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Module 3, Video 16: The importance of considering sex differences in pharmacology when designing preclinical studies

Outside of industry-based drug development, which has a rigorous process for drug selection based on multiple aspects of pharmacology, basic researchers in academic labs are increasingly conducting translational studies, including the use of pharmacological agents as a means to gain mechanistic insights in rodent models of human diseases. But most published preclinical studies fail to justify the chosen drug dose or simply cite a dose from a published study, even when the cited paper lacks drug exposure data. Further, many studies do not show evidence of target organ engagement. This, coupled with the fact that most biomedical scientists do not receive sufficient training in the application of PK and PD principles, means that many preclinical studies do not satisfy basic pharmacology principles when drawing conclusions about their findings.

There is a misconception that if a referenced drug dose produces a measurable effect—either physiological or behavioral—in an animal model, then insights can be drawn about the ability of the drug to disrupt disease-relevant mechanisms at the level of the target organ. In addition, many parameters can alter the relationship between the dose, exposure, and measured response—including the route of administration, vehicle, species, strain, age, genetic modifications, time course, dosing duration, target organ, and, relevant to this video series, the sex of the study animal [1].

In this video, we will focus on how sex as a biological variable may influence study design related to dose selection, timing and target organ engagement.

As we have demonstrated throughout this video series, sex hormones have documented modulatory effects on many basic pharmacology principles, as well as on many of the hormones and enzymes that influence these drug properties. As females are included more widely into preclinical research, the knowledge of sex differences in pharmacology is expected to grow. Thus, to avoid producing exposures that are either too low or too high to interpret a mechanistic hypothesis, the effect of sex hormones on PK, PD and even toxicology should be considered as you design your experiment, particularly with regard to drug dose selection, timing, and target engagement.

So how should dose selection proceed? First, a thorough literature search should be conducted to look for existing evidence for a modulatory role of sex hormones by your drug of choice OR within your organ of interest. You should also look for baseline sex differences in your intended

outcome measure, including variations across the estrous cycle. However, as mentioned previously, the lack of published data on sex differences does not suggest they don't exist. In contrast, as a good rule, you should always assume sex differences exist until thoroughly proven otherwise.

In the absence of comprehensive published data showing the use of the intended drug under similar biological and experimental parameters—such as the same strain, route of administration, disease model—sex differences in basic pharmacology should be considered during drug dose selection. Ideally, basic pharmacology studies would be performed in conjunction with individuals with specialized knowledge of pharmacology. But some basic principles can be followed by all preclinical scientists. One of the simplest things to do is a basic dose-response curve. Dose-response relationships are often used to examine the relationship between exposure to a substance in increasing doses (2-3) and the effect on a parameter of choice, such as the desired effect or target receptor engagement. Dose-response relationships can tell us two important things about potential sex differences in the PD actions of a drug: the first is sex differences in the potency of the drug. Potency is the amount of drug required to produce a particular effect. More specifically, it is the concentration or dose required to produce 50% of the maximal effect, or EC_{50}. The same drug administered to males and females may show differences in potency such that a higher amount is necessary in one sex to produce the same effect as that observed in the other.

The second concept is efficacy. Efficacy is the maximum effect that can be achieved by a drug. Two drugs may show different efficacies, or maximum responses, in males and females, leading to drastic misinterpretations of their effects on the outcome measure of interest. Sex differences in drug efficacy may be related to sex differences in pharmacokinetics, such as the expression or binding affinity of the receptor in one sex versus the other. Sex differences in the mechanism of action of a drug are best studied *in vitro*.

Another consideration when administering a drug is ensuring it makes it to the target organ. One of the most challenging organs to reach is the brain due to the blood brain barrier. A 2016 review of preclinical studies of drugs targeting the brain found that nearly three quarters of the studies selected a dose without ever measuring the brain concentrations at the intended site of action [1]. Without measuring the concentrations in the brain, you may never know if the same dose of a drug reaches different concentrations in the brain in males and females or has a different time course of action. For example, suppose you design a sufficiently powered experiment in which you give a drug with known actions on enzyme A at a concentration 500 nanomolar to both males and females. Behaviorally, you observe a change in the behavior of

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the male animals but not in the females, which you conclude to be the result of sex differences in the effects of the drug on your behavior of interest. However, when you measure the concentration of the drug in the brain, you find that in males, the drug concentration is 500 nanomolar, enough to inhibit enzyme A, whereas in females, the concentration in the brain is too low to be effective. Thus, a higher dose of the drug must be administered to females to achieve the same brain concentration as that in males before a comparison of its effect on the behavior of interest can be made.

The drug concentration at the site of action may also be time dependent. In other words, a drug might show sex differences in the speed in which it is metabolized. For example, brain concentrations might be initially similar between males and females but then decrease at the target organ faster in one sex compared with the other. Ideally, you should test 5-6 time points, including the maximal concentration and the wash out phase, when no drug is in the system. This will give you insights into the time course of your drug's effect and whether multiple doses are needed to maintain the desired effect [2, 3].

There are a number of methods to assess drug distribution in the brain [4, 5]. Certain drug properties are also known to enhance crossing of the blood brain barrier including the size of the molecule, its solubility and whether it is hydrophilic [6-8]. However, little is known about sex differences in these properties or whether they vary with the estrous cycle.

Drug dose selection and target engagement are two areas that may show significant effects on your experimental outcomes and the interpretation of your data. They are also largely underexplored areas in terms of potential sex differences. As more females are included in basic research, the potential effects of sex hormones on different pharmacology principles will become clearer. Keeping these potential differences in mind during study design will ensure that conclusions about the effect of a drug on a particular outcome can be adequately made.

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