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Alternative Method to Replace Animals Use in Toxicological Testing for Drug Development

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Abstract: Advances in cell culture have given researchers a viable alternative and/or a supplement to testing on live animals. *In vitro* investigations are specialized cell cultures which have been used to clarify the mechanism of toxic action of chemicals on a particular target organ. Cell culture is the technique by which prokaryotic or eukaryotic cells are grown under controlled conditions. In practice, the term "cell line" refers to the culture of cells derived from multicellular eukaryotes, in particular from animal cells. Cell systems used in toxicity studies include primary cells, genetically modified cells, immortalized cells, stem cells and cells in different stages of differentiation and transformation, sub-cultures of different types of cells, etc. Emphasis is placed on cellular models used to study chemistry, toxicity, specific toxicity endpoints at levels including molecular, and the extrapolation of data obtained from *in vitro* models in the context of *in vivo*. Thus, *in vitro* toxicology has the potential to replace the use of animals in toxicological evaluations to a very large extent.

Keywords: *In vitro*, *In vivo*, Toxicology, Sub-culture, CPCSEA

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Introduction

The use of non-animal test methods, including computer-based approaches and *in vitro* studies, provide important tools to improve our understanding of the hazardous effects of chemicals and to predict these effects in humans. The *in vitro* tools are primarily used for screening purposes and to generate comprehensive toxicological profiles, to obtain information derived from the mechanisms, and to provide important non-invasive tools for improving extrapolation from *in vitro* to *in vivo* in humans (Adler *et al.*, 2008, 2010, 2011; Aardema *et al.*, 2010; Doke and Dhawale, 2015; Taylor, 2019).

Over the past decades, an increasing number of test systems to assess the possible toxicological risks of chemical compounds have been developed. Many systems do not rely on the use of intact animals, but use biological systems such as lower level organisms, isolated organs, cell cultures, and sub-cellular systems. These *in vitro* systems have proven to be extremely useful for studying the molecular basis of the biological

activity of a chemical, including its mechanisms of toxic action. Other important developments have been made in the prediction of biological reactivity based on the physicochemical properties of compounds, such as structure, molecular size, reactive groups, etc. One application of this knowledge is the construction of structure-based activity relationships. The increasing possibilities of using cell and tissue cultures to measure these biomarkers of effect are now complemented by the potential use of information derived from micro-array analyses, genomics, transcriptomics and proteomics (Scholz *et al.*, 2013; Badyal and Desai, 2014; Cheluvappa *et al.*, 2017; Meigs *et al.*, 2018).

Advantages of in vitro assay using primary cell culture:

In vitro tests are primary cells used to routinely assess organ-specific toxic effects, for example, the use of primary culture cells to assess cytotoxicity. Human and animal organs from cultured primary cells have been developed and have long been used for the evaluation of drug toxicity (Santha, 2020). Primary culture cells are particularly important as experimental systems for human-specific drug properties. Applications of drug development in human cells include assessment of metabolic stability, profiling and identification of metabolites, drug-drug interaction potential, and cytotoxic potential (Van Norman, 2019). The use of intact human cells, due to their complete and undisturbed metabolic pathways and cofactors, provides developmental data relevant to humans *in vivo*. Incorporation of key *in vivo* factors with the *in vitro* cell culture may help to predict the in vivo drug interaction properties (Shapiro *et al.*, 2008).

Ethical recommendations involved in animal use in toxicological testing:

Bioscience research contributes to the quality of life by expanding knowledge of living organisms. This improvement in the quality of life stems in part, from progress towards improving human disease and disability, in part from advances in animal welfare and veterinary medicine, and in part from a steady increase in knowledge of human and animal life's abilities and potential (Shapiro *et al.*, 2008). Continued progress in many areas of biomedical research requires the use of live animals. Animals serve the humanity in different ways-- serve as pet, they are used as food etc., and they are also used as experimental tool. So we should take proper care of these animals and maintain them ((Goh *et al.*, 2015; Wang *et al.*, 2020).

History:

The earliest reference to animal experiments is found in the writings of the Greeks in the third and fourth centuries BC. Aristotle (394-322 BC) and Erasistrauss (304-258 BC) were among the first to perform animal experiments (Bayne *et al.*, 2015). Galen, a second-prone country doctor who dissects pigs and goats, is known as the "father of vivisection".

Ethical Recommendations:

Animal testing should cause animals as much suffering as possible and animal testing should only be done when necessary. The “three Rs” (reduction, replacement and refinement) are guiding principles to guide the use of animals in research in many countries.

Outbreed stock:

Random mating to maintain a relatively constant maximal genetic variation.

Inbreed strains:

Those which exhibit genetic variation as a result of brother x sister mating for at least 20 successive generation or the equivalent.

Congentic strains:

This term is given to inbreed strain into which a single mutant gene has been introduced by a series of back cross mating.

Housing, environment ventilation, temperature and humidity:

These are interacting factors that are controlled at

the level of facility and room, but should also be monitored within the cages. Evenness of ventilation in the micro-environment will depend on cage and rack arrangement and airflow patterns (Fig. 1). The temperature and humidity ranges from 20-25 C (68-77 F) and 50-55%, respectively (Bayne *et al.*, 2015).

Fig. 1: Breeding and Maintenance of Laboratory animals.

Laboratory animal – Rabbit:

General characteristics:

The Rabbits life expectancy in the laboratory or breeding colony will rarely exceed four or five years. Although under natural conditions they may particularly in case of males, live at least twice that long (Adler *et al.*, 2008, 2010, 2011; Aardema *et al.*, 2010; Doke and Dhawale, 2015; Meigs *et al.*, 2018; Taylor, 2019).

Handling:

When removing or picking up a rabbit from a cage, the loose skin on the shoulders can be grabbed with hands. Rabbits should never be wedged by the ears as they are easily injured, the ears are sensitive organs that play a role in regulating body temperature, as well as hearing in animals of this family.

Sampling and manipulation:

Marginal ear vein puncture can readily be used to obtain venous blood samples. The hair directly over the vein is plucked or shared and 70% alcohol is applied to clean the area and wet the surrounding hair, making visualization of the vein easy. Petroleum jelly is then applied to the site (Qadri and Newcomer, 2014; Bayne *et al.*, 2015; Retnam *et al.*, 2016; Qadri and Ramachandra, 2018). A small nick may be made with scalpel through the vein from which blood may be collected directly into the pipette or into a tube held below the cut. Care must be taken not to cut through the entire ear edge while making the nick in the vein (Fig. 2).

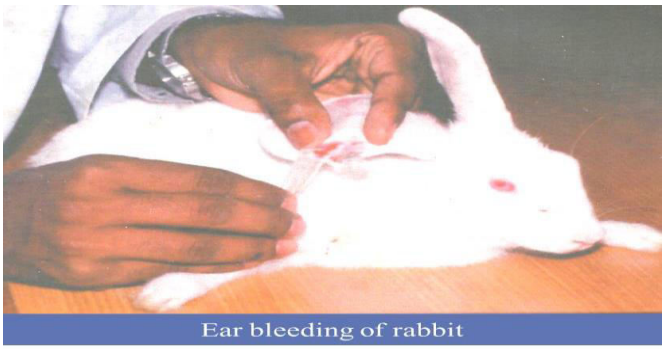

Fig. 2: Ear bleeding of Rabbit.

Governing bodies of laboratory animal maintenance:

CPCSEA's (Committee for the Purpose of Control and Supervision of Experiments on Animals) mission is to ensure that animals are not exposed to unnecessary pain or suffering. To this end, the Committee prepared the Rules for Animal Husbandry and Experiments (Control and Monitoring) from 1998, which were amended in 2001 and later in 2006 to regulate animal experiments.

In vitro toxicity assay as an approach to define human-specific xenobiotic toxicity:

Human-specific xenobiotic toxicity, be it due to drug metabolism or the sensitivity of the target cells, cannot be accurately assessed using in animal models. *In vivo* and *in vitro* toxicity testing using experimental systems with low, relevant human-specific properties is the only practical preclinical approach for obtaining human-specific information to accurately predict xenobiotic toxicity in humans (Bayne *et al.*, 2015; Retnam *et al.*, 2016; Wang *et al.*, 2020).

The use of dedifferentiated cell lines such as transformed or immortalized mouse or human fibroblasts may not make sense because a network of the important properties mentioned above is present. The use of primary cells from human organs as a monogenic culture only enables the evaluation of the effect of xenobiotics on a particular cell type which may or may not have significant human xenobiotic metabolic pathways (Wang *et al.*, 2020).

A cell system that is target cells and human cells has human metabolic capacity. This cell system has the following advantages:

Human xenobiotic metabolism:

Fresh isolates or cryo-preserved fresh isolates of human cells are known to contain most, if not all, of the *in vivo* xenobiotic metabolism capacity.

Human target cells:

Hepatocytes are the cells in the human liver that are damaged by hepatotoxicants, leading to liver failure. However, a commonly used cell line does not have these properties and therefore, would not represent a relevant *in vitro* model for the investigation of cytotoxicity.

In vitro assays contribute to use of lesser number of animals in toxicity testing:

Last but not the least *in vitro* system addresses the ethical and pedagogical issues of animal use in biomedical testing and research. Several in vitro methods have adequately replaced the classical in vivo toxicity testing. The use of cell systems to measure responses to xenobiotics has been widely investigated in the quest for alternative methods in toxicity testing and have proved to be accurate for some testing like in ecotoxicity, skin corrosivity test, ocular irritancy/corrosivity test etc. (Qadri and Newcomer, 2014; Qadri and Ramachandra, 2018).

Conclusion

In vitro toxicity testing is the scientific analysis of the effects of toxic chemicals on cultured micro-organisms or mammalian cells. *In vitro* testing methods are primarily used to identify hazardous chemicals and to ensure that they contain beneficial new ingredients, such as treatments, pesticides, live foods, and cosmetic development. There are no toxic properties at the initial stage. In addition to animal testing, there are many moral and economic benefits. These tests have previously been used for tubular poisoning, often establishing risk assessments and/or controls that ultimately save the animal's life.

Introduction models are useful for understanding the biological processes involved in toxic reactions that rely on visual inspection of animals. *In vitro* technology is a tool that toxicologists can use to design and select compounds at various stages of drug development, to perform special assessments, and to address any major or clinical problems that have arisen. The use of *in vitro* technology contributes to the toxicologist's commitment to the scientific quality and economics of the safety assessment process, as well as a three-point reduction, purification and replacement, and a fourth responsibility.

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