PRECLINICAL SCREENING OF DRUGS

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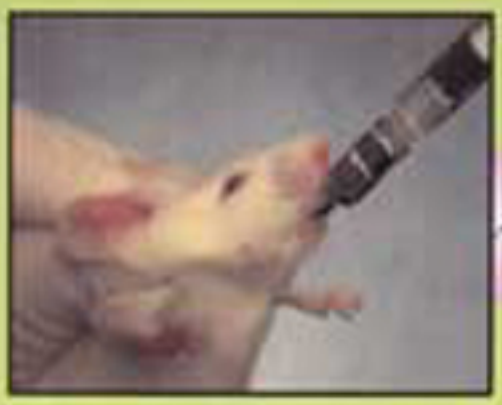

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PRECLINICAL SCREENING OF DRUGS

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Foreword

Preclinical Pharmacology i.e. Study of agent/drug on experimental animals has become a critically important determinant towards assessing the drug's safety profile and ensuring its safety and efficacy.

In keeping with the latest norms, it is required to decipher the adverse effects of a drug are crucial to chalk out its therapeutic effects and also decide the course of therapy. As against this, the integral aspects of this field viz. animal handling, care, conduct of experimental procedure needs to be done with minimum pain and discomfort to the animal and also the post experimental rehabilitation or disposal of animal as per the case is.

In view of the above, the authors have compiled a comprehensive database elucidated in lucid language and an easy going comprehensible design.

I am sure that this book will achieve its target of providing the current updates of the need and importance of Preclinical experiments and also the ethical roadmap to execute it.

My best Wishes for the fruitful and intellectual exchange through this book.

Col. (Retd.) S. K. Joshi
Campus Director
Dr. D. Y. Patil Educational Complex, Akurdi, Pune

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■■■

Preface

Preclinical screening is an important stage of drug development process which involves the evaluation of safety and efficacy of new chemical entity using suitable laboratory animals. The data collected from preclinical screening act as a base for clinical trials, hence preclinical studies carry immense importance.

The purpose of this book is to provide comprehensive information ranging from fundamental knowledge to recent updates of all aspects of experimental Pharmacology and animal models regarding screening of various categories of drugs. This book has also covered the basic CPCSEA guidelines especially towards care as well as handling of laboratory animals and conduct of the experiment not merely as a part of mandatory requirement but to enhance accuracy and precision of the data.

We are sure that, the information summarised in this book will act as a true guide for the students, teachers and research scientists to develop expertise in the area of Preclinical Pharmacology.

We have made a sincere attempt to amalgamate the required aspects of basic and advanced preclinical screening. We have also provided additional information pertaining to alternative drugs and their marketed preparations which is to achieve refinement in experimental design as suggested by the CPCSEA.

We are hope that this book will serve the purpose of preclinical screening requirements to suit the need of the hour.

14th September, 2016 **Authors**

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Contents

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Chapter 1...

New Drug Discovery Process

New drug discovery is the most challenging and expensive task of the pharmaceutical industry. It takes years together for a new drug to enter into the market.

In the recent years, with the advancement of various techniques and technologies like biotechnology, nanotechnology, nano-biotechnology, genetic engineering, recombinant DNA technology, biological screening procedures etc., the time required for the process of discovery of lead molecules has been markedly decreased. On the other hand, the cost incurred in new drug discovery process has been increased with the use of advanced techniques. The reduction in duration has become an advantage for bigger pharmaceutical industries, while cost has been a major limitation for comparatively smaller setup.

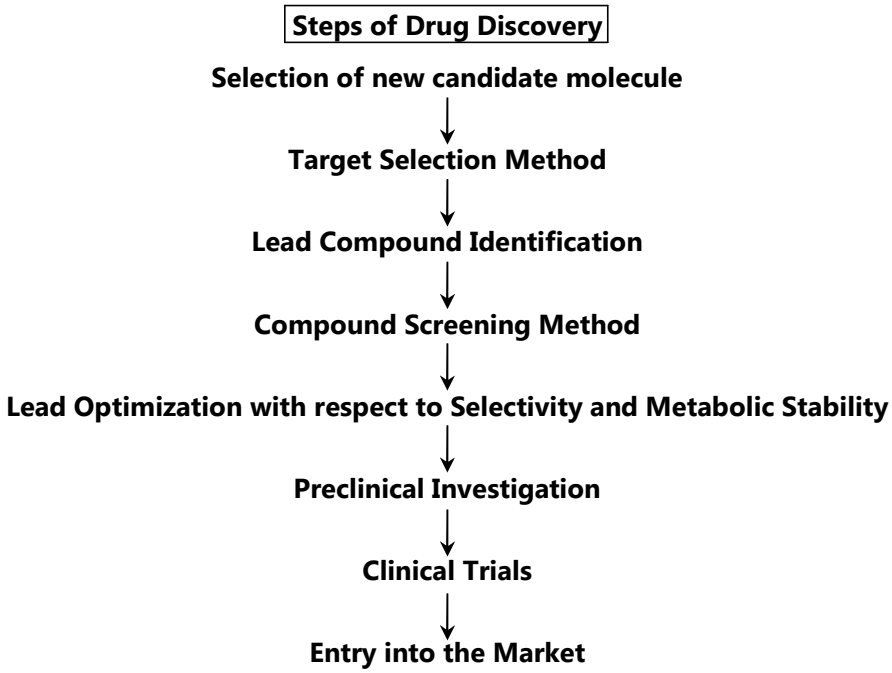
The main objectives of drug discovery are

- To develop clinically effective and safe drug.
- To explore new clinical uses and modify chemical configuration of the existing drug for better therapeutic outcome.
- To develop entirely new class of drugs.
- To determine the pharmacokinetic and pharmacodynamic properties and mechanism of action of various drugs.

Sources of drug discovery

New drugs can be discovered either from natural sources or it may be synthesized in the laboratory by several chemical modifications. During past several years, there has been growing interest among the researchers and clinicians in the use of various phytochemicals for the treatment of various ailments, due to their lesser side effects as compared to synthetic drugs. Furthermore, it is also possible to isolate particular phytochemical from plant sources followed by its structural elucidation, to develop its semi synthetic analogues. This process leads to desired alterations in the pharmacological activities compared to that of the original isolated phytochemical with the aim to improve its therapeutic utility.

Steps involved in drug discovery process:



1. Selection of new candidate molecule:

In the drug discovery process, candidate molecules are selected on the basis of their pharmacological properties. Some drugs may be found by accident (called serendipity), while most of the drugs are discovered as a result of systematically assigned screening programs and use of mechanistic approach. There are various techniques available and selection of technique entirely depends upon the type of drug under process.

2. Target selection:

One of the important aspects of drug discovery process is the target selection because without identification of target, several hindrances may be produced. Drug targets include functional proteins like receptors, enzymes, transport proteins etc. The targets are mainly chosen on the basis of knowledge about mechanism of disease or disorder, its chemical signaling pathway including involvement of new proteins (if any) and role of genes involved.

3. Lead compound identification and compound screening method:

After the selection of targets, the next step is to find lead compounds. Lead compounds are those compounds which need some chemical alteration by means of molecular modification, for the purpose of enhancing their usefulness as a drug. Molecular modification may enhance the specificity of lead compounds for particular drug targets, increase its potency, modify its pharmacokinetic parameters and reduce its toxicity. Certain new techniques, like high throughput assay system in association with combinatorial chemistry, allow the synthesis and screening of several hundreds or thousands of related compounds simultaneously and thereby reduce the time taken to find the lead compound for specific pharmacological activities.

4. Lead optimization

To increase the potency of the compound on its target and to optimize it with respect to various properties i.e. selectivity and metabolic stability, a technique called lead optimization is used. It encompasses various types of assay methods, study of in vivo biological activity, absorption studies along with evaluation of side effects associated with the use of drug. Lead optimization is mainly carried out with an objective of identification of one or more drug candidates suitable for further development.

Natural products could be used as a source of lead compound, but they are associated with several disadvantages. They possess complex structures which are difficult to synthesize or modify by the use of conventional synthetic chemistry; hence lead optimization may be difficult making the process of drug production much expensive.

Once a potential new drug has been identified, it is then subjected to a range of investigational stages in order to characterize it in terms of its likely safety and effectiveness in treating its target disease/disorder. Among these investigational stages, two main stages are:

(i) Preclinical trials

(ii) Clinical trials

In order to get approval for marketing, a new drug should be thoroughly screened for its safety and efficacy towards its intended use. After the discovery of new agent, it is characterized for various pharmacological as well as toxicological effects and potential therapeutic application to produce the evidences for its safety and effectiveness. Whenever the preclinical studies demonstrate adequate safety and effectiveness, drug discoverer files an Investigational New Drug Application (IND) seeking permission of its testing in human beings. Once the clinical trials are successfully completed, the discoverer of the drug may file a New Drug Application (NDA) to respective drug control authority (i.e. FDA: Food and Drug Administration as in India and USA).

It is the responsibility of drug sponsorers to test the drugs efficacy and safety on animals and, later, on human subjects through controlled clinical trials. NDA consists of proposed labeling for the new drug, entire history of drug development and testing, results of preclinical (animal) studies and clinical trials, drugs composition along with explanation that how the drug acts in the body, details of manufacturing, processing and packaging of drug with a special emphasis on quality control. The extensive review of NDA is carried out by FDA officials (which includes physicians, statisticians, chemists, pharmacologists and other scientists). If the drug fulfills the criteria of safety and effectiveness, the FDA approves it.

5. Preclinical Investigation:

The aim of preclinical investigations is to fulfill all the criteria that have to be complied before a new compound is deemed ready to be tested for the first time in humans. Regulatory authority approval to commence clinical trials is largely based upon preclinical pharmacological and toxicological assessment of the potential new drugs prescribed in animals. Such preclinical investigations usually take up to 3 years to complete and its expenses ranges between costs around 10-30 million USD (i.e. 60-200 Cr. INR). On an average, approximately 10% of potential new drugs pass preclinical trials. According to the guidelines of the regulatory authority, following tests are undertaken during preclinical trials.

(i) Pharmacological Studies:

Pharmacological tests mainly include all-round description of all the pharmacological effects of a drug, e.g. effect of drug on various systems. These tests are carried out on experimental animals (like rats, mice, guinea pigs, rabbits, dogs, monkeys). The selection of animal is based upon the type of activity to be screened, related resemblance of animal with human beings, identification of its overall desired therapeutic action and associated untoward effects.

(ii) Pharmacokinetic and Pharmacodynamic Studies:

Pharmacokinetics encompasses the study of absorption, distribution, metabolism and excretion (ADME) pattern of the drugs. The results of pharmacokinetic studies help to identify most appropriate method of its administration, most likely effective dosage regimen to employ and extent of toxicity (if any). On the other hand, pharmacodynamic studies are carried out to determine the effect of drug on the body with special emphasis on its mechanism of action and interaction with cell/organ type or receptors, associated side effects with respect to dose response relationship.

(iii) Toxicity Studies:

Toxicity studies are carried out using laboratory animals to determine presence and extent of any short term, long term toxicity, genotoxicity if any. These studies are also helpful to ascertain the maximum tolerated dose of the drug. Toxicity studies are highly expensive and time consuming, but essential to extrapolate possible toxicity in humans. However, they do not provide full proof data to extrapolate (2-5 years). The collection of toxicity data from maximum possible species in consultation with institutional animal ethics committee is an ideal way for providing a higher predictive value for its toxicity in humans. The animals under toxicity studies are sacrificed at the end of experiment to check for different histological and biochemical evidences of tissue and/or organ damage also called as post mortem analysis. Different types of toxicity studies performed on animals include: (a) acute toxicity studies, (b) sub-acute toxicity studies and (c) chronic toxicity studies.

(a) Acute toxicity studies: These studies are also known as single dose studies, since a single high dose of the test drug is usually administered. Both mice and rats (either sex) are usually employed. Single dose of a test drug is given in 2 species of animals by two routes of administration, one of which should represent the proposed therapeutic method of administration. The animals are then supervised for the period of 7-14 days for gross behavioral changes and fatalities finally undergoing extensive post mortem analysis. These studies require calculation of LD_{50} (i.e. lethal dose which is expected to produce mortality in 50% of the test animals) for the determination of maximum tolerated dose of test drug. Earlier large quantities of animals were used in such studies. But now as per CPCSEA guidelines, revised procedures have been implemented which have reduced their requirements to a greater extent.

(b) Sub-acute toxicity studies: These studies are also called as repeated dose studies, wherein, 03 different doses of test drug are administered in minimum 02 different species up to 06 months. This duration of sub-acute toxicity studies increases for drugs like antidiabetics, antihypertensives etc., which are intended for long term clinical use. After the completion of sub-acute toxicity studies, evaluation of different physiological signs, hematology, histology, autopsy etc. examination is carried out for the drugs producing toxicity.

(c) Chronic toxicity studies: These studies require comparatively large number of animals and may last up to 2 years. Most chronic toxicity study protocols include demand daily administration of test drugs. Minimum two different animal species are used. Usually rats and dogs are preferred. Routine clinical examination, periodic biochemical investigations (e.g. blood and urine analysis), extensive histopathological examinations at the termination of study is usual pattern of this study.

Table 1.1: Types of toxicity studies

Type	Description and objectives	Duration
Acute toxicity (Single dose toxicity studies)	• Single dose of a drug is administered in minimum 2 species of animals by two different routes • Determination of LD_{50} • Determination of maximum tolerated dose	7-14 days

contd. …

Sub-acute toxicity studies (Repeated dose toxicity studies)	• Evaluation of three doses of drug in 2 different species	Up to 6 months
Chronic toxicity (Long term toxicity)	• Daily administration of drug in different animal species. (Usually rats and dogs are preferred.)	Up to 2 years

(iv) Carcinogenicity Studies:

Carcinogenesis is the process of induction of malignant characteristics in cells. If the test drug is intended for its long term use (up to 1 year and more), then carcinogenicity test has to be performed. In addition, if there is any reason to suspect that the active molecules could be carcinogenic, then it is recommended even if it is intended to be used for less than one year. In this test, the test drug is administered at 3 dose levels in 2 animal species (which are known to have low incidence of spontaneous tumor) for periods of up to 2 years, and at the end of the test, various tissues are examined histologically for carcinogenesis and related changes.

(v) Mutagenicity Studies:

Mutagenicity tests are performed to determine the ability of test drug to induce changes in the genetic material of animals (DNA) like alterations in chromosomal structures along with modification in various nucleotide sequences. These tests are usually carried out by both methods i.e. in vitro and in vivo. The drug which induces mutagenicity upon administration may show variable effects ranging from abnormalities in mating to loss of embryo or several deformities in fetus.

(vi) Reproductive Toxicity Studies:

In reproductive toxicity studies, a test drug is administered at three different dose levels (ranging from non-toxic to slightly toxic) to different groups of the target species (usually rodents). These studies aim to assess the nature of any effect of test drug on male or female reproductive functions. The drugs are mainly administered to males (on full spermatogenesis cycle) for at least 60 days; whereas, females are dosed for at least 14 days before they are mated. Following these, specific tests like assessment of male spermatogenesis, female fertilization and follicular development, implantation and early fetal development are carried out to assess the effect of drug on reproductive system.

6. Clinical Trials:

Clinical trials are defined as a systemic study of new drugs in human volunteers called subjects to generate comprehensive data especially to determine safety and effectiveness of new drugs.

A clinical trial requires the prior approval of an Investigational New Drug Application (IND) which has been filed by the discoverer of new drug. Further, protocols for clinical trial testing are typically developed by researchers and are subjected to the approval of an FDA review board. These protocols clearly describe the type of people which may participate in trial, the schedule of tests and procedures, dosage and types of medications and length of total clinical trial study. Throughout various phases of clinical trial, the trial participants are monitored for the safety as well as effectiveness of drug along with associated adverse effects.

Clinical trials of a new drug proceeds through following phases:

(i) Phase 0 trials

(ii) Phase I trials

(iii) Phase II trials

(iv) Phase III trials

(v) Phase IV trials

(i) Phase 0 Trials:

Phase 0 trials are the recent designation for exploratory, first in human trials conducted in accordance with United States Food and Drug Administration (US FDA). These trials are also called as human micro dosing studies and are designed to speed up the development of promising drugs by establishing very early on whether the drug behaves in human subjects as was expected from preclinical studies. Distinctive feature of Phase 0 trial includes the administration of single sub-therapeutic dose of the study drug to a small number of subjects (10-15) to gather preliminary data on agent's pharmacokinetic and pharmaco-dynamic parameters.

Phase 0 studies give no data on safety and efficacy of therapeutic agents. Pharmaceutical companies carry out Phase 0 trials to rank drug candidates in order to decide which has the best pharmacokinetic parameters in humans to take forward into further development.

(ii) Phase I Trials:

These trials are also known as human or clinical pharmacology trials. The main objective is to determine the safety of the maximum tolerated dose in healthy adults of both sexes. At least two subjects should be administered each dose to establish the safe dose range,

pharmacokinetic, pharmacodynamic effects and adverse reactions, if any, with their intensity and nature. The duration of time lapsing between two trials in the same volunteer should be minimum of 03 months. The volunteers should preferably be covered under some insurance scheme. Usually, Phase I trials are carried out on test population of 20-100 healthy volunteers.

Phase I trials are further categorized into different studies as follows:

(a) SAD studies: Single Ascending Dose (SAD) studies are those in which small groups of subjects are given a single dose of drug while they are observed and tested for a period of time. If they do not exhibit any adverse side effects, and the pharmacokinetic data is roughly in line with predicted safe values, the dose is escalated and a new group of subjects is then given a higher dose of test drug. This is continued until pre-calculated pharmacokinetic safety levels are reached or intolerable side effects start developing up. This helps to determine maximum tolerated dose of a drug.

(b) MAD studies: Multi Ascending Dose (MAD) studies are conducted to understand the pharmacokinetics and pharmacodynamics of multiple doses of drug. In these studies, a group of patient receives multiple low doses of drug, while samples (of blood and other body fluids) are collected at various time intervals and analyzed to understand how the drug is processed within the body. The dose is subsequently escalated for further group upto a predetermined level till the intolerable side effects start developing up again in the group.

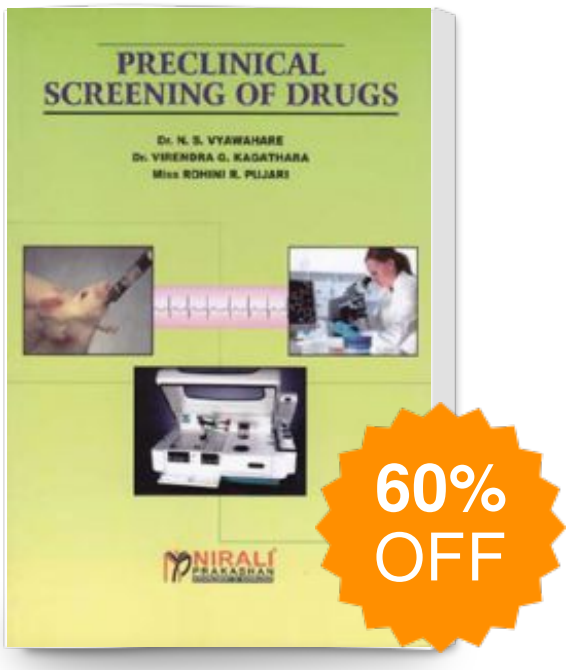
(c) Food effects: A short trial designed to investigate any differences in absorption of the drug, caused by eating before the drug is given. These studies are usually carried out as crossover study, with volunteers being given two identical doses of drug on different occasions; one while fasted, and other one after being fed.

(iii) Phase II trials:

These are controlled studies conducted in a limited number of patients (usually 100-500) of both sexes to determine therapeutic uses, effective dose range, further evaluation of safety and pharmacokinetic parameters. These trials are also called as exploratory trials and the ultimate goal is to determine the therapeutic efficacy and tolerability of the drug by patients.

The drug is mainly studied in the patients, who have the condition for which it is intended to be used. It is mainly useful to determine whether the drug has a favorable effect on the diseased state or not. A placebo (an inactive substance that has no treatment value) or positive control drug is included in a single blind or double blind study.

Preclinical Screening Of Drugs



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