



## Some Alternative Methods to the Use of Laboratory Animals in Medical Research

### Tıbbi Araştırmalarda Laboratuvar Hayvanlarının Kullanımına Alternatif Bazı Yöntemler

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#### ABSTRACT

Experimental animals have long been used for research and educational purposes. However, in recent years, ethical debates regarding the rights of animals to life have also become important. It is advocated that animal experiments can be carried out for scientific goals that can contribute to human and animal welfare in the long term and in cases where there are no alternative methods. The principles of “replacement”, “reduction”, and “refinement”, known as the 3R rule, have been updated as 4R with the addition of the “responsibility” principle. These principles provide basic guidance for the ethical use of animals in scientific experiments. Techniques that can be alternatives to animal experiments in research and biological effect studies to test synthesized drug candidate compounds and various chemicals have been developed. In vitro cell culture techniques and bioimaging methods are very important alternatives to in vivo animal experiments. The micro-dose technique is another alternative method that reveals the effects of drug candidate compounds on volunteer individuals at very low doses without using animals. Software databases and in silico computer simulations are also gaining importance in research institutions as an alternative to animal experiments. In this review, the fundamentals of animal research ethics and emerging alternatives to reduce animal use in medical experiments were discussed.

**Keywords:** Experimental animals; ethics; alternative methods.

#### ÖZ

Deney hayvanları uzun süredir araştırma ve eğitim amaçlı olarak kullanılmaktadır. Ancak son yıllarda hayvanların yaşam haklarına ilişkin etik tartışmalar da önem kazanmaya başlamıştır. Uzun vadede insan ve hayvan refahına katkı sağlayabilecek bilimsel amaçlar doğrultusunda ve alternatif yöntemlerin bulunmadığı durumlarda hayvan deneylerinin yapılabileceği savunulmaktadır. 3R kuralı olarak bilinen replacement (değiştirme), reduction (azaltma) ve refinement (iyileştirme) ilkeleri, responsibility (sorumluluk) ilkesinin de eklenmesiyle 4R olarak güncellenmiştir. Bu ilkeler, hayvanların bilimsel deneylerde etik kullanımına ilişkin temel rehberlik sağlar. Sentezlenen ilaç adayı bileşiklerin ve çeşitli kimyasalların test edilmesine yönelik araştırma ve biyolojik etki çalışmalarında hayvan deneylerine alternatif olabilecek teknikler geliştirilmiştir. İn vitro hücre kültürü teknikleri ve biyogörüntüleme yöntemleri, in vivo hayvan deneylerine çok önemli alternatiflerdir. Mikro-doza tekniği ise ilaç adayı bileşiklerin gönüllü bireyler üzerindeki etkilerini hayvan kullanılmadan çok düşük dozlarda ortaya koyan bir diğer alternatif yöntemdir. Yazılım veritabanları ve in silico bilgisayar simülasyonları da araştırma kurumlarında hayvan deneylerine alternatif olarak önem kazanmaktadır. Bu derlemede, hayvan araştırma etiğinin temelleri ve tıbbi deneylerde hayvan kullanımını azaltmak için geliştirilen alternatif yöntemler tartışılmıştır.

**Anahtar kelimeler:** Deney hayvanları; etik; alternatif yöntemler.

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## INTRODUCTION

Laboratory animals are used in basic biological and medical research, in the development of diagnosis and treatment methods of diseases, in the reliability testing of drugs and chemicals, and for educational purposes in the fields of biology and medicine. However, in these areas where animals are used without their consent, animals are often exposed to interventions that may cause pain, distress, or permanent damage, and this causes serious ethical problems and debates. According to scientific society, it is advocated that in the presence of scientific goals that have a high probability of being achieved and that can contribute to human and animal welfare in the long term, experimental studies on animals can be carried out in cases where there is no alternative method (1,2).

Reports of scientific experiments on animals have existed for years, but their primary use has become more frequent in recent years. Animal welfare was an issue that was not taken into consideration for many years while these experiments were ongoing (3). In recent years, interest in ethical principles has increased in the scientific community. Within the scope of the legislation regarding animal experiments enacted within the framework of animal protection laws in developed countries, national and international scientific journals do not publish studies that are not approved by local ethics committees (4).

In vitro cell culture techniques and bioimaging methods are very important alternatives to in vivo animal experiments. In this review, the fundamentals of animal research ethics and emerging alternatives to reduce animal use in medical experiments were discussed.

## THE 4R PRINCIPLES

The basic principles of scientific ethics originated from the Universities Federation for Animal Welfare (UFAW) project and were first put forward by Russell and Burch in 1959; it is briefly defined as 3R, due to the initials of the terms, “reduction”, “replacement”, and “refinement”. These defined basic principles explain the qualities that can guide researchers in animal experiments (5). The reduction principle, in its original definition in Russell and Burch’s study, is expressed as “reducing the number of animals used to obtain a certain amount and precision of information” (6). In terms of animal ethics, reduction means obtaining reliable data by using the least number of animals possible in experiments. The term refinement refers to the comfort of experimental animals in their living environments during the period between birth and death and to ensure that they are exposed to minimal pain and suffering in the procedures applied during experiments. In its original definition, this principle is defined as “reducing the incidence of violence in inhumane procedures applied to animals that have to be used” (6). For each animal species, the optimal living conditions must be defined throughout the experimental period. Replacement refers to the use of alternative materials instead of experimental animals, if possible, in scientific research. This principle is defined as “the use of insensitive materials that can replace methods using conscious living vertebrates” (6). In 1985, The International Foundation for Ethical Research (IFER) created the principle of responsibility and developed the principles in the form of 4Rs. This principle envisages the use of animal models for scientific courses and studies,

increasing the society's level of awareness and awareness about the use of animals in scientific research, education, and testing of products, and increasing personal responsibility by developing new methods and realizing new technologies (7). In accordance with this principle, individuals and institutions that will conduct research with experimental animals must comply with legal regulations and ethical rules.

## ALTERNATIVE METHODS

Some techniques have been developed that can be alternatives to animal experiments in research and biological effect studies where it is necessary to test synthesized drug candidate compounds and various chemicals. These alternative methods include cell and tissue cultures, the use of microorganisms, the use of plant materials, and non-invasive models such as human clinical studies. In addition, physicochemical techniques, various software and computer simulations, mathematical models, and nanotechnological methods can also be used. These techniques are cost-effective compared to animal use and can effectively replace animal models. The fact that these alternatives can address ethical concerns is an important advantage (2).

### In-vitro testing with Animal Cell Cultures

In vitro tests allow the effects of drug candidate compounds and chemicals on the organism to be examined in laboratory conditions as an alternative to animals by monitoring their effects in the in vivo environment. In this method, cells are isolated from the target organ and grown in special flasks to create a growth environment for a specific tissue type (8). It is advantageous that this technique is repeatable and more economical. It is also easy to maintain and follow. These tests also allow preliminary screening as to whether further experiments will be required. Thus, the efficacy and toxicity of a drug candidate compound can be determined by these in vitro tests (9,10). Experiments based on cell culture may reduce the use of animals, but serum obtained from the animal is required for the continuity of the cultures of these cells. Cell culture models are performed in a growth medium containing fetal calf serum. Fetuses removed from pregnant cows are used to obtain serum from fetal blood. Fetal calf serum is used in cell culture media as it is a rich natural medium and significantly increases cell growth. Alternatively, there are advanced studies on the ability to culture many cell lines in a chemically synthesized medium without the use of animal products. Additionally, chemically synthesized media may be more effective than animal serum media because animal serum varies depending on the gender and genetic makeup of the animals. However, at least the cell culture method is an important alternative in terms of reducing animal use, and this point makes this technique important (11,12).

### 3D Cell-Culture Models and Organs-On-Chips

As a result of the advancement of known classical cell culture techniques, tissue models can be created with 3D cell culture technology. 3D models of multiple organ systems and skin and muscle are produced in microfluidic channels (13). In 3D cell culture models, cells are grown as 3D spheroids or aggregates on a matrix scaffold or without a scaffold. 3D cell culture modeling conditions can

be modified through the addition of proteins and other factors present in a tumor microenvironment or specific tissues. The cellular environment can be accurately mimicked in vivo through extracellular matrix components such as proteins and glycosaminoglycans, which are found in the structure of these matrices and play a role in communication and cell-to-cell contact through intercellular signaling pathways. It is thought that this method will accurately reflect drug screening experiments and reduce the use of laboratory animals for research purposes (14).

#### **In Silico Methods**

Computer-based tests use various advanced software and mathematical equations to create imitations of the real functioning of organ functions and metabolic processes in the human body. In this way, drugs can be designed and verified by simulation on special computer models and software programs. More importantly, these simulation models are used to predict the toxicity level of an experimental drug candidate chemical without harming any animals (15). In simulation studies, any biological effect is shown in an equational form, providing more accurate and predictable results compared to data obtained from experimental animals. The most successful example in this regard is the design of protease inhibitors for HIV patients. In these studies, for a more precise verification, protease inhibitors were designed on computers, and the necessary tests were carried out in a simulation environment. In this way, successful results were obtained without using animal models in drug efficacy trials (16). Various computer models have been created to examine atherosclerotic plaque formation, which is an important risk in the cardiovascular system (17).

To be mentioned again, the most important point in bioinformatics simulations is that they significantly reduce the use of animals in biological effect assessment studies. In addition, computer models developed specifically for the field of anatomy may make it possible to reduce or completely eliminate the need for animal dissection for educational purposes (18).

#### **Micro-Dosing**

Micro-dosing testing is an effective method developed to test the effectiveness level and usability of candidate drugs in drug development studies. In this method, metabolism data of the human body is obtained in the analysis of the drug that is in the testing phase. Studies have shown that the high failure rate observed in phase I clinical trials, the long duration of the trials, and their high cost have increased the importance of this technique. Micro-dosing can screen targeted drugs more quickly, and economically. Additionally, the accuracy of predicting metabolic effects is also quite high. In this method, the effectiveness at the metabolic level is investigated by applying the compound to be tested at a dose that is high enough to affect the cellular level but relatively low. However, full dosing in animals is still needed to obtain definitive data for drug use in humans (19).

#### **Imaging Methods**

With technological developments in the field of radiology, it has become more possible to reveal the functions of body parts, the structures of internal organs, and possible disease diagnoses compared to previous years. It is also possible to examine the biological effects of drugs and the changes

that occur with new radiological equipment (20). Since it is possible to image animals multiple times in these methods, animal welfare is protected by reducing the number of animals used in research. Non-invasive imaging technologies such as X-rays or ultrasound allow observation of the skeletal system and organ functions of living animals. Thus, disease mechanisms or treatment methods can be studied in real time without harming the animal. Imaging is smaller than devices used for humans and is widely used for research purposes with equipment adapted to animals. During the imaging procedure, anesthetic drugs are used, unlike humans, to ensure that the animal is immobilized so that they do not panic and suffer. (21). Among these methods, computed tomography is functional in investigating bone structure, magnetic resonance imaging is functional in examining internal organs and soft tissues, and ultrasound is functional in monitoring organ movements and heartbeat (21,22).

#### **CONCLUSION**

The use of animals in education and research still causes many disagreements due to ethical concerns. However, it is clear that the development of vitally important drugs and vaccines in medicine will continue to develop thanks to these experiments. Despite this, the benefit level of the studies to be carried out should still be taken into account because animals have the right to life and this should always be taken into consideration.

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