

PCO 351

The evaluation of a drug candidate molecule involves processes, which include biological screening, animal toxicity studies and clinical trials in humans.

Sources of a new drug

Most new drugs can be discovered through the following processes

1. Identification of a new drug target
2. Chemical modification of a new drug
3. Screening for biological activity in a large number of natural products, collections of previously discovered agents with medicinal properties and large collections of peptides, nucleic acids and other organic molecules.
4. Repositioning of a drug for new therapeutic or the combination of drugs to produce synergistic or additive effects
5. Biotechnology and cloning using genes to produce peptides and proteins,
6. The design of a new drug based on knowledge on biologic mechanism, drug receptor interaction and drug structure

Biological screening of a new drug

This is the evaluation of the biological activity of a drug candidate molecule at the molecular, cellular, organ system and whole organism levels. Drug candidate molecules are screened at the molecular levels to assess receptor binding affinity to cell membrane containing receptor and binding sites on enzymes. At the cellular levels a drug is evaluated to know if it is an agonist, partial agonist or antagonist at the receptor site. Whole animal studies are performed to evaluate the effect of a drug on organ systems and disease models. In whole animals, candidate drug molecules can be evaluated for effects, which include hypoglycaemic, analgesic, anti-inflammatory. The selection of molecules for further studies is best performed with animal models of human disease. However, some pre-clinical animal models of human disease are not available. Biological screening is categorized into three; simple screening, blind screening

and programmed screening. Simple screening involves the use of one or two tests to evaluate if a substance has a particular property. Blind screening is used for new chemical substance that is available, however, with no information on its pharmacological activity. Blind screening is used to provide clues to potential activity and to indicate field of activity. In addition, blind screening ought to show pharmacological inertness if it exists. Programmed screening is used when series of new compounds are to be investigated for some pharmacological effects (for example, effects on the heart), a program of testing is required to provide information on the compounds.

Preclinical safety and toxicity testing

Toxicity testing is a branch of science that deals with toxins and poisons and their effects and treatments. A drug candidate molecule that survived biological screening and profiling procedures must be assessed for potential risks (toxicities) preclinically in animals and clinically in humans. Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. The toxic effects of chemicals, food substances, pharmaceuticals, etc., have attained great significance in the 21st century.

Institute ethics committee

Before conducting any toxicological testing in animals or collecting tissue/cell lines from animals, the study has to be approved by the Institute Animal Ethics Committee (IAEC) or the protocol has to satisfy the guidelines of the local governing body. The guidelines for conducting experiments and regulatory requirements vary from region to region.

Safety and toxicity evaluation of a candidate drug in animals

This is divided into acute toxicity test, sub-acute toxicity test and chronic toxicity test.

Acute toxicity test

Acute toxicity test is carried out to determine the effect of a single dose of a candidate drug on a particular animal species. In general, it is recommended that acute toxicity test be carried out with two different animal species (one rodent and one non-rodent), two routes and a single dose. Animals are observed for mortalities caused by the investigational product during the experimental period. Also, morphological, biochemical, pathological, and histological changes in the dead animals are investigated. Acute toxicity test permits the 50% lethal dose (LD_{50}) of the investigational product to be determined. The LD_{50} was used as an indicator of acute toxicity previously. LD_{50} is the dose of a drug that is required to kill 50 % of animals in a study group or population. Some of the methods used for the determination of LD_{50} include Lorkes' method, arithmetical method of Karber, and graphical method of Miller and Tainter. The determination of the LD_{50} involves large numbers of animals, and the mortality ratio may be high. Because of these limitations, modified methods were developed which include; fixed dose procedure (FDP), acute toxic category (ATC) method, and the up-and-down (UDP) method. Despite the limitations of LD_{50} it can be used to calculate the therapeutic index (TI) of a drug. TI gives an idea of the margin of safety of a drug and shows the relationship between the effective dose and the toxic dose. TI is calculated as the ratio of LD_{50} and ED_{50} . ED_{50} is the dose that is effective in 50% of animals in a study group or population.

Lorke's Method

This method of acute toxicity study has two phases based on the outcome of the study. The first phase, consist of three groups of 3 animals' per group which are administered with respective doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of the test substance based on the selected route. The animals are monitored for 24 hour for behavioral changes and mortality after the

administration of the test substance. If there is no mortality observed in the first phase, the study goes into the second phase. This phase consists of 3 animals of 1 animal per group administered with 1600 mg/kg, 2900 mg/kg and 5000 mg/kg body weight respectively of the test substance. The animals are observed for behavioral changes and mortality after the administration of the test substance. If mortality occurred in any of the groups the LD₅₀ is determined by calculating the geometric mean of the lowest dose that produced mortality (minimum toxic dose) and the highest dose that produced no mortality (maximum tolerated dose). The LD₅₀ is calculated by the formula below. However, if there is no mortality in phase two it shows that the test substance is safe up to a dose of 5000 mg/kg body weight. The determination of LD₅₀ has some limitations which include (a) observations in animals may not be accurately applied to humans (b) it does not take cognisance of idiosyncratic reactions (c) some toxicities in humans depend on the clinical stage of a disease.

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D₀ = Highest dose that gave no mortality

D₁₀₀ = Lowest dose that produced mortality

The fixed dose procedure (FDP)

The FDP is used to assess the non-lethal toxicity rather than the lethal dose. The investigational product is administered at fixed dose levels of 5, 50, 500, and 2000 mg/kg and the experimental animal is observed for a specified period.

Acute toxic category (ATC) method

The ATC method is a sequential procedure in which three animals of the same sex are used in each step. In the ATC screening method, four pre-identified starting doses may be used and animals observed for toxic effects.

Up-and-down (UDP) method

The UDP method is also known as the staircase design. This is method most recommended by various regulatory agencies because it reduces the number of vertebrate animals in research. The UDP screening method involves dosing single animal sequentially at 48 h intervals. A dose less than the best-estimated LD₅₀ dose is selected and administered to an animal, and the animal is observed for 48 h. If it survives, the study is continued with a higher dose (twice the original dose); if the animal dies, testing is conducted with a lower dose with another animal of the same sex as the original animal. UDP method is limited to doses up to 2000 mg/kg.

Sub-acute toxicity test

Two species (Rodents and non-rodents), two routes and three doses are used for acute toxicity testing. A candidate drug is normally administered for 4 weeks to 3 months. The longer the duration of expected clinical use in humans the longer the sub-acute test. The body weight variations, biochemical and cardiovascular parameter changes and behavioural changes are observed. At the end of the study, the experimental animals are sacrificed. Gross pathological changes are observed, and all the tissues are subjected to histological analyses.

Chronic toxicity test

It is the evaluation of the toxicological effect of a candidate drug for a long period about 1-2years. It is very essential if the drug will be used for a very long duration in humans. Chronic toxicity studies are conducted with a minimum of one rodent and one non-rodent species. The test compound is administered for a long period (1-2yrs) and the animals are observed periodically. A chronic toxicology study provides inferences about the long-term effect of a test substance on animals, and it may be extrapolated to the safety of the test

substance in humans. During the study period, the animals are observed for normal physiological functions, behavioural variations and alterations in biochemical parameters. At the end of the study, tissues are collected from all parts of the animal and subjected to histological analyses.

Mutagenicity test

Mutagenicity test is used to assess changes in the base sequence of DNA, chromosomal aberrations, and structural aberrations in DNA including duplications, insertions, inversions, and translocations. Certain types of mutations result in carcinogenesis. The determination of the mutagenicity is essential in the development of a candidate drug molecule. *In-vitro* test is carried out in two or three different bacteria and mammalian cells to cover the end points of gene mutations, clastogenicity, and aneuploidy. An example of mutagenicity test is bacterial reverse mutation assay.

Carcinogenicity test

This is the evaluation of the ability of a candidate drug to induced cancer or tumour in animals. Both rodents and non-rodent animal species are used in carcinogenicity test. The tests are carried out over the greater portion of an animal's lifespan. During and after exposure to test substances, the experimental animals are observed for signs of toxicity and development of tumors. If these are not found, a test may be terminated after 18 months in the case of mice and hamsters and after 24 months in the case of rats. If the animals are healthy, hematological analysis is performed after 12 months and 18 months, respectively, and the study is terminated. The animals are sacrificed, and gross pathological changes are noted on all the tissues.

Reproduction toxicity test

It is the toxicological evaluation of the effect of a candidate drug on the reproductive function of animals. The test compound is administered to both male and female animals. Administration is for the duration of one complete spermatogenic cycle in male animals and for two complete estrous cycles for female animals. Rodents are preferred for the one-generation reproduction toxicity testing. After the completion of the specified duration of drug administration, the animals are allowed to mate. The test compound is administered to the female animals during the period of pregnancy and nursing. The sperms of male animals are collected, and analysed. During the study period, the animals are observed for signs of toxicity, parturition, the number of offspring and their sexes are recorded. The numbers of dead and live pups and the weights of the live pups are noted. After the termination of the study, the animals and pups are sacrificed and subjected to a histopathological examination.

Neurotoxicity studies in rodents

This is the toxicological evaluation of the effect of a test substance on the central nervous system. Neurotoxic studies may be employed to evaluate the specific histopathological and behavioural neurotoxicity of a chemical substance. The observations are used to characterize neurotoxic responses such as neuropathological lesions and neurological dysfunctions (loss of memory, sensory defects, and learning and memory dysfunctions). Usually neurotoxicological studies are carried out in adult rodents. The test substance may be administered for 28 days or even more than 90 days, and neurological changes are evaluated. In 1998, the *in-vitro* model for neurotoxicity was developed, and various regulatory agents now recommend *in-vitro* neurotoxicity test.

Developmental toxicity/embryotoxicity studies

This study evaluates the toxicological effect of a candidate drug on an embryo. It can be performed using both *in-vivo* and *in-vitro* methods. Rodents are preferred for *in-vivo* toxicity screening. The compound is administered between the 8th and 14th day of pregnancy, and embryo lethal effects are studied. At the end of the study or on the 21st day of the study, a caesarean section is performed and parameters such as fetuses with hemorrhagic bullae, limb malformations, exencephaly, cleft palates, open eyelids, and tail deformities as well as the mortality and the numbers of dead and live pups are noted. Embryotoxicity studies can be performed using *in-vitro* methods such as the embryonic stem cell test for embryotoxicity, micromass embryotoxicity assay, and whole rat embryo embryotoxicity assay.

Genetic toxicity test

Genetic toxicity tests are used to identify gene mutations, chromosome changes, and alterations in the DNA sequencing. These tests are usually conducted in various species including whole animals, plants, micro-organisms, and mammalian cells. In the whole animal model, rodents are preferred. Genetic toxicity is assessed using the rodent chromosome assay, dominant lethal assay, mouse-specific locus test, micronucleus test, heritable translocation assay, and sister chromatid exchange assay.

Investigation toxicity

This involves the determination of the sequence and the mechanisms of observed toxicological effects in animal studies. The genes, proteins and pathways involved are determined.

Limitation of preclinical testing

1. Toxicity test is time consuming and expensive
2. Large number of animals may be required to have valid preclinical data or result
3. Extrapolation of data from animal study to human may be predictive for many, but not for all toxicities
4. For statistical reason rare adverse effect may not be detected

Regulatory requirements

Before conducting any clinical study, the safety of the test substance has to be assessed using animals. The target organ toxicity, relationship between the dose and response, relevant human effects, and any complications arising during treatment (adverse drug reactions) should be established through preclinical evaluations. The toxicity study should be carried out with a minimum of three doses viz. low, medium, and high doses in the experimental animals and the toxic effect compared with data from a control group of animals. From the toxicological evaluation, the no observed effect level (NOEL) or NOAEL, which may be useful for human studies, may be established. The low dose, intermediate dose, and high dose used in a toxicity test provide the NOEL, dose–response relationship, and target organ toxicity in animals.

Once acceptable results are obtained from the animal toxicology studies and the candidate drug is determined to be potentially safe and effective in humans, application for the Claimed Investigation exemption for a New Drug (IND) is submitted to the drug regulatory agency e.g food and drug administration (FDA) in the USA for permission to try the drug in humans. The IND requires specific information about the drug, including its chemical composition, pharmacologic nature, and the results of preclinical tests. The IND also requires a complete protocol and method for the initial human clinical studies. IND regulations

further include institutional review board approval for clinical trials. There are 2 types of INDs: commercial and non-commercial. A commercial IND authorizes the drug company to collect the safety and effectiveness data required for a New Drug Application (NDA), which if approved by the FDA, allows the drug to be marketed. A noncommercial IND permits the Drug Company or sponsor to use the drug in research or early clinical trials for the sole purpose of advancing scientific and medical knowledge.

Testing of a new drug in humans

Clinical human trials are allowed to begin after IND approval. These trials are divided into 4 sequential phases, which are simply labeled phase 1, 2, 3, and 4.

Phase 1 clinical trials (Clinical pharmacology)

Phase 1: This is the first time the new drug is used in humans. The subjects are healthy volunteers, not patients, and usually are male. However, if significant toxicity is expected from the new drug as in the case of AIDS or cancer patients with the relevant disease are used for phase 1. For ethical reasons, women of childbearing age are banned from participating in phase 1 and phase 2 trials, simply to protect a fetus from exposure to an experimental chemical. Typically, 25 to 50 subjects are enrolled in phase 1. The primary concern in phase 1 is the safety of the new drug. Phase 1 involves monitoring of pharmacokinetics in humans: absorption, distribution, metabolism, and excretion of the new drug. In this phase, the volunteers are kept within the investigation facility under close supervision and monitoring by specially trained clinical pharmacologists. Generally, this phase lasts several months and if the outcome is successful the study moves to phase 2. It is not a blind trial 'an open trial' because the volunteers and the investigators know what is been administered.

Phase 2 clinical trials (Exploratory clinical trials)

In phase 2, investigators give the new drug to patients who have the particular condition or disease the drug is intended to treat, but who are relatively free of coexisting illnesses or disorders. About 100 to 300 volunteers are used for this phase of study. In this phase, the safety and effectiveness of the drug is assessed. Phase 2 is divided into phase 2A and phase 2B. The phase 2A trials are open trials, because patients, and clinicians know the drugs been administered (the experimental drug, or an older drug already approved and known to be effective and the placebo. Phase 2B studies are double-blinded studies, meaning that neither physicians nor patients know whether the new drug or the placebo is being dispensed. Phase 2 lasts several months to 2 years. The results of the phase 2 studies are put through exhaustive statistical analyses to warrant advancement to the phase 3 trials. Phase 2 trials are performed in special clinical centers e. g university hospital

Phase 3 clinical trials (Multicentre clinical trial)

Successful phase 2 trials lead to the initiation of phase 3 trials. Phase 3 is intended to validate the new drug's creation and its ability to cure or treat an ailment. The purpose of phase 3 is to validate the statistical results of phase 2 and to further assess safety, dosage, and effectiveness of the drug in a targeted population. This phase of the study is carried out at multiple centers. Phase 3 involves several hundred to several thousands of patients, and lasts 1 to 4 years. This phase is usually performed in settings similar to those which the drug will be used and the investigators are specialists in the disease been treated. The drug approval process at the completion of phase 3, involves the submission of a new drug application (NDA) to the required drug regulatory agency. The NDA captures a clear and comprehensive picture of the drug, from its chemical structure to the results of the phase 3 trials. It includes specific technical information and scientific data such as:

1. A description of the chemical and physical composition of the drug.

2. An explanation of the methods, facilities, and controls used to manufacture, process, and package the drug and how the drug will be shipped and stored in bulk supply.
3. Actual samples of the drug with examples of labeling and package inserts.
4. Proof that neither the manufacturing process nor the drug itself is harmful to the environment.
5. A description of the pharmacologic actions and toxicological effects of the drug, including effects on reproduction and the unborn fetus.
6. Data from all preclinical animal studies and clinical human trials, with a comparison of the results and statistical evaluations.
7. A summary of the benefits and risks and convincing evidence that as long as the drug is used as prescribed, the benefits outweigh the risks.

Phase IV clinical trial (Post marketing surveillance)

If the appropriate drug regulatory agency gives approval for the NDA, the study moves to phase IV. Post marketing surveillance refers to the process of obtaining information on drug experience after a drug is approved for use by the general population. It involves several thousands of people who have the disease the drug is intended for, but are free of co-morbidities. In this phase of study an adverse reaction that was not identified in phases 1-3 clinical trials might surface once the drug is used by the general public. For example, an untoward reaction might occur in 1 out of 5,000 people administered the new drug or 1 in 10,000. Phase 4 study has no fixed duration. This study is instituted to assess the 3 most outstanding post marketing concerns. First, the nature of the drug is explored more closely, with an intimate focus on the incidence and severity of adverse reactions. Second, the drug's long-term effects on morbidity and mortality are ascertained. And third, the consequence of use in populations not well represented in premarketing studies eg pediatrics and geriatrics. The

time for the filing of a patent application to approval for the marketing of a new drug can take up to 5 years or more. The life time of a patent granted to the manufacturer of a new drug (a pharmaceutical company) may last for 20 years. After the expiration of the patent any pharmaceutical company can produce the drug, file an NDA with the drug regulatory agency and market the drug as a generic product without paying license fees to the original patent owner. However, the trade mark (the drug's proprietary trade name) will be legally protected.