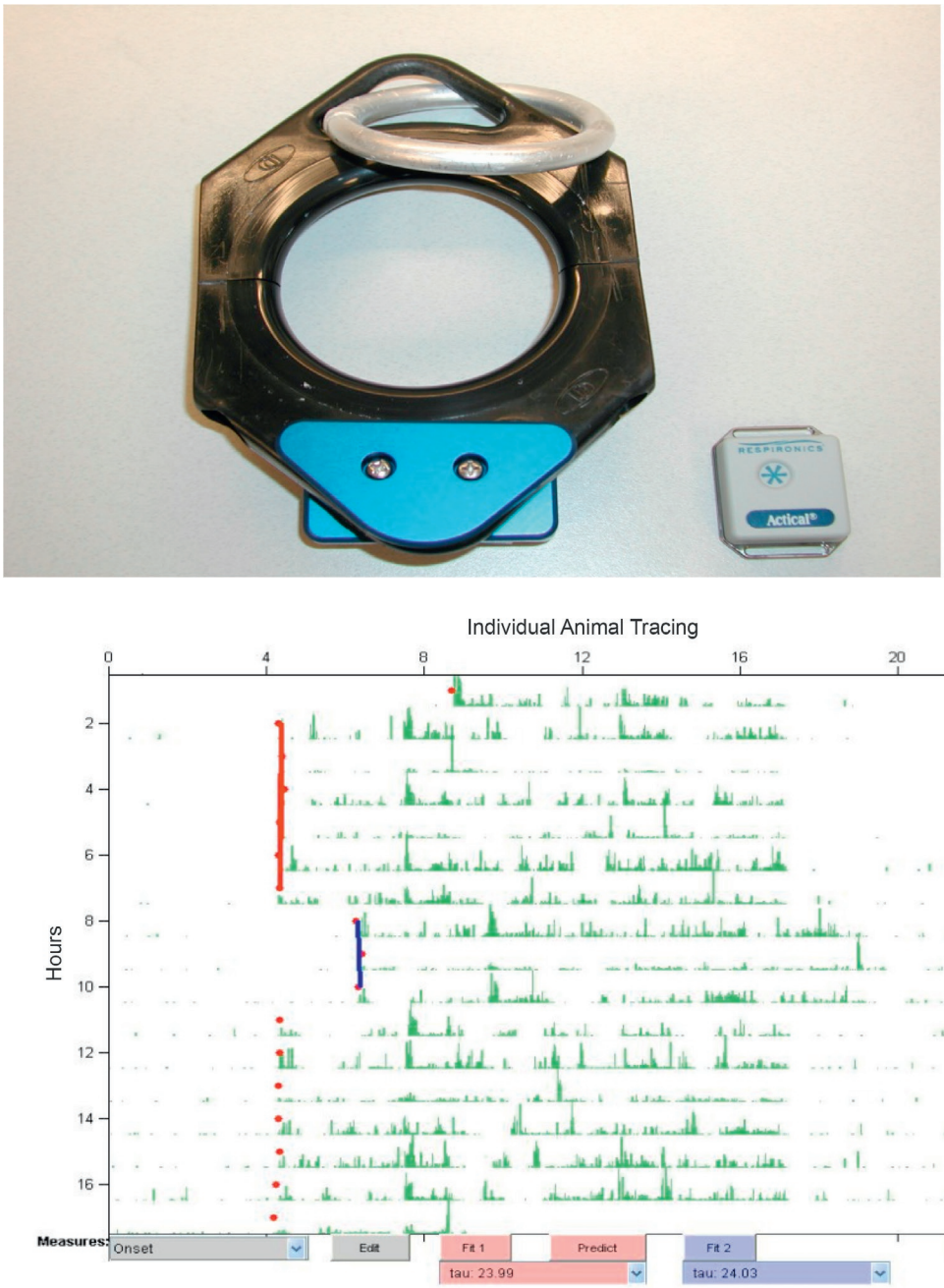


FIGURE 31.1 Top: the Actical device for recording activity. This transmitter fits into the water-resistant case that is counted on the collar work by the primate. Bottom: this tracing shows that these transmitters can record circadian fluctuations in activity coincidental with environmental lighting changes. Each horizontal line represents a different individual animal tracing across the 24 hour day.

VetBooks.ir

points can cause important problems for the development of new molecules for therapeutic use which have CNS and non-CNS therapeutic indications. Seizure or convulsion problems are viewed by the regulatory agencies as a critical component of preclinical understanding of safety requiring detailed preclinical understanding of the convulsive effect with mapping to the clinical plan for drug development. Usually, a 10-fold margin of safety is required to pursue clinical trials or the report of convulsive activity is sufficient to place a hold on clinical trials until the effect is better understood preclinically. Peripheral neuropathies are most frequently associated with oncolytic drug development or are associated with drug treatments for diabetes. Because of the importance of understanding or predicting the margin of safety for both EEG/convulsion and peripheral neuropathies, the primate is one of the important animal models for developing margins of safety. The pharmacokinetic similarity of humans and primates provides extra reassurance that the margin of safety estimates will be appropriate. A second reason for the emphasis on the primate is the increased industry emphasis on drug development of oncolytic and large molecules where the primate is often the best model for understanding the drug of interest. In addition, pharmacokinetic and toxicology data are available to provide context for interpreting the data collected.

EEG recordings for the purpose of establishing a potential link to seizure/convulsion are recorded by surface electrodes that record a summation of multineuron activity. This application of EEG assessment has been extensively used in rodents [9,10] and is generally the same scientific approach for primate assessment [11,12]. Although EEG can be used to record area or nuclei specific activity to identify specific brain site damage or various stages of sleep progression, detection of seizure activity is a more general approach to monitoring EEG. Pharmaco-EEG, or the more granular analysis of detailed changes in activity, is different from the investigation of spiking activity or prespiking activity that is associated with various convulsion syndromes. One of the primary uses of EEG assessment is to distinguish between peripheral muscular alterations that are unrelated to seizure activity but have been documented as convulsion in the clinical signs data for a study. Muscular fasciculation may be peripherally mediated and generate no seizure-like activity in the brain.

Two approaches to recording EEGs in primates have been used in safety assessment. The first approach requires training primates to sit in a chair. Once the monkey is trained to this situation, scalp needle electrodes can be inserted and EEG recorded in the animal facility for approximately 5 minutes. These acute and short-duration recordings can be used to qualify monkeys and document baseline proconvulsive activity before placing animals in toxicology studies. An EEG tracing that is free of movement is required to make definitive evaluation of the presence of spiking activity in the recorded signal. Although the number of monkeys that have baseline proconvulsive EEG patterns is small, those animals could be problematic for a toxicology study of a known convulsive compound. The acute EEG tool can also be effectively used to evaluate the congruency between the behavioral observation of convulsion and actual EEG activity or seizure activity. A second approach requires surgical preparation of monkeys to implant electrodes for recording purposes. EEG signals can then be captured by telemetry for more long-term capture of data. The monkeys are not restrained and are in a free-moving situation. The DSI telemetric system can capture data continuously for 24 hours/day up to 3 months without replacing the transmitter or the electrode placement for collecting data. The more work intensive telemetry model should only be used when continuous data is required to understand the progression of EEG changes over a 24-hour period or over weeks

VetBooks.ir

of compound exposure, as in the case of a delayed-onset phenomenon. Selection of the more costly telemetry approach should be justified based on study needs because the approaches for collecting EEGs have different cost profiles.

In the clinic, an estimate of the safety margin of the compound to a measure of efficacy or therapeutic window is important. It may also be important to understand if any preseizure activity is present at lower plasma concentrations of the compound or earlier in time as in the case of delayed onset convulsions. If these earlier changes can be reliably reported and recorded in the clinic, they may be used to avoid unwanted convulsions in the early clinical trials before the availability of actual human data. Figure 31.2 depicts the progression of EEG changes for a compound with delayed onset convulsion at about 10-12 weeks of continuous dosing. The four frames in the figure illustrate the progression of EEG changes that eventually represent seizure activity. During weeks before the development of convulsive activity there are reliable changes in EEG that can be documented. This progression of events is valuable information for the conduct of clinical trials for this compound. Although most clinicians are skeptical about the reliable detection of pharmaco-EEG changes across human subjects to make dosing decisions, they are more confident that seizure-like activity can be reliably detected in the clinic if it is present.

Sleep analysis to better understand the efficacy of compounds for treating various sleep disorders is another application of EEG recordings, the subset of EEG referred to as rapid-eye movement sleep or dreaming. This requires quantification of EEG frequency data into detailed stages or staging analysis. Evoked potentials are also a subset of EEG activity and refer to an evoked response to typically auditory, somatosensory, or visual stimuli where the activity becomes entrained to the stimulus. Evoked potentials are beyond the scope of this chapter, but they have typically been applied to understanding sensory functioning or as a tool for evaluation of compound efficacy for learning and memory. Please refer to the chapter references to better understand this evaluation tool [13–15].

A second electrophysiological model that has become important in drug development and CNS assessment in the primate is nerve conduction to evaluate potential peripheral neuropathies. Peripheral neuropathies affect approximately 20 million people in the United States, and this affected population is broadly divided into the elderly, diabetics, the obese, those taking cancer chemotherapy compounds that target mitochondria, and those taking other drugs. The symptoms of peripheral neuropathies include diminished thermal and vibratory sensation, sensory loss, muscle weakness, and pain [16]. Degeneration of the peripheral nerve fiber is affected first, often with progression to the dorsal root ganglion, and in some cases to the CNS. Early detection is integral to reversal of effects because progression of the damage toward the CNS reduces the prognosis for reversibility. Understanding the relationship between the functional loss, nerve electrophysiology, and the pathologic changes are all components of tracing the progression of neuropathies. Traditional pathology assessments as part of a standard toxicology evaluation do not detect early signs of peripheral nerve degeneration since the traditional evaluation includes assessment of the large fiber sciatic nerve near the spinal column. Typically, the more distal portions of the smaller fibers are affected first, and these changes may produce detectible and functional changes before visual detection of changes in the larger portion of peripheral nerve. Probably the most sensitive and certainly the most translatable measure for clinical monitoring is the assessment of nerve conduction, although evaluations of novel compounds may include

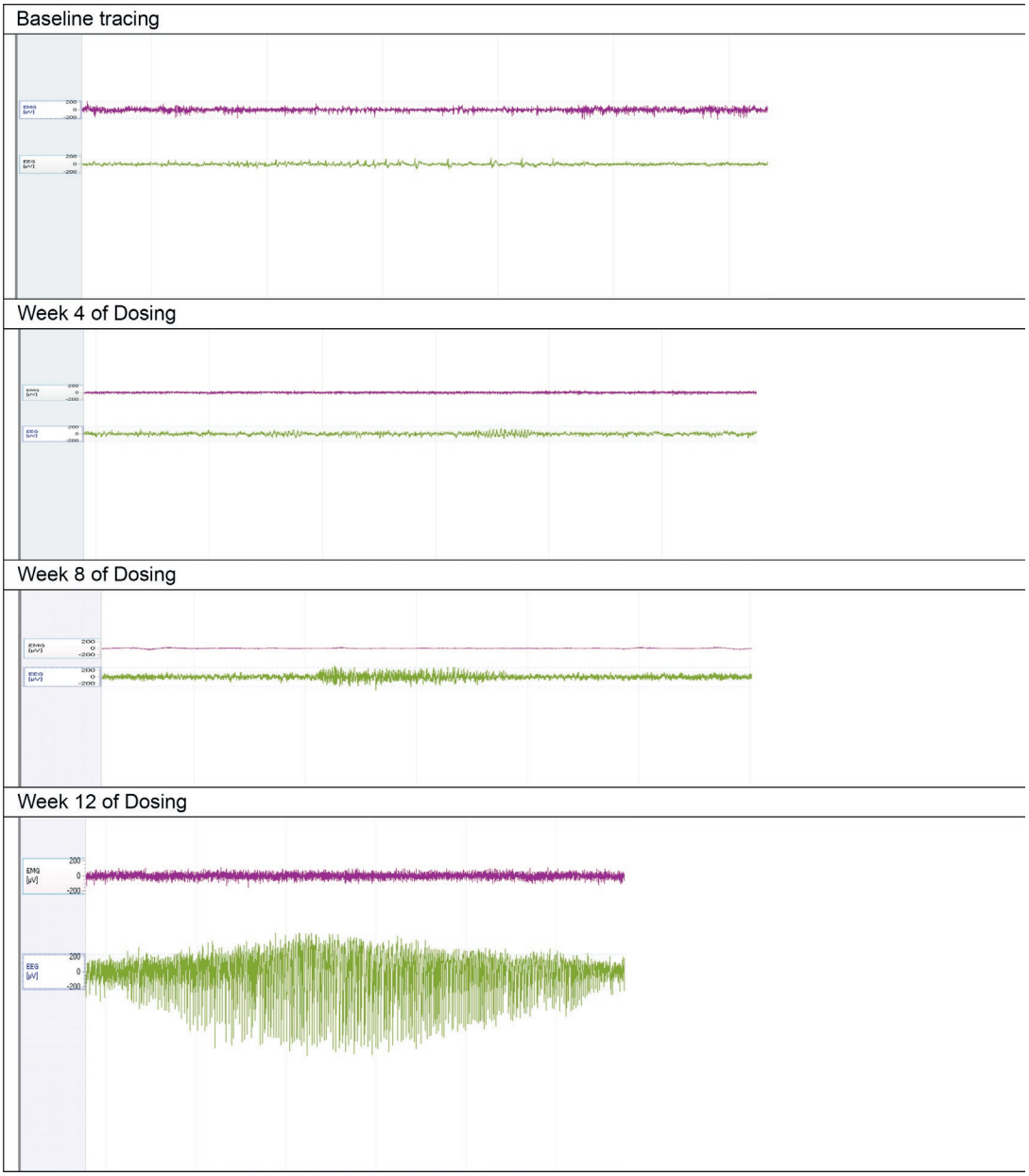


FIGURE 31.2 Progression of electroencephalography signals across time. The first tracing is at baseline before drug administration, and the subsequent three tracings illustrate the progression across 12 weeks of time to frank seizure activity, which occurs after 12 weeks of dosing.

VetBooks.ir

the overlapping evaluation of pathology, functional changes, and electrophysiology. The multiple assessment approach does not always provide the same temporal characterization of neuropathy progression [17].

Nerve conduction assessment is accomplished by providing electrical stimulation of a sensory or motor fiber and recording the evoked electrical response in the fiber. The nerve conduction velocity is the dependent measure of primary interest (Figure 31.3). Reduction in the nerve conduction velocity is predictive of signs associated with peripheral neuropathies. Laboratory assessment of the small diameter fibers has been possible more recently because of improvements in recording capabilities [18], which supports earlier detection of electrophysiological changes.

The primate is an ideal subject for evaluation of the potential for peripheral neuropathies because of their similarities to humans with respect to pharmacokinetic of development compounds and the similarities in the modeling of the peripheral and CNS. The neuroanatomic makeup of the dorsal root ganglion is most similar to humans, and the potential for recovery, one of the most valued outcomes of progression mapping, is also similar between primates and humans. It is the potential for recovery for early detected neuropathies that make monitoring crucial to prevent irreversible damage. Because of the current drug discovery focus on oncolytic compounds, nerve conduction assessments will continue to be important in developing new standards for care and the delivery of well-established drug delivery routines.

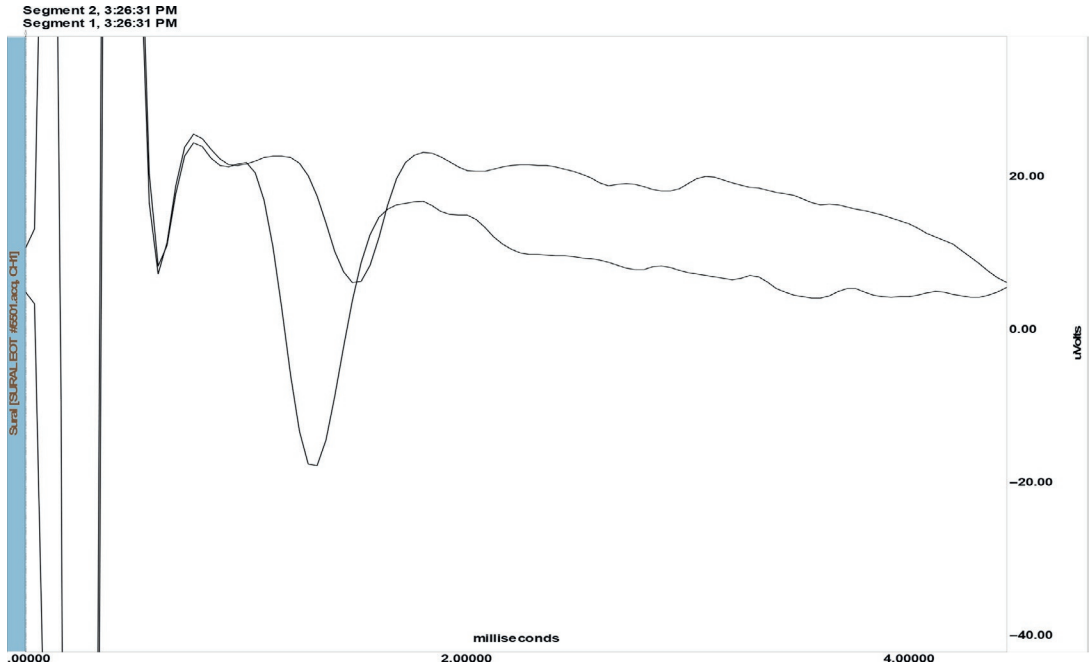


FIGURE 31.3 Recording of primate nerve conduction response to electrical stimulation. The larger tracing is recording during baseline or the normal condition. The smaller signal represents reduced conduction velocity indicative of a compound producing a peripheral neuropathy recording.

COGNITIVE ASSESSMENT

VetBooks.ir

The increased interest in pharmaceutical drug development in the identification of therapeutic treatments for Alzheimer's disease and other neurodegenerative diseases has increased the importance of primate models for detecting changes in cognition. Rodent models for detecting changes in cognition do not have a strong concordance rate with human cognitive changes; therefore data documenting the effects of novel compounds on primate cognition are more convincing of the potential for efficacy to improve human cognition. The primary rodent models for assessing cognition include both land (radial arm maze, Barnes maze) and water mazes (Morris water maze, Biel maze). Other rodent assessment procedures include: the five-choice serial reaction time task, delayed matching-to-sample task, reaction time tasks, and various operant procedures that require learning and memory for performance and object recognition. Because of the poor predictability of the rodent models, primates are the preferred subjects for evaluating changes in cognition.

The primate models can be time-consuming because they require training the primates on the tasks before assessing the compound. The most common test procedures used in primates are delayed matching-to-sample, object retrieval, and the Cambridge Neuropsychological Test Automated Battery (CANTAB). The CANTAB package of test routines is a development of the famous Wisconsin General Test Apparatus that was used for primate assessment [19,20]. CANTAB is a computerized software package for evaluating multiple cognitive functions. Table 31.1 lists the assessment procedures that are integrated into the CANTAB package. The list of test procedures included in CANTAB is varied and requires different cognitive capabilities, some of which are reflective of the old two-process learning theory where short- and long-term memory were considered different brain processes. These procedures are directly translatable to human testing and evaluation procedures in current use. Since the procedures are identical to those used for human assessment, the predictability of primate CANTAB data is excellent. The most common application of CANTAB is in identification of the progression of Alzheimer's disease [21] and schizophrenia [22] or the pharmacological treatment of these medical conditions. One approach for conducting these evaluations is to build a colony of aged monkeys and to test drug efficacy in improving cognitive functioning in these animals. This approach has the drawback of difficulty in obtaining elderly monkeys for these studies

TABLE 31.1 CANTAB Routines for Testing

Attention/vigilance
Working spatial memory
Visual paired learning
Reaction time
Recognition/recall memory
Emotional recognition
Reasoning/problem-solving
Visual planning

VetBooks.ir

and maintaining an elderly primate colony. An alternative to this aged monkey approach is to use a drug to induce cognitive disruption and then to test improvements in the disruption by novel compounds. The most common approach to disrupting cognition is with a cognitive disrupting drug like scopolamine.

Delayed matching to sample is an operant testing procedure that has been used outside of the CANTAB testing. This procedure requires the monkey to remember an object displayed on a video screen and select that object from a group of objects [23,24]. Various duration screen blackout delays are used to make the task more difficult and require that the test subject recall information for longer and longer time frames. Much of the validation work with this model was conducted with nicotine as a positive control compound for enhancing memory. For a review of this work, see the publication by Webster et al. [25].

The object retrieval task is also used for the evaluation of problem-solving, and it requires less training time than the CANTAB modules [26]. Figure 31.4 depicts a common apparatus that can be placed on a tray for primate access or affixed to the cage. Typically, a raisin is placed inside of the cube, and the monkey must problem-solve to figure out the open side of the cube from which the raisin can be retrieved. By rotating the cube, various configurations can be presented for monkey resolution. The time needed to obtain the raisin or other food stuff is the dependent measure in this assay. The advantage of this assessment technique is that it is quick and requires little training.

Other cognitive assessment procedures that have received less validation attention in the lab include pairing imaging techniques with various executive monkey functions such as recognition of familiar objects or pairing with actual problem-solving tasks. Simple learning tasks have also been paired with the recording of evoked auditory or visual responses where alterations in various components of the evoked response correlate with learned events or previous experience with sensory stimuli. Prepulse inhibition is a technique that has been used most often for evaluating schizophrenic-like responses. A simple auditory response measured by jumping in response to a loud noise can be damped down by giving a warning stimulus where the primary response to noise is less. Schizophrenics by definition have difficulty tracking sensory information and are less effective using the prepulse information in this paradigm to modify the full-blown startle response. This approach for assessment has been applied less to understand cognitive impairment due to Alzheimer's or other degenerative diseases.

FIGURE 31.4 Figure of a primate object retrieval apparatus for testing primate problem-solving skills. The monkey must identify which side of the cube is open to retrieve a raisin from the center of the box. The open side can be rotated on different trials.

ABUSE LIABILITY POTENTIAL

VetBooks.ir

Since the issue of the U.S. Food and Drug Administration guidance [27] and the European Medicines Agency guideline [28] on preclinical abuse liability testing, an increase in abuse potential testing has occurred. These studies are required for compounds in drug development that have CNS activity. Identification of CNS activity for new molecules is based on binding assays done early in drug development that identify CNS receptors, brain circulation of the compound, and activity of the compound in traditional CNS pharmacodynamic assays such as locomotor activity, EEG, and operant performance. Abuse potential assessment is required for the scheduling of new drugs and the decision-making process at the time of the new drug application (NDA) submission and before clinical use of the compound. These studies are used to provide a drug scheduling recommendation at the time of the NDA submission. The three studies that are required include a dependence/withdrawal study, a drug discrimination study, and a self-administration study. All three of these study types have been used predominately for the preclinical characterization of compounds with known abuse liability potential or to characterize chemically similar molecules.

The dependence/withdrawal study evaluates the consequences of repetitive drug administration for 2-4 weeks and the effects of drug withdrawal for 1-2 weeks after the termination of drug dosing. Although considerable laboratory investigations have been conducted to evaluate precipitated withdrawal where an antagonist is administered to precipitate rapid withdrawal effects, this design is not used in studies to meet the abuse liability guideline or guidance. Animal observation, food intake, body weight, and body temperature are monitored to determine the effects of a compound when withdrawn from repetitive use. Other end points would be added to known toxicology or pharmacology end points for this compound identified earlier.

Drug discrimination studies are designed to determine if the compound produces an effect that the monkey can perceive and use to modify their behavior. Monkeys are tested on a two-lever panel where they are trained to respond on one lever when they receive saline before the session and on the other lever when they are given the drug before the session. Monkeys do learn to perform this discrimination and to respond appropriately on the two levers based on the drug stimuli administered before the session. When humans or animals self-administer drugs, they perceive the effect of the drug, and they develop a preference for a specific drug effect and even a specific dose of the drug. This is similar to human drinking behavior, where the number of beers consumed in a bar during happy hour is specifically titrated by the drinker, who knows the effects of alcohol consumption and modifies their consumption based on the internal effect of the alcohol. The drug discrimination assay is used to determine whether the novel compound is similar to a known drug of abuse or whether the drug can produce a unique cue. The drug discrimination assay is a true pharmacological assay as the monkey discriminates different doses of the test compound dose responsively. Many drugs produce reliable discriminative cues, and although centrally active drugs work well in this model, other drugs can be discriminated that are not abused compounds.

The most powerful assay for predicting the potential for abuse by humans is the self-administration assay. For a good review of self-administration paradigms see the work by Balster [29] and Balster and Bigelow [30]. This assay determines whether a monkey will work to get intravenous injections of the drug. First, the monkeys are trained to respond to

VetBooks.ir

injections of a known abused drug. This is usually cocaine or morphine. Once they successfully work to get access to the known compound, the novel compound is substituted for the known compound. If the monkey works to get the injection of the novel compound, then how much they will work (a question about how hard the animals will work relative to a known compound such as cocaine) to get the novel compound would be evaluated. This is assessed with the progressive ratio schedule, where the behavioral requirement increases so that more and more responses are required to get drug injections for the novel compound. At some point, the monkey will quit responding, and this is called the *breakpoint*. The breakpoint value can be compared to known compounds such as cocaine to the comparative reinforcing strength of the new drug. One unique feature to the primate abuse potential studies is that primates are typically reused after drug washout (five or six half-lives) and reestablishing a baseline to conserve animals. Because of their shorter lifespan, rodents are rarely reused for additional drug testing.

The rat is the preferred species for these studies, but, under some circumstances, the primate is the species selected for evaluation. The scientific databases in the drug discrimination and dependence models are stronger for the rodent, but the self-administration database in the rat is not as extensive as the primate information. The primate data offer only slightly better concordance with the human abuse profile than those the rat data provide [31,32]. The sophistication of the primate brain and some of the unique drug effects in the primate does provide an argument for conditions for their selection as subjects [33]. Some questions that should be answered when choosing between the primate and the rodent for abuse-potential assessments include (1) "Are the receptors for the therapeutic activity of the compound similar in the rodent and primate?" (2) "Are the metabolite profiles similar in rats and primates?" (3) "Are the toxicology profiles similar in rodents and primates?" (4) and "Are the pharmacokinetic profiles similar or not?"The primate may be the best model for predicting abuse liability and recommending scheduling for the new compound if the rodent is a poor predictor of the compound's effect. Compounds that produce sedation are probably best characterized in the primate since the rodent model has been more difficult to use as a predictor of abuse liability. One weakness of these assays in both primates and rodents is that they fail to predict anabolic steroid and hallucinogen human abuse.

CONCLUSION

The primate is a critical model for CNS drug development and the resolution of clinical neurotoxicology issues. The behavioral profile of the primate is complex, and in the situation of cognitive capability it is more like human cognition than can be modeled in the rodent. Many of the models that are used to understand neurotoxicology, especially EEG and nerve conduction, are directly translatable to the techniques used in the clinic for evaluation. The primate is invaluable to understanding the progression and prevention of seizure effects and peripheral neuropathies. The CNS scientific data available for understanding the effects of novel compounds that have not been previously characterized is more extensive in the primate than the dog, a preferred large-animal species for toxicology characterization. To conduct drug development related to CNS issues would be extremely difficult without primate models.

References

[1] Long G, Works J. Innovation in the biopharmaceutical pipeline: a multidimensional view. Boston, MA: Analysis Group, Inc.; 2013, Available at http://www.analysisgroup.com.

[2] Gavin D, Baird T. A functional observational battery in non-human primates for regulatory-required neurobehavioral assessments. J Pharmacol Toxicol Methods 2008;58:88–93.

[3] Korte S, Fuchs A, Weinbauer G, Moscardo E, Giarola A. A modified Irwin test as a diagnostic tool to monitor neurobehavioural changes in cynomolgus monkey. Int J Toxicol 2006;.

[4] Haggerty G. Strategy for and experience with neurotoxicity testing of new pharmaceuticals. J Am Coll Toxicol 1991;10:677.

[5] O'Keeffe R, et al. Nonhuman primates in neurotoxicity screening and neurobehavioral toxicity studies. J Am Coll Toxicol 1989;8:127.

[6] Porsolt R. The usefulness of non-human primates in central nervous system safety pharmacology. J Pharmacol Toxicol Methods 2013;68:23–9.

[7] Moscardo E, McPhie G, Fasdelli N, Dorigatti R, Meecham K. An integrated cardiovascular and neurobehavioural functional assessment in the conscious telemetered cynomolgus monkey. J Pharmacol Toxicol Methods 2010;62:95–106.

[8] Rose C, deHeer RC, Korte S, van der Haarst JE, Weinbauer GF, Spruijt BM. Quantified tracking and monitoring of diazepam treated socially housed cynomolgus monkeys. Regul Toxicol Pharmacol 2012;62:292–301.

[9] Durmuller N, Guillaume P, Lacroix P, Porsolt R, Moser P. The use of the dog electroencephalogram (EEG) in safety pharmacology to evaluate proconvulsive risk. J Pharmacol Toxicol Methods 2007;56:234–8.

[10] Easter A, Bell M, Damewood J, Redfern W, Valentin J-P, Winter M, et al. Approaches to seizure risk assessment in preclinical drug discovery. Drug Discov Today 2009;14:876–84.

[11] Authier S, Paquett D, Gauvin D, Sammut V, Fournier S, Chaurand F, et al. Video-electroencephalography in conscious non human primate using radiotelemetry and computerized analysis: Refinement of a safety pharmacology model. J Pharmacol Toxicol Methods 2009;60:88–93.

[12] DeBoer E, Friedrichs G. EEG telemetry models in the rodent and non-human primate. J Pharmacol Toxicol Methods 2009;60:21.

[13] Meijer E, Selle N, Elber L, Ben-Shakhar G. Memory-detection with the concealed information test: a meta analysis of skin conductance, respiration, heart rate, and P300 data. Psychophysiol 2014;51:879–904.

[14] Osinsky R. Should I stay or should I go? Frn and p300 differientally reflect the integration of activity versus passivity in the evaluation of decision outcomes. Psychophysiol 2014;52:S73–4.

[15] Shim M, Kim D, Lee S, Im C. Disruptions in small-world cortical functional connectivity network during an auditory oddball paradigm task in patients with schizophrenia. Schizophr Res 2014;156:197–203.

[16] Brell J. Animal models of peripheral neuropathy: modeling what we feel, understanding what they feel. ILAR J 2013;54:255–60.

[17] Arezzo P, Litwak M, Zotova E. Correlation and dissociation of electrophysiology and histopathology in the assessment of toxic neuropathy. Toxicol Pathol 2011;39:46–51.

[18] Zotova E, Arezzo J. Non-invasive evaluation of nerve conduction in small diameter fibers in the rat. Physiol J 2013;2013:1–18.

[19] Weed M, Taffe M, Polis I, Roberts T, Koob G, Bloom F, et al. Performance norms for a rhesus monkey neuropsychological testing battery: acquisition and long-term performance. Brain Res Cogn Brain Res 1999;8:185–201.

[20] Taffe M, Weed M, Gutierrez T, Davis S, Gold L. Modeling a task that is sensitive to dementia of the Alzheimer's type:individual differences in acquisition of a visuo-spatial paried-associate learning task in rhesus monkeys. Behav Brain Res 2004;149:123–33.

[21] Nagahara A, Bernot T, Tuszynski M. Age-related cognitive deficits in rhesus monkeys mirror human deficits on an automated test battery. Neurobiol Aging 2010;31:1020–31.

[22] Barnett J, Robbins T, Leeson V, Sahakian B, Joyce E, Blackwell A. Assessing cognitive function in clinical trials of schizophrenia. Neurosci Biobehav Rev 2010;34:1161–77.

[23] Rodriguiz J, Nvrcher N, Bartlett T, Nathanielsz P, Mijland M. CANTAB delayed matching to sample task performance in juvenile baboons. J Neurosci Methods 2011;196:258–63.

[24] Myers T, Hamilton L. Delayed match-to-sample performance in African green monkeys (*Chlorocebus aethiops sabaeus*): effects of benzodiazepine, cholinergic, and anticholinergic drugs. Behav Pharmacol 2011;22:814–23.

[25] Webster S, Wilson C, Chich-Hung L, Mohler E, Terry A, Buccafusco J. The acute effects of dimebolin, a potential Alzheimer's disease treatment, on working memory in rhesus monkeys. Br J Pharmacol 2011;164:970–8.
[26] Rutten K, Basile J, Prickaerts J, Blokland A, Vivian J. Selective PDE inhibitors rolipram and slidenafil improve object retrieval performance in adult cynomolgus macaques. Psychopharmacology 2008;196:643–8.
[27] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation, Research (CDER). Guidance for Industry Assessment of abuse potential of drugs. Draft Guidance. 2010.
[28] EMEA. Guideline on the non-clinical investigation of the dependence potential of medicinal products. Evaluation of Medicine for Human Use. London: European Medicane Agency (EMEA); 2006.
[29] Balster R. Drug abuse potential evaluation in animals. Br J Addict 1991;86:1549–58.
[30] Balster R, Bigelow G. Guideline and methodological reviews concerning drug abuse liability assessment. Drug Alcohol Depend 2003;70:S13–40.
[31] O'Connor E, Chapman K, Butler P, Mead A. The predictive validity of rat self-administration model for abuse liability. Neurosci Biobehav Rev 2011;35:912–58.
[32] Horton D, Potter D, Mead A. A translational pharmacology approach to understanding the predictive value of abuse potential assessments. Behav Pharmacol 2013;24:410–36.
[33] Weerts E, Fantegrossi W, Goodwin A. The value of nonhuman primates in drug abuse research. Exp Clin Psychopharmacol 2007;15:309–27.

VetBooks.ir

CHAPTER

32

Skeletal Assessments in the Nonhuman Primate

John L. Vahle, Yanfei L. Ma, David B. Burr

Lilly Research Laboratories, Indianapolis, IN, USA

OUTLINE

Introduction to Skeletal Assessments in the Nonhuman Primate NHPs 606

NHP Bone Morphology and Physiology 607
Cortical Bone 607
Trabecular Bone 607
Bone Modeling and Remodeling 608
Comparison to Rodent Models 609

Bone Densitometry 610

Bone Biomechanics 612

The Role of Biomarkers in Skeletal Assessment 615
Bone Biomarkers 615
Mineral Ion Homeostasis 615

Bone Histology and Histomorphometry 617
Bone Histology—Qualitative Light Microscopy 617
Bone Histomorphometry 618

Implementing Skeletal Assessments in Drug Development Programs 620
Characterization of Detrimental Effects on the Skeleton 620
Ovariectomized NHP Model in the Preclinical Evaluation of Osteoporosis Therapies 622

References 623

http://dx.doi.org/10.1016/B978-0-12-417144-2.00032-9
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION TO SKELETAL ASSESSMENTS IN THE NONHUMAN PRIMATE NHPs

The skeleton is composed of bone and cartilage, which are highly specialized connective tissues. They function together to provide multiple functions, including (1) locomotion, by providing a structure and attachment sites for muscle; (2) protection, by encasing vital organs and tissues; and (3) metabolic, by serving as a reserve of mineral ions such as calcium and phosphorus. When viewed under a light microscope, many observers may consider the skeleton to be a relatively inert structure; however, the skeleton is quite labile and subject to pharmacologic effects that can be either beneficial or detrimental. The degree to which skeletal effects are assessed in drug development can vary widely and are based on the specific mechanism of action and therapeutic intent of the potential therapeutic. In general, there are three basic scenarios to consider:

(1) Routine toxicity testing: For therapeutics that do not include the skeleton as part of their mechanism of action, skeletal evaluation would typically be limited to routine light microscopic evaluation in repeat-dose toxicity studies that enable clinical development. These evaluations are useful in detecting overt bone pathology and can detect prominent changes in bone architecture; however, they are relatively insensitive to detecting minor but potentially important changes in bone mass.

(2) Efficacy and safety testing of a bone-targeted agent: For potential therapeutics being developed for a bone indication, studies of both rodents and nonrodents use quantitative techniques to evaluate the effects of the molecule on bone mass, structure, and function. A variety of animal models may be utilized depending on the disease state under consideration, and they could include ovariectomized animals to simulate bone loss (osteopenia) or fracture models in which a defect is surgically introduced and the effects on bone-healing are studied. A long-term study of ovariectomized nonhuman primates (NHPs) is typically conducted to assess bone quality specific to molecules being developed for an osteoporosis indication.

(3) Specialized bone toxicity assessments: For potential therapeutics in which bone effects have been observed in routine toxicity studies or for which the mechanism of action suggests a potential for undesirable effects on the skeleton, additional characterization of skeletal effects may be conducted either in the context of routine toxicity studies or as specialized studies. These studies often need to include quantitative assessments of bone mass and structure to detect effects that would not be evident in routine gross and microscopic observations of the skeleton. Other goals of these assessments might include characterizing potential biomarkers for monitoring clinical safety or mechanistic insights. Because routine toxicity studies typically use animals that are not skeletally mature, these specialized studies may include comparisons of immature versus mature skeletons.

Many agents whose mechanism of action involves the skeleton utilize the monkey for bone efficacy and safety assessments because of the similarities between the skeletons of NHPs and humans. While rodent models have important roles in both bone research and general toxicity testing, there are distinct differences in skeletal physiology that may render rodent data less predictive of the effects observed in humans. The purpose of this chapter is to provide scientists engaged in drug development programs an understanding of the unique role

VetBooks.ir

of the NHP in characterizing skeletal effects. The chapter provides an overview of the basic principles of skeletal physiology and a summary of how the NHP skeleton compares to both humans and rodents. Evaluation methods summarized include densitometric measures to assess bone mass, noninvasive biomarkers of bone formation and resorption, biomechanical assessments to understand functional aspects, and histology and histomorphometry to aid in understanding both bone structure and the mechanism of effect.

NHP BONE MORPHOLOGY AND PHYSIOLOGY

Because of their taxonomic similarity to humans, NHPs are often considered a better surrogate for bone assessments, both morphologically and physiologically, than other animal models. The most commonly used monkeys are macaques, primarily cynomolgus macaques (*Macaca fascicularis*) and, less frequently, rhesus macaques (*Macaca mulatta*). Although these animals do demonstrate greater similarities to human morphology and skeletal physiology than do rodent models, they are not necessarily more comparable to humans skeletally than other midsized (rabbits) or large animal models (dogs, pigs). However, the NHP has several other advantages compared to some of these animal models. For example, the structure and length of the femoral neck tends to be much more similar to that in humans than it does to that in rabbits. Moreover, ovariectomy in NHPs leads to modest bone loss, unlike dogs and adult pigs, which are insensitive to estrogen deficiency. Mineral metabolism in Old World primates such as macaques and baboons is similar to human mineral metabolism, but vitamin D and calcium metabolism in New World monkeys such as squirrel monkeys is not similar to that in humans.

Cortical Bone

The adult human cortical bone is largely composed of Haversian systems, or osteons, and complete osteons with intact Haversian canals occupy about 45% of the total cortical area. This is a reflection both of longevity and of the rate at which cortical bone turnover occurs. In contrast, the percentage of osteonal bone in the most commonly used species of adult macaques is 5-7% [1]. Cortical bone morphology in dogs, pigs, and rabbits is very similar to humans, with substantial portions of osteonal bone in the cortex (Figure 32.1), so cortical bone morphology in NHPs is not necessarily a better model for humans than is cortical bone in other large animal models.

Trabecular Bone

Unlike cortical bone, the volume and structure of vertebral cancellous bone is very similar in humans and NHPs. Whereas normative values for fractional bone volume range between 13% and 30% in human iliac crest biopsies, bone volume in intact cynomolgus iliac crest and lumbar vertebrae average about 25% and 27%, respectively. Estimates for other static cancellous parameters in the lumbar vertebrae are also consistent with human values, although the trabecular number may be 25% higher than in human iliac biopsies. Bone formation rates (BFRs) in macaque lumbar vertebrae tend to be somewhat higher than those in human iliac

VetBooks.ir

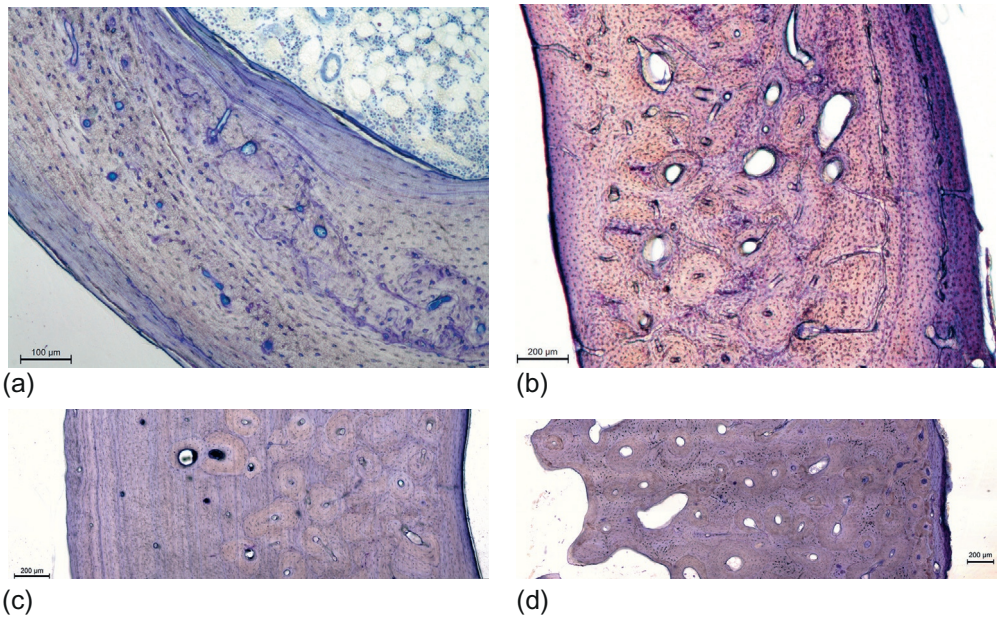


FIGURE 32.1 Histological cross sections of femoral cortical bone (stained with Toluidine blue) from rat (a), dog (b), monkey (c), and human (d). Rats have very little osteonal bone. Macaques have more but large regions of the cortex have no osteons, and they are composed of primary lamellar bone. Dogs have substantial amounts of osteonal bone spread throughout the cortex and are more similar to humans. *Photomicrographs courtesy of Dr. Keith Condon.*

crest biopsies, although activation frequency (1-2 year^{-1}) is similar (Table 32.1). Values for the cynomolgus iliac crest are very similar to those for their lumbar vertebrae.

Bone Modeling and Remodeling

Osteoclasts, osteoblasts, and osteocytes have coordinated functions that result in two distinct processes integral to the development and maintenance of the skeleton. Bone modeling is the primary process responsible for developing and maintaining bone shape. During bone modeling, bone resorption by osteoclasts and bone formation by osteoblasts is not locally coordinated or "coupled" and occurs on different bone surfaces. In contrast, bone remodeling is the primary process that provides for turnover and adaptation of the mature skeleton and is a coordinated and sequential process of cellular activation (A), osteoclastic resorption (R), and osteoblastic formation (F), also sometimes known as the ARF system. The remodeling period, the time over which these processes occur at a given site, requires about 4-6 months for completion in the cancellous bone in humans. It is very similar but perhaps a little more rapid in NHPs. Although estimates vary, the best estimates suggest that the remodeling period in NHP cancellous bone ranges between 3 and 6 months in both the lumbar vertebrae and the femoral neck. This is much slower than the estimate reported in the U.S. Food and Drug Administration's 1994 "Guidelines for preclinical and clinical evaluation of agents used in the prevention or treatment of postmenopausal osteoporosis," [16] but research since 1994

TABLE 32.1 Comparison of Selected Trabecular Bone Parameters in Various Species

	Humans		Macaques[c] [2–5]			Rats[e]	
Parameter	Postmenopausal women[a] [9,10]	Healthy subjects[b] [11]	Intact	OVX	Dogs[d] [6–8] (intact)	Intact [12–15]	OVX [12,14,15]
BV/TV (%)	14-30	13-28	26-28	21-25	22-25	19-26	~6
Tb·Th (μm)	93-105	100-225	107-127	97-122	96	40-60	53
Tb·(N/mm)	1.2-2.0	1.0-1.8	2.1-2.6	2.1-2.5	2.6	3.89-5.62	1.10
Tb·Sp (μm)	480-850	800-1500	282-359	306-388	302	133-286	883
MS/BS (%)	1.0-13.5	5-20	11-14	13-22	7.63-21.5	4.6-15.4	8.2-29.4
MAR (μm/day)	0.5-2.0	0.83-0.98	0.61-0.66	0.65-0.80	1.04-1.56	0.62-1.56	0.68
BFR/BS ($\mu m^3/\mu m^2$/year)	1-25	7-15	26-31	31-67	36-122	103-232	85
BFR/BV (%)	3-52	6-14	49-51	62-141	—	52-232	96
Rm·P (days)	22-131	120-180	153	67	90	28	~20
Ac·f (cycles/year)	0.15-0.43	1-2	0.57-1.43	1.57-2.74	0.79-1.74	~0.7	0.9-1.6

[a]*Based on iliac crest biopsies from Recker et al. [9] and Hauge et al. [10].*
[b]*Based on iliac crest biopsies from 25 healthy subjects, age 19-46 years (for static data); dynamic data are normal values for 13 white adults. From Weinstein [11].*
[c]*Based on measurements of lumbar vertebrae from Jerome et al. [2,3], Stroup et al. [4], Jerome, unpublished data, and Cabal et al. [5]. Data either are averaged from several sources or presented as ranges.*
[d]*Mashiba et al. [6], Allen et al. [7], and Allen and Burr [8]. Dogs ranged in age from ~2 to 5 years.*
[e]*Based on measures from both the proximal tibia and lumbar vertebra in 3- to 19-month-old female rats. Mori et al. [12], Jee and Li [13], Chen et al. [14], and Ma et al. [15].*
BV/TV = Cancellous Bone Volume; Tb-Th = Trabecular Thickness; Tb.N = Trabecular number; Tb.Sp = Trabecular separation; MS/BS = Mineralizing surface; MAR = Mineral apposition rate; BFR/BS = Bone Formation Rate, tissue level; BFR/BV = Bone formation rate, volume referent; Rm.P = Remodeling period; Ac.f =Activation frequency

suggests that the longer period is probably a better estimate. With estrogen deficiency following ovariectomy, the remodeling period in cynomolgus monkeys is shortened to 2-3 months, comparable to the 1-4 months measured from human bone [9].

Comparison to Rodent Models

Rodent models are frequently used to characterize skeletal effects of pharmacologic agents, and it is important to be aware of some of the interspecies differences in skeletal physiology that can impact model selection and data interpretation. With regard to an induced model of bone loss, rodents have an advantage over larger animal models as they lose substantial amounts of bone quite rapidly following ovariectomy. In part, this is because of the shorter remodeling period (28 days) compared to the longer period in postmenopausal humans (approximately 2-3 months, on average) or estrogen deficient NHPs (about 2 months). Bone volume fraction in rat proximal tibia and vertebral cancellous bone is similar to that in humans and macaques, but rats achieve this by having more but thinner trabeculae. One major difference between rodents NHPs is that rats and mice do not remodel cortical bone except under

VetBooks.ir

conditions of matrix damage or severe calcium deficiency. Thus, although NHPs do not have the robust intracortical remodeling of humans, they still provide a better model for cortical processes than do rats and mice. Rodents do, however, remodel cancellous bone, but the formation rate is 2-4 times faster than that in macaques. However, in the typical toxicology study, younger rodents generally are used, and in these young animals, unlike more mature monkeys, modeling is the predominant activity.

BONE DENSITOMETRY

Components of bone include organic matrix, inorganic mineral, cellular elements, and lipids. The ratio of these components varies depending on the specific bone, bone surface, age, and the species being studied. The inorganic mineral, principally carbonated hydroxyapatite, is embedded in an organic matrix consisting predominantly of type I collagen with lesser quantities of types III, V, and X collagen as well as other specialized noncollagenous proteins. Radiographic densitometric techniques are of major importance in the clinical evaluation and measurement of changes in bone mass. Bone mass is defined by bone mineral content (BMC), which can be measured by the amount of X-ray energy that is absorbed by the mineral in a section. BMC divided by the area or volume of the bone defines the bone mineral density (BMD). Dual-energy X-ray absorptiometry (DEXA) and peripheral quantitative computed tomography (pQCT) are commonly used to evaluate the BMC and BMD changes in humans and NHPs.

DEXA remain the most common densitometric tool to diagnose osteoporosis and other bone diseases in humans. DEXA scans in NHP studies provide data that are directly transferable to clinical end points in humans. DEXA can provide whole-body scanning for assessment of the entire skeleton as well as specific, clinically relevant sites such as the lumbar spine, femoral neck, total hip, radius, or other specific bone sites of interest. The main limitation of DEXA is that it only provides a two-dimensional projection when bone is three-dimensional. In drug development, studies may be conducted in growing animals, and it is important to understand that apparent changes in BMD, as measured by DEXA, may just reflect changes in growth and bone size but not true change in bone density. An additional limitation of DEXA is that changes in body composition can confound interpretations of changes in bone density. Furthermore, DEXA cannot differentiate between changes in bone density caused by increased porosity (as in osteoporosis) from those caused by mineralization defects (as in osteomalacia) or rapid bone turnover that may result in undermineralized bone (as in Paget's disease, or following anabolic treatments such as with teriparatide). From a methodologic standpoint, slight variations in the region of interest between animals, or the same animal at different study time points, can significantly influence the measurements [17]. Standardized procedures and experienced operators ensure reproducible data and correct analyses.

pQCT is another noninvasive densitometry technique used in humans and animals [18,19]. The key advantage of pQCT over DEXA is the ability to capture a three-dimensional image of a fixed scan thickness. Therefore, pQCT provides separate three-dimensional cortical and trabecular BMC and BMD analyses. pQCT also provides more detailed information regarding bone geometry and structure such as total, cortical, and trabecular area, marrow space area, cortical thickness, and periosteal and endosteal circumferences. Unlike DEXA, pQCT is not influenced by changes in body composition. Because of the limitation of scanner gantry

size, pQCT is limited to long bones such as the proximal tibia or distal radius. The emergence of high-resolution pQCT has made it possible to evaluate more precisely three-dimensional bone microstructure and volumetric BMD for human and animals. However, the long scanning time and radiation exposure required to achieve sufficient resolution of trabecular microstructure [20] renders it less useful for repeated in vivo scans.

Microcomputed tomography (μCT) analysis provides low double- or single-digit micrometer resolution of bone structure (Figure 32.2). As such, μCT can be used to assess the

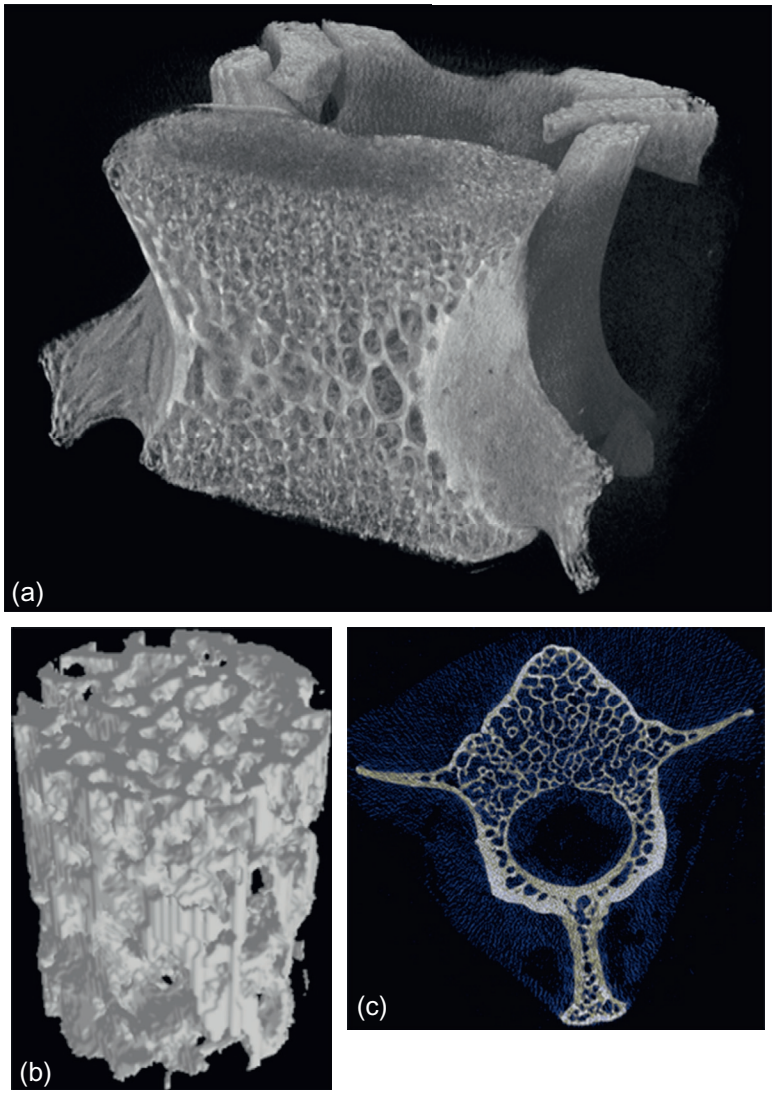


FIGURE 32.2 Fifth lumbar vertebra (L5) from a 3-year-old male cynomolgus macaque. Images obtained by microcomputed tomography and include a frontal view (a), central column (b), and cross section (c).

three-dimensional microarchitecture of the bone and to evaluate individual trabecular number, thickness, and separation, as well as trabecular connectivity, orientation, and volumetric BMC and BMD. It is a powerful tool that can only be used ex vivo in NHPs to complement static histomorphometric analyses.

NHP skeletons are similar to humans in that they go through phases of rapid growth, followed by attainment of peak bone mass, and subsequent age-related bone loss. These phases respond to mechanical and hormonal influences. Studies of bone mass in 56 male rhesus monkeys 4-34 years of age revealed increasing BMC and BMD at all sites from 4 to 10 years of age, with peak bone mass achieved at that time. After 10 years of age, age-related bone loss occurred at the lateral spine and distal radius [21]. Most NHP toxicology studies use 2- to 6-year-old growing monkeys, and the clinical significance of bone effects can be confounded by growth-related processes.

Studies have shown that decreases in bone mass observed in ovariectomized [22,23] and orchidectomized [24] monkeys closely resemble those observed in humans following menopause. In ovariectomized monkeys, significant BMD loss occurs in the lumbar spine, in the total hip as early as 3-4 months and in the femoral neck and radius by 6-9 months after surgery [23,25,26]. Bone losses reached steady state around 9-12 months for most of the bone sites [23,27,28]. pQCT analyses of proximal tibiae revealed earlier (3-4 months after surgery) and larger percentage volumetric bone losses in both cancellous and cortical bone [23,25,27,28]. Three-dimensional analyses of cancellous and cortical compartments separately by CT improves the ability to more sensitively detect changes of bone mass in these models.

BONE BIOMECHANICS

Bone strength and propensity to fracture are derived from a combination of bone mass or density, its architecture/geometry, and the material properties of the bone matrix itself. There are compensations among these various factors to achieve target bone strength, and none of these factors alone are sufficient to fully describe the strength, or other mechanical characteristics, of the bone. For instance, although BMD is highly correlated to bone strength, it explains only about 50-60% of the strength of the bone under normal (nonpathological) conditions, and it is not a particularly good indicator of whether bone may fail in fatigue (i.e., by a slow deterioration of properties caused by repetitive loading, as in a stress fracture) or when subjected to high impacts, as in a fall on the hip.

Many biomechanical properties can be used to characterize the mechanical fitness of bone, which can be described as weak or strong, ductile or brittle, stiff or compliant, depending upon the results of a mechanical loading test (Figure 32.3). An accurate picture of these characteristics of bone can be obtained through a few simple measures derived from tests that are relatively easy to perform. Typically, bone properties can be divided into structural (extrinsic) or material (intrinsic) properties. Extrinsic properties are determined by density, architecture, and tissue properties and are characterized by the strength, stiffness, and energy required to fracture the bone as an organ. Intrinsic properties describe the same characteristics of the bone tissue (strength, stiffness, and energy to fracture) independent of how much bone one has or its architecture. Both types of measurements can be useful in interpreting the ability of bone to withstand particular kinds of loads.

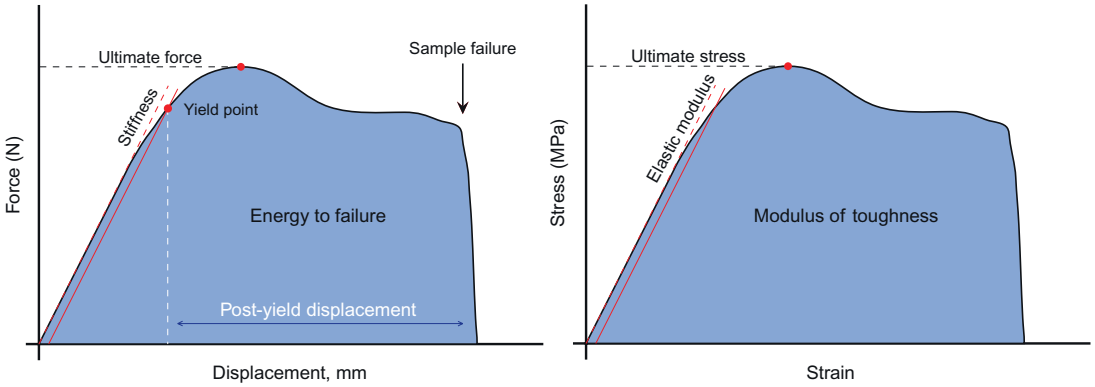


FIGURE 32.3 Load-deformation and stress-strain curves collected from mechanical tests of bone. The load-deformation curve (a) provides extrinsic (structural) mechanical parameters such as strength (maximum load), stiffness (slope of the linear portion of the curve), and energy to fracture (the area under the curve). When converted to a stress-strain curve (b) by normalizing for bone mass and architecture, similar properties of the bone matrix material can be calculated. The yield point is the point at which irreversible damage to the bone begins to occur.

Extrinsic properties can be measured by simply placing a load on a bone and measuring from a load-displacement curve how much it deforms before it breaks (Figure 32.3). The maximum load that a bone can sustain before it fractures is its strength; the slope of the linear portion of the load-displacement curve provides a measure of the bone's stiffness; and the area under the curve indicates how much energy is required to cause the bone to fail. If one normalizes these measurements for the amount and distribution of the bone, a stress-strain curve is obtained. Stress is the load per unit area, and strain is the fractional or percentage change in length; it is clear that these measures are just normalized derivatives of load and deformation. The stress-strain curve provides the same measurements as the load-displacement curve but for the material, rather than for the whole structure. Thus the maximum or ultimate stress is one measure of material (tissue) strength. The point at which the stress-strain curve begins to bend is the yield point, and this can be used as yet another measure of strength (yield strength). The slope of the linear region of the stress-strain curve is called the modulus of elasticity. This represents material stiffness; the greater the slope, the stiffer the material, and the more gentle the slope, the more compliant the material. The area under the stress-strain curve is the amount of energy the tissue can withstand before failure. This tissue property is called the modulus of toughness, or simply toughness. Toughness is important in bone biomechanics because a tough bone is more resistant to fracture. Toughness is increased more by increasing the amount of strain that occurs after the yield point than it is by increasing either its strength or stiffness. A bone that can sustain little strain after yield is considered brittle. Interestingly, osteoporosis is commonly called the "brittle bone disease." Even though it may be weaker and less rigid, there is little or no evidence that osteoporotic bone is actually more brittle than normal.

From a biomechanical standpoint, a drug is considered effective if it significantly improves extrinsic properties without impairing the intrinsic properties of bone. For NHPs, tests of the lumbar spine, long bone diaphysis, and femoral neck should be performed in those cases warranting biomechanical testing. Different kinds of biomechanical tests may be more

VetBooks.ir

appropriate than others, depending on the structure and size of the bone. Also, the properties of bone are different depending on the direction in which testing is performed (anisotropy) and depending on whether the test measures properties in tension, compression, torsion, or shear. Because bone from cynomolgus monkeys is not very large, true material property tests cannot be performed. These tests require removing a piece of bone and machining it to a standard size so that bone from all animals is geometrically identical. This allows one to compare the material properties of bone from different animals without regard to size, mass, or geometry (although it does not control for variations in porosity). For the smaller bone from cynomolgus monkeys, standard load-displacement and stress-strain curves are the best approach. For long bones, testing can be performed either in three-point bending (in which load is applied to the bone at one point with the bone sitting on two supports) or four-point bending (in which load is applied at two points simultaneously). Three-point bending is simple, but it has the disadvantage that it creates high shear stresses near the midsection of the bone. Four-point bending eliminates transverse shear stressors between the loading points but requires that the force be equal at each loading point. This can be difficult to achieve if the bone is curved. For studies of fracture healing, however, four-point bending is more desirable; three-point bending conflates the material properties of the callus, which is being indented with the more important structural properties (strength) of the healing bone. For bending tests, the span between the loaders should be about 16-20 times the thickness of the specimen, but there has been success in the laboratory testing NHP bone with ratios on the order of 10:1. For example, if the diameter of a long bone from a cynomolgus monkey is about 6.0 mm, then a span diameter of about 55-60 mm is probably appropriate to minimize shear stressors within the specimen.

Vertebrae cannot be tested in bending because vertebral bodies are relatively short and a sufficient distance between the loading points cannot be achieved. Moreover, vertebral bodies tend to be under greater amounts of compression than bending. Consequently, vertebral bodies are generally tested in conditions of compression, which also allows the use of relatively small specimens. Preparation of vertebrae for compression testing is aided by the removal of the posterior elements and transverse processes and by the use of a pivoting platen into the load train to correct for misalignment, which creates stress concentrations between the loading platens and the bone that alter the results and make them inaccurate.

Because of the clinical relevance of hip fractures, the femoral neck is a site that should also be mechanically tested. Changes in strength of the femoral neck subsequent either to ovariectomy or drug treatment may not always be self-evident because changes in cancellous bone as a consequence of these interventions can be compensated by alterations in the cortical shell, which plays a significant role in the strength of the femoral neck. Tests of the femoral neck can be performed either using an axial loading configuration in which the load is applied to the superior aspect of the femoral head, or in a fall configuration that places the femoral neck in 15° of internal rotation and 10° from the horizontal. In both cases, the proximal end of the femoral diaphysis must be embedded in plastic or clamped in place while a force is applied until the neck breaks. Although the femoral neck in these tests is placed in a bending configuration, significant shear force can be responsible for bone failure.

For long-bone diaphysis that contains primarily cortical bone, it is relatively easy to obtain an estimate of the rigidity (structural stiffness) of the bone without performing a mechanical test at all. The cross-sectional moment of inertia (CSMI) is a measure of the distribution of

material around a given axis, and it is proportional to its rigidity in bending. The polar moment of inertia is by defined collagen and cross-linking characteristics that do not vary. These properties can be calculated for the femoral neck from information provided by a DEXA scan [29,30]. Although these parameters can provide an estimate of bone strength, variations in the material and physical properties of bone that may occur as the result of estrogen deficiency or treatment with a therapeutic agent render comparisons based solely on CSMI less accurate. More detailed information about mechanical testing of bone can be found reviews by Turner and Burr [31,32].

THE ROLE OF BIOMARKERS IN SKELETAL ASSESSMENT

Bone Biomarkers

Bone as a dynamic and living tissue undergoes constant renewal throughout life. The balance of bone resorption and formation increases or decreases or bone mass is maintained. Bone remodeling activity can be evaluated by measuring osteoblast or osteoclast enzyme activities or assaying fragments of type I collagen that are resorbed from the bone matrix and released into the circulation or excreted in the urine. Markers of bone formation or resorption are typically collected at various time points to provide a snapshot of overall bone resorption or formation activities. This information is complementary to bone density data and together reflects the net bone balance that occurs over the course of remodeling cycles spanning weeks or months. In preclinical studies bone biochemical markers are used to assess disease progress and to monitor treatment-induced effects to bone turnover. Because bone growth (and consequently resorption and formation) slows with skeletal maturation, the effect of age should be considered when interpreting the drug effect on changes in bone biochemical markers.

Human bone biochemical marker assays are readily available for the NHP and are summarized in Table 32.2. Bone formation markers include bone specific alkaline phosphatase, intact osteocalcin, and N-terminal or C-terminal propeptides of type I procollagen. Bone resorption biomarkers include C-terminal or N-terminal telopeptides and pyridinoline crosslinks. It is well known that both bone formation and resorption biomarkers are elevated shortly after ovariectomy of the NHP, indicating high turnover bone loss with resorption exceeding formation, similar to human postmenopausal osteoporosis [23,28]. Bone formation and resorption biomarker response to osteoporotic drugs in NHPs are similar to what have been seen in humans, reflecting similar mechanisms of action for stimulating bone formation [2,28,33] or suppressing bone resorption [25,34,35]. Because bone resorption and formation are coupled during bone remodeling, molecules that affect bone resorption can also impact bone formation. Therefore, biomarker measurements must be interpreted cautiously. For an overview of bone biochemical marker use and clinical significance, see reviews by Vasikaran et al. [36], Biver [37], and Smith et al. [23].

Mineral Ion Homeostasis

In addition to assessing markers of bone formation and resorption, it is important to characterize effects on mineral ion homeostasis by measuring serum and urine calcium and phosphorus and calcitropic hormones such as parathyroid hormone (PTH) and

VetBooks.ir

TABLE 32.2 Selected Examples of Cancellous Bone Histomorphometry Parameters

Parameter	Abbreviation	Units	Comment
Cancellous bone volume	BV/TV	%	Percentage of the total tissue volume evaluated that is composed of bone
Trabecular thickness	Tb·Th	μm	Mean thickness of the trabeculae
Trabecular number	Tb·N	mm^{-1}	Mean density of the trabeculae in a unit area
Trabecular separation	Tb·Sp	μm	Mean distance between trabecular edges in a unit area
Osteoid surface	OS/BS	%	Percentage of the total bone surface that is occupied by unmineralized osteoid
Osteoblast surface	Ob·S/BS	%	Percentage of the bone surface lined by osteoblasts; increases with active bone formation
Osteoclast surface	Oc·S/BS	%	Percentage of bone surface lined by osteoclasts; increases in conditions of increased bone resorption
Bone formation rate, surface referent	BFR/BS	$\mu m^3/\mu m^2/day$	Provides a measurement of bone formation rate over a unit of time

1,25-dihydroxyvitamin D. While these parameters could be included in any evaluation of skeletal effects, they are particularly important when the mechanism of action of the molecule has known effects on mineral ion homeostasis or in those cases where bone findings suggest altered mineralization (osteomalacia). Serum calcium and phosphorus are routinely measured in repeat-dose toxicity studies; however, these are typically obtained at a limited number of time points and generally not accompanied by the determination of urinary phosphorus and calcium [38]. For programs where more rigorous evaluation of calcium and phosphorus metabolism is warranted, evaluation of both serum and urinary calcium at multiple time points during the study should be considered. The collection of blood and urine should be performed consistently throughout the study, most often as fasting samples obtained early in the day. Depending on the mechanism of action of the molecule being studied, a time-course study evaluating multiple samples over a 24- or 48-h period may be needed. Routine serum calcium assays detect total calcium that includes the active ionized calcium as well as protein-bound or complexed calcium. If precise measures of ionized calcium are required, detection of ionized calcium via ion specific electrodes would be warranted. The samples used to assess urinary calcium and phosphorus can be compromised and significant variability introduced by contamination of the urine sample by drinking water or feces. One solution to this issue is to collect a single sample at a defined time point ("spot" sample by cystocentesis). Alternatively, a closely controlled collection over a defined period of time (e.g., 16 h) provides a more accurate reflection of overall urinary excretion of calcium and phosphorus. In most cases concurrently determining urinary creatinine and normalizing urinary calcium and phosphorus values to creatinine values is advantageous. The primary calcium regulating hormones that are measured are PTH and both 25-hydroxyvitamin D and the active

VetBooks.ir

1,25-dihydroxyvitamin D. The net effect of PTH is an increase in serum calcium, a decrease in serum phosphorus, and an increase in urinary excretion of phosphorus mediated via PTH's actions on the bone and kidney and its role in the formation of active vitamin D. Metabolically active vitamin D also acts to increase serum calcium via its actions to promote calcium and phosphorus absorption from the intestinal mucosa. For an overview of calcium and phosphorus metabolism in animals, see the review by Rosol et al. [39].

BONE HISTOLOGY AND HISTOMORPHOMETRY

Bone Histology—Qualitative Light Microscopy

For programs in which the bone is not a tissue of interest, skeletal evaluations would be performed only in repeat-dose toxicity studies and would be limited to evaluation of formalin-fixed, decalcified, paraffin-embedded bone sections stained with hematoxylin and eosin. The standard sections of bone vary between laboratories, they but most often include the sternum and a long bone, usually distal femur and/or proximal tibia. In addition to the routine collection of a prespecified bone, any gross abnormalities of the skeleton identified by imaging should be collected and prepared for histologic examination. In cases where there is a specific concern for focal abnormalities of bone, plain film radiographs obtained before the start of dosing and before necropsy can aid in the detection and collection of bone lesions. Trimming the long bone should allow for evaluation of an articular surface, the epiphysis, physis, the metaphysis, and a region of diaphyseal bone. Given the size of the tibia or femur, the laboratory needs to be attentive during trimming that all regions are represented on the slide. Following histologic processing, the pathologist assesses the specimens for both overt bone lesions but also performs a qualitative assessment of metaphyseal bone volume or thickness of the physis. Consistent orientation and trimming is important to maximize the pathologist's ability to detect changes in the relative quantity of the various components. Examples of the types of changes that might be detected during the routine light microscopic evaluation include:

(1) Focal abnormalities: Focal lesions such as bone cysts, bone tumors, or focal bone necrosis require light microscopic evaluation for diagnosis.
(2) Physeal effects: A variety of agents that impact pathways important in endochondral ossification can cause either a relative increase or decrease in the thickness of the physis or cause overt dysplasia of the physis. In the NHP, inhibitors of angiogenesis cause this [40].
(3) Generalized increases in bone: While quantitative measures such as densitometry or histomorphometry are much more sensitive, agents that cause a prominent change in the amount of cancellous or cortical bone can be detected with routine histology. For example, potent bone anabolic agents such as teriparatide [2,22] and sclerostin antibody [33] can cause an increase in bone that is detectable by qualitative light microscopy.

In addition to repeat-dose toxicity studies, developmental toxicity studies include skeletal assessments to detect malformations in the offspring of dams administered the compound. For the majority of programs these studies will be conducted in the rabbit, rat, or mouse. In some

VetBooks.ir

cases (refer to Chapter 28), assessing developmental toxicity in the NHP may be necessary, and in these cases radiographs are often used to assess skeletal defects [41]. Drug development programs intended to support pediatric development may require separate juvenile animal toxicity studies that in many cases necessitate a more detailed skeletal examination. In these cases, it is important to determine whether there are effects on longitudinal skeletal growth, and femoral length data can be determined from either standardized plain film radiographs or from specimens collected at necropsy.

While decalcified, paraffin-embedded specimens stained with hematoxylin and eosin may be suitable for standard toxicity studies, in certain circumstances evaluating other types of histologic preparations or stains may be necessary to better characterize the effects of the test article on the skeleton. Some of these techniques are identical to those that are used for bone histomorphometry (as described later in this section). For example, in routine decalcified sections it is not possible to assess the degree and quality of mineralization. As such, it is necessary to prepare undemineralized sections by embedding in a resin such as poly-methyl methacrylate. Special stains may be utilized to aid in visualization of certain components of the bone and the selection of evaluation methods should be based on the specific objectives of the experiment. Examples of special stains used for bone include:

(1) Trichrome or van Gieson stains to highlight collagen
(2) Safranin O, Alcian blue, or toluidine blue to highlight proteoglycans. Toludine blue has the advantage of staining nuclei to aid in identification of osteoblasts and osteoclasts and also stains osteoid
(3) Alizarin red or von Kossa to stain mineral components (un-decalcified sections only)
(4) Alkaline phosphatase to stain osteoblasts or tartrate-resistant acid phosphatase to stain osteoclasts
(5) Goldner's Trichome or Villanueva to stain osteoid on un-decalcified sections.

Bone Histomorphometry

Bone histomorphometry yields valuable quantitative information on bone structure and bone formation and resorption. While the methods are highly standardized [42], it is technically demanding because of the need to use undemineralized specimens and is time consuming because of the exacting nature of the measurements obtained. Following preparation of the histologic sections, precisely defined regions are measured using an image analysis system. Static histomorphometry refers to measurement of the bone matrix architecture and bone cells. Dynamic histomorphometry allows characterization of the level of bone turnover and the rate of bone formation and requires in vivo administration of bone-seeking labels such as tetracycline, Calcein green, or alizarin complexone at a defined interval (often two labels, 10 days apart in monkey studies). The bone labels are then visualized and measured to allow assessments of the rate of bone formation (Figure 32.4). While a large number of static and dynamic parameters can be measured, a few of the more common ones are listed in Table 32.3. Several reviews provide a complete listing of the structural and dynamic parameters measured or derived for cancellous or cortical bone [42–44]. Bones collected at necropsy for histomorphometry frequently include the lumbar vertebra, proximal femur (femoral neck), radius, and tibia. Analyses are commonly completed on

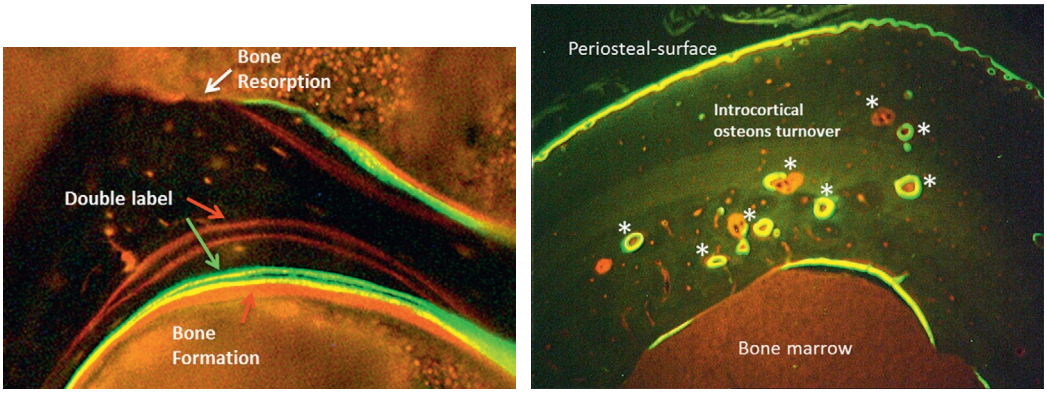


FIGURE 32.4 Histological fluorescent image of a 3-year-old male cynomolgus monkey midfemur (right) and Trabecular bone from the fifth lumbar vertebra (left). Double alizarin complexone (red) or Calcein (green) indicates active bone formation. Note active Haversian systems, or osteons, that indicate active turnover throughout the cortex (*). In the trabecular bone, the scalloped surface with interrupted fluorescent labeling is consistent with a region of bone resorption.

TABLE 32.3 Common Bone Biochemical Markers Used in the Nonhuman Primate

Name	Abbreviation	Source	Clinical significance
Serum bone-specific alkaline phosphatase	BALP	Synthesized in osteoblast, there is a 15-20% cross with liver alkaline phosphatase in adult humans	Bone formation Ubiquitous enzyme, regulates osteoid formation and mineralization
Serum osteocalcin	OCN	Osteoblast secreted or matrix degradation	Intact OCN: bone formation N-midterm OCN: bone turnover
Serum procollagen type I N-terminal or C-terminal propeptide	PINP, PICP	Derived from N-terminal or C-terminal extension type I procollagen	Bone formation
Serum/urine C-telopeptide type I	CTX-1	Bone matrix mature collagen degradation	Bone resorption
Serum/urine N-telopeptide type I	NTX-1	Bone matrix mature collagen degradation	Bone resorption
Urine hydroxypyridinium crosslink	DPD	Bone matrix mature collagen degradation, free moieties or peptide bound	Bone resorption

cancellous, endocortical, and periosteal surfaces, respectively. It is also possible to obtain information from animals during the course of the study by procuring a bone biopsy from the ilium or rib and conducting histomorphometric analyses. This procedure is typically reserved for long-term bone safety and efficacy studies and is not used for investigative studies of bone toxicity.

VetBooks.ir

IMPLEMENTING SKELETAL ASSESSMENTS IN DRUG DEVELOPMENT PROGRAMS

For many drug development programs, skeletal evaluations are limited to the standard histologic assessments completed in the context of the repeat-dose toxicology programs; however, there are several instances where more extensive skeletal evaluations may be warranted. Programs in which the NHP is already a component of the development package have the advantage of being able to characterize effects in an animal model that has a high degree of relevance with respect to human skeletal structure and function. A few of the scenarios where the types of skeletal analyses discussed in this chapter may be applicable are considered in the next section.

Characterization of Detrimental Effects on the Skeleton

There are several different sources of data that may drive the need to conduct a more thorough evaluation of the NHP.

- Theoretical mechanistic concerns: An assessment of the drug target and mechanism of action may suggest potential effects on the skeleton even in programs where bone effects are not the intended pharmacologic effect. This data may arise from either in vitro or in vivo studies and a weight-of-evidence assessment should be conducted to determine if more detailed skeletal analyses are warranted to either confirm or refute the potential of the agent to impact the skeleton. In general, careful evaluation of the skeleton is more warranted when there are clear indications of a phenotypic change in animal models such as data from relevant transgenic models or from in vivo studies of molecules with a similar mechanism of action. If the only signals are from genomic analyses or in vitro systems, additional skeletal analyses may not be warranted.
- Characterizing an observed bone effect: If skeletal effects have been observed in prior studies of the molecule, additional characterization and quantitation of the effect are often warranted. Examples might include histologic observations of an apparent reduction in cancellous bone volume, the occurrence of nontraumatic fractures suggestive of increased bone fragility, tinctorial alterations of the bone matrix suggestive of defective mineralization states, and alterations of the physis such as thickening or dysplasia of the physeal growth plate. In these cases, additional analyses may be needed to determine appropriate biomarkers for clinical use, understand potential mechanisms, and characterize and quantify the relative magnitude of the effect.

Once the need for additional skeletal evaluations have been established, careful consideration should be given to which end points are likely to be most informative. While this chapter has summarized an extensive array of end points that can be considered to provide a comprehensive evaluation of bone mass and structure, a case-by-case approach should be taken to determine which end points best fit the experimental objectives. Important factors to consider in determining appropriate end points include the nature and potential mechanism of the effect being characterized and the feasibility of including the end point into the NHP study being designed. If the primary objective is to determine whether there are changes in bone formation or resorption with resultant alteration of bone mass, the evaluation of

VetBooks.ir

formation and resorption markers in conjunction with an ex vivo assessment of bone density may be sufficient. If the objective is to determine if previously observed changes in bone mass result in altered biomechanical competence, than it including an assessment of biomechanics in samples collected at necropsy is essential. If the objective is to determine whether the changes may be secondary to changes in mineral ion homeostasis, detailed measurements of urinary phosphorus and calcium combined with analysis of key bone regulating hormones such as PTH and vitamin D may be needed.

Consistent with approaches to reduce and refine the use of animals, serious consideration should be given to incorporating the desired skeletal end points into either an existing or planned NHP toxicology study. Two key principles must be considered when determining if and how to incorporate bone end points into NHP toxicology study:

(1) The small group size ($n = 3$ or 4 per sex) of most toxicology studies limits the ability to detect a change if only a single measurement at study termination is obtained. The power to detect an effect is greatly aided by characterizing a parameter longitudinally over the course of the study in the same animal. Serum and urine biomarkers readily lend themselves to this approach. In addition, it is possible to incorporate bone densitometry by including an in vivo determination of bone mass before starting the study and then include an ex vivo measurement at study termination.

(2) The skeletal evaluations should not compromise the collection of routine toxicology end points. Each experimental procedure added to a study increases study complexity and introduces factors that could confound the primary objective of the study, which is the detection of target organ toxicities. For example, while performing multiple in vivo densitometry assessments (DEXA or pQCT) greatly facilitates the ability to characterize the effects on bone mass and structure, and this advantage should be weighed against the fact that scan procedures require anesthesia that may last from 20 min to 1 h. It is also important to understand the general toxicity profile of the molecule in determining if additional in vivo procedures are advisable. If the molecule causes either generalized high-dose toxicity or has specific effects on the nervous system, the scanning procedure may present an unacceptable degree of risk to the conduct of the study.

With the considerations in mind, it is often feasible and advantageous to characterize skeletal effects in the context of a general toxicology study. A useful case example was published by Smith et al. [34] in which skeletal end points were included in toxicology studies of osteoprotegerin. Osteoprotegerin is a secreted glycoprotein of the tumor necrosis factor receptor superfamily and been shown to block differentiation of osteoclasts and increase bone mass. In a 13-week toxicity study, the investigators assessed biochemical markers of bone turnover before starting the study and at weeks 4 and 13. Bone densitometry was determined by pQCT assessments of distal radius and proximal tibia before the study and near week 13. These analyses identified clear decreases in biochemical markers of bone resorption and a resultant increase in bone density.

It is often necessary to assess the effects of a molecule in either a developmental toxicity setting or in the context of juvenile toxicity studies. If these studies include an assessment of skeletal development, bone lengths can be determined from plain film radiographs and bone densitometry can be used to provide measurements of bone mass and geometry.

VetBooks.ir

Ovariectomized NHP Model in the Preclinical Evaluation of Osteoporosis Therapies

For agents intended to prevent or treat osteoporosis, a rigorous evaluation of bone quality in relevant animal models is necessary. While clinical development programs define the effects of agents on biomarkers of formation and resorption, bone density, and fracture risk-reduction rates, clinical studies have limited ability to characterize various aspects of bone quality. Although bone biopsies can be obtained in the clinical trial program and provide important information, they are derived from a limited number of subjects. Animal models provide the advantage of being able to include invasive assessments such as histomorphometric assessment of multiple skeletal sites and, importantly, determination of bone strength via biomechanical testing. The most commonly used large animal model that provides a definitive preclinical evaluation of the skeleton is the ovariectomized NHP model. A complete discussion of this model can be found in excellent reviews by Smith et al. [23] and Jerome et al. [3,26,45], and a summary of some of the key design features are provided in Table 32.4. These large, complex studies are typically performed concurrent with the phase III clinical program and are generally required for marketing authorizations. The model uses surgical ovariectomy of skeletally mature NHPs to induce a high state of bone turnover and a degree of bone loss. While no animal model completely models the clinical state of postmenopausal osteoporosis, the elimination of cyclic reproductive hormone exposure due to ovariectomy in the mature NHP provides a reasonable approximate of the clinical condition. The rate of bone loss following ovariectomy in cynomolgus monkeys is very similar to that in human postmenopausal osteoporosis. On average, cynomolgus monkeys lose 2-6% of the cancellous bone in the lumbar vertebrae per year, compared with 2-5% in women immediately

TABLE 32.4 Study Design Elements for Ovariectomized Nonhuman Primate Bone Quality Studies for Osteoporosis Agents

Animal selection	**Sexually and skeletally mature female nonhuman primate**[a]
Treatment duration	16-18 Months for agents intended for chronic administration; shorter designs may be possible, depending on clinical use
Treatment initiation	Dependent on indication and mechanism of action of molecule; can vary from shortly after ovariectomy (prevention) and up to 9 months after ovariectomy (treatment)
Group size	12-20 Animals per group; careful consideration of the mechanism of action and existing data are important in determining appropriate group size
Dose selection	Minimum of two dose concentrations: a low dose that approximates human exposure and/or pharmacodynamic response on bone mass and a high dose approximately five times greater than the low dose to maximize ability to detect detrimental effects on bone quality
Key end points	• Bone formation and resorption biomarkers • Densitometry (DEXA, pQCT, μCT) • Histomorphometry and qualitative histology • Biomechanical analysis

μCT = microcomputed tomography; DEXA = dual-energy X-ray absorptiometry; pQCT = peripheral quantitative computed tomography.
[a]*Typical age range of 9-12 years. A detailed reproductive, skeletal, and general health screening is required before entering the study.*

VetBooks.ir

after menopause. Given such a slow rate of loss, it can be difficult to create significant bone loss after even 2 years of estrogen deficiency. Consequently, some studies that use a surgical ovariectomy (OVX) NHP model to demonstrate the efficacy of a therapeutic treatment suffer from the inability to demonstrate conclusively that significant bone loss has occurred in the estrogen deficient animal. The duration of treatment used in these studies was originally based on regulatory guidance from the U.S. Food and Drug Administration. The guidance indicated that 16 months of treatment in the monkey would cover approximately 4 years of human treatment in terms of bone turnover cycles, although more recent evidence indicates this is probably equivalent to less than 3 years in the human [2–4]. Therefore 16-18 months of treatment were commonly used in the model. The model is sometimes described as having a prevention mode in which treatment is initiated shortly after ovariectomy and a treatment mode in which treatment is initiated following a period of bone loss (up to 9 months). As noted in Table 32.4, relatively large group sizes are used and essentially all of the evaluation techniques described in this chapter are used. Depending on the specific needs of the program, the study design can include targeted incorporation of other end points to address key safety or mechanistic questions. For example, if prior animal toxicology or clinical data suggest a potential concern for effects on the renal system, both biochemical and tissue-based analysis could be incorporated in a study that provides for a long duration of exposure and large group sizes. The ovariectomized monkey studies are technically demanding and should be conducted in laboratories with a thorough understanding of the model and the end points being collected. Examples of the use of this model in support of marketed osteoporosis agents include, but are not limited to, denosumab [25], ibandronate [35], teriparatide [2,22], and PTH 1-84 [28].

References

[1] Schaffler MB, Burr DB. Primate cortical bone microstructure: relationship to locomotion. Am J Phys Anthropol 1984;65:191–7.

[2] Jerome CP, Johnson CS, Vafai HT, Kaplan KC, Bailey J, Capwell B, et al. Effect of treatment for 6 months with human parathyroid hormone (1–34) peptide in ovariectomized cynomolgus monkeys (*Macaca fascicularis*). Bone 1999;25:301–9.

[3] Jerome CP, Carlson CS, Register TC, Bain FT, Jayo MJ, Weaver DS, et al. Bone functional changes in intact, ovariectomized, and ovariectomized, hormone-supplemented adult cynomolgus monkeys (*Macaca fascicularis*) evaluated by serum markers and dynamic histomorphometry. J Bone Miner Res 1994;9:527–40.

[4] Stroup GB, Kumar S, Jerome CP. Treatment with a potent cathepsin K inhibitor preserves cortical and trabecular bone mass in ovariectomized monkeys. Calcif Tissue Int 2009;85:344–55.

[5] Cabal A, Jayakar RY, Sardesai S, Phillips EA, Szumiloski J, Posavec DJ, et al. High-resolution peripheral quantitative computed tomography and finite element analysis of bone strength at the distal radius in ovariectomized adult rhesus monkey demonstrate efficacy of odanacatib and differentiation from alendronate. Bone 2013;56:497–505.

[6] Mashiba T, Turner CH, Hirano T, Forwood MR, Johnston CC, Burr DB. Effects of suppressed bone turnover by bisphosphonates on microdamage accumulation and biomechanical properties in clinically relevant skeletal sites in beagles. Bone 2001;28:524–31.

[7] Allen MR, Iwata K, Phipps R, Burr DB. Alterations in canine vertebral bone turnover, microdamage accumulation, and biomechanical properties following 1-year treatment with clinical treatment doses of risedronate or alendronate. Bone 2006;39:872–9.

[8] Allen MR, Burr DB. Three years of alendronate treatment results in similar levels of vertebral microdamage as after one year of treatment. J Bone Miner Res 2007;22:1759–65.

[9] Recker RR, Kimmel DB, Parfitt AM, Davies KM, Keshawarz N, Hinders S. Static and tetracycline-based bone histomorphometric data from 34 normal postmenopausal females. J Bone Miner Res 1988;3:133–44.
[10] Hauge E, Mosekilde L, Melsen F. Missing observations in bone histomorphometry on osteoporosis: implications and suggestions for an approach. Bone 1999;25:389–95.
[11] Weinstein RS. Clinical use of bone biopsy. In: Coe FL, Favus MJ, editors. Disorders of bone and mineral metabolism. New York: Raven Press; 1992. p. 455–74.
[12] Mori S, Jee WS, Li XJ. Production of new trabecular bone in osteopenic ovariectomized rats by prostaglandin E2. Calcif Tissue Int 1992;50:80–7.
[13] Jee WS, Li XJ. Adaptation of cancellous bone to overloading in the adult rat: a single photon absorptiometry and histomorphometry study. Anat Rec 1990;227:418–26.
[14] Chen HK, Ke HZ, Jee WS, Ma YF, Pirie CM, Simmons HA, et al. Droloxifene prevents ovariectomy-induced bone loss in tibiae and femora of aged female rats: a dual-energy X-ray absorptiometric and histomorphometric study. J Bone Miner Res 1995;10:1256–62.
[15] Ma YF, Li XJ, Jee WS, McOsker J, Liang XG, Setterberg R, et al. Effects of prostaglandin E2 and F2 alpha on the skeleton of osteopenic ovariectomized rats. Bone 1995;17:549–54.
[16] FDA. Guidelines for preclinical and clinical evaluation of agents used in the prevention or treatment of postmenopausal osteoporosis. Rockville, MD: FDA Good Guidance Practices; 1997.
[17] Kanis JA, Gluer CC. An update on the diagnosis and assessment of osteoporosis with densitometry. Committee of Scientific Advisors, International Osteoporosis Foundation. Osteoporos Int 2000;11:192–202.
[18] Valentinitsch A, Patsch JM, Burghardt AJ, Link TM, Majumdar S, Fischer L, et al. Computational identification and quantification of trabecular microarchitecture classes by 3-D texture analysis-based clustering. Bone 2013;54:133–40.
[19] Genant HK, Jiang Y. Advanced imaging assessment of bone quality. Ann N Y Acad Sci 2006;1068:410–28.
[20] Cheung AM, Adachi JD, Hanley DA, Kendler DL, Davison KS, Josse R, et al. High-resolution peripheral quantitative computed tomography for the assessment of bone strength and structure: a review by the Canadian Bone Strength Working Group. Curr Osteoporos Rep 2013;11:136–46.
[21] Colman RJ, Lane MA, Binkley N, Wegner FH, Kemnitz JW. Skeletal effects of aging in male rhesus monkeys. Bone 1999;24:17–23.
[22] Jerome CP, Burr DB, Van Bibber T, Hock JM, Brommage R. Treatment with human parathyroid hormone (1–34) for 18 months increases cancellous bone volume and improves trabecular architecture in ovariectomized cynomolgus monkeys (*Macaca fascicularis*). Bone 2001;28:150–9.
[23] Smith SY, Jolette J, Turner CH. Skeletal health: primate model of postmenopausal osteoporosis. Am J Primatol 2009;71:752–65.
[24] Seidlova-Wuttke D, Schlumbohm C, Jarry H, Dullin C, Wuttke W. Orchidectomized (orx) marmoset (*Callithrix jacchus*) as a model to study the development of osteopenia/osteoporosis. Am J Primatol 2008;70:294–300.
[25] Ominsky MS, Stouch B, Schroeder J, Pyrah I, Stolina M, Smith SY, et al. Denosumab, a fully human RANKL antibody, reduced bone turnover markers and increased trabecular and cortical bone mass, density, and strength in ovariectomized cynomolgus monkeys. Bone 2011;49:162–73.
[26] Jerome CP. Primate models of osteoporosis. Lab Anim Sci 1998;48:618–22.
[27] Kostenuik PJ, Smith SY, Jolette J, Schroeder J, Pyrah I, Ominsky MS. Decreased bone remodeling and porosity are associated with improved bone strength in ovariectomized cynomolgus monkeys treated with denosumab, a fully human RANKL antibody. Bone 2011;49:151–61.
[28] Fox J, Miller MA, Newman MK, Turner CH, Recker RR, Smith SY. Treatment of skeletally mature ovariectomized rhesus monkeys with PTH(1–84) for 16 months increases bone formation and density and improves trabecular architecture and biomechanical properties at the lumbar spine. J Bone Miner Res 2007;22:260–73.
[29] Beck TJ, Ruff CB, Warden KE, Scott Jr WW, Rao GU. Predicting femoral neck strength from bone mineral data. A structural approach. Invest Radiol 1990;25:6–18.
[30] Yoshikawa T, Turner CH, Peacock M, Slemenda CW, Weaver CM, Teegarden D, et al. Geometric structure of the femoral neck measured using dual-energy X-ray absorptiometry. J Bone Miner Res 1994;9:1053–64.
[31] Turner CH, Burr DB. Basic biomechanical measurements of bone: a tutorial. Bone 1993;14:595–608.
[32] Turner CH, Burr DB. Experimental techniques for bone mechanics. In: Cowin SC, editor. Bone mechanics handbook. Boca Raton: CRC Press; 2001. p. 1–35.

VetBooks.ir

[33] Ominsky MS, Vlasseros F, Jolette J, Smith SY, Stouch B, Doellgast G, et al. Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. J Bone Miner Res 2010;25:948–59.
[34] Smith BB, Cosenza ME, Mancini A, Dunstan C, Gregson R, Martin SW, et al. A toxicity profile of osteoprotegerin in the cynomolgus monkey. Int J Toxicol 2003;22:403–12.
[35] Smith SY, Recker RR, Hannan M, Muller R, Bauss F. Intermittent intravenous administration of the bisphosphonate ibandronate prevents bone loss and maintains bone strength and quality in ovariectomized cynomolgus monkeys. Bone 2003;32:45–55.
[36] Vasikaran S, Eastell R, Bruyere O, Foldes AJ, Garnero P, Griesmacher A, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int 2011;22:391–420.
[37] Biver E. Use of bone turnover markers in clinical practice. Curr Opin Endocrinol Diabetes Obes 2012;19:468–73.
[38] Weingand K, Brown G, Hall R, Davies D, Gossett K, Neptun D, et al. Harmonization of animal clinical pathology testing in toxicity and safety studies. Fundam Appl Toxicol 1996;29:198–201.
[39] Rosol TJ, Chew DJ, Nagode LA, Capen CC. Pathophysiology of calcium metabolism. Vet Clin Pathol 1995;24:49–63.
[40] Ryan AM, Eppler DB, Hagler KE, Bruner RH, Thomford PJ, Hall RL, et al. Preclinical safety evaluation of rhuMAbVEGF, an antiangiogenic humanized monoclonal antibody. Toxicol Pathol 1999;27:78–86.
[41] Weinbauer GF, Fuchs A, Niehaus M, Luetjens CM. The enhanced pre- and postnatal study for nonhuman primates: update and perspectives. Birth Defects Res C Embryo Today 2011;93:324–33.
[42] Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 2013;28:2–17.
[43] Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, et al. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 1987;2:595–610.
[44] Recker RR, Kimmel DB, Dempster D, Weinstein RS, Wronski TJ, Burr DB. Issues in modern bone histomorphometry. Bone 2011;49:955–64.
[45] Jerome CP, Peterson PE. Nonhuman primate models in skeletal research. Bone 2001;29:1–6.

VetBooks.ir

VetBooks.ir

CHAPTER

33

Considerations for Special Administration Techniques

Helen Palmer, Virginia Fisher

Huntingdon Life Sciences Ltd, Huntingdon, United Kingdom

OUTLINE

Introduction 628

Major Considerations 628
- *Social Groupings and Isolation* 628
- *Training and Restraint* 629
- *Sedating/Premedicating Primates* 629
  - Anesthesia 630
  - Recovering from Anesthesia 631
- *Pain Assessment* 631

Considerations in Practice 632
- *Intravenous Infusion* 632
- *Surgical Infusion Models* 632
  - Equipment Required 633
- *Anesthesia* 633
  - Premedication 633
  - Surgical procedure 634
  - Postprocedural Considerations 635
  - Administration of Test Formulation 636
- *Restrained Infusion Models* 637
  - Procedure 637
  - Potential Complications 638
- *Capsule Administration* 638
- *Intradermal and Subcutaneous Injections* 639
  - Restraint 639
  - Infection 640
  - Pain 640
  - Logistics 640
- *Intravitreal Injections* 641
  - Logistics 641
  - Administration Requirements 641
  - Postprocedural Considerations 642
- *Imaging* 643

Conclusion 645

References 645

http://dx.doi.org/10.1016/B978-0-12-417144-2.00033-0
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

Drug development and toxicological models are constantly evolving. This evolution drives continuing change and development in the requirements for novel and special administration and monitoring techniques in toxicological testing. First and foremost, all special administration techniques must be developed within the remit of the "3 Rs" (replace, reduce, refine). Once an effective administration technique is established, this should be reviewed regularly and refined as new technologies become available.

When special administration techniques are required, there are several considerations that must be assessed before establishing that a technique is achievable:

- Cost-benefit analysis of the technique: Will it achieve the scientific outcome? Is the scientific outcome of sufficient value to warrant the cost to the animal?
- Refinement: Can the technique be refined further to lessen the cost?
- Legislation: Is the procedure enabled by correct licensing and local law?
- Logistics:
  - Is the required equipment available?
  - Is the procedure cost prohibitive?
  - Are the required animals available and are they fit for their required purpose?
  - Does the procedure require specialist training (animal as well as scientific)?
  - Is there sufficient provision for adequate monitoring?
  - Can the required volume of compound necessary be placed in the target area without causing adverse effects that will affect the study's validity?
- Staffing: Are there suitably trained and proficient personnel available to perform the technique?
- Monitoring: Are specialist equipment/monitoring techniques required for the procedure?
- Training: Do staff require training? Is specialist expertise required from an external source? Is cadaver work necessary to achieve competency in/refinement to the technique?
- Animal welfare: Are adequate provisions for welfare assessment provided to monitor and minimize pain, suffering, and distress? Does the facility promote positive welfare? How is this monitored?
- Pilot study: If this is a novel technique, does it require validation and the use of minimal animals to establish it as a valid technique before performing a full-scale toxicological study?
- Special requirements according to the target organ and possible adverse effects (e.g., ocular administration and irritancy, pancreatic administration, and pancreatitis).

Effective planning and resourcing are essential to a successful outcome for implementing novel and special administration techniques.

MAJOR CONSIDERATIONS

Social Groupings and Isolation

Macaques are social animals, living in large familial groups in the wild and generally held in large single-sex groups at breeding colonies. Although replicating these types of environments is difficult due to experimental design factors, macaques should generally be housed in

VetBooks.ir

groups of two or more animals. It has been shown that attending to the social environment of the animals reduces the impact of stress and that the animals show more normal behaviors [1].

It is essential that the animals remain as calm as possible, particularly when unusual procedures are to be used, so that data generated can be compared against background data without too much extrapolation for the physiological effects of stress. Therefore, consideration of possible effects on the animals' social pattern is key.

There are relatively few procedures that require animal to be totally isolated to fulfill the scientific objective, and these should be minimized. Unless unavoidable, animals should be maintained in social groups as much as possible, and, when this is not possible, environmental and experimental design should be optimized to enable visual and verbal contact between peers. Where animals are removed for brief periods, they should be habituated to the procedure before the start of the experiment.

Training and Restraint

Positive reinforcement training for routine husbandry, and procedures within the animal facility can be extended to incorporate habituation to novel or less common procedures. Animals that are accustomed to regular positive human interaction and handling, for example, will acclimate more easily to the procedures required for administration. In this laboratory (Huntingdon Life Sciences), general training and habituation to staff and general procedures take place from arrival, whereas training for study-specific techniques generally takes place in the last 2 weeks before the start of treatment. By this point, the animals are well used to handling, voluntary removal from the cage, offering limbs for catching, moving around the cage, and so on. Training uses a short list of verbal commands with hand signals and treats. The same commands and signals are used throughout the facility for consistency. The animals quickly learn to move around the cages on command and present their arm for catching or blood sampling. Mature animals which may be initially resistant can be trained by a more gradual approach; target training, for example, rather than expecting the animals to move around the cages to earn a reward. This gives a small demonstration of the extent of possibility when training animals to volunteer. In terms of welfare considerations, training primates is a huge improvement compared to untrained animals, and offers them control over a situation while achieving the study objectives using positive welfare.

Training for more unusual techniques may include learning to sit in a restraint chair for short periods, to be dosed with sham treatment by having a catheter touch the back of the tongue, to become accustomed to wearing a weighted jacket, or one of many other specific situations. Once the initial training of several weeks, which is used to build trust between the animals and the handlers, is completed, including these study-specific procedures is a simple extension of the training [2] (Chapters 14 and 15 in this volume).

Providing training as a stimulus for these highly intelligent primates has the added benefit of occupying them in what can be an unchanging and routine environment in the laboratory setting. In addition, habituation to handling is likely to minimize aggression and hence possible injury to other animals and staff.

Sedating/Premedicating Primates

Anesthetics used in primates must be carefully planned and tailored to the individual institution, primate (species and individual), and operator experience. There are many options

VetBooks.ir

available for sedation in primates, and these options are extensively published in the literature. Our preferred sedation method in macaques is ketamine, usually in combination with another drug such as an opioid analgesic and an antisialogogue to help reduce salivation. Ketamine produces reliable sedation and immobilization in primates, and it is the drug of choice in larger primates for many [3,4]; at low doses ketamine has the added benefit of removing the biting reflex in this species, and has relatively minimal effects on cardiovascular parameters [3–6].

All animals that are to be administered an anesthetic and/or undergo a procedure requiring anesthesia should have a full preprocedural health evaluation to confirm their suitability for the anesthetic and procedure. This can include but is not restricted to physical examination, particularly heart and lung auscultation and cardiorespiratory assessment; blood biochemistry; and hematological assessment to assess general health and ensure no subclinical issues are present. Body weight should be measured as close to the day of the procedure as possible but without adding any stress to the procedure. This enables accurate calculation of the drugs required for the anesthetic and procedure and ensures that the anesthetic is administered at a therapeutic and safe dose.

Macaques should have food withheld for at least 8 h before anesthesia to allow the stomach to empty and prevent the risk of vomiting during anesthesia/sedation. To reduce stress, animals should preferably be sedated with a suitable premedication in the home environment. Training animals to come forward to the handler to be caught manually greatly reduces the stress of the procedure. If the primate is too large to handle, then training it to enter a transport box and to volunteer a limb for injection is achievable in the vast majority of primates [7,8].

The primate should be restrained manually or in the transport box until it is immobilized. It should never be returned to a pen, where it may be in danger of falling when it becomes sedated; macaques' natural instinct is to escape vertically from danger, and, if returned to a cage, they climb to the top, where they may be at risk of falling when they become sedated. A sedated animal should never be returned to a home pen with other animals while the sedation takes effect. Macaques have a strict social hierarchy, and a sedated animal is at risk of being attacked by other macaques. If the animal must be returned to an area for the sedation to take effect, then precautions must be taken to ensure the safety of the animal, including removing all climbing opportunities and ensuring there are no open bodies of water into which the animal may fall.

If the premedication is allowed to take effect while the animal is in a transport box, the animal must be monitored continually to ensure that no adverse effects occur, such as apnea, or that the airways become occluded should the animal relax into an awkward position against the box wall.

Anesthesia

There are many specific considerations that must be made when anesthetizing macaques to ensure that they are safely anaesthetized and homeostasis is maintained. These include some practical aspects such taking care when positioning the nonhuman primate's (NHP's) head for intubation to prevent any damage to the neck. Local anesthesia should be applied to the larynges before intubation in NHPs to help prevent laryngeal spasm (e.g., lidocaine). Maximum volumes of local anesthetics should be calculated per animal before surgery to ensure that these are not accidentally exceeded through liberal splash blocks. Endotracheal (ET) tubes should be premeasured to ensure that they are not inserted too far into the trachea and inadvertently occlude a bronchus. If a cuffed ET tube is used, it can be inflated for positive pressure ventilation or when inhalation anesthesia is used to prevent escape of anesthetic gases into the

VetBooks.ir

theatre atmosphere. If the cuff is inflated, it should be regularly deflated and the tube moved slightly before reinflation throughout the procedure to reduce pressure irritation to the trachea.

Within the many choices of anesthetics, based on our experience we prefer to use total intravenous propofol and remifentanil. The recovery from anesthesia using propofol and remifentanil is very smooth in comparison to isoflurane anesthesia, with animals regaining motion control in a much more coordinated way.

Monitoring and maintaining homeostasis can be challenging in NHPs. They can have a small body mass and therefore are prone to losing body temperature rapidly if care is not taken to minimize evaporative, conductive, and radiation heat loss.

Monitoring blood oxygenation with a pulse oximeter can also be problematic in small NHPs, particularly in cynomolgus macaques. The pigmentation of the skin and access to a hairless part of the body presents challenges to finding a good spot for the probe.

Recovering from Anesthesia

SOCIALIZATION/ACCLIMATIZATION

Always be cautious and take adequate measures to prevent bite injuries to staff when primates are recovering from anesthesia. Well-socialized primates are less likely to bite during recovery and generally are less stressed by the human contact while they are recovering from anesthesia. For larger NHPs, with which it is a true risk to be in close contact while they recover, measures should be introduced to remotely monitor the animals. For example, a pulse oximeter probe can be left attached to the lip, which can be pulled back through a gap in a closed door so hands can be kept away from the animal and sudden escape prevented. Similarly, an ET tube can be left in place until the anesthetist is satisfied that the animal is recovered sufficiently to maintain its airway, and then it can be removed by withdrawing a preplaced long tie attached to the tube.

ENVIRONMENT

Once the NHP is recovered sufficiently to move around, keep its head aloft, and stay alert, it may be preferable to return the animal to its home unit to recover fully in a familiar environment.

SOCIAL STATUS

Return macaques to their home enclosures with other animals only after they are fully recovered and no longer uncoordinated or drowsy. This is primarily to prevent any falls and injuries and to avoid upsetting their strong social hierarchy. The hierarchy can be affected if the animals are returned when they are not fully able to fend for themselves, and it can lead to fighting when the animals try to reestablish their position in the social group. Animals should always be housed in compatible, stable social groups for several weeks before any procedure or anesthesia. Returning animals into a group whose hierarchy is still being established can cause more disruption, stress, and probable fighting [9].

Pain Assessment

Where procedures may be expected to cause pain, for example, those that may require a surgical element, adequate provision must be made for analgesia and pain assessment. Pain assessment is complex when primates are concerned and is an area of continual development [10]. All projects should include a recent literature review to ensure the latest information is available to scientists

VetBooks.ir

to fulfill the analgesic requirements of the study and to minimize pain suffering and distress. Analgesia treatment should be tailored to the study to ensure the objectives of the study are not compromised. A multimodal approach is most effective [3,4] and should include a combination of local anesthesia, nonsteroidal anti-inflammatory, opioid, and additional types of anesthesia as appropriate. For example, tramadol and paracetamol can be included in the analgesia protocol.

In our experience, primates should always be given the benefit of the doubt regarding pain. We receive far more reliable pain assessment from experienced animal care staff who know their animals well and can detect subtle changes in behavior than from any pain score assessment. These subtle changes would not necessarily trigger an analgesia requirement on pain scoring assessments, yet the behavioral changes resolve with additional analgesia, indicating a requirement for it.

CONSIDERATIONS IN PRACTICE

The following examples demonstrate these considerations in practice.

Intravenous Infusion

Considerations for selection of central vessel surgical cannulation versus superficial vein temporary cannulation include:

- Physicochemical characteristics of the test article: Is it necessary to infuse into a stronger blood supply for buffering or distribution of the test formulation? It is necessary to consider primarily the objective of the investigation: if a potential exists for irritation of smaller vessels because of increased contact time; if instability of the test formulation could lead to deposition in smaller, superficial vessels with less blood flow.
- Duration and frequency of infusion administration: There are inherent welfare costs to the primate in general anesthesia, surgery, or restraint [7,8,11]. Thus general anesthesia, surgery, and the consequent recovery are a significant cost to the animal on a single occasion, whereas repeated or long-term restraint is well known to cause stress reaction and physiological as well as behavioral consequences. If the two choices are equal in terms of physicochemical parameters, a choice must be made based on welfare: thus, in this laboratory (Huntingdon Life Sciences) a short, intermittent infusion is more likely to be carried out under restraint, whereas a longer daily infusion is more likely to warrant surgical preparation to allow ambulatory infusion in the home cage. Where continuous (24 h/day) infusion is required, surgical preparation is always deemed necessary.

Surgical Infusion Models

Animals that are to receive ambulatory infusion are implanted with a subcutaneous venous access port (VAP) and wear a jacket to carry the infusion equipment. Training and habituation to the jacket within the group housing environment are essential to prevent undue stress to the animals and potential damage to the infusion equipment once administration is underway. Once acclimated, there is no reason why a macaque cannot remain in a group environment during recovery from implantation and the subsequent treatment phase.

VAP implantation is a straightforward surgical procedure, and the animals recover quickly postoperatively.

VetBooks.ir

Equipment Required

- Suitable VAP and cannula (e.g., SWIRL-MID SMALL V-A-P with 5Fr-polyurethane cannula by Access Technologies), a division of Norfolk Medical Inc. This comes with moveable beads to secure the cannula into the femoral vein, and it facilitates securing of the cannula as it exits the vein to minimize movement of and kinks in the cannula. Research into the latest developments in this technology should be carried out because devices and cannulas are being continually refined.
- Standard surgical kit, including instruments for vascular surgery (e.g., vessel dilators) and surgical loupes if necessary
- Suture material to secure the VAP and cannula (e.g., Nurolon) and for wound closure (e.g., Monocryl)
- Fine fur clippers to prevent injury to the macaque's skin while preparing the surgical sites
- Suitable surgical aseptic scrub
- Suitable equipment to maintain a sterile operating environment (e.g., theater maintenance, surgical-site drapes, and autoclave for surgical instruments)
- Suitable anesthetic equipment specific for the NHP, including:
  - suitably sized ET tube and mask
  - anesthetic machine with medical-grade oxygen and anesthetic gas vaporizer and circuit appropriate for the NHP's size.
- Anesthetic monitoring equipment, including stethoscope, body temperature monitoring equipment, and blood pressure monitoring equipment.
- Temporary venous cannula for induction of anesthesia and to provide fluid support during procedure (and anesthesia if required); 24 gauge for small primates, 22 gauge for larger NHPs is sufficient in our experience
- Syringe/drip driver if total intravenous anesthesia is to be used; must be suitable for calibrations for smaller animals. A drip driver should be used for administering intravenous fluids throughout the procedure for improved accuracy versus drip lines because the fluid rate is low for small animals.
- Supplementary heat source (e.g., hot bed, warm pads)
- Flush for the cannula (heparinized saline, 50 IU/mL)
- A "lock" solution to maintain the cannula's patency at the end of surgery (e.g. Taurolidine-Citrate Solution - trademark TCS by Access Technologies, TCS)
- Appropriate carrier/environment for induction and maintenance of anesthesia
- Perioperative medications; antibiosis (e.g., potentiated amoxicillin), analgesia (e.g., methadone, buprenorphine, meloxicam), local anesthesia (e.g., lidocaine, bupivacaine)
- Ocular lubricant (e.g., Viscotears) to prevent corneal drying while primate is under anesthesia

Anesthesia

Premedication

The primate should be sedated in the home unit to reduce anxiety. A suitable combination such as atropine with ketamine and methadone given intramuscularly provides sufficient sedation to transport the animal to the surgical theater and gain intravenous access to induce anesthesia.

VetBooks.ir

Anesthesia should be induced by an appropriate route. For example, propofol is widely used as an agent to induce anesthesia in many species including primates. It has a wide safety margin and has relatively little effect on the patient's cardiorespiratory function [3,4]. This makes it the induction agent of choice for many clinical and regulatory situations. Always take into consideration the drugs used in the premedication because the total dose of anesthetic drugs is reduced when used in combination with sedatives and analgesics.

Anesthesia may be maintained with propofol, and when it is used in combination with an opioid such as the short-duration remifentanil, it provides excellent analgesia to reduce the total dose of propofol required and produce a well-balanced anesthetic.

The NHP should have an airway secured for the procedure using an ET tube of appropriate size. Oxygen should be supplemented and the anesthetic and vital signs monitored carefully throughout the procedure. Providing supplementary heat sources that are monitored to ensure that they are not overheating the animal are necessary to maintain body temperature.

Placing an intravenous catheter before surgery is good practice to allow for quick venous access should it be needed during the procedure. Both the cephalic and saphenous veins provide good access sites for the NHP—be careful not to catheterize the same limb that will have the VAP catheter inserted, however, because the lower limb circulation will be affected by the procedure. Providing appropriate intravenous fluid therapy to maintain perfusion and minimize effects on the blood pressure is good practice. The procedure itself should not result in more than a slight blood loss when performed correctly. Ensure that fluids are warmed before infusion to help prevent body temperature reduction. Providing preemptive analgesia and antibiosis is advocated at an early stage of the preparation for surgery.

The surgical sites are prepared aseptically—hair is clipped away and thoroughly prepared with an appropriate surgical preparation (e.g., diluted chlorhexidine). Care must be taken to minimize evaporative heat loss.

Surgical procedure

Isolate a short segment (approximately 1.5 cm) of the left femoral vein via an incision along the center of the femoral triangle of the medial thigh. Insert a trochar beneath the skin around the leg and up the primate's back to exit at a small incision site between the scapulae, where the port will be situated, then tunnel the cannula up to the port site.

Local anesthesia should be applied to all surgical wounds; a splash block would be appropriate for this procedure. Bupivacaine currently provides the longest duration of action (up to 8 h) for local anesthesia; longer-acting preparations are being prepared for marketing. Personal licensees must be diligent in keeping up to date with the most recent developments in anesthesia and analgesia.

Prime the cannula with heparinized saline (50 IU/mL). Tie off the distal end of the femoral vein and occlude the proximal end of the isolated length (Figure 33.1). In our experience, a multifilament artificial, nonabsorbable suture material such as Nurolon provides the best securing grip on the cannula combined with minimal tissue reaction.

Incise the femoral vein using either small sharp, pointed scissors or a bent 22-gauge hypodermic needle if the vessel is collapsed and small. The use of surgical loupes may

VetBooks.ir

FIGURE 33.1 Insertion of a venous cannula into the femoral vein.

be necessary when operating around the femoral vessels to help prevent damage to the nearby femoral nerves and to help visualize the intima of the femoral vessel when it is empty of blood.

Insert the cannula proximally (toward the heart) until the end is situated in the caudal vena cava; premeasure this distance to ensure that it is inserted to the correct length. Using a heparinized cannula is advocated to try and prevent clot formation within the cannula and reduce the patency period of the model.

When the catheter is secured, attach the port and secure this to the tissue between the shoulder blades using nonabsorbable sutures again. In our experience, the skin of cynomolgus macaques may be prone to ulceration around tension points over VAPs, so placing the port in an area with the most mobile skin is advisable [12].

In NHPs closing the wounds with absorbable subcuticular sutures (e.g., Monocryl) and burying all knots well is important to remove all temptation of patient interference with the suture material. Tissue glue can be used to provide another layer of security against wound interference; however, care should be taken not to over-apply this because it may cause more irritation.

The VAP and cannula must be "locked" with an appropriate solution (e.g., Taurolidine citrate solution), taking into account the port, cannula, and needle volumes.

Postprocedural Considerations

PREVENTION OF INFECTION Strong consideration should be given to providing animals with postoperative antibiosis; when placing surgical implants, these can act as a nidus for infection.

PAIN ASSESSMENT All animals should be provided with appropriate pain relief postoperatively. Multimodal pain relief is most effective, such as meloxicam with buprenorphine in addition to the local anesthesia applied at surgery. More recent alternatives such as tramadol may also be considered. Animals should be monitored for any signs of pain by staff who are familiar with the animals. Subjectively scoring pain is notoriously difficult in NHPs, and, in our experience, the unit staff observing subtle changes in the primates is more reliable than any pain scoring method that has been suggested. NHPs should always be given the benefit of the doubt. If a subtle change in the animal's behavior resolves after provision of further analgesia, we conclude that this change had been due to pain.

VetBooks.ir

POTENTIAL COMPLICATIONS Complications can arise following any surgical procedure and anesthetic. Duly considering these should help to minimize the occurrence of these adverse effects. Specific issues relating to the implantation include:

- Animal interference—occurs in animals that developed ulceration and wounds that precluded further use in the study. The importance of port placement away from access of animals' hands and the importance of jackets, which are secure from primates being able to insert their arms inside each other's jackets, have been demonstrated.
- Loss of cannula patency—this can occur for several reasons such as clot formation in the cannula or kinking of the cannula as scar tissue forms. The importance of using an appropriate lock solution and volume of lock solution is demonstrated, and reducing pocket formation and tissue trauma and suitably placing the cannula are also good surgical techniques. Using a heparinized cannula, if available, can be considered. The use of moveable beads in the cannula can help to prevent kinking as the cannula exits the femoral vein.
- Needles falling out of the ports—demonstrates the importance of using well-fitting adjustable jackets to prevent friction and a suitable choice of port. A "gridlock" port should reduce the movement of the needle within the port itself.

Administration of Test Formulation

- Once the subcutaneous port is sufficiently healed to be considered stable, the animals need to be acclimated to wearing a jacket, which contains a pump and dose reservoir (Figure 33.2). Administration to the surgically prepared animal occurs by means of a small pump and dose reservoir that are carried by the animal in the home cage. The animal is removed from the home cage to allow a gripper needle to be passed through the subcutaneous port that is held in place by the membrane of the port. Test formulation passes through the port and internal cannula directly into the central venous system (Figures 33.3 and 33.4). Dose volume and rate must be chosen to provide sufficient rate to maintain patency and provide a significant exposure but must not be

FIGURE 33.2 Acclimation to jackets.

VetBooks.ir

FIGURE 33.3 Vascular access port in situ.

FIGURE 33.4 Vascular access port with gripper needle inserted.

so great that physiological stress to the kidneys or heart ensue. In our laboratory, rates of up to 5 mL/kg/h have been administered successfully, although for continuous infusion, a rate of 2.5 mL/kg/h would be considered preferable (Figure 33.5).

- When intermittent infusion is carried out via the port, a locking solution (e.g., Taurolidine citrate solution) is used between dose administrations to maintain patency of the equipment. In this case, the animals may continue to wear a jacket with a small weight instead of the infusion apparatus while off dose to maintain familiarity.

Restrained Infusion Models

Procedure

The biggest consideration in restrained infusion is stress on the animal and the physiological consequences thereof. The primate is retrained in a suitable device (usually a chair or recumbent seat, depending on the anticipated length of infusion) and the selected vein is cannulated using a soft indwelling cannula with Luer lock or membrane lock. It is important that the animal is not restrained too firmly but that it is sufficiently secure that no damage to the dose site or equipment is possible.

FIGURE 33.5 Ambulatory pump in a jacket pouch.

At our laboratory, dose sites for intermittent restrained infusion are generally peripheral veins such as the saphenous, cephalic, and lateral tail vein. Depending on the frequency of treatment, these may be used in rotation to allow healing.

Potential Complications

Complications for the retrained infusion technique are generally limited to localized damage at the dose site veins either by mechanical or chemical means. Selection of soft cannulae, good technique, secure restraint, and particular attention to appropriate dose vehicles limit the incidence of such findings.

Capsule Administration

Oral capsule administration is generally used to mimic the intended route of administration in the clinical setting. It is important that the administration technique is well executed without causing undue discomfort or necessitating prolonged restraint of the animals. The administration must be performed correctly so that the animal cannot regurgitate the capsule. Although considering anesthesia or pain relief for such a procedure is not necessary, minimizing stress and adequate training and preparation are essential to guarantee a positive outcome.

VetBooks.ir

Positive reinforcement training within the animal unit can be supplemented with procedure-specific training to habituate the animals to handling and dosing. There are three basic methods of efficient and accurate administration, depending on the required outcome of the administration:

- When a capsule is required to break down in the stomach, it may be given with a food snack as part of the reinforcement process. The animal is removed from the cage by its usual handlers and is held facing the dose administrator. The capsule is applied to the very back of the throat using a "piller device" designed for administering tablets to household pets, essentially a short rubber cannula on a syringe with a pusher inside. The capsule is pushed out of the end of the soft catheter directly behind the soft palate, avoiding the gag reflex. The animal is immediately rewarded with a small food treat that pushes the capsule down the esophagus.
- When the capsule is enteric coated and must pass into the intestine before breaking down, not adding calories or solid food material to the stomach at the same time as dosing is preferable because this delays gastric emptying. In this case, the capsule may be placed into the end of a gavage cannula and inserted directly into the stomach followed by a few milliliters of water.
- When the capsule is being given as part of a medication regime rather than a testing protocol, the exact amount of material may be less important. In this case, training the animals to self-medicate by taking a capsule inside a piece of fruit or with a drink may be possible. This is generally only possible for long-term treatment regimes where the animals become wholly acclimated to the procedure and for medications that the animals do not associate with feeling unwell (i.e., no clear side effects).

Dose capsule size and dose frequency are generally linked to animal size and required systemic exposure. At our laboratory, we found that up to three size 0 capsules may be given satisfactorily to young adult cynomolgus or rhesus macaques on a single or on intermittent occasion(s). For frequent or daily administration, a single capsule at a time may be preferable and less stressful for the animals, but it may be given on several occasions during each 24-h period.

Intradermal and Subcutaneous Injections

Intradermal and subcutaneous injections are particular specialisms for the laboratory primate because of the thin skin and low pelt mobility of these animals. The necessity for total restraint during the procedures also provides an opportunity of great stress to the animals and a negative relationship between animal and handler if the handling, training, and procedures themselves are not carried out well.

Restraint

For intradermal and subcutaneous bolus injection, restraint may be of short duration, but the animals must be held immobile for the duration of the procedure. For short-duration administration, it is generally preferable to hold the animals manually on a soft surface, allowing the dose administrator easy access to the dosing site. Being held prostrate is generally very stressful for primates and, consequently, training for the procedure is of paramount importance. This is especially so for intradermal injection, where positioning of the dosing needle between the layers of skin warrants particular care and that the animals not be struggling at any time.

VetBooks.ir

Infusion is not generally carried out intradermally, but for subcutaneous infusion the animal may be held in a half chair so that a soft indwelling cannula may be applied to a defined area of the back. Animals must be monitored continuously during the procedure to prevent damage to the dosing apparatus and site. Enrichment toys and screens may be used to entertain the animals during this time.

Infection

Use of sterile needles, dosing solutions, and antisepsis of the dosing area immediately before administration is generally enough to prevent local infection.

Pain

Minimal discomfort during the procedure itself is not usually regarded warranting of analgesia. Subsequent inflammation of the dose site, either due to the test article itself or from some irritation of the dose vehicle, may be treated with simple analgesic when it would not interfere with interpretation of the study results.

Logistics

Equipment required:

Smallest-gauge needle considered appropriate for the dose volume to be administered. The decision of minimal discomfort upon insertion against the possibility that administration may be prolonged by a very fine needle should be considered.

Intradermal: A microfine insulin syringe and needle (gauge 30) is suitable for areas of thin skin, whereas up to 26 gauge may be suitable for dose sites that are easier to isolate.

Subcutaneous: 25/26-gauge needles of at least 15 mm are suitable for all potential dose sites.

Technique:

Intradermal: the needle should be held almost parallel to the surface of the skin to ensure that the point does not traverse all the layers. A defined bleb (Figure 33.6) will be seen if the technique has been performed correctly.

Subcutaneous: A dose site is selected with sufficient skin that it will be possible to lift it slightly to allow accurate placement of a needle tip in the subcutis. The area is prepared by removing hair and wiping with an anti-infective. The skin around the area is gently lifted

FIGURE 33.6 Intradermal bleb.

VetBooks.ir

(pinched) to allow insertion of the needle. The needle should be inserted at a slightly downward angle to avoid seepage and then moved slightly under the skin to ensure that the needle has gone to the appropriate level. Upon removal of the needle, the area may be dabbed with gauze or cotton wool but should not be rubbed or pressure applied. The material should be allowed to disperse naturally.

Intravitreal Injections

Intravitreal injection is used to administer substances directly into the posterior chamber of the eye, which is required for regulatory toxicological testing when drugs are developed for use by this administration route in medicine. The technique is relatively straightforward to perform. However, as with all regulatory work carried out in animals, adequate provision must be made to ensure that all legal requirements, competencies, equipment, and resources are in place before commencing a toxicological study.

Logistics

Equipment required:

- Small-volume sterile syringe and small-gauge sterile needle (e.g., single-use insulin syringe).
- Sterile-lid speculum of the appropriate size.
- Local anesthetic agent for the eye (e.g., 0.5% proxymetacaine hydrochloride).
- Pupil dilator (e.g., 1% tropicamide).
- Surgical scrub agent for asepsis suitable for use in eyes (e.g., povidone iodine 10%).
- Suitable anesthetic agents and equipment for the size and species of the NHP and a secure area in which to perform the procedure out of the sight of the other animals.

Sterility is of utmost importance; all formulations, equipment, and handling of the test article must be sterile. The compound is to be injected directly into the eye and therefore any microbial contaminants will also be injected directly into the eye, which may result in infection. This causes adverse effects that are likely to invalidate the test results.

Administration Requirements

ANESTHESIA

For the test article to be administered effectively into the vitreous, the eye must be immobilized. to achieve this, and to ensure the animal is not aware of the procedure for welfare purposes, the NHP must be anesthetized. Any agents that are proposed should be assessed for suitability to be used with regard to interactions with the test article that may occur and for effects on intraocular pressure. Agents with the least effect on intraocular pressure should be used.

The procedure is quick, and the anesthetic agents used should reflect this. For example, a suitable premedication combination such as a low dose of ketamine (8 mg/kg) with an opioid (e.g., methadone 0.3 mg/kg) and an antisialogue (e.g., atropine 0.05 mg/kg) should be used to sedate the animal and reduce anxiety and salivation. General anesthesia may then be induced in the immobilized animal by administering intravenous propofol to effect. Because the procedure is quick, an adequate plane of anesthesia can be maintained with propofol given via a temporary venous catheter. The airway should ideally be secured by placing an ET tube for the duration of the anesthesia to administer oxygen.

VetBooks.ir

PREPARATION OF THE EYE

The eye should be examined thoroughly for any clinical disease once the animal is anesthetized to ensure that it is still suitable for the purpose. The pupil must be dilated using an appropriate agent and local anesthesia applied to the ocular surface.

The eye must then be prepared aseptically using a suitable agent. Sterility of administration is essential to prevent introduction of microbial agents into the eye that may invalidate the results should an infection then ensue.

A sterile-lid speculum can be inserted to immobilize the eye in preparation of the injection placement. Careful use of sterile atraumatic forceps to gently grip the bulbar conjunctiva to secure the eye further can be used when the intravitreal injection is made.

ADMINISTERING THE INTRAVITREAL INJECTION

The procedure must be performed aseptically. Ensure that you familiarize yourself with the anatomy of the eye and are aware of the relative location of structures when performing the procedure. This is essential to minimize any inadvertent damage to ocular structures.

- Insert the needle via the bulbar conjunctiva approximately 1-2 mm lateral to the junction of the cornea and the conjunctiva.
- Angle the needle at approximately 45° to the surface of the eye to direct it into the body of the vitreous.
- Advance the needle to the appropriate depth (e.g., 5 mm in a 3-kg cynomolgus macaque). Inject the compound slowly to prevent a rapid increase in intraocular pressure.
- Withdraw the needle, taking care not to alter the angle during withdrawal it to prevent accidental damage to intraocular structures.

Postprocedural Considerations

MONITORING THE EFFECTS

Visual monitoring of ocular effects is a useful adjunct to the standard toxicological investigations (histopathology, toxicokinetic screens, etc). Using an indirect binocular video ophthalmoscope, a pretreatment assessment can be recorded of the ocular structures. Staged examinations after treatment can be scheduled to assess the ocular structures and any effects of the compound on them. This is particularly important in assessment of the lens; slit-lamp biomicroscopy in addition to examination with the indirect ophthalmoscope can aid in this assessment. Assessing changes to the lens on histopathology is difficult, and visual assessment of the eyes by an experienced person in these studies is essential.

PAIN ASSESSMENT

Animals should be monitored for signs of pain—pain assessment is complex when primates are concerned and is an area of continual development. Overt signs of ocular pain such as blepharospasm should be addressed with appropriate analgesia. Analgesia treatment should be tailored to the study to ensure the objectives of the study are not compromised. A multimodal approach is most effective and can include a combination of the following:

- Topical nonsteroidal anti-inflammatory preparations (e.g., Votarol, 0.1% diclofenac sodium) or systemic nonsteroidal anti-inflammatory drugs (e.g., meloxicam, 0.2 mg/kg orally/subcutaneously every 24 h for macaques [13])

VetBooks.ir

- Systemic analgesia with opioids for severe pain (e.g., buprenorphine 0.015 mg/kg for macaques)
- Painful increases in intraocular pressure can be addressed with medical therapy (e.g., provide carbonic anhydrase inhibitors systemically or topically and administer miotics to facilitate aqueous outflow [14])
- If intraocular pressure reaches more than 40 mmHg, performing anterior chamber paracentesis is recommended [14].
- End points for the study should be defined in advance to ensure that appropriate amelioration of discomfort or pain (including terminating the study, if necessary) are initiated at set points, thereby allowing flexibility in treatment of the animals while ensuring fulfillment of the scientific objectives of the study.

PREVENTION OF INFECTION

Medication should be prescribed to prevent postprocedural infection. Topical agents such as gentamicin have been administered at a frequency suitable for the preparation to achieve this successfully [15].

POTENTIAL ADVERSE EFFECTS

Adverse effects can arise from any regulated procedure, and it is important to be prepared and to anticipate what these may be. Amelioration and action plans can be formulated in advance of any adverse effect occurring. This should ensure rapid implementation of effective amelioration and minimize any pain, suffering, and distress.

With intravitreal dosing following the previously discussed procedure, adverse procedural effects can also include [16]:

- adverse reaction to anesthetic agents,
- anesthetic complications,
- retinal detachment,
- intraocular inflammation,
- hemorrhage within the eye or ocular tissues, and
- physical injury to the lens by the injection.

Suitable monitoring procedures should be implemented to observe for these adverse effects in addition to any compound-related effects.

Imaging

As toxicological testing becomes more demanding with more target organ specific administrations (e.g., intrathecal), so does the requirement for accurate administration and monitoring after the procedure.

With special administrations, general anesthesia is required in the vast majority of cases. Should this also require specialist imaging, then consideration for this must be made. For example, if the imaging requires remote operation for health and safety reasons, such as with magnetic resonance imaging and flow magnetic resonance imaging, then provision must be made for remote maintenance and monitoring of anesthesia for the duration of the imaging. This can be a significant issue because anesthetic stations invariably consist of metal components that

VetBooks.ir

cannot be kept in close proximity to the animal and conventional equipment and so must be tailored to allow extensions without compromising the quality of the anesthesia.

Adaptation of the circuiting needs to be made if extensions are placed because the tubing adds to the expiratory resistance of the circuit, and, therefore, tubing should be wider bore. This is especially important for smaller animals, and the use of intermittent positive pressure ventilation is usually advisable.

In addition, personnel should also maintain a distance from the imager and so remote monitoring must be adequate to ensure that anesthesia is safely maintained. If the imaging procedure is lengthy, adjustable supplemental heating must be provided to maintain the body temperature of the animal within the appropriate thermoneutral zone. All monitoring that is used must be applied with common sense in this environment. For example, if end-tidal carbon dioxide is measured at the end of the 6 m of tubing away from the patient, then the value will be completely different than that measured next to the animal. It can, however, still provide useful information on trends of increasing or decreasing values. Monitoring leads need to be compatible with magnets, and these are available for human medicine such as carbon-fiber or fiber optic leads.

Computerized tomography scanning provides similar challenges, and therefore careful choice of anesthesia should be made. Total intravenous anesthesia with propofol and an opioid such as remifentanil provides a stable anesthetic. However, the anesthetist must preempt changes in advance because it may take several minutes for a change in the infusion rate to take effect—this is particularly important because there is no direct access to the animal possible during imaging.

Equipment required for the procedure such as stereotaxic frames can also intrude on anesthesia equipment. Limited access for the anesthetic tubing and access to the animal to monitor reflexes is often inhibited by the surgical field. Special consideration needs to be made for the use of these frames as they are painful to fit and require peri- and postoperative analgesia for the pressure that is applied to the orbital ridges and ears [5]. Maintaining the animal's body temperature can be challenging when the animals are positioned upright. The procedures, imaging, and recovery involving stereotaxic frames tend to have a long duration of several hours. To prevent muscle contractures, limbs should be massaged or moved regularly throughout the procedure.

Where prolonged anesthesia is used in any procedure, then special considerations should be made for these factors to maintain the animal's homeostasis and to minimize adverse effects. These should include maintaining body temperature and preventing hypotension. Adequate ventilation should be provided, including an occasional artificial supranormal lung inflation to prevent microatelectasis. Measures should be used to prevent inflammation of the trachea due to prolonged contact with an ET tube, particularly if it is cuffed and the cuff is inflated. Fluid balance must be maintained and considerations made for the fact that the urinary bladder will continue to distend over time.

These are all in addition to the usual considerations of anesthetizing an animal for a toxicological procedure such as choice of anesthetic agent, minimizing physiological effects on the target system (e.g., cardiovascular), and preventing drug interaction with the test article. Choosing a suitable anesthetic for the operator is important and based mainly on experience, equipment availability, and safety of the anesthetic used. An anesthetic regime should also be tailored to the procedure, including duration and depth of anesthesia required.

VetBooks.ir

CONCLUSION

Consideration of primary concerns such as the complex behavioral requirements of NHPs, their physiological needs, and in particular the amelioration of potential adverse events during procedures is essential for all techniques undertaken. These considerations are essentially similar for all techniques, although emphasis on different considerations is necessary for each differing procedure.

References

[1] Koban TL, Schapiro SJ, Kusznir T, Lambeth SP, Ogbin J, Garofalo E. Effects of international transit and relocation on cortisol values in cynomolgus macaques (*Macaca fascicularis*). Am J Primatol 2010;72:51.

[2] Prescott M, Buchanan-Smith H. Training non-human primates using positive reinforcement techniques. J Appl Anim Welf Sci 2003;6:157–261.

[3] Flecknell P. Primates: pre-anaesthetic medication. In: Laboratory animal anaesthesia. London: Academic Press/Elsevier; 2009. p. 231.

[4] Flecknell P. "Multi-modal" pain therapy. In: Laboratory animal anaesthesia. London: Academic Press/Elsevier; 2009. p. 173–4.

[5] Flecknell P. Anaesthesia in difficult situations. In: Course proceedings laboratory animal anaesthesia and post-operative care. Newcastle University; 2011.

[6] Baskerville M. Old world monkeys: laboratory procedures: handling, restraint and training. In: 7th ed. The UFAW handbook on the care & management of laboratory animals, vol. 1. Oxford: Blackwell Science Ltd. 2006. p. 628–9.

[7] Reinhardt V, Reinhardt A. Positive reinforcement training. In: Environmental enrichment and refinement for nonhuman primates kept in research laboratories: a photographic and literature review. 3rd ed. Washington: Animal Welfare Institute; 2008. p. 46–58.

[8] Reinhardt V, Reinhardt A. Involuntary restraint. In: Environmental enrichment and refinement for nonhuman primates kept in research laboratories: a photographic and literature review. 3rd ed. Washington, DC: Animal Welfare Institute; 2008. p. 9–10.

[9] Bernstein IS. Social housing of monkeys and apes, group formations. Lab Anim Sci 1991;41:329–33.

[10] Gebhart GF, Basbaum AI, Bird SJ, Flecknell P, Goodly L, Karas AZ, et al. Pain assessment: species-specific clinical signs: nonhuman primates. In: Recognition and alleviation of pain in laboratory animals. National Research Council of the National Academies. Washington, DC: The National Academies Press; 2009. p. 57.

[11] Wolfensohn SE, Honess P. Handbook of primate husbandry and welfare. Oxford: Blackwell Publishing; 2005.

[12] Saunders M, Lawrence R, Mitchell D. Development of a group housed, ambulatory, surgically cannulated, intravenous infusion model in cynomolgus macaques *(Macaca fascicularis)*. In: Society of toxicology annual meeting 11th–15th March 2012 San Francisco, California, USA; 2012.

[13] Carpenter JW. Chemical restraint/anesthetic/analgesic agents used in primates. In: Exotic animal formulary. St Louis, MO: Elsevier Saunders; 2013. p. 633.

[14] Gelatt KN. Medical and surgical treatment of the canine glaucomas. In: Veterinary ophthalmology. 4th ed. Iowa, USA: Blackwell Publishing; 2007. p. 791–7.

[15] Peters S, Heiduschka P, Julien S, Ziemssen F, Fietz H, Bartz-Schmidt KU, et al. Ultrastructural findings in the primate eye after intravitreal injection of bevacizumab. Am J Ophthalmol 2007;143(6):995–1002.

[16] Doshi RR, Bakri SJ, Fung AE. Intravitreal injection technique. Semin Ophthalmol 2011;26(3):104–13.

VetBooks.ir

VetBooks.ir

PART IX

FUTURE TRENDS IN PRIMATE TOXICOLOGY

VetBooks.ir

VetBooks.ir

CHAPTER

34

Considerations Regarding the Future Use of Nonhuman Primates to Support the Clinical Development of Biopharmaceuticals

Joy A. Cavagnaro[*], *Beatriz Silva Lima*[†]

[*]Access BIO, Boyce, VA, USA

[†]iMED.ULisboa, Universidade de Lisboa, Faculty of Pharmacy, Lisboa, Portugal

OUTLINE

Introduction 650
Background 650
Regulatory Guidance 651
Improving Predictive Value 653
Species Selection 655
Study Design: General Toxicity 656
Reproductive/Developmental Toxicity 658
Immunogenicity 660
Product Class-Specific Considerations 661
Oligonucleotide-Based Therapeutics 661
Enzyme Replacement Therapies 662
Disease-Specific Considerations 662
Parkinson's Disease 662
Osteoporosis 664
Potential Impact of Scientific Innovation on the Use of NHPs 664
Conclusion 665
References 666

http://dx.doi.org/10.1016/B978-0-12-417144-2.00034-2
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

INTRODUCTION

The goal of preclinical safety evaluation is to support clinical development by providing relevant safety information to permit initiation of clinical trials and support subsequent clinical investigations. The most efficient way to achieve this goal is to design studies to ask specific questions, discerning what needs to be known and making acceptable assumptions for the intended clinical population. The basic principles of toxicology are applicable across all product classes of pharmaceuticals and biopharmaceuticals. However, it is the specific attributes of the product that have the greatest influence on the practice of biopharmaceutical toxicology. Ideally, preclinical studies should be science based, data driven, and practical to obtain maximum information without compromising the scientific objective of the study, human safety, or regulatory acceptance. Over the years, optimal preclinical development of biopharmaceutical products have benefited from experience. This is because the strategy for design is influenced not only by the amount of existing knowledge on the specific product and/or product class but also by the expanding knowledge base across various product classes. Unfortunately, the speed at which clinical development is expected to take place to bring novel therapies to market is challenging not only for small virtual companies but also for large multinational companies. The pace is confounded by competing pressures for improving the predictive value of toxicity testing while reducing the numbers of animals in general and nonhuman primates (NHPs), in particular. Although optimizing the design of preclinical safety evaluation programs is consistent with the global initiatives to facilitate successful clinical development programs, ensuring consistency in program designs is inconsistent with a case-by-case approach. The case-by-case approach is a science-based approach in which studies are designed based on product attributes, and, although it is not a minimalist approach, it is one where regulatory acceptance is often less predictable.

BACKGROUND

In the early 1980s neither industry toxicologists nor regulatory scientists were sure of what constituted an appropriate toxicological assessment program for biopharmaceuticals. In 1986 the Biotechnology Working Party was established in Europe to focus on specific issues related to the development of biotechnology-derived pharmaceuticals. In July of that year, a satellite symposium to the IV International Congress of Toxicology was held in Tokyo, Japan. Approximately 135 scientists from various countries, including government regulatory scientists, university scientists, industrial scientists, and research managers, gathered—all with an interest in the development of new biotechnology products. At this meeting, Anthony Dayan argued that there is no place for detailed programs of rigidly predefined tests to be applied automatically to all products. Instead, useful information will only be obtained if the need for any experimentation at all is decided according to the specific properties and planned use of each compound. As such, the nature of the studies should be adapted to those individual circumstances. Dyan [37] argued that toxicity testing in this area is most similar to a series of pharmacological explorations and should not be expected to follow conventional rigid guidelines. In support of this approach (defined later as the case-by-case approach), Finkle [38] prompted the audience to consider focusing on the goal behind testing to identify and

VetBooks.ir

understand the potential toxicity of the agent rather than focusing on procedures (length of studies, numbers of groups, numbers of animals, numbers of species, etc.). Zbinden [39] acknowledged that for many areas of potential toxicity, there were no satisfactory safety tests available and most of the existing models were not yet properly validated. Nevertheless, since progress can be made only through an accumulation of experience, Zbinden proposed that an effort be made to develop and use scientifically sound experimental systems and to refrain from following traditional routine toxicity testing. Based on experiences gained over the intervening years, the U.S. Food and Drug Administration (FDA) proposed a concept paper in 1995, under the auspices of the International Conference on Harmonisation of Technical Requirements for Pharmaceuticals (International Conference on Harmonisation, ICH), for a new safety topic specifically relating to the preclinical safety assessment of biopharmaceuticals. An agreement was reached in July 1997 by the Expert Working Group for the final draft of the ICH S6 Guideline Preclinical Safety Evaluation of Biotechnology-derived Products, advocating a rational science-based approach [1,32,33].

REGULATORY GUIDANCE

The challenge in developing regulatory guidelines is making the guidance general enough for wide applicability but specific enough to be useful for a particular application. Guidelines often fall short on the latter. The initial ICH S6 was a unique safety guideline intended primarily to recommend a basic framework for preclinical safety evaluation rather than a prescriptive set of study designs to follow. The guidance applied to products derived from characterized cells through the use of a variety of expression systems. The active substances included proteins and peptides such as cytokines, plasminogen activators, recombinant plasma factors, growth factors, fusion proteins, enzymes, receptors, hormones, and monoclonal antibodies (mAbs). The guidance also suggested that the principles may also be applicable to recombinant DNA protein vaccines, chemically synthesized peptides, plasma-derived products, endogenous proteins extracted from human tissues, and oligonucleotide drugs [2]. The guidance emphasized that a flexible, case-by-case, science-based approach was needed to support clinical development and marketing authorization, but it also cautioned that there would need to be continuing dialog (among the regions and between industry and regulators) to ensure a common understanding in this rapidly evolving scientific area in order to reduce the uncertainty of regulatory acceptance. Although the guidance preceded the establishment of the "3Rs" (replace, reduce, refine), many sections were notably consistent with the principles.

The language in ICH S6 [2] explicitly stated that safety evaluation programs should normally include two relevant species, but, in certain justifiable cases, one relevant species may suffice (e.g., when only one relevant species can be identified or where the biological activity of the biopharmaceutical is well understood). The guidance acknowledged that because of the species specificity of many biotechnology-derived pharmaceuticals, it was important to select relevant animal species for toxicity testing. The guidance intentionally did not specify use of the most relevant in order to avoid the routine consideration of use of higher NHP species (e.g., greater homology of a protein or a better affinity of a receptor to chimpanzees or baboons). More important, the guidance explicitly stated that toxicity studies in nonrelevant species may be misleading and were therefore discouraged. Alternative approaches

VetBooks.ir

were suggested in cases where there were no relevant species, including consideration of animal models of disease as an acceptable alternative to toxicity studies using normal animals. Suggestions were made to improve the ability to detect toxicity, especially in cases in which there are limitations of sample size, as is often the case for NHP studies, by increasing the frequency and duration of monitoring. An increase in numbers and group was not recommended. Although both sexes should generally be used, justification could be provided for specific omissions, notably the "3Rs". Safety pharmacology end points could be investigated in separate studies or incorporated in the design of toxicity studies (n.b., "3Rs"). Investigations could also include the use of isolated organ or other test systems not involving intact animals to better allow for a mechanistically based explanation of specific organ toxicity and an opportunity for further refinement and reduction in animal use. The duration of animal dosing for general toxicity was generally 1-3 months. For those biopharmaceuticals intended for chronic indications, studies of 6-month duration were generally appropriate (providing an opportunity for 2 vs. 3 repeat-dose studies). With respect to developmental and reproductive toxicity (DART), the guidance identified cases where the only relevant species was the NHP and where extensive public information was available regarding potential reproductive and/or developmental effects of a particular class of compounds (e.g., interferons). Mechanistic studies indicate that similar effects are likely to be caused by a new, but related, molecule that may obviate the need for formal DART studies, which is another potential opportunity to reduce NHP animal use.

In 2005 the Preclinical Toxicology Working Group of the Academy of Medical Sciences Forum endorsed the case-by-case approach for pharmaceuticals in general. The authors cautioned that implementation of the case-by-case approach was dependent on acceptance by both regulators and the industry, that the interpretation of the data has to reflect best scientific practice, and that no study using experimental animals can predict with certainty the outcome when a drug is given to humans (The Academy of Medical Sciences Forum, 2005).

Two years later, in 2007, ICH S6 was proposed for updating (maintenance) under the auspices of ICH. Since the optimal design of preclinical programs is based on experience, the revisions would reflect accumulated experiences as well as advancements in science over the past decade. However, despite the flexibility afforded by the guidance published 10 years earlier and the increased scrutiny and ethical concerns over using NHPs in biomedical research, there was competing pressure to improve the predictive value of preclinical safety testing to reduce risks to patients and healthy volunteers in clinical trials. During this time, there was also a deliberate effort by both industry practices and regulatory expectations for better alignment, including consistency of preclinical development programs of biopharmaceuticals with pharmaceuticals without consideration of specific product attributes [1,32,33]. Thus, instead of the expected decrease in animal use, there was actually an increase in the numbers and types of studies, including an increase in the numbers of NHPs per study, driven by concerns by companies about anticipated and/or actual regulatory demands, fears of delays to marketing authorization, and the view from both industry and regulatory authorities that NHP data provides a gold standard that often cannot be adequately achieved using alternatives [3].

It was therefore deemed important that ICH S6 (R1), the addendum to ICH S6, confirm, clarify, and update study expectations to facilitate the timely conduct of clinical trials now also with the explicit intent of reducing the use of animals in accordance with the "3Rs" [4]. Specific topics especially relevant to NHP use include species selection, study design, immunogenicity, and DART.

IMPROVING PREDICTIVE VALUE

VetBooks.ir

The relevance of preclinical data is often the subject of debate among innovators, developers, clinicians, and regulators. Opinions range from those who consider preclinical data to have high predictive value and relevance to those who believe they have low predictive value or even no relevance at all. Of particular concern is when severe adverse effects are not anticipated from the currently available preclinical screening tools and animal models [5].

Paradoxically, judgment of relevance is often correlated with the end point and test species. For example, results of animal efficacy studies are often believed and interpreted as having high predictive value, whereas the opposite is true of toxicity data, even when using the same model. When toxicology studies are positive (i.e., toxicity is observed in the animal model), the first question that is often asked is whether the finding could be species specific and therefore not relevant to humans. When toxicity studies are completely negative (i.e., no observed adverse effect level [NOAEL]) at the higher dose tested, concerns with regard to the responsiveness of the test species are raised. If efficacy data are positive, the tendency is to "want to believe," but if toxicity data are positive, the tendency is "to explain away." It is generally more difficult to argue lack of relevance of NHP safety data. As previously mentioned, the ultimate goal of preclinical toxicity studies is to identify and characterize toxicities to subsequently identify the optimal patient population, dose, route, treatment regimen, and safety monitoring to avoid toxicity in humans. Ironically, if the goal was achieved and toxicity was not observed in humans, it could be reasoned that the animal studies were not predictive. Likewise, when a phase III trial fails or unexpected toxicity is observed during the postmarketing period, it could also be reasoned that humans do not predict humans—notwithstanding the results or availability of any animal data.

It is important to acknowledge that no animal model can mimic the subtleties of human disease. One important limitation in the extrapolation of preclinical safety when informing clinical risk is that normal animals are generally used to assess safety, whereas the patient population is generally being treated for some type of disease or injury. The use of animal models for disease have also been challenged based on the inherent variability, minimal historical data on general health or spontaneous disease pathology, potential limitations of the model in only mimicking one aspect of disease, difficulty in distinguishing treatment-related toxicity from disease effects, increased sensitivity that may not be relevant to humans, increased expense, resource intensiveness, limits in supply, or their otherwise unavailability to meet the aggressive timelines of the clinical development plan [6].

The added value of NHPs has recently been questioned when pharmacology-mediated adverse effects (e.g., mAbs) are so highly predictive from in vitro testing [7]. Although the authors acknowledge the need for NHPs when confirmation of a novel target is needed and the value that NHP data have added to the body of knowledge on mAbs, pharmacokinetic (PK), pharmacodynamic (PD), efficacy, and toxicity, they question the scientific justification and value of extensive NHP use in routine safety and efficacy studies. This is in contrast to the opinion of [40] in their recent review "Translating co-stimulatory blockade to the clinic: lessons learned from three pathways," in which they conclude the critical importance of NHPs as an intermediate step between murine screening and mechanistic models and clinical trials.

Although it is true that antidrug antibodies (ADAs) that clear mAbs of interest or neutralize mAb function can compromise the utility of NHP studies [7], such information can only be

VetBooks.ir

determined retrospectively. More important, the presence of neutralizing antibodies in some cases has provided critically valuable information (e.g., anti-thrombopoietin antibodies).

ICH S6 (R1) explicitly states that consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation. ICH S6 advocated the use of in vitro cell lines derived from mammalian cells to predict species specific aspects of in vivo activity and to assess quantitatively the relative sensitivity of various species (including human). Batteries of human-based tests provide reliable and relevant information on which to base further research and speed the translation of research to the bedside [8]. NHP-oriented research is not expected to be more predictive and reliable than human-based tests.

It has been suggested that the most exciting technology with regard to drug development is microdosing, and the use of microdosing in humans in place of NHPs should be encouraged [8]. One assumption is that the first-in-human trial is in normal volunteers, which is not the case for many biopharmaceuticals. More important, the trial design is not only very limited, but very little information can be gained with respect to safety. Another approach could be to initiate the clinical trial in subjects with disease by starting with very low doses; however, lacking any additional information, the dose escalation would have to go slowly because monitoring would likely be extensive as there would be no information on what to monitor or how long to monitor for exacerbation or reversibility. It is unclear whether there is added value with this approach for facilitating clinical development.

Protein-based biopharmaceuticals carry the inherent risk of immune reactions (e.g., acute anaphylaxis or generation of antibodies). In most cases toxicity of biopharmaceuticals has been related to exaggerated pharmacology, which is related to their specific targets (e.g., serious infections, malignancies, cytokine release syndrome [CRS], tumor lysis syndrome, and autoimmunity). This is often based on an understanding of the pharmacology/mechanism of action and data from preclinical studies in pharmacologically responsive species [9].

Although the mode of action of the immunomodulatory biologic is a major cause of CRS induction, the contribution of patient-specific factors and disease states to the induction of CRS is not immediately apparent. In general, the immune system of NHPs is considered to be the most similar to the human immune system and is therefore thought of as the most appropriate model to evaluate immunomodulatory biologics for adverse reactions. Immunomodulatory biologics induce responses that are characterized by trigger pharmacology or steep or bell-shaped dose-response curves. When these responses are combined with the varying immune activation thresholds of the patient, the therapeutic could either have a safe outcome or induce an exaggerated immune response. In addition, captive cynomolgus monkeys that are used in safety assessment are often derived from different geographic origins and have diverse NHP genetics and spontaneous pathologies, and such intrastrain (dependent on geographic origin) genetic variation in cynomolgus monkeys could contribute to the differential phenotype responses observed during in vivo and ex vivo safety assessments [10].

With regard to the selection of the relevant in vivo models for evaluating the risk of developing serious infections upon receiving an immunomodulatory biologic, NHPs may often be the most appropriate. Opportunistic infections in NHPs are not unexpected following immunomodulation by some immunomodulatory biologics. However, because the incidence of serious infections are very low and studies of NHPs are not sufficiently statistically powered to detect this, the utility of NHPs in predicting these adverse reactions remains uncertain [10].

SPECIES SELECTION

VetBooks.ir

Repeat-dose toxicity studies in two species (one nonrodent) are expected to support administration of more than a single dose of pharmaceuticals in humans. It is also generally expected that in vitro metabolic plasma protein binding data for animals and humans and systemic exposure data in the species used for the repeat-dose toxicity is available before initiation of clinical trials (ICH M3 (R2) [11]). Dogs are the usual nonrodent species in most cases, unless they have a significantly different metabolic profile. Although in some cases rabbits have been acceptable and more recently there has been an increasing use of minipigs, NHPs are generally (only) selected if dogs show unique sensitivity to the test article and/or there is limited availability of the test article.

For biopharmaceuticals, ICH S6 recommended that safety evaluation programs should normally include two relevant species. However, in certain justified cases, one relevant species may suffice (e.g., when only one relevant species can be identified or where the biological activity of the biopharmaceutical is well understood). If two species were deemed relevant to characterize toxicity in short-term studies, it could be possible to justify the use of only one species for subsequent long-term toxicity studies. ICH S6 (R1) later clarified that there are two pharmacologically relevant species (one rodent and one nonrodent) that should be used for short-term (up to 1 month) general toxicity studies, noting that two nonrodent species are not appropriate. For the longer-term general toxicity studies, the rodent species should be considered unless there is a scientific rationale for using nonrodents. Historically, most biopharmaceuticals have only been active in NHPs.

ICH S6 recommended that the selection of the animal species for toxicity testing should be based on the use of in vitro cell lines from mammalian cells to predict specific aspects of in vivo activity and to assess quantitatively the relative sensitivity of various species (including humans). Studies could be designed to determine, for example, receptor occupancy, receptor affinity, and/or pharmacological effects. A relevant species would be one in which the test material is pharmacologically active due to the expression of the receptor or an epitope (in the case of mAbs). A variety of techniques, including immunochemical or functional tests, could then be used to (further) identify a relevant species. Relevant animal species for testing of mAbs are those that express the desired epitope and demonstrate a similar tissue cross-reactivity (TCR) profile as for humans to optimize. The explicit intent of the TCR was to optimize the ability to evaluate toxicity arising from the binding to the epitope and any unintentional (unexpected) tissue cross-reactivity. The reason to select an animal species (in particular the NHP) based on TCR was only in those cases where an animal species did not express the desired epitope but where comparable unintentional tissue cross-reactivity to humans was demonstrated. The combined results from the in vitro and in vivo studies would ultimately assist in extrapolating the findings to humans.

Cross-reactivity, or lack thereof, can be predicted by an in silico analysis of sequence and structural homology/identity between the human antigen protein or targeted epitopes and the cognate proteins in conventional species used for toxicology studies [9]. However, for most mAbs, TCR is generally used. The limitations of TCR have been recently reviewed [12,13]. TCR studies should be viewed as screening assays, not unlike the Cerep assays conducted for pharmaceuticals and should not be overinterpreted. TCR can be used to identify potential target organs and may also be used to confirm selection of species for toxicity studies. The presence

VetBooks.ir

of ex vivo binding does not necessarily mean that there will be any association with functional activity or toxicity. Developing a TCR assay for some products may not be feasible, and there also may be challenges in interpreting data in other cases.

Per ICH S6 (R1) for mAbs and other related antibody products directed at foreign targets (e.g., bacterial, viral targets), a short-term safety study (see ICH S6) in one species (choice of species to be justified by the sponsor) can be considered. In most cases it is assumed that the short-term rodent toxicity studies will be adequate. However, based on the limitations of the TCR assay, it may be important to consider a more mechanistic approach, single-dose concentration, and single gender if NHPs are identified as the only species with nonspecific/unexpected binding.

ICH S6 (R1) reconfirmed the key considerations that should be taken into account when determining species relevancy. The document clarified that comparisons of target sequence homology between species can be an appropriate starting point, which should then be followed by in vitro assays(s) to make qualitative and quantitative cross-species comparisons of relative target binding affinities and receptor/ligand occupancy and kinetics, as well as a demonstration of in vitro and/or in vivo functional activity in species-specific cell-based systems or in vivo pharmacology or toxicology studies respectively.

Although ICH S6 (R1) suggests that these data be considered in the context of the intended dosing regimen and that there should also be a consideration of potential differences in normal versus disease implied, there is no guidance as to how comparable they have to be. For example, when should dosing be adjusted based on differences in binding affinities? When are species considered irrelevant based on binding affinities, especially when NHPs are the only relevant species (e.g., if 10-fold differences are deemed relevant what about 25,100, or 400 times)? Of note, some companies have specifically used NHP binding assays for lead candidate selection to ensure that there would be at least one relevant species to evaluate safety.

The most commonly used NHPs for preclinical development are cynomolgus monkeys and to a lesser degree rhesus monkeys and marmosets based on unique specificity. Despite their preferred use, there is relatively little information on the genome of cynomolgus monkeys in public domain databases. Differences in the genetic sequences of some target proteins within cynomolgus monkey sources from different geographic origins (e.g., China, the Philippines, Mauritius) have raised concerns that certain genetic variants might result in a loss or gain of pharmacological responses in the cynomolgus monkey colony used for toxicology studies. Phenotypic differences in cynomolgus monkeys of diverse geographic origin are well established, in particular for immune system functions. Collectively, these observations suggest that a prospective assessment of target gene variation may be warranted for cynomolgus monkeys (and other NHP species) in pharmacology and toxicology studies. A suggested complementary strategy would be to systematically archive blood/tissue DNA from each individual animal to enable retrospective investigation of a potential genotypic basis for unanticipated or variable pharmacological response [9].

STUDY DESIGN: GENERAL TOXICITY

ICH S6 did not provide specifics regarding the number of animals to be used in studies, despite cautioning that the number of animals per dose has a direct bearing on the ability

VetBooks.ir

to detect toxicity and that a small sample size may lead to a failure to observe toxic events because of observed frequency alone regardless of severity. However, the guidance acknowledged the limitations that are imposed by sample size, especially in the case of NHP studies that in part could compensate by increasing the frequency and duration of monitoring. Since the late 1980s, studies using NHPs were routinely designed with control, low-, mid-, and high-dose groups, with three animals per sex per group in the main study and two animals per sex per group in the control and high-dose groups for recovery. These designs were routinely accepted by regulatory authorities. However, as previously discussed, the size of the studies including the number of animals has gradually increased over the years in part to be more aligned/consistent with the design of the repeat dose studies using dogs, where four to six animals per sex per group are routinely used for the main study and two or three animals per sex per group for recovery in all dose groups. This increase in the size of NHP studies was recently confirmed in a review by Chapman et al. [3].

ICH S6 recommended that the route and frequency of administration should be as close as possible to that proposed for clinical use and consideration could be given to the effects of volume, concentration, formulation, and site of administration suggesting the acceptability of a maximum feasible dose. Dose concentrations should be selected to provide information including a toxic dose and a NOAEL. Although the number of dose concentrations was not addressed, the need for at least two is implied. The guidance acknowledged that in some cases because of lack of toxicity, defining a maximum dose may not be possible, but rather that a rationale should be provided for projected multiples of human exposure. In cases where the product showed lower affinity or potency in the cells compared to human cells, testing of higher doses may be more important. It was suggested that the multiples of the human dose that were needed to determine adequate safety margins may vary with each product class and clinical indication. ICH S6 (R1) emphasized the importance of PK/PD principles in dose selection and identified an acceptable high dose of 10-fold exposure multiple over the maximum anticipated human exposure. The guidance also clarified the purpose of recovery animals is for understanding reversibility rather than delayed toxicity. Demonstration of complete recovery was not required nor was there a need to extend study groups to better understand immunogenicity.

Buckley et al. [14] captured the opportunities to minimize (but not avoid) the use of NHPs provided by ICH S6 guidance. These included (1) incorporating safety pharmacology end points into repeat-dose toxicity studies, (2) minimizing the number of dose groups in chronic toxicity studies, (3) conducting a maximum of two general toxicology studies (i.e., 1 month and 3 or 6 months), (4) avoiding the conduct of multiple chronic toxicity studies (which have been requested based on the lack of achieving toxicity), and (5) the use of only the rodent for chronic toxicity studies when appropriate [34]. Additional opportunities based on ICH S6 (R1) include (1) the inclusion of recovery animals in fewer dose groups and (2) utilization of study designs that minimize the use of NHPs used in DART studies.

Recognizing the unique properties of mAbs when compared with other types of drugs (e.g., toxicity being predominantly mechanism-based) Chapman et al. [3] recommend similar and additional opportunities for refining NHP study designs, including (1) better use of PK/PD analysis to identify the dose that gives the maximal pharmacological effect and the dose that gives up to 10 times the exposure multiple over the maximum anticipated human dose, (2) inclusion of recovery groups in short-term studies based on preliminary data (when

VetBooks.ir

available) as well as known effects and biology, and (3) consideration of only two treatment groups in long-term studies in cases where the high or highest dose concentration in the short-term study is the NOAEL. Some of these suggestions could be applied more broadly to other biopharmaceutical classes.

While one dose group plus a control has been proposed, the use of this design would likely be very limited for assessment of general toxicity [15]. Additional animals would likely be needed in a pilot toxicity study to ensure that the dose selected would be a NOAEL. Cases where a single dose concentration may be relevant include a small mechanistic study that was designed to confirm lack of toxicity based on unexpected tissue cross-reactivity in humans and NHPs. Another consideration could be in a DART study where, depending on the variability of antibody formation, a single dose concentration could be used and exposure assessed retrospectively after treatment. One case in which it could be used is a focused study (limited numbers of animals) to confirm lack of toxicity of an unexpected TCR finding.

REPRODUCTIVE/DEVELOPMENTAL TOXICITY

Neither the ICH S6 guidance document nor the ICH S5 (R2) guidance document for the DART testing of medicinal products specifically addressed how developmental studies should be conducted when NHPs are the only relevant species. Both guidance documents emphasized a case-by-case scientific approach and state that the actual testing strategy should be determined by the anticipated clinical use, the form of the substance and routes of administration intended for human use, and making use of any existing data. The ICH S5 (R2) guidance document states that NHPs are best used when the objective of the study is used to characterize a relatively certain reproductive toxicant rather than detect a hazard.

As was true for general toxicity assessment, the principles of DART testing for biopharmaceuticals are similar to those for pharmaceuticals and in general follow the regulatory guidance outline in ICH S5 (R2) [16]. However, because many biopharmaceuticals are species specific (i.e., they often show limited cross-reactivity with traditional toxicology species used in DART testing [rat and rabbit]), alternate approaches are needed to evaluate DART potential as initially outlined in ICH S6 [17].

Prior to issuance of the ICS S6 guidance document, the preclinical assessment of biopharmaceuticals generally followed the same paradigm of assessment as pharmaceuticals, especially for developers of pharmaceuticals. Hoffmann La Roche and Schering-Plough, developers of the first alpha interferons, conducted conventional studies using multiple species, including standard reproductive/toxicity studies in rat and rabbits. Additional studies conducted in monkeys were identified as the most sensitive/relevant species.

During the early development of interferons, confirming that the reproductive/developmental toxicity findings that were observed with alpha interferons were not subclass specific was of interest; hence, companies were encouraged by regulators to conduct developmental toxicity studies of NHPs. Embryo-lethal and abortifacient effects were observed at multiples of the human dose. It is unclear whether Avonex was actually tested because the product label refers to the testing of interferon beta but the package insert refers to the testing of Avonex. An embryo-fetal development study in NHPs was conducted for Rebif and approved in the United States in 2002; Rebif was approved for marketing in the European Union in 1998. Murine interferon-γ

VetBooks.ir

was used in several safety studies by Genentech, including a developmental toxicity study, in an effort to determine potential toxicity for humans. Murine interferon-γ increased resorptions in mice [18].

The accumulated embryo-fetal development study data in monkeys with interferons was taken into account during the drafting of ICH S6. In addition, studies of nonpregnant females showed menstrual cycle abnormalities following administration of various interferons. Suggestions were being made as to potential correlations of changes in certain hormones and abortifacient activity. This information led to the following note in ICH S6 specific to interferons:

> Note 2. There may be extensive public information available regarding potential reproductive and/or developmental effects of a particular class of compounds (e.g. interferons) where the only relevant species is the NHPs. In such cases, mechanistic studies indicating that similar effects are likely to be caused by a new but related molecule may obviate the need for formal reproductive/developmental toxicity studies. In each case, the scientific basis for assessing the potential for possible effects on reproductive/development should be provided.

In 2009, Enright et al. published the comparative effects of interferon-α2b and pegylated interferon-α2b on menstrual cycles and ovarian hormones in cynomolgus monkeys. They concluded that administration of both products at high doses resulted in similar effects on menstrual cycles, estradiol, and progesterone profiles, and that the previously observed high-dose interferon-α effects on the maintenance of pregnancy in monkeys are likely the result of altered ovarian function [19].

In November 2008 an ICH Expert Working Group discussed specific topics regarding DART testing, resulting in more detailed recommendations for assessment of biopharmaceuticals, especially in cases where monkeys were the only relevant species. ICH S6 (R1) endorsed the concept of knowledge of the mechanism of action in a weight of evidence approach and also acknowledged class effects, even suggesting that additional preclinical studies may not be warranted. Although not explicitly stated, based on the accumulated data, interferons could be considered as a class where additional studies may not be warranted.

ICH S6 (R1)

> Reproductive toxicity studies should be conducted in accordance with the principles outlined in ICH S5 (R2) Guideline; however, the specific study design and dosing schedule can be modified based on an understanding of species specificity, the nature of the product and mechanism of action, immunogenicity and/or pharmacokinetic behavior and embryo-fetal exposure.
>
> An assessment of reproductive toxicity with the clinical candidate in a relevant species is generally preferred.
>
> Developmental toxicity studies should only by conducted in non-human primates (NHPs) when they are the only relevant species.
>
> When the weight of evidence (e.g., mechanisms of action, phenotypic data from genetically modified animals, class effects) suggests that there will be an adverse effect on fertility or pregnancy outcome, these data can provide adequate information to communicate risk to reproduction, and under appropriate circumstances additional nonclinical studies might not be warranted.

When the NHP is the only relevant species, it is recognized that mating studies to directly assess fertility are not practical or ethical in NHPs. If sexually mature NHPs are used in the earlier repeated-dose general toxicity studies, then male and female fertility end points can be

VetBooks.ir

included and the need for stand-alone fertility studies can be avoided. However, there are significant challenges in inclusion of fertility end points in toxicity studies, including irregular cycling, making cycle times difficult to calculate, effect of housing conditions on cycling, the long time required to establish baseline menstrual cycle, and the lack of information to aid decisions on what constitutes a significant effect [36]. There is also a concern that the increasing use of sexually mature primates for general toxicity studies for the sole purpose of assessing fertility will put a strain on animal supply and may not be sustainable in the long term [9].

The need for toxicity in juvenile toxicity studies is an ongoing discussion even in situations where species other than NHPs are used. The decision is based on a number of considerations, including the age of children to be included in trials, the risk/benefit to the intended juvenile patients, the duration of dosing, the mechanism of action and expression of the target in adults and juveniles, existing pharmacology/toxicology data in animals and humans (including the data from target knockout mice if available), and the timing of the trials during development. Other triggers for juvenile studies may include whether the target is known to be involved in postnatal development or known to be expressed in the developing tissue and whether any target organs identified in adult toxicology or clinical studies are developing organs in the proposed pediatric populations [9]. Cynomolgus monkeys aged 2-3 years (8- to 12-year-old human equivalents) used frequently to support adult clinical trials may not be sufficient to support early juvenile populations.

It will be interesting to see whether the FDA's new Pregnancy and Lactation labeling requirement will impact the current scope of NHP studies and whether additional studies will be needed. More complete information will be expected to inform the use of drug and biological products by women of childbearing age, pregnant women, and women who choose to breastfeed. The new labeling format includes a risk summary and information about available data on use of the drug during pregnancy and while breastfeeding to help healthcare providers counsel pregnant women, nursing women, and all women of reproductive age about the use of prescription drugs and biologics [20].

IMMUNOGENICITY

While the consequences of ADAs are not frequently associated with adverse events, some have produced serious consequences, most notably erythropoietin and thrombopoietin. ADAs can cause some general adverse events, including administration reactions such as systemic infusion reactions, localized injection reactions, or acute hypersensitivity reactions, although the underlying mechanisms are unclear. In most cases, however, clinical adverse events have not been directly linked to the presence of ADAs [41].

As previously discussed, the predictive value of animal models depends on both the type of animal model and what needs to be predicted. Similarity in amino acid sequence does not guarantee that an animal model will display similar immunogenicity compared to humans. Various patient-specific factors also contribute toward an immunogenic response, including immunological state and disease type or severity. The predictive value of animal models for assessing immunogenicity depends on the similarity in processes underlying immunogenicity compared with those in humans. According to this assumption, rats and mice would have the least predictive value, followed by transgenic immune tolerant animal models, and

VetBooks.ir

NHPs would have the highest predictive value. What needs to be predicted includes (1) neoepitopes on modified proteins, (2) relative immunogenicity between products, (3) breaking of tolerance, (4) immunogenicity in patients, (5) incidence of immunogenicity in patients, and (6) clinical consequences of antibody development. However, because the mechanisms underlying immunogenicity differ between all of these models and humans (i.e., the models generally overpredict), they thus are limited to predicting clinical consequences [21,35].

Most studies show that conventional animal models overestimate immunogenicity, making them unsuitable to predict the incidence clinically. It is acknowledged in ICH S6/ICH S6 (R1) that many biopharmaceuticals intended for humans are immunogenic in animals and that induction of antibody formation in animals is not predictive of a potential for antibody formation in humans. In fact, the detection of antibodies should not be the sole criterion for early termination of a study unless the immune response affects the pharmacological/toxicological effects of a large portion of the animals. The rationale for conducting preclinical immunogenicity assessments is to assist in the interpretation of toxicity study results (e.g., immune-mediated reactions such as complement activation, anaphylaxis, vasculitis) and possible pathological changes related to immune complex formation and deposition.

The development of binding, sustaining, and/or neutralizing ADAs and subsequent safety or efficacy liabilities have been adequately identified in NHPs, in most cases without serious consequences. However, as rodents are increasingly being defined as relevant species in early development programs, there has been a higher incidence of unexpected fatal acute infusion reactions. The emergence of the presumed immune reactions in rodents often cannot be definitively attributed to immunogenicity. Plausible mechanisms of ADA-independent immune response in rodents are being explored. However, there are limitations in available tools to evaluate, characterize, and/or confirm immune reactions specific to mice and rats. Defining the mechanism is important as the results can adversely affect clinical development (e.g., in defining a NOAEL, especially in those cases where there are no adverse immune affects identified in NHPs). More important, if rodents were used initially to assess toxicity and the presumed immune effects were not convincingly confirmed mechanistically, it may be difficult to justify the use of NHPs. The increased immunogenicity in rodents compared to that observed in NHPs may also decrease the option of selection of a relevant species to assess long-term toxicity.

NHPs have predictive value for some therapeutic proteins for assessing relative immunogenicity, neoepitopes, and development of neutralizing (cross-reactive) antibodies. The predictive value is, in addition to the model and research questions, also depend on the protein [21,22].

PRODUCT CLASS-SPECIFIC CONSIDERATIONS

Oligonucleotide-Based Therapeutics

For oligonucleotides, the catabolic pathways for degradation of oligonucleotides are similar from species to species. Because metabolism is not an issue, the ability of the test species to mimic response in humans became the driving force for the selection of NHPs. Mice were generally used as the rodent species for early studies of the toxicity of oligonucleotides because many of the pharmacology models are in mice, and therefore surrogate

VetBooks.ir

molecules were readily available. In hindsight, this was a fortuitous decision. NHPs were selected originally because they were thought to be more representative of humans than dogs. At similar doses on a milligram per kilogram basis, plasma concentrations of a given sequence administered by the same route of administration are nearly identical in monkeys and humans. More important, compared to rodents, the toxicity profiles in monkeys may be more predictive of those in humans [23]. Dogs do not seem to have the signature complement activation response with phosphorothioate (PS) oligonucleotide-based therapeutics as NHPs do—with the acute toxicity associated with complement activation characterized in the most sensitive species (monkey), chronic studies could be performed in dogs. Dogs have been used successfully to support investigational new drugs for locally and orally administered oligonucleotide-based therapeutics [23].

Enzyme Replacement Therapies

Enzyme replacement therapy is a therapeutic approach in which the specific enzyme that is absent or inactive in infected individuals is replaced with a functional enzyme molecule. There is an emerging recognition that substrate load in animal models and patients significantly complicate the translation of preclinical safety findings into a human risk-benefit equation. Successful preclinical programs demonstrate the importance of flexibility based on the individual disease and the availability of relevant animal models to address specific safety concerns [24]. In addition to the use of rodent animal models that mimic the disease, repeat-dosed toxicity studies using the NHP generally are required to support clinical trials. Although most NHPs develop an immune response in 3-month toxicity studies, the rationale to terminate the study has not been considered scientifically justified by some regulatory authorities unless the immune response shows adverse effects on clinical observations, clinical pathology parameters, and histopathology. On the other hand, humanized mouse models in which a mouse's own enzyme is knocked out by a transgenic procedure to expresses an enzymatic inactive enzyme may better represent the patients' conditions. The added value of chronic toxicity studies using NHPs for enzyme replacement therapies is currently being debated.

DISEASE-SPECIFIC CONSIDERATIONS

Parkinson's Disease

Animal models have provided significant contribution to the understanding of the pathophysiology of neurodegenerative diseases. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration to NHPs is the experimental model that more closely resembles the pathologic features of Parkinson's disease (PD), including nigrostriatal dopamine depletion and extrastriatal dopamine, noradrenalin, and serotonin changes observed in patients with PD [25].

Recombinant human glial cell line-derived neurotrophic factor (GDNF) is a potent neuronal growth and survival factor that has been considered for clinical use in the treatment of PD. However, translating successful animal studies into effective clinical therapy for PD has proven difficult [26]. Significant differences have been reported in

VetBooks.ir

results from three clinical trials evaluating intraputamenal (IPu) infusion of GDNF for the treatment of PD [27].

On September 1, 2004, Amgen halted clinical trials and withdrew treatment from participants in all study groups citing lack of clinical efficacy and safety concerns in NHPs. In addition, GDNF-neutralizing antibodies were found in five study subjects, and there was a concern that these might lead to a reduction of naturally occurring GDNF in the brain. Clinical studies were originally supported by a comprehensive panel of nonclinical intracerebroventricular (ICV) toxicology studies. To support clinical development via IPu route the original ICV package was supplemented with a series of IPu infusion toxicology studies that culminated in a pivotal 6-month study in rhesus monkeys. The spectrum of drug-related effects observed in the pivotal IPu toxicology study was largely equivalent to that seen in shorter-term IPu infusion studies and in the bolus ICV administration studies. However, cerebellar neuron loss was observed in animals treated with the highest evaluated dose (100 µg/day) of recombinant human GDNF. Cerebellar damage is known to be associated with abnormalities in gait and posture, limb movement deficits (including ataxia and intention tremor), dysarthria, and oculomotor disturbance across species. No observations of this nature were noted in affected animals. However, the extent of the observed cerebella damage was generally limited (at least three of the four affected animals), and the study was not designed to assess subtle changes in motor function [28].

Gash et al. [26] used rhesus monkeys to analyze two variables hypothesized for achieving efficacy: dose and GDNF distribution in target tissue. The results suggested that after achieving a critical threshold, GDNF-tissue distribution is more important than dose for trophic stimulation.

To determine whether problems in drug bioavailability could have contributed to the discrepancies between studies, Salvatore et al. [27] analyzed the distribution of IPu-infused GDNF in the rhesus monkey brain using the delivery system and infusion protocol followed in a phase II clinical trial that failed to achieve its primary end point. I125-GDNF was unilaterally infused into the putamen of three adult rhesus monkeys for 7 days. Three age- and sex-matched animals received vehicle infusions following identical procedures. GDNF concentrations in the brain, peripheral organs, blood, and cerebrospinal fluid were quantified and mapped by GDNF immunocytochemistry, GDNF enzyme-linked immunosorbent assays, and I125 measurements. Infused GDNF was found to be unevenly concentrated around the catheter, with tissue concentrations dropping exponentially with increasing distance from the point source of the single opening in the catheter tip. The distribution of GDNF around the catheter, as determined by immunocytochemistry, varied over fourfold between animals, ranging from 87 to 369 mm^3. The concentration of GDNF around the catheter tip and limited diffusion into surrounding brain parenchyma support the hypothesis that drug bioavailability was limited to a small portion (2-9%) of the human putamen in the clinical trial using this catheter and infusion protocol.

MedGenesis has now licensed Amgen's GDNF, proposing that the reason for clinical failure of GDNF was that the delivery technology was not sophisticated enough to allow for fully targeted delivery, thereby supporting a rationale for their proprietary convection-enhanced delivery platform.

NHPs have also been useful in assessing gene therapy-based delivery of neurotrophic factors, GDNF, and neurturin. Bartus et al. [29] showed that studies of rats and NHPs provided

VetBooks.ir

an empirically supported approach to properly scale the dose to account for the volume of the human substantia nigra, thus providing a rationale for selecting a clinical dose in subjects with PD that might be expected to be equally safe and effective.

Osteoporosis

NHPs, particularly macaques, have been more extensively characterized than any other large animal species as animal models for human skeletal biology. They have been used extensively to evaluate hormonal as well as nonhormonal therapies for osteoporosis, with results that are entirely consistent with clinical data. Although postmenopausal changes occur more rapidly in the monkey surgical ovariectomy model than in humans, they are comparable in magnitude, and experiments must be appropriately designed and powered to be successful.

Trends in NHP bone research are aimed toward relatively short-term studies to establish proof of concept or preliminary efficacy dose-ranging with novel compounds. The short-term models have been made possible by the availability of a range of serum and urine biomarkers for bone resorption and formation that have become available for clinical use, and most of these assays can be applied to NHP samples. Because of the relatively limited experience to date, how predictive a short-term biomarker dose-response relation is of the longer-term bone mass or strength dose-response relationship is not yet established. A second, and even less well-understood difficulty is that the biomarkers provide a measure of global (whole-body) bone resorption and formation rates, whereas some therapeutic agents may depend on site-specific effects for their efficacy. Jerome [42] speculated that in the future, NHPs would become more important as models for the evaluation of new osteoporosis strategies with combined antiresorptive/anabolic capability, but this assumption remains to be confirmed.

POTENTIAL IMPACT OF SCIENTIFIC INNOVATION ON THE USE OF NHPs

Although the ICH process has revealed enormously successful results in providing guidance to support preclinical development programs to support clinical development, the attrition rates in late development persist at high levels, for which solutions are needed and investigations continue. Some of the failures are related to deficiencies in clinical trial design, including subject selection, study size, and optimization of dose and dosing regimen. However, as discussed, there is also the opportunity to improve the success rate with a more targeted approach to preclinical safety evaluation, including a more judicious use of animals. The standardized animal-based preclinical paradigm, although having provided a highly relevant asset on scientific knowledge, may be reaching a plateau in its predictive capabilities for human safety. It is difficult to imagine which additional or improved information can be extracted in the future from animals, including from NHPs, or which innovative approaches can still be ethically created based on large animals. From this perspective, if the success of drug development needs to be increased, then new strategies—either as an add on or as a replacement for animal-based developments—have to be created.

Adjustments had to be made to the development plans of biopharmaceuticals compared with small molecules based on their attributes and on the need for often using NHPs as the

VetBooks.ir

only relevant species. This chapter has highlighted how modifications have been possible and appropriate (e.g., the number of animals per dose group, the reduction of species for assessing chronic toxicity, duration of studies, combined reproductive/toxicity studies, the prediction rather than testing of carcinogenicity). The development of other therapeutic concepts such as cell or gene therapies have illustrated well how rodents can be used in large part to advance to the clinic and how NHPs are used in a more restricted sense in certain cases, primarily for extrapolation for dose confirmation. Alternatives are clearly needed to avoid a false sense of reassurance, which may emerge from negative studies performed in nonrelevant systems. The development of in silico predictive approaches (for activity, drug metabolism and pharmacokinetics (DMPK), etc.), the important evolution of molecular and systems biology, and the science of human cell culturing, differentiation/regression, and cell coculturing is under enormous pressure and seems to be delivering an important asset in test systems. These emerging systems (e.g., the organs on chips, the human in a chip), if appropriately built, characterized, and standardized, might constitute a critical step toward the increase in success rates for developing drugs, based on the hopefully improved predictive capabilities of such systems when appropriately used and integrated. The achievements by multidisciplinary scientific groups incorporating academics, industry scientists, and regulators seem enormously promising in the multiple regions of the world, including the United States, Japan, and Europe, and they are being actively followed and supported by the respective governments. Building test systems to mimic human organs based on human cells derived from different healthy and/or diseased patients is paving the road toward more personalized and hopefully more efficient therapies. A rethinking of the clinical investigation concepts is occurring in parallel to the preclinical aspects. In particular the (safe) early introduction of human subjects in the research to help with candidate selection (e.g., DMPK) or pharmacological or safety markers, based on sophisticated monitoring technologies is expected to improve the early understanding of the effects of drug candidates in humans, allowing an earlier and more efficient "go/no go" decision. Given the amount of data that might be generated in the future with those human-based in vitro tools, where interconnected organotypic systems will be used, it is hard to believe that the role and importance of animals, including the NHP, in the prediction of human safety will remain unchanged, especially in situations of divergent outcomes.

CONCLUSION

No primate model is completely predictive, including human primates, for predicting clinical safety. In all cases, limitations of any model should be identified before conducting a study. The ethical concerns for the use of NHPs is being translated into regulatory guidelines, which now carry legal implications for scientists to provide greater justification for the use of NHPs in their research [3]. In Europe, the new Directive 2010/63/EU [30] on the protection of animals used for scientific purposes further restricts the use of NHPs and states that their use should be avoided unless justified on a case-by-case basis. The European Commission will also monitor relevant scientific developments to see where these could provide opportunities for phasing out the use of NHPs and will undertake periodic reviews of the implementation of the replacement, refinement, and reduction of animals in research (the "3Rs"), paying particular attention to NHP use. Therefore justification should be provided when designing

VetBooks.ir

studies to ask specific questions to support clinical development. If questions have been previously answered and data are available (e.g., in the case of more relevant animal models, biosimilars, known product class-specific toxicities) these data could be a rationale for a more targeted approach (e.g., either no study or a more targeted study using a few groups, fewer animals per group, single gender, an so on).

Although healthy animals represent the standard model test system used to conduct toxicology studies, study designs using animal models of disease/injury are frequently modified to incorporate important safety parameters that allow for assessment of the potential toxicology (i.e., hybrid pharmacology-toxicology study design). Such data can supplement, or possibly be used in lieu of, toxicology studies using healthy animals. This is an approach outlined in the FDA's recent Preclinical Guidance on Investigational Cell and Gene Therapy Products [31] that could be more generalizable to protein-based biopharmaceuticals to improve predictive value. In some cases, the model may be a NHP disease model (e.g., osteoporosis and neurodegenerative disease); in other cases a rodent model of disease may be more relevant (e.g., estrogen replacement therapies).

Current ICH guidance documents allow for flexibility in study designs that are in accordance with the "3Rs". Development practices for preclinical safety assessment of biopharmaceuticals have been and will continue to be a dynamic process that is strongly controlled by the expanding knowledge and the innovations in product design. NHPs have provided important data to support clinical development. There is also a need to develop and refine appropriate human in vitro systems to aid safety assessment in cases where reliable animal models do not exist but also to address specific limitations of animal studies. It is imperative that we continually learn from each case using animals in general and NHPs in particular if we are to adequately rely on preclinical safety assessments. It is hoped that preclinical evaluation programs will evolve and mature concurrently with more novel products and will focus on improving the predictive value to facilitate clinical development, challenging toxicologists to provide information from the most appropriate studies.

If and when they are appropriately developed and accepted by regulatory authorities, it is anticipated that human-based systems, emerging from healthy and/or specific disease situations, will gain an advantage with their predictive value. It is clear that implementation of a more judicious use of animals will require the concerted efforts of scientists in the diverse scientific areas with input from the different concerned stakeholders for improvements in predictive value to be realized.

References

[1] Cavagnaro J. The principles of ICH S6 and the case-by-case approach. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals: a science-based approach to facilitating clinical trials. Hoboken, NJ: John Wiley & Sons; 2008. p. 45–65.

[2] ICH S6. International Conference on Harmonisation (ICH) S6: preclinical safety evaluation of biotechnology-derived pharmaceuticals, July 1997.

[3] Chapman KL, Andrews L, Bajramovic JJ, Baldrick P, Black LE, Bowman CJ, et al. The design of chronic toxicology studies of monoclonal antibodies: implications for the reduction in use of non-human primates. Regul Toxicol Pharmacol 2012;62:347–54.

[4] ICH S6 (R1). International Conference on Harmonisation (ICH) S6 (R1): S6 addendum to preclinical safety evaluation of biotechnology-derived pharmaceuticals. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S6_R1/Step4/S6_R1_Guideline.pdf; May 2012 [accessed January 12, 2015].

[5] Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. Nat Rev Drug Discov 2010;9:325–38.
[6] Cavagnaro JA. Preclinical safety evaluation of biotechnology-derived pharmaceuticals. Nat Rev Drug Discov 2002;1:469–75.
[7] Van Meer PJK, Kooijaman M, van der Laan JW, Moors EHM, Schellekens H. The value of non-human primates in the development of monoclonal antibodies. Nat Biotechnol 2013;31:882–3.
[8] Bailey J. Non-human primates in medical research and drug development: a critical review. Biog Amines 2005;19:235–55.
[9] Brennan FR, Cauvin A, Tibbitts J, Wolfreys A. Optimized nonclinical safety assessment strategies supporting clinical development of therapeutic monoclonal antibodies targeting inflammatory diseases. Drug Dev Res 2013;75:115–61.
[10] Sathish JG, Swaminathan S, Bielksy M-C, de Haan L, French NS, Govindappa K, et al. Challenges and approaches for the development of safer immunomodulatory biologics. Nat Rev Drug Discov 2013;12:306–24.
[11] ICH M3 (R2). International Conference on Harmonisation (ICH) M3 (R2): nonclinical safety studies for the conduct of human clinical trials in marketing authorization for pharmaceuticals. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2__Guideline.pdf; 2014 [accessed January 12, 2015].
[12] Bussiere JL, Leach MW, Price KP, Mounho BJ, Lightfoot-Dunn R. Survey results on the use of the tissue cross-reactivity immunohistochemistry assay. Reg Tox and Pharm 2010;59:493–502.
[13] Leach MW, Halpern WG, Johnson C, Rojko JL, MacLachlan TK, Chan CM, et al. Use of tissue cross-reactivity studies in the development of antibody-based biopharmaceuticals: history, experience, methodology, and future directions. Toxicol Pathol 2010;38:1138–66.
[14] Buckley AL, Chapman K, Burns-Naas LA, Todd MD, Martin PL, Lansita JA. Considerations regarding nonhuman primate use in safety assessment of biopharmaceuticals. Int J Toxicol 2011;30:583–90.
[15] Baldrick P. Safety evaluation of biological drugs: what are toxicology studies in primates telling use? Regul Toxicol Pharmacol 2011;59:227–36.
[16] ICH S5 (R2). International Conference on Harmonisation (ICH) S5 (R2): determination of toxicity to reproduction for medicinal products & toxicity to male fertility. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S5_R2/Step4/S5_R2__Guideline.pdf; November 2005 [accessed January 12, 2015].
[17] Martin PL, Breslin W, Rocca M, Wright D, Cavagnaro J. Considerations in assessing the developmental and reproductive toxicity potential of biopharmaceuticals. Birth Defects Res (Part B) 2009;86:176–203.
[18] Henck JW, Hilbish KG, Serabian MA, Cavagnaro JA, Hendrickx AB, Agnish ND, et al. Reproductive toxicity testing of therapeutic biotechnology agents. Teratology 1996;53:185–95.
[19] Enright BP, Compton DR, Collins N, Davis T, McIntyre BS. Comparative effects of interferon alpha-2b and pegylated interferon alpha-2b on menstrual cycles and ovarian hormones in cynomolgus monkeys. Birth Defects Res (Part B) 2009;86:29–39.
[20] FDA. FDA proposed rule pregnancy and lactation labeling. http://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/labeling/ucm093307.htm; 2008 [accessed January 12, 2015].
[21] Brinks V, Jiskoot W, Schellekens H. Immunogenicity of therapeutic proteins: the use of animal models. Pharm Res 2001;28:2379–85.
[22] Zwickl CM, Cocke KS, Tamura RN, Holzhausen LM, Brophy GT, Bick PH, et al. Comparison of the immunogenicity of recombinant and pituitary human growth hormone in rhesus monkeys. Fundam Appl Toxicol 1991;16:275–87.
[23] Levin AA, Henry SP. Toxicity of oligonucleotide therapeutics. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals: a science-based approach to facilitating clinical trials. Hoboken, NJ: John Wiley & Sons; 2008. p. 537–74.
[24] Andrews L, O'Callaghan M. Enzyme replacement therapies. In: Cavagnaro JA, editor. Preclinical safety evaluation of biopharmaceuticals: a science-based approach to facilitating clinical trials. Hoboken, NJ: John Wiley & Sons; 2008.
[25] Ribeiro FM, da Silva Camargos ER, de Souza LC, Teixeira AL. Animal models of neurodegenerative diseases. Rev Bras Psiquiatr 2013;35:S82–91.
[26] Gash DM, Zhang Z, Ai Y, Grondin R, Coffey R, Gerhardt GA. Trophic factor distribution predicts functional recovery in parkisonian monkeys. Ann Neurol 2005;8:224–33.

VetBooks.ir

[27] Salvatore ME, Ai Y, Zhang AM, Grondin RC, Zhang Z, Gerhardt GA, et al. Point source concentration of GDNF may explain failure of phase II clinical trial. Exp Neurol 2006;202:497–505.
[28] Hovland DN, Boyd RB, Butt MT, Engelhardt JA, Moxness MS, Mam MH, et al. Six-month continuous intraputamenal infusion toxicity study of recombinant methionyl human glial cell line-derived neurotrophic factor (r-metHuGDNF) in rhesus monkeys. Toxicol Pathol 2007;35:1013–29.
[29] Bartus RT, Brown L, Wilson A, Kruegel B, Siffert J, Johnson EM, et al. Properly scaled and targeted AAV2-NRTN (neurturin) to the substantia nigra is safe, effective and causes no weight loss: support for nigral targeting in Parkinson's Disease. Neurobiol Dis 2011;44:38–52.
[30] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, 2010.
[31] FDA. FDA guidance for industry preclinical assessment of investigational cellular and gene therapy products. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm376136.htm; November 2013 [accessed January 12, 2015].
[32] Cavagnaro J. Considerations in the preclinical development of biopharmaceuticals. In: McQueen CA, editor. Comprehensive toxicology. 2nd ed. Toxicity testing and evaluation, vol. 3. Oxford: Elsevier Ltd, UK; 2010. p. 29–52.
[33] Cavagnaro J, Sims J. ICH S6 preclinical safety evaluation of biotechnology-derived pharmaceuticals. In: van der Laan JW, DeGeorge JJ, editors. Global approach in safety testing: ICH guidelines explained. NY: Springer; 2013. p. 215–42.
[34] Clarke J, Hurst C, Martin P, Vahle J, Ponce R, Mounho B, et al. Duration of chronic toxicity studies for biotechnology-derived pharmaceuticals: is 6 months still appropriate? Regul Toxicol Pharmacol 2008;50:2–22.
[35] Frings W, Cavagnaro JA. Predicting clinical immunogenicity: intended or unintended. In: Weinbauer GF, Buse E, Muller W, Vogel F, editors. New developments and challenges in primate toxicology. Münster: Waxmann; 2005. p. 9–21.
[36] Martin PL, Weinbauer GF. Developmental toxicity testing of biopharmaceuticals in nonhuman primates: previous experience and future directions. Int J Toxicol 2010;29:S52–68.
[37] Dayan AD. Rationality and regulatory requirements-a view from Britain. In: Graham CE, editor. Preclinical safety of biotechnology products intended for human use, vol. 235. NY: Alan R. Liss; 1987. p. 89–106.
[38] Finkle BS. Genetically engineered drugs: toxicology with a difference. In: Graham CE, editor. Preclinical safety of biotechnology products intended for human use, vol. 235. NY: Alan R. Liss; 1987. p. 161–7.
[39] Zbinden G. Biotechnology products intended for human use, toxicological targets and research strategies. In: Graham CE, editor. Preclinical safety of biotechnology products intended for human use, vol. 235. NY: Alan R. Liss; 1987. p. 143–59.
[40] Ford ML, Larsen CP. Translating co-stimulatory blockade to the clinic: lessons learned from three pathways. Immunol Rev 2009;229:294–306.
[41] Shankar G, Pendley C, Stein KE. A risk-based bioanalytical strategy for the assessment of antibody immune responses against biological drugs. Nat Biotech 2007;25:555–61.
[42] Jerome CP. Hormonal therapies and osteoporosis. ILAR J 2004;45:170–8.

VetBooks.ir

CHAPTER

35

Gaps and Future Challenges

Joerg Bluemel[*], *Sven Korte*[†], *Emanuel Schenck*[*‡], *Gerhard F. Weinbauer*[†]

[*]Genentech, Inc., San Francisco, CA, USA

[†]Covance Laboratories GmbH, Münster, Germany

[‡]Medimmune, Gaithersburg, MD, USA

The importance and relevance of nonhuman primate (NHP) models has changed dramatically in the last two decades. More than 15 years ago the use of NHP models more or less centered on academic research, and the use of these models for drug development was considered only in special cases. This paradigm has since changed in that NHP models are quite regularly used as preclinical animal models for biopharmaceutical drug development. The 35 chapters in this book that cover various aspects relevant to drug development and safety assessment are clearly strong testimony to the expansion of the field. Overall, knowledge and experience have advanced within the field and numerous complete drug development programs have successfully used the NHP model. As a consequence, NHPs are now considered less "exotic," and drug developers are more knowledgeable about their use in general. Many are also more acquainted with using these models on a regular basis. Conducting a regulatory pre- and postnatal study, for example, was not considered feasible in macaques for quite some time, but now an (enhanced) pre- and postnatal study—under appropriate and defined circumstances—is in fact recommended through the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidance.

Notwithstanding these positive developments, significant gaps and challenges remain around the use and value of NHP models for drug development and safety assessment. In many countries some of these challenges are related to public and political attention toward NHP use that often does not seem to take into account the purpose or merit of these studies (e.g., academic vs. drug development research). In Europe animals used for scientific purpose are protected by a specific directive [1]. This directive acknowledges the necessity of NHP models for basic and biomedical research, but it also states that "…the use of non-human primates should be permitted only in those biomedical areas essential for the benefit of human beings, for which no other alternative replacement methods are yet available." Furthermore, it explains, "their

http://dx.doi.org/10.1016/B978-0-12-417144-2.00035-4
© 2015 Elsevier Inc. All rights reserved.

VetBooks.ir

use should be permitted only for basic research, the preservation of the respective non-human primate species or when the work, including xenotransplantation, is carried out in relation to potentially life-threatening conditions in humans or in relation to cases having a substantial impact on a person's day-to-day functioning, i.e. debilitating conditions." The latter statement has caused substantial concern expressed with regard to potentially severe restrictions for NHP use for drug development. However, it is important to realize that a debilitating clinical condition—for the purpose of this directive—means a reduction in a person's normal physical or psychological ability to function. In that context NHPs can be used for the development of new medicines that help restore normal human ability and function.

There is also an active call to investigate the feasibility of self-sustaining breeding colonies to phase out the capture of feral monkeys for breeding purposes. Primate supply continues to be a challenge, and this is largely related to transport issues (e.g., airlines refusing to transport NHPs rather than issues at breeding colonies). In the meantime many of the industrial primate breeding centers have become accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and have defined quality standards. In this context of primate supply it is important to note that international regulations govern the age at which NHPs can be transported and that the availability of NHPs for juvenile toxicity studies can therefore be a challenge if animals younger than 9-12 months of age are required. Another relevant challenge is the practice of using minimal instead of optimal animal numbers, which is sometimes taken to the extreme by running studies without control groups to reduce the numbers of animals. These approaches can lead to success, but they might also be fraught with significant issues requiring repeat and/or follow-up studies that then seriously undermine the original goal of reducing animal numbers. Some efforts to determine the statistical power of various study parameters applied to NHP toxicity testing are currently underway. The hope is that this work will help optimize the use of NHPs; consideration of their use is important as the center of our animal welfare efforts, but it is also in the interest of drug development science. Another challenge lies in the increasing demand to add more sensitive readout parameters (e.g., for functional assessment of vital organs) and in the need to characterize the pharmacodynamic response consistently and in more detail.

The devastating incidence with TGN1412 underscores how important it is to characterize target biology and to establish translational relevance of the model and the observed effects. Administration of TGN1412, a CD28 superagonistic antibody, led to a massive and uncontrolled release of proinflammatory cytokines (cytokine storm) in healthy human volunteers during a clinical phase I trial [2]. It was established retrospectively that cynomolgus monkey T-cell biology is different from humans in a way that dramatically reduces the susceptibility of monkey T effector memory cells to respond to TGN1412 in a manner similar to humans [3,4]. The occurrence of severe adverse clinical events that were not predicted in preclinical safety testing with TGN1412 prompted a revision of European guidelines for first-in-human clinical trials with biopharmaceutical drugs [5]. These tragic events should also serve as a constant reminder to nonclinical safety scientists that close phylogenetic proximity of the NHP and humans do not automatically imply predictability and translational relevance of the model.

Overall, the NHP is still considered a very consistent pharmacologically relevant species, especially for biopharmaceutical drug development, and it can be an important animal model

VetBooks.ir

used to establish and validate sensitive safety biomarkers that can ensure early detection of toxicity and suitable monitoring in clinical studies. This approach often requires the use of very sensitive and technically challenging methodologies. These methods are often available in basic research or clinical practice, but the implementation can be quite challenging due to the specific nature of studies with NHPs (small group size, high interanimal variability, profound influence of handling and environment, etc.).

The aging process of NHPs and humans is comparable, and because NHPs develop many similar age-related diseases, the use of geriatric NHPs as models is feasible in principle. New technologies, on the other hand, have recently become available that might permit the generation of genetically modified NHPs [6,7] with acceptable rates of success. This work is still at its infancy but holds great promise for at least targeting very specific and selected diseases.

Recent discussions focus on the relevance of healthy animal models for safety assessment of compounds intended to treat a pathological condition [8]. This concern is of particular relevance in case the target is not expressed or only poorly expressed in a healthy animal model but is highly overexpressed in a disease state or if signaling pathways or tissue architecture are altered under pathophysiological conditions. However, discussions of approaches to using alternative disease models as first-tier models are controversial. Unknown or inevitable differences in pathobiology in the animal model of disease compared to humans and lack of model validation or relevant background data may compromise data analysis, and it could have an impact on translational relevance of findings or even compromise overall safety assessment of the molecule. This is especially true for the NHP, where both ethical concerns and practical difficulties limit the use as animal model of disease. First-tier safety testing in NHPs will therefore most likely continue to rely mostly on healthy animals. Potential impacts of pathophysiological alterations need to be taken into account by the safety scientist in a weight of evidence approach during the overall risk-benefit assessment.

Considerable genomic research efforts over the past ten years have revealed that NHP models such as macaques are frequently hybrids with varying degrees of inter- and intraindividual genetic variation and that it might become very important to understand geographic macaque origin [9]. An improved understanding of the genomics of the main NHP species might provide an opportunity to better use NHP models for the development of specific classes of compounds. This obviously requires massive genomic sequencing efforts, but given the rapid progress in this field, it might become a practical approach. Another gap is the limited availability of NHP disease and efficacy models compared to rodent models.

NHP models provide for opportunities and challenges just like any experimental species used in preclinical testing. Currently, the NHP, especially the cynomolgus monkey (*Macaca fascicularis*), has proven its value and importance for nonclinical safety testing and translational biomedical research. The choice of species should be based on thorough scientific considerations (gaps and opportunities) regarding relevance to human safety.

References

[1] Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Published in the Official Journal of the European Union of 22 September 2010.

[2] Farzaneh L, Kasahara N, Farzaneh F. The strange case of TGN1412. Cancer Immunol Immunother 2007;56(2):129–34.

[3] Pallardy M, Hunig T. Primate testing of TGN1412: right target, wrong cell. Br J Pharmacol 2010;161(3):509–11.
[4] Hunig T. The storm has cleared: lessons from the CD28 superagonist TGN1412 trial. Nat Rev Immunol 2012;12(5):317–8.
[5] Stebbings R, Poole S, Thorpe R. Safety of biologics, lessons learnt from TGN1412. Curr Opin Biotechnol 2009;20(6):673–7.
[6] Liu H, Chen Y, Niu Y, Zhang K, Kang Y, Ge W, et al. TALEN-mediated gene mutagenesis in rhesus and cynomolgus monkeys. Cell Stem Cell 2014;14(3):323–8.
[7] Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, et al. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell 2014;156(4):836–43.
[8] Dixit R, Boelsterli UY. Healthy animals and animal models of human disease(s) in safety assessment of human pharmaceuticals, including therapeutic antibodies. Drug Discov Today 2007;12(7–8):336–42.
[9] Haus T, Ferguson B, Rogers J, Doxiadis G, Certa U, Rose NJ, et al. Genome typing of nonhuman primate models: implications for biomedical research. Trends Genet 2014;30(11):482–7.

Index

VetBooks.ir

Note: Page numbers followed by *f* indicate figures and *t* indicate tables.

A

Abuse liability testing, 601–602
Acquired immunodeficiency syndrome (AIDS), 42, 45, 89, 266, 348
Actical device, 594*f*
African cynomolgus monkey. *See* Mauritius-origin cynomolgus monkey
African green monkeys, 5, 220–221, 266, 317, 317*t*
Aging, 262–263
Airway resistance, 581, 586–588
Albumin, 369
Alkaline phosphatase (ALP), 73, 119–122, 150, 448
Alpha-amylase 1A gene *(AMY1A)*, 57–60
Alzheimer disease, 262–263, 350–351, 599
American Society for Veterinary Clinical Pathology, 177
Ames test, 53–55
Anesthesia, 630–631
  environment, 631
  pain assessment, 635
  potential complications, 636
  premedication, 633–634
  prevention of infection, 635
  socialization/acclimatization, 631
  social status, 631
  surgical procedure, 634–635
Anesthetized models, 582–585
Antibody-dependent cell cytotoxicity (ADCC), 520
Antidepressant therapy, 55
Antidrug antibody (ADA), 330, 491, 523
Apneic time, 581
Association for Assessment and Accreditation of Laboratory Animal Care International, 283, 406, 670
Atheroma, 259, 260
Atherosclerosis, 259, 261–262, 571
Avonex, 658–659
Azithromycin, 370

B

Baboons
  Ab2, 370
  airway constriction, 158–159
  endometriosis, 266
  genomic studies, 24
  menopause, 265
  mineral metabolism, 607
  neutrophil, 220–221
  nonclinical toxicity studies, 317, 317*t*
  type 2 diabetes mellitus, 259
Bacterial infections
  Helicobacter pylori, 200
  moraxella catarrhalis, 200
  mycobacterium tuberculosis, 199–200
Balantidium coli, 81, 202–203
Barbary macaque, 3–4, 8–9, 88
Baroreceptor, 565*f*, 568–569
Basophile function assays, 529
Bayesian analysis, 175, 176–177
Bazett correction, 558
B-cell lymphomas, 190, 195–196, 228–229, 230
Beagle pain syndrome, 53–55, 344
Beagles, 53–55, 343–345
Bergmann's rule, 69–70
Bioimpedance method, 586–588
Biomarkers, 529–532
Biopharmaceuticals, clinical development, 503
  enzyme replacement therapy, 662
  general toxicity, 656–658
  immunogenicity, 660–661
  oligonucleotide-based therapeutics, 661–662
  predictive value, 653–654
  regulatory guidelines, 651–652
  reproductive/developmental toxicity, 658–660
  scientific innovation, NHPs, 664–665
  species selection, 655–656
Blood-brain barrier, 507
Blood pressure
  marmosets, 122, 122*t*
  rhesus macaques, 93, 94*t*
Bone biomarkers, 615
Bone biomechanics, 612–615
Bone densitometry, 610–612
Bone histology, 617–618
Bone histomorphometry, 618–619
Bone mineral content (BMC), 150, 610
Bone mineral density (BMD), 150, 610
Bone morphology and physiology
  bone modeling and remodeling, 608–610
  cortical bone, 607
  rodent models, 609–610
  trabecular bone, 607–608

Brazleton-based human neonatal behavioral assessment scale, 486
Bupivacaine, 634

C
Cage mates, 296, 311, 489*f*
Callithrix jacchus, 244–245, 398, 438, 448–449. *See also* Marmosets
Calponin 3 *(CNN3)*, 55–57
Cambridge Neuropsychological Test Automated Battery (CANTAB), 599–600, 599*t*
Cancer, 228–229, 267, 351
Canis lupus familiaris. *See* Dogs
Capra aegagrus. *See* Goats
Carbamazepine, 369
Cardiac arrhythmia assessment, 555–558
Cardiac baroreflex curve, 565*f*
Cardiothoracic ratio (CtR), 78
Cardiovascular disease
  clinical and investigative assays, 261–262
  hormone therapy, 264–265
  multiorgan vascular pathology, 259
  myocardial and cerebral infarction, 260
  NHP models, 570–571
  pharmacological options, 260–261
  risk factors, 261–262
Cardiovascular safety assessment
  anesthetized primates, 563–567
  circulatory system, control of, 554
  congenital and cardiovascular pathology, 569–570
  echocardiography, 561–563
  heart, 553
  in vitro/ex vivo methodologies, 569
  left ventricular pressure and dp/dt_{max} end points, 560–561
  specialty cardiovascular assays, 568–569
  toxicology studies, 567–568
  vascular system, 554
Cardiovascular system
  cardiac structure and function, 423–424
  cynomolgus monkey, 77–78
  hemodynamics and ECG, 422–423
  limitations, assessments, 424–425
  marmosets, 122–123
  rhesus macaques, 91–95, 92*t*, 94*t*, 95*t*, 96*t*
Cats, 348
Cell-based assays, 320, 529
Central nervous system (CNS)
  abuse liability testing, 601–602
  cognitive assessment, 599–600
  drug development, 591–592
  electrophysiological assessment, 593–598
  observation/general behavior, 592–593
Cerebral malaria, 268–269
Charcot-Leyden crystal galectin *(CLC)* gene, 55–57
Chimpanzees
  growth rate, 146
  immune-mediated thrombocytopenia, 460–461
  menopause, 265
  neurobiology, 159, 160
  neutrophil predominance in, 220–221
  nonclinical toxicity studies, 317, 317*t*, 322–323
  phylogenetic relationships, 5
  type 2 diabetes mellitus, 259
Chinese-origin cynomolgus monkey, 69, 78, 83
Cholinesterase inhibitors, 262–263
Chorionic gonadotropin (CG), 141, 438, 473–474
Chronic enteropathies, 409–410
Chronic inflammation, 240–241
Clara cells, 156–158
Class II-associated invariant peptide (CLIP), 39–40
Clawed New World monkeys. *See* Marmosets
Cocaine, 601–602
Codeine, 55
Cognitive assessment
  aged monkey approach, 599–600
  Alzheimer's disease, 599
  CANTAB package, 599–600
  primate problem-solving skills, 600*f*
  rodent models, 599
Comparative genomic hybridization (CGH) array, 61
Complement-dependent cytotoxicity (CDC), 391, 520
Complement system, 530–532
Complete blood count
  marmosets, 119–122, 120*t*, 121*t*
  rhesus macaques, 91
Condyloma acuminatum, 80
Conscious nonrestrained models, 585–588
Conscious restrained models, 585
Copy number variation (CNV)
  CYP2D6 CNVs, 55
  definition, 55
  gene expression, 57–60, 61*f*
  genome-wide analysis, 57, 58*f*
  geographic clustering, 57–60, 60*f*
  homozygous deletions, 55
  ideogram, 57, 59*f*
  SNP, 57–60
  tiling array, 55–57, 56*f*
Crab-eating macaque. *See* Cynomolgus macaques
Creatine phosphokinase (CPK), 73
Critical process parameters (CPPs), 524
Critical quality attributes (CQAs), 524
Cross-sectional moment of inertia (CSMI), 614–615
CtR. *See* Cardiothoracic ratio (CtR)
Cyclooxygenase-2 (COX-2) inhibitor, 260–261
Cynomolgus macaques, 11, 18

amyloid, 249–250
ancestral divergence and disease susceptibility, 23–24
blood sampling procedures and volumes, 410–411, 410*t*
cardiomyopathy, 248–249
cardiovascular assessment, 411
cognitive test, 159
CYP450 protein, 30–32, 30*t*
Fc receptors, 390
functional allele, 28
gastric ulceration, 249–250
genetic diversity in, 22–23
gene variation, 29, 383–384
genomic sequence analysis, 25, 27
genomic studies, 24
GPCR variants, 28–29
idiopathic chronic colitis, 249–250
immune response variation, 32
introgression of, 26–27
kidney glomerular lesions, 251
lymphoid, 253–254
mononuclear cell infiltrates, 245–248
origin and dispersal of, 18–20, 19*f*
placentation, 143
pulmonary pigment, 250–251
reproductive and developmental toxicology, 252, 386–388
skeletal muscle, 252
SLCO sequence variants, 29–30
thromboembolisms, 391
Toll-like receptors, 390
upper gastrointestinal tract hemorrhage, 249–250
Cynomolgus monkeys, 473
appearance and morphology, 69–70, 71*f*
behavior, 82–83
cardiovascular system, 77–78, 79*f*
CDC import data, 67–68, 68*f*
Chinese-origin cynomolgus monkey, 69
CNS studies, 592–593
CNV (*see* Copy number variation (CNV))
corpora lutea development, 142–143
digestive system, 80
echocardiography, 154
electrocardiography, 153–154
endemic diseases and susceptibility, 81–82
fluorescence angiography, 545*f*
genetic analysis, 136–139
genital lesion, 80
geographic dispersal of, 67–68
growth rate, 146
hematology, 70–73, 72*f*
hepatic and urinary, 78–80
immunotoxicity, 328–329
insular Asian cynomolgus monkey, 69
insulin treatment, 570–571
lactation and early nutrition, 146–147
Mauritius cynomolgus monkey (*see* Mauritius-origin cynomolgus monkey)
microRNAs analysis, 154–155
nonclinical toxicity studies, 317, 317*t*
ocular pathology, 80
ovarian cycle, 142
pharmacokinetic studies (*see* Pharmacokinetic (PK) studies, NHPs)
QT effects, 152–153
regulatory toxicity testing, 338
reproduction and development, 75–77, 76*f*
serum chemistry, 73–75, 74*f*
sexual maturation, 147
Cytochrome P450 protein (CYP450), 30–32, 30*t*
Cytokine release syndrome (CRS), 459–460, 530, 654
Cytokines, 530
Cytomegalovirus, 194–195
Cytotoxic T-lymphocyte (CTL), 529

D

Dacliximab, 371–372
Dentition, 149–150
marmosets, 116, 117, 118*t*
rhesus macaques, 88*t*, 89–90
Developmental and reproductive toxicity (DART), 327–328, 490–491, 533
biopharmaceuticals, 472
NHP model, 472
three-segment approach, 472
Developmental immunotoxicology (DIT) testing
biopharmaceuticals, 534–535
clinical translation, 539
DART, 533
DTH, 536–537
eEFD study, 533
immunopathology, 539–540
isotype skewing, 535
maternal dosing, 534
methodological considerations, 538–539
PPND, 533
rituximab, 535–536
study designs, 537–538
TDAR, 535
ustekinumab, 535
DEXA. *See* Dual-energy X-ray absorptiometry (DEXA)
Diarrhea, 409–410
Direct ophthalmoscopy, 544
Dogs, 53–55, 343–345
cardiovascular safety evaluation, 152–153
chest wall compliance, 158
microRNAs analysis, 154–155
QT effects, 152–153

Doppler technology, 562
Drug discrimination assay, 601
Drug safety testing, 29, 32
Dual-energy X-ray absorptiometry (DEXA), 610

E
Ebola virus, 82
Echocardiography (ECG), 154, 561–563
  in marmosets, 122–123
  rhesus macaques, 95, 95*t*, 96*t*
Eculizumab, 386
Efalizumab, 386
Electrocardiogram (ECG), 151–152, 555
  conscious models, 555–558
  hemodynamic assessment, 558–559
  rhesus macaques, 93–95, 94*t*
Electroencephalography (EEG), 593–595, 597*f*
Electroretinography (ERG), 547–549
ELIspot, 529
Embryo-fetal development (EFD), 327–328, 533
  nonhuman primate EFD toxicity study, 479–482
  organogenesis, 479
  toxicokinetic/ADA analysis, 481–482
  ultrasound analysis, 481–482
Endemic diseases, 81–82
Endocrine system, rhesus macaques, 108
Endometriosis, 265–266
Endotracheal (ET) tubes, 630–631
Epidermal growth factor receptor (EGFR), 367–368
Epstein-Barr virus (EBV), 195, 226, 382–383
Ethnovison software, 592–593
European Medicines Agency (EMA), 472, 601
Exon-capture technology, 28
Experimental autoimmune encephalomyelitis (EAE), 260–261, 384
Extramedullary hematopoiesis (EMH), 254, 450–451, 450*t*
Eye, rhesus macaque, 100

F
Felis catus. *See* Cats
Ferrets, 346–347
Fertility and early embryonic development
  NHPs reproductive toxicity study, 475–478
  physical mating, 475
  sexual maturity determination, 478–479
Figitumumab, 491–492
Flow cytometry assays, 528–529
Fluorescein angiography, 544–546, 545*f*
Forced expiratory flows (FEF), 583–584
Forced oscillation technique, 584–585
Frank-Starling mechanism, 554
Functional residual capacity (FRC), 158, 584–585
Fundus photography, 544–546
Fungal infections
  Candida albicans, 203–204
  Pneumocystis carinii, 204
  Pneumonyssus simicola, 204

G
Gastrointestinal system, 430–431
  marmosets, 117–119, 119*t*
  rhesus macaque, 89–91
Genetically modified animal models, 350–351
Genetic diversity, 22–23
Genome-wide expression analysis, 53–55
Genomic sequence analysis, 25, 27
Glial cell line-derived neurotrophic factor (GDNF), 662–663
Gloger's rule, 69–70
Goats, 347–348
Gonadotropin-releasing hormone (GnRH), 139–142, 509
Good manufacturing process (GMP), 524
Göttingen minipig, 340–343
G protein-coupled receptors (GPRCs), 28–29
Grade sleep-disordered breathing, 581
Granulocyte colony-stimulating factor (G-CSF), 360–365
Guinea pig, 152–153, 339

H
Heart rate (HR), 151, 554
  marmosets, 122, 122*t*
  rhesus macaques, 93
Helicobacter pylori, 200
Helmet plethysmograph system, 586*f*
Hematology, 70–73, 72*f*
Hepatitis B virus (HBV)
  cynomolgus monkeys, 81
  woodchuck, 351
Hepatobiliary system, 430
Hepatocyte growth factor (HGF), 360
Herpes B virus, 192–193, 409–410
Homo sapiens
  adrenal androgen production, 148
  adrenarche, 148
  Cox-2, 156
  electrocardiography, 153–154
  fetal growth, 145*f*
  gene expression, 154–155
  GnRH receptor, 141
  growth rate, 146
  heart and anatomy, 151
  IgG concentrations, 144
  lactation and early nutrition, 146–147
  menarche, 149
  microchimerism, 139
  physiological parameters, 145

VetBooks.ir

placentation, 143
postnatal development, 160–161
renal physiology, 155–156
respiratory physiology, 156–157
sexual maturation, 147–148
skeletal and dental maturation, 149–150
visual system, 161
Human immunodeficiency virus (HIV), 382–383
Human leukocyte antigen (HLA), 38, 41–42
Husbandry refinements
housing design and environmental enrichment, 293–295
social groups, 296–297
staff-animal interactions, 298–299
study design and scientific procedures, 297–298
Hypersensitivity, 330–333, 332*t*
Hypothalamic kisspeptin, 509

I

Immunogenicity, 330, 532
cyclosporine, 462
endogenous proteins, 461
immune-mediated hematologic disorders, 460–461
pharmaceutical proteins, 461
PK and PD effects, 458–459
prophylactic uses, 462
protein therapeutics, 462–465
toxicities, 461
type I hypersensitivity, 459–460
type III hypersensitivity, 460
Immunology, 389–392
Immunosuppression, 266–268
bone marrow smears, 227
clinical dosing, 217
definition, 216
differential diagnosis, 224–228
gross pathology and histopathology, 222
hematology, 220–221
hyperplastic lesions and tumors, 226
ICH S8 guidance, 216–217
identification, 231–232
immune function assays, 227–228
infections, 225–226
lymphoid tissue, 226–227
pharmacodynamic (PD) effects, 217
rhesus monkey *(Macaca mulatta)*, 219
serum chemistry, 221
serum globulins, 227
specialized immunologic assessments, 222–224
stress effects, 224–225
Immunotoxicity, 328–329
Immunotoxicology testing, monoclonal antibodies, 413–414
antidrug antibodies, effect of, 523
EMEA, 520–521
Fc-mediated effects, 522–523
FDA guideline, 521
human immunogenicity response, 523–524
in silico, in vitro and *in vivo* studies, 525
mAb product and process factors, 524
nonclinical and clinical studies, 522–523
preclinical immunotoxicology testing, cynomolgus NHP, 525–526
STSs, 521
weight-of-evidence approach, 520–521
Indirect ophthalmoscopy, 544
Indocyanine green (ICG), 545–546
Infliximab, 386
Institutional animal care and use committee (IACUC), 406, 410*t*
Insular Asian cynomolgus monkeys, 69
International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 316, 552, 669
International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) guidelines, 241
Intraocular pressure (IOP), 100, 546–547
Intravitreal injection, 641–643

J

Jacket technologies, 567
Juvenile animal toxicity studies
NHPs, use of, 502–504
nonclinical study, NHPs, 511–512
regulatory considerations, 502

K

Ketamine, 629–630
Keyhole limpet hemocyanin (KLH), 527–528
Kidney glomerular lesion, 251
Konno-Mead method, 586–588

L

Lactation, 146–147
marmosets, 126–127
rhesus macaques, 102, 103
Laser scanning ophthalmoscope, 546
Learning and memory test, 486
Left ventricular end systolic and end diastolic pressure (LVEDP), 560–561
Leptin, 260
Leydig cells, 509
Linkage disequilibrium (LD), 25–26

VetBooks.ir

Lododoxorubicin, 371
Long-tailed macaques, 11–13, 473
  CNV (*see* Copy number variation (CNV))
  in vitro test, 53–55
  in vivo test, 53–55
  vs. Mamu MHC Class I genes, 41
  vs. Mamu MHC Class II genes, 41–42
  MHC genetic variability, 42, 43*t*, 44*t*
  microarrays, 53–55
  SIV infection, 45
Lower-order species, 352
Lung compliance, 580*f*, 581–582, 583–584
Luteinizing hormone (LH), 101–102, 124, 473–474
Luteolysis, 142–143, 444
Lymphatic system, rhesus macaques, 107–108

M

Macaca
  M. arctoides, 9–10
  M. fascicularis (*see* Cynomolgus macaques)
  M. mulatta (*see* Rhesus macaques)
  M. radiata, 156–157
  M. silenus, 9
  M. sylvanus, 3–4, 8–9
Macacine herpesvirus 4, 195–196
Macaque
  alveolar type II pneumocyte differentiation, 157–158
  Barbary macaque, 3–4
  bone, 506
  brain, 506–508
  chest wall compliance, 158
  cynomolgus macaques (*see* Cynomolgus macaques)
  cytogenetic changes, 139
  electrocardiography, 153–154
  estrogen and progesterone, 143
  evolutionary history, 6–11
  feeding, 4
  fetal growth, 144–145, 145*f*
  gang housing system, 294*f*
  gastrointestinal tract, 505
  gene expression, 154–155
  genetic analysis, 136–139
  growth rate, 146
  growth spurt, 149
  heart and anatomy, 151, 505
  hormonal changes, 146
  immune system, 510–511
  kidney, 504–505
  lactation and early nutrition, 146–147
  LH concentrations, 148–149
  menstrual bleeding, 149
  multifunctional box, urine collection, 295*f*
  neurobiology, 159
  osteoporosis, 150
  ovarian cycle in, 139–141
  ovulation, 4
  peripheral vasculature, 154
  phylogenetic position, 5
  physiological parameters, 145
  placentation, 143
  postnatal development, 160–161
  pulmonary deposition, 158–159
  renal physiology, 155–156
  respiratory physiology, 156–157
  respiratory tract, 504
  retinal vascularization, 505
  rhesus macaques (*see* Rhesus macaques)
  sexual dimorphism, 4
  sexual maturation, 147, 508–509
  skeletal and dental maturation, 149–150
  taxonomic and genetic diversity, 11–13
  visual system, 161
Major histocompatibility complex (MHC), 390
  antigen II, 37–38
  H-2 complex, 38
  H genes, 37–38
  Ir genes, 38
  long-tailed macaques (*see* Long-tailed macaques)
  polymorphism, 38–39, 40
  rhesus macaques (*see* Rhesus macaques)
  SIV infection, 45
  structure and function, 38–40, 39*f*
Malaria
  cerebral malaria, 268–269
  chloroquine and primaquine, 201
  diagnosis of, 201
  in human, 268–269
  infection, in cynomolgus monkey, 81
  life cycle of, 201, 268–269
  prevalence, 81, 200–201
  prevention, 201
  rhesus macaques, 269
Marmosets, 244–245, 254, 473–474
  age effects, in captivity, 442
  blood pressure and heart rate, 122, 122*t*
  blood sampling procedures and volumes, 410–411, 410*t*
  body temperature, 123
  canakinumab, 384–385
  cardiovascular assessment, 411
  CG concentrations, 144
  challenges, 384–385
  clawed New World monkeys, 116
  complete blood count, 119–122, 120*t*, 121*t*
  contraception, 443–444
  dentition, 116, 117, 118*t*
  diabetic and obesity research, 571
  diagnostic parameters, 448

dosing techniques and design, 449
drug administration, 384–385, 449
echocardiography, 122–123
electrocardiography, 153–154
embryo transplantation, 444–445
endocrine regulation, 440
evolutionary origin, 439
fetal growth, 144–145, 145*f*
fetal zone, 146
fluid collection, 447
FSH concentrations, 147–148
gastrointestinal tract and dietary requirements, 117–119, 119*t*
genetic analysis, 136–139
GnRH receptor, 141
growth spurt, 149
heart and anatomy, 151
housing and husbandry, 445–446
immunotoxicity, 328–329
implantation stages, 143
infectious disease research, 384
lactation and early nutrition, 146–147
life history, 116, 118*t*
male contraceptive regimen, 440
male primate reproduction, 128
menstruation, 149
microchimerism, 139
monogamy, 118*t*, 123–124
morphology, 116, 117*f*
neuroscience research programs, 384
nonclinical toxicity studies, 317, 317*t*
ophthalmoscopic examinations, 448
osteoporosis, 150
ovarian cycle, 118*t*, 124–126, 125*f*, 142, 444
pathology/histology, 450–452
pharmacokinetic and toxicological screening, 384–385
pregnancy and lactation, 126–127
QT effects, 152–153
renal physiology, 155–156
reproductive and developmental toxicology, 388–389
reproductive biology, 438, 441–443
respiratory system, 123, 156–157
senescence, 127
sexual maturation, 124, 147
skeletal and dental maturation, 149–150
spermatogenesis, 128–129
spontaneous mortality, 448–449
stem cell research, 442–443
subordinate females, 126
taxonomy, 115–116, 116*f*
triplet and quadruplet births, 447
weight and length, 116, 118*t*
Mature monkeys
  acclimation and socialization period, 304–305
  affiliative behavior, 308
  animal husbandry tasks, 306–307
  animal-related aspects, 304
  behavioral assessments, 311
  cage mates, 311
  clicker training, 306
  desensitization, 305
  facility and staff-related aspects, 304
  facility conditions and study requirements, 307
  focal sampling/focal behavioral assessment, 308
  full-scale systematic ethograms, 309–310
  hierarchical behavior, 308
  pair assignments, 311
  positive reinforcement training, 304
  ranking behavior, 308
  self-confidence and cooperation, 304
  sequence of behaviors, 305, 306*f*
  snapshot ethogram, 308, 309*f*
  social ranking, 308
  stimulatory effect, 308
  stress avoidance and housing conditions, 303
  toxicology facility, 305
Mauritius-origin cynomolgus monkey
  cardiovascular system, 77–78, 83
  dispersal of, 18–20
  gene expression variation, 29
  genital lesion, 80
  hepatitis B virus infection, 81
  liver and kidney function, 78–80
  malaria, 81
  population of, 68, 69
  subclinical gastritis, 81
  tuberculosis, 82
Measles (Rubeola) virus, 191–192
MedGenesis, 663
Menopause
  endocrine markers of, 103–104
  macaque models of, 263
  occurrence, 103–104
  and perimenopausal transition, 264–265
Metabolic syndrome
  definition, 259
  macaque model, 259
  numerous clinical and investigative assays, 261–262
Michaelis-Menten model, 370, 372
Microarray analysis, 31–32
Micropig, 340
Mineral ion homeostasis, 615–617
Minimal anticipated biological effect level (MABEL) approach, 352
Minipigs, 340–343
Minor allele frequencies (MAFs), 28–29

Minute volume, 581
MNHs. *See* Multinucleated hepatocytes (MNHs)
Monoclonal antibodies (mAbs), 472, 520
  DART evaluation, 472
  ICH S6 (R1), 656
  immunomodulatory effects, 520
  immunotoxicology study designs, 526
  neonatal Fc receptor, 368–369
  renal elimination, 369
Moraxella catarrhalis, 200
Morphine, 601–602
Multinucleated hepatocytes (MNHs), 78–80
Multiple sclerosis (MS), 384
Mustela putorius furo. *See* Ferrets
Mycobacterium tuberculosis, 199–200
Myocardial degeneration, 83

N

Natural killer (NK) cells, 38–39, 520–521
Necrosis, 83, 191–192, 203*f*, 345
Nerve conduction assessment, 598
Nervous system
  automated activity analysis, 427–428
  body/core temperature, 428–429
  global neurobehavioral assessment, 427
Neurogenesis, 507
Neurologic system, 98–100, 98*t*, 99*t*
New chemical entities (NCEs), 316, 384–385
New drug application (NDA), 601
Next-generation sequencing technologies, 24
Nonclinical biotherapeutics development
  carcinogenicity assessment, 329
  chimpanzee, 317, 322–323
  GLP repeat-dose toxicity studies, 324–326
  homologous protein, 323
  hypersensitivity reactions, 330–333, 332*t*
  ICH regulatory guidelines for, 316, 317–319, 318*t*, 334
  immunogenicity and antidrug antibody formation, 330
  immunotoxicity, 328–329
  in vivo pharmacology studies, 323–324
  NHP species, characteristics of, 317, 317*t*
  non-GLP exploratory studies, 324
  pharmacologically relevant species, 319
  recovery groups, *in vivo* studies, 326
  stand-alone safety pharmacology studies, 326
  tiered approach to species selection, 319–321, 320*t*
Nonhuman primates (NHPs)
  acclimation, 401–402
  aging process, 671
  animal sourcing, 398–400
  animal specification, 407–408
  animal transportation, 400–401
  animal welfare, care, and use, 406
  biopharmaceutical development, 472, 669
  blood sampling procedures and volumes, 410–411, 410*t*
  cardiovascular assessment, 411
  clinical pathology, 413
  DART studies, 472
  data evaluation, 415
  dosing, 411
  EEC guidelines and regulations, 406–407
  fertility assessment, 412–413
  first-tier safety testing, 671
  food and water consumption, 409
  genomic studies, 24
  graphic representation, 474*f*
  healthy animal models, 671
  immunology, 389–392, 526*t*
  immunotoxicology, 413–414
  juvenile animals, use of, 408–409
  legal provisions, 286*t*
  NCE development, 316
  neurobehavioral observations and activity monitoring, 412
  nonclinical safety testing and translational biomedical research, 671
  nonclinical toxicity studies (*see* Nonclinical biotherapeutics development)
  ophthalmology, 414–415
  pharmacokinetic (PK) studies (*see* Pharmacokinetic (PK) studies, NHPs)
  pre-and postnatal study, 669
  preclinical animal models, 669
  pregnant NHPs, 493
  primate supply, 670
  quarantine procedures, 400, 401
  randomization procedures, 415
  reference intervals (*see* Reference intervals)
  socialization, 402
  species-specific differences, 380–381, 381*t*
  study group size, 415
  study selection, 402
  TGN1412, 670
  veterinary considerations, 409–410
  xenotransplantation, 669–670
Nonrodent species, 503
  cats, 348
  dogs, 343–345, 360
  ferrets, 346–347
  guinea pig, 339
  minipigs/pigs, 340–343
  placental mammalians, evolutionary tree of, 339*f*
  rabbits, 345–346
  sheep and goats, 347–348
  tree shrews, 348–349

Nurolon, 634
Nutrition
  marmosets, 117–119, 119*t*
  rhesus macaque, 89–91

O
Odanacatib, 371
Oligonucleotides, 349, 661–662
Ophthalmological examinations
  ERG, 547–549
  fundus photography and fluorescein angiography, 544–546
  IOP, 546–547
  laser scanning ophthalmoscope, 546
  OCT, 546
  pachymetry, 547
  scheimpflug photography, 547
  specular microscopy, 547
  standard investigations, 544
Optical coherence tomography (OCT), 546
Orthostatic hypotension, 568
Oryctolagus cuniculus. *See* Rabbits
Osteoporosis, 622–623, 664
Ovariectomy (OVX), 622–623
Ovis aries. *See* Sheep

P
Pachymetry, 547
Pain assessment, 631–632
Panitumumab, 367–368
Parasitic infections
  Balantidium coli, 202–203
  Cryptosporidium muris-like protozoa, 202–203
  malaria, 200–201
  Toxoplasma gondii, 202
  Trichomonas spp., 202–203
  Trypanosoma cruzi, 201–202
Parathyroid hormone (PTH), 615–617
Parkinson's disease, 662–664
PCA. *See* Principal components analysis (PCA)
Peri-and postnatal development study (PPND), 533
Peripheral neuropathies, 596–598
Peripheral quantitative computed tomography (pQCT), 610
Pertuzumab, 491–492
Pharmacokinetic (PK) studies, NHPs, 523
  Ab2, 370
  azithromycin, 370
  concentration profiles, 370
  designs of, 360, 361*t*
  hepatic metabolism, CYP450 enzymes, 369–370
  human PK, prediction of, 371–372
  mAbs, interaction with FcRn, 368–369
  Michaelis-Menten model, 370, 372
  panitumumab, EGFR-mediated clearance, 367–368
  pegylated G-CSF clearance, 368
  proteins, 360–365, 366*t*, 367
  renal elimination, 369
  small molecules, 360–365, 366*t*, 367
  species differences, 371–372
  therapeutic antibodies, 360–367, 365*t*
  TMDD model, 370
  two-compartment model, 370, 372
Pigs
  cortical bone morphology, 607
  genome sequencing of, 341
  hormonal and dermal testing, 342
  immune system, 341
  olfactory sense, 340–341
  organ transplantation, 340–341
  placental morphology, 342–343
  QT effects, 152–153
  teeth of, 340–341
Plasmodium spp., 268–269
  P. coatneyi, 81
  P. knowlesi, 81
Plexiglas panels, 311
Pneumocystis carinii, 204
Pneumonyssus simicola, 204
Pneumotachometer, 582–583
Pre-and postnatal development (PPND), 327–328
Preclinical Toxicology Working Group of the Academy of Medical Sciences Forum, 652
Prenatal and postnatal development (PPND)
  infant evaluation, 482–486
  nonhuman primate ePPND toxicity study, 482–486
  TDAR, 486
Prepulse inhibition, 600
Principal components analysis (PCA), 18–20, 20*f*, 23
Pseudocarcinomatous hyperplasia, 80
Puberty
  adrenal androgen production, 148
  definition, 441–442
  female rhesus, 101
  male reproductive development, 128
  marmosets, 441–442
  spermatogenesis, 128–129

R
Rabbits, 345–346
Rats
  C-cell hyperplasia, 451
  DART, 472
  fetal growth, 145*f*
  gene expression, 154–155
  microRNAs analysis, 154–155
  postnatal development, 160–161

VetBooks.ir

Reference intervals
Bayesian analysis, 175
bootstrapping, 174–175
clinical pathology test, 179
concurrent controls, 176
control and treated animals, 181–182
correlative findings, 181–182
data interpretation, 178, 180–181
historical controls, 175–176
historical data, 174–175
iatrogenic anemia, 180–181
in-house reference intervals, 179
interanimal variability, 178
quality control function, 179–180
robust methods, 174–175
scientific and statistical justification, 174
variance component analysis, 176–177
veterinary medicine, 178–179
Wilcoxon test, 176–177
Regulatory toxicology
cats, 348
conventional rodent species, 349–350
dogs, 343–345
ferrets, 346–347
genetically modified animals, 350–351
guinea pig, 339
lower-order species, 352
MABEL approach, 352
minipigs/pigs, 340–343
rabbits, 345–346
sheep and goats, 347–348
tree shrews, 348–349
Renal system, 429–430
Replacement, refinement, and reduction (3Rs), 282–283
husbandry and procedures (*see* Husbandry refinements)
legal provisions, 284*t*
Reproduction
cynomolgus macaques, 75–77, 76*f*, 386–388
endometriosis, 265–266
marmosets, 123–129, 125*f*, 388–389
menopause and perimenopausal transition, 264–265
rat, 349–350
rhesus macaques, 100–105, 101*f*, 382–383
statistical power, 487–488
vehicle-exposed and test article-exposed groups, 487
Resistin, 260
Respiratory function
anesthetized models, 582–585
conscious nonrestrained models, 585–588
conscious restrained models, 585
strategy, 580–582
Respiratory inductive plethysmography (RIP), 586–588
Respiratory system
marmosets, 123
rhesus macaque, 96–97, 97*t*, 98*t*
ventilation and lung resistance parameters, 425–426
Restrained infusion models
capsule administration, 638–639
complications, 638
intradermal and subcutaneous injections, 639–641
procedure, 637–638
RETHINK project, 342
Retina cameras, 544–545
Rhesus macaques, 10, 18, 67, 263, 269, 473
ancestral divergence, 24
candidate risk alleles, genomic discovery of, 27
cardiovascular system, 91–95, 92*t*, 94*t*, 95*t*, 96*t*
chromosomal assignment of, 40
CYP450 protein, 30–32
endocrine system, 108
functional allele, 28
gastrointestinal tract and nutrition, 89–91, 91*t*
genetic diversity in, 22–23
genomic sequencing, 25, 27
genomic studies, 24
geographic range of, 89
GPCR variants, 28–29
hematological and leukocyte parameters in, 92*t*
immunology, 382–383, 391
immunotoxicity, 328–329
introgression of, 26–27
linkage disequilibrium, 25–26
lymphatic system, 107–108
vs. Mafa MHC Class I genes, 41
vs. Mafa MHC Class II genes, 41–42
MHC genetic variability, 42, 43*t*, 44*t*
neurologic system and special senses, 98–100, 98*t*, 99*t*
nonclinical toxicity studies, 317, 317*t*
origin and dispersal of, 20–22, 21*f*
pharmacokinetic studies (*see* Pharmacokinetic (PK) studies, NHPs)
physical characteristics and life history, 88–89, 88*t*
reproduction, 100–105, 101*f*, 382–383
respiratory system, 96–97, 97*t*, 98*t*
SIV infection, 45
urinary system, 105, 105*t*, 106*t*
Rituximab, 535–536

S

Sanger sequencing, 53–55
Sarcocystosis, 252
Sarcopenia, 262, 263
Scheimpflug photography, 547
Schizophrenia, 599–600
Scopolamine, 599–600

Senescence
marmosets, 127
rhesus macaques, 103–104
Sertoli cell proliferation, 508
Serum alkaline phosphatase (ALP), 73
Sexual maturity
adrenal maturation, 508
determination, 478–479
females, 509
males, 508–509
Sheep, 347–348
Simian adenoviruses, 196–198
Simian betaretroviruses, 190–191
Simian immunodeficiency virus (SIV)
long-tailed macaques, 45
rhesus macaques, 45, 383–384
Simian parvovirus, 198–199
Simian virus 40, 198
Single nucleotide polymorphisms (SNP), 24, 25, 26–27, 32, 53–55, 57–60
Skeletal assessments
bone morphology and physiology, 607–610
drug development programs, 620–623
efficacy and safety testing, 606
routine toxicity testing, 606
specialized bone toxicity assessments, 606
SLC. *See* Solute carrier (SLC)
SLCOs. *See* Solute carriers of organic anions (SLCOs)
Sleep analysis, 596
Slit-lamp biomicroscopy examinations, 544
SNPs. *See* Single nucleotide polymorphisms (SNP)
Social housing, 488–489
adult male cynomolgus macaques, 311
cage mates, 307, 311
pregnant animals, 310
Socialization, 402
Society of Toxicologic Pathology (STP), 236
Solute carrier (SLC), 29–30
Solute carriers of organic anions (SLCOs), 29–30
Special administration techniques
effective planning and resourcing, 628
intravenous infusion, 632
sedating/premedicating primates, 629–631
social groupings and isolation, 628–629
surgical infusion models, 632–633
test formulation, 636–637
training and restraint, 629
Specialty cardiovascular assays, 568–569
Spermarche, 478
Spermatogenesis, 104
marmosets, 128–129
puberty, 128–129
Standard toxicity studies (STSs), 521
Streptozocin (STZ), 570
Surgical implantation, 586–588
Surrogate molecules, 349
Sus scrofa. *See* Pigs
Synaptogenesis, 507

T

Target-mediated drug disposition (TMDD), 369, 370
T-cell-dependent antibody response (TDAR), 223, 392, 486, 520–521, 527–528
T-cell receptor, 389–390
TDAR. *See* T-cell-dependent antibody response (TDAR)
Telemetric chip, 593
Tetanus toxoid (TTX), 535
Thromboembolisms, 391
Timed matings, 473
Tissue cross-reactivity (TCR) assay, 320
Tissue Doppler techniques, 562
Tonometer (TonoVet), 546
TonoPen, 546
Torsades de pointes (Tdp), 152–153
Torsadogenic risk, 556–557
Toxicologic pathology
blinding procedure, 238
drug development, 236
histopathologic finding, 239–244
microscopic historical control data, 242–243
organ weight, microscopic evaluation, 237–238
origin and interpretation, microscopic findings, 241–242
pathology community, 236
quality control, 243–244
terminology, 240–241
thresholds, 239–240
Toxoplasma gondii, 202
Transgenic animal models, 350–351
Tree shrews, 348–349
Tricarbocyanine dye, 545–546
Trichomonas spp., 202–203
Tropicamide, 544
Trypanosoma cruzi, 201–202, 245–248, 562
Tuberculosis (TB), 81–82
Type 2 diabetes mellitus (T2DM)
atherosclerosis, 259
clinical and investigative assays, 261–262
obesity and insulin resistance, 259
spontaneous T2DM, 259
treatment options, 260–261
tumor necrosis factor and RBP-4, 260

U

Ulcer, 240–241
Urinary system
cynomolgus monkey, 78–80
rhesus macaques, 105, 105*t*, 106*t*

VetBooks.ir

US Food and Drug Administration (FDA), 521, 601
Ustekinumab, 536–537

V
Venous access port (VAP), 632
Viral infections
  cytomegalovirus, 194–195
  herpes B virus, 192–193
  macacine herpesvirus 4, 195–196
  measles (Rubeola) virus, 191–192
  simian adenoviruses, 196–198
  simian betaretroviruses, 190–191
  simian parvovirus, 198–199
  simian virus 40, 198
Visual system, 161

W
Weight-of-evidence approach, 475
Whole-body plethysmography, 585–586
Wilcoxon test, 176–177
Women of child-bearing potential (WoCBP), 385–386

Z
Zebrafish, 352

VetBooks.ir