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Module 3, Video 17: The need for improved animal models to study sex differences during drug development

An overarching aim of preclinical biomedical research is to develop better ways to diagnose and cure human diseases. The term "animal model" can mean many things but for the purpose of this video, an animal model refers to the use of a nonhuman animal, usually a mammal or vertebrate, to obtain information about a human disease and its prevention, diagnosis, and treatment [1]. Animal models play an important role in the drug development process. Many proponents for the inclusion of females into basic biomedical research argue that it will ultimately lead to improved health outcomes for both sexes. However, a significant gap still exists whereby many animal models, including those aimed at modeling sex differences and diseases specific to females, fail to demonstrate sufficient predictive power. This leads to rates of translation from bench to bedside that are dismal, regardless of sex. In this video, we will provide a rationale for the selection and validation of more predictive animal models that can answer important drug development questions [2].

Skepticism about whether or not studying both sexes in animal models will result in improved public health is often framed as a translational problem. The success rates for drugs in clinical trials based on preclinically-derived evidence alone is low, a phenomenon referred to as the "Valley of Death".

Animal models serve two primary purposes to aid drug development. The first is to better understand fundamental mechanisms and processes. A key assumption here, however, is that the chosen animal species must be comparable enough to reasonably extrapolate the results to human biology and disease. The second purpose is during essential parts of the traditional drug development process that occur prior to clinical trials in humans. This includes: the preclinical stages of drug compound testing, ascertaining the safety of a drug or treatment, such as its toxicity or adverse events, and establishing the therapeutic index, or dose, that produces the desired effect without being toxic or lethal [3]. For both of these purposes, it is critical that the right animal model is chosen, one that shows the most homology to humans for the particular organ affected or behavior of interest. This is particularly important when studying sex differences because females are physiologically, biologically and genetically distinct.

Choosing the right species for drug development research improves the translation potential. It is true that the best model for human is human, but many parts of the drug discovery process cannot be achieved in humans. Greater divergence from humans across the phylogenetic scale leads to greater gaps in genetic and physiological homology [4]. Currently, preclinical research relies heavily on lower order species such

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as rats and mice, with one meta-analysis highlighting that rodents were used in 85% of studies in neuroscience, pharmacology, immunology and physiology [5].

But many other species can and are used in preclinical research, including ferrets, sheep, pigs and non-human primates. These animals have a higher degree of genetic, physiological and biological homology to humans compared to rats. They also have gyrencephalic brains, making their choice for CNS studies particularly ideal.

The high level of similarity between non-human primates and humans makes them an attractive model for research on human disease, especially for understanding the biological and evolutionary roots of sex differences [6-13]. But their inclusion in research is costly, requires specialized training and facilities and is accompanied by numerous ethical considerations [14]. Thus, model development in non-primate gyrencephalic mammals should be a priority. One such model is the pig. The pig has become an increasingly popular model for preclinical research because of its physiological, anatomical, and genetic similarity to humans [15-19]. There is also high homology between the reproductive cycles of the pig and that of humans, making them a potential model for sex differences research. Both species show a similar cycle mean length and hormone fluctuation pattern [20], particularly with regards to estrogen, and both show spontaneous ovulation and continuous cycling. Thus, broadening model development to other species with more homology to humans may improve the translational findings of preclinical results of sex differences.

In addition to choosing the right model in terms of species homology, it is also important to ensure that the chosen model has appropriate external validity—or generalizability. In 1984, Willner proposed three criteria of external validity: face validity, predictive validity, and construct validity—which have remained the benchmark for describing a model's purpose for the clinical condition, particularly during target identification [21]. But few animal models go beyond face validity. This is particularly relevant when considering how females and males fair in later clinical studies as animal models are used at many stages of drug development [22]. The characterization and validation of mechanisms in females related to many diseases is far from complete, and as we have demonstrated in this video series, there is a sufficient basis for assuming that sex differences ALWAYS exist between males and females, at some level. Thus, ensuring that models are fit-for-purpose when investigating female-specific mechanisms will, in turn, improve the likelihood of developing advanced precision medicine approaches that take the sex of the patient into account. This will only come about when females are more routinely included into all lines of early drug development research [23-25].

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In this video, we have discussed two factors related to animal model selection that may impede translation. These two factors are also particularly relevant when considering that females are often left out of many of the steps in the R&D process, including model validation and selection. Thus, ensuring that sex inclusion is taken seriously at both the fundamental and clinical ends of the drug development pipeline is an essential step towards improving translation.

References

1. Shanks N, Greek R, Greek J. Are animal models predictive for humans? Philosophy, ethics, and humanities in medicine : PEHM 2009;4:2-2. DOI
2. Seyhan AA. Lost in translation: the valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles. Translational Medicine Communications 2019;4:18. DOI
3. Institute of Medicine (US) Forum on Neuroscience and Nervous System DIsorders. Improving the Utility and Translation of Animal Models for Nervous System Disorders: Workshop Summary. Washington (DC): National Academies Press (US). 2, Evaluation of Current Animal Models. Available from: https://www.ncbi.nlm.nih.gov/books/NBK158935/, 2013. DOI
4. Rizzo P, Altschmied L, Ravindran BM, et al. The Biochemical and Genetic Basis for the Biosynthesis of Bioactive Compounds in Hypericum Perforatum L., One of the Largest Medicinal Crops in Europe. Genes (Basel) 2020;11. DOI
5. Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. Neuroscience and biobehavioral reviews 2011;35:565-572. DOI
6. Meredith SL. Comparative perspectives on human gender development and evolution. American Journal of Physical Anthropology 2015;156:72-97. DOI
7. Lonsdorf EV, Markham AC, Heintz MR, et al. Sex differences in wild chimpanzee behavior emerge during infancy. PLoS One 2014;9:e99099. DOI
8. Simpson EA, Nicolini Y, Shetler M, et al. Experience-independent sex differences in newborn macaques: Females are more social than males. Sci Rep 2016;6:19669. DOI
9. Kulik L, Amici F, Langos D, et al. Sex Differences in the Development of Social Relationships in Rhesus Macaques (Macaca mulatta). Int J Primatol 2015;36:353-376. DOI
10. Primate psychology. Cambridge, MA, US: Harvard University Press, 2003. DOI
11. Young GH, Coelho AM, Bramblett CA. The development of grooming, sociosexual behavior, play and aggression in captive baboons in their first two years. Primates 1982;23:511-519. DOI
12. Wang X, Li B, Ma J, et al. Sex differences in social behavior of juvenile Sichuan snub-nosed monkeys Rhinopithecus roxellana at Yuhuangmiao, Mt. Qinling, China. 2007:939-946. DOI

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13. Eaton GG, Johnson DF, Glick BB, et al. Development in Japanese macaques (Macaca fuscata): Sexually dimorphic behavior during the first year of life. Primates 1985;26:238-247. DOI
14. Feng G, Jensen FE, Greely HT, et al. Opportunities and limitations of genetically modified nonhuman primate models for neuroscience research. Proc Natl Acad Sci U S A 2020;117:24022-24031. DOI
15. Bode G, Clausing P, Gervais F, et al. The utility of the minipig as an animal model in regulatory toxicology. J Pharmacol Toxicol Methods 2010;62:196-220. DOI
16. Turk JR, Henderson KK, Vanvickle GD, et al. Arterial endothelial function in a porcine model of early stage atherosclerotic vascular disease. Int J Exp Pathol 2005;86:335-45. DOI
17. Squier CA, Mantz MJ, Schlievert PM, et al. Porcine vagina ex vivo as a model for studying permeability and pathogenesis in mucosa. J Pharm Sci 2008;97:9-21. DOI
18. Kinder HA, Baker EW, West FD. The pig as a preclinical traumatic brain injury model: current models, functional outcome measures, and translational detection strategies. Neural Regen Res 2019;14:413-424. DOI
19. Meurens F, Summerfield A, Nauwynck H, et al. The pig: a model for human infectious diseases. Trends Microbiol 2012;20:50-7. DOI
20. Lorenzen E, Follmann F, Jungersen G, et al. A review of the human vs. porcine female genital tract and associated immune system in the perspective of using minipigs as a model of human genital Chlamydia infection. Vet Res 2015;46:116. DOI
21. Willner P. The validity of animal models of depression. Psychopharmacology (Berl) 1984;83:1-16. DOI
22. Denayer T, Stöhr T, Van Roy M. Animal models in translational medicine: Validation and prediction. New Horizons in Translational Medicine 2014;2:5-11. DOI
23. Solomon MB, Herman JP. Sex differences in psychopathology: of gonads, adrenals and mental illness. Physiol Behav 2009;97:250-8. DOI
24. Bangasser DA, Valentino RJ. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. Front Neuroendocrinol 2014;35:303-19. DOI
25. Shansky RM. Sex differences in PTSD resilience and susceptibility: Challenges for animal models of fear learning. Neurobiol Stress 2015;1:60-65. DOI