Exploring Innovative Approaches for Advancing the 3 R's in Research: Moving Beyond Animal Testing through Biological, Biochemical, Ethical, and Multi-Cellular Strategies – Can We Eliminate the Need for Animal Testing?

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Abstract

With the further development of medical technology research and development, the number of animals used in research is increasing. Every year, millions of laboratory animals are used around the world. The pain, suffering, and death of animals during scientific experiments have long been a controversial issue. In addition to major ethical concerns, animal experiments also have several drawbacks, such as the need for qualified personnel, time-consuming protocols, and high costs. A 3R strategy (i.e., reduce, purify, substitute) is employed for the use of animals in laboratories. Various methods and alternative organisms are used to carry out this strategy. These methods are alternatives to drug and chemical testing in specific fields, and this review describes and provides examples of these alternatives and their associated advantages. Integrated application of these approaches will minimize the use of animals in scientific experiments.

I. Introduction

The use of animals for various purposes, such as food, transportation, pets, sports, recreation, and society, is as old as humans, and the use of animals in research is one of its extended uses. Various animals such as mice, rats, hamsters, rabbits, fish (e.g. zebrafish, trout), birds (mainly chickens), guinea pigs, amphibians (Xenopus laevis), primates, dogs and cats have been used in research for many years (CULABBR, 1988). The primary goal of such studies is to conduct drug testing and toxicity screening to aid in the development of new treatments for infectious and non-communicable diseases. Animals also serve as tools for making sense of medical procedures and surgical experiments. In addition, it is used to obtain vaccines, antibiotics and other products that are used both diagnostically and therapeutically (Giacomotto and Segalat, 2010; Hendriksen, 2009, 2007). With the further development of medical technology, the number of animals used in research has also increased. Every year, millions of laboratory animals are used around the world. For example, in the UK, 3.71 million animals were used in research in 2011 (www.rspca.org.uk). The total number of animals used was estimated at 1,131,076 he in the United States in 2009, while in Germany it reached a maximum of 2.13 million in 2001 (Rusche, 2003). This large experimental animal population typically comes from various university breeding centers and national breeding centers. These are all referred to as Class A dealers, while intermediaries who obtain animals from various sources (such as auctions and animal shelters) are referred to as Class B dealers. In some cases, wild animals such as monkeys and birds are also used (Baumans, 2005). In clinical laboratories, animals are isolated from their group and used as tools regardless of their natural instincts. Whole animals or their organs and tissues are used in experimental procedures. For this purpose, animals are euthanized (slaughtered) according to established methods. Animals that survive clinical trials are often euthanized at the end of the experiment to avoid subsequent pain and suffering (Rusche, 2003). In some cases (e.g. LD 50 analysis), animals die as a result of experiments.

The pain, suffering, and death of animals during scientific experiments has long been a controversial issue. The argument is that if animals are alive, they have the right to alleviate pain and suffering, and therefore the use of animals in experiments is unethical and should be stopped (Rollin, 2003). Various laws and regulations have been enacted to regulate the unethical use of animals and to minimize animal suffering during experiments. For example, animal rights organizations were founded in 1824 by the Royal Society for the Prevention of Cruelty to Animals. In 1876 Britain passed a law to prevent cruelty to animals (Balls, 1994). Founded in 1960, 1963, and 1966 in India, Franc,e and the United States. Many rules and laws are now followed internationally to protect animals from cruelty and cruelty. Besides major ethical concerns, animal experiments also have drawbacks, such as the need for qualified and

trained personnel and time-consuming protocols. Furthermore, the very high cost of breeding, rearing, and lengthy animal testing protocols is another drawback. (Balls, 1994).

II. Three Rs: reduction, refinement, and replacement

Alternatives to animal testing have been developed to address some of the difficulties of animal testing while also avoiding unethical activities. The 3R strategy is applied. This means reducing, improving, or replacing the use of animals in laboratories (Ranganatha and Kuppast, 2012). Various methods and alternative organisms are used to carry out this strategy. The concept of animal substitution was first discussed by Charles Hume and William Russell at the Union of Universities for Animal Welfare (UFAW) in 1957 (Balls, 1994). Russell and Burch (1959) proposed several ways of humanizing animal experiments, later called the 3Rs. This approach motivates the use of minimal numbers of animals. H. "Reduction" in the total number of animals used in the experiment. Animal use must be carefully planned and 'refined' to minimize pain and distress during the experiment. Animal substitution is defined as "any scientific method of using non-sensory substances that can replace the use of conscious living vertebrate animals in animal experiments". He distinguished two kinds of substitutions, ``relative" and ``absolute". Animals are used as relative surrogates but are not subjected to stress during the experiment. Withdrawal of the animal at any point in the experiment has been identified as an absolute alternative strategy (Balls, 1994).

2.1. Reduction

Experiments can produce relevant scientific results with the use of statistical support and appropriate research design. For example, in vitro cell culture is a good way to test compounds in the early stages. Human hepatocyte cultures provide information about how drugs are metabolized and cleared from the body. Incorporating such methods into the research plan can help preempt inappropriate compounds and minimize the use of animals in further testing (Kimber et al., 2001). The effects of some compounds on embryonic development have been studied in live animals and embryos. In vitro studies using embryonic stem cell cultures help reduce the number of live embryos used and the number of compounds toxic to developing embryos (Gipson and Sugrue, 1994; De Silva et al., 1996). Additionally, the transferability or availability of the data obtained (e.g. excipient properties of the study drug) avoids the need for animal testing.

2.2. Refinement

Reduce animal stress by enriching the cage environment through animal care. Scientists need to improve animal husbandry practices to reduce pain, discomfort and stress during animal life and scientific procedures. Additionally, any stress or discomfort can create an imbalance in the animal's hormone levels, which leads to variability in results. Therefore, it is necessary to repeat the experiment, and the number of experimental animals increases. For example, when mice genetically engineered to study HD were given a complex cage environment with the ability to nest, hide, gnaw and feed, they observed that the disease progressed more slowly than mice housed in sterile cages. It was also found that such mice mimic the

human disease course better. Such improvements provide a very good model for treating disease and also minimize stress on animals (De Silva et al., 1996). 2.3.

2.3. Replacement

Various alternatives to the use of animals have been proposed, including in vitro models, cell cultures, computer models and new imaging/analytical techniques (Balls, 2002). In vitro models offer the opportunity to study cellular responses in a closed system where experimental conditions are maintained. Such models provide preliminary information about the outcome of in vivo experiments. For example, computer models have been used to study cardiac function and select potential drug candidates (Gipson and Sugrue, 1994). In many countries, in vitro cell cultures have replaced skin irritation and Draize eye irritation tests, as well as the use of animals. Another example is the extraction of insulin from porcine or bovine pancreas, now extracted from bacterial cultures, making it an essential medicine for diabetics. This extracted insulin must be checked for purity, potency and dosage. Animals were routinely used for such checks, but chromatographic techniques are now used to check purity, potency, and drug dosage calculations (Foreman et al., 1996). Overall, the alternatives significantly reduce the use of animals in various processes.

III. Alternative methods

Various methods have been proposed to avoid the use of animals in experiments. These methods offer an alternative to drug and chemical testing in specific areas, with the benefits of time efficiency, personnel reduction, and cost efficiency. Details of these methods are as follows:

III.1 Computer Models

Computers help us understand many basic principles of biology. Special computer models and software programs help develop new drugs. Computer-generated simulations are used to predict various potential biological and toxic effects of chemicals or potential drug candidates without the need for animal dissection. Only the most promising molecules from the primary screen are used for in vivo experiments. For example, in vivo experiments are required to know the receptor binding site of a drug. A software called Computer Aided Drug Design (CADD) is used to predict receptor binding sites for potential drug molecules. CADD identifies potential binding sites and avoids testing for unnecessary chemicals that have no biological activity. Furthermore, with the help of such software programs, new drugs can be tailored to specific binding sites and subjected to final-stage animal studies to obtain confirmatory results (Vedani, 1991). This reduces the total number of experimental animals and fulfills Russell and Birch's 3R goal.

Another popular tool is the structure-activity relationship (SAR) computer program. Predict biological activity of drug candidates based on the presence of chemical moieties attached to the parent compound. A quantitative structure-activity relationship (QSAR) is a mathematical description of the relationship between the physicochemical properties of a drug molecule and its biological activity (Knight et al.,

2006). His QSAR software now provides better results while predicting the carcinogenicity of each molecule. Speed and relatively low-cost procedures are two benefits of computer models over conventional animal models (Matthews and Contrera, 1998). A very good example is the study by Dewhurst et al. (1994) investigated the effectiveness of computer models compared to conventional laboratory practice. In this comparative study, two groups of students conducted experiments using traditional wet lab approaches and computer-assisted learning (CAL), respectively. CAL is an interactive computer-aided learning (CAL) program that does not require real lab tools. At the end of the study, both groups' knowledge acquisition was assessed (through test questionnaires, calculations and interpretations).

III.2 Cells and tissue cultures

The use of in vitro cell and tissue culture to grow cells outside the body in a laboratory setting can provide an important alternative to animal testing. Cells and tissues, such as liver, kidney, brain, and skin, are harvested from animals and can be stored in vitro in an appropriate growth medium for days, months, or even years. In vitro culture of animal/human cells separates the cells from each other and grows as a monolayer on the surface of culture plates/flasks. Cellular components such as membrane fragments and cellular enzymes can also be used. Different types of culture are used depending on the purpose, such as cell culture, callus culture, tissue culture, and organ culture. The advantages associated with this technique are that it is easy to follow, quick and inexpensive. These methods are routinely used for preliminary screening to assess the toxicity and efficacy of potential drug molecules/chemicals (Shay and Wright, 2000; Steinhoff et al., 2000). These tests test the toxicity and efficacy of nearly all cosmetics, pharmaceuticals, and chemicals. For example, eye irritation test. In the past, the Draize test, which requires animals (mainly rabbits), was used to confirm the irritant potential of chemicals. “Every time I use a new animal it is very painful” (Ke Ping Xu et al.), proposed an alternative using bovine corneal organ cultures. Bovine corneas are cultured in the laboratory for up to 3 weeks, and various analytical methods are used to assess the toxic effects of the irritating effects of test chemicals in vitro (Xu et al., 2000).

III.3 Stem cell research:

As a complementary option to using animals for toxicological studies and disease modeling in vitro, stem cells may be useful. Disease genes are inserted into embryonic stem cells, which are then induced to differentiate into human disease tissue that can be used for drug screening. ES cells proliferate in petri dishes and differentiate into various cells that make up human organs. These in vitro versions of human tissues are superior to single-cell type shells when evaluating the toxicological effects of drugs. These provide a human impact profile, not a mouse impact profile. Researchers used genes from Parkinson's disease patients to create embryonic stem cell lines that exhibit degenerative manifestations of the disease. Diabetes and Alzheimer's disease have been found to be associated with a combination of genetic and environmental causes, and stem cells are being used to test new drugs to treat these common diseases. Embryonic stem cell-derived mouse models of two spinal cord disorders, spinal muscular atrophy and Lou Gehrig's disease, have been developed to test new drugs.

Mammals are not always good models, especially when it comes to delineating a drug's potential hepatotoxicity and cardiotoxicity. Animal models are expensive and time-consuming to obtain results. Stem cells offer a better alternative material for studying various types of cancer, as well as liver and heart toxicity.

III.I Results - Alternative Organisms

Ethical concerns impose many restrictions on the experimental use of higher model vertebrates such as guinea pigs, rats, dogs, and monkeys. Therefore, the use of alternative organisms has been proposed. Various model organisms are used as an alternative to experimental animals (Table 1).

Table I: Selected Examples of Organisms

Alternative Organism (s)	Specific Cellular Function
Escherichia coli	**Model for molecular/genetic studies (prokaryote)**
Bacillus subtilis	**Model for cellular differentiation (prokaryote)**
Saccharomyces cerevisiae	**Model for gene expression (cell cycle, fungi)**
Genus ascomycetes	**Model for population genetics (fungi)**
Aspergillus nidulans	**Model for genetic / cell biology (fungi)**

3.3.2.2. Example – Caenorhabditis elegans

Caenorhabditis elegans is a eukaryotic nematode. This multicellular organism is about 1 mm long and has a very short generation time. The entire life cycle of this hermaphrodite is about 2-3 weeks. Embryogenesis occurs in 12 hours and adults develop in 2.5 days. It is transparent, genetically accessible, and exhibits simple cellular complexity. It was therefore chosen as a model organism by Nobel laureate Brenner (Barr, 2003; Strange, 2007). The C. elegans life cycle progresses through various complex developmental stages, including embryogenesis, morphogenesis, and adult development. This is he one of the most commonly used model organisms for research purposes. The information obtained may also be applicable to more complex organisms such as humans. C. elegans is used as a model to study various neurological diseases such as Huntington's disease, Parkinson's disease, and Alzheimer's disease. Various immune diseases, cancer, diabetes, etc.

3.3.3. Microorganisms

3.3.3.1. Example – Saccharomyces cerevisiae

The brewer's yeast Saccharomyces cerevisiae is the most popular and important model organism due to its rapid growth, ease of replication plating and isolation of mutants, dispersed cells, well-defined genetic system, and highly versatile DNA transformation system. Yeast can be grown in solid or liquid culture and isolated as colonies derived from single cells on solid media. The very short generation time of about 90 minutes makes breeding and analysis of large populations very easy (Mell and Burgess, 2002). The entire genome of this unicellular fungus was sequenced in 1996. The nuclear genome contains approximately 16 chromosomes with over 13 million base pairs. Mitochondria also contain an additional nuclear genome. Reproductive yeast carries genetic information in the form of 6000 genes. The number and size of genes are relatively small and the gene density is very high. S. cerveisiae is one of the most

well-characterized and studied genomes, making it one of the most ideal eukaryotic microorganisms for biological research. Having a similar cellular structure and basic life cycle to multicellular eukaryotes is another advantage. Numerous membrane-bound organelles such as the nucleus, peroxisomes, mitochondria and secretory pathway organelles also mimic mammalian cell function (Mell and Burgess, 2002). This brewer's yeast has been used to understand human programmed cell death and cell death regulators, and is very useful for cancer research (Madeo et al., 2002). By studying the endogenous or heterologous proteins that underlie neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases, S. cerevisiae helps us understand fundamental aspects of cell biology in these diseases (Pereira et al., 2012; Siggers and Lesser, 2008).

IV. Findings/Significance - Ethics of Animal Testing – ***How is the alternative more effective?***

According to the Humane Society International, 100,000–200,000 animals suffer and pass away each year as a result of aesthetic experimentation. In these tests, chemicals are poured down the animals' throats, into their eyes, and onto their shaved skin to record how they react and confirm that they are safe for human consumption. Your safety through cosmetics may be improved by these studies and testing, but is a chemical reaction in an animal the same as a chemical response in a human?

You might argue that this study and testing is beneficial for your health, but can they anticipate how various consequences will affect various individuals? Do people ever suffer any consequences? \However, because human beings cannot use the results of animal experimentation, doing so has put human lives in jeopardy. In many instances, animal research can injure people in addition to harming animals, according to a fact sheet published by PETA. Some medications were evaluated as safe after being tested on animals, but they nevertheless had negative effects on people. Have we all considered the potential causes of this? The solution is really quite easy. This is due to the stark differences between animals and humans.

With modern technology that we have created these days, animal testing is really unreliable, unscientific and unnecessary experimentation. Nowadays, we have plenty of alternatives which have a much higher percentage of success than animal testing. Instead of animal testing, we can use human cell culture systems; instead of animal testing, we can use computer mathematical models; instead of animal testing, we can use artificial human skin and eyes that mimic the body’s natural properties. "While rabbit tests misclassified 10 of the 25 test chemicals, the company's EpiDermTM approach accurately recognized all irritating substances," according to the article "Cell Culture Beats Animal Tests for Irritancy Accuracy.” This and different investigations show that strategies, for example, EpiDerm or growing cells in vitro can yield results that are identical to creature testing, yet in addition more exact, and even cheaper.

Not only is animal testing ineffective and unreliable, but it is immoral as well. Treatment and cruelty in U.S. laboratories display ethically concerning worries of injustice. Many rabbits, for example, are subjected to the “Drazie Test,” which involves injecting concentrated doses of a test substance into an animal's eye (while its lids are clipped open) or applying a chemical to an area of the animal's skin that is shaved ("Animal testing is Unethical, Unreliable and Unnecessary"). According to Lee, Miri, et al., animals experience "ulceration, inflamed/bleeding skin, enlarged eyes, and blindness" when taking part in an experiment. Lab animals are culled from the wild or produced specifically for scientific research. They are forced to inhale toxic gasses, imprisoned in withdrawal devices, have holes bored in their heads, or

have their flesh burnt off (PETA). These types of confinements reveal how immoral animals are handled "behind the scenes." Furthermore, a substantial number of animals in American laboratories demonstrate the same torture behaviors as a person would (Akhtar, Aysha). In psychological distress, many animals howl, scream, break their backs in writhing pans, and wrap their arms around their bodies for solace, or rock ("Animal testing is Unethical, Unreliable and Unnecessary"). This demonstrates how animal ethics are ignored when testing is carried out on them. Carried out experiments are out in the name of technical development or pure curiosity at the price of an animal's ability to exist in peace, a right that animals have. Ultimately, this expresses just how immoral animal experiments are.

V. Conclusion (II)

Animal ethics is as important an issue as human welfare. Further efforts are needed to effectively implement the 3 Rs in laboratory animal use. Various alternatives to animal use have been proposed and need to be effectively implemented. This integration requires a variety of computer models, bioinformatics tools, in vitro cell cultures, enzyme screens and model organisms. Analyzing alternative protocol results using modern analytical techniques, data collection, and statistical methods ensures reliable results. These integrated approaches minimize animal involvement in scientific procedures.

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VI. References

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