

Mini Review

Laboratory animal research of metabolic bone diseases in Greece: Laws and ethics

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Abstract

In the present article the main legal and ethical considerations that form the framework within which a research laboratory in Greece conducts animal testing for research into metabolic bone diseases are discussed. Preclinical testing procedures with animal models are specifically defined by EU law - and in the case of Greece - by its transposition into Greek legislation by the relevant Presidential Decree. This legislation describes the principle of the three Rs (Replacement, Reduction and Refinement) and turns ethical considerations into firm legal requirements, necessary for both the protection of animal welfare, as well as the safeguarding of the validity of clinical protocols that research - among others - some of the most widespread endocrine diseases of our time, i.e. metabolic bone diseases.

Keywords: Animal testing, Animal welfare, European legislation, Metabolic bone diseases, Three Rs

Introduction

"Metabolic bone diseases" (MBDs) is an umbrella term for a variety of disorders that are characterized by anomalies in calcium metabolism and/or bone cell function¹. The most common metabolic bone diseases are osteoporosis, Paget's Disease and osteogenesis imperfecta. Most metabolic bone diseases are asymptomatic "silent diseases" and only present clinical manifestations at a late stage in the patient's life².

According to the International Osteoporosis Foundation, metabolic bone diseases affect more than 200 million people worldwide, and pose an immense personal and economic toll. In Europe, it has been estimated that the disability due to osteoporosis alone is graver than that resulting by most cancers. In Greece, the cost of new and prior fractures has been estimated to approximately €680 million each year, and is expected to increase by 20% and reach €814 million within the next five years³.

The serious effects of MBDs on a personal and state level, in combination with the prevalence of the diseases due to the higher life expectancy in the developed world, call for continued research into their pathogenesis, prevention and treatment options⁴.

Laboratory animals are commonly used in preclinical prevention and treatment research into MBDs. The purpose of preclinical tests with animals is the establishment of the safety and efficacy of new methods or drugs before they are used on humans. Various factors such as toxicity,

reproduction issues, fertility, mutations, cancers etc. are tested in different protocols. Small laboratory animals such as rats, mice and rabbits are the most common animal species for these trials due to their short life span and reproductive cycle length⁵.

During the last three decades, the increase of public awareness in animal testing, and the interest shown by the scientific community on the issue of the health and wellbeing of laboratory animals, have led to the establishment of specific legal, and ethical considerations that clearly define the use of laboratory animals, in order to safeguard both the wellbeing of the animals as well as the quality of the research results⁶.

In the following sections we will examine the focal points of the European and Greek legislation on animal testing that preclinical trials have to abide by, as well as the main points of the ethical validation of the proposed projects.

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Method

For the purposes of this article, an extensive search was carried out in electronic libraries, databases and official journals (PubMed, Science Direct, Cochrane, Medline, Google Scholar, EUR-Lex, Official Journal of the EU, Greek Government Gazette) and scientific books with the use of the key words: *European legislation, animal testing, three Rs, animal welfare, metabolic bone diseases*, in English and in Greek, in various combinations. From the 157 sources retrieved in our search, 24 met our inclusion criteria and were used in the present review, either in English or in translation from Greek into English. Inclusion criteria were the specific European and Greek legislation pertaining to the use of laboratory animals for scientific and other purposes, review articles, bioethics review articles and guidelines, as well as metabolic bone diseases research review articles. Exclusion criteria were articles written before 2002, clinical trials, articles on rare MBDs, research articles in diseases other than MBDs, reviews and legislation on specific industries (e.g. chemical industry), articles on proposed improvements of legislation, animal welfare legislation other than European and Greek, and articles on developing countries.

Results

Legal framework in Europe

The first effort to establish a harmonized legal framework in Europe for the use of lab animals for research and other purposes within the field of science was made in 1986 with the then EEC Council Directive 86/609/EEC of 24 November 1986⁷, and the signing of the corresponding European Convention of the Council of Europe, ETS 123⁸. This first harmonized legislation aimed to set procedures for the registration of the facilities that housed, supplied and used lab animals, for the licensing of research protocols when animals were used and for the proper training of the person(s) involved with lab animals.

In 2010, after rapid developments in lab animal science and numerous consultations with scientific and social entities, the European Union issued the Revised Directive 2010/63/EU for the protection of Laboratory Animals (9). This Directive focuses on the principle of the Three Rs, i.e. to Replace, Reduce and Refine the use of animals used for scientific purposes, as they have been described in the - now classic - 1959 book "Principles of Humane Experimental Techniques" by W.M.S. Russell and R.L. Burch¹⁰. The Directive presents a widened scope to include all animals used for basic research, higher education and training, defines minimum standards for the housing and care of laboratory animals and regulates how animals are used via systematic project evaluations that include - among others - the assessment of pain, suffering, distress and lasting harm caused to the animals. It furthermore sets firm rules for regular risk assessment inspections and promotes transparency with measures

such as non-technical summaries and retrospective assessments. It promotes the way alternative methods of research are developed, validated and implemented by establishing a Union Reference laboratory that validates alternative methods and is supported by Member State reference laboratories⁹.

Legal framework in Greece

The EU Directive 2010/63/EU was transposed into the Greek legislation by the Presidential Decree 56/2013¹¹. According to the Decree, every research protocol that includes the use of lab animals has to acquire a license to conduct experiments. In order to obtain the license to experiment, the Principal Investigator has to file an application to the appropriate veterinary district authority with the following documents attached:

- The suggested research protocol with a detailed project plan
- The procedures and species of animals that are to be used, their origins and the facilities in which the trial will be conducted
- A non-technical summary of the protocol
- The positive recommendation of the Research Protocol Evaluation Committee
- A personal declaration signed by the head researcher, stating that he/she takes full responsibility for the proper execution of the protocol

The formation of Research Protocol Evaluation Committee is mandatory for every facility and it meets on the premises. Every Research Protocol Evaluation Committee comprises of:

- A biomedical research scientist (President) and his substitute
- The designated Veterinarian of the facilities and his substitute
- A biostatistician and his substitute
- A representative of the relevant veterinary district authority

The Presidential Decree describes all the necessary elements for the proper description of a research protocol, such as the methods of euthanising the laboratory animals, the minimum requirements with regard to education and training of the personnel involved with the protocol, the severity classification of the procedures and others.

In addition to the Research Protocol Evaluation Committee, every research laboratory has an Animal Welfare Body that consists of:

- The person responsible for the animals' care and welfare
- The Designated Veterinarian
- A scientist with a related research background.

In addition to the above committees that are in place in every research laboratory that uses lab animals in their studies, the laboratory itself must be approved and licensed, according to the provisions of Article 19, Presidential Decree 56/2013¹¹. The establishment license is held by the

laboratory director who is responsible for compliance to the legal framework.

Ethical validation of proposed projects

In the past, researchers justified the necessity of the research protocol based on the scientific value of its results and the benefit for humans. Nowadays, ethics play a major role in the use of animals for experimentation¹². The researchers have to be fully aware of the ethical scope of using animals for experimentation and also be in a position to validate the use in every phase of their proposed protocol, making certain that it abides by all the relevant laws that govern the use of animals for experimental use. All the people involved in the trial have to cooperate and continuously evaluate the progress of the protocol, making improvements, where needed. More specifically, the ethical validation of a research proposal must include the following:

Clear definition of the goal of the research protocol and the probability of success

The main goal of the research team is to set the goals and to validate the necessity of experimentation, the probability of success and the expected benefit the specific protocol will present for humans, animals or the environment. The avoidance of the repetition of research protocols is of paramount importance and is inextricably linked to the level of scientific knowledge of the research team on the scientific area it proposes to work on¹³.

The validation of the necessity to use animals

The researchers involved in the project have to prove that they have researched the available bibliography and have concluded that the project cannot be carried out with an alternative research method that does not necessitate the use of laboratory animals. A cost/benefit analysis has to be carried out, whereby “cost” is defined as the expected pain, distress and death of the lab animals and by “benefit” the expected benefit for humans, animals or the environment. The benefits should always outweigh the cost^{12,14}.

The implementation of the Principle of the Three Rs

The implementation of the principle of the Three Rs (Replacement, Reduction and Refinement), as described by Russell and Burch¹⁰ forms the crux of the validation of the research project.

• *Replacement*

The concept of replacement refers to all the approaches, methodologies and courses that do not include the use of animals and can be attained by means, such as *in vitro* methods that use tissues, cells or part of them, biochemical “*in chimico*” approaches, *in silico* models, technologies such as metabonomics, transcriptonomics etc. or approaches such as the “read-across” that does not involve testing¹⁵⁻¹⁸.

• *Reduction*

Reduction is defined as any course of action that will result in the use of fewer animals for the achievement of the same goal, i.e. the attainment of maximum information per

animal, the reduction of the number of animals used in the procedure and the limitation or avoidance of future use of additional animals for the same project. The reduction of the number of animals in any given project can be achieved by the performance of several procedures on the same animal when it does not endanger the scientific purpose or result in poor animal welfare^{16,18,19}.

• *Refinement*

Refinement refers to changes in the procedures or husbandry and care practices of animals throughout their lifetime in order to achieve minimum pain, suffering and distress, ensuring the animals’ well-being and limiting the physiological changes that might affect the project’s results due to distress. Additionally, refinement can be achieved by changing the animal species used if a less sentient species can produce similar results, as well as choosing the appropriate anesthetic or analgesic method. Special justification is required for the cases of genetically modified animals which will likely have serious health problems, need increased monitoring and additional welfare measures to be taken (lab animals used for the research into arthritis, diabetes, etc.)^{20,21}.

The design of a pilot study

The aim of the pilot study is the collection of information for the implementation of the research proposal, its methodology and the definition of possible problems that may arise during the main study. Conclusions drawn from the pilot study can result in improvements made in the protocol regarding the number of animals used, the severity of the damage incurred, the necessity for additional drugs, the appropriate sample withdrawal and storage, and the successful collaboration with the other research partners. The pilot studies should always be designed within the framework of the relevant legislation in force^{9,11,21}.

Systematic review

The use of systematic reviews can limit the number of animals by pinpointing studies that have already been carried out, as well as validate the effectiveness and the validity of new experimental methods that make no use of laboratory animals²².

The capacity to reach the goal set in the protocol within the premises of the research facility with the available personnel

The research team has to provide validation that its facilities are appropriate, the equipment is sufficient and the personnel has the expertise necessary for the implementation of the proposed study. Furthermore, the facility can guarantee the necessary standards relating to animal accommodation, care, use and husbandry^{23,24}.

Discussion

The prevalence of MBDs, the long-term complications, their mortality rates and the personal and state costs, create the need to fully understand them and call for the development and testing of preventive and therapeutic methods and drugs

that are studied with the use of laboratory animals in specific MBD-targeted research laboratories.

The use of laboratory animals for experimental purposes, when deemed unavoidable, after through research of available alternative methods and appropriate justification of their inevitable use, should always undergo rigorous ethical validation within the relevant legal framework. The ethical justification of animal experimentation is a dynamic process that should be implemented with responsibility and diligence throughout the duration of the research study by all the parties involved, i.e. the researchers, the animal carers, the designated Veterinarian and the authorities, in order to safeguard the well-being of the animals and the validity of the research results.

The ethical validation of research studies performed with animal models reflects the genuine interest and the responsibility of the research team, and builds a relationship of mutual respect and trust between the researcher and the public. Furthermore, it paves the ground for the proper further education and training of new scientists within a framework of respect towards the well-being of animals and proper work ethics. Adherence to the legal framework requirements, in combination with the conscientious monitoring of the animals' welfare throughout the research projects on MBD, can result in significant scientific findings, beneficial to human patients.

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