Animal ethics in Cardiovascular Research

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EDITORIAL

Animal ethics in *Cardiovascular Research*

1. Background

More than 100 million animals are used for research purposes around the world annually, most of them in Europe and the USA. Rats, mice, and other rodents make up 85–90% of all research animals. 1–1.5% correspond to dogs and cats and less than 1% is represented by non-human primates. Despite a significant decrease in the numbers of animals used in biomedical research and testing in the last two decades, in part due to more restrictive policies and development of alternative methods (*in vitro* tests, availability of human cells and tissues, computational models, and others), the magnitude of these numbers, together with an increasing social sensitivity towards animal pain and suffering, makes the debate about the use of animals in experimental research an important and indisputable ethical issue among scientists, scientific journals, regulatory committees, and local, national, and international agencies.

The use of animals in research has historically represented an irresolvable moral dilemma between the value of the expected benefits and the suffering, pain, confinement, and death to which it subjects sensitive living beings. Scientists using animals have to work on the assumption that the resulting increase in knowledge or improvement in human health outweighs all these associated costs, which in some cases has been arguable. The Cartesian view that prevailed up to the second half of the last century tried to circumvent this ethical conflict by depriving the animals of any sensitive and intellectual capacity. This view of animal status was extended throughout Europe for centuries due to the important impact the French philosopher Descartes (1596–1650) had on our concept about animal consciousness. Non-human living beings were considered to lack cognitive abilities and could not feel pain or distress but only automatic reactions in response to external stimuli. This doctrine had a pernicious effect on animal welfare and gave rise to extremely cruel and inhumane practices with unanaesthetized dogs, cats, and other mammals.

An overwhelming amount of scientific evidence about the emotional and social abilities of a growing list of animal species has moved the debate on animal ethics towards a more complex scenario. Individual rights and care cannot be based solely upon intellectual abilities. The capacity of certain animals to experience pain, rather than the ability to think, is enough to guarantee ethical consideration, and this capacity can be ascribed to all vertebrate species. This new perception has been adopted by policymakers not necessarily involved in scientific activities but with the right to discuss how animal research should be conducted. Inflicting pain or causing avoidable suffering and distress to sentient beings may give rise to a serious moral conflict, according to our current knowledge, and has led to questioning of the utility of some routine lethal toxicological tests—among other equally aggressive interventions—by scientists and non-scientists. Society is the primary recipient of the benefits derived from biomedical research, which is significantly funded by public resources, and has the right to decide whether animal experimentation must be humane as well as scientifically justifiable. The public demands that only minimal pain be inflicted on animals involved in experiments and disapproves of their indiscriminate use. In some instances, discrepant views between the public and scientists regarding what may be considered essential for the progress of knowledge has ended up in a complete lack of understanding between the two parties. In other cases, open and constructive debate has resulted in an improvement of animal welfare and the quality of science.

2. The use of animals for biomedical research: new requirements internationally and locally

It was in 1959 that William Russell and Rex Burch developed for the first time the innovative concept that excellence in scientific research is linked to the humanitarian use of animals.[1] They clearly defined what has become the prevailing ethical rule for any scientific project: to *reduce* the number of animals used to test a hypothesis, to *replace* them with alternative experimental models as much as possible, and to *refine* the experimental procedures in order to minimize sources of pain and distress. The principle of the so-called 'three Rs' has been implicitly adopted by all scientific journals.[2]

On 22 September 2010, the EU adopted Directive 2010/63/EU on 'the protection of animals used for experimental and other scientific purposes', which updates and replaces Directive 86/609/EEC, in force from 1986 and initially conceived to eliminate disparities between national laws and local regulations of the Member States regarding the protection of animals used in experimentation. The aim of the new Directive is to strengthen former legislation in line with the latest scientific developments, improving animal welfare, firmly imposing the principle of the three Rs in EU countries, and further reducing the discrepancies between different European countries. However, the new European legislation allows for maintaining national laws provided they increase the protection requirements agreed on in the Directive, as some nations have a higher sensitivity towards animal welfare. The Directive also sets minimum standards for housing laboratory animals and for training personnel responsible for handling them and supervising the experiments.

This new legislation is based upon published scientific evidence on factors influencing animal welfare, highlighting the capacity of animals to sense and express pain, suffering, and distress. Importantly, for the first time in this type of legislation, the Directive covers cephalopods

and advanced foetal forms of mammals, restricts the use of animals to procedures resulting in human or animal health improvement (therefore, highly discouraging animal use for educational purposes), and underscores the importance of selecting an appropriate method of euthanasia by a competent person (a list of allowed methods is provided in the text). The use of non-human primates is only permitted in very specific and restrictive biomedical areas. The Directive demands the classification of the estimated level of pain, suffering, and distress inflicted to animals using a severity score and considers an upper limit above which an animal procedure should be prohibited. Animal suppliers have to receive permission from the appropriate authority and should meet the housing requirements for each animal species. In addition to all these specifications, it is mandatory that each institution using animals for scientific purposes (hospitals, universities, public, and private research centres) undergoes a detailed and constructive scrutiny of the scientific projects by a competent ethics committee. Local or university ethics committees have to approve and supervise all the studies carried out with animals, providing the necessary advice to implement the latest scientific knowledge and to ensure good practice, as demanded by the legislation. It is important to note that the European legislation is not the source of a complete list of accepted or discouraged procedures used in biomedical scientific publications, but it provides the basic rules and refers to available scientific literature for more detailed information on specific issues. A more practical and periodically updated guideline on accepted pharmacological approaches for laboratory animal experimentation can be found in the textbook by Paul Flecknell *Laboratory Animal Anaesthesia*.[3] This book has recently incorporated all the current knowledge on this topic and constitutes a basic working tool for ethics committees and scientists.

3. The quest for uniformity

Improving the welfare of animals as well as the design of animal studies has consequences that go beyond humanitarian considerations, as it increases the quality of scientific papers and ultimately human health care and patient safety.[4] In past years, many journals' demands for the description of the details regarding animal handling were too limited to allow a reliable reproduction of the experiments, rendering reviews or meta-analysis of animal studies impossible. This highlights the double standard applied to animal vs. human studies when one considers the high standards of quality and uniformity present in human clinical trials.[5] In order to resolve this limitation, some journals have recently adhered to the so-called ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines, produced by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). These guidelines are designed to improve the reporting of animal experiments by means of a 20-point checklist of the essential information that should be included in publications reporting animal research.[6] In parallel, a Gold Standard Publication Checklist (GSPC) was developed to increase the standardization of animal experimentation in accordance with welfare principles, reduce the number of animals, and allow others to replicate previously published experiments.[7] Overall, these types of initiatives reflect the increasing interest and concern aroused by editors and publishers about the way animal studies are carried out.

4. Editorial policy of *Cardiovascular Research*

Since November 2010, *Cardiovascular Research* has adopted a more active role to ensure that manuscripts submitted for publication are in line with new international regulations. As specifically demanded by the European Directive, the instructions to authors in *Cardiovascular Research* have raised the ethical standards for protection of animals used in scientific procedures, following the most recent published evidence and recommendations. The new requirements refer to recognition of inflicted pain, palliative measures, potential drug combinations, and accepted euthanasia methods. Painful procedures have to be carried out under general or local anaesthesia, and analgesia should be used to ensure that suffering is kept to a minimum. Accordingly, authors are expected to provide a more explicit and detailed description of procedures involving animals, from initial anaesthesia to euthanasia, indicating generic names, routes of administration, and doses of the drugs used in each step, and they should describe the methods that are used to monitor the adequacy of anaesthesia. Hence, a description of animal management can no longer be restricted to a brief sentence stating that the procedure is in agreement with current legislation, as this will not allow a proper evaluation of the ethical aspects of the study (which is always carried out prior to scientific evaluation).

With the revision of the editorial policy, 6% of the total articles received in the past year for evaluation in *Cardiovascular Research* were rejected for ethical reasons. One of the most frequent causes of rejection on ethical grounds is the improper choice of anaesthetic drugs for major surgical procedures. This problem is exemplified by barbiturates used without adjuvant analgesia for surgery. While traditionally a popular and convenient veterinary anaesthetic, pentobarbital has lately come under scrutiny for its poor analgesic properties at doses usually used in surgery ($<$60 mg/kg, depending on the species).[8–12] At high-range doses ($\geq$100 mg/kg), it provides sufficient analgesia, but it is not advisable for the associated increased risk of mortality and haemodynamic instability.[13] Therefore, barbiturates are only suitable for terminal procedures (high dosing) and are not approved for painful manipulations unless they are co-administered with opiate derivatives or non-steroidal anti-inflammatory drugs. Similarly, diethyl ether, a highly flammable and toxic substance largely used in the past for anaesthetic and euthanasia purposes, has been excluded from current recommendations not only for human safety reasons but also for its insidious effect on animal suffering. Also, as part of the new recommendations, neuromuscular blocking or paralytic agents should never be used without general anaesthesia. Dissociative agents, e.g. ketamine, have to be combined with appropriate muscle relaxant analgesia, whereas hypnotic drugs, e.g. chloral hydrate or alpha-chloralose, are considered to have inadequate analgesic properties and are no longer acceptable for anaesthesia. In some cases, problems arise by the inadequate use of otherwise standard and safe drugs (use of short-lasting agents for long procedures, major surgery with local anaesthesia, extrapolation of a recommended procedure from one animal species to another, or use of the wrong route of administration).

Cardiovascular Research also advocates the incorporation of compassionate endpoints in the study design as part of authors' ethical responsibility. This may sometimes imply a modification of what was

previously considered an acceptable protocol. The fact that a certain procedure involving animals has already been published cannot be used as an argument to refuse to modify it in accordance with latest recommendations. A reluctant attitude towards the adoption of new recommendations can eventually give rise to unreliable data, as an excess of stress or pain is associated with a non-controlled, undesirable physiological response. This is particularly important in the field of cardiovascular medicine in which the potential effect of pain-relieving drugs on cardiovascular function has been systematically considered an argument to reduce their use, while the negative consequences of inadequate anaesthesia on the quality/reproducibility of the results have tended to be dismissed. In animals, post-surgical pain reduces food and water consumption, decreases a whole range of 'self-maintenance' behaviours, produces immobility-related complications, and is responsible for several neuro-vegetative and inflammatory disorders.[14] However, assessment or detection of animal pain is not always trivial because animals' manifestation of pain depends on several factors, including species-specific, phylogenetically selective behaviour.[15] Thus, to fulfil editorial ethical requirements, investigators should revise their list of available pharmacologic tools and get the latest advice from the veterinarian responsible for their animal facility.

5. Conclusion

Studies involving animals contribute importantly to scientific progress in cardiovascular medicine. However, it is imperative that more standardized animal welfare considerations become an essential aspect of the experimental design, as they may increase the quality and relevance of the data, reduce the variability of the results, facilitate the potential translation to humans, and ultimately decrease the number of unnecessary replications, fulfilling the principle of the three Rs. It is time to reconsider the usefulness of some aggressive or lethal tests and to systematically include humanitarian endpoints (euthanasia) as part of the study design. Avoidance of recommended analgesia/anaesthesia due to its undesirable contribution to the empirical results can no longer be sustained, as it is extremely unlikely that alternative drugs are not available or that an adequate control group cannot help circumvent this limitation.[16] Animal selection should be more specific, reducing the profusion of models with questionable relevance, in an attempt to more specifically mimic human pathologies.[17] For better of for worse, animals are similar to humans and suffer from similar conditions, sharing with us underlying pathophysiological mechanisms with comparable levels of pain and suffering, and this is fundamental not only for science but also for ethics.

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NOTE ADDED IN PROOF

At the time this editorial went to press, we performed a quick assessment of the fate of some of the papers that were rejected by *Cardiovascular Research* for ethical reasons. Of about 60 articles, 24 have been published in various journals to date. Surprisingly (or perhaps not so surprisingly), 6 of these no longer give information about the method of anaesthesia used. However, most disturbing is that 5 papers now contain anaesthetic protocols that have been changed from the original version submitted to *Cardiovascular Research*! Obviously, plain and simple fraud may be a tempting, although deplorable, way out. Only widespread and homogeneous improvement of journal policies on animal ethics may help to limit this problem.