Biotechnology: An answer to alternatives for animal model testings

S. Indu Kumari and Kaiser Jamil

Following Peter Singer's version of Animal Liberation and the movement of animal rights activists, there has been a resurgence of new and alternate testing procedures for toxicological evaluation. Scientists and researchers have long been involved in the search of alternate testing methods; however, the regulatory toxicologists, FDA and EPA of several countries do not accept in vitro methods as a substitute for safety tests or alternate methods. This is because the new products are for human consumption. Extrapolating the in vitro methods to animal models is therefore not always acceptable. This review discusses issues raised by activists and the available scientific technologies which could serve as alternate methods for toxicological evaluation.

TOXICITY studies are generally performed to determine drug-related effects that cannot be evaluated in standard pharmacology profile or occur only after repeated administration of the agent. Theoretically, toxicity profiling in animals would be most useful if the test model responded in a fashion that was identical to the human. However, such is seldom true in practice, even when the route of administration and vehicle used are identical to the clinical use because deposition (pharmakokinetics) can vary dramatically among species, and even between strains[2]. Therefore, most toxicity tests are performed in two species – a rodent and a non-rodent – to ensure that any unexpected adverse effects are not overlooked before new chemical entities (NCE) are introduced into man. The sequence of toxicity testing proceeds from the simple to the complex. This is because toxicity testing is desirable at an early stage in the development process but large quantities of drug samples are usually not available at that time, limiting the advancement of medical science during the last 100 years which largely depended on research with animals[3]. Animal studies have provided the scientific knowledge that allows health care providers to improve the quality of life for humans and animals by preventing, treating diseases and disorders, and easing pain and suffering[4].

The debate regarding animal research and testing

Use of animals in scientific research and testing has raised controversy and criticism for long. The use of animals in medical research has been objected to and a number of legislative initiatives have been proposed from time to time to limit animal research, or ensure proper treatment of animals. Animal protection movement began in England during the nineteenth century. Antivivisection groups, sometimes referred to as abolitionists, opposed all forms of animal research. Animal welfare groups, or reformers, opposed various forms of animal research due to the increased use of animals for developing drugs and safety tests for pesticides. However, use of non-animal alternatives is also being developed wherever possible to meet the mandatory regulation of animal experimentation[5,6]. A third element of animal rights arose in 1975 following publication of Peter Singer's book *Animal Liberation*[1]. The new animal rights activists began to question the use of animals for any purpose and this movement picked up a number of antivivisectionists. Active proponents of responsible animal use in scientific research and testing include organizations, such as the Research Defence Society in UK and the National Association for Biomedical Research (NABR) in USA. These groups actively support the rights of scientists to use animals in their research, and they monitor the activities of animal rights groups. As a result, groups like the Scientists Center for Animal Welfare (SCAW) have come together to address the humane and responsible use of animals. This organization often serves as an information resource but does not represent the research, animal rights as antivivisection communities.

The medical community, the regulatory agencies, and the legislators have responded positively to this problem, as evidenced by the fact that the extent and use of laboratory animals decreased by 40% between 1968 and 1986. In addition, various amendments to the Animal Welfare Act of 1966 ensured the quality of animal care research institutes[7,8]. All institutions or laboratories performing animal research now have institutional animal

The authors are in the Biological Sciences and Toxicology Division, Indian Institute of Chemical Technology, Hyderabad 500 007, India.
*For correspondence. (e-mail: kaiserjamil@iict.ap.nic.in)

care and use committees (IACUC) to determine the ethicality of experiments to be performed to ensure that the procedures are as humane and painless as possible. Many technical and procedural changes have taken place since the mid-1970s in drug development and specifically in toxicological assessment of drugs. The elimination of calculated LD_{50} study from regulatory requirements, and the environmental regulations for lab animals promulgated by amendments to the Animal Welfare Act are two examples of procedural changes brought about by social and ethical pressures. Technical advances made in analytical chemistry have drastically changed the procedures used in safety assessment and the impact of the changes in toxicology studies is conducted and evaluated[9].

As computers become more powerful, their use in drug design, analytical chemistry, microscopy and data analysis will increase, in turn spawning additional inventions and procedural changes. However, despite technical innovations and enhancements discussed above, the basic toxicology procedures performed today have changed little over the years; for example, acute and repeat dosage toxicity studies, developmental toxicity studies and carcinogenecity studies in 2 species are still with us. This will change when *in vitro* procedures become more refined and the methods employed by biotechnology become more popular[10]. However, until now tests and assays used in toxicology have been in *in vivo* type. Yet a number of inroads have been made and more and more *in vitro* assays will be used in future to assess the safety pharmaceutical products.

Tissue culture methods are becoming more sophisticated and computer modelling techniques for drug disposition will make correlations between *in vivo* and *in vitro* techniques easier to assess. Cultures of human liver cells have already been used in toxicology screens for many years. Therefore, it is not unreasonable to envisage that use of such systems will grow and eventually much of the basic toxicology may be determined in human cell culture systems, making extrapolation from species to species almost unnecessary.

The impact of biotechnology

Biotechnology in its broadest sense is the use of living organisms (cells, microbes, plants and animals) to make useful products. The application of biotechnological knowledge and in particular gene technology is especially important to pharmaceuticals in the manufacture of vaccines, development of more diagnostic aids and therapeutic agents[11], and ultimately in gene dosing and expression of genes; peptide engineering generation and use of various antibodies, mammalian cell cultures; and transgenic animal techniques. These tools are already being used to some degree by pharmaceutical firms, but as their use expands and knowledge accumulates, many disciplines, including toxicology will undergo substantial changes. Diagnostic methodology will be infinitely enhanced as techniques are developed to monitor various pathogenic proteins and enzymes, and fluorescent antibody techniques become more common for identifying sites of drug or metabolite deposition. Transgenic animals for mutagenecity testing are already on the market[12]. Transgenic animal models of carcinogenesis, as well as a number of human diseases are also available, and increasing use of such models will provide a powerful tool for assessing the potential toxicity of drugs and chemicals[13].

Safety evaluation of biotechnology products also challenges the ingenuity of the toxicologist. These products are usually large molecules of peptides or proteins that have to be administered parenterally. Since they are expensive to produce, only small quantities of material are available to the pharmacologist or toxicologist. Furthermore, many of the products are species-specific and engineered for human use, and neutralizing antibodies are often a problem in subchronic animal studies.

To circumvent or minimize the problems associated with non-clinical testing of biotechnology products, many standard methods of testing had to be abandoned or replaced. Thus mutagenicity assays, sub-chronic studies over 30 days duration or testing in standard lab species may not be relevant and may be replaced by short biochemical assays that are more relevant. It has also fostered a close working relationship between the pharmocologist and the toxicologist, both have limited test article with which to conduct their work, and therefore a short study in a few monkeys may have to answer simultaneously a multitude of questions. The Food and Drug Administration has also recognized the problems, and the Center for Biologics Research has attempted to make its judgements on a scientific case-by-case basis rather than requiring submission of standard protocols that would be of limited relevance.

Animal alternatives

It is not possible to replace whole animal models with *in vitro* systems to evaluate drug effects on major organ systems. However, techniques can greatly reduce the number of animals needed, and refined protocols can improve the design efficiency and quality of studies, and lessen stress and discomfort experienced by lab animals[14]. In order to monitor physiological functions in conscious animals, survival surgery may be performed to implant catheters, electrodes, flow probes or other devices. While chronically instrumented animal models can reduce the numbers of animals used per study and reduce numbers associated with acute procedures, these models are resource-intensive to prepare and maintain.

Generally instrumented animal models can be reused in major organ systems toxicology (MOST) for studies to evaluate more than one drug. Precautions have to be taken to allow for drug washout and to ensure the integrity of the organ systems before restudy. This is accomplished by establishment of requalification procedures for reuse of instrumented animal preparations, based upon normal blood biochemistry and haematology parameters, a veterinary examination, and consideration of previous study history. With requalification of instrumented animals, the total numbers of animals and resources needed to complete MOST evaluations can be substantially reduced. The rapid expansion of telemetric techniques for monitoring physiological functions has further decreased human and animal resources necessary to collect, process, and report pharmacodynamic international requirements for pharmacodynamic evaluation. This approach reduces the number of studies to be conducted and saves animals and resources.

Impact on animal testing

Public concerns about use of animals and activist group campaigns have definitely had an impact on the nature of biomedical research and testing. New laws and regulations have altered the way that protocols are written and have promulgated new levels of review. Public pressures have caused companies to be concerned about animal testing in particularly sensitive areas, for example, cosmetics, and to be concerned about animal testing in particularly sensitive issue disclaimers that their products have not been tested on animals. The concept of alternatives has gained in level respectability and has attracted the interest of scientists and the attention of funding agencies. The modern toxicologist has certainly shared this impact. The profession of toxicology is now one of the most scrutinized of all the disciplines. In USA, for instance, animal use in the average laboratory regulatory toxicology work is monitored by the United States Department of Agriculture (USDA), the Food and Drug Administration (FDA), the American Association for the Acreditation of Laboratory Animal Care (AAALAC) as well as quality assurance unit and other organizations. The time spent in preparing and responding to studies lessens the stress and discomfort experienced by laboratory animals. Using this technology, animals previously prepared for telemetric monitoring need only be dosed and their home cages where monitoring can continue on virtually any schedule for up to six months or more. Telemetered animals may be reused to study additional drugs, so long as they can be requalified for each subsequent study based upon preestablished criteria. With this technology it is feasible to consider periodic or continuous organ function monitoring in repeat-dose animal toxicity studies.

Currently, telemetry systems are available which monitor blood pressure, heart rate, ECG and other biopotential body temperature, gastrointestinal pH and gastric empty, intestine transit time, and relative activity. When developing a safety pharmacology programme, it is useful to consider the entire animal safety programme. Clinical observations and functional assessments made in conjunction with animal toxicology studies may provide data for the basic central nervous system, behavioural, and/or gastrointestinal pH and gastric emptying, and intestinal transit time. In these cases, it may only be necessary to package the observations from toxicology studies as separate reports to fulfil to this monitoring are considerable. There is also a personal impact because toxicologists are a part of the public community. Now, more than ever, they must justify the use of animals in their experiments to those outside their laboratories[15].

Alternative techniques: definition and overview

Rowan[16] used the term 'alternative' to refer to those techniques or methods that replace the use of laboratory animals altogether, reduce the numbers of animals required, or refine an existing procedure or technique to minimize the level of stress endured by the animal. The concept of alternative techniques is now widespread throughout the scientific community. This is due largely to regulations and standards which require consideration and support to alternatives. However, the field of alternatives study particularly *in vitro* toxicology has evolved into a respected discipline and is attracting competent and motivated scientists around the world. The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) was founded in 1981 and is structured to support four core programmes. The Extra-Mural Grants Programme provides pilot grants to scientists primarily for developing *in vitro* approaches to evaluating cellular and development of *in vitro* parameters for extrapolation of risk. The validation programme attempts to define and standardize protocols for validation of *in vitro* test methodologies, co-ordinates efforts in a wide range of laboratories to validate existing methods, and serves as a central co-ordinating resource to achieve regulatory acceptance and the implementation of alternative techniques.

Alternative techniques in toxicological testing and evaluation replacement

In vitro models have been used in toxicology for many years. However, the upsurge of interest in alternatives since mid-1980s has resulted in a number of methods for studying mechanisms of toxicity using cell, organ, and

tissue culture. Some *in vitro* methods show promise as screening tools for specific areas of toxicity, for example, eye and skin irritation. There are numerous and complex interactions within the living animal which cannot be duplicated *in vitro*[17].

Structure activity relationships are expressions of biological effects of a test material in quantitative terms. Correlation of the materials, chemical properties with its biological effect is accomplished by a mathematical equation. Advances in computer technology have greatly aided the development of these relationships but, except for certain classes of carcinogens, their broad application to general toxicity has not been established[18].

Reduction

Reduction in numbers of animals used in toxicology testing has been accomplished by a number of routes. Improved methods of conducting acute toxicity studies, for example, approximate lethal dose, and use of fewer animals compared with the classic LD_{50} test. Timed-pregnant animals provide opportunity for using fewer animals in reproductive toxicology studies[19]. Harmonization efforts and regulations between different countries also result in less animal use since fewer studies have to be done overall.

Refinement

Refinement of toxicology studies has resulted in reduced stress or distress for the animals. This was accomplished by many ways. For instance, limits on tumour size and guidelines for declaring animals moribund can reduce distress during long-term carcinogenicity studies. Improved cage design and environmental enrichment techniques can enhance the overall welfare of the study animals. Group-housing, another refinement alternative technique, is becoming more common in toxicology studies. It has been shown, for instance, that beagles can be successfully housed in groups during GLP regulated toxicology studies[20]. Proper acclimatization of animals to study techniques and thorough training of personnel can also contribute to refinement of toxicology studies.

Challenges for the future

Thus the impact of biotechnology and the use of alternative techniques will take mankind ahead in its endeavours in a humane manner without causing suffering to animals for selfish human needs. While it is risky to make predictions in print, it seems certain that the future of animal research and testing in toxicology will be both exciting and challenging. Mechanistic *in vitro* and computer-based research will provide a host of new screening tools which will further reduce the number of animals used and improve the overall efficiency and effectiveness of testing. Biotechnology will present toxicologists with new test materials, for example, human-specific hormones, which will have to be tested in human cell lines rather than animals. Animal models will be further improved and model choices expanded. The credit for this goes to advances in biotechnology. Legislation for implementation of alternate methods will change to reflect the new technology and the new and increased public sensitivities.

The regulatory organizations and researchers must be prepared to change during these developments. There will be new challenges, frustration opportunities, and satisfaction to ensure a higher quality of life for humans and animals alike.

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