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***Nonhuman Primates and Translational Research***

**Harding. Nonhuman Primates and Translational Research: Progress, Opportunities, and Challenges, pp. 141-150**

Domain 3: Research, Knowledge required to perform tasks (animal models, K3)

SUMMARY: This review serves as an introduction to the ILAR issue on primate models. Harding asserts that NHPs are critical for translational research because they are sometimes the only relevant animal models for human conditions due to of their close genetic, physiological, and behavioral similarity to humans versus other species, including rodents. Harding does indicate that research from NHP models can be combined with data from other species for a complete picture. Harding first gives credit to others who have published recent reviews on NHP translational models and provides several electronic references, such as links to the various National Primate Research Centers.  Additionally, he highlights the recent report of the “NIH Workshop on Ensuring the Continued Responsible Oversight of Research with NHP.” Harding defines ‘translational research’ to “include both efforts aimed at developing or validating NHP models that are directly relevant to studies of human disease as well as the use of NHPs in later stages of translation that help validate drug targets; evaluate the efficacy of therapies, vaccines, or devices; and test toxicity of pharmaceuticals before testing in human clinical trials.” Additionally, Harding defines ‘model’ as “a simplified representation of a phenomenon, and the very process of simplification means that some of the complex reality is lost.” Primate models have been instrumental in the design of vaccines and therapeutics for various infectious diseases, particularly HIV/AIDS where vaccines are tested and refined, and new antiretroviral therapies are assessed for safety. Harding points out the crucial role of large NHP centers in translational studies because they contribute to the availability of subjects, characterization of husbandry and welfare, improvement of breeding protocols and genetic assessment, and the development of assays and technologies. Harding mentions the role China is playing in primate breeding and contract research. The regulations governing use of NHPs in research are mentioned, but not discussed in depth.  Harding discusses challenges to primate research such as reproducibility, revised NHP requirements for grant funding, changes in supply and demand and associated costs, emerging pathogens and zoonotic agents, transportation of animals, lack of ability to use chimpanzees as a model, and reduced public support for NHP-based research.  Harding introduces the other reviews in the issue by briefly outlining the susceptibility of primates and humans to similar diseases and the usefulness of primates as models for genetic modification, HIV/AIDS, hepatitis, tuberculosis, substance abuse and neurodegenerative disease, metabolic diseases such as diabetes, cardiovascular disease, respiratory disease, female reproductive health, and defects in vision. Finally, Harding introduces the topic of environmental enrichment for primates because best practices are covered elsewhere in the ILAR issue.

N.B.  This is an introductory article.  The relevant definitions are indicated in the summary.  The other articles in the issue discuss specific topics in more detail.

QUESTIONS

1.  This use of this species has been instrumental for the development of vaccines and therapeutics for Hepatitis A, B, and C viral infections.

a.  Macaca mulatta

b.  Macaca nemestrina

c.  Macaca fasciularis

d. Pan troglodytes

e.   Chlorocebus atheiops

2.  In addition to the challenges listed above in the summary, can you think of additional challenges with working with primates in research?

3.   How many National Primate Research Centers are there?  Can you name (the location of) 4 of them?

4. In addition to the NPRCs, can you name at least one other large primate research center?

5.  True/False:  Both rodents and NHP are important species for TB research.  In fact, both display highly structured granulomas, similar to those seen in humans.

ANSWERS

1.   d. Pan troglodytes (Chimpanzees)

2.  Challenges:  High costs, variability due to genetic differences, low N in most studies, incidence of certain diseases (such as diabetes, endometriosis, etc.) that may impact research models, ethics of working with/confining intelligent primate and high rates of behavioral disorders, dealing with final disposition of healthy senior animals (e.g., finding funding and space for sanctuary), ethics of genetic models and ramifications for human medicine (e.g., animal cloning), high level training of staff required when working with NHP, and compassion fatigue when subjects require euthanasia.

3.  7; California, Oregon, Southwest, Tulane, Washington, Wisconsin, and Yerkes (Georgia) (Note the closure of the NE Primate Center in 2015)

4.  Michale E. Keeling Center, Caribbean Primate Research Center, Wake Forest University Primate Research Center, New Iberia Research Center

5.   False: One advantage over mice is that primate disease more closely mimics human disease, including the aspect of granuloma structure.

**Foreman et al. Translational Research in the Nonhuman Primate Model of Tuberculosis, pp. 151-159**

Domain 5: Regulatory Responsibilities

SUMMARY: Most Mycobacterium tuberculosis in Nonhuman Primates predominantly establishes subclinical latent infection over the lifetime of an individual.  A fraction of infected individuals rapidly progressing to active disease.  In this article it covers advanced techniques to assay various clinical outcomes of the natural progression of infection as well as therapeutics in development and novel preclinical vaccines. The article surveys the translational aspects of nonhuman primate research and argue the urgent need to thoroughly examine preclinical therapeutics and vaccines using this model prior to clinical implementation.

QUESTIONS

1.  Manifest the gamut of clinical and pathological findings in human Mycobacterium tuberculosis infection, including the ability to co-infect with Simian Immunodeficiency Virus to model HIV co-infection.

a. Zebrafish

b. Rhesus macaques

c. Mice

d. Cynomolgus macaques

e. b and c

2.  The gold standard model of Mycobacterium tuberculosis (Mtb) infection?

a. Zebrafish

b. Rhesus macaques

c. Mice

d. Cynomolgus macaques

e. b and c

3.  In comparison to human tuberculosis and in contrast to mouse models, macaques

a. Form highly structured granulomas with readily-apparent lymphocytic and macrophage tropic layers

b.  Central caseation

c. Severe neutrophilic immunopathology

d. All the above

4.  As studies delineate the subtle differences in the progression of Mtb infection in the rhesus macaques and cynomolgus macaques provide insights into the different aspects of human disease syndrome that must be protected against

a.  Rhesus macaques model for high doses of low virulence strains like CDC1551 or low doses of high-virulence strains like Erdman

b.  Cynomolgus macaques model has been exploited for vaccine studies which used moderate doses of Erdman

c. a and b

d. a only

5. Vaccination with BCG provides increased protection in cynomolgus macaques when challenged with a high dose of Erdman. T

6.  Which of the following statement is/are false?

a. Cynomolgus macaques model general exhibit lower bacterial burdens relative to BCG-vaccinated rhesus macaques.

b.  Cynomolgus macaques has been rapidly exploited for vaccine studies, which used moderate doses of Erdman.

c.  Cynomolgus macaque model use both high doses of low-virulence strains like CDC1551 or low doses of high-virulence strains like Erdman, has been used in vaccine development

d.  All above are false

7.  It has been shown in lower animal models, as well as in rhesus macaques more recently, that \_\_\_\_\_\_\_ - delivered BCG provides increased protection when compared to \_\_\_\_\_\_\_\_\_ delivery.

a. Aerosol, intradermal

b. Intradermal, aerosol

c. Sublingual, oral

d. Intramuscular, intradermal

8.  Reason why NHP maybe a better tuberculosis model than murine counterparts

a.  MTb bacilli experience greater stress in primate tissues relative to their murine counter parts

b.  NHP model can additionally be used to screen mutant strains of Mtb, particularly in genes that may not be particularly necessary in the mouse model

c.  NHP model can further be utilized to assay multiple gene knockouts via infection with transposon mutant libraries

d.  All the above

9.  Reason why murine maybe a better tuberculosis model than NHP counterparts. Is because of the ability to test novel PET tracers more effectively in mice.  True or False

10. One highly unique aspect of the NHP model of tuberculosis is the ability to co-infect macaques with SIV, as a model of human HIV co-infection.  True or False

11. Example of nonpathogenic model of SIV co-infection using\_\_\_\_\_\_\_\_.

a.  *Cercocebus atys*

b.  *Cercopithecus aethiops*

c. *Macaca mulatta*

d.   *Macaca fascicularis*

e. a and b

12. \_\_\_\_\_\_\_ plays a critical role in mediating protection from SIV-induced reactivation.

13. What technological advance in the study of NHP model of tuberculosis has emerged as a pertinent tool for measuring granuloma dynamics, determining study data output and consistent multisite comparison of data?

ANSWERS

1.  e

2. e

3. d

4. c

5. True

6.  c

7.  a

8.  d

9. T

10. T

11. e

12. CD4+ T-cells

13. Pet/CT

**Veazey and Lackner. Nonhuman Primate Models and Understanding the Pathogenesis of HIV Infection and AIDS, pp. 160-171**

Domain 3

SUMMARY:  1981 was the year of the first published cases of HIV in humans.  It was later found that HIV-1 had evolved from either Gorillas (Group O) or Chimpanzee (Group M) and that HIV-2 came from the Sooty Mangabey.  SIV infections in NHPs has been vital to our understanding of the pathogenesis of the human disease.  There have been difficulties with the use of NHPs as models of HIV in humans, but this have been due to a lack of understanding of the virus and its mechanisms rather than a failure of the model itself.

Early work with HIV infections in Chimpanzees did not account for the SIV that these animals may or may not have already been infected with.  Past or current SIV infections greatly alter the host’s response to the HIV infection.  This did help researchers to understand that HIV evolved from SIV.  It was initially thought that chimpanzees resisted progression to AIDS after experimental inoculation with HIV strains, yet this may have been a premature conclusion. Much like HIV-infected humans, some of these chimpanzees did succumb to AIDS after a long incubation period, both from experimental inoculations with HIV and from their naturally occurring SIV infections

Because of their relevance, as well as greater availability, rhesus macaques (Macacca mulatta) are the most widely used NHP model for HIV and AIDS research, especially rhesus macaques of Indian origin. Macaque susceptibility to SIV was found accidentally when they were cohoused with the Sooty Mangabey.  Rhesus macaques of Indian origin in US primate centers more consistently progress to AIDS compared to the Chinese subspecies.  Many African NHPs exhibit no AIDS like disease despite a SIV infection.

It is known that both SIV and HIV infect CD4+ cells expressing CCR5 in the GI tract very early in the infection.  As the CD4+ cells in the mucosal lining are depleted many more CD4+ cells are recruited to this area and are infected soon after they arrive.  As the viral load increases with the new population of cells coming into the tissues there is an explosion of viral replication which causes the virus to slightly mutate, thereby causing it to be more difficult for the immune system to recognize and destroy.  HIV not only recruits its own target cells for enhancing local viral replication, it also prevents an effective immune response from developing against its critical antigens by targeting effector, proliferating, and responding / homing CD4+ T cells, which are all critical for mounting primary immune responses. In addition to the CD4+ cells, the virus also infects Treg and T helper cells.

Retroviruses have been continually infecting primates for an estimated 30 million years as evidenced by the fact that the human genome and the genome of NHPs have retroviral elements.  Almost 8% of the human genome is retroviral DNA.  It is thus conceivable that retroviruses similar to SIV have guided primate evolution and the subsequent evolution of humans, perhaps more so than any other single factor.  Studying the immune system of “natural hosts” to SIV (Sooty Mangabey and African Green monkey) can help us understand how they are “winning” the evolutionary arms race against this virus.  For example, both species have lower levels of CCR5 on their cells which limits the level of infection.

Antiretroviral Therapies (e.g. tenofovir) was first shown to be effective in SIV infected macaques.  Monoclonal antibodies have also been shown to be effective in preventing transmission from mother to infant.  Macaques are the species of choice for testing vaccines against SIV and HIV.

QUESTIONS

1. Which of the following is considered to be a “natural host” for SIV?

a. *Pan troglodytes*

b. *Callithrix jacchus*

c. *Chlorocebus aethiops*

d. *Cebus albifrons*

2. What is the predominant cell type that is infected by the SIV and HIV viruses?

a. CD8+

b. CD23-

c. CD17+

d. CD4+

3. What cellular receptor is crucial for HIV infection and virus replication?

a. CCR5

b. CWW3

c. KRT7

d. TLR9

ANSWERS

1. c

2. d

3. a

**Lanford et al. The Chimpanzee Model of Viral Hepatitis: Advances in Understanding the Immune Response and Treatment of Viral Hepatitis, pp. 172-189**

Domain 3: Research

SUMMARY:Chimpanzees have been used in research since the 1930s and have been essential to many biomedical research breakthroughs.  In 1995 the NIH established a moratorium on breeding of NIH-owned chimps and in 2011 began the initial halt on research in chimpanzees.  In 2015 captive chimps were designated as endangered by the U.S. Fish and Wildlife service, which would require special permits for their use in research. Chimpanzees have been used as animal models of human diseases due primarily to their close genetic relationship with humans. Chimpanzee models have been used extensively for research on vaccines and therapies for hepatitis viruses, particularly hepatitis A, B, and C, because they represented either the only model (for hepatitis B and C) or the most appropriate model (for hepatitis A).

Hepatitis A (HAV) is a nonenveloped, single-stranded, positive-sense RNA virus belonging to the family *Picornaviridae*.  It is transmitted via the fecal-oral route and causes acute inflammation of the liver, however unlike other hepatitis viruses, HAV cannot establish long-term persistence in humans or NHPs.  Human HAV has been shown to infect several species of NHPs, including chimpanzees, marmosets, tamarins, macaques, African green monkeys, and owl monkeys. HAV was first isolated in cell culture following serial passage of virus in tamarins and marmosets. Studies in chimpanzee and other NHP models of HAV have led to the characterization of virologic and immunologic features of infection, the development of HAV vaccines, and demonstration of HAV’s unique ability to exist as both a nonenveloped virus and a membrane-cloaked, quasi-enveloped virion.

Hepatitis B (HBV) is a double stranded DNA virus belonging to the family *Hepadnaviridae*.  It is transmitted primarily via blood. Over 250 million people are chronically infected with HBV, which can progress to liver cancer and cirrhosis over time.  Research with the chimpanzee model led to the development of two vaccines for HBV, however chronic HBV infection remains a problem.  The chimp model of HBV has been essential to understanding this disease.

Hepatitis C (HCV) is an enveloped, single-stranded, positive-sense RNA virus belonging to the *Flaviviridae* family. It is transmitted via exposure to blood and blood products.  An estimated 170 million people worldwide are chronically infected with HCV; while the disease may be asymptomatic for decades, up to 20% of those infected will develop cirrhosis and are at an increased risk for hepatocellular carcinoma. End-stage liver disease due to HCV is the leading cause of liver transplantation in the United States, and HCV-associated liver cancer cases are continuing to climb. HCV liver disease is a leading cause of morbidity and mortality in HIV-infected individuals whose HIV infection is under control with antiviral medication but who will experience a more rapid progression to liver disease.  Chimps were essential to the discovery of HCV and in demonstrating that blood product contamination was a risk for HCV transmission. Chimp models of HCV infection were also used to develop methods to inactivate HCV in blood products to render them safe for use in humans, to validate molecular tools for drug discovery, and to understand the immune response to HCV infection which has aided vaccine development. Most notably, work in chimp models has led to the development of FDA-approved antivirals that can cure HCV infection in 12 weeks.  It represents the first cure of a chronic viral disease.

The advances in hepatitis virus research have been heavily dependent on the chimpanzee model, and the loss of this model will impact the development of cures for HAV and HBV, and the development of an effective HCV vaccine.

QUESTIONS

1.   Which hepatitis virus is a bloodborne pathogen, double-stranded DNA virus that can lead to liver cancer with chronic infection?

a.   Hepatitis A

b.  Hepatitis B

c.  Hepatitis C

2.  Which organization implements the Endangered Species Act within the United States?

a.  Food and Drug Administration (FDA)

b. United States Department of Agriculture (USDA)

c.  U.S. Fish and Wildlife Service (USFWS)

d.  World Wildlife Fund (WWF)

3.  Work in chimpanzee models of viral hepatitis were instrumental in the development of a cure for:

a.  Hepatitis C

b.  Hepatitis A

c.  Hepatitis B

d.  Hepatitis F

ANSWERS

1.  b

2.  c

3.   a

**Emborg. Nonhuman Primate Models of Neurodegenerative Disorders, pp. 190-201**

Domain 3: Research

SUMMARY:The report reviews Nonhuman Primates (NHPs) models of neurodegenerative disorders and gives emphasis to the main characteristic of Alzheimer’s (AD), Huntington’s (HD) and Parkinson’s (PD) disease, the current available therapies and the contributions that NHPs studies have brought to the treatments and to the process of understanding the causes of the above mentioned diseases. NHPs are considered of great interest when studying neurological disorders, thanks to their highly developed cerebral cortex, their cognitive functions, complex motor skills and neuroanatomy.

Clinical trials based on NHPs have shown to have a higher rate of translational success compared to rodent ones, especially when studies to assess efficacy and safety of a treatment are required.

Despite different and characteristic clinical signs associated to the 3 disorders, the author underlines the points of similarities at the basis of the mechanism of neurodegeneration, that are identified with inflammation, oxidative stress and protein aggregation.

AD is a progressive neurodegenerative disorder associated with memory loss, spatial disorientation, gradual deterioration of intellectual capacity and psychiatric symptoms. A combination of genetic, environmental and lifestyle factors have been proposed as a cause.

Current treatments are symptomatic and work by enhancing cholinergic transmission by inhibition of the enzyme acetylcholinesterase (for cognitive loss), by using N-methyl-D aspartate receptor antagonists (to improve memory and learning) as well as antipsychotic and antianxiety medications (for psychiatric symptoms).

Several pathological changes have been described in the post mortem brain of AD patients: synaptic and neuronal loss, oxidative damage, activated inflammatory cells and amyloid plaques, mainly composed of the β-amyloid peptide and neurofibrillary tangles, comprised of hyperphosphorylated aggregated of protein tau (the latter two considered pathological hallmarks).

As for the use of NHPs as an animal model of AD, aging or targeted lesioning of cholinergic brain areas through stereotaxic injections of the cytotoxin ibotenic acid into the basal forebrain are considered well established methods to induce such disease; however, new models are based on the intracerebral delivery of β-amyloid plaques.

Therapeutic approaches can be distinguished into neurorestorative and neuroprotective.

In the past, neurorestorative approaches that worked by using fetal tissue or stem cells to replace lost cholinergic neurons, have been proposed but currently new approaches have been developed and are rather based on neuroprotective strategies. Lifestyle modifications can decrease the risk to develop AD, in particular, with a healthy diet and exercise; studies on NHPs in caloric restriction (CR) have shown that individuals following a CR diet had better glucose regulation, better preservation of grey matter and better learning of motor tasks. As of today, nerve growth factor (NGF) represents a good neuroprotective treatment as it seems to be correlated to a decreased amyloid burden and to protective effects to cholinergic neurons.

There are other therapies that are currently tested for AD treatment and target β-amyloid such as vaccines, antibodies and inhibitors as well as modulators against ɣ and β secretase and vaccines against tau protein.

HD is a rare neurodegenerative, autosomal-dominant disorder of the central nervous system characterized by unwanted choreatic movements, behavioral and psychiatric disturbances as well as dementia. Mean age of onset of symptoms is 30-50 years but, in some cases, symptoms start before the age of 20 years with behavioral disturbances and learning difficulties (Juvenile Huntington’s disease). Treatments are symptomatic and have limited efficacy and severe side effects. Tetrabenazine (TBZ, a catecholamine depleting agent) and antipsychotic drugs are used in the treatment of the movement disorder and are useful for the severe anger that can be present in HD patients. Cognitive disorders are difficult to manage and do not respond to cholinesterase inhibitors.

Pathologically, HD is characterized by progressive loss of GABA producing neurons in the striatum; as the disease progresses, neuronal loss and atrophy are observed in the globus pallidus, thalamus and subthalamic nucleus, substantia nigra and cerebellum.

The gene responsible for HD has been identified in chromosome 4 in 1993 and a diagnostic test is currently available. The HD mutation consists of an expansion of a cysteine-adenosine-guanine repeat encoding a polyglutamine tract, in the N-terminus of the protein huntingtin.

Several NHPs models of HD have been proposed: neurotoxin models can be induced by stereotaxic injections into the striatum of N-methyl-d-aspartate receptor agonists (quinolinic acid, for example); in these models, a systematic administration of apomorphine will elicit diskinesias; an alternative option is represented by chronic administration of the mitochondrial complex II inhibitor 3-nitropropionic acid (3-NP). 3-NP intoxication induced dyskinesia and cognitive decline when tested in baboon monkeys and capuchin monkeys.

The identification of the mutated huntingtin gene opened the door to the possibility of generating genetic HD models (through lentiviral vectors in macaques) and in 2008 the first transgenic monkey model for HD was reported (Yang et al 2008).

Unfortunately, HD is still considered an aggressive disease, characterized by a certain progression of severity. Neuroprotective treatments are not available and HD patients have been treated with riluzole (a glutamate antagonist): this drug has shown to improve chorea intensity by preserving brain glucose metabolism and increasing the production of neurotrophins, but it does not improve other motor, cognitive, behavioral and functional components of the disease.

Currently, gene therapies with the potential of silencing the expression of mutated huntingtin are investigated in NHPs and represent a hope for future treatment in HD patients.

PD is characterized by the presence of motor symptoms, such as tremor, bradykinesia and postural instability. The progression of the disease may lead to incapacity to perform simple tasks, talking and walking. The onset of non-motor clinical signs can precede the motor disfunction and are often present as depression, sleep troubles and hypotension. Despite the cause of PD is still considered unknown, several risk factors have been identified and include aging, genetics and exposure to environmental toxins. As in other neurodegenerative diseases, in PD the mechanisms that are known to contribute to the neurodegeneration include inflammation, mitochondrial dysfunction, oxidative stress and protein aggregation.

Pathologically, the main characteristic is the loss of dopaminergic neurons in the *substantia nigra pars compacta* and the presence of Lewy bodies (LBs) and Lewy neurites (LNs).

As for the treatment, dopamine replacement (dopamine agonists and anticholinergics) represents the main strategy for motor symptoms, but to treat early stage diseases, other treatments are suggested, as for example monoamine oxidase B inhibitors.

Several NHP’s models have been used to study PD and are mainly represented by: aging NHPs (age-related disfunctions, similar to humans); neurotoxin models, in which PD is induced either by the administration of 6-OHDA (that impairs mitochondrial function and elicits inflammation) or MPTP that directly induces motor symptoms by conversion into its toxic metabolite by the MAO-B enzyme; Intracerebral injections of AVV and lentiviral vectors encoding for the protein a-syn in cynomolgus and rhesus macaques; creation of transgenic PD rhesus macaques, through injection of lentiviral vectors encoding for human A53T SNCA (⍺-syn gene) into fertilized oocytes.

NHPs research has been essential in order to develop new therapeutic approaches: Nigral fetal grafts were tested in monkeys and results were translated to human trials, with controversial effects.

Gene therapy has been proposed and used as an alternative to deliver enzymes responsible for dopamine production as well as for GABA synthesis. There is an ongoing phase I trial in which the viral vector suspension with AAV2-AADC is infused in the putamen of PDs patients (this has been previously tested both in rodent and NHPs models).

New therapies are now aiming to address synucleinopathy and include silencing the ⍺-syn gene or increasing ⍺-syn clearance. This has been tested in vervet monkeys with interesting results. Neuroprotective strategies are still needed, considering the progressive nature of PD; despite the fact that several promising compounds have been tested, as for example, MAO B inhibitors, dopaminergic agonists, gangliosides and nicotinic agonists, none of them as shown clear clinical efficacy.

Although NHP models of neurodegenerative disorders continue to be improved, they are not perfect and many still need to be validated. Neurodegenerative disorders are complex, and their presentation varies between individuals, so one model will no provide necessarily all needed answers.

QUESTIONS

1.  It has been shown that at the base of neurodegeneration there are similar mechanisms, common to the three diseases; name the main ones.

2.  AD patients present both cognitive/intellectual symptoms as well as psychiatric ones. Provide some examples.

3.  True or False: the loss of cholinergic, serotoninergic and catecholaminergic neurons is thought to be a leading factor in the decline of cognitive function seen in AD.

4.  Explain why NGF is considered among the favorite tropic factors in the neuroprotective strategy for AD.

5.   True or False: HD is characterized by progressive loss of GABA producing neurons in the striatum.

6.   What are the main motor symptoms observed in HD patients?

7.  What is considered to be the mainstay therapy for PD motor symptoms?

8.   Why are Lewy bodies and Lewy neurites mentioned when talking about PD?

ANSWERS

1.   Inflammation, oxidative stress, protein aggregation.

2.   Amnesia, aphasia, apraxia, agnosia (cognitive and intellectual); personality changes, depression, hallucinations, delusions (psychiatric).

3.   Loss of cholinergic neurons is the leading factor; loss of serotoninergic and catecholaminergic neurotransmission are proposed as contributing factors.

4. It protects cholinergic neurons from degeneration, sustains cholinergic function; safety, toxicity and efficacy of fibroblasts genetically modified to deliver NGF have been tested in primates with positive results.

5.  True

6.   Dystonia, involuntary choreiform movements of the arms, legs, head, face and upper body.

7.  Dopamine replacement. This is usually combined with carbidopa.

8.   Lewy bodies (LBs) are intracytoplasmic inclusions and with Lewy neurites (LNs) are found in neurons of the central and peripheral nervous system. Together with the loss of dopaminergic neurons in the substantia nigra they are considered as the pathologic hallmarks of PD. LBs and LNs are composed of over 70 different proteins and α –syn is their main component.

**Banks et al. Utility of Nonhuman Primates in Substance Use Disorders Research, pp. 202-215**

Domain 3: Research

SUMMARY: This ILAR Review article highlights three advantages of using NHPs as preclinical substance abuse research subjects, as opposed to rodents and other laboratory animal species.

ONE: NHPs are larger than rodents, offering the use of larger, double lumen catheters for multi-drug studies, the catheters can be placed in multiple peripheral and internal vessels, and the catheters are able to remain patent for longer.   These advantages in size allow NHPs to be used to facilitate drug development strategies for substance abuse disorders over long periods of time, more parallel to human studies.

TWO:  Non-invasive imaging techniques such as MRI or PET can be used in NHPs to help understand the mechanistic problems and consequences of substance abuse, both acute and chronic, as well as sexual dimorphism effects of substance abuse.

THREE: NHP that are used in substance abuse studies provide more concordant results with behaviors in humans, which allows for varying pharmacokinetic studies to translate to human substance abuse studies.

The authors conclude that NHPs should continue to be used for substance abuse disorder studies.

QUESTIONS

1. Which species of monkeys have been predominantly used for substance abuse research?

a.  *Macaca mulatta*

b.  *Macaca fascicularis*

c.  *Saimiri scirureus*

d.   *Papio anubis*

e.   All of the above

2. True/False: In addition to the reasons stated in the introduction, NHPs are more ideal research subjects for substance abuse disorders than rodents for the following reasons.

a.  NHPs and humans share behavioral phenotypic similarities, including social behavior and early life stress

b.  NHPs and humans produce different metabolite of 3,4 methylenedioxrmethamphetamine, but assimilation of the drug into the system is similar

c.   Imaging studies correlate closely in NHPs and humans due to phylogenetic similarities of brain organization

d. Immune function similarities between NHPs and humans have allowed candidate immunopharmacotherapies to be developed for substance abuse disorders

3. When is a drug considered to produce “reinforcing” effects?

a. When some dose of the drug maintains constant pharmacokinetic levels in the blood stream

b.  When the animal consistently performs the behavioral task at 80% completion or better

c.   When some dose of the drug maintains significantly higher rates of behavior than vehicle

d.   When the substance abuse behavior lessens by >50%

4. Why is it advantageous to have a long catheter life in NHPs involved in substance abuse research?

a.  Facilitates repeated measures within subject testing

b.  Facilitates use of relatively complex behavioral tasks that require extensive training

c.  Allows evaluation of long-term dosing regiments with test compounds

d.  All of the above

e.   None of the above

5. Which species is the only research animal for which novel cannabinoid compounds are robustly self-administered?

a.  *Macaca fascicularis*

b.   *Rattus rattus*

c.  *Saimiri sciureus*

d.   *Sus scrofa*

ANSWERS

1. a. In addition,*Chlorocebus sabaeus aiethops* (African Green Monkey), has been used

2. a. True; b. False; c. True; d. True

3. c

4. d

5. c

**Mustari. Nonhuman Primate Studies to Advance Vision Science and Prevent Blindness, pp. 216-225**

Domain 3: Research

Primary Species: Macaques (Macaca spp.)

SUMMARY: Most primate behavior is dependent on high acuity, binocular vision. To see objects clearly, the image must be on or near the fovea of each eye, a structure specific to simian primates among mammals. Other equidistant elements fall on the Vieth-Müller horopter and impinge on corresponding points of each retina. The oculomotor system must maintain precise eye alignment and track moving targets, called motor fusion, to produce a single image for later stages of the visual system, known as sensory fusion. Sensory fusion allows for the percept of stereotopic depth for targets slightly in front or distant to the horopter (Panum’s area). If a target is outside of Panum’s area, it is perceived as two distinct objects.

The retina derives from an outpouching of the embryonic forebrain, and, thus, is a part of the central nervous system containing multiple neuronal elements: photoreceptors, bipolar neurons, and retinal ganglion cells (RGCs). The fovea contains the highest density of retinal neurons, so visual acuity is highest at that central point. Peripheral aspects are for visual orienting and motion perception but have lower acuity. A “blind spot” exists where the RGC axons leave the
eye at the optic disc. Information is delivered via distinct visual pathways from the nasal retina to the contralateral primary visual cortex (V1) and the superior colliculus (SC) via partial decussation at the optic chiasm. The temporal retina RGCs project ipsilaterally. The different layers of visual information enter the lateral geniculate nucleus (LGN) for combination in V1. LGN neurons are sensitive to visual information from either the left or the right eye, and different classes of RGCs project to different LGN layers. A large portion of V1 is dedicated to central vision, so lesions in V1 can lead to cortical blindness in the contralateral hemifield. Visual
information from V1 is sent via two streams, dorsal and ventral, to extrastriate cortical areas where it is extracted. The dorsal stream is made of V1 neurons and projects to the middle temporal visual area (MT) and deals with visual motion, including depth. The ventral stream
involves projections to V2 and V4, which play a role in determining the color and identity of an object.

In primates, the visual and oculomotor systems are immature at birth and sensitive to quality of binocular visual and eye movement experience in the first weeks to months. Disruption during that critical period can lead to improper alignment (strabismus), amblyopia, unsteady gaze (nystagmus, or defective eye movements. V1 neurons, for example, require binocular visual experience to develop normally, and early restriction can lead to strabismus or amblyopia.
This can be demonstrated in the different patterns of right or left eye “dominance columns” in V1, which don’t develop normally in the absence of binocular experience. If one eye is compromised during development, it will “lose ground” in V1, which may contribute to
amblyopia. In humans and other primates with strabismus, oculomotor neurons may be driving cross axis eye movements rather than the strabismus being a primary muscular problem. That cross-axis movement can send a signal to the paramedian pontine reticular formation and
brainstem areas, which code the retinotopic movement and abnormal saccades and continue the misalignment. Optical methods to create these defects in NHP include atropine to defocus one eye, daily alternating monocular occlusion with an opaque lens, and wearing prism
goggles to deviate the optical axis of one eye during the first three months of life. Surgical methods include resection or recession of extraocular muscles, or the use of botulinum toxin to weaken them. Growth factor IGF-1 may also be used locally to strengthen individual muscles.

Treatment modalities tested in non-human primates include intraocular lens implant to treat congenital cataract, gene therapy using subretinally injected viral vectors for retinal defects such as Leber’s congenital amaurosis-type 2 and color blindness, human embryonic stem cell (hESC) therapy for Stargardt’s macular dystrophy and retinitis pigmentosa, and neuroprosthetic arrays to activate visual pathways via the retina or V1 in blind individuals. Future directions may include further stem cell treatments, gene editing therapies with Crispr-Cas9 technology, and incorporation of optogenetic modalities to neuroprosthetics.

QUESTIONS (T/F)

1. V1 neurons are spatially organized in layers corresponding to information from each eye, which are completely developed at birth in primates.
2. Strabismus in primates can be induced either by oculomotor muscle strengthening or resection or by providing monocular stimulus during the developmental period.
3. The retina, like most layers of the eye, is derived from embryonic endoderm.
4. Visual acuity and normal oculomotor movements in primates depend on neonatal experience in binocular vision.
5. The fovea is a retinal structure in primates that allows for central visual acuity.

ANSWERS

1. F
2. T
3. F
4. T
5. T

**Capitanio. Naturally Occurring Nonhuman Primate Models of Psychosocial Processes, pp. 226-234**

Primary Species: Macaques (Macaca spp.)

Domain 3: Research

SUMMARY

Introduction: In contrast to induced nonhuman primate models of human psychosocial processes, naturally occurring models may more closely parallel situations in humans.  For instance, unlike an induced model in which a behavioral phenotype is generated using known mechanisms linked to the development of that phenotype, naturally occurring phenotypes in nonhuman primates may mirror that of humans by being the result of a heterogeneous set precipitating conditions.  Disadvantages of naturally occurring models, though, may include large sample size requirement as well as the necessity of identifying naturally occurring phenotypes in the first place.  This review article provides three examples of the development of naturally occurring models of psychosocial phenomena in nonhuman primates.

Loneliness and Health: Loneliness in humans is a risk factor for mortality and poor health outcomes.  Biologically, loneliness may be considered an aversive state that motivates behavior change to satisfy a biological imperative, similarly to hunger.  Induced models of loneliness in nonhuman primates, e.g. isolating an animal and observing changes in behavior and physiology, may be inadequate because they cannot distinguish the objective alteration of the animal’s social environment from the animal’s subjective experience.  A naturally occurring model, on the other hand, may be able to delineate why some individuals find a specific social situation fulfilling while others do not.   The authors described a nonhuman model of loneliness that was developed by having animals rated on a series of personality traits, then identifying animals that had low scores for sociability.  Lonely animals were differentiated by introverted animals based on the frequency of social initiations with animals considered more likely to be “safe,” such as juveniles or adult females; lonely animals should have higher scores for social initiations (an indication that lonely monkeys might want more social interaction than they currently have), while introverted animals should have lower scores (indicating they are not interested in social interactions).  While this trend was observed for simple social initiations, e.g. approaches and walkbys, lonely monkeys and introverted monkeys exhibited similar frequencies of complex interactions such as contact and grooming.  The authors suggested that this model of loneliness revealed potential underpinnings of human loneliness, such as fear of rejection.  The authors went on to demonstrate some physiological parallels between lonely monkeys and lonely humans – pattern of leukocyte gene expression, downregulation of glucocorticoid receptor function, and upregulation of proinflammatory transcription factor.

The authors described the establishment of an assessment program referred to as Biobehavioral Assessment (BBA) in order to provide guidance to colony management staff to identify animals with different behavioral phenotypes for the purposes of experimental design, colony management, and assessment of experimental outcomes.

Behavioral Inhibition and Asthma: Psychosocial components of asthma have been demonstrated, but many human-based studies rely on self-reporting rather than physiological lung function.  As temperament inhibition has been proposed as a potential risk factor for asthma in children, a nonhuman primate model of inhibited temperament was developed from BBA.  A retrospective analysis showed that emotionality, vigilance, and plasma cortisol concentrations predicted which monkeys showed airway hyperresponsiveness, a critical feature of asthma.  One caveat, however, was that this model appeared to be more for nonatopic asthma than atopic asthma.

Social Functioning and Autism: Variations in levels of sociability were also utilized to develop a naturally occurring model of autism spectrum disorder.  Ability to recognize faces and ability to respond appropriately to social signals were assessed in the BBA program using a test for novel face recognition and a video playback test that gauged responses to aggressive and neutral behavior.  These measures were correlated to different levels of sociability.

QUESTIONS

1. According to the Animal Welfare Regulations, the ambient temperature in an indoor housing facility for nonhuman primates must not fall below \_\_\_\_ or rise above \_\_\_\_ for more than 4 consecutive hours when nonhuman primates are present.

a. 45 and 75

b. 45 and 85

c. 55 and 75

d. 55 and 85

2. According to the Animal Welfare Regulations, nonhuman primates weighing 10-15 kg are considered Group \_\_\_ and require \_\_\_ ft2 of floor space.

a. 2, 3.0

b. 3, 4.3

c. 4, 6.0

d. 5, 8.0

ANSWERS

1. b

2. c

**Cox et al. Nonhuman Primates and Translational Research – Cardiovascular Disease, pp. 235-250**

Domain3: Research; K3 - Animal models (spontaneous and induced)

SUMMARY: Studies describing metabolic and physiologic aspects of CVD, and studies investigating genetic and epigenetic mechanisms influencing CVD initiation and progression, have been conducted in baboons (Papio hamadryas), rhesus macaques (Macaca mulatta), cynomolgus macaques (Macaca fascicularis), snow monkeys (Macaca fuscata), and vervet monkeys (Chlorocebus aethiops sabaeus).

Major advantages of studying CVD in NHPs are their genetic, metabolic, and physiologic similarities with humans, and equally important is the ability to control diet, environment, and breeding. In addition, these NHP species are genetically and phenotypically heterogeneous, providing opportunities to study gene by environment interactions that cannot be feasibly studied in inbred animal models.

Baboon plasma cholesterol and triglyceride (TG) concentrations respond to dietary cholesterol, fat, and carbohydrates similar to those of humans. Chronic feeding of a high-fat diet leads to fatty streaks and atherosclerotic plaque. In addition, a diet high in simple carbohydrates and fat causes baboons to develop increased body fat and TG concentrations, altered adipokine concentrations, and altered glucose metabolism, consistent with observations in humans.

Rhesus macaques, cynomolgus macaques, vervet monkeys, and snow monkeys also develop atherosclerosis when fed a high-fat diet long term

There are also challenges to studies of CVD in NHP: longer lifespans and longer health spans; more expensive to maintain, require greater amounts of experimental reagents for testing of therapies and drugs, NHP genomes are less well annotated than mouse and rat genomes, which poses challenges for identifying and validating genetic variants that influence CVD initiation and progression. Some of these challenges can be overcome by using comparative genomic analysis and annotation tools, such as those available through the UCSC Genome Browser.

Studies to identify genetic loci and genetic variation that are correlated with CVD risk have been conducted with the pedigreed baboon colony at the Southwest National Primate Research Center (SNPRC). Initial studies identified correlations of variation in apolipoprotein(a) isoform frequencies with lipoprotein(a) serum concentrations, genetic loci related to hypertension, loci related to low density lipoprotein (LDL) size phenotypes, and loci regulating plasma levels of gamma glutamyl transferase and albumin, genetic variants that regulate high density lipoprotein cholesterol (HDL-C) plasma concentrations and to identify novel candidate genes that regulate LDL-C plasma concentrations. Plasma HDL-C concentrations are also heritable in rhesus macaques

Addition of whole genome sequence data for the SNPRC pedigreed baboon colony, as well as a pedigreed snow monkey colony (Oregon National Primate Research Center) and pedigreed rhesus macaque colonies at the other NPRCs, will accelerate the identification of functional genetic variants that influence CVD initiation and progression.

NHP models such as the baboon, with their strong similarity in dietary adaption with humans (both being highly omnivorous) as well as the large extent of genetic conservation and the ability to control diet and breeding, presents a unique opportunity to investigate gene by diet interactions under tightly controlled experimental conditions.

Many experiments (see article for more details) have shown that experimental dietary manipulations of NHPs have biological impacts that closely resemble those of humans whose diets have high levels of cholesterol and fat and that there is considerable individual variation in response to these dietary components.

MiRNAs have been implicated in a plethora of biological pathways, including cholesterol metabolism, and contribute to the progression of a number of diseases. Because many genes can be regulated by a single miRNA and different miRNAs can target one gene, miRNAs fine-tune gene expression and coordinate genetic networks underlying common complex human diseases, such as CVD.

Investigation into the role of miRNAs on LDL-C variation in half-sib baboons discordant for serum LDL-C concentrations, challenged with HCHF diet for 7 weeks, revealed 226 differentially expressed miRNAs. Overlaying these miRNAs onto genetic networks to identify molecular mechanisms that may regulate variation in LDL-C revealed seven candidate miRNAs These findings demonstrated that liver miRNAs are responsive to diet, that miRNA response to a HCHF diet challenge differs among baboons with different LDL-C serum concentrations, and that miRNAs may regulate LDL-C variation.

Serum HDL-C concentrations are inversely associated with CVD, and HDL-C particles are essential for sequestering cholesterol from organs and macrophages. MiR-33 and miR-27b have been implicated in cholesterol synthesis and fatty acid oxidation.

A study of 200 healthy individuals showed a positive correlation between OSBPL6 hepatic expression and plasma HDL-C concentrations. These findings suggest that miR-33 and miR-27b regulate variation in HDL-C metabolism

In NHPs, only three studies have prospectively investigated the effects of prolonged calorie restriction (CR) on metabolism. In these three experimental paradigms using Indian-origin rhesus, Chinese-origin rhesus, and cynomolgus macaques, CR consistently positively impacted insulin sensitivity. Although diabetes and CVD are tightly linked, CR did not consistently show a similar positive impact on lipoprotein and triglyceride profiles or extent of atherosclerotic lesions. It is possible that genetic variation plays a role in these differences, supporting the need for further studies to understand genetic mechanisms central to cardiovascular health

*Suboptimal maternal nutrition during pregnancy* alters fetal development. A baboon model of fetal intrauterine growth restriction (IUGR) has been developed to study tissue, cellular, and molecular changes in the developing fetus and the impact on offspring health. Fetal studies showed that maternal CR (MCR, 30% reduction of controls fed ad libitum) influences the fetal kidney transcriptome and renal tubule structure

In addition, MCR impacts the fetal liver transcriptome and energy storage with epigenetic changes in the energy management enzyme PEPCKI. Similar findings have not been reported in non-primate models of IUGR, suggesting fundamental molecular and cellular differences between primate and non-primate response to a suboptimal in utero environment. Furthermore, IUGR juvenile offspring in this model show signs of hypertension, insulin resistance, and metabolic syndrome.

The effects of cardiac function on IUGR persist postnatally and baboons exhibit cardiac abnormalities similar to humans. One study found differences in cardiac dysfunction by comparison with non-primate models, indicating either variation in IUGR protocols or differences between primate and non-primate cardiac physiology.

A growing body of evidence links early-life nutritional adversity to the development of long-term metabolic disorders. Supporting the programming hypothesis is the increasing prevalence of maternal obesity and excess maternal weight gain associated with increased risk of obesity in offspring and metabolic-related diseases such as diabetes and CVD. Maternal obesity-induced developmental programming has been validated in mice, rats, sheep, and NHPs.

In addition, some studies observed accumulation of lipid in NHP fetal livers, which may be an upstream event that influences CVD and metabolic disease risk in offspring of obese pregnancies. Also, of interest is the observed disruption of the methionine cycle in obese pregnant baboons, suggesting an epigenetic mechanism by which obesity during pregnancy may impact fetal development.

Nonalcoholic Fatty Liver Disease (NAFLD): Recent studies suggest that NHP species develop NAFLD-like symptoms similar to humans. A NHP model would have benefits over current rodent models due to extensive human-NHP genetic, physiologic, and nutritional behavior similarities. Baboons also develop significant liver steatosis, initial signs of hepatic inflammation, and insulin resistance on a HCHF diet. Steatosis can be observed within a few weeks, much more rapidly than reported in other NHP species.

The rate of steatosis is variable among animals, suggesting that genetic factors also influence the development of steatosis, similar to humans. Therefore, it is likely that baboons develop all characteristic features of NAFLD and NASH, including the hepatic pathologies and the systemic complications when exposed to a HCHF challenge diet for extended periods.

Chagas Disease:like many other wild mammals, monkeys serve as a reservoir for T. cruzi. Although T. cruzi in nature is confined to the Americas, ranging from central and the southern half of the United States to the deep south of South America, Old World monkeys also can be experimentally infected with the parasite. Old World monkeys, which are phylogenetically and physiologically more similar to humans. than are New World monkeys, are the species of choice for translational research on Chagas disease. The species that are most frequently used are baboons, rhesus macaques, and cynomolgus macaques.

Clinical therapeutic drug trials have been conducted with great optimism after stunningly successful results from experiments with mice, and none has produced in humans the favorable results that were anticipated. The results of clinical trials on Chagas disease are particularly difficult to interpret, because it is not possible to determine with certainty if the parasites have been eliminated from heart tissues. Only in animal models can the absence of parasites in tissues be determined; first, by immunosuppression, which often leads to increased blood parasitemia, and then by direct examination of tissues by PCR after euthanasia.

Moreover, the course of the disease in humans is gradual over many years, so it is not possible to ascertain in a follow-up of only a few years if candidate therapies lead to a reduction in disease progression or not. However, Old World monkeys can provide reliable information in regard to disease progression in an experiment of only a few years of duration for two reasons:

First, While the average duration from the time of infection to the development of clinically detectable symptoms might be many years, the accumulation of lymphocytes in the heart and consequent inflammation (myocarditis) occur continuously as the immune system responds to the presence of parasites. Therefore, in the NHP model, euthanasia and histological examination of the heart can determine if disease is progressing

Second, just as Old World monkeys and their immune systems age about three times faster than humans, so also Chagas disease progresses about three times faster in Old World monkeys, therefore results would probably be observed in two to three years in Old World monkeys.

Microbiome—Diet Responsiveness:Most animal studies of the gut microbiota have been done in a rodent model. recent studies have shown that diet has a profound impact on the microbial composition of the NHP gut microbiota.

Metabolic and gut microbiome development is different in formula-fed infants from breast-fed infants and that the choice of infant feeding may have future health consequences, such as an increased susceptibility to CVD.

Studies show that the NHP microbiota is species-specific and altered by environmental factors (e.g., diet, geography, and social factors). They also reveal that the abundance of Firmicutes and Bacteroidetes in the NHP microbiota is similar to that of healthy humans. Also, chimpanzees (Pan troglodytes), have similar gut microbiota. Although these findings make a compelling case for using NHPs to study how diet affects the gut microbiota of humans and influences CVD, a recent study suggests that such research should be done with caution. Their findings indicate that host-gut microbe interactions, genetic and physiological response differ in humans and vervets

Stem cell therapies are being developed to treat CVD by two fundamental mechanisms: the stimulation of angiogenesis to develop improved vascularization of damaged heart or vascular tissue, and the stimulation of myogenesis to repair damaged cardiac muscle. Mouse models have pioneered preclinical research, but the immunological and physiological differences between mice and humans limit the capacity to translate many results obtained from mice directly to human subjects. Moreover, it is impossible to scale many of the results obtained from mice to human applications.

The most widely used NHPs in stem cell therapies research are rhesus and cynomolgus macaques and baboons.

Male baboons can weigh as much as 30 to 40 kg, so this species among all NHPs used in research is the most appropriate for investigations where scaling to human size is an important factor. Pluripotent embryonic stem cell (ESC) lines, as well as induced pluripotent stem cell (iPSC) lines, have been developed from all three of these species, and these species are all used in research aimed at developing stem cell therapies and interventions. Old World NHPs also are invaluable models for research on multipotent adult stem cells, which exist throughout the body after development.

*Potential Therapy Involving Bone Marrow Stem Cells (BMSCs)*: BMSCs are continuously released from bone marrow and serve to repair damage to the vascular endothelium, and they are released in large quantities in response to acute, severe damage such as occurs during myocardial infarction. They also are released in large numbers in response to some cytokines, particularly granulocyte colony-stimulating factor (G-CSF). A baboon model has shown that BMSCs mobilized by GCSF do not have the same therapeutic capacity as those mobilized naturally in response to acute, severe vascular damage suggesting that alternative strategies for inducing the release of BMSCs for clinical purposes might be more therapeutically effective than administration of G-CSF. This result illustrates the value of NHP models for developing applications of adult stem cell therapies for cardiovascular therapies.

*Potential Therapy for Repair of Vascular Endothelium*:Damage to the arterial endothelium is a critical early event in atherogenesis and peripheral vascular disease, and deterioration of the vascular endothelium occurs naturally throughout life. A baboon model has given proof-of-concept for in vivo experiments in which the arterial endothelium has been damaged by a balloon catheter. Although the inoculated stem cells will be broadly distributed throughout the vasculature (unlike in the ex vivo model, which involved only a segment of an artery), it is expected that the stem cells will home to and preferentially attach to the injured site, as occurs when BMSCs are released into the circulation after vascular injury.

*Construction of Bioengineered Arterial Segments:*Much more ambitious potential use of pluripotent stem cells is the construction of bioengineered arterial segments that could be used to replace diseased sections of arteries. The concept is to develop separate lineages of endothelial progenitor cells and smooth muscle progenitor cells, which can be used to seed the inside and the outside, respectively, of a segment of tubular scaffold placed in a bioreactor. Progress toward this goal has been made (unpublished data) using baboon ESCs, although the segments produced in initial in vitro experiments were not sufficiently robust for transitioning to in vivo experiments.

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Detection and Prevention of CVD: Despite recent scientific advancements, treatment options, including statins, angiotensin-converting enzyme inhibitors, beta blockers, and other drugs, the prevalence of CVD continues to increase, underscoring the need for new therapeutic strategies.

*HMG-CoA Reductase Inhibitor (Statins)*: Statins are synthetic molecules that bind and decrease the activity of HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis pathway. Statins are the most widely used therapy for lowering plasma LDL-C. Despite the positive effect of statins on cardiovascular endpoints, there are concerns regarding the adverse effects of statin therapy. NHP models of atherosclerosis provide an opportunity to comprehensively investigate the side effects of statins and the underlying mechanisms.

*PCSK9 Inhibitor (Evolocumab)*: The proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor Evolocumab is a recently discovered monoclonal antibody therapy for lowering plasma LDL-C. PCSK9 asserts its function by binding to and degrading the LDL receptor (LDLR), impacting the LDLR recycling process necessary for cholesterol absorption in hepatocytes. The potency of Evolocumab has been evaluated in NHPs, it was shown to lower LDL-C in healthy and in diet-induced hypercholesterolemic cynomolgus macaques (Liang et al. 2012). In addition, Evolocumab therapy significantly lowered LDL-C in monkeys treated with statins. Currently there is inadequate information about PCSK9 therapy, including the long-term CVD effects, the age range for treatment, and interaction with other therapies. NHP models provide an opportunity to evaluate the age range of treatment, drug interaction, toxicity, and effects on atherosclerosis.

Other lipid-lowering therapies that have not been evaluated in NHPs include apoB and microsomal triglyceride transfer protein and NPCILI inhibitor (Ezetimibe). These therapies are designed to target genes involved in lipid metabolic pathways.

Genome Editing:Recent studies demonstrate feasibility of gene knockout by CRISPR/Cas9 in rhesus macaques, indicating that this system can be used to test candidate functional variants in NHPs, overcoming one of the major limitations of working with NHP models by comparison with mice. However, the quality of NHP genome annotations currently limits the ability to design guide RNAs for genetic manipulation. With improved quality of NHP genome annotation, NHP genomic manipulation studies will likely progress rapidly.

QUESTIONS

1. T/F: Age-related comorbidities such as dyslipidemia and diabetes are accelerated proportionally 3 to 4 times faster in NHP than in humans.
2. Of the usual models of CVD, which one can develop it spontaneously?
	1. Baboons
	2. Rhesus and cynomolgus macaques
	3. Snow monkeys
	4. Vervet monkeys
	5. All of the above
3. Which of the following has the closest lipid metabolism with regard to humans?
	1. Baboons
	2. Rhesus and cynomolgus macaques
	3. Snow monkeys
	4. Vervet monkeys
	5. All of the above
4. T/F: Rodents models like the Zucker rat develop obesity, dyslipidemia, and insulin resistance but fail to develop the entire complement of liver pathologies (Nonalcoholic fatty liver disease), except when on a challenge diet.
5. T/F: Evolocumab (PCSK9 Inhibitor) has been evaluated in NHPs, showing to lower LDL-C in healthy and in diet-induced hypercholesterolemic cynomolgus macaques

ANSWERS

1. T
2. e
3. a
4. F - they do not develop them even on a challenge diet
5. T

**Havel et al. Use and Importance of Nonhuman Primates in Metabolic Disease Research: Current State of the Field, pp. 251-268**

Domain 3: Research

SUMMARY: Nonhuman primates are a valuable model for the research of obesity and its metabolic consequences. Given that NHPs are metabolically similar to humans, they also bridge the gap between rodent models and clinical studies in humans. The genomes of several NHP special such as rhesus macaques and baboons have been sequenced, enabling comparative genetic studies with humans. As the prevalence of obesity increases on a global scale, there is a demand for novel ways to prevent and treat these widespread metabolic conditions including dyslipidemia, atherosclerosis, type 2 diabetes, hepatic steatosis, steatohepatitis, and hepatic fibrosis. The NHPs most commonly used for metabolic research include the rhesus macaque (*Macaca mulatta)*, cynomolgus macaques (*Macaca fascicularis),*baboons (*Papio spp)*, marmosets (*Callithrix spp)*, and African Green Monkeys (*Chlorocebus spp).*They have been valuable in understanding the regulation of food intake by gastrointestinal peptides including cholecystokinin, glucagon-like peptides 1 agonists and peptide-YY. Studies performed in NHPs have also proven important for establishing the role of the autonomic nervous system in post-prandial insulin secretion and the regulation of glucagon secretion during insulin-induced hypoglycemia as well as compensatory insulin secretion in glucocorticoid-induced insulin resistance. Experimental diets that are high in fat and/or simple sugars are being increasingly used to induce rapid weight gain and accelerate metabolic disease progression in NHPs. One common diet is a high-fat, high-sugar (HFHS) diet, ranging from 30%-40% of calories from fat and increasing percentages of calories from simple sugars. These diets have been useful in investigating the effects of resveratrol supplementation on a number of metabolic outcomes in rhesus macaques, including preservation of beta-cell differentiation.

Studies using NHPs have also provided important insights into the genetic and epigenetic factors that also contribute to the risk for developing obesity. Impaired glucose tolerance during a dynamic intravenous glucose tolerance test (IVGTT) is also observed in rhesus macaques with diet-induced obesity/metabolic syndrome with a relatively modest (20%) increase of the glucose area under the curve over a 60-minute period following i.v. glucose administration. Obese NHPs are insulin resistant as demonstrated by the use of hyperinsulemic-euglycemic clamps, IVGTTs, and fasting insulin concentrations. The prevention of insulin resistance in diet-induced obese and insulin-resistant rhesus macaques by dietary omega-3 fatty acids from fish oil and an improvement in metabolic responses to pharmacological interventions is evident by both reductions of fasting plasma insulin concentrations and reduced first and second phase glucose-induced insulin secretion during dynamic metabolic testing. In an NHP model of diet-induced metabolic syndrome, fasting TG concentrations increase from an average prediet baseline level of 80mg/dL into the metabolic syndrome range(>150mg/dL) within 1 month on the high-fructose diet. The increase in fasting TG and decrease in HDL-C levels are much more marked in animals that develop overt diabetes after 1 year on the high-fructose diet; this is indicative of diabetic dyslipidemia and has been previously reported during the natural history of the progression from insulin resistance to overt type 2 diabetes in middle-age rhesus macaques.

NHPs have also been used to evaluate the pharmacological profiles of several new compounds for the treatment of type 2 diabetes such as the insulin secretogogue nateglinide and gemigliptin, a novel dipeptidyl peptidase-4 inhibitor. Although much of the current research is focused on type 2 diabetes, there is an increasing prevalence of type 1 diabetes around the world.  Although autoimmune diabetes is not well recognized in NHPs, chemically induced islet cell lesions using the beta cell toxin streptozotocin (STZ) or surgical pancreatectomy have been used to model type 1 diabetes in rhesus and cynomolgus macaques, baboons, and vervet monkeys. Diabetes associated with insulin resistance can also be induced by administering nicotinic acid to baboons with reduced beta-cell mass produced with a low dose of STZ. Measurements of glucose tolerance and insulin sensitivity are central in the investigational evaluation of metabolic status in humans and in NHP models. Hyperinsulinemic-euglycemic clamps, often considered to be the optimal method for assessing insulin sensitivity, have successfully been used in NHP species. Intravenous glucose tolerance tests (IVGTTs) have also been used to compare parameters of insulin sensitivity and glucose effectiveness in control and energy-restricted rhesus.

NHP models have also successfully been used to evaluate the hemodynamic effects of existing and novel therapies aimed at treating hypertension. Extensive preclinical studies in a cynomolgus monkey were recently performed to demonstrate the early clinical profile of a highly selective oral inhibitor of aldosterone synthase. NHP models have also been used to reveal the mechanisms underlying how poor maternal health affects fetal programming and imparts risk for future metabolic disease in offspring. Specifically, a Japanese macaque model has demonstrated that consumption of a maternal Western-style diet causes placental dysfunction, tissue-specific changes in the mitochondria in the offspring, widespread inflammation, hepatic steatosis, and broad developmental changes in the liver, skeletal muscle, brain and pancreas. New approaches for managing fetal nonalcoholic fatty liver disease(NAFLD) as well as nonalcoholic steatohepatitis (NASH) are needed. Studies in the baboon model show that defects in the long-chain fatty acid metabolism along with increased hepatic TG accumulation resulted in significant hepatic insulin resistance. Recent studies in vervet monkeys also show that dietary exposure to fructose also leads to increased fat in the liver and hepatic damage.

The anatomical structure and relatively larger body mass make NHPs well-suited for a number of metabolic imaging studies such as DEXA scanning, CT, MRI, and contrast-enhanced ultrasound. In addition, the two major approaches for assessing energy expenditure in NHPs are the calorimetry chamber technique for measure oxygen consumption and carbon dioxide production. A recent study demonstrated that antagonism of a microRNA expressed in the liver in vervets targeting genes involved in lipogenesis could provide a potentially novel therapy to treat dyslipidemia in humans. An important future direction for the use of NHPs is the investigation of the relationships between diet-induced metabolic dysfunction, cognitive decline and dementia.

QUESTIONS

1. T/F: We have not yet used hyperinsulinemic-euglycemic clamps on NHPS in a research setting

2. T/F: Type 1 diabetes is well recognized in NHPs

3. T/F: We have sequenced the rhesus macaque genome

4. Extensive preclinical studies on hypertension in a cynomolgus monkey were performed to demonstrate the early clinical profile of a highly selective oral inhibitor of which of the following:

a. Aldosterone synthase

b. Calcium channels

c. Cholecystokinin synthase

d. None of the above

ANSWERS

1. False

2. False

3. True

4. a

**Miller et al. Nonhuman Primate Models of Respiratory Disease: Past, Present, and Future, pp. 269-280**

Domain 3, K3

**SUMMARY**: Although genetically modified rodents continue to be very important to the research of respiratory diseases, nonhuman primates are essential for understanding the progressive pathogenesis of conditions that are initiated during early life.  Vaccine trials in nonhuman primates have been crucial for confirmation of safety and protective efficacy against infectious diseases of the lung in a laboratory animal model that recapitulates pathology observed in humans.  Significant progress in the development of nonhuman primate models of respiratory disease have been made over the past several decades, but viable pharmaceutical candidates for permanent restoration of lung function and mucosal immunity remain elusive.  The prolonged nature of chronic respiratory conditions presents a major obstacle for this animal model but expanded use of new world monkey species with accelerated lifespans may potentially yield critical information on the natural progression of obstructive airways and lung function decline in the human population.

**COMPARATIVE ANATOMY AND PHYSIOLOGY OF THE NONHUMAN PRIMATE LUNG**

In comparison with other laboratory animals, the nonhuman primate lung most closely resembles the human lung in structure, physiology, and mucosal immune mechanisms.  In both humans and rhesus macaques, the left lung is divided into two distinct lobes.  In the rhesus, the right lung is divided into four lobes, whereas the human right lung is divided into three lobes.  Identifying the mechanical factors within the pulmonary system that contribute to ventilator dysfunction is critical for understanding and ultimately treating the underlying cause of chronic airways disease.  The size of nonhuman primates commonly used in biomedical research permits characterization of pulmonary disease processes utilizing techniques common to the study of human pulmonary disease.  The rhesus monkey chest wall is relatively stiff in comparison to small domestic animals but similar to the human chest wall, which gives rise to a relaxed lung volume called functional residual capacity (comprises a large percentage of total lung capacity).  A cynomolgus macaque model was found to respond to bronchoactive agents primarily with changes in airway resistance indicating a large airway contribution, while other studies suggested that peripheral airways contribute about one-third of lung resistance in response to bronchoactive agents.  This laboratory animal model is particularly relevant to the study of pulmonary disease processes in humans, especially where small airways play a significant role.

**ROLE OF NONHUMAN PRIMATES IN INHALATION TOXICOLOGY**

Ozone: Acute and chronic inhalation studies in nonhuman primates have played a critical role in defining ozone-induced injury and inflammatory and repair responses.  More recent nonhuman primate studies have focused on the effects of early-life ozone exposure on lung development.  The development of chronic bronchiolitis with prolonged exposure to high ambient concentrations of ozone has been demonstrated in multiple nonhuman primate studies, providing evidence for prolonged structural and functional airway changes that could increase the risk for chronic lung disease as the animal ages.

Tobacco Smoke: Nonhuman primates have been key in advancing our understanding of the mechanisms of smoke-induced lung disease due to early-life exposure to second-hand smoke (environmental tobacco smoke - ETS).  Studies suggest that normal immune system development can be significantly compromised by in utero and postnatal exposure to ETS to contribute to ETS-related childhood diseases.

Other Inhaled Toxicants: Primate models where results have differed from rats include chlorine and formaldehyde gases (rhesus) and long-term diesel and coat dust exposure (cynomolgus).  Adolescent rhesus macaques have recently been used to study the effects of ambient wildfire smoke on lung function.

Future Studies: Primates may be needed to study the effects of electronic cigarette vapor (ECV), as it may contribute to lung disease.

NONHUMAN PRIMATE MODELS OF AIRWAYS INFLAMMATION

Asthma: Cynomolgus monkeys naturally infected with the nematode *Ascaris suum* proved to be a good representation of human atopic asthma and became widely used by the pharmaceutical industry.  The first nonhuman primate model of experimentally induced asthma using house dust mite (*Dermatophagoidesfarine*) was reported in the rhesus monkey and has been used in preclinical trials for asthma therapeutics.

COPD: Cynomolgus macaques have recently been used to study the effects of heavy smoking with pronounced pathological changes (inflammation, mucus metaplasia, glandular hypertrophy/hyperplasia, peribronchial fibrosis, and a “pre-emphysemetous” profile.  An earlier study trained baboons to smoke and resulted in blunted bronchial reactivity and preserved residual lung volume upon exposure to the cholinergic agonist methacholine.

NONHUMAN PRIMATE MODELS OF RESPIRATORY INFECTION

The immune system of the nonhuman primate shows significant homology with that of humans, which is a critical characteristic for understanding host-pathogen responses that lead to identification of therapeutic targets.  Nonhuman primate models provide a critical biomedical resource for accelerating preliminary safety and efficacy testing of vaccines as well as other pharmaceutical interventions that cannot be ethically evaluated in human subjects prior to clinical trials.  Experimental studies in nonhuman primate models have focused on respiratory viruses that have evolved from domesticated or wild animal hosts, and fungal and bacterial respiratory infections.

Tuberculosis (*Mycobacterium tuberculosis*): One of the oldest experimental nonhuman primate models of human respiratory pathogen infection was developed to address the imperfections of murine models of *Mycobacterium tuberculosis*, the causative agent for TB in humans.  The cynomolgus monkey was an important first step in establishing models to demonstrate the feasibility of infection by intratracheal administration of either high or low doses of *M. tuberculosis*.  High doses of M. tuberculosis elicited fulminant conditions, and low doses resulted in chronic symptoms that were similar to what has been described for human patients.  Rhesus macaques have been key in the development of a promising vaccine using a *M. tuberculosis* strain engineered with a mutant SigH region.  In addition to serving as a critical laboratory animal model for human TB, the nonhuman primate has been critical for the development of blood diagnostics for detection of interferon production in repose to *M. tuberculosis*, a step that has virtually eliminated the need for the less sensitive and specific tuberculin skin test.

Measles: Measles virus is highly contagious and readily spread by airborne transmission following infection within the respiratory tract, resulting in significant morbidity and immunosuppression.  Nonhuman primates are highly susceptible to infection with measles virus, and experimental inoculation can result in acute clinical signs that are comparable to that observed in humans.  A study in cynomolgus macaques clearly demonstrated that alveolar macrophages and dendritic cells were the initial sites of viral replication, followed by spread to lymphoid aggregates in the oral cavity.  Nonhuman primates have also been useful in development of a microneedle patch vaccine that does not require injections.

Influenza: Experimental animal models of viral infection using the nonhuman primate have played an essential role in understanding the pathogenesis of the pandemic influenza A strain H1N1 as well as other highly pathogenic strains such as avian H5N1.  Primates have also been important for defining immunomodulatory pathways that dictate health outcomes in vulnerable populations with weakened immunity toward vaccine preparations (very young and geriatric individuals).  Both infant rhesus macaques and African green monkeys are more susceptible to the H1N1 virus, revealing delayed type 1 interferon synthesis and impaired mucosal immunoglobulin responses following infection.

Viral Bronchiolitis (Respiratory Syncytial Virus): Infants are particularly susceptible to the development of viral bronchiolitis, and childhood infection is frequently a predictor for development of asthma later in life.  Nonhuman primate models include the rhesus macaque, Bonnet monkey, and baboon.

SARS/MERS (SARS CoV/MERS CoV): In the first reported outbreak of SARS, confirmation of SARS coronavirus as the etiologic agent was determined by infection of rhesus macaques, resulting in similar symptoms to humans.  African green monkeys were used to study the effect of immune deficiencies associated with aging on morbidity.  MERS coronavirus can infect both the rhesus macaque and common marmoset, resulting in clinical symptoms.  The marmoset has been reported to develop lethal pneumonia following infection.

Fungal and Bacterial Pneumonia (*Pneumocystis jirovecii, Streptococcus pneumoniae, Klebsiella pneumoniae):* Confirmation of the role of immune compromise in the pathogenesis of Pneumocystis pneumonia was experimentally determined in the macaque model of human immunodeficiency virus infection using the simian immunodeficiency virus (SIV).  Early models of *S. pneumoniae* and *K. pneumoniae* included squirrel monkeys, which elicited a lobar pneumonia more comparable to humans in contrast to previous rodent models.  A rhesus macaque models of *S. pneumoniae* infection was developed and successfully used for evaluation of a novel protein-based pneumococcal vaccine.  Baboons have recently been used for identifying new biomarkers of early stage infection, with the goal of rapid diagnosis and treatment.

Whooping Cough (*Bordetella pertussis*): The Taiwanese macaque develops clinical symptoms to *B. pertussis*, but its availability for research is limited as it is now considered endangered.  Rhesus macaques are not a favorable model as only about 25% display clinical symptoms consistent with human disease.  Baboons appear to be a much more reliable nonhuman primate infection model for *B. pertussis*.

QUESTIONS

1.  Name the right and left lung lobes of the macaque.

2. What is the volume of air present in the lungs at the end of passive expiration?

a.  Total lung capacity

b.   Functional residual capacity

c.  Pulmonary resistance

d. Dynamic compliance

3.   Cynomolgus monkeys naturally infected with this nematode proved to be a good model for human atopic asthma.

a.  *Ascaris suum*

b.  *Strongyloides stercoralis*

c.  *Strongyloides fulleborn*i

d. *Oesophagostomum bifurcum*

4.  Which vaccine was developed in the early 1920s using a weakened strain of the bovine Mycobacterium bovis, which in later years has shown to have a highly variable efficacy?

a.  Rubeovax vaccine

b.   Bacille Calmette-Guerin vaccine

c.  Lirugen vaccine

d. b-CAPSA 1 vaccine

5.  Measles virus is classified in the genus \_\_\_\_\_\_\_\_\_\_\_\_\_.

a.   Orthopoxvirus

b.   Erythroparvovirus

c.   Morbillivirus

d.   Lentivirus

6.   Influenza virus is classified in the family \_\_\_\_\_\_\_\_\_\_\_\_.

a.   Herpesviridae

b.   Poxviridae

c.   Paramyxoviridae

d.   Orthomyxoviridae

7. Which of the following primates are more susceptible to the H1N1 virus?

a.  Baboon

b.  Common Marmoset

c.  Cotton-top tamarin

d.   African green monkey

8. Which of the following primates have been used as models of respiratory syncytial virus infection?

a.   *Macaca radiata*

b.  *Callithrix jacchus*

c.   *Macaca cyclopis*

d.  *Saguinus Oedipus*

9.  Severe acute respiratory syndrome (SARS) and Middle East Respiratory syndrome (MERS) are pulmonary diseases caused by a virus from what family?

a.   Coronaviridae

b.   Paramyxoviridae

c.   Pneumoviridae

d.   Flaviviridae

10. Which of the following bacteria is a highly contagious gram-negative bacterium that blocks normalmucociliary clearance in the lung by production of a cytotoxin?

a.  *Streptococcus pneumoniae*

b.  *Klebsiella Pneumoniae*

c.  *Bordatella pertussis*

d.  *Mycobacterium tuberculosis*

11. Which of the following primates has been reported to develop lethal pneumonia following infection with MERS?

a.  *Macaca radiata*

b. *Callithrix jacchus*

c.   *Macaca cyclopis*

d.   *Saguinus Oedipus*

ANSWERS

1. Right – cranial, middle, accessory, caudal; left – cranial (divided into 2 parts – cranial and caudal) and caudal

2.  b

3.  a

4.   b

5.  c

6.  d

7. d

8. a

9.  a

10. c

11. b

**Stouffer and Woodruff. Nonhuman Primates: A Vital Model for Basic and Applied Research on Female Reproduction, Prenatal Development, and Women’s Health, pp. 281-294**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Domain 3: Research

**Summary:** The need for applied research related to women’s health remains great. There is an unmet need for low-cost effective contraceptives and infertility treatments (10% of couples). Current artificial reproductive technologies (ARTs) have max 30-40% success rate. Lab rodents (first rats, now mice) and ag spp (sheep) have contributed greatly to understanding of female reproduction function and regulation. But NHPs have similar hormonal, neural, and local reproductive control to women, including: neuroendocrine hypothalamic pituitary activity; cyclic function of ovary and reproductive tract; maternal-placental unit; and reproductive aging to menopause. Recent discoveries in NHPs include the role of leukemia inhibiting factor in ovulation and identification of non-classical MHC class I genes in NK cells and macrophages, both comparable to women. Rhesus have traditionally been used in repro research, cynos are recognized as valuable due to more docile nature and lack of summer anovulatory cycles. Baboons have been used in studies of transcervical agent delivery due to cervix being similar to humans (Rhesus too tortuous). NWPs (common marmoset) smaller size and shorter developmental interval are advantageous in some studies. This paper discusses many recent advances in women’s reproductive health (infertility, ARTs, developmental defects) made through NHPs, with a focus on reproductive tract biology/disorders as opposed to neuroendocrine aspects of reproduction/aging.

Polycystic Ovary Syndrome (PCOS): Phenotype is very heterogenous; must meet 2 of 3 criteria: clinical (e.g. hirsutism, menstrual irregularity) or biochemical evidence of hyperandrogenism; oligo/anovulation; and/or abnormal # of small to medium antral follicles in the ovaries. PCOS affects up to 15% of women but etiology is unknown (there is a component of complex, polygenetic inheritance). Recent G-WAS showed mutations/polymorphisms in 20 different genes but functionality of only one (DENND1a) has been documented. There is a lack of info on PCOS as it develops in utero and in children, and a lack of naturally occurring animal models. NHPs are used (in addition to rodents, sheep), with macaques being valuable due to genetic and menstrual function (monovulatory) similarity to humans. In Rhesus, early-to-mid gestation exposure to testosterone led to PCOS-like characteristics in 2/3 of female offspring in adulthood (42% were clinical using above criteria, 25% had milder PCOS); PCOS is also inducible in sheep. Other findings in Rhesus show that excess fetal testosterone results in early changes in metabolic processes (hyperinsulinemia, visceral fat accumulation) important to PCOS phenotype and onset of obesity. Further studies in Rhesus are ongoing to look at long-term (up to 5y) effects of testosterone and Western-Style Diet (WSD) on neuroendocrine, reproductive, and metabolic parameters.

Mitochondrial DNA-Based Diseases from the Oocyte: Mitochondrial activity involves products of a limited # of genes (37 in humans) interacting with nuclear genes; currently over 700 mutations are known leading to myopathies, neurodegeneration, diabetes, cancer, and infertility. Oocytes have a high # of mitochondria and maternal inheritance can potentially cause disease at a frequency of 1:200 children. Recently, a Mitochondrial Replacement Therapy (MRT) called spindle transfer (ST) was developed/validated in Rhesus. Fifteen ST-derived blastocysts were transferred into 9 females, leading to three pregnancies of four live births. Clinical studies suggest it is possible to transfer a patient’s nuclear genome without mutant mtDNA to an enucleated egg containing normal mtDNA, allowing for sperm fertilization and the usual genetic features of both partners (except for donor mtDNA). These have been coined “three-parent babies” and they are not without ethical and regulatory issues. The Human Fertilization and Embryology Authority and UK Parliament provided support and human studies are proceeding. NHP studies are needed to further study efficacy and safety of MRT, including developmental features of subsequent MRT-derived generations of offspring.

Uterine Disorders – Endometriosis: Affects 10% of reproductive age women. The most common symptom is chronic pelvic pain, but also causes painful intercourse and menstruation, and subfertility/infertility. Health care costs from morbidity rival those of diabetes and rheumatoid arthritis. There are no curative or preventative therapies for endometriosis, only palliative (laparoscopic removal of lesions) therapies that result in 40% recurrence. Treatments like progestin or GnRH analogs may relieve pain but eliminate fertility. Etiology is difficult to study due to advanced disease at time of presentation, so *in vitro* and animal models are needed. Benefits of NHPs (old world monkeys and baboons) are monthly cycles in ovarian and uterine structure-function comparable to women, and several species develop disease spontaneously. Inducible models (transplanting menstrual tissue intraabdominally) allow for study of pathogenesis and preclinical trials of drug therapies. Baboon studies of ectopic tissue have shown dysregulation of steroidogenic enzymes and hormone receptors leading to cell proliferation, as well as increased numbers of immune cells and cytokines promoting inflammation. It is also suspected that ectopic endometrial lesions lead to changes in ectopic endometrium causing progesterone resistance and altered expression of genes required for decidualization and implantation. Recent promising therapies tested in baboons include an aromatase inhibitor, letrozole (suppresses conversion of androgen to estrogen), and a JNK inhibitor, bentamapimod.

Uterine Transplantation: Uterine-Factor Infertility (UFI) describes the inability to maintain an intrauterine pregnancy due to non-functional or absent uterus. The most common cause is leiomyoma (fibroids) and hysterectomy may be indicated (as with cervical cancer). “Nonvital, quality of life” organ transplant, such as uterine transplant, is being investigated. Transplants have been performed in macaques and baboons and allow for optimization of surgical procedures and the study of uterus viability, immunosuppression, tissue rejection, pregnancy, and fetal development. Autologous uterine autograft has been reported in a cynomolgus macaque. In baboons, autologous uterine transplanted resulted in resumption of menstruation in 60% of animals, although no pregnancies after mating (possibly due to occluded oviducts). To date, allogenic transplants in NHPs have been less successful, but the insight gained was sufficient to start clinical trials in humans, and 11 attempts at uterine transplants have resulted in 2 live births.

Pregnancy Disorders, Placental Dysfunction: Environmental Influences: Little info on maternal-embryo interaction from implantation to placental development due to ethics of risk. NHPs important models because much of maternal recognition and control and placentation (hemochorial with villous structure within regional cotyledons) are similar to women. One goal of research is to develop means of noninvasively monitoring placental structure/function, as supported by the NIH “Human Placenta Project”. Contrast-enhanced ultrasonography and MRI have been used to quantify/qualify blood flow. Rhesus studies have also shown HFDs are associated with decreased uterine blood flow and increased uteroplacental insufficiency and fetal death. Similarly, chronic nicotine exposure led to reduced placental blood flow and abnormal villous histology (both effects mitigated by ascorbic acid).

Preterm Labor and Delivery: Intrauterine infections (ureaplasma) are a well-recognized feature of preterm labor and delivery and the leading cause of very-low birth weight babies born before 30 weeks. These babies have highest rates of neonatal complications and deaths. Causality of ureaplasma has been difficult to prove due to polymicrobial nature of reproductive tract. A study in Rhesus using *Ureaplasma parvum* and *Mycoplasma hominis* showed causative premature labor and delivery, upregulation of leukocytes, proinflammatory cytokines, and prostaglandins, uterine contractions, and fetal lung and brain disease. Maternal administration of azithromycin eliminated U parvum from amniotic fluid within 4 days, placental and fetal tissues were negative at delivery, and onset of delivery was delayed by at least 1 week.

Studies are ongoing to assess whether neural changes seen result in behavioral or cognitive deficits.

Emerging Opportunities, Fetal Development and Birth Defects: Controversy with fetal tissue research in the US and abortion restriction laws in other countries make NHPs important for studying factors that impair fetal development, such as Zika virus (flavivirus). Following inoculation, all Rhesus (n=8, 2 pregnant) tested positive for Zika in saliva, urine, and CSF within 1-day, remaining viremic for 3 weeks (non-pregnant); reinoculation at 10 weeks did not result in second round of viremia. However pregnant females remained viremic for study duration despite activation of NK and T cells and neutralizing antibodies, and fetal brain tissue contained 108copies/mL of tissue. NHPs will be used for vaccine development, and a study showed an East African strain conferred protection when later challenged with an Asian strain. Cynomolgus have been used to study pregnancy complications from *Listeria monocytogenes*. Although complications from listeriosis are commonly associated with 3rd trimester, this study showed the highest bacterial burden in the first trimester, broadening clinical attention to early pregnancy. Fetal Growth Restrictions (FGR) are seen in 5-10% of pregnancies, are a major cause of perinatal morbidity/mortality, and lead to increased risk of neurologic, cardiovascular, and metabolic disease later in life. Baboons have been used to demonstrate that moderate nutrient insufficiency (30% decrease) results in suboptimal development of organs, including placenta, kidney, liver, heart, and brain. This is providing insight to the necessity of clinical nutrition during pregnancy.

New Disease Models by NHP Genome Editing: Compared to rodents, NHPs could be better disease models due to similarities in genetics, developmental biology, and neural structure/function. The first transgenic (TG) NHP (a Rhesus expressing GFP) was reported in 2001. A Huntington’s Disease model was created in 2008. There is a general lack of progress on TG NHPs because of low numbers of mature oocytes available for microinjection and low efficiency of transgenesis. Recently TG marmosets (EGFP-expressing) were created with germline transmission and IVF, yielding health offspring. The CRISPR/Cas 9 system and TALENs technique are currently being applied to NHPs. TALENs has been used to create loss-of-function in MECP2 gene (Rett Syndrome) in macaques. In cynomolgus macaques, CRISPR has been used to simultaneously disrupt two genes (PPAR-gamma and Rag1). Additionally, dystrophin (Duchenne muscular dystrophy), Dax1 (adrenal hypoplasia, hypogonadism), and p53 genes have been knocked out. Generation of embryonic and induced pluripotent stem cells combined with genome editing will allow for in vitro and in vivo studies to repair or replace diseased tissues.

Conclusion: Challenges of NHPs include high costs and need for special resources, but they have led to advancements in fertility and infertility research that directly benefitted women and their reproductive health. Ethical debates on the appropriate use and limits of experimentation are also important and should frame future research.

QUESTIONS

1. True/False: The most common cause of very-low birth weights in babies born prior to 30 weeks gestation is maternal malnutrition.

2. Which of the following is not one of the criteria for diagnosing Polycystic Ovarian Syndrome (PCOS)?

a. Abnormal number of small to medium antral follicles in the ovaries

b. Inability to establish or maintain pregnancy

c. Clinical or biochemical evidence of hyperandrogenism

d. Oligoovulation or anovulation

3. True/False: A recent UK regulation supported by the Human Fertilization and Embryology Authority recently banned Mitochondrial Replacement Therapy (MRT).

4. True/False: Recent studies in Rhesus have shown that High Fat Diets (HFDs) are associated with decreased uterine blood flow and increased uteroplacental insufficiency and fetal death.

5. True/False: Uterine transplant studies in NHPs have led to insight that has allowed for successful uterine transplants in humans, some of which have resulted in successful pregnancies and live births.

ANSWERS

1. False - It is intrauterine infections, most commonly caused by *Ureaplasma parvum.*

2. b

3. False - They support this evolving area of research.

4. True

5. True - Two live births have been reported

**Coleman and Novak. Environmental Enrichment in the 21st Century, pp. 295-307**

Domain 1

SUMMARY: Environmental enhancement has been mandated by the Animal Welfare Act for many years and should promote the psychological and physiological well- being of captive nonhuman primates used in biomedical research. Therefore, research facilities must develop an appropriate enrichment plan to enhance the quality of captive animal care. Enrichment plans should be species-specific and based on currently accepted professional standards and scientific evidence which requires constant updating. Another important consideration is that environmental enhancement plans are consistent and systematic, so as not to become a source for uncontrolled variations to the research. Because of their common usage in biomedical research, this journal reviews environmental enhancement plans along with relevant outcomes in Old World monkey species including Macaques.

In order to meet the primary goal of environmental enhancement, various enrichment tools have been used in nonhuman primate facilities, like adding perches for structural enrichment, social housing of conspecifics, occupational enrichment like toys and foraging devices, and sensory enrichment like sounds and odors. The overall goals of environmental enrichment programs are to minimize abnormal behavior, to facilitate adaptation to environmental changes, to reduce any stress responses and maintain physical health. All these objectives can be measured. There are some challenges to select appropriate enhancement strategies for an individual animal. Thus, it is important to have sufficient knowledge and understand the species natural environment in addition to examining the animals’ sensory, cognitive, and behavioral capacities which should be assessed to evaluate the efficacy of particular types of enrichment. Whether the enrichment objective promotes species-specific behavior or reduces abnormal behavior can be assessed by extensive behavioral observations. If the environmental objective is to reduce stress of laboratory environments, then one way to assess stress is to measure Cortisol levels as a stress response. Another novel approach to understand effects of environmental enhancement is the cognitive bias test. This test assumes that animals will be pessimistic in their judgements when exposed to adverse conditions. This means that nonhuman primates show optimism after a positive event and pessimism after a negative event. This test may be useful to determine emotional responses to situational events, but it is unclear if it can be used for long term evaluations of enrichment tools. Despite its value, this test is very labor intensive and requires extensive training. The challenges of an enrichment plans are that one size doesn’t fit all, since there are individual differences between nonhuman primates. The presence of
individual differences requires a monitoring program to ensure that the environmental enrichment plan meets its goal for as many animals as possible.

Every enrichment device carries a potential risk. Treats for example can increase body weight leading to health-related problems. Toys can be chewed and ingested with the potential of gut impact action. Monkeys can entrap limbs or even heads in chains or ropes. Social housing is the default condition for nonhuman primate housing making it more challenging when animals are or become incompatible which is in most cases determined by different temperaments. Enrichment strategies as mandated by the Animal Welfare Act include physical cage structure, tactile stimulation, visual stimulation, auditory stimulation, taste, cognitive stimulation, exercise, social housing and human interaction. Cages can contain a variety of structural enrichment such as perches, nest boxes, additional exercise cages, natural flooring in outdoor enclosures to promote species- specific behavior. There are many tactile enrichment devices like toys, foraging devices or cardboard boxes which are rotated on a regular basis and reduce stereotypic behavior. Visual devices have the most effect to reduce abnormal behavior when they can be changed frequently and controlled by the subject. Auditory stimuli can mask stressful sounds and can lessen startle reflexes. Treats or novel food are common enrichment strategies, but carry the risk of increasing weight, because they are high in empty calories. Cognitive stimulation through tablets and apps solving problems have shown a beneficial effect on reducing stereotypic behavior. Socialization with conspecifics is as beneficial as interaction with humans through positive reinforcement training.

The enrichment of captive primates requires constant innovation taking advantage of emergent and novel technology. Individualized enrichment plans should be tailored to unique behavioral patterns of each individual species taking individual differences in account. Temperamental differences among individuals may also influence the enrichment enhancement plan for that particular species. Some individuals do better with familiar than with novel enrichment offerings. These animals might be in need of desensitization or habituation. Human interaction should be performed consistently and by multiple people rather often only occurring when there is time and only by those tasked with enrichment.

Effective enrichment programs should provide the animal with a sense of control over its environment, reduce stress and promote physical health without compromising the integrity of research.

QUESTIONS

1. True or False: Environmental enrichment should not provide the animal with a sense of control over its environment.

2. Environmental enrichment is enacted by \_\_\_\_\_\_\_\_\_?

a. Federal legislation

b. State legislation

3. True or False: The Animal Welfare Act mandates research facilities to develop and follow a plan to promote the psychological well-being of captive nonhuman primates used in biomedical research

ANSWERS

1. False

2. a

3. True