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2nd Edition

Practical Manual of Experimental and Clinical Pharmacology



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CHAPTER

3

Alternative to Animal Experimentation

INTRODUCTION

Drug placed an important role in our life and modulating our life style. Sometime drugs are lifesaving material and hence it is very difficult to invent and process (explained drug development process in *Chapter 1*); we have to establish the safety and efficacy of drug before it is use clinically. As per the ethics and regulatory requirement we can't use the new drug as such. In the drug development process animal (rodents/non-rodents) plays an important role, or we can say without their participation it is an impossible to bring any new molecule to the market for the human use. But, in the present scenario, use of experimental animals (rodents/non-rodents) in experiments or research is a highly debatable topic. However, we know that without their use we can't led to discoveries and understanding of new molecule and their efficacy pathway. Their effectiveness and safety studies may only possible, if we use the ideal animal models. However, use in few areas of research need to be justified and must follow the ethical and handling and care guidelines before, during and after the experimental procedures. To protect the rights of animals used in the R & D, national and international regulatory bodies had made laws to govern the use of animals in drug development R & D.

However, alternative methods are also shout to reduce the use of numbers of experimental animal during the experiment and research. But, the question is that whether these alternative methods can be reproducing the similar type of result as, animal experimentation do. We all know that 100% *in vivo* condition can't be reproduced in *in vitro* condition. The alternative methods and the ethics of experimental animal use, all of which are based on the principles of the 4Rs – (now 5"Rs") *refinement, reduction, replacement and rehabilitation*. But, as per the ethics of animal use on the top of all 4Rs, the **5thR**, i.e.**responsibility** of a researcher or scientific staff to use the experimental animals plays vital role.

As per the *Committee for the Purpose of Control and Supervision of Experiments on Animals* (*CPCSEA*) guideline, it is mandatory to all institutes who perform the animal experimentation must process the animal ethical review which promote a good animal care and welfare practices.

THE 5 Rs OF ANIMAL ETHICS

Reduction

As per the ethical background, the first choice is to use the justified number of animals in experiment which means that pre-planning of experimentation includes, justified aim and objectives, experimental methods including animals distribution improving protocol and statistical design. The handling and care of experimental animals must be taken as per CPCSEA guidelines.

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Refinement

It is a process of refining the protocol for the quality evaluation and assessment of experimental results. It may include improved protocol with more refined methodology, instrumentation and experimental procedures. Moreover, invasive procedure and postoperative care should be improved and followed by the experimenter as per handling and care of experimental guidelines (CPCSEA).

Replacement

The main and alternative mode of protocols which do not includes experimental animals and use other *in vitro* cell cultures method or computational methods which may mimic the same results. Hence, this can able to reduce the use of animals in the experimentation and R & D.

Rehabilitation

The new inclusions in the ethical ground, which says about the re-use of experimental animals, if they are not treated with any toxic or harmful, or radioactive hazards compound and must be washed out within a certain period of time. If the invasive surgery have made, it must be recovered with the no anatomical or physiological differences than before. Rehabilitation must be followed by the ethical committee for each protocol wherever it needs to be followed.

Responsibility

The concept of 5th 'R' is most important to be followed by any research fraternity i.e. 'responsibility' for the experiment and experimental animals. In new era, there is need of performance-based outcomes of any good protocol which includes the scientifically justified objectives, reasonable use of animals and most importantly integrity and honesty of a researcher for their work and must understand the necessity of life of a experimental animals for a sound justified outcomes.

So, keeping in mind all ethical consideration and protest against experiments on animals by several societies like PeTA (People for the ethical treatment of animals), it is always better to known about the alternatives of animal experiments and adopt it as much as possible. These methods are economical and simple as compared to animals experimentation. However, its applicability and reproducibility is still questionable since cannot able to reproduce the same result as *in vivo* condition. But, now-a-days extensive discussion is going on the alternative methods of animal experimentation. Hence, it is useful to known which are the possible alternatives of animal experimentation.

ALTERNATIVE METHODS TO ANIMAL EXPERIMENTATION

Apart from different animals model for the screening of new drugs, the availability of many other alternatives methods which insure and accelerate the drug discovery and development process which additionally secure the lives of experimental animals.

1. *In silico* or computational studies
2. Mathematical analysis or modeling
3. Observational studies:
   - Epidemiological surveys: Retrospective and prospective studies
   - Post-marketing surveillance (PMS)
   - Drug surveillance
4. Microdosing
5. *In vitro* studies
   a. Human tissue and organ culture preparation
      - Human dopaminergic neurons
   b. Stem cell research
      - Stem cells in toxicological research
   c. DNA chips
   d. Microfluidics chips
6. Physicochemical techniques

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In Silico or Computational Studies

Computer modeling or *in silico* studies play an important role to predict the result before conducting the animal experiment. However, it may be seen as alternative rather than to replacement of the experimental animals in research.

Computer based simulations or model of physiology, organs, biochemistry is an prediction rather than the actual values hence, *in vivo* research even needed with the *in silico* studies. But, yes, it is the economical alternatives as compared to the animal experimentation.

Mathematical Analysis or Modeling

Same as the computational modeling, mathematical modeling is also a better alternatives, but be all known that representing a biological system, where many factors play the role into a mathematical equation can be predictable rather than assuming a confirmatory observations. However, mathematical mode allows researchers to understand the complex biological system and help researchers to analyze systematically the systems without using the biological system. It allows to develop hypotheses to guide the design of new experimental tests, and ultimately assess the suitability of specific molecules as novel therapeutic targets as the target identification and validation.

Observational Studies

Now days, the role of observational studies are well planned and executed. Observational studies are the additive study plan rather than to use it as the replacement for the animal experimentation. These studies only predict about the confounding variables in the outcome of the study. However, these study are not marked as standard due to the lack of the cohort of interest which includes epidemiological surveys like retrospective and prospective studies, post-marketing surveillance (PMS) and drug surveillance.

Microdosing

Microdosing is the gift of technology based science, which allows measuring very small doses of potential new medicines and giving complete pharmacokinetics profile of medicine. On these dose there is no pharmacodynamics activity, hence, microdosing in humans gives an good alternative to reduce the numbers of animals used. But, since we are using very small doses (in micron), it can't predict the toxicity or side effects that may occur at higher or therapeutic doses. Hence, it may be used with the animal experimentation to reduce the animal use in the research.

In Vitro Studies

In vitro studies are conducted in the laboratory condition, which may mimic the *in vivo* condition, but not exactly the same, because of complexity to understand the biological system of the body, where several factors plays the active role like heart, liver, spleen, brain, and nervous system. Therefore, the aim of the *in vitro* studies is to *refine* the animal models and *reduce* the number of animal in experiments rather than to replace it.

Physicochemical Techniques

This is the process of understand the basic structures and its resemblance with the toxic compound and can characterize a wide range of materials, natural and synthetic compounds. It may include the sophisticated instrumentation like scanning electron microscope with chemical microanalysis, HPLC, LC-MS, thermogravimetric Analysis etc. In addition, these techniques also help in study of interaction with the target receptor, which governed by major two factors i.e. the size and shape of the compound.

Hence, the aim of the physicochemical optimization of a compound is to understand the fundamental on absorption and distribution of compound *in vivo* and secondly, provides an important insight into the *in vivo* transport processes.

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Matter of Discussion

Organization who favors the animal rights like *PeTA*, have a long discussion with a hypothesis that animal research may be replaced by the numbers of "alternatives methods of experimentation" as discussed above in the chapter.

But, still number of doubts in mind as per the scientific background, i.e.

1. Why the group have quoted "alternatives method" instead of "replacement"?
2. Computer simulation and modeling is a useful tool in drug discovery and development, but can it be reproduce the complexity of body in to the model developed?
3. In *in vitro* studies, cells works good in test tube and give good result, but, are the condition in test tube similar as *in vivo* condition?
4. How we can assess the "therapeutic dose" and toxicity and side effects of compound through the alternative methods of animal experimentation?

But yes, we can reduce and refine the number of animal use in the research with the alternatives and follow the principles of 5 "R" concept.

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Practical Manual of

Experimental and Clinical Pharmacology

New Prospects in History of Pharmacology

- Brief history on pioneers of pharmacology
- Drug development and experimental animals: Covers complete drug developmental process from lead compound to clinical trial
- Details on experimental animals including zebra, fish, pig, chicken, etc.
- Drug dose calculation for experimental animals
- Blood collection from the experimental animals
- Ethical consideration in animal use
- Illustrated with the help of figures and tables

Experimental Pharmacology

- Bioassay in detail with several examples
  - Added points like human and cytokine bioassay
- Practical aspect of cell culture
- Preclinical to clinical drug dose calculation
- Protocol and thesis writing for postgraduate students
- Biomedical waste disposal
- *In vitro* and *In vivo* animal experiments

Clinical Pharmacology Practical

- Includes guideline-based statement
- Experiments on CVS, CNS, respiratory and kidney
- Pharmacokinetics experiments

Conceptual Basis of Recent Advantages in Clinical Pharmacology

- ❑ Pharmacokinetic and pharmacodynamic modeling
- ❑ Translational medicine
- ❑ Reverse pharmacology
- ❑ Microdosing
- ❑ Evaluation of pharmaceuticals product literature
- ❑ Analytical toxicology (Identification of general unknown sample)

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