

PRECLINICAL STUDIES IN THE DRUG DEVELOPMENT PROCESS: PROSPECTS AND CHALLENGES

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ABSTRACT

The process of drug discovery is lengthy and tortuous, spanning several years. These years are characterized by different stages of differing development processes. A major stage in this development process is the preclinical stage, which is characterized by testing the drug candidate in animal models as a predictor of its efficacy and tolerability in humans. Preclinical drug trial is froth with challenges, which range from choice of the appropriate animal species to generation of the relevant data for filling application for IND.

A successful pre-clinical trial is still not a guarantee of the drug product scaling through the clinical stage. These have been attributed to heterogeneity differences in the animal and human species. Effort in genomic research to circumvent this challenge is presently on-going.

INTRODUCTION

Drug discovery according to Steven, et al. (2010), is still a lengthy, expensive and inefficient process with low rate of new therapeutic discovery. Discovering and bringing one drug to the public, typically costs a pharmaceutical or biotechnology company a budget range of \$800 million to more than \$1 billion and takes about an average of 10 – 15yrs, according to Pharmaceutical Product Development Inc (2012), a pharmaceutical research laboratory. This is supported by a similar study by Hughes et al,(2011) in which they maintained that a range of 12-15ys is required for a new drug product to successfully reach the market for clinical application.

Only preclinical studies can last for 1 – 5yrs. In addition to the cost implication and rigors of the development process, the efficiency or success rate is a great challenge. Only five in five thousand or 10% of the drugs that begin pre-clinical testing ever make it to human testing. Only one of these five is ever approved for human usage (Hughes et al, 2011).

The journey of finding a new drug for an identified disease process involves high thorough-put screening (HTS) where large number of chemicals is tested for ability to influence the target and achieve desired effect (Fox et al. 2006) This screening also helps to determine the selectivity of the chosen compound to the target. The more selective a molecule is to the target the better. This implies that it interacts with only the target and less with other related targets. A successful identification of an active compound or intended new drug sets the stage for pre-clinical trials.

This paper on the types of pre-clinical studies conducted during drug trial will be discussed by reviewing generally pre-clinical drug development, the types of preclinical drug trials conducted and the choice of animal species. The rising controversy over the use of animals for pre-clinical drug trials and the possible way forward will also be discussed.

PRE-CLINICAL DRUG DEVELOPMENT

Pre-clinical drug development (trials) as pointed out by Steinmetz & Edward, (2009), involves all the activities that link drug discovery in the laboratory to initiation of human clinical trial. They also postulated that preclinical studies can be designed to identify a lead candidate from several target hits, develop the best procedure for new drug scale-up, select the best formulation, determine the route, frequency and duration of exposure, and support the subsequent clinical trial design. Pre-clinical testing therefore analyzes the bioactivity, safety and efficiency of the formulated drug product, according to Pacific biolabs, (2012); a pharmaceutical development research laboratory.

During the pre-clinical stage of drug development process, plans for clinical trials and an investigational drug (IND) application are prepared. The Wikipedia; free encyclopedia, (2012), puts it as the stage of research that begins before clinical trial; (the testing of drugs in humans), can begin. It is during this period that important feasibility iterative testing and drug safety data is collected. Many project development teams find it helpful to develop a target product profile (TPP) to guide preclinical development. The TPP, according to Steinmetz & Edward, (2009), is a useful tool for delineating the required and/or desired attributes of the new drug product, critical milestone and metrics to success.

The TPP is also affirmed by Curry & Brown, (2003) as a framework that ensures that the preclinical development programme supports the intended clinical trial design and therapeutic use. The content of a TPP depends on the drug product or research team. However, the general profile includes therapeutic indication, expected clinical use, drug target and mechanism of action, market size, competition and differentiators, drug target route, form and frequency of administration, patient's age, bioavailability and duration of action, safety precaution and contraindications, chemistry, manufacturing and controls profile, including solubility, manufacturing process, formulation, storage conditions and stability, patent status and modifiers of exclusivity of usage, such as in orphan drug status.

The main aim of pre-clinical drug trial or studies is to determine a product's safety profile. Products may be new or iterated or like-kind medical devices, diagnostic devices, drugs; gene therapy solutions etc. All products, as noted by Wikipedia; free encyclopedia,(2012), don't necessarily undergo the same process of preclinical studies. Drugs may undergo pharmacokinetics, pharmacodynamics, absorption; distribution, metabolism and excretion (ADME), and toxicity testing using animals. This enables researchers to estimate the safe starting dose of the drug for clinical trial in humans.

Medical devices will not pass through these processes if they do not have any drug component. They are subjected to Good Laboratory Practice (GLP) testing for safety of the device and its components. Some devices may undergo compatibility testing which will demonstrate if the

device and all its components are compatible and sustainable in a living model. Some of such devices are sophisticated and are of high technology like in medical imaging sciences. Here, such devices may be computed Tomography Scan, Magnetic Resonance Imaging (MRI), Ultrasonography, Positron Emission Tomography, Photoacoustic Tomography (PAT) etc. Preclinical imaging versions of these devices abound for research involving animals. Imaging modalities as pointed out by a Wikipedia Imaging Website, (2012), have long been crucial to the researcher in observing changes, either at the organ, tissue, cell or molecular levels, in animals responding to physiological and environmental changes. These imaging systems were broadly categorized by William et al. (2008), into two major groups. These are those used primarily for morphological/anatomical studies and those for molecular imaging techniques.

These devices such as high frequency micro-ultrasound, magnetic resonance imaging (MRI) and computed tomography are usually used for anatomical imaging while positron emission tomography (PET) and single photon emission computed tomography etc are usually used for molecular visualization. Micro-ultrasound device using high frequency (15MHz – 80MHz) harmless sound waves from transducers can insonate living tissues from which the reflected waves are converted by the transducer to produce 2D and 3D images of the living structures. In a study by Foster et al, (2009), imaging of up to 30µm is possible; which makes visualization of tiny vasculature in cancer angiogenesis a reality. In clinical application in humans, frequency ranges of 2.50 MHz – 15MHz are used.

Most pre-clinical studies must adhere to the Good Laboratory Practice (GLP) standard, as stipulated in the International Conference on Harmonization (ICH) guidelines, in order to satisfy acceptability for submission to the regulatory agencies. Information collected from pre-clinical studies is vital in determining the safety of clinical trials in human beings. It is equally a prerequisite for a new drug application.

CHALLENGES

Despite careful planning, most drugs that enter clinical trial stage fail. According to Kareen & Edward, (2009), the reasons for failure include poor solubility, life threatening or undesirable side effects, poor bio-distribution by the proposed clinical route of administration, prohibitive scale-up and manufacturing costs, market competition, and poor efficacy in early clinical trial. In the modern drug discovery pipeline, as noted by Radloff et al,(2008), the assessment of the efficacy and toxicity of the therapeutic agents are based on relatively homogeneous cell or animal model.

The heterogeneity issue is encountered once the most expensive clinical trials are underway in human subjects. The poor success rate, especially in some daunting disease conditions such as in cancer, to drug development informs Janghui's,(2010) suggestion that the standard preclinical models are failing to predict how the drug candidate works in clinical trials. This failure rate is attributed to inherent heterogeneity between the animal models and human beings involved in clinical trial. Furthermore, recent results from comprehensive genomic efforts such as The Cancer Genome Atlas (TCGA), (2008) have highlighted the marked heterogeneity of genetic alterations in patient population. It suggests that the intrinsic heterogeneity in genetic and /or

epigenetic alterations which are driving the tumorigenesis might be one of the main causes for the observed discrepancies between clinical and standard preclinical models.

Thus, efforts according to Radloff et al,(2008) and Kamb, (2005), are presently geared towards establishing new animal models which will mimic heterogeneous patient population. This is considered a challenging task, in view of the multi-stage scientific intricacies required.

CHOICE OF ANIMAL SPECIES

In animal testing of drugs during pre-clinical drug trials, two animal species are involved. The most commonly used models are the rodent and non-rodent species; the murine and canine. However, porcine and primate species, according to Wikipedia; free encyclopedia can also be used. The choice of a specie is guided by which specie will give the best correlation to the human trial. The pre-clinical trial environment should be close to the actual clinical trial environment as much as possible. The choice of the specie is also guided by the characteristics of the drug candidate, the organ or system involved, formulation, route of administration, site of activity, metabolites to be produced, among other factors.

In oral dosage form of a preclinical research, canines may not be good models because the characteristic carnivore intestine is underdeveloped and cannot be compared with that of the omnivores. In carnivores, gastric emptying is faster. In antibiotic drugs, rodents are disqualified due to the state of their intestinal flora, which may cause significant adverse drug reactions.

Medical equipments or devices testing are best performed using larger animal models. Such animals may be dogs, pigs etc. To illustrate further the consideration of organ or body system type in choice of specie model, swine are more suitable for coronary stent and dermatological studies while goats or cow are good for mammary implant testing. Gastric studies are appropriate in dogs.

However, there is growing challenge against the use of animals in research by animal rights activists. Animal studies are currently in decline in spite of the implementation of the three 'Rs' (Replacement, Reduction and Refinement) principle in the use of animals in researches. The ethics of animal testing is currently becoming a world-wide controversy. Many non-governmental organizations and rights activist exist in different countries, fighting against ill-treatment of animals whether for research purposes or as delicacies on family dining tables. In the United States of America, according to Foster et al,(2009), People for the Ethical Treatment of Animals (PETA) and the Humane Society, believe that animal testing amounts to cruelty and injustice to the animal kingdom . They see no justification in the use of animals in research despite its contribution to quality of life of the human race.

Counter organizations like the Americans for Medical Progress (AMP) feels that the benefits of use of animals in researches has lead to the betterment and longevity of the human race. The pro-animal groups claim that millions of animals are experimented on and killed annually; for food, clothing and entertainment. In reality most of the animals used for experiments are reared or bred in the laboratory for that purpose. They spare no thought on the impact the absolute restriction of the use of animals for research would have on the scientific progress of the human race.

In view of these controversies, alternative to animal testing in preclinical research is being explored. One of such is the exploration of the possibility of predicting the effect of biochemical preparations in humans from its chemical/pathophysiological characteristics in in-vitro studies. This is presently a tall order in preclinical research trials. However, a compromise reached so far among 'agonists and antagonists' of animal testing is that animal testing should cause as little suffering to animals as possible, and as suggested by William et al,(2008), tests should only be performed where 'necessary'

The principle of Three Rs' has been adopted as a guide in the use of animals in research. Many countries; especially where animal right issues are prominent have adopted this principle. The 'Three Rs' stand for: -

1. *Replacement*:- This advocates use of alternative methods instead of animals testing procedures, provided same scientific aim is achievable. This principle is otherwise requesting for total replacement of animals in the preclinical trials of drug discovery process. The current introduction of automation and computational biotechnology in the drug discovery process may hopefully offer a window of opportunity in this direction.
2. *Reduction*:- this principle advocates the application of methods that employ less number of animals in an experiment while achieving the same or more level of information if more number of animals were used. It therefore encourages less number of animals for comparable level of information or use of same number of animals for more level of information.
3. *Refinement*: - This preaches the principle that methods adopted should be such that pain, suffering and distress is minimized for the animals that are still in use in the preclinical research process. It encourages animal welfare and care.

These principles are still being contested by the extreme animal rights protectionists. This has led many governments of some countries to legislate on the Regulatory Requirements/Guidelines for the use of animals in laboratories. The guidelines differ from one country to the other but the underlying principles are the same. Though the choice of a specie for trial is made to be as close to the human as possible, there exists, differences which has to be taken into consideration when evaluating the findings from the study.

To account for differences in species and individual, an uncertainty factor or safety margin of $1/100$ is given. Inter-specie safety margin is $1/10$ while inter-individual differences is also $1/10$.

The documentation of the result of the pre-clinical study, according to the Pharmaceutical product development Inc, (2012), should provide information about the pharmaceutical composition of the drug, its safety, formulation and manufacture process. Further required information is the route of administration to the human subjects, packaging and handling guidelines; including storage, among other factors.

PRE-CLINICAL STUDIES

Pre-clinical studies involve in-vitro and in-vivo experiments involving the identified drug compound.

In “Vitro” (glass) tests are experiments carried out in the laboratories using test tubes, beakers etc., while in “Vivo” (life) are experiments conducted in living cell cultures or animal models. Pre-clinical drug studies are appropriately called toxicity studies. These studies are carried out in animal species in which the following among others are evaluated: -

1. Acute Toxicity Effect in a single large dose.
2. Sub-acute toxicity; involving multiple doses.
3. Chronic toxicity in case of prolonged usage.
4. Reproductive Toxicity of possible effect on Reproduction function.
5. Genetic toxicity for possibility of mutagenicity and;
6. Carcinogenicity

Acute Toxicity Effect in a single large dose

This study involves the use of two animal species, of which one should be a non-rodent specie. Acute toxicity study may involve the administration of a single dose or more over a period of up to twenty four hours. The goal is to determine the toxic dose levels and observe indications of toxicity on the study animals. Animals should be observed for fourteen days. Post-administration mortality (if any) and morbidity (clinical signs and Symptoms) are recorded. Other factors documented are time of onset of any reaction, duration and reversibility. The histopathological changes are also recorded.

Sub-acute toxicity involving multiple doses

This study involves a minimum of fourteen days daily drug administration of three or more doses in at least two animal species. The effects of this prolonged multiple doses are evaluated and recorded for use as a pre-requisite for the human clinical trial. Similar findings as in the acute single dose study are documented.

Chronic toxicity in case of long term application

Certain medications for some disease conditions last for up to one week or more. Such drugs may also be used in the treatment of chronic diseases. Such drugs require animal studies of three to six months and up to one year or more for any chronic diseases to demonstrate its safety. The specific duration should determine the length of the trial of the new drug. Here also, at least two animal species are required. Allotted effects are noted and documented.

Reproductive Toxicity

Reproductive toxicity study is a requirement for any new drug compound before administration to any female of child bearing age. The studies are used to determine any effect of active ingredients on the reproductive system. This study may be divided into three segments:

Segment I reproductive toxicity study documents effects of the drug on fertility. Segment II and III documents such effects as on the embryo, pre and post natal developments. Multigenerational effects and teratology are also studied.

Genetic Toxicity

These tests are aimed at ruling out mutagenic effects of a new drug compound. Any effects on the chromosomes or the DNA strands may be a precursor for gene mutation.

Salmonella typhimurium is usually used in assays to detect mutation.

Carcinogenicity Studies

Testing for carcinogenicity usually applies to drugs for treatment of chronic disease conditions. The duration of this test is about 18 – 24 months. The surviving animals are killed and studied at specified weeks during the period of the study. Data collected are animal deaths, tumour incidence, type and site of tumour appearance (if any) and pre-neoplastic tissue changes etc.

CONCLUSION

Pre-clinical studies constitute an important segment in the drug development process. A successful pre-clinical trial provides extensive information for perfection of the drug development and subsequent trial in human beings. It is a pre-requisite for investigational new drug application. However, preclinical drug trial has mainly relied on animal species for testing prior to application on humans. This is greatly being challenged and hampered by the animal right activists who are advocating for total ban on the use of animals for research and experimentation. Though efforts are ongoing to find alternatives to animal testing of drugs prior to clinical trial, the use of animals may still go on for some time to come.

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