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In vivo drug screening on C. elegans and zebrafish using dose response plots and automated tracking programs

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abstract

In vivo drug testing is an expensive and rigorous process where model organisms can offer significant advantages prior to clinical trials. In vivo testing can reveal any deficiencies in the safety and efficacy of chemicals or drugs at the pre-clinical stage of drug development. Model organisms such as C. elegans and zebrafish offer the design flexibility and experimental guidance at the pre-clinical stage in laboratory settings The drug screening protocol is well-established for these model organisms. In this article, we review the protocols for drug screening of new and existing chemicals using model organisms, methods of measuring end points of experiments, and automation tools to plot and characterize the dose response of the drugs. A number of engineering design and automation tools are available to design the microfluidic chips, automation electronics, and worm tracking algorithms which have been commercialized for several applications. We discuss the performance and parameters defining the drug screening process using the dose response. In addition, we discuss the limitations of simplifying the drug screening process using model organisms, such as underestimating the biological complexities of nature, temporal variations in drug effects, and small variability in the experiments which may hinder the transition to clinical trials.

Introduction to In Vivo Testing

In vivo drug testing refers to the evaluation of pharmaceutical compounds or substances within living organisms, typically animals, to assess their pharmacokinetics, efficacy, safety, and potential toxicity before advancing to human clinical trials [1-10]. In vivo drug testing is crucial for establishing the safety and efficacy profiles of potential therapeutics, informing subsequent clinical trial designs, and ultimately advancing treatments for human diseases [2-9]. Ethical considerations and stringent experimental protocols are paramount to ensure the reliability and ethical integrity of the research conducted in this field.

There are several steps in In Vivo Drug Testing using model organisms [1-5]. First, we choose an appropriate animal species and strain that best represents the disease or condition being studied and has relevance to human physiology. We need to ensure compliance with ethical guidelines and obtain necessary approvals for animal experimentation. The dose selection is key to determining the initial dose range based on preclinical data, pharmacokinetic profiles, and safety considerations. Then we select the route of administration (e.g., oral, intravenous, subcutaneous) based on the drug's properties and intended clinical application. We consider the practicality and relevance of the chosen route to mimic human administration. We monitor animals for physiological parameters,

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behavioral changes, and adverse effects. We perform systematic assessments of efficacy endpoints relevant to the disease model (e.g., tumor growth inhibition, symptom relief) [10-14].

We collect biological samples (e.g., blood, tissues) at specified time points to analyze pharmacokinetics, distribution, metabolism, and excretion of the drug. We utilize analytical techniques (e.g., HPLC, mass spectrometry) to quantify drug concentrations and metabolites in samples. We analyze data to determine pharmacokinetic parameters (e.g., clearance, half-life) and pharmacodynamic responses (e.g., efficacy, toxicity). We interpret results in the context of safety margins, therapeutic index, and potential translatability to human trials. We document findings in comprehensive study reports adhering to regulatory standards (e.g., FDA, EMA).

Considerations for In Vivo Testing

There are several considerations in In Vivo Drug Testing [3-10]. First is related to animal welfare where we ensure humane treatment and minimize animal suffering throughout the study. We validate the chosen animal model to accurately reflect the disease pathophysiology and predict human responses. We plan the clinical studies with adequate sample sizes to achieve statistically significant results and reduce variability. We implement rigorous quality control measures for experimental procedures, data collection, and analysis. We conduct studies with sufficient replicates and validate results through independent experiments to ensure reproducibility.

Drug Screening in Model Organisms

Dose-response studies are a fundamental part of drug screening, used to determine the relationship between the dose of a drug and its biological effect [12-20]. Drugs are prepared in series of dilution, typically in a logarithmic scale (e.g., 1 μM, 10 μM, 100 μM). It is ensured that the solutions are sterile and prepared fresh or stored appropriately. We next choose the developmental stage of the model organism (e.g., zebrafish embryo, larva, or adult) based on the study objectives. We administer the drug to the model organism by putting in the drug solution, microinjection, or feeding, depending on the nature of the compound and the study design. We then decide the appropriate exposure duration (e.g., hours to days) to observe the effects.

We monitor and record the responses at regular intervals, and use imaging techniques to observe phenotypic changes, behavioral assays for neuroactive drugs, or molecular assays for specific biomarkers [20-28]. We measure the relevant endpoints (e.g., mortality, developmental abnormalities, behavioral changes, gene expression levels). We next plot the dose-response curves, typically with drug concentration on the x-axis and the measured response on the y-axis. We fit the data to a suitable model (e.g., logistic or sigmoidal function) to calculate key parameters like the EC50 (effective concentration for 50% of the maximal effect) or IC50 (inhibitory concentration for 50% of the maximal effect).

Behavioral Assays for Drug Screening

Caenorhabditis elegans (C. elegans) is another model organism widely used in drug screening due to its simplicity, well-characterized biology, and ease of genetic manipulation [11-20]. C. elegans

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has a simple, well-mapped nervous system with over 300 neurons, making it ideal for neuroscience and pharmacology studies. C. elegans is used to evaluate the toxicity of compounds at various developmental stages. Models of diseases like Alzheimer's, Parkinson's, and Huntington's have been developed in C. elegans. C. elegans is extensively used in studying the effects of drugs on lifespan and age-related diseases. C. elegans is used to study host-pathogen interactions and screen for antimicrobial compounds.

Behavioral assays are available for C. elegans to quantify the effects of drugs or other chemicals [5-18]. Behavioral studies in C. elegans are essential for understanding the effects of drugs on nervous system function, sensory perception, and overall organismal behavior. Locomotion assays measure the body bends per second, speed and direction of worm movement, or the thrashing frequency of the worm in a liquid medium. A dose response curve can be generated from each locomotion parameter that determines the effect of the drug on the worm motion. The egg-laying behavior can also be studied in the presence of various drug solutions to understand the drug mechanisms on the reproductive health of the worms.

For locomotion assays, the worms are synchronized to have a population of a certain age group. The worms are transferred to agarose plates with nutrients in the presence or absence of the drug under test. The worms are allowed to acclimate to the drug environment, and their locomotion is recorded by images and video recording. Some sort of locomotion tracking software is used to process the raw videos to extract the locomotion parameters, such as speed, direction, and thrashing speed.

Antibiotics and C. elegans Assays

Antibiotics have been used routinely in C. elegans research [7-20]. Using antibiotics in Caenorhabditis elegans research is common for maintaining bacterial food sources, controlling bacterial infections, and studying the effects of antibiotics on C. elegans physiology and behavior. By carefully selecting and using antibiotics, researchers can effectively manage bacterial cultures, control contamination, and study the effects of antibiotics on C. elegans in various experimental settings.

The common antibiotics used in C. elegans research are ampicillin, tetracycline and kanamycin [20-28]. Ampicillin prevents any contamination in bacterial cultures used in C. elegans plates (e.g., E. coli OP50) usually in the range of 50-100 µg/mL. Kanamycin can be used in applicatios to select E. coli strains that carry the kanamycin resistance genes and are also in the range of 50-100 µg/mL. Tetracycline is used to prevent contamination and selecttetracycline-resistant bacterial strains, and are usually used in the range of 10-20 µg/mL. Streptomycin is used in the selection of streptomycin-resistant E. coli strains and prevention of contamination, and is used in the range of 50-100 µg/mL.

Antibiotics are used to keep E. coli cultures free from contamination by other bacteria [20-27]. Antibiotics are also used to select for E. coli strains carrying plasmids with specific antibiotic resistance genes. Antibiotics are also used in gene knockdown and expression studies to ensure the presence of plasmids in bacterial strains used for RNA interference (RNAi) or gene expression

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studies. C. elegans can be infected with pathogenic bacteria, and antibiotics are used to study infection dynamics and host responses. C. elegans are aso used to test the efficacy of antibiotics in testing the effectiveness of antibiotics against bacterial infections in C. elegans. Antibiotic resistance tests study the development of antibiotic resistance in bacterial pathogens using C. elegans as a host.

There are several considerations for using antibiotics in C. elegans studies. It is critical to use the lowest effective concentration to minimize any potential indirect effects on C. elegans. It is also important to monitor for any toxic effects on C. elegans. Some antibiotics, like tetracycline, are light-sensitive and should be protected from light.

It is important to include agarose plates without antibiotics to serve as controls. We also should include both resistant and sensitive strains of E. coli to ensure the antibiotic is working as intended. It is vital to monitor the bacterial growth to ensure consistent bacterial lawn coverage and quality across all experimental plates.

Conducting Dose Response Studies on C. elegans

In Caenorhabditis elegans (C. elegans) research, conducting dose-response studies involves exposing these nematodes to varying concentrations of substances (e.g., drugs, chemicals) to observe how their biological responses change in relation to the dose [11-20]. There are several steps in conducting dose-response studies with C. elegans. By following these steps and considerations, researchers can effectively conduct dose-response studies with C. elegans, providing insights into the biological effects of substances and their potential applications in various research fields. First, it is important to select the experimental design, choose the drug or chemical and determine its solubility and stability in the experimental medium (e.g., NGM agar, liquid media). Then we decide on a range of concentrations to be tested, covering sub-effective to potentially toxic levels. The drug or chemical is dissolved in a suitable solvent (e.g., DMSO, water) to prepare stock solutions of varying concentrations. We want to ensure the solvent does not interfere with the assay or have unintended effects on C. elegans.

There are several ways to administer the drug or chemical to the worms. In the feeding assay, we incorporate the substance directly into the bacterial lawn (e.g., E. coli OP50) on NGM plates. This method is common for drugs and chemicals that affect C. elegans through ingestion. In the soaking assay, we immerse C. elegans in a solution containing the substance. This method is useful for substances that can penetrate through the cuticle of the worms. The experimentalist determines the duration of exposure to the substance. This can vary depending on the substance's pharmacokinetics and the biological processes being studied (e.g., developmental stages, lifespan). We monitor and record relevant endpoints such as growth rates, developmental milestones (e.g., larval development, reproduction), behavior (e.g., locomotion, feeding), and physiological markers (e.g., stress responses, fluorescence in transgenic strains). Lastly, we measure and quantify the responses observed in each concentration group, and plot the dose-response relationship, typically with the concentration of the substance on the x-axis and the measured response on the y-axis. Then

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we determine the effective concentration (EC50) or inhibitory concentration (IC50) that produces a specified response (e.g., 50% of maximal effect).

It is important to maintain consistent environmental conditions (e.g., temperature, humidity) throughout the experiment to minimize variability. We also want to interpret dose-response curves to understand the substance's potency, efficacy, and potential toxicity. The dose response could provide the biological relevance of observed effects and their implications for further research or application.

Plotting the Dose Response Curve

A dose-response plot is a graphical representation that illustrates the relationship between the dose or concentration of a substance (such as a drug or chemical) and the biological response it induces. By visualizing and analyzing dose-response relationships, researchers can make informed decisions about optimal dosing, safety margins, and potential therapeutic efficacy of substances, contributing to the advancement of drug development and Creating a Dose-Response Plot

To obtain a dose response curve, we start by conducting experiments where a biological system (e.g., cells, organisms, C. elegans) is exposed to varying doses or concentrations of the substance [9-18]. We measure the biological response at each dose, which could be efficacy (e.g., growth inhibition, enzyme activity), toxicity (e.g., cell death), or any other relevant endpoint. We arrange the data in a table format where one column represents the dose or concentration levels and another column represents the corresponding biological response observed. We use a graphing software or tool (e.g., GraphPad Prism, Excel, R) to create a scatter plot or line plot. We plot the dose or concentration on the x-axis (independent variable) and the biological response on the y-axis (dependent variable). For curve fitting, we fit a mathematical model to the data points to visualize the trend and estimate parameters like the EC50 (effective concentration 50%) or IC50 (inhibitory concentration 50%). Common models include sigmoidal curves (e.g., Hill equation, logistic equation) that describe the typical shape of dose-response relationships. Next, we analyze the shape of the curve, wherein a steep curve indicates a potent response at low doses, while a shallow curve indicates a gradual response across a wider range of doses.We determine the EC50 or IC50 values, which represent the concentration or dose at which 50% of the maximal response or inhibition is achieved.

Limitations and Future Considerations

Dose-response curves are valuable tools in pharmacology and toxicology research for understanding the relationship between the dose or concentration of a substance and its biological effect [11-19]. However, they do come with several limitations and considerations. By recognizing these limitations and adopting appropriate experimental designs and methodologies, researchers can enhance the robustness and applicability of dose-response curve analyses in advancing drug discovery, toxicology, and therapeutic development. The first limitation is regarding the assumed simplicity of the biological organism. Dose-response curves often represent a simplified view of complex biological systems. Biological responses can be influenced by multiple factors such as

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genetics, environment, and physiological conditions, which may not be fully captured by a single curve. Furthermore while dose-response curves describe the relationship between dose and response, they may not provide direct mechanistic insights into how a substance exerts its effects at different concentrations. Understanding the underlying mechanisms often requires additional experimental approaches beyond dose-response studies. In addition, dose-response curves assume a continuous relationship between dose and response. However, some substances may exhibit threshold effects, where responses only occur above a certain dose threshold. These effects may not be adequately represented by standard curve fitting models. Lastly, biological responses can vary significantly between individuals due to genetic differences, age, sex, and health status. Dose-response curves derived from population averages may not accurately predict responses in specific subgroups or individuals. Dose-response curves typically measure responses at a single time point or over a short period. However, biological responses can change over time (e.g., acute vs. chronic exposure), and dynamic changes may not be fully captured by static dose-response assessments.

While sigmoidal curves (e.g., Hill equation) are commonly used for dose-response modeling, some substances may elicit non-linear or biphasic responses that require alternative modeling approaches [12-21]. Linear dose-response assumptions may not hold true in all cases. We should consider using multiple models or dose-response approaches (e.g., concentration-response curves, time-course studies) to capture different aspects of substance effects. Dose-response relationships established in preclinical models may not always translate directly to clinical settings due to differences in species-specific responses, pharmacokinetics, and disease states. Human variability and safety considerations may necessitate additional studies. The accuracy and reliability of dose-response curves can be influenced by experimental techniques, assay sensitivity, and limitations in quantifying complex biological endpoints. Variability in experimental conditions and techniques can affect curve reproducibility. We should supplement dose-response curves with mechanistic studies (e.g., molecular assays, imaging techniques) to elucidate underlying pathways and modes of action. We should conduct studies across diverse populations and conditions to account for inter-individual variability and potential threshold effects. Lastly, we should evaluate temporal dynamics and long-term effects through longitudinal studies or chronic exposure assessments.

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