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Preclinical Evaluation of Cardiovascular, Hepato-protective, Anti-cancer, Analgesic, Anti-inflammatory and Anti-oxidant Agents Using Animal Models: An Over View

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Abstract

It is very important for an innovator to select appropriate animal models for the preclinical evaluation. The preclinical evaluation cannot be accomplished with the proper animal care and maintaining a good laboratory practices. This study illustrate the various factors involve in the animal study and role of various regulatory bodies in controlling the animal studies.

Keywords: Analgesic; Preclinical examinations; Toxicity; Hypertension; Cell counting

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Introduction

The utilization of animal models in the development of new drug and research is normal in the Pharmacology. Animal model for new medication discovery and adequacy testing for preclinical research is investigated in pharmacology. Researchers have customarily utilized animals in biomedical research to show the remarkable highlights of biologic reactions actuated by particular classes of drugs. The legitimacy of conclusions drawn from these tests as a rule is weighed precisely against the virtue, course of organization, amount, and recurrence of administration of the drug. The timeframe (post exposure period) before tissue inspecting, the quantities of test and control animals, the strategy for factual examination, and the sort, species, and sex of the animals are basic variables for thought before translating research information [1-3].

Pre-clinical trial

A laboratory test of a new drug, usually done on animals, to see if the hoped-for treatment really works and if it is safe to test on humans.

The animal studies are designed for evaluation of the activity of the novel drugs with reference innovator product and objective of study is to determine the differential biologic activity.

The necessities of preclinical examinations may fluctuate depending on the clinical parameters, for example, therapeutic value, the sort also, number of signs connected and so on. The way to deal with is embraced ought to be completely legitimized in the preclinical study, wherein every candidate must submit the accompanying clinical data:

- Known/proposed clinical utilization of the drug, a particular signs proposed to be focused on and thought of age, sex, pregnancy, lactating, and infants and so on [4,5].
- Dosage plan including amount of each dosage, number of measurements/day, recurrence and interims and aggregate length of treatment, mode of drug dose administration, details of definite detailing including adjuvants, added substances and so forth - Information about diluents, other ingredients.
- Available toxicity studies in human/animals on trailblazer recombinant item and toxicology information of adjuvants and added substances as relevant. The researcher is likewise required to outfit data about the proposed test and faculty to be engaged with leading these examinations at the test site [6,7]. e.g. Research team lead, principal investigator, pathologist, pharmacologist and quality assurance team.
- The statutory endorsements from Institutional Biosafety Board of Committee (IBSC) and Institutional Animal Ethics Committee (IAEC) must be submitted, by and large. The

examinations ought to in a perfect world be led as per good laboratory practices (GLP) and the researcher ought to educate about the status of accreditation of test site, assuming any.

- Preclinical examinations ought to be done with the final drug dose formulation of the comparative reference drug expected for clinical use. The researcher should clearly indicate the expected adverse drug reaction and toxicity of the drug to the ethical committee. The accompanying investigations are required for preclinical assessment:

(I) ***In vitro* study:** Assays like receptor restricting examinations or cell based measures (e.g. cell expansion examines) ought to be directed, when proper to build up equivalence of pharmacological action of the comparative drug and reference drug. Such information is normally accessible from biological assays portrayed in the pharmacopoeias.

(ii) ***In vivo* study:** Regarding animal studies have to administer, at least on repeated dose of toxicity, to ensure the toxic dose. The comparative toxicity dose studies ensure that there will no toxic side effects on the particular animal species. The toxicity studies of the test drug must be compared with the reference drug. The length of the investigation would be for the most part at least 28 days with 14 days recuperation period. However, the study period may depend on various parameters.

Animal models

Animal model is a living, non-human animal utilized amid the examination and examination of human disorders, with the end goal of better understanding the disorder without the additional danger of hurting a real human throughout the procedure. Animal models serving in research may have a current, inbred or induced disorder or damage that is like a human condition. The animal picked ordinarily meets similar taxonomical order equivalence to the human, in order to respond to infection or its treatment in a way similarly like human physiology as required. The utilization of animal models enables researchers to work on disease states in ways which would be out of reach in a human patient, performing techniques on the non-human creature that infer a level of mischief that would not be viewed as moral to dispense on a human. Animal models are used to study the physiology, attention, pathological states that are resembles to human environment [8-11].

Animal's procurement

Before obtainment of animals, thought ought to be given to the kind of animals required, the capacity to animal house and look after those animals, and any potential effects on different animals in the animal house. In the studies where the animals require high health status has to procure very well. Animals that are used in the short period e.g. acute, sub-acute studies have to be procured separately with different resources.

Before storing the animals, precaution should be given to the conditions and facilities available in the animal house pertaining to both new animals and already existing animals. Previous storing conditions should be taken into consideration, while introducing animals in new house. The capacity to isolate the animals on arrival ought to be considered, especially as far as space, office outline and staff, to permit isolate for quarantine, inoculation, testing, and so on. Maybe then again, plans ought to be made to do this off-site [12-15].

A portion of the components to be considered in deciding if animals ought to be reproduced or raised in house or outsourced include:

- Space accessible (Relies upon the quantity of animals required);
- Office appropriateness (e.g., Capacity to isolate from different animals);
- Staff accessibility and information of particular cultivation and breeding;
- Cost;
- Measure of time required to raise the animals to an age suited to their proposed utilize;
- Accessibility of a dependable provider that can give animals on request; and
- Capacity to contract out breeding or raising animals to required age.

In the event that the choice is made to breed animals inside, assist thought ought to be given to the desired qualities of the animals and accessibility of animals. For instance, residential swine are not a uniform populace in business generation. Proper documentation must be kept up for all animals obtained by an organization or its specialists. Animals ought to be acquired from legitimate providers.

In-house animal breeding should be conducted when there is specific requirement, and ought to be proficiently managed, steady with foreseen requires for the breeding of animals. When getting animals of obscure beginning or from stockyards or homesteads, an evaluation of the health status of the crowd should first be made. Once the animals are gotten, they ought to be isolated as per built up SOPs, keeping in mind the end goal to limit the spread of maladies to different animals in the office. By and large, animals utilized for logical purposes ought not to be gotten from pet stores or their providers because of the potential wellbeing dangers related with disease transmission and the potential medical issues for these animals.

Dogs and cats particularly breed for logical purposes must be the model of decision for examines where characterized hereditary, natural and wellbeing statuses are important. While thinking about the obtainment of animals from another logical foundation or from another animal house inside a similar organization, a demand ought to be made for documentation itemizing the first wellspring of the animal and the history of the animal must be documented. (e.g., nutrition, storage conditions, drugs used in the study, and so on).

For exchanges between organizations, a heath testament for the animals ought to be given, and the animal care services at the getting foundation ought to be advised of the exchange and be given a duplicate of the health status declaration. Animals

subjected to invasive surgery must not be utilized as a part of extra investigations, without unequivocal endorsement of the animal ethical committee. For procurement of any animals from the field, researchers should watch and pass on to understudies and representatives a strict ethic of natural surroundings protection and deferential treatment of the animals. Research objectives will for the most part direct the proper testing technique; however, investigators should select the method that has the least impact on the animals and on the local ecosystem, and is the safest for all concerned.

The main aim of the any transportation of animals ensures the security, relief and the safety, while transporting to its target location. All animals documents should carried while transporting to the international borders, knowledge of the appropriate procedures and documentation is essential to preventing unnecessary delays. A proper standard operating procedure should be maintained for the transportation of animals by its researchers, organizations etc. there should be a special consideration to the pregnant animals and younger aged animals while transportation. Transportation of animals through the private vehicles are prohibited because there will be an increased risk of accidents, lack of vehicle safety and poor maintenance of animals, appropriate storage set ups, chance of transmission of infections. While transportation one has to maintain the proper storage container requirements, ventilation, temperature both the container and the environment, labelling the animals and proper documentation. All Standard Operating Procedures regarding the transportation of animals must include instructions describing emergency responses, in line with the mode of transportation to be used.

Institutions receiving animals should be prepared for accepting the animals by providing proper facilities and appropriate handling by trained, experienced personnel. Institutions should be responsible for ensuring records are kept for all animals received. Institutions should have Standard Operating Procedures for conditioning animals upon receipt that take into account the species and background of the animals. After transportation and before use in any experiments, animals should be acclimatized to the experimental conditions. Quarantine areas should be subject to extra vigilance in monitoring the animals and in maintaining good records, in order to detect and respond to any health problems in quarantined animals. Duration of quarantine should be appropriate to ensure that the health of the animals under quarantine and that of the conspecifics already resident at the research facility is assured [16].

Different animal models used in research

Animal models utilized as a part of biomedical research, especially those utilized as a part of the investigation of infections and other conditions in people, can be gathered as follows:

- Spontaneous models or natural models
- Experimental models
- Genetically-engineered models
- Negative models
- Orphan models

Spontaneous models or natural models

In this type of models the naturally occurring diseases conditions of animals are as similar like the humans. So we are using these animals for the experimental purpose. e.g.: hypertension, diabetes, obesity, eating disorders etc.

Experimental models

In this type of models the disease conditions is induced experimentally with the help of chemicals and are used for the research. The induction of disease state to be regulated by the controlled use of the inducing agent. If the induction dose is higher there will be death of the animal.

Genetically-engineered models

In this type of models the disease condition is induced by the deletion or insertion genetic code. The genetic code gets manipulated to produce the disease state in animals. The genetically changed animals may contain foreign gene. Some of the animals have no genes, these types of animals are called as knock out animals and the models are described as knock out animal models. These types of animal models useful in the study of genetic disorders.

Negative models

Some animals are resistant to a particular condition or disease. For instance, certain strains of mice are resistant to some infectious agents while others are susceptible.

Orphan models

Orphan models are conditions appearing naturally in an animal, for which there is no known human counterpart [17].

Selection of suitable animal model for the research

The principal investigator should search for the alternatives to use the live animals in his experiment (alternatives of Replacement, Reduction and Refinement).

The appropriate choice of an animal model can be considered a reduction alternative, as it should minimize variation in the experiment and hence the numbers of animals that are required. Using an appropriate model can be considered a Reduction alternative when:

• The use of isogenic, or genetically identical, strains (inbred strains and F1 hybrids) results in less experimental variability than would occur when outbred strains are used.

• The model has been validated and shown to meet the objectives of the research and/or be predictive of the endpoint of interest.

• The model has been fully characterized so that all aspects of the animal's life cycle and their potential

impact on the experiment are understood (for example, to ensure the animal will survive long enough

to complete the experiment, or whether the rapid growth of a young animal is appropriate to model an adult human).

• The model is being continuously improved to decrease variability and improve its productivity.

• The research facility ensures it is able to properly house and care for the species, including accommodation of the species' behavioural and companionship needs.

• Competent welfare assessments of the species can be made, including knowledge of pain and pain management in the species.

• Purpose-bred animals are used rather than those caught in the wild.

Discussion

The most obvious choice of animal species and model for a specific research program may be the same model used by other researchers for the same research. However, with ever increasing numbers of animal models available including new spontaneous mutations, and genetically modified animals constantly being developed, the investigator must consider all factors when selecting the best model for his/her research.

Some of the factors that will influence which animal model the investigator selects are:

1. Appropriateness of the model or organ system for the proposed study

2. Genetic aspects of the model

3. Natural vs. experimentally produced models - both natural models and induced models of disease are

4. Useful depending on the objectives of the study

5. Responses of the animal to procedures

6. Environmental aspects important to that particular animal model

7. Background information available on the animal and specific model

8. Species availability

9. Numbers needed, according to statistically appropriate design [18].

There are various animal model techniques are used and they are widely differentiated based upon their use. Some of the important techniques were described below. The animal models used to screen the cardiovascular activity includes-

Screening of vasoconstrictor and vasodilator activity

The contraction and relaxation of blood vessel depends on the intracellular calcium ion concentrations. The activity can be tested on isolated aorta of rodents. The aortal rings are contracted with different agents and the concentration of ions that are present at intracellular ion channel [19-32].

Experiment: We can use any rodents (guinea pigs, rabbits, rats) and they are properly weighed.

Animals are sacrificed using cervical dislocation, stunning methods. The heart and pulmonary artery are quickly removed and placed in physiological salt solution at room temperature. The artery is dissected into rings and gently rubbed for removing the any cells. Cut the artery spirally of 15-20 mm length and they are placed in the organ bath congaing 20 ml physiological salt solution at room temperature. Changes in the length are calculated with the lever [22-34].

To test the effect of the experimental drug compound, the drug is placed in the organ bath. The normal functioning of the aorta is calculated at first. The acetylcholine application on aorta results in the relaxation of the vascular smooth muscle. After the equilibrium period of 1 h the contractile agents (adrenaline, noradrenaline and isoprenaline) are administered into the physiological salt solution of organ bath. When a stable line appeared, then calculate cumulative drug dose response curves, the curves can be obtained at 1 h time interval till the saturation point. There are some modifications in the method in which the different contractile agents were used.

Mean values of relaxation ± SEM are calculated. The height of contraction before the first drug administration is taken as 100%. IC50 values are determined from the individual dose response curves. Statistical evaluation is performed by means of the t-test [32-35].

Screening of anti-hypertensive activity

The male Sprague Dawley rats were anaesthetized and further back side of the area get shaved and skin gets disinfected. In the left lumbar region an incision was made along the parallel axis of the rat. The renal pedicel get exposed and retracted along with the kidney to the abdominal area. The renal artery get separated and clipped with U-shaped silver clip. The diameter of the clip can adjusted with the help of forceps. The right kidney is removed through a flank incision after tying off the renal pedicle. The skin incisions are closed by wound clips. After 4-5 weeks completion the blood pressure values are measured and they are noticed with 150 mm hg of higher blood pressure [19-34].

The blood pressure reading were measured before the experiment and then after they were administered with the test drug concentration. Drugs are administered orally in volumes of 10 ml/kg. The rats are divided into 4 animals per dose and each animal is used as his own control. Compounds are administered for 3 days and pre-drug and 2 h post-drug blood pressure readings are taken [36-53].

The changes in the blood pressure were evaluated and the activity of the experimental drug was known.

Screening of hepato-protective activity

The prolonged administration of carbon tetrachloride to rats causes the hepatotoxicity by forming the covalent bond with the hepatocytes.

The male wistar rats of 20 divided into groups. The rats with 100-150 g weight are to be selected for the experiment [54,55]. The animals are administered orally with 1 mg/kg carbon tetrachloride dissolute in olive oil, weekly twice. The procedure should be done for a period of 8 weeks. The animals are regularly

feed with standard diet ad libitum at room temperature. The 20 animals are administered orally with olive oil and the group is classified into control. 40-60rats were administered with carbon tetrachloride only [56,57].

The group of 20 rats were administered in different doses of carbon tetrachloride and the drug of investigation. After the completion of experiment (after 8 weeks), the blood samples were collected from the in orbital vein and the serum was separated.

The following parameters were calculated using the separated serum:

- SGOT
- SGPT
- Total bilirubin and the results were compared with the control.

Screening of anti-cancer activity

The CFPAC-1 cell line originally collected from the pancreatic ductal adenocarcinoma of 26 year old male patient and they are cultured, incubated under the standard conditions. The cell suspension was prepared and diluted to a concentration of 5×10^6 cells/ml. The cell suspension was used for the tumour implantation in the nude mice [58-61].

Three nude mice were injected subcutaneously with 0.2 ml of cell suspension and there by mice serve as tumour donor. After the completion of 4 weeks the implanted tumours developed up to 5 mm in diameter. The tumours were separated from the mice. The tumours samples were cleaned from the necrotic tissue and blood vessels and are cut down into small pieces of 8 mm. the mice were anaesthetized and then implanted with the pieces of tumours. These mice were divided into groups with 10 each. The mice were administered with the innovator drug for 7 days and tested for the activity. The treatment was continued until 25 days.

The growth of the tumours was measured using calliper at 3-4 days for a period of 25 days. The growth curves could be drawn based upon the growth measurement. The tumour growth can be corresponds to its volume and value can be calculated by the formula:

Tumour volume (tumour growth) = length×width^2 × 0.5

The time required for the tumour to grow from 50 mm^3 to 100 mm^3 for the control group is called as tumour volume doubling time and the for test 35 mm^3 to 70 mm^3.

Cell counting

CFPAC-1 cells collected from 60–70% confluent cultures are used for this study and seeded to 24-well culture plates. After the cells are cultured in IMDM containing 10% FCS for 48 h, the medium is replaced by IMDM supplemented with 2.5% FCS and various concentrations of bombesin, bombesin antagonist, or a combination of both. The same volume of medium but without peptides is added to control wells [62-73]. Following another 24 h of incubation, the culture is terminated by aspiration of the medium from the wells and washing with PBS (0.5 ml/well). The cells are trypsinized by a 10-min incubation with (0.5 ml/well) 0.25% Trypsin-EDTA. The detached cells are dis-persed by repeated pipetting using a G-22 needle and syringe. The number of cells is counted by Coulter Counter Model. All the data was calculated as the mean ±SEM of the results [74-76].

Screening of analgesic activity

The analgesic activity of the innovator drug can be studied with the help of hot plate method. The paws of the mice and rats are very sensitive to the temperature. The response towards the heat can be observed are licking of the paws, jumping and withdrawal of the paws. The time until prolongs the responses occur after the administration of the innovator drug indicates its analgesic activity [77-80].

The mice were divided into groups 10 each and their body weight is in range 18-22 g. the hot plate contains the electrically heated surface and it can be heated copper plate or the glass surface. The temperature should be maintained at 55-56^{0}C. The animals were placed on the hot surface and time period until licking, jumping responses is calculated by the stop watch.

At a time interval of 20 min, 60 min, 90 min the responses were calculated and compared. The before and after application of the drug the responses were recorded and they are compared by using standard t- test [81-90].

Screening of anti-inflammatory activity

There so many methods in determining the anti-inflammatory activity of the drug, but among all the commonly used method is rat paw oedema method. The activity is calculated as the rate of inhibition of the oedema. The oedema in rats was induced by the inflammatory agents such as brewer's yeast, formaldehyde, egg albumin, kaolin, dextran etc.

The male albino rats with a body weight of 100-150 g are chosen for the study. The animals are fasted overnight for the better results. The animals were administered with the 5 ml of water for control and along with the drug were administered to the test group. 30 min later the rats were injected with the 0.05 ml of carrageenan solution at the hind paw region. The paw is marked, immersed in the mercury of plethysmometer for calculating the paw volume. The changes were observed at time interval of 3 h, 6 h, 9 h [78-90].

The changes in the paw volume was calculated and compared with the control group.

Screening of anti-oxidant activity

There are several methods of antioxidants among all most widely used method is free radical scavenging assay. The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) is the chemical used for the identification of the anti-oxidant activity. The innovator drug compound was added to 4 ml of 0.004% methanol solution of DPPH, the bleaching of the purple colour of the DPPH indicates anti-oxidant activity. After the incubation period of 30 min, the absorbance of the solution was calculated against the blank solution. Inhibition of free radical formation by DPPH was calculated and reported [91,92].

The activity of the innovator drug can be influenced by the various factors

- Animals related factors (age, sex, genetic makeup, health status of the animal, etc.)
- Environmental and physical factors (food, humidity, bedding, ventilation, temperature, lighting, etc.)
- Animal care and handling factors.

The pre-clinically evaluated drug undergoes to clinical evaluation in which the drugs ADME (absorption, distribution, metabolism and excretion) was studied in detailed. It's each and every pharmacist [93] responsibility to monitor the any adverse drug reactions of the marketed drug whether it's a generic or branded one.

Conclusion

The animal studies must be carefully done by reducing, refraining, and replacing the animal use. The innovator and team must follow the ethical guidelines laid down by the ethical committee. Finally maintain a good animal care and get good results and help the society by giving quality drugs.

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