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Animal toxicity testing: Innovations, Challenges and Future Directions

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Abstract

An essential first step in assessing the possible effects of chemicals and medications on human health is testing for animal toxicity. Animal testing has limitations and presents ethical questions despite its significance. The goal of this paper is to give a general overview of the background, procedures, and guidelines utilized in animal toxicity testing, together with an analysis of the difficulties and constraints involved. We also talk about the innovations and future paths that toxicity testing will take, such as the application of computational models and in vitro methods. This review's objective is to draw attention to the necessity of a well-rounded strategy for animal toxicity testing that considers both ethical obligations and scientific advancements.

Keywords: Toxicity, Animal models, Lethal Dose, In- vivo studies

Introduction

Toxicity testing is the process of determining potential risks. A test chemical may likely be generated and its effects shown as a drug's short and long-term toxic effects on animals. Most toxicity testing is done on experimental animals including rats, pigs, rabbits and dogs Arome and Chinedu (2013). Potential new medication must undergo safety and tolerability testing in animals before moving on to first-in-human (FIH) studies by (Prior et al 2018) as per global regulatory requirements.

Animal toxicity testing for drugs offers various benefits. These benefits include the potential for a genetic constitution that is clearly defined, the ability to be exposed for a controlled amount of time and the ability to thoroughly examine every tissue after necropsy. Pharmacology in humans and other species demonstrates how medication responses can differ significantly between species. According to certain reports, (Oslon et al 2000) showed that, improper pharmacological reactions account for 29% of drug withdrawals throughout development. The outline for significant study designs by Randhawa (2009) and Hendriksen (2005) includes repeated dosage acute toxicity tests, fixed-dose procedures, up and down procedures and investigation of potential side effects that could arise from the first exposure to a single dose of chemical (acute toxicity studies).

Toxicological classification of chemicals is now based on the LD50 value, which is defined as the statistically calculated dose that, when supplied in an acute toxicity test, is likely to cause

death in 50% of the treated animals in a particular period, Walum (1998). These animal-based toxicity studies might not produce consistent data due to the various endpoints in repeated tests, which could lead to a sizable number of false-positive and false negative results, statistical and biological errors as suggested by (Fischer et al 2020) Toxic indications of medicine are determined through toxicity testing using doses over its therapeutic range. A drug's toxic effects might range from minimal to severe as to hinder the compound's continued growth. Animals in toxicity testing allow researchers to examine a whole functional creature, this practice is likely to continue in further literature research.

The goal of this review is to draw attention to the necessity of toxicity testing evaluations of drugs to guarantee the welfare of both humans and animals.

Testing in animals

In order to safeguard both human health and the environment, regulatory studies involving animals have historically been necessary for evaluating the safety of novel chemicals and medications. Given their significance, the applicability of animal models for predicting human safety should be periodically assessed as scientific knowledge and technological advancements progress, (Chapman et al 2013). Since the primary goals of toxicity testing are to define safety and identify potential concerns for humans, regulatory toxicology must have different objectives from academic toxicology, Heywood (1987).

It is proposed that the concepts of "reduction, replacement, and refinement" serve as a unifying theme for the global scientific community and animal welfare organizations.Studies by Gauthier and Griffin (2005), have shown that public approval of using animals in research have generally indicated that 85% of people are in favour of using animals in research as long as there is strict monitoring over that use and that the animals' pain and suffering are kept to a minimum.

For regulatory purposes, as well as to confirm favorable in vitro test results, in vivo genetic toxicology assays quantify direct DNA damage or the occurrence of gene or chromosomal changes. They are also used to anticipate the mutagenic and carcinogenic potential of chemicals. These experiments are extensively employed and include a significant number of animals, with a discernible rise due to the EU's REACH chemicals law, (Pfuhler et al 2009).

Historical perspective & Evolution of animal testing

Since ancient times, animal studies by Santha (2020), have helped us learn more about illnesses and their treatments. Animal experiments are still a common tool in preclinical testing, toxicology, safety investigations, and drug development in the fields of medicine, biomedicine, and veterinary medicine.

The Past

Since the beginning of medicine, humans have used other vertebrate animal species—henceforth referred to as animals—as models of their anatomy and physiology by Franco, (2013).

In 1918, almost 150 years after Joseph Priestley documented the effects of gas on study animals, techniques for subjecting animals to chemicals were introduced. Methods became more refined as researchers like Weideman (1993), linked hazardous exposure to ailments like black lung in miners and scrotal skin cancer in kids who frequently swept soot off eighteenth-century chimneys.

In the early and mid-1950s, as pesticides and antibiotics became more prevalent in post-World War II American society, guidelines for standardized methods of toxicity testing started to emerge in the form of acute, subacute, and chronic toxicity studies in animals. In 1962, the Food, medicine, and Cosmetic Act underwent significant modifications following the thalidomide tragedy, which resulted in the deformity of 7,000 infants born in Europe, Canada, and Britain to mothers who took the medicine. The FDA's clearance of clinical trials, informed consent criteria, and the crucial necessity that newly approved pharmaceuticals prove both their safety and efficacy were all clarified by the recently passed Food, Drug, and Cosmetic Act.

In 1978, the National Toxicology Program was formed by the federal government, which centralized toxicity testing. NTP has evaluated over 450 compounds so far, and the Carcinogenic Potency Database (CPDB) now includes the results of those tests.

The Present

Advanced genetic and molecular biology methods, such as gene editing and transgenesis, are accessible for the experimental modification of mice's genomes. The artificial introduction of harmful microorganisms (fungi, viruses, mycoplasmas, and bacteria) into experimental animals also modifies the pathomechanism of infectious disorders. Exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a notable example of this, as it preferentially destroys dopaminergic neurons, allowing for the modelling of Parkinson's disease. An alternative

strategy to modelling and studying different illnesses would be to use surgical techniques (such as artery or vein ligations) to create pathological abnormalities (e.g, to investigate ischemic lesions, brain hypoxia, etc.). Viral, chemical, or physical inducement, graft transplantation, or genetic engineering are the methods used to create animal models of human malignancies.

Animal models in toxicity testing

Alternative approaches have been developed in an effort to decrease, replace, and improve the number of animals used in laboratory experiments, thereby lowering the costs associated with the purchase of animals used in research, as a result of growing interest and ongoing advancements within the scientific community , Araujo, (2014). Non-mammal vertebrates, such as fish, amphibians, reptiles, and birds that share some traits with mammals, are being researched in place of conventional animal models, Winn (2001).

Now, Stokes is examining the utility of the FETAX (frog embryo teratogenesis assay xenopus) system. The teratogenic potential of chemicals is evaluated by Sklarew, (1993) using fertilized frog eggs, which in 96 hours mature into free-swimming tadpoles

Zebrafish (Danio rerio) are currently the most commonly used small fish model species in biomedical laboratories worldwide, with killifish species like the Japanese Medaka (Oryzias latipes) being employed less frequently. (Bauer et al, 2021) used Zebrafish as disease models and comparison models in many different fundamental research fields, including immunology, cancer, bone studies, cardiovascular problems and immunology.

At the end of the 20th century, mice were used in drug research more often than rats. Numerous factors support the widespread use of mice in animal research: (a) their relatively cheap breeding costs; (b) their quick reproduction (20-day gestation); (c) their large number of offspring (8–12); (d) their early maturity (4–6 weeks); (e) their well-characterized genome (the entire genome sequence is known); and (f) the fact that 99% of mouse genes are homologous to human genes.

Genetically engineered mice, including knock-out, knock-in, and transgenic models, are quickly becoming recognized as valuable instruments for mechanistic studies and target biology validation. They also hold great promise as specialized models with significant toxicological implications. Knockout mice have been by (Bussiere et al 2009) to test treatment candidates for mutagenic and carcinogenic properties, evaluate medication selectivity, and look into toxicity causes.

Finding new drugs needs a significant financial commitment. Many protocols need to be followed in order to make a discovery. One can use in-silico model techniques in place of animal experimentation to lessen the workload. Basic biological concepts serve as the foundation for in silico models. New medication development is aided by computer software and specially created computer models. It anticipates potential drug binding sites and receptors, which lowers the quantity of animals employed. This is made possible by the use of computer-aided drug discovery (CADD) software, which forecasts a medication's likelihood to bind receptors as well as its likely pharmacological and therapeutic actions as suggested by (Ajmera and Shendge 2017).

Methodologies & protocols in toxicity assessment

Techniques used to assess Acute toxicity

Miller-Tainter approach

The usual technique for obtaining LD 50 is the Miller-Tainter approach. Plotting the dosage vs the probit value is done. The graph will be used to estimate the LD50. The experiment shows how to calculate the LD50 of neostigmine in the experimental animals and compare that value to the LD50 of neostigmine that is considered standard. Information on toxicology by Arya and Bist [21], have reported that LD50 oral Rat: 7,500 mg/kg IV LD50 Mice: 0.3 mg/kg SC LD50 Mice: 0.54 mg/kg IMD50 Mouse: 0.395 mg/kg IV LD50 Rat: 0.315 mg/kg SC LD50 Rat: 0.445 mg/kg Rat's IM LD50: 0.423 mg/kg.

Reed and Muench's arithmetical technique

This approach uses a cumulative analysis of values derived from the study's findings. It is often believed that administering a larger dosage of the test chemical to the animals would have resulted in their deaths. A list of all the deceased and living people is kept. Both the LD50 and the percentage of survival are computed.

The Arithmetic technique, or Karber's technique:

To get the LD50 value, the product's total was divided by the number of animals in a group, and the quotient was then deducted from the least lethal dosage.

LD50= LD100 − ∑ (a×b÷n)

whereby the median lethal dose (LD50) LD100 = Minimum dosage necessary to kill 100%A= Dose difference B= Mean mortality N = Population of the group.

Lorke's Method

The procedure that Lorke outlines consists of a preliminary assessment where nine animals are divided into three groups. The three animal groups get varying doses of the research chemical. Phases 1 and 2, the two steps of this procedure, are as follows:

Phase 1: Nine animals are needed for this phase. There are three groups of three creatures each out of the nine species. Different test drug dosages (10, 100, and 1000 mg/kg) are given to each group of animals. For a whole day, the animals are kept under watch to track their behaviour and determine whether any will die.

Phase 2: Three animals are used in this phase, and they are divided into three groups of one animal each. Higher dosages of the test substance—1600, 2900, and 5000 mg/kg—are given to the animals, and they are then monitored for 24 hours to look for signs of behaviour and death. The formula used to compute the LD50 is as follows: D0 = highest dosage that did not cause mortality, D100 = lowest dose that did cause mortality.

Alternative methods

Fixed Dosage Procedure (FDP):

The British Toxicology Society introduced this technique in 1992. It's a method that may assess the acute oral toxicity of a substance. This approach yields similar findings with fewer animals and less pain and suffering than the previous LD50 test, which was conducted in 1927. 1992 Under OECD Test Guideline 420, the Organization for Economic Co-operation and Development suggested this test as a substitute for the LD50 test. Instead of using death as the end point, this technique evaluates the LD50 by seeing clearly defined insignias of five different degrees of toxicity at one of a series of preset dosage levels.

Acute toxic class method (ATC):

The ATC technique is an alternative to the LD50 trial, when we desire to decline the number of experimental animals under test. The use of a considerably scarcer number of animals is needed for the classification of substances proposed by the German Federal Health Authority,

and it provides alternatives to the LD50 test for grading substances by their acute oral toxicity confirmed by animal try-outs and biometric determination. The advantage of this approach is that it employs toxicity symbols in a stepwise fashion to get the LD50 rather than using death as the end goal.

Procedure in an up-and-down manner:

Using this novel approach, the test substance is given to experimental animals one at a time while the animals' survivorship is monitored. The amount for the following experiment is increased if it is discovered that an animal can tolerate the tested concentration of the substance. On the other hand, the dosage is often reduced for the subsequent animal in case of an animal death. Before treating the following animal, each animal is usually observed for one to two days. Animals that are dose-tolerant are watched for delayed mortality for a total of ten to seven days.

Ethical considerations and regulatory frameworks

Designing animal studies should only happen after carefully weighing the possible effects on animal welfare against the growth of information about people or animals, as well as the health of the animals. Researchers have to treat animals with sentiment, take into account the best ways to care for and utilize them and make sure that any discomfort, anguish, or agony is avoided or minimized as much as possible. All animal research in this field must take into account the three "R" principles: Before beginning any technique involving the use of animals, it is necessary to explore replacing animal testing with other approaches that either complement or replace the use of animals, such as mathematical models, computer simulation, and in vitro biological systems. Improvement of initiatives and methods to reduce animal impact, which entails: (a) The animals selected for the scientific studies involved must belong to the correct species and be of a suitable quality, considering their unique biological characteristics such as genetic makeup, behaviour, microbiological, nutritional, and overall health state, (Naderi et al 2012)

Challenges and Limitations of Animal toxicity studies

Animal research comes at a tremendous cost, both monetarily and in terms of lost potentially useful medications for human use and delays in prescription licensing as per Van Norman (2019).

Determining which study results in animal toxicity studies are truly unfavourable versus non-adverse and which ones are pertinent for producing a no observable adverse effect level (NOAEL) to evaluate human risk presents a difficulty for all toxicologists. The existing toxicity testing system has many flaws like the high cost of toxicity testing, the difficult moral and ethical questions which are raised due to the injury to animals are some of the challenges, (Baldrick et al 2020).

The reproducibility of preclinical research is a problem that many researchers report. 4-6 Preclinical research has a significant economic impact on the world at large; estimates of the irreproducibility of data from this type of study range from 51% to 89%. In addition to these significant challenges, humane practices, animal welfare, and the ethics of animal experimentation are also vital considerations.

The Mission of the Committee for Control and Supervision of Experiments on Animals (CPCSEA) is responsible for measures of safety to animals and ensuring that animals are not subjected to unnecessary pain or suffering before, during, or after the performance of experiments on them." The Prevention of Cruelty to Animals Act (PCA Act-1960)1 in India was enacted to "prevent the infliction of unnecessary pain or suffering on animals", (Singh et al 2016)

Future directions and Innovations in toxicity testing

Toxicological evaluations of environmental substances by (Shula et al 2010), traditionally mostly depended on animal models from which potentially detrimental human events might be extrapolated. These models have been created to assess particular toxicological endpoints, including immunotoxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and toxicity to the mouth, skin, and eyes.

Utilizing in vitro techniques and a range of novel technologies, several significant new initiatives have recently started to create in vitro signatures and computational models indicative of in vivo response. Scientists should be able to find a range of *in-vitro* tests capable of detecting cellular pathway alterations that are predicted to cause or contribute to unfavourable health outcomes. These efforts also mark a welcome departure from conventional *in- vivo* high-dose hazard investigations. By switching from the current costly and time-consuming *in-vivo* testing with qualitative endpoints to *in-vitro* toxicity pathway assays on human cells or cell lines using robotic high-throughput screening with mechanistic quantitative

parameters, it envisions increased toxicity testing efficiency and decreased animal usage as reported by (Krewski et al 2010). With the general goals of improving public health, lowering environmental risks, and streamlining, improving, and encouraging innovation in the chemical sector, the European Union has implemented a comprehensive program known as Registration, Evaluation, Authorization, and Restriction of Chemical substances, or REACH, (Locke et al 2017)

The goal of alternate approaches or the traditional 3Rs has been to completely replace animal testing. The scientific understanding of the pathophysiology or an analysis of the data obtained, such as what was actually observed in the guideline tests that guided the classification (e.g., which organ toxicities are actually driving regulatory decisions) or is observed in intoxicated patients (human-relevant manifestations), can inform the selection of key events. Similar to their *in-vivo* counterparts, *in-vitro* experiments are not without limitations according to (Basketter et al 2004).

Conclusion: Balancing scientific advances & ethical responsibility

Determining the hazardous impact and characterizing the test material both depend heavily on toxicity testing. Human toxicity is comparable to that seen in animal research in terms of frequency and severity. Due to the advantages of using animals for toxicity testing in studying a complete functional organism, this practice is expected to persist for some time to come. When applying threshold exposures, maximum allowable exposures, and hazardous consequences to humans, care must be taken in their interpretation. The most effective approach for assessing the impact of environmental toxicants on humans is still through well-conducted epidemiological research.

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