Experimental animal models and alternatives in research

Suhas, Y.S., Jayakumar, K., Rajeshwari, Y.B. and Ramesh, N.

Dr. Suhas, Y.S.,
Dr. Jayakumar, K.,
Dr. Rajeshwari, Y.B.
and
Dr. Ramesh, N.
University of Agricultural Sciences
Veterinary College, Hebbal,
Bangalore – 560 024

In spite of the public criticism level led at experimental work on animals there seems little doubt among the majority of the informed public that there is a need for the use of animals not only for drug testing and development but for basic research too. The object of using animals in basic research need justification, on the grounds that without such an understanding there would be no rational basis for medical, dental or veterinary practice. It is clear that our difficulties increase when this basic knowledge is lacking, and makes even more important the use of a battery of simple test models referred to as a screen, in drug development (Lewis, 1982).

The majority of animals used in the pharmaceutical industry are rats and mice and the great advantage of using these rodents is that they can be bred and kept in very large numbers in a fairly small space. This, of course, does not mean that the greatest laboratory animal care should not be exercised in their breeding and maintenance. It is quite clear that the researcher cannot risk having to repeat laborious and time-consuming work with numerous animals because of some undetected infection of deficiency. One such factor in animal care is nutrition (Lewis, 1982).

The animal models are indispensable in drug research (Ohsawa, 1982). In spite of the public criticism leveled at experimental work on animals, there seems little doubt among the majority of the informed public that there is a need for the use of animals not only for drug testing and development but for basic research too (Lewis, 1982).

In order to assure reproducible results in animal experiments, the experiments must be performed by appropriate methods using defined laboratory animals, specific pathogen free animals and specific strains of animals (Nomura and Tajima, 1982). Hence appropriate animal models are continuously being developed.

Alternatives for animal models should confirm to the three Rs-Reduction, Refinement and Replacement (Bruckner *et al.*, 2000). Alternatives to the use of animals in toxicology, pharmacology, education and the testing of medical devices are being developed (Brown *et al.*, 1999).

Classification of Experimental Animals

Experimental animals are of various species and strains, and they are reared in different environments. Their quality with respect to back ground and history is not always constant.

Experimental animals can be divided into three groups: Laboratory animals developed for research purposes and produced under controlled conditions, domestic animals produced without the controls required for research purposes, and animals obtained in the wild state from nature.

Classifiation of experimental animals:

Laboratory Animals which are domesticated because of importance or need in research. They are bred and produced under controlled conditions for research purposes.

Domestic animals Animals which are domesticated for use in human society. They are bred and produced without the control required for research purposes.

Animals obtained from nature Animals which have been in nature. They are not produced or reproduced by humans.

The reasons for dividing experimental animals into these three groups are as follows. The results of animal experiments are affected by the origin and rearing environment of the animal used. Therefore, it is important that this origin and environment be clear and controllable. However, these factors clearly differ among the three groups. Laboratory animals satisfy all of these factors while those obtained from nature do not. Domestic animals are intermediate between laboratory animals and those obtained from nature. Therefore, it is essential that the group to which the animals belong be known if the results of the experiments are to be evaluated precisely (Tajima, 1973). Defined laboratory animals are the most strictly controlled types of all laboratory animals.

Development of New Animal Models

The analysis of the present status of laboratory animal science indicates that the development of new experimental animals and disease models is necessary for the establishment of the principle and the methods of the extrapolation of the laboratory animal data to humans. There are several methods of developing new experimental animals. The following are the methods of development of new animal models:

1. From domestic and wild animals
2. From mutants
3. From toxicology studies
4. By developmental engineering

1) Development of new experimental animals from domestic and wild animals

This is the most traditional way of developing new animal models which includes the discovery of a useful feature through a wild survey and the development of animals with suitable size for experiments. Pigs are evaluated to be extremely useful experimental animals, especially for arteriosclerosis research and immunological studies. The wide application of ordinary pigs, however, has been hampered by their large size. ***Gottingen miniature pigs*** are extremely small and proved to be suitable for routine experiments (Beglinger and Co-workers, 1975).

Marmosets Monkeys are very small primates of the new world, which are easy to handle. The marmosets are useful for the studies of drug safety and some viral diseases. Since ***Macaca Monkeys*** widely used as experimental primates are now in extremely short supply, marmoset monkeys are expected to become substitutes for macaca monkeys.

Pica (*Ochotona rufescens rufescens)* is a small non-rodent animal of approximately the same size as rats. Since picas have similar characteristics to rabbits, they are expected to become a small and easily handled substitute for rabbits as a non-rodent experimental animal (Puget, 1973).

Ordinary pig, Omini-pig, Callithrix jacchas are the other animals which are used for research and experimental purposes.

2) Development of New Experimental Animals from Mutants

Many mutant animal models including nude mutants are now widely used but still many more new mutants are needed. A careful search for mutants by animal breeders with the collaboration of clinical researchers is expected to be fruitful for the new discovery of various mutant animals (Nomura and Tajima, 1982).

3) Development of New Experimental Animals from Toxicology Studies

During toxicology studies, prolonged administration of large quantities of new chemical substances results in the development of various adverse effects in test animals. Some of them might be useful models for human diseases. For instance, oral administration of cyproheptadine, an anti-serotonin substance, causes diabetes in rats (cyroheptadine diabetes rat). This diabetes mellitus is caused by a unique disturbance in insulin secretion from the rats pancreatic islets and is useful for studies on the mechanisms of insulin secretion (Nomura and Tajima, 1982).

4) Development of New Experimental Animals by Developmental Engineering

One of the fascinating approaches of developing new experimental animals is the use of the technique of microsurgery of mammalian eggs or embryos to produce artificially developed animals. The following are the

developmental engineering techniques.

Techniques:

- Aggregation chimeras
- Intraspecies (mouse « mouse, rat « rat)
- Interspecies
- Injection chimeras
- Mouse embryo « mouse embryonal cell
- Mouse embryo « mouse teratoma cell
- Uniparenteral homozygous diploid animal
- Parthenotes
- Nuclear transplantation

Aggregation chimeras: Two embryos of a different mouse or rat were aggregated *in vitro* and developed to a single blastocyst which was transplanted into the uterus of pseudopregnant foster mothers to obtain congenitally chimeric animals (Mclaren, 1976; Mintz, 1971).

Injection Chimeras: Mouse embryonal cells or teratoma cells are injected into a mouse blastocyst. Injected cells are organized into the blatocyst and developed into normal tissues, including gonadal tissues. When teratoma cells are developed into sperms or eggs within the chimeric mouse, normal F1 mice can be obtained. If teratoma cells become mutant cells by irradiation or mutagens and develop into sperms or eggs in injection chimeras, a mutant normal F1 hybrid mouse can be obtained. Using these procedures, a new mutant mouse can be induced artificially from mutant cells (Dewey and coworkers, 1977).

Uniparenteral homozygous diploid animals: A mouse embryonal cell is obtained before two pronuclei of sperm and ovum are fused and onepronuclei is removed by a micropipette to form a haploid cell. The cell is treated with cytochalasin B to induce a karyokinesis without cytokinesis which produces a uniparenteral complete homozygous diploid embryonal cell. The embryo is caused to develop into a blastocyst. The blastocyst is transplanted to the uterus of a pseudo-pregnant foster mother and a uniparenteral homozygous diploid animal is obtained (Hoppe and Illmensee, 1977). The technique will be extremely useful in developing completely homozygous diploid animals within a short period. These microsurgical techniques are expected to open a new field of experimental animals.

In the course of development of new drugs, animal experiments are indispensable in evaluation of the efficacy and the safety of the drug which can be extrapolated to humans. Reproducibility of the results of such experiments is essential but the results are often difficult to reproduce because of the complexity of the factors involved, such as the genetic and microbiological background of the animals themselves, the environment in which the animals are reared and the experimental environment and techniques used. To assure reproducible animal experiments, the experiments must be performed by appropriate methods using defined laboratory animals (Nomura and Tajima, 1982).

In order to assure reproducible animal experiments, the experiments must be performed by appropriate methods using defined laboratory animals. The site and the course of the host response in animal experiments must be understood to make sure that a certain treatment always produces the same responses. When an animal is considered on the basis of its genes it is called a genotype and when the material effects are added, is then known as a phenotype.

It was formerly considered that the results of animal experiments were the direct response of the phenotype to the experimental procedure, but it has since become clear that the same results are not always obtained when the environment of the phenotype is varied and, therefore, a new type, the dramatype which is the result of the effects of the proximate environment on the phenotype is necessary. The dramatype must be uniform to achieve reproducible animal experiments and animals having such uniform dramatypes are defined laboratory animals. When considering the effects of these proximate environmental factors on the phenotype, it is necessary to introduce a new concept the dramatype which is determined by the action of proximate environmental factors on the phenotype (Nomura and Tajima, 1982).

Therefore, it is absolutely essential to carefully control the genotype, the material effects on the genotype, the phenotype, and the proximate environment of the genotype which are the decisive factors for the dramatype. In other words, animals which have a uniform dramatype can be considered as defined laboratory animals.

Fig.2 Factors influencing the physiological status of animals (Nomura and Tajima, 1982)

The factors influencing the physiological status of animals are given in Fig.2. These factors should be taken care of while achieving uniform physiological status of the animals. Proper and reproducible results can be

obtained when the animals are maintained with uniform physiological status. The mentioned proximate environmental factors have more influence on the phenotype and genotype which are decisive factors for the dramatype. The animals which have uniform dramatype can be considered as defined laboratory animals. To check the invariability of physiological status, peformance test or quality assurance test is done. In producing defined laboratory animals, the dramatype of the animals should be monitored by performance as quality assurance test.

Absolute essentials to produce defined laboratory animals

a) Careful control of genotype

b) Careful control of maternal effects on the genotype

c) Careful control of maternal effects on the phenotype.

d) Careful control of proximate environment on the phenotype

The international council for Laboratory Animal Science (ICLAS) is promoting a genetic and microbiological monitoring centre system program as one aspect of the international standardization of laboratory animals, and this programme includes the preparation of an international genetic and microbiological monitoring manual (Radzikowski and Nomura, 1981). This genetic and microbiological monitoring system involved periodic checks of the genetic and microbiological quality of the animals to determine if their quality remains invariable.

The aim of genetic monitoring is to assure the genetic quality of laboratory animals, mainly inbred mice, for the early detection of genetic contamination and mutations, and thus improve the accuracy and reliability of the results of animal experiments. Genetic markers which are located on loci of the chromosomes are tested by morphological, biochemical and immunological methods.

The aim of microbiological monitoring is to make possible the correct analysis of experimental data by permitting the experimenter to know the microbiological background of laboratory animals. Microbiological monitoring is also a system for checking the accuracy of control of microorganisms in the experimental environment.

Specific Pathogen Free Animals (SPF)

The International Committee on Laboratory Animals (ICIA) has defined specific pathogen free (SPF) animal as the animals which are free of specified micro organisms and parasites but not necessarily free of the ones which are not specified. For example, *Pasteurella* free SPF animal. SPF are also called as disease free animal, healthy animal, pathogen free animal, clean animal or caesarean derived animal.

The SPF animals are obtained by caesarean section in a sterile and clean environment and the animals are maintained in specialized unit called SPF units which are designed to discourage the entry of pathogenic organisms.

SPF animals are used in immunological studies and ageing process of individual.

Germ Free Animals (GFA)

Germ free animals are defined as the animals whose life is well defined and well known. GFA is one which is isolated from all demonstratable living microorganisms like bacteria, virus, fungus, parasite, algae and yeast. The aim of GF work is isolation and prevention of contamination from air, water, food and environment. Rats, mice, guinea pigs and chicks are used in production of GF animals.

The GFA can be produced by methods of isolation and the equipment is Isolator. GF animals are also called as Axenic animal (free from strange) or Gnotobiotic animal (known life). GF animals are used in studying wound healing process, studying secondary complications, immunological studies and nutritional studies.

ALTERNATIVES TO ANIMAL MODELS IN RESEARCH

Alternatives are those techniques which reduces the number of animals used in biomedical research, decreases the sufferings of the animals used in research and substitutes the animals by other techniques. The alternatives to animals involves 3R's concepts: Refinement, Reduction and Replacement.

Replacement

The concept of alternatives based on the principle of the Three R"s which was defined by William Russell and Rex Burch (1959) in their book "Principles of Humane Experimental Technique". The alternatives provide a rational approach to decrease the suffering caused by animals use and to decrease the number of

animals in research and experimentation. This also ensure the use of non-animal alternative in biomedical research without compromising with the quality of research work. The full text of Principles of Humane Experimental Technique written by William Russell and Rex Burch can be accessed at Altweb site of the Center for Alternatives to Animal Testint, Johns Hopkins University, Baltimore.

Refinement

Refinement concept involves the modification of any macro and micro environment of a laboratory animal from its birth and throughout its life span enhancing its well-being by decreasing the pain and distress to the animal. Apart from the ethical point of view, refinement also involves proper care and management of laboratory animals. Any changes in the environment provided to laboratory animals that causes pain and distress alters the physiological status of the animals which may lead to the variability of experimental results.

The best possible living condition should be provided to animals used for the research purpose. Normally the care of animals should be under the supervision of veterinarians or a person having adequate experience in laboratory animal care. The investigator and other personnel should treat the animals with kindness and should take proper care by avoiding or minimizing discomfort, distress or pain.

The techniques which assess the end points and measures the parameter early without having effect on the study and are least invasive are employed to minimize the suffering of animals and duration of the research work.

Some of the advanced techniques like Nuclear magnetic Resonance, Electorn Spin Resonance, Magnetoencephalograph and Positron Emission Tomograph provides non-invasive observations of the changes occurring in internal organs and even some techniques enables observations of the events occurring intracellularly in the whole living animal.

At the end of or when appropriate during an experiment the animal that would otherwise suffer severe or chronic pain, distress, discomfort or disablement that cannot be relieved or repaired then most humane method of euthanasia should be chosen.

Reduction

The reduction concept signifies the strategies that will result in the use of reduced number of animals in research to obtain the same amount of information or in maximizing the information obtained per animal thus avoiding the use of more number of animals.

There are several approaches to reduce the number of animals use. The following are some of the strategies for reducing the number of animals:

1. Alerting other researchers in the laboratory when the animals are sacrificed. If one investigator intends to carry out a work on kidneys, other investigators may be able to make use of liver, brain, bone marrow, intestines, stomach, blood, serum or other parts of the animal.

2. The animals selected for an experiment should be of an appropriate species and quality and minimum number should be obtained to get scientific and statistically valid results.

3. Before conducting actual research on animals, a small preliminary or pilot study can be carried out. This indicates the species of animals, number of animals and whether to carryout or not to carry out the major research work and also to modify the research work to use reduced number of animals.

4. Wherever possible, in vitro systems or techniques are employed to reduce the number of animals.

Replacement

The concept of replacement involves replacing the animals in the experiment or research which does not require the use of whole living animal.

Replacement alternatives acts as relative replacement of animals. In some studies where the whole living animal use is not required, the cells, tissues and organs obtained by humane sacrificing of an animal can be used. The cells, tissues and organs from the sacrificed animal can be cultured and obtained in large number thus avoiding the use of live animals research and experimentation.

Replacement alternatives can also be employed as a total replacement of animals in a study which requires only biological material derived from the animals. In such studies the cultured cells, tissues and organs may be used.

In some studies the replacement alternatives will complement the animal experiments and reduces the number of animals used.

In recent times, there is a need for the development of new models, methods and alternatives suitable for obtaining scientific and significant statistical results.

Replacement alternatives can be classified into 6 categories

Information, Computer based systems, Physico-Chemical techniques, Use of lower organisms and embryo stages, Human studies and Cell, Tissue and Organ cultures, Each category acts as a total replacement, relative replacement or complement replacement to the use of animals in research or experimentation.

1. Access to information

Access to information involves the collection of information on the compound to be screened for its efficacy and toxicity potential. The information can be obtained from text books, journals, records, epidemiological data, colleges, institutes and universities. This prevents the duplication of the animal research which are already done.

Access to information provides information on the effects and biological interactions in a particular species of animal but does not provide information on other species of animals. Such information may be of commercial importance and cannot always be found in public domain.

2. Computer based systems

Computer modeling and expert systems are used for the prediction of efficacy, biological interactions and toxicity potential in drug discovery and development. This method prevents the use of live animals for research and experimentation.

Computer simulations and multimedia presentations are often used to replace the use of animals in research work.

The protease inhibitors in AIDS triple therapy, the viral enzyme and the types of chemical that would block its action was developed by using powerful computers. Physiologically based Pharmacokinetic modeling (PBPM) predicts the pharmacokinetics of a compound.

However, these systems requires lot of data collected from literature, in vivo and in vitro studies and integrated into the program.

These methods do not simulate a real experimental situation for which huge amount of information and data is required.

3. Physico chemical techniques

The physico-chemical data such as color, matter state, pH, molecular weight, solubility, stability partition co-efficient, net charges along with structure activity relationship are used to predict the biological activity of a compound.

The proprietary reagent solution composed of proteins, glycoproteins, lipids and low molecular weight components used in commercial irrigation ocular assay system from a complex macro-molecular matrix when it is added to a potential chemical to imitate the eyes.

This system is used to screen many potential irritants by cosmetic companies without testing on animals.

4. Lower organisms and embryo stages

Many of the studies can be conducted in lower organisms such as invertebrates, plants and micro-organisms or in invertebrates at early stages of development.

The Ames test for genotoxicity studies uses Salmonella bacteria to detect mutagenic activity has been validated and accepted for screening of compounds in regulatory toxicology. The LAL test which detects the presence of fever inducing endotoxins has also been validated and accepted in regulatory toxicology. The use of horse shoe crab for research on vision has also been accepted. The teratogenicity of compounds can be tested on hydra which has the capacity to produce malformations in the embryo. Yeast cells and tobacco plant pollen can be used for toxicity studies.

Advances in genetic engineering by the addition of human drug-metabolising capacity to bacterial test systems and nematodes carrying human disease gene can be used to discover and develop new drugs. HET-CAM test carried out on fertilized chicken eggs predicts eye irritancy from the effects of a chemical on the chorioallantoic membrane of the egg.

5. Human studies

The studies in human can replace the use of animals in some studies. While carrying studies in humans, a deep ethical consideration should be given to the safety issues. As mentioned in the refinement concept, the non-invasive methods of analysis can be employed in human for investigations. More human volunteers are being used for cosmetic skin testing.

6. Cell, Tissue and Organ culture

The in vitro systems have been effectively used in virology, parasitological and bacteriological studies to a greater extent and also in Immunology, production of vaccines and drug development. These systems do not come as replacement alternatives but for the studies at

cellular and molecular level they become relative alternatives. Many of the studies require freshly isolated materials and the animals are used more economically because a single animal will provide tissues for large number of cultures.

There are several types of in vitro system in which sub cellular fractions of one cell component, primary cell culture, cell line culture. Cell lines, tissue culture and organ cultures can be used.

The in vitro systems have many advantages in addition to elimination of animals. These are economical, time saving and does not occupy unnecessary space.

However, the data obtained from the in vitro studies involving these techniques tends to be partially related. The in vitro systems under the study are free from rest of the animal. The confounding factors such as chemicals, enzymes, plasmabinding, hormones etc. may have the systemic influences. These aspects makes it to be disadvantage when the confounding factors have crucial effect on the system.

Future strategies in research

The basic medical research and production of vaccines would come to a halt if the experimentation and testing of compounds involving the use of laboratory animals is banned.

Many research involving the use of animals may lead to scientific breakthroughs in effective treatments for many of the life threatening diseases of animals and mankind. There could be an increase in the number of tests and experimentation involving animals. With due ethical consideration, proper care and management the number of animals used in research can be reduced.

The 3 R"s approach would provide a platform for scientists and animal welfare activists to work together in bringing out the best possible remedies for many of the diseases.

Essential of animals use in research

To test safe, discover and to produce effective products, animal models will always be essential in research work. It would be impossible to develop many life saving drugs without animal testing.

Many of the alternative techniques can help in a limited way to predict the biological activity and potential toxicity of a compound. These techniques do not accurately predict the biological effects of a compound in the whole organism. Many of the complicated interactions, stability of the compound, metabolic pathways and consequences of the fate and therapeutic value of a compound and its effect on the whole body system information cannot be obtained from the alternative techniques.

Conclusion

Defined laboratory animals must be used which are bred and produced under controlled conditions and maintained in a controlled environment to assure correct experiments. For obtaining reproducible results in animal experiments, the use of such animals is a prerequisite.

The use of alternative to animal experimentation is a complicated issue because of differences among species of animals. The alternative methods does not produce expected things and they are less likely to reveal the complex interactions that occur in a living animal. Even though there is considerable efforts to develop alternatives to animal research and testing, the use of animals continue to be essential for the development of newer products. Therefore, while the use of animals will continue to be necessary, it should be assured that all the animals used in the product development, experimentation, research and studies due consideration should be given for the ethical care and management of animals. The animals should be treated with ethical consideration, most respect and humane care.

REFERENCES

Beglinger, R., Backer, M., Eggenberger, E. and Lombard, C. (1975). Das Gottinger Miniaturschwein als Versuchstiere. *Res. Exp. Med.*, 165: 251-261.

Bruckner, L., Bongers, J., Castle, P., Flore, P.H., Halder, M., Jungback, C., Xavier, F., Gross, L., Tollis, M., Nair, V.K., Wilhelm, M., Zeegers, J. and Zigterman, G. (2000). Three Rs approaches in the production and quality control of avian vaccines. *Alternative to Laboratory Animals*, 28: 241

Hoppe, P.C. and Illmensee, K. (1977). Microsurgically produced homozygous diploid uniparenteral mice. *Proc. Natl. Acad. Sci. USA* 74: 5657-5661.

Mclaren, A. (1976). *Mammalian Chimeras*. Cambridge University Press, Cambridge.

Mintz, B. (1971). In J.C. Daniel, Jr. (Ed.), *Method in Mammalian Embryology*. W.H. Freeman and Company, San Francisco, pp. 186-214.

Nomura, T. and Tajima, Y. (1982). Defined Laboratory Animals. *Advances in Pharmacology and Therapeutics*. 5: 325

Radzikowski, C.Z. and Nomura, T. (1981). ICLAS Monitoring Centre System Programme. ICLAS Bull., No. 48: 6-8