REVIEW - A FORWARD-THINKING: NEW METHODOLOGIES TO UNDERSTAND DISEASE BIOLOGY IN HUMANS

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ABSTRACT

It is the goal of all medical researchers to use as few animals as possible. Ultimately it would be ideal if the use of animals could be totally replaced by non-clinical methods. In the last 20 years, the number of animals used annually has decreased significantly and the search for validated alternatives continues. It is a common misconception that animals are used because they offer a "cheap alternative" to non-animal techniques. The reverse is in fact true. Research animals are very expensive to purchase, house, feed, and care for. Computers and laboratory equipment in the long run would be much less expensive, and much easier to care for. Many areas of research have already committed to replace all animal use with scientifically better alternative methods. Examples include the use of modern in vitro methods to replace the testing of caustic chemicals and the production of monoclonal antibodies, both procedures that are known to cause great pain when done on live animals. The main aim of this article is to focus on non-animal models, even though it cannot completely eliminate the use of animals in testing. However, several non-animal models have helped reduce the number of animals used in the field of biomedical and pharmaceutical research.

Key words : *Animal, Alternative, drug testing.*

INTRODUCTION

Alternatives to animal testing was a substitute method for animal testing, which are used to develop new methods and performance of non-animal testing methods avoids the use of live animals.[1]

"Alternatives" or substitute is defined as replacement of laboratory testing animals in research, for the purpose of minimizing the level of stress endured by the laboratory testing animal.[2]

In 1995 Russell and Burch introduced the definition of alternatives as the 4 R's - Replacement, Reduction, Refinement, and Responsibility. These ideas at the moment are accompanied in lots of checking out institutions.[3]

1. Replacement: Animal model to be replaced by non-animal techniques whenever possible.
2. Reduction: Reduction includes usage of less number of animals.
3. Refinement: refers to techniques that alleviate or decrease capability ache, suffering or distress and enhance animal welfare for the animal's used.[1]
4. Responsibility: it is the additional responsibility to the unique 3 R's. It has grown into a new generation of overall performance, primarily based outcomes, which displays integrity, honesty, and clinical correctness in the proper and reasonable use of laboratory animals. This guarantees that animal existence is needed and necessary for biomedical development.[3,4]

In this review, the different disciplines not over biomedical and pharmaceutical research are surveyed in order to focus the observation on disease biological studies on human by alternative testing methodology without much use of animal models.

Advantages of alternative testing methods:

1. Reduces the usage of number of animals in research.
2. Reduces the animal pain, suffering, and experimental insult.
3. Time saving.
4. More quick result.
5. Reduce the research cost
6. Reduce the error from inter individual species variability.

Disadvantages:

1. No chance to study about the organ (Like histopathological study)
2. No chance to study about metabolic pathway.
3. No chance to recover the organ damaged tissue.
4. Don't have ability to study the organism's growth process.
5. Reduced potential to take a look at the conduct.
6. Decreased capability to examine the interplay among the organism and its surroundings;
7. Reduced ability to study idiosyncratic or species-precise responses.
8. Reduced capacity to differentiate among male and girl-specific phenomena.[1,5]

What are those alternate testing methods or animal substitutes?

Non-living testing methods can be

* Physico-chemical techniques
* Computer stimulation or Mathematical data

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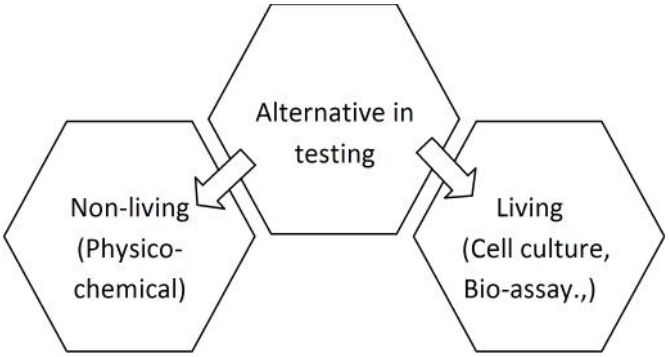
Classification of alternative animal testing models



* Non-invasive imaging techniques
* molecular docking studies
* Micro-array
* Pyrogenicity

Living testing methods can be

* Proteomics
* Genomics
* Tissue or organ culture
* Micro-organism
* Micro dosing
* Bio-assay.[1,2,4,6,7,8]

Non-living Animal substituents methods

1. Physico-chemical techniques:

These techniques help to identify human responses by using chemicals and biological substances example: In GC-MS, gas chromatography is used to separate complex substance and basic elements from the solution which is further characterized by mass spectra. It is repeatedly done in vitamin and drug research.[9,10]

Analysis of physico-chemical properties of test substances such as pH, absorption spectra, partition co-efficient and other parameter can often indicate potential toxicity. OECD guidelines state that substance with a pH of < 2 or>11 do not need to be tested in vivo for irritancy potential. Physico-chemical test is fast, quick and cheap and transferable to laboratories easily.[11,12]

2. Computer simulation or Mathematical models:

Mathematical and computer aids are all helpful in the initial stages of research. Simulation means researcher can manage the parameters at will and observe the consequent effects on the model. This way, computer simulation is a beneficial tool for studies and specially for implying new mechanisms or hypotheses to the research work.[13,14] Few classes, the scientists have an idea of using models and unique resources is furnished within the development of simulation software program.[15,16]

The computers stimulation or mathematical model can help to learn the different basic principles of biology. Specialized computer models and software programs which helps to modifying the structure of drug already existed drug molecule and design new molecule. Computer produced simulations model helps to conclude the feasible toxic and biological effects of molecule, drug candidate without animal dissection. The most effective molecules collected from the initial screening are used for in vivo experimentation. Softwares like Structure Activity Relationship (SARs), Computer Aided Drug Design (CADD), Computer Assisted Learning (CAL), etc. are available plenty, with the help of such software programs we can tailor the results and then if necessary in-vivo test can be performed in animals which helps to reduce the total number of animals used in the actual study methodology.[17,18]

Mathematical models are conceptual model that uses mathematical languages, alternative ordinary languages to represent a particular scientific context. Mathematical models help to improve experiment design, conclude the organism's response to varying levels of exposure to a particular chemical. By using the processes of trial and error, a relationship start to be understood and may be described via mathematical expressions. By collecting research data points, it is easy to fit into mathematical models. Many of the research component may involve kinetic statistics expressed by means of different mathematical equations.[19]

3. Non-invasive imaging (NII) techniques

It is a technique to create photocopy of the human structure for clinical use such as medical procedures find to reveal, diagnose or medical science it includes the work of normal human body. Imaging technology such as the diffusion tensor imaging (DTI), magnetic resonance imaging (MRI), magneto encephalography (MEG), computed tomography (CT) scan, accelerator mass spectroscopy (AMS), nuclear imaging and ultrasound. Above mentioned imaging methods are substituted to make it to irresponsible animal models to give results specific to humans. NII techniques,real-time measure-ments, very modern and accurate.[39]

4. Molecular docking

Docking studies, mostly conducted in the area of drug design. "It is simply defined as the best or perfect-fit orientation of the ligand molecule to the particular target site result forms a complex". Now a day molecular docking is routinely used to understand the drug-receptor interaction, and it provides the useful information about the binding orientation of drug molecules so that researcher get knowledge about the affinity and activity of drug molecules.[20,21,22,23]

Docking approaches

In molecular docking community, two approaches are very popular

1. Matching technique
2. Simulates the actual docking

Both approaches have significant advantage and some limitations.

Structure can be determined by some biophysical technique such as nuclear magnetic spectra or x-ray crystallography method. In docking program structure of

known protein molecules and potential ligand from data base serve as input. Two things are success of docking program. One is search algorithm and another is scoring function.[24]

Docking studies and binding free energy calculations of human pancreatic alpha-amylase (HPAA) with Embelin and its metal complexes like Copper and Zinc embelin complexes were investigated. Results strongly suggested that the above studied embelin and its metal complexes were potential candidates for human pancreatic alpha-amylase inhibitory activity.[25]

Another study has shown 14 ligands of natural compounds against Human neutrophilelastase (HNE) inhibitors represents a good therapeutic target for the treatment of inflammatory diseases.[26]

5. Micro-array

It is also called DNA/ RNA chips, Bio-chips or Gene-chips. The basic technique of this micro-array is hybridization of nucleic acid. The definition of a micro-array is ordered collection of micro-spots and that each spot consists of a single defined species of nucleic acids based on base pairing rules. In this technique, between two single-stranded of nucleic acid molecules hybridization occurred, it causes sequence complementary.[27][28][29] Expression of many gene was quickly concluded by single reaction with efficient manner, this technology helps researchers gain more about various diseases. Microarray expression analysis, microarray for mutation analysis, comparative genomic hybridization are the different types of microarray.[30]

6. Pyrogenicity

This experiment was designed for the purpose of to identify the potential bacterial endotoxin substance present or contaminated to pharmaceutical injectable products. Last twenty years rabbits was used for testing pyrogen in pharmaceutical injectable so that millions of rabbits died. One of the alternative method was developed called pyrogen testing or bacterial endotoxin test or Limulus Amoebocyte Lysate (LAL) test. The amoebocytes isolated from horseshoe crabs to identify the immune response of pyrogens. Several Invitro models are currentlyunder investigation.[31,32]

Living testing methods can be Research on '-omics' methods-which include genomics, transcriptomics, proteomics and metabonomics focused on trying to identify a critical alternations in the genomic, proteomic or metabolic activities. This would lead to understanding how a chemical might damage genetic code or disturb the functionality of a cell.[33]

1. Proteomics

"Proteomics" is part of drug discovery or testing technology. Proteomics is the investigation of the proteome and includes the innovation used to distinguish and measure the different proteins, protein-protein and protein-nucleic corrosive collaborations inside of the proteome, and in addition to the post-translational alterations that influence protein movement. Proteomics innovations with computational strategies have been progressed as of late over numerous other corresponding methods. This empowers researchers to screen extensive quantities of proteins inside clinically unmistakable specimens that finds sickness bio-markers, recognize and approve drugs targets, outline more successful medications, appraisal of medication viability and patient reaction, i.e., to meddle with practically every progressions in cutting edge drug revelation process. Proteomic methodology of medication revelation incorporates finding a precarious protein that is creating an undesirable influence and afterward use of a particle to alter its impact. Proteomics joins parts of science, science, designing and data science and apply them to all regions of medication revelation. Presentation of more secure, more successful and savvier medications will be a definitive result of change of this innovation.[34,35]

2. Genomic

Pharmacogenomics is a study of how the drug is responding to a particular person gene. Objective of the pharmacogenomics is to optimize the drug therapy with respect to patient gene. This technique involves gene sequencing, gene analogy analysis, etc. Main focus of pharmacogenomics is single drug- single gene interaction. Pharmacogenomics is applied on several area of medicine such as oncology, forensic pathology, cardiology etc.[33]

3. Tissue or Organ culture

Tissue or Organ culture is a wide concept. On drug testing point of view tissue or organ culture gives the details about pharmacological and toxicological information on new drug such as the LD50 value of drug substances. It is the most successful method because in animal model, 50% of animal was killed and remaining animals are getting severe pain. Human cell was artificially grown or maintained in sterile culture media containing cell growth regulator and promoter of known concentration so that we get the details regarding absorption, distribution and bio-transformation of drug product. By using this technique, scientist can get the most accurate and faster result.[36,37]

4. Micro-organism

In pharmaceutical drug testing point of view, microorganism are used to evaluate the efficacy, safety and potency of drug product. Broad spectrum anti-biotic of gentamycin sulphate drug potency was tested by using Staphylococcus epidermis, Bacillus pumilus. Even the vitamin cyanocobalamin also tested by micro-biological method.Antibiotic susceptibility test (AST) is to determine which antibiotic will give successful treatment for bacterial infection in vivo.[32]

5. Micro-dosing

This is a method to study the behavior of drugs in humans. It is done before Phase I clinical trial to find whether the drug is efficient for the next level of testing. Micro-dosing is used to decrease the resources spent in

in effective drugs and the frequency of testing which is done on animal models. In phase I clinical trials 40 percentage of drug will fail, which takes 18 months and it is expensive. By using micro-dosing, screening of drugs is done prior, results are obtained faster and are less expensive.

Micro-dosing requires 4-6 months, inexpensive and it shows excellent accuracy at predicting human metabolism. It is reported that largest pharmaceutical companies have used micro-dosing now-a-days in drug development. Micro-dosing has been provisionally endorsed by the USFDA and European Medicines Agency. [35]

6. Bio-assay

Bio-assay is a type of scientific experiment which is conducted to measure the effect or potency of drug substance and it's compared with standard one by using of live animal part and it also used to find out the concentration of a particular constitution of a mixture.[36]

"The determination of the relative strength of a substance (e.g., a drug or hormone or toxicant) by comparing its effect on a test organism with that of a standard preparation." is called bioassay.[33,34]

When compared to other methods of assay such as chemical or physical, bio-assay is less accurate, less elaborate, more laborious, more troublesome and more expensive. The active principle of the drug is unknown or cannot be isolated or chemical method not available orthe chemical composition is not known, or chemical composition of drugs differs, but have the same pharmacological action. Hence bio-assay is very useful method to evaluate the drug substance and determine the relative strength of drugs.[37,38]

CONCLUSION

In addition to this, Epidemiological surveys, Stem cell research, New imaging technologies, DNA chips will help in reducing use of animals in experiments. Wherever feasible, alternative method can be devised and used. It cannot be possible to eliminate fully animal model but can be reduced in experiments and for research purpose.

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