Macaque Models of Human Disease

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Disclaimer

• Veterinary education “generally provide[s] great breadth but little depth” ¹

• Laboratory Animal Medicine Veterinarian: “Knowledge a mile wide and an inch deep.”

• Caveat: Some “deep dive” areas, variable from one vet to another ...

Overview

• General information about the use of NHPs in research
• Macaque models of human disease
  • Research areas (not covering in depth)
• Challenges
• Lessons learned

Caveats:
1. Based on my experiences (and/or experiences of veterinary colleagues) with providing veterinary support for these macaque models
2. Not a comprehensive review of all macaque models of human disease
NHP Models

• Do you need to use an animal model? (3Rs – “Replacement”)
• Can you use a less sentient species (lower on the phylogenetic scale) like a fish, rodent, etc.?
• If you must use an NHP model, is it a valid model?
  • Does it recapitulate all or part of the disease that I am studying?
  • Is it the appropriate model to provide the answers that I am looking for?
  • Are there ways to refine the model to get better data?
    • 3Rs – “Reduction and/or Refinement” examples:
      • Less invasive
      • More effective analgesics
      • Better environmental enrichment
      • Acclimating and Training (PRT)
      • Genetic modification
Positive Reinforcement Training (PRT)

- Subjects receive rewards (e.g., food) for doing behaviors we ask them to perform
- Allows animals to voluntarily cooperate with research and/or husbandry procedures
- Reduces stress associated with these procedures
Training to Cooperate with Procedures

Figure 14.17 “The least distressing method of handling is to train the animal to co-operate in routine procedures. Advantage should be taken of the animal’s ability to learn” (Home Office, 1989, p. 18). Here a female rhesus macaque cooperating during in-home cage injection.

Figure 14.18 The typical, significant cortisol response to traditional blood collection involving enforced restraint is absent in rhesus macaques trained to voluntarily cooperate (Reinhardt, 2003).
Infusion Systems with Jacket

Tether and Swivel
(Remote Pump)

Ambulatory
(Pump in Jacket)

Note: Should acclimate NHP to jacket before study
Social Housing

• AAALAC International Position Statement:

“The Guide [ILAR] states that single housing of social species should be the exception. Social housing will be considered by AAALAC International as the default method of housing unless otherwise justified based on social incompatibility resulting from inappropriate behavior, veterinary concerns regarding animal well-being, or scientific necessity approved by the IACUC (or comparable oversight body). . . ”
“The Association of Primate Veterinarians supports the responsible use of nonhuman primates in biomedical research. Ideally, primates should be housed in a manner that provides for expression of species-typical behaviors. Thus, it is essential to have an understanding of the behavioral biology for each species being housed in a facility. Institutions should design and implement a plan to provide for the social needs of nonhuman primates in a way that promotes their psychological well-being while being consistent with the objectives of the research. This is best achieved by supporting an active, engaged behavioral medicine program to help direct socialization, enrichment, and training of captive nonhuman primates. These guidelines are intended to provide basic information for veterinarians, animal caregivers, scientists, and institutional animal care and use committee (IACUC) or ethical review committee members to consider when designing and implementing this program.”
Social Housing

Compatible Pair
“Social Housing”

- Partial contact
- Grooming bar divider with solid section

**Grooming contact pair**: Allows two animals in adjacent cages to have social contact (e.g., groom), but still gives privacy and separation
The Association of Primate Veterinarians strongly recommends the use of humane endpoints to prevent, alleviate, or reduce pain, distress, and suffering of nonhuman primates in biomedical research. **Endpoint criteria should be developed for every research project** to identify when a nonhuman primate should be removed from a study, provided with supportive treatment, or euthanized. **Endpoints should also be developed for all colony nonhuman primates** to ensure that animals with untreatable conditions are euthanized in a timely fashion.

[https://www.primatevets.org/education](https://www.primatevets.org/education)
Why Nonhuman Primates?

• Susceptibility to human infectious agents,
• Similarities in physiological responses,
• Developmental biology,
• Response to experimentally induced disease

• Critically important to advancements in biomedicine
Educate the Public

Tours at ONPRC
### Assessing the Validity of Models

Proposed validity scoring system

<table>
<thead>
<tr>
<th>Value</th>
<th>Score</th>
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</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>4</td>
</tr>
<tr>
<td>Non-human primate</td>
<td>3</td>
</tr>
<tr>
<td>Non-human mammal</td>
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<tr>
<td>Non-mammal</td>
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<tr>
<td><strong>Disease simulation</strong></td>
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<tr>
<td>True</td>
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<tr>
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</tr>
<tr>
<td>No</td>
<td>1</td>
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<tr>
<td><strong>Face validity</strong></td>
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</tr>
<tr>
<td>&gt;1 core symptom</td>
<td>4</td>
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<tr>
<td>1 core symptom</td>
<td>3</td>
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<tr>
<td>1 symptom</td>
<td>2</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td><strong>Complexity</strong></td>
<td></td>
</tr>
<tr>
<td><em>In vivo</em></td>
<td>4</td>
</tr>
<tr>
<td>Tissue</td>
<td>3</td>
</tr>
<tr>
<td>Cellular</td>
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</tr>
<tr>
<td>Sub-cellular/molecular</td>
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<tr>
<td><strong>Predictivity</strong></td>
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<tr>
<td>Graded for all pharmacology principles</td>
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<tr>
<td>Graded for certain pharmacology principles</td>
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<tr>
<td>principles</td>
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<tr>
<td>No or not shown</td>
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<table>
<thead>
<tr>
<th>Species</th>
<th>No. (%) of facilities</th>
</tr>
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<tbody>
<tr>
<td>Rhesus</td>
<td>21 (80.8%)</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>19 (73.1%)</td>
</tr>
<tr>
<td>African Green</td>
<td>4 (15.5%)</td>
</tr>
<tr>
<td>Squirrel monkey</td>
<td>4 (15.4%)</td>
</tr>
<tr>
<td>Baboon</td>
<td>4 (15.4%)</td>
</tr>
<tr>
<td>Pigtailed macaques</td>
<td>3 (11.5%)</td>
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<tr>
<td>Capuchin monkey</td>
<td>2 (7.7%)</td>
</tr>
<tr>
<td>Stump-tailed macaques</td>
<td>1 (3.8%)</td>
</tr>
<tr>
<td>Titi monkeys</td>
<td>1 (3.8%)</td>
</tr>
<tr>
<td>Marmosets</td>
<td>1 (3.8%)</td>
</tr>
</tbody>
</table>
Macaques in Research
Rhesus

• Common name: Rhesus
• Species: *Macaca mulatta*

• Weight
  • Male 6.8 – 10.0 kg
  • Female 4.1 – 9.0 kg

• Research use:
  • *Polio Vaccine*
  • Behavior, Neuroscience
  • Substance abuse
  • Reproductive study – (twin not common)
  • Infectious disease: HIV, SHIV, SIV
  • Transgenic - including for Huntington’s disease
Rhesus Macaques

• Largest home range of any nonhuman primate
• Indian origin and Chinese origin are very different!
  • Indian
  • Chinese
  • Hainan Island
• Hybridization occurs with cynomolgus macaques in Southeast Asia (Vietnam, Laos, Burma, etc.)
Abie, an American-born rhesus were launched on May 28, 1959 in the nose cone of Army Jupiter missile for 300 miles altitude. He was accompanied by Ms. Baker, a squirrel monkey onboard. Both were back safely to earth and recovered unharmed.

Patricia, Michael, and Sam (named after the Air Force School of Aviation Medicine), also rhesus monkeys, and Ham, a Chimpanzee, were among 20 NHP launched into space within 50 years.
Rhesus Macaque

• Susceptible to SIV and excellent model for HIV model, vaccine research and development
• Diploid number of 42
• In the wild multimale-multifemale group
• Seasonal breeder (mid September to mid February)
• Gestation period: approximately 164 days
• Mostly used for research: India and China origin
  • Behavior
  • Sexual dimorphism and temperament
  • Major histocompatibility complex
Macaques in Research
Cyno

• Common name: Cynomolgus/ longtail/ crab eating macaque
• Species: *Macaca fascicularis*
• Weight
  • Male 4.5 – 8.5 kg
  • Female 2.5 – 6 kg
• Research use:
  • Polio Vaccine
  • Behavior and Neuroscience
  • Substance abuse
  • Atherosclerosis, diabetes, asthma
  • Safety and efficacy evaluation: DMPK, toxicology
  • Reproductive: hormonal, menopause: osteoporosis
  • Infectious disease: TB, retroviral
Cynomolgus macaques

Genetics vary by origin

Continental
   Cambodia
   Vietnam
   Burma
   Etc.

Insular
   Philippines
   Indonesia
   Mauritius

Chinese????

Very temperature sensitive...tails can get frost bite and necrose due to cold...
Macaques in Research
Pigtailed

• Common name: Pig-tailed macaque (Brunei, Indonesia, Malaysia and southern peninsular Thailand.)

• Species: *Macaca nemestrina*

• Weight
  • Male 6.2 – 14.5 kg
  • Female 4.7 – 10.9 kg

• Research use:
  • Virus study: HIV, dengue
  • Vaccine study
Macaques in Research
Stumptailed

• Common name: Stump-tailed monkey
  (Cambodia, south-western China, north-eastern India)
• Species: *Macaca arctoides*
• Weight
  • Male 9.9 – 10.2 kg
  • Female 7.5 – 9.1 kg
• Research use:
  • Male pattern baldness model
  • Discovery of minoxidol (Rogaine®)
Macaques in Research
Japanese

• Common name: Japanese/ snow monkey (Japan)
• Species: *Macaca fuscata*
• Weight
  • Male average 11.3 kg
  • Female average 8.4 kg
• Research Use (Japan, ONPRC):
  • Behavior
  • Neuroscience
  • Transplant surgery
  • Obesity
Japanese Macaque
(ONPRC Outdoor Corral)
Macaques in Research
Bonnet

• Common name: Bonnet monkey (India)
• Species: *Macaca radiata*
• Weight
  • Male average 6.7 kg
  • Female average 3.9 kg
• Research use (India):
  • Behavior
  • Neuroscience
Macaques in Research

• Common name: Barbary Macaque, Rock Apes, (Gibraltar, Morocco and Algeria)
• Species: *Macaca Sylvanus*
• Weight
  • Male average 14.5 kg
  • Female average 9.9 kg
• Research use:
  • Social Organization
  • Hepatitis B research (France)
Macaques in Research

• Common name: Celebes Ape, Celebes Crested Macaques, or Celebes Black Macaque (Sulawesi)

• Species: *Macaca nigra*

• Weight
  • Male average 9.9 kg
  • Female average 5.5 kg

• Research use:
  – Diabetes mellitus
    • Chronic hyperglycemia
    • Amyloidosis in islet cells
Selected Examples of Macaque Models of Human Disease
Challenges/Lessons Learned

• Cognition
• Cardiovascular Telemetry
• Pancreatic Islet Cell Transplant
• Infectious Disease (Zika virus)
• Mandibular Reconstruction
Cognition Testing
(Learning and Memory)

- Rhesus (ONPRC), Cynos (Singapore)
- Touch Screen – Pellet Reward System

Challenges/Lessons Learned

• Training macaques to use the test system takes months
• Temperament Screening (Coleman et al) *
• Temperament predicts trainability
• Inhibited versus Exploratory
  • Less success training inhibited macaques
  • Significantly greater success training exploratory macaques
• Screened cynos for temperament at vendor’s facility
• Purchased only exploratory cynos

1. Initial response to observer:
2. Predominant response to observer:
3. Initial response to direct eye contact:
   Fear; threat; aggressive; no response; avert eye contact; other
4. Predominant response to direct eye contact:
   Fear; threat; aggressive; no response; avert eye contact; other
5. Initial response to novel food:
   Take food; inspect food; fear; threat; aggressive; other
   Latency to: Inspect _____ Touch _____ Manipulate _____
6. Initial response to novel object:
   Grab object; inspect object; fear; threat; aggressive; other
   Latency to: Latency to: Inspect _____ Touch _____ Manipulate _____

Coleman, et al
Cardiovascular Telemetry

Model Summary

• Implants designed for chronic physiologic monitoring
• Implant transmits continuous EKG, Arterial Blood Pressure
• Safety pharmacology studies to address regulatory core battery requirements in cardiovascular (CV) applications.
• Awake, freely moving cynos in cage
• Receiver on cage
Challenges Lessons Learned

• Early CV telemetry devices required single housing of NHPs with 1 meter between cages

• Latest devices allow social housing

• Failure of blood pressure sensor in Femoral artery (e.g., bubble or thrombus)

• Learned to salvage with minor surgery

• Most definitive solution for bacterial contamination/infection is to remove the implant

https://www.datasci.com/docs/default-source/case-studies/casestudy_ptd_biotrial.pdf?sfvrsn=0
Pancreatic Islet Cell Transplant

Model Summary

- Cynos
- Partial pancreatectomy
- Culture islets
- Implant in anterior chamber of eye
- Serial imaging to study revascularization, re-innervation, and blood flow

Imaging Islets with Confocal In vivo Microscopy

High-Resolution In Vivo Longitudinal Imaging of Islet Re-vascularization in the anterior chamber of cynos using Confocal in vivo microscope
Challenges/Lessons Learned

• Poor survival rate with total pancreatectomy
• Partial pancreatectomy improved survival rate
• Refined surgical method by using laparoscopy
• NHP islet blood flow different from rodent
• NHP is superior model

Diez, et al
Study Summary

• Wild-caught Cynos
• Experiment using “Zika-infected live mosquitoes”
• Continuation phase for previous trial of infection via Intravenous and subcutaneous route of viral administration.
• *The first phase without live mosquitoes showed that virus is neutralized just 2-3 days post infection.*

Challenges/Lessons Learned

• Difficulty sourcing cynos that are negative for Flavivirus antibodies from the wild caught colonies (source of experimental animals).
• Management of infected mosquitoes and seeking regulatory approvals and critical process of project risk assessment.
Mandibular Reconstruction

Study Summary

- 10 – 15 years old female cynos at 4 -6 kg body size
- Comparison of 3D printed titanium vs milled titanium implant
- Implantation of Endoprosthesis secured by 3 titanium plates (40 mm)

Challenges/Lessons Learned

- Pain management during post-operative care - providing high dose of analgesics while managing the analgesic effect of respiratory depression.
- Supplemental nutrition for 3-5 days post-op while animal has difficulty chewing even the softened chow and fruit treats.
- Fed soft diet 1 month pre-surgical procedure to get familiarity and acceptance.
- Dental hygiene and management of oral infection post surgery
Conclusion

• Continue to promote 3 R’s (Replacement, Reduction, Refinement) with all animal models, especially nonhuman primates/Macaques.

Veterinarian’s Oath

“... I solemnly swear to use my scientific knowledge and skills for the benefit of society through the protection of animal health and welfare, the prevention and relief of animal suffering, the conservation of animal resources, the promotion of public health, and the advancement of medical knowledge. ...”
Thanks and Acknowledgements

• Arthur Stewart Hall, DVM (Mentor, Pioneer in NHP Medicine)
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• World Veterinary Association
? Questions ?