Basic research: Issues with animal experimentations

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ABSTRACT
In vivo studies using the animals are helpful in developing the treatment strategies as they are important link between the successful in vitro testing and safe human use. Various research projects in the field of fixation of fractures, development of newer biomaterials, chemotherapeutic drugs, use of stem cells in nonunion of fractures and cartilage defects etc., have hugely depended on animal experimentation. The employment of animals in experiments is both scientific and ethical issue. There must be reasonable reasons to show that it will significantly advance the present knowledge and lead to improvement in care. The regulatory bodies exist for humane use and care of animals used for experiments e.g., International Council for Laboratory Animal Science, Council for International Organizations of Medical Sciences, International Union of Biological Sciences, International Committee on Laboratory Animals. In India, Indian National Science Academy, Indian Council of Medical Research, National Centre for Laboratory Animal Sciences promote high standards of laboratory animal quality, care and health. The Committee for the Purpose of Control and Supervision on Experiments on Animals guidelines are well defined and is a must read document for any one interested to carry out research with animal facilities.

Key words: Animal experimentations, basic science, orthopedic research

INTRODUCTION
In vivo studies using the animals provide invaluable information about various disease processes and help in development of treatment strategies. Virtually every medical achievement in the twentieth century relied on the use of animals in some way. The Institute for Laboratory Animal Research of the U.S. National Academy of Sciences argue that even sophisticated computers are unable to model interactions between molecules, cells, tissues, organs, organisms and the environment, making animal research necessary in many areas. Animal studies have been used in the past and shall continued to be used in the future for a number of applications in the field of orthopedics. Human testing cannot be done for any novel treatment unless adequate safety has been ensured. Any new implant material should match various safety standards in terms of biocompatibility, mechanical stability, lack of local and systemic toxicity. In vitro studies are quintessential initial step in testing new implant materials but results from in vitro studies are hard to apply to the in vivo situation. Many aspects of biocompatibility and safety like tissue reaction to implant, systemic toxicity, long term safety, late carcinogenicity, effect of controlled physiologic loading etc., cannot be tested in an in vitro situation. Hence, extensive in vitro, as well as in vivo studies [animal studies] are essential, prior to clinical application in humans. Animal studies bridge the knowledge gap between successful in vitro testing and safe human use. Innumerable advances in the field of orthopedics and allied sciences have been critically dependent on animal experiments. Advances in internal fixation of fractures, newer implant materials, chemotherapeutic drugs for various neoplastic conditions, use of stem cells in nonunion of fractures and cartilage defects etc., have hugely depended on animal experimentation.

SCOPE
By law, any new drug has to pass through a series of toxicological analysis in animals before being introduced to clinical research and usage. The results from animal studies can be extrapolated to human since the internal milieu of humans are similar to animals. After a successful in vitro tests of a new treatment strategy [stem cells, drugs etc.], it has to show its effect in an animal model before it is introduced into clinical research. Healing of bone gaps by
stem cells, characterization of new implant materials all have to undergo animal trials prior to human application. Healing potential of tissue engineered stem cells, chondrogenic construct has to be tested in cartilage defect model in rabbits or dogs to demonstrate its efficacy prior to human trials.\(^6\)

Biodegradable materials are analyzed in vitro and in vivo to determine various characteristics of the material like rate of degradation, enzymatic reactions, immunological reactions, effect of physiological loading etc. Larger animals [sheep, goat] are used for testing of joint replacement components and surface and to study the innovative methods of fixation of bone fragments.

**Selection of an animal model**

An ideal animal model is one in which the anatomy and physiology match the specific study design. The pathogenesis and disease progression should parallel that of humans and so should the histopathology response.\(^4\) The in vivo milieu should closely mimic the human clinical situation. Invertebrate animals are preferred over vertebrate animals for experiments; however, commonly used animals for experiments are rats, mice, rabbits, goats, sheep, pigs, dogs, cats and primates.\(^7\)

Rabbits are commonly used animal in tissue engineering research and in orthopedic practice. They are vertebrate animals with an appropriate size for surgical operation, hence are suitable for studies on healing of gap nonunion in bones, repair of damaged articular cartilage, reconstruction of ligaments and tendons, and spinal fusion.\(^8\)\(^–\)\(^11\) Rat is also in use for tissue engineering research due to its low cost and easier maintenance.\(^4\) It is used for studies on fracture healing,\(^12\) biocompatibility studies,\(^13\) and repair of bone defects.\(^14\) Mouse, a small rodent has been extensively been used as its genome can be easily manipulated and because of its ease of handling. Regular mice or immuno compromised nude mice have been used in studies on bone\(^15\) and cartilage healing.\(^16\) Generally, larger animals impose more housing and handling difficulties than small animals such as rats and rabbits, and are more expensive. The factors involved in selection of the animal model includes the study design, cost, ability of the facility to handle the animals, resistance of the animal to diseases [specially in long term studies] and transportation.

**Ethical Issues**

Before an animal experiment is designed there must be reasonable expectation that it will significantly advance the present knowledge and understanding about the subject and lead to improvement in care.\(^5\) Public opinion polls have consistently shown that a majority of people approve the use of animals in biomedical research that does not cause pain to the animal and leads to new treatments and cures.\(^2\)

Animals should be used in research only when there are no suitable alternatives. The designing of research projects should be such that minimal required number of animals should be used and in a manner that conform to established standards of animal care. Animal care and ethical committee should be formed in all institutes and its due approval should be made essential prior to starting the animal studies.

**Guidelines for care of animals**

Many national bodies have been formed in various countries for humane use and care of animals used for experiments through recognition of ethical principles and scientific responsibilities. International Council for Laboratory Animal Science (ICLAS), with a membership of about 100 countries is the apex body. The ICLAS draws guidelines on animal husbandry, experimental procedures, teaching and training of researchers and professionals in the field world over.\(^17\) ICLAS is dedicated to advancing human and animal health by promoting the ethical care and use of laboratory animals in research worldwide. ICLAS promote high standards of animal care and use in education, research, testing and diagnosis, to promote good science and foster humane practices in scientific research. The aims of ICLAS are compatible with the highest possible standards of animal research internationally.

Indian National Science Academy (INSA) issued such guidelines in 1992 and recently revised it in 2000. Indian Council of Medical Research (ICMR)\(^5\) also set up a unit called Laboratory Animal Information Service (LAIS) at the Indian Cancer Research Centre now called Cancer Research Institute, Mumbai. This unit subsequently got shifted to National Institute of Nutrition, Hyderabad, wherein in 1986 it received additional support from Department of Biotechnology, and developed into a National Centre for Laboratory Animal Sciences (NCLAS). India enacted its first animal law as early as 1960, called the ‘Prevention of Cruelty to Animals Act’. It was subsequently amended in 1982, which provided for the prevention of cruelty to animals in general. Chapter 4 of act deals with control of experimentation on animals. The act also empowers committee formed under it to take care of the legal and ethical aspects of experimental animals being used in research and enact preventive actions when there is violation of the law.\(^5\) The ministry of social justice and empowerment, New Delhi, issued a notification\(^18\) on fifteenth February, 2001, where some guidelines were defined. In this guidelines, the word “experiment” means any program/project involving use of an animal for the acquisition of knowledge of a biological, psychological, ethological, physical or chemical nature. The establishment on registration for the purpose of performing experiments
on animals shall comply with the conditions as may be specified at the time of registration by the committee and every registered establishment shall maintain a register of particulars about the animals used for conducting experiments. A detailed specifications for housing, feeding and maintenance of various species to be used in animal experimentation as notified by the committee, shall be adhered to by the registered establishment. It also specifies that the experiments shall be performed in every case by or under the supervision of a person duly qualified, that is, degree holders in medicine or veterinary science, post graduate and above in life sciences/pharmaceutical sciences or any other natural sciences, degree or diploma holders in pharmacy, diploma or certificate in laboratory animal techniques sciences from a recognized institution as identified by committee. It also mentions that experiments shall not be performed for the sole purpose of attaining or retaining manual skill. The Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines\textsuperscript{19} are well defined and is a must read document for any one likes to carry out research with animal facilities. It provides the guidelines regarding the animal procurement, animal care, requirement of physical facilities and the environmental issues. The guidelines also deal with the issues of their food, water, sanitation, and transportation. The guidelines regarding anesthesia, standard operating procedures, euthanasia are helpful for any orthopedic surgeon or research institutions if they wish to carry the research using the animals.

In order to reduce the impact of research on animals, the principles of three Rs as advocated by Russel and Burch (1959)\textsuperscript{20} are still considered as good laboratory practice. The three Rs are: Reduction, Refinement and Replacement. Reduction means reducing the number of animals used in experiments. Refining the experiment to reduce their suffering and whenever possible, replacing experiments on animals with alternative techniques.

**The Indian scenario**

In India animals have been considered sacred. The animal experimentations are in primitive stage in India due to poor infrastructure in most institutes, lack of expertise in handling animals, dearth of adequate funding and lack of interest in basic research among orthopedic surgeons. Most experiments involve small laboratory-bred animals and are approved by the Institutional Animal Ethics Committee (IAEC). These committees are formed as per the guidelines of the Indian National Science Academy, and include a nominee of the CPCSEA. The current rules (2000) empowers IAECs to allow experiments only on small laboratory bred animals- guinea pigs, rabbits, rats, mice, hamsters, and invertebrates.\textsuperscript{21} For all other animals, permission must be sought from a subcommittee of the CPCSEA.\textsuperscript{19} The Medical Council of India (2010) has allowed medical colleges to use software instead of animals for teaching and training undergraduate students. The condition can be improved by more focus on animal experiments at medical colleges, better awareness about need and methodology of animal experiments, more funding, stringent laws regarding unnecessary harm to animals and their strict implementation.

**Challenges in the field**

Though animal experiments have contributed immensely towards our understanding of various disease processes and in developing better treatment methods, the opposition to use of animals for research purposes has always existed. This movement is gaining a strong foothold in our country too. Organizations like PETA [People for Ethical Treatment of Animals], IFAW [International Fund for Animal Welfare] etc., have protested against animal experiments. Appropriate permissions from animal welfare boards, maintenance of highest standards of care of animals and being abreast with the current law on animals and their use is essential to avoid trouble from the animal rights activists.

The set up for conducting animal studies is costly and faculty must be trained in handling animals well. In the West, 1 or 2 year diploma courses are available to train biology, medical and veterinary graduates. In India, short term courses ranging from six to twelve weeks are run by national bodies for those involved in animal husbandry and animal studies. National Centre for Laboratory Animal Sciences (NCLAS) at Hyderabad under the ICMR and Central Drug Research Institute (CDRI), Lucknow under the Council for Scientific and Industrial Research (CSIR) host such training programs on a regular basis.\textsuperscript{5}

The animal experiments are relevant to an average orthopedic surgeon. An average orthopedic surgeon may not come into direct contact with animal studies but he should know the importance of animal studies in his day to day practice. New drugs for osteoporosis, osteoarthrosis, antibiotics, cancer chemotherapy etc., have been derived from animal research. New implant materials for fracture fixation, bearing surfaces for joint replacement, biodegradable interference screws for ligament reconstruction, stem cell therapy for nonunion of bones are only a few examples to show the bearing animal studies have on the day to day practice of an average orthopedic surgeon. Thus, all of us as the orthopedic community should be aware of the issues associated with animal studies, recent developments, legal and ethical aspects associated with the same. All of us should pay due respect and be deeply indebted to the animal kingdom, our fellow species for making our daily practice of orthopedics so much more better and this world a much better place to live in.
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