EDITORIAL

The use of animal models in disease research

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Abstract

Animal experimentation has always been an important issue in scientific practice. However, in recent years the use of animals in biomedicine and toxicology has become even more relevant not only for the scientific community, but for the public of non-specialists as well. New considerations in relation to animal welfare and ethics of research has brought to fundamental legislative changes, reflected in the publication of the recent Directive 2010/63/EU on the protection of animals used in scientific procedures. This legislation has now been implemented by the 28 Member States.

It is not our intention here to discuss the pros and the cons of animal experimentation in the eyes of the different stakeholders taking part in this discussion, but rather point out some important elements regarding the use of animals in research. We believe that this can be of some interest for the study of rare diseases because, as we will see at the end of this contribution, animal models can be of great help in this particular branch of biomedical research. We apologize with our readers, because some of the concepts are very well known and embedded in experimental biomedicine, but we will mention them as useful for our reasoning.

Key words

Animal experimentation, animal models, Directive 2010/63/EU, ethics of research, 3Rs principle.

Quantitative aspects of animal experimentation in Europe

First of all, we would like to illustrate the numbers of animal experimentation. The last set of complete official data comes from 2011. It must be pointed out that these numbers could increase following the new reporting rules of Directive 2010/63 [1, 2], where animals not counted before will be now reported in the EU statistical tables.

In the EU, the total number of animals used for experimental and other scientific purposes from the data collected in 2011 is just under 11.5 million. This is a reduction of over half a million animals used in the EU from the total number reported in 2008. As found previously, rodents and rabbits accounted for 80% of the total number of animals used in the EU. Mice were the most commonly used species with 61% of the total use, followed by rats with 14%. The second most used group of animals was, as in previous years, the cold-blooded animals which represent almost 12%. The third largest group of animals used was birds with 6% of the total use. As stated in the previous three statistical reports, no 'great apes' were used in experiments in EU in the last years. The highest increase is noted for fish (+310,300) in comparison to 2008 and for rabbits (+25,000). The largest decrease observed in 2011 for the more commonly used species is for rats, with a reduction of more than 500,000 animals. The increase in the use of fish in area of fundamental research was attributed to studies on fish production, genetics, cancer research, physiopathology and diagnosis. Fish have also been used for neurological and cardiovascular studies and in selection according to bioenergetic properties of their cardiac cells, and their use is expected to rise in the forthcoming years. Non-human primates represent a very little percentage of the total animals used every year in Europe in research laboratories.

Both fundamental biological research and research and development in human and veterinary medicine are the areas using by far the highest number of animals in the EU.

The number of animals used for toxicological and other safety evaluation amounts to 8% of the total [3, 4].

Scientific aspects and use of animal models

We have seen that animal experimentation is a scientific reality characterized by important numbers. Is the use of all of these animals scientifically sound and justified?

A fundamental concept in animal experimentation is the notion of animal model. An animal model can be described as a condition which permits to study in an animal the fundamental biological and behavioural processes, at least under a certain aspect, of the same pathological phenomenon observed in humans, or in other animal species. In research laboratories the use of animal models is common practice. Animal models are commonly used for the study of human diseases, aimed at shedding light on aetiology, potential risk factors and natural history of the disease. They are important tools also in the discovery and development of new diagnostic and therapeutic approaches; the marketing authorization process of new drugs requires preclinical studies on animal models to test new compounds as for toxicology, teratology and mutagenesis, before passing to phase II and III clinical studies in humans for the evaluation of efficacy. Animal experimentation is even more important in the study of rare diseases, where very few patients, usually scattered worldwide, are available [5]. Very small populations of patients do not allow to test different doses, routes and timing of treatment administration, nor to detect clinical and demographic characteristics of subgroups who may benefit from the specific therapy. In case of rare diseases all these issues, which are usually dealt with in clinical trials, must be addressed in preclinical studies. Most orphan drug applications submitted to the Committee for Orphan Medicinal Products (COMP) include such animal studies as a basis for initiating clinical trials in patients with the specific rare disease [6]. Moreover, due to the small number of patients, pivotal clinical trials on orphan drugs are frequently single-group, non randomized, unblinded, and rely on surrogate markers of disease response to assess efficacy. These study characteristics raise concerns about the robustness of clinical trial findings [7], and make sound animal study results necessary to support claims of efficacy.

Appropriate and reliable animal models must be carefully selected, so as to present close physiological similarities and better mimic the target disease. It can be advantageous to use more animal models to cover different aspects of the same disease (transversal/integrative approaches), and to use one or more small animal models before the use of large animal models in the early stage of clinical applications. At these conditions, animal models can allow in vivo validation of a proof of concept, mainly concerning mechanism of action and efficacy of candidate compounds through the use of selected biomarkers.

However, in general, it is important to point out some aspects of animal models. In some cases it is simply impossible to replicate in another animal the complexity of a particular human pathology. For example, in the case of the study of Parkinson's disease, no animal model can usefully replicate all of the characteristics of this condition, ranging from molecular to psychological aspects. The Parkinson's disease is studied in a myriad of animal models, using species such a fruit fly (*Drosophila melanogaster*) up to the marmoset monkey (*Callithrix jacchus*). Therefore, we could argue that the "animal model for Parkinson's disease" is the sum of all of the models provided by all of these different species. Each one tells us about a particular aspect of the disease. What we are saying is that for some complex conditions the concept of animal model is a relative one, not absolute, related to a particular aspect we want to investigate. This is an important aspect to underline when explaining the concept of animal experimentation to a public of non specialists.

Another important aspect is that a particular animal model is not valid for all purposes. A model could be especially appropriate to replicate the symptoms of a certain pathology ("face validity"); another one could be valid to elucidate the mechanisms underlying a certain condition ("construct validity"); finally, a particular model could be used to study an appropriate therapy ("predictive validity"). It is difficult to find a model that can satisfy at the same time the three requirements. The point here is to be clear from the start what we require from a particular model.

However, the same question remains: "Are animal models informative for human health?". Detractors of animal experimentation argue that this is not the case, because "a mouse is not a man" (this is definitively true, but also a chimpanzee is not a human, although very similar). We think that they miss an important point. The concept of animal model is a Darwinian one. The Darwinian theory of evolution affirms that the morphological and physiological level of similarity between the human species and another species is inversely related with the time passed from the existence of a common ancestor of the two species. Therefore, for example, humans and non-human primates are more similar than humans and rodents. If we adopt this methodological and theoretical framework we can legitimise, on scientific ground, the use of animals in experiments whose aim is to increase the knowledge on the biology of the human species. We are looking for those characteristics shared, in a Darwinian sense, between a non-human species and the human one. Even an animal phylogenetically distant from humans can be extremely useful. For example, the mollusc *Aplysia californica* is a very useful model for studies aimed at understanding the molecular aspects involved in animal learning processes, because this invertebrate is characterised by a very simple and accessible nervous system. However, due to its limited behavioural repertoire and the phylogenetic distance from the human species, this animal is not very useful as a model for identi-

fying the neuro-psychological aspects related to the display of certain behaviours typical of mammals, such as *Homo sapiens*. In this case we are looking for "more complex" species, such as a non-human primate, for example.

Ethical aspects

Animal experiments are like any other form of human activity in having costs and benefits. Public acceptance of the benefits of findings that might emerge from such work and public tolerance of the possible costs involved in carrying it out, however well informed, are both subjective and both subject to change over time. There is no universal agreement about either the benefits or the costs, nor is there ever likely to be. The right answer to such an ethical question can never be reached by a purely scientific method but will depend upon our ethical emotions to matters whose factual characters should be understood as fully as possible.

The ethics of animal experimentation is a widely debated topic and it is part of a more general discussion about the ethics of human/animal relationships, and the use of animals for research seems to be the field where the debate is most radicalized. In the public discussion opinions tend to be polarized in "against" and "pro" views. At the same time, the academic debate does not seem less radicalized and somehow simplistic. As a matter of fact, the most influent animal ethics theories aim at resolving the moral problems of experimentation on animals by applying one single principle to it (e.g. the principle of equal consideration of suffering in Singer's utilitarianism). Likewise, supporters of animal research defend it recurring to analogous simple principles (like the sovereign good of humanity). The attempt to "solve" the moral problem of animal research reducing it just to simple moral calculations (i.e. human benefits vs harms to animals) or making appeal to general principles (i.e. absolute right to life of animals) misrepresents the complexity of moral reality. Animal research is a multifaceted practice embedded in the wider scenario of the whole of human/animal relationships.

Ethically speaking, if we were confident of the fact that animals do not suffer, there would be no problem. Animal experimentation would bring just benefits to the human species, with no cost inflicted upon other species. Unfortunately we have plenty of indications, both direct and indirect, that animals do suffer: from the physiology of pain to behavioural signs of distress and anxiety. Even if we are not sure about animal sufferance we should always adopt Jeremy Bentham's thought, when he says that if we behave towards animals thinking that they are able to feel pain and/or sufferance, and if we are wrong in this belief, we do not cause them sufferance; on the contrary, if we treat them thinking that they do not feel pain and/or sufferance, and we are mistaken, we are likely to cause pain and sufferance. We have to give the animals the benefit of doubts [8].

If the animals do suffer, is it ethically justified to use them in experiments for our own good? Philosophers help us a great deal in trying to focus such question. Animal ethics is part of moral philosophy, and deals with the moral aspects of our way to treat the other animals. It is an approach characterized by many different points of view, but all of the positions have two points in common: the critique to the notion of "specism", and the elaboration of "moral status".

Specism is morally wrong, as the same as racism or sexism. If racism calls for the colour of the skin to provide for the reasons to deny rights, and sexism does it with gender, to be *Homo sapiens*, rather than *Macaca nemestrina* or *Mus musculus*, does not automatically mean that we are more morally relevant. It follows that no other animal should be experimentally exploited for the sake of human health.

The second point concerns the notion of moral status. If to belong to a particular species does not automatically assign a moral relevance, what does? In the philosophical literature a great deal of different opinions can be found on this issue. For philosophers such as Peter Singer and Tom Regan (father founders of animal ethics), most of the animals are sentient beings, able to experience pain and pleasure, and entitled to be "moral patients". For this reason we simply cannot use them for our good. Animal experimentation then becomes a problem, for which is difficult to find an ethical justification [9, 10].

Singer and Regan's conclusions are rationally and philosophically sound. But not everybody thinks this way. A discordant voice is Nell Noddings, who holds that morality ultimately stems from the emotion of caring [11]. Noddings incorporates the caring model into a general ethical theory and asserts that moral sensitivities are rooted in interpersonal relationships. Because Noddings is concerned with the ethical responsibilities incumbent on caregivers, her ideas are particularly relevant to the ethical implications of human-animal bonds. Noddings believes that we do have moral obligations toward some animals, the ones with whom we have personal relationships. Predictably, Noddings does not think she has a general moral duty directed toward other species; for example, she feels absolutely no obligation to rats. Noddings' idea is not immune to criticism, because it offers a vision of ethics perhaps too personalised. However, it offers an alternative and interesting point of view, perhaps more close to everyday reality than other more classical philosophical positions.

At the end, what moral status the other animals have? Following Noddings' thought: "the one we give them". How can all this make animal experimentation more acceptable? A Dutch ethologist-philosopher, Tjard de Cock Buning, has expressed his idea that humans have a natural tendency to feel more attached to other humans, rather than to members of another species (indicated as "affinity for people"). This attitude, right or wrong it does not matter here, determines our everyday moral choices: as a matter of fact, we are specists. Our cognitive abilities al-

low to feel empathic beyond time and space, towards humans far away and not even born yet, that we will never meet. No other species is able to feel this way (for the current knowledge of other species' cognitive abilities). That could be one of the reason why, in general, other humans for us have a higher moral status than other animals. In this sense animal experimentation, when searches for the cure of diseases which will eventually hit future generations, could become morally justified.

The principle of the 3Rs

Even if we are able to find a moral justification for animal experimentation, this does not automatically mean that we are legitimated to do what we want with our experimental subjects. There is a law, the already mentioned Directive 2010/63, which regulates the activity of animal researchers. The Directive is heavily inspired by the principle of the 3Rs, which is directly mentioned in several articles of the European normative. In particular, the Article 4 of the Directive is dedicated to this principle.

In 1959 William Russell and Rex Burch, members of the Universities Federation for Animal Welfare, published a book destined to be fundamental for the future of animal experimentation. The book was entitled *The principles of humane experimental technique*, and introduced a series of recommendations, that any researcher should follow when planning an experiment involving animals [12]. These recommendations are called the 3Rs. It is very important to remember that this principle does not undermine the importance of animal experimentation at all, but instead it suggests a more careful approach to the use of animals. The first "R" is intended as "Replacement", that is, first of all the researcher should evaluate the possibility to substitute his/her animal model with a non-sentient being; the second for "Reduction", suggesting he/she should try as much as possible to reduce the number of individuals used in a particular experimental protocol; finally, the third "R" stands for "Refinement", where the researches try as much as possible to reduce the amount of sufferance and distress experienced by the animal during all of its life in captivity [13] (see refs 14 and 15 for a particular application of the principle of the 3Rs).

There is a vast literature concerning each one of the "3Rs", and it is important to point out that the term "alternative" does not imply only not to use animals, but also to use them in a different way (we suggest to visit the website of the National Centre for the 3Rs based in London).

As well as being a methodological recipe for animal experimentation, the principle of the 3Rs should be considered a sort of *forma mentis* for each researcher in order to make use of a more ethically and scientifically sound concept of animal experimentation (see, for example, ref. 16). Respect for the quality of the data must go hand in hand with the respect for the quality of life of the animals that we utilised in our research laboratories.

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