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Review Article

A review on humane endpoints in animal experimentation for biomedical research

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ABSTRACT

Introduction: The use of animals in experiments and their role in the development of medical sciences are undeniable. Humane endpoints terminate pain and distress in laboratory animals, which are experimented in painful procedures and an involuntary manner. This study was going to review studies published in this area to assist researchers in developing their approach.

Methods: Articles used in this review study were obtained from relevant databases including Pubmed, Scopus, Science Direct, OVID, SID, Magiran and Google scholar.

Results: "Humane endpoints" or killing the animal humanely means the point at which an experimental animal's pain and/or distress is terminated. This pain and distress are not necessarily accompanied by clinical symptoms and it can also be recognized by biochemical, physiological and molecular biomarkers testing.

Conclusion: Regarding the extensive use of laboratory animals, the aim is not only to take care of animals but also to develop knowledge and prevent unintentional animal suffering and death. Increasing awareness of ethical issues regarding research animal use needs scientific information and designing experiments, which are terminated immediately after achieving main goals. Otherwise, it threatens the life of animal and leads to the animal suffering.

Keywords:
Laboratory animals
Humane endpoints
Animal welfare
Biomedical research

Introduction

Research on live organisms is necessarily carried out to understand complex diseases and to find new and more effective methods for the diagnosis and treatment of diseases. In spite of being the first step toward understanding diseases, in vivo experiments are the main links between in vitro studies and clinical trials in humans (Faustino-Rocha et al., 2019). In medical research, the use of laboratory animals is common and in these researches, procedures such as injection of drugs, toxins and pathogenic microorganisms are used. These experiments subject the animals to a challenging condition between suffering and death (Araujo and Paixão, 2019). All individuals, who use animals to achieve scientific

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and experimental goals, have ethical and legal responsibility to minimize animal pain and/or distress. Although the use of animal in experiments is an important case, animal care should be taken in laboratories. The humane endpoints (HEP) is the only appropriate way to observe ethical principles in research if the aim of study is valuable and achievable and there is not alternative method. HEP can be defined as the earliest indicator of experimental pain and distress in the animal and can be implemented based on the ethical and scientific guidelines to restrict or terminate pain and suffering through actions such as killing the animal humanely (Howard et al., 2016). Moreover, HEP is going to use animals for several times (Dunlap, 2015). If the aim is to determine the positive effect of a drug or vaccine on an infection, animal death should not occur as an endpoint of research, because information will be lost and the death will be the only thing remain (Dunlap, 2015; Wright and Phillpotts, 1998). In designing experiment, the point at which the animal is killed humanely should be defined. Canadian Council on Animal Care (CCAC) issued guidelines on choosing endpoints in experiments by which the pain and suffering are minimized regarding the aims of the research protocol. In addition to general guidelines, CCAC also issued guidelines for in vivo experimental models of cancer, based on the macroscopic appearance, dimension and weight of tumors (Guidelines Committee U of PIAC and U, 2016).

The number of animals in the implementation of a research project, the time assigned to each experiment to achieve definite results and the animal suffering should be minimal (Rezende et al., 2008). These principles and guidelines led to the establishment of the 3R campaign. Currently, the term 3R indicating three words including «replace», «reduce» and «refine» is a part of animal research regulations all over the world and has been used in improving animal welfare in research (Russell et al., 1959, Changizi-Ashtiyani, 2013).

Regarding the importance of HEP application in animal experimentations, the present study was going to review humane endpoints in animal experimentation for biomedical research and to discuss preventing pain and suffering in the animal without obstacle in discovering new therapy and producing knowledge.

Materials and methods

Articles used in this review study on humane endpoints in animal experimentation were obtained from relevant databases including Pubmed, Scopus, Science Direct, OVID, SID, Magiran and Google scholar. The keywords used in this study were «principles of bioethics», «animal rights», «humane endpoint» and «laboratory animal care». This study included all articles related to animal experimentations and ethical principles and excluded imperfect studies.

Results and discussion

Historical background of the use of animals in biomedical research and the guidelines governing it

The use of laboratory animals in medical research has had high importance and value during history (Cornett et al., 2019). Aristotle (384-322 BC) and Erasistratus (304-258 BC), ancient Greek physicians, Avenzoar (1094–1162) (Hajar, 2011), a twelfth-century physician, Claudius Galenus, a famous Greek philosopher, Rhazes (854-925 AD), and Andreas Vesalius, the founder of modern human anatomy (1514-1564) had many discoveries in medicine by using animals in their experiments. The use of animals in laboratories increased after publishing the theory of biological similarities between humans and animals in the 18th century by Charles Darwin. Ethical issues in animal experimentation have been discussed since the 17th century and the use of animals in biomedical research has been severely criticized by animal rights groups in recent years (Changizi-Ashtiyani and Cyrus, 2010). Critics claimed that although the use of animals in experiments benefits human societies, it is not a good reason to harm these animals. Many others also believed that animals are very different from human and consequently the result of these experiments are not valid for human. On the other hand, individuals who agree with these experiments believed that these experiments are necessary to develop medical and biological sciences. Claude Bernard believed that «results of experiments on animals with the aim of toxicology and human health have been completely approved and its effect on animals is similar to humans». Bernard considered animal experiment as a part of the scientific method. The importance of using animals in pharmacology has also increased in 20th century (Hajar, 2011).

Regarding the validation of issues involving cruelty to animals and the ethical behavior with them, some laws (guidelines) were enacted for the use in animal experimentation, the first of which was passed in the UK in

1976 (Ashtiyani et al., 2013). Afterward, these guidelines were also enacted in many European countries to observe ethics in animal experimentations, according to which only researchers, who had certificates of animal experimentation, could perform animal testing with some limitations (Van Zutphen et al., 2001). In the 18th Meeting of the Eastern Mediterranean Advisory Committee for Health Research (EEM/ACHR) and the National Research Ethics Committee, the National Ethical Guidelines in clinical research was enacted and implemented (Ashtiyani and Sirous, 2010).

Declaration of Helsinki, Article 12, implies that animal experiment is prerequisite for clinical trials in human with the emphasis on observing animal rights (Fox, 2015). Eventually, all these affairs led to the 3R campaign involving the replacement of animals with non-living organisms, the reduction in the number of animals used and the refinement of methods used in the animal experiment. Regarding the type of animal experimentation, deciding about the state of the animal is very important. In order to predict the imminent death in experimental animal models, objective data-based approaches are able to facilitate the implementation of timely euthanasia and consequently reduce animal pain and distress. According to the Animal Welfare Act and Institutional Animal Care and Use Committees (IACUCs), killing the animal is necessary to minimize their suffering. In fact, HEP or killing the animal humanely was directly obtained from regulations. A state at which experimental animals cannot escape from or adapt to the external or internal stressors and leads to negative effects on animal life is considered the endpoint (Mobasher et al., 2009).

Principles of humane endpoints

HEP was firstly enacted in the European Union in January 2013 and EU member countries have to implement the guideline (EU/63/2010) and to protect experimental animals (Sharon-Schneidleder et al., 2016). «Distress» is mostly described as the state at which an animal experiences stress more than that in the normal life condition. The International Association for the Study of Pain defined «the pain» as an unpleasant sensory experience along with actual or potential tissue damage. Based on this definition, pain is always subjective (Gauvin et al., 2018).

HEP is carried out to decrease pain and distress or to provide a normal condition for the animal (Directive, 2010). This process is not necessarily accompanied by clinical symptoms and it can also be recognized by biochemical, physiological and molecular biomarkers testing (Council National Research, 2000). Some biochemical researches such as cancers, toxicity, vaccination and infectious diseases are accompanied by high stress and pain (Directive, 2010). According to guidelines for animal care and use, the minimum level of pain in an animal experiment is equal to inserting the tip of needle into the animal body. HEP as the earliest indicator in the animal experiment is going to observe ethics in the experiment of animals and to control their pain (Council National Research, 2000).

This process should be carried out in a scientific and ethical manner. In order to relieve pain, two methods are used including the use of analgesics and improvement of the condition after recognizing the cause of pain and distress. In order to prevent the loss of animals during the experiment, the selection of an appropriate animal in terms of age, species and genus is usual before the experiment (Council National Research, 2000; Council National Research, 2010).

Guidelines for humane endpoints involve providing a precise definition and important point in the regulation of HEP, identifying supervisors of experiment and the number of animal experiments based on its pain and health (excluding the animal if the pain and distress are high), evaluating the availability of animal species and designing experiments based on the once use of animal before its removal. Moreover, the following evaluations should also be considered in animal: paralysis and paresis (such as evaluating the body weight), disability in respiratory action, agonal breathing, neoplasia and infection. Evaluation of the body condition, weight loss, dehydration, disability and dyspnea in the experimental animal should be evaluated in acute studies. Pain, redness, swelling, secretion in the surgical site and wound healing are necessary to be evaluated in surgeries. Moreover, body weight gain, hypothermia and paralysis should also be considered in studies involving infection and shock (Council National Research, 2010; Directive, 2010).

The signs of pain or distress should be recorded in the implementation of HEP and the selection should be carried out based on the importance of the signs and the validity of the experiment. Moreover, it should provide

easy assay, the re-use of animal, the correct prediction of disease duration as well as decreased pain and distress. The method used in the therapy and autopsy is very important in HEP and a few animals should be tested if there is not enough data to prove the progression of the disease or its factors. All individuals participating in the experiment should be aware of physiological principles, animal normal behavior and its expected deviations before the experiment. Moreover, researchers are assured to receive counseling during unpredicted accidents or death for the animal. During the experiment, the accurate implementation of HEP should be proved (Council National Research, 2010).

The scientific argument for the death

The painful killing of an animal is not ethical and a program should be designed to monitor animal life. Before the implementation of HEP, some requirements should be provided including the consideration of replacements and the determination of best time for the use of these replacements, the number of animals and easy exclusion of the dying animals. Animals, which may experience death, should constantly be monitored in terms of skin lesions, inflammation around the eye and nose, breathing abnormalities, reduced water/food intake, violence against oneself and head tilt. The frequency of evaluations should be increased with the frequency of symptoms. Researchers should be aware of these symptoms immediately, animals which are dangerous for other animals should be transferred to other cages and dead animals be excluded. In addition, evaluations should be recorded (Council National Research, 2010).

Criteria indicating the humane endpoints are as the following (Berkeley, 2018; Council National Research 2000; Directive, 2010):

1. Evaluation of five factors including weight, physical appearance, behavior, response to external factors and appearance symptoms.
2. Clinical evaluation including changes in the appearance of skin, eye, nose, breathing and urine.
3. Depending on the severity and duration, clinical symptoms can include diarrhea, progressive dermatitis, thick hair, curling, chronic anemia, cough, dyspnea, nasal discharge, neurological symptoms, hemorrhage, spontaneous trauma, difficulty swallowing and hypo/hyperthermia
4. Before and after each change in these conditions, a veterinarian, who was instructed for identifying clinical symptoms, should carry out the animal monitoring according to the program, even on holidays.
5. Worsening the physical condition of the animal.
6. The significant weight loss in animal (15 to 20% during a few days).
7. Paralysis.
8. Large tumor and its metastasis.
9. Dyspnea.
10. Dehydration in the animal body (losing skin plasticity).
11. Necrosis or infection in the wound.

Ethics in the implementation of humane endpoints

Nowadays, researchers have shown that animal suffering leads to the extensive change of biomarkers in the animal body, affecting data obtained from the experiment (Gauvin et al., 2018). Since the results of research are evaluated based on the decreased suffering in the animal, new methods including pre-clinical and non-clinical procedures were introduced for HEP. In pre-clinical procedures, the symptoms are diagnosed before the onset of pain. For instance, the animal can be excluded by the diagnosis of tumor cells (by labeling pathogen or cancer cell and measuring its growth level or dispersion) or infection. In non-clinical procedures, a new method is replaced with the old method in which animal is sickened. For instance, the effectiveness of a vaccine can be evaluated by measuring antibodies instead of the injection of a pathogen (Russell et al., 1959).

According to the guidelines regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (1960), experiments should be designed and implemented based on the improvement of methods and the decrease of pain, suffering and the number of animals (Morton, 1999). The improvement of methods also minimizes the pain and suffering of the animal and improves its life quality (Ahmadi-Noorbakhsh, 2018; Council National Research, 2010; Changizi-Ashtiyani, 2016)

In some studies, painful protocols can be reduced instead of reducing the number of animals tested. Sometimes invasive studies can lead to valuable scientific goals; however, the use of large numbers of animals is associated with an inappropriate increase in the cost of optimal animal care. (Singh et al., 2019) Sufferings

have different qualities and range from mild to severe with more than one sign. Based on the aim of a study, some points are obvious at which suffering is prevalent in the life of the animal and it is not possible to suppress suffering by the use of common procedures. At these points, the animal is dominated by fear and it is not possible to escape from depression. Such measures can prevent pain and suffering in most cases (Singh et al., 2019; Stokes, 2015). Thus, old thought implying that only sever endpoints are acceptable is definable no longer.

Regarding the disability of animals to express their pains and sufferings, implementing the principles of humane endpoints is dependent on evaluating the capacity of animals to experience suffering. Some animals have more ability to terminate the painful and short-term event and suffer more pain (Stokes, 2015). Instead of reducing the number of animals to prevent pain and suffering, its duration can be decreased. Invasive procedures including the re-use of animals and the choice of endpoints at an appropriate time lead to the achievement of scientific goals using the less number of animals. However, it is linked to the increase in maintenance costs of animals (Ahmadi-Noorbakhsh, 2018; Council National Research, 2010; Bhasin, 1998).

Conclusion

In ancient civilizations, animals were classified based on the sanctity and value and each one had a mythical symbol. Medical experiments should be carried out based on a design in which minimum mortality occurs in animals, unnecessary killing of the animal is prevented and avoided from cruelty to the animal.

Killing laboratory animals based on ethical principles, which prevent pain or distress, are trustworthy and in agreement with the aim of the research. In the normal condition, when animals show illness signs, the experiment should be terminated. Determination of endpoints in the experiment and animal life has high importance because minimizes pain and suffering in the animal. HEP is used when the animal is in painful condition and its improvement is not possible, leading to death. In fact, an imminent death occurs, indicating a special time for tissue or organ damages in the animal. In order to determine the cause and time of death, some biological and behavioral data are necessary. However, these biological indicators should not ignore the ability to recognize pathologies for the decision- making based on no repetition of experiment and more use of animals, according to the 3Rs principle. Nowadays, these three principles form the scientific basis of ethics in animal experimentation in many countries and are the criteria for the performance of Animal Care and Use Committees.

Appropriate use of HEP can lead to the improvement in human and financial resources and accelerate the scientific process. Along with promoting knowledge, the choice of methods to determine the appropriate time for the endpoint is recommended. Researchers should consider the best endpoint for their experiment, requiring precise attention to scientific aims and possible negative effects on animals. Animals should be tested by instructed individuals and the surgical team should be able to reduce the time of anesthesia and surgery and assist animal to recover faster.

Therefore, the observance of animal rights and ethics in the experiment has strong theoretical and scientific foundations and researchers can make informed decisions that meet current animal care standards and research goals.

Conflict of interests

Authors declare no conflict of interest.

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