

Animal Experimentation: An Absolute Necessity

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ABSTRACT

The preclinical study is the backbone of most of the drug discovery processes, although it has been debated by both scientific and animal welfare organizations on the use or not to use animals from experimental procedures completely. The human body because of complex physiological systems makes it difficult to be reciprocated into nonliving models, thus animal models being conscious and living somehow share a level of similarity being. The animals need to show parallelism with human anatomy and physiology, particularly with the vital organs of the body to study pathophysiology or devise the treatment of any disease. For decades animals are being exploited for the benefits of mankind, from devising new antibiotics to many transplants (hip transplant, pacemaker, knee, etc) thus they cannot be replaced completely. The pharmacological models (autoimmune encephalomyelitis – multiple sclerosis model, collagen induced arthritis, digoxin induced arrhythmias, etc.) have been proven to be of great relevance not only in devising new target for the drugs but also for the repurposing of drugs. The selection of proper methodology with the sound knowledge of the type of animal to be used for a particular disease can positively shift the whole process one step ahead.

Keywords: Screening, An animal Model of Human Disease, Allometric Modeling, Toxicity Studies

1. Introduction

Preclinical studies are performed to evaluate the pharmacological and toxicological response of any novel compound in animals for the first time to extract the data to be extrapolated on human beings. These are the studies done specifically on animals from lower to higher vertebrates (e.g. rats, mice, guinea pigs, rabbits, horses, monkeys, dogs, etc). The animals chosen for the research must show parallelism with human anatomy and physiology, particularly with the vital organs of the body to study pathophysiology or devise the treatment of any disease. For instance, omnivores show a level of similarity in intestinal physiology (similar gastric emptying rate, etc.) compared to a human, they can be the best models to study oral solid dosage forms, whereas canines (carnivore) cannot be used for the same because of their underdeveloped intestine with increased gastric emptying rates. Similarly, antibiotics cannot be tested in rodents (mice, rats, hamsters, etc) because the microflora in the intestine can get altered, which can altogether change the outcome. Sometimes, the functional groups present in the drug determine the choice of the animal, because of interspecies variations the drugs opt different metabolic pathways as humans that eventually affects efficacy and toxicology. For example, both amphetamine and ephedrine are the drugs that are metabolized principally via oxidative deamination in both humans and rabbits, whereas in rats, the same opt aromatic oxidation route as the major route of metabolism.

1.1. Screening

In general, screening means examining or filtering out the potential compound from less efficient compounds with respect to drugs and diagnosing the underlying disease and its cause when explained with respect to disease.

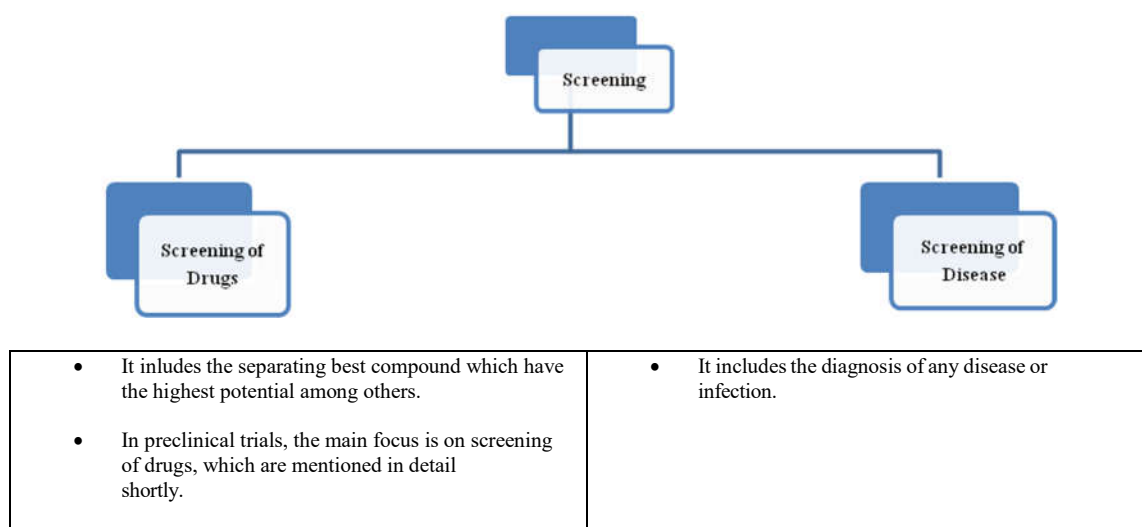


Fig.1 Types of screening

2. Stages of Preclinical trials

The time taken to complete the preclinical trials is 3-6 years this is because the testing of a newly discovered compound encompasses every aspect from its pharmacological response to toxicities studies are performed to ensure that the drug is liable to be used in human beings. Thus, the preclinical studies are performed at different stages which are as follows:

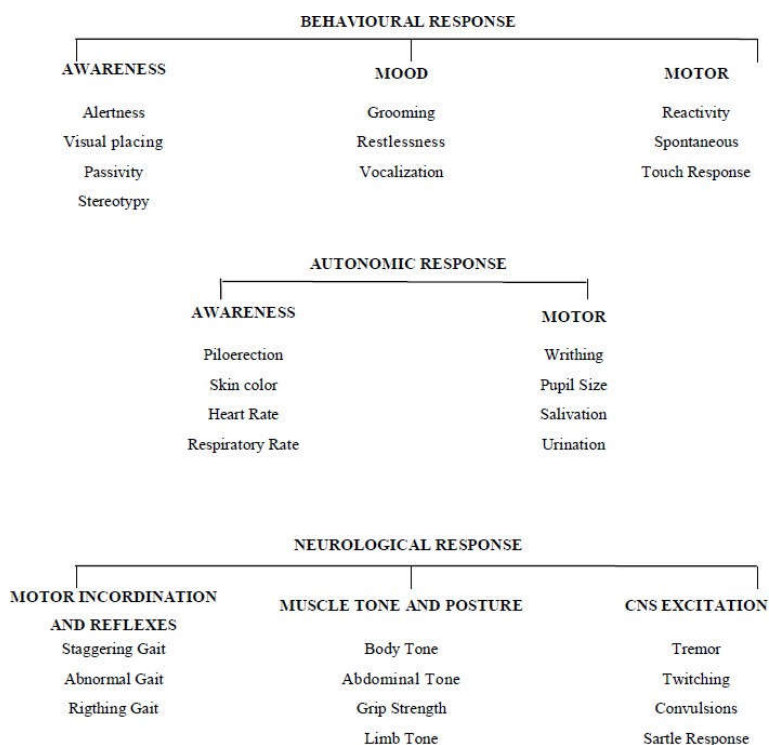


Fig.2 Irwin's Primary Tests for Pharmacological Activity.

2.1 General Observational Tests

It involves visual observation of a novel chemical entity in a live or conscious animal. One of the best ways to carry out the general observation test was best described and designed by Irwin called “Irwin’s Primary for Pharmacological Activity.” The new chemical entity is injected intraperitoneally and the response is assessed based on its behavioral, autonomic, and neurological responses. The observations enable us to compare the test with the reference drug of known activity. The observation includes is shown in Fig 2

2.2 Screening Tests

The word screening means the test substance or the novel chemical entity is exposed to the set of procedures that will segregate the substance having the potential to produce a desired therapeutic response from the ones which show the response submaximally or below. The compounds which are discarded in the first screening procedure are exposed to the secondary screening test, if they pass the stage further they can be taken into the next stages. In short, screening is nothing but the process of filtering the best or the potential compounds from a large number of chemicals. Principally the screening is of three types:

- *Simple or Single Screening* - This particular test is done whenever the new drug is developed by altering the structure either by addition or deletion of the functional groups from the parent molecule of known pharmacological response. The efficacy of the newly formed compound is detected to ensure that the new molecule is producing the same pharmacological response as that of the parent molecule. For example – the hypoglycemic test is performed for new substances that measure the ability to decrease the blood sugar level.
- *Blind or Random Screening*:- When the entirely new compound without any previous data of its pharmacological activity, is screened is called blind screening. It entails a series of tests to identify the nature of the drug and its activity. The only purpose is to determine the fact if the molecule is efficacious enough to process further. If at this stage any pharmacological activity is determined, the compound is sent for further processing.
- *Programmed or Rational Screening*:- This screening employs the identification of drug action not only its principal pharmacological effect but also the effects are seen on other organs of the body. It gives the idea regarding potential side effects caused by drugs at very early stages. It is generally employed when the new drug of a specific type is to be screened. It employs screening of certain drugs on CVS, kidney, CNS, etc.

2.3 Tests on Animal Model of Human Disease

An animal model is a living, non-human approach to study the effect of drugs by inducing the diseased conditions, particularly by inducing the conditions equivalent to the symptoms seen in humans during any pathological condition, this is done to ensure the efficacy of any new drug without the involvement of human beings in the process. The animal preferred for this purpose must react to the treatment in the same way as humans this is only possible if the animal has some similarities with the human genome or if animals share the same anatomy and physiology with humans. Many treatments, implants, etc have been devised by the use of animal models. The animal models used can be classified as follows:

- *Chemical models:*

The chemical models are developed by the induction of chemicals within the animal’s body to generate the diseased condition. Those chemically act on certain cells and destroy them thus producing the pathological conditions within them which is similar to the human pathological condition. For instance – to develop the parkinsonism with the animal, MPTP (1-methyl 4-phenyl-1,2,3,6 tetrahydropyridine) is injected within the animal’s body. Similarly, streptozotocin induces diabetes in mice by lowering the blood sugar level and digoxin is used to produce cardiac arrhythmia in rats. To analyze the anti-inflammatory activity of the compound, the edema is induced in rats by the introduction of some chemicals like carrageenan, formaldehyde, etc.

- *Physical models:*

The symptoms of certain diseases or disorders are induced within the animals by physical factors such as induction of convulsion in rats by electric shock, to study the anticonvulsant activity in the compound. Similarly, the heat generated analgesia is produced within the rats to study the analgesic potential of the compound.

- *Surgical models:*

These are the surgery generated models, it includes either surgical removal to any organ within the body for example removal of the pancreas from the rabbit to induce diabetes-like condition, to study the antidiabetic property of the drug or it includes or inducing the pathological conditions within the body without taking out any organ from the body. For example, pyloric ligation to induce ulcer within the animal to test for the antiulcer property of the drug.

- *Genetic models:*

Genetic manipulation is done in various ways and without using the above-mentioned method we can create any diseased condition within the animal. The genetic models are prepared by the introduction of a DNA sequence of the desired set of a gene with codes for a very specific and unique characteristic, using recombinant DNA technology, the desired DNA is incorporated within the germ cell line with the thought that via germ cell lines the characters are directly and easily be passed on to the next generation with normal reproduction. The method employed for the DNA transfer involves the addition of certain genes to the host genome (knocked-in) and sometimes deleting or silencing the specific set of a gene from the host genome (knocked-out), thus causing mutation within the organism. An example of a gene knockout model is ob/obknockoutmicemodeltostudyobesitywhereas the db/dbknockoutmicemodeltostudydiabetes. This particular type of mutation is termed as induced or targeted mutation which is different from the naturally occurring mutation within the body. Another way is the disruption or degradation of a particular gene at the time of development, this is achieved by the Flox-P system. The models can be made by different approaches:

Retr viral and lentiviral techniques

The foreign gene of interest is incorporated into the genome of a retrovirus, as the retrovirus has the ability to convert its genetic material which is RNA directly into viral DNA inside the host cell because of the presence of the special enzyme called reverse transcriptase. The viral DNA then incorporates into the host DNA and replicates. This specialty of the retrovirus is exploited in the production of transgenic animals. This method was successfully used for the very first time in 1974 when an SV-40 (simian virus) was inserted into mice embryos. The problem with the retrovirus is that it often damages the cell to which it is incorporated thus lentiviral transfection has replaced the retrovirus over these years. It produces stable transgenic cell lines by the introduction of lentivirus into the perivitelline space of zygotes.

Chemical technique In this technique, chemicals are used to facilitate the entry of DNA into the cells. The chemicals used for this purpose are diethyl aminoethyl dextran calcium or phosphate.

Pronuclear injection

The transfection done by this technique involves the microinjection of foreign DNA directly to the male pronucleus. It is done right after the fusion of egg and sperm cells when both pronuclei are visible distinctly. This technique is not the primary choice for transfection because of its low efficiency and variable expression pattern. The incorporation of foreign DNA can be checked by PCR (polymerase chain reaction) or green fluorescent proteins.

Electroporation

The cell membrane becomes polarized and loses its integrity upon the introduction of an electric field which makes cells become leaky and allow the permeation of exogenous DNA molecules inside the cell.

Embryonic stem cells

The embryonic stem cells are pluripotent that means they have the ability to differentiate into any organ of the body, these cells are being modified according to the need to produce genetically modified organisms with the desired set of characters. The embryonic stem cells along with primordial germ cells allow the development of producing transgenic animals through gene insertion technology.

Transplantation of Cultured Spermatogonia

The method involves the introduction of genetically modified germ cell lines into the testis of recipient male, the desired characters are seen in the next generation by normal reproduction.

Sperm Mediated Gene Transfer

The sperm cells can bind exogenous DNA readily. These modified sperm cells when fuses with oocytes either by artificial insemination or in-vitro fertilization carry the characters to the next generation. This technique is widely exploited in cattle.

RNA Interference(RNAi)

In this method, small interference RNAs miRNA (micro RNA) siRNA (small interfering RNA), which are 20-25 nucleotides long, bind to their complementary sequences on target in principle mRNAs and make a particular gene translationally silent. This is employed as a gene knocked out technology.

Extrapolating the data from animal model to humans

Allometric modeling

In this approach, the bodyweight of the animal is taken into consideration while determining the normal physiological parameters. This method cannot be applied if the elimination of the same drug is occurring through different routes in animals and humans. It can predicate the average values of the pharmacokinetic parameters. This is not a reliable method to extrapolate the data as there might be unexpected variations. FDA recommends accurate extrapolation of experimental animal dose to a human dose based on body surface area (BSA) in mg/m^2 .

The human dose can be accurately calculated using the following formula:

$$\text{HED} = \text{Animal dose} \times \text{Animal Km} / \text{Human Km}$$

Where:

HED - is the human equivalent dose determined in mg per kg body weight.

Animal dose - is determined in mg per kg of body weight

Table 1: Conversion of animal dose into human equivalent dose (HED) based on BSA

Species	Weight (kg)	BSA (m ²)	Km Factor
Human			
Adult	60	1.6	37
Child	20	0.8	25
Baboon	12	0.6	20
Dog	10	0.5	20
Monkey	3	0.24	12
Rabbit	1.8	0.15	12
Guinea Pig	0.4	0.05	8
Rat	0.15	0.025	6
Hamster	0.08	0.02	5
Mouse	0.02	0.007	3

Pharmacokinetic modeling (PK)

The pharmacokinetic models provide whole data of the drugs within the body drug absorption(its possible mechanism of absorption) distribution, drug binding ratio (tissue or blood proteins), metabolism (via phase I or phase II), clearance, excretion, etc). According to the preclinical data, human pharmacokinetics can be predicted up to an extent. Since this method provide a lot of data which a level of accuracy this is a widely used and accepted method in the extrapolation of data to humans

2.4. Bioassay and Confirmatory Tests

It is also termed as Biological Assay or Biological Standardization. Bioassay comparative assessment of a test substance with an internationally accepted standard compound of the same class, it is done to determine the potency or concentration of any agent (physical, chemical, or biological) by measuring the magnitude of response produces. It can either be performed directly on animals (in vivo) or on isolated cell lines or tissue (in vitro) done by measuring the pharmacological response of both tests and standards under the same conditions.

Scope of bioassay:

- It is the best option for the substances which degrade on exposure to the chemicals (for example hormones) or if any chemical assay is present it is very complex to perform, insensitive, or require a very high dose of the sample, for example, acetylcholine.
- When the active principle of the drug is not known or cannot be isolated, for example, posterior pituitary extract.
- It is best to perform when the sample to be analyzed is in a very small amount.
- Bioassay is best to evaluate the concentration of endogenous mediators present in tissue extract such as serotonin, prostaglandins, acetylcholine, etc.
- The chemical composition of the drug differs but has the same pharmacological actions and vice-versa e.g. cardiac glycosides and catecholamines.
- Bioassay has a huge role in environmental sciences also, it is done to monitor sewage discharge into the water bodies which have high human exposure.

- It is especially used to standardize drugs, vaccines, toxins or poisons, disinfectants, antiseptics, etc.
- Its contribution to the drug discovery process is huge as it is done to validate the SAR (structure-activity relationship) studies, comparing the potency and the effect of the molecule when different functional groups are added or removed on an intact animal.
- Bioassay is also done to determine the type of antibiotic that must be given for the patient's quick recovery by sputum analysis.

The bioassay can be classified as:

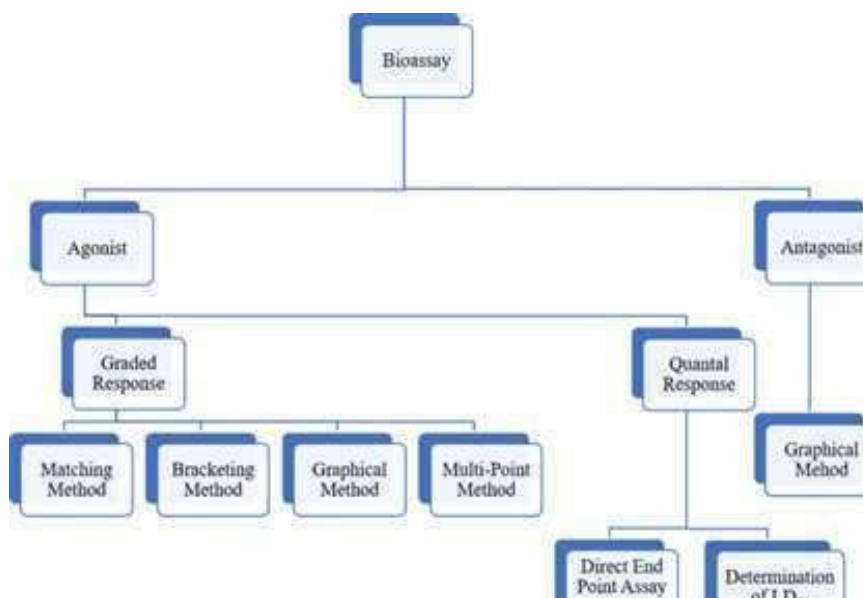


Fig. 3. Types of bioassay

2.5. Determination of the mechanism of action and systemic pharmacology

This approach is generally employed when the drugs are extracted from the natural source (plants, microbes, animals). As the drugs are prepared by techniques like CADD, combinatorial chemistry the mechanism of action is predetermined, as the drug is formed with prior knowledge of its receptor or target. Thus such compounds' mechanism of action is already known.

There are some extensively used techniques to determine mechanisms which are mentioned below:

- Radio Ligand Binding Assay
- Confocal Microscopy
- Scintillation Proximity Assay (SPA)
- Fluorescence Correlation Spectroscopy (FCS)
- Fluorescence Resonance Energy Transfer (FRET)
- Homogeneous Time-Resolved Fluorescence Technology (HTRFT) etc.

The effect of a particular drug on vital organs (brain, heart, gastrointestinal tract, kidney, liver, respiratory organs) is equally important, apart from its principal effect. Thus complete pharmacological profiling of the drug is done.

2.6. Quantitative tests:

The quantification of the response of any compound is very essential as dose determines the fate of any chemical substance that it will act as a drug or poison. The effect can be quantified in two ways:

- *Graded Effect:*

The principle involves is to evaluate the response by increasing the concentration dose. It is observed with increasing dose there is a proportionate increase in the response. The response produced is gradual and continuous. For example, in the assay of compound showing histaminic activity, the degree of contraction in smooth muscle varies with the increasing concentration of the compound.

- *Quantal Effect:*

The principle of this is based on the “All or None” phenomenon. The compound is checked for some degree of response or no response at all. For example to check the efficacy of compound to produce cardiac arrest or not.

2.7. *Determination of Pharmacokinetic parameters*

The pharmacokinetics is the study of the movement of the drug throughout the body, it includes the phases which almost every drug has to face that are absorption, distribution, metabolism, and excretion. This stage is important to tell if the compound is qualifiable to go further or not and many compounds are rejected in this stage of the preclinical trial due to undesirable pharmacokinetic parameters. The following parameters are determined during pharmacokinetic studies:

- *Absorption Parameters:*
 - Absorption of the compound via various routes.
 - Mechanism of absorption.
 - Various factors affecting absorption.
 - Rate and extent of absorption (Bioavailability).
- *Distribution Parameters:*
 - Tissue permeability of compound.
 - The volume of distribution.
 - Protein and tissue binding of the compound.
 - Factors influencing distribution.
- *Metabolic Parameters:*
 - Pathways of metabolism.
 - First Pass Metabolism.
 - Factors influencing metabolism.
 - Enzyme induction / Enzyme inhibition.
 - Bioactivation and other parameters.
- *Excretion Parameters:*
 - Routes of excretion. Clearance.
 - Dose Adjustments.
 - Factors influencing excretion.
- *Interactions:*
 - Pharmacokinetic interactions.
 - Pharmacodynamic interaction.
- *Other Parameters:*
 - Plasma Concentration – Time profile.
 - Bioavailability studies
 - Therapeutic Concentration Range studies.
 - Design of dosage regimen.

2.8. *Toxicity studies*

Toxicity studies are done to estimate the adverse effect of the novel compound on animals and also predict the mechanism of toxicity. The main aim is to determine the safety of the molecule within the animal species which are extensively used in laboratories. The toxicity study comprises of multiple-dose level studies to determine NOAEL (No Observable Adverse Effect Level) value, through which a safe starting dose is evaluated for Phase I clinical trials. Types of toxicity studies that are done during preclinical studies are:

Table 2. Types of toxicity studies that are done during preclinical studies.

STUDY	TIME	DESCRIPTION
Acute Toxicity Studies	1-3 days	It is a single-dose study. Animals are observed for any adverse effect and mortality. LD50 value is determined.
Subacute Toxicity Studies	3 days – 1 month	Repeatedly dose based on LD50 and ED50 are given for over a month, the animals are observed for any adverse effects, along with that their daily food intake, body weight, and hematological parameters are assessed.
Chronic Toxicity Studies	6-12 month	The animals are given a small volume of doses for more than 6 months, then the adverse effects are monitored, along with the other parameters mentioned in the subacute toxicity study.
Teratogenicity and Fertility Studies	-	In teratogenic studies, the effect of drugs taken by mothers during pregnancy is observed in developing fetuses. The effect of drugs is examined on the spermatogenesis in males and ovulation in females.
Mutagenicity Studies	-	The ability of a compound to produce alteration at the human genome, thus causing mutation.
Carcinogenicity Studies	-	The compound is checked if it is interfering with the cell cycle, due to which the regulation of the cell cycle gets disturbed causing excessive proliferation of cells within the body, thus producing cancer or tumor.
Immunotoxicity Studies	-	The ability of the compound to induce immune response within the body is studied.

3. Screening of Disease

The search for unrecognized disease or defect utilizing rapidly applied tests, examinations, or other procedures in apparently healthy individuals. Screening is testing for infection or disease in a population or in individuals who are not seeking health care facilities.

There are three types of screening.

- *Mass Screening:*

Mass screening involves assessing the whole population or a distinct sector of the population, for example examining all adults or elderly individuals for a particular disease such as AIDS, diabetes, tuberculosis, etc.

- *High risk or selective Screening:*

This type of screening is employed in a particular sector or group who are more likely to have the disease, these groups are made based on the epidemiological survey and research. For example, cervix cancer is prevalent in the people of lower social groups living in unhygienic conditions and is more susceptible to catch infection thereby ending up having the possibility of cervix cancer as compared to the upper social sector.

- *Multi phasic Screening:*

It involves the screening of more than one parameter with every single person in a particular population. This may include different tests like hematological, urine analyses, liver function tests, kidney function tests, audio and visual tests, etc along with the test the clinical history, questionnaires are also included in this type of screening.

4. Conclusion

The animals have always been of great relevance in the field of medical sciences. The success of the preclinical research is based on selecting the animal showing the best co-relation to humans otherwise most of the drugs fail in clinical trials. According to the statistics generated by federal organizations around 50% of drugs are rejected in preclinical trials only because of poor pharmacokinetics and some toxicological issues. Thus the selection of proper methodology with the sound knowledge of the type of animal to be used for a particular disease can positively shift the whole process one step ahead. If any drug qualifies the preclinical stage, the data generated by testing is very useful in devising the proper dosage form, route of administration, which can be used in the first phase of clinical trials.

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