Humane endpoints and the importance of training

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Summary

Continuous refinement of experimental protocols resulting in the introduction of the earliest possible endpoint requires competence, commitment and the collaboration of all staff categories associated with animal care and animal experimentation. An intimate knowledge of the normal biology and behaviour of the species in question is necessary for the development of relevant observation checklists on which to base the endpoint. Education and training are thus essential prerequisites for continued optimization of animal welfare during experimental procedures.

When an experiment or test leads to the death of an animal as the endpoint and is preceded by suffering or pain, the design of the experiment needs to be scrutinized for possible changes allowing the introduction of euthanasia prior to suffering and pain perception. Euthanasia—or the humane killing of animals—may obviously be essential when using animals in biomedical research. However, the term *humane endpoint* indicates that certain endpoints in biomedical research are not humane and that it is possible to introduce more humane endpoints in certain experiments by terminating them at an earlier stage, such as when clinical or physiological changes indicative of the effect of the treatment or procedure become identifiable. In other words, the search to minimize pre-death suffering in those biomedical experiments where it occurs may also be termed *refinement* in accordance with the Three Rs of Russell and Burch (1959).

Replacement and reduction are more easy concepts to deal with than refinement. Consequently, refinement has been termed the *Cinderella* of the Three Rs (Hau & Carver 1994), a term later adopted by van Zutphen (1998), because refinement in laboratory animal experimentation requires competence, commitment and the collaboration of all staff involved in the care of animals and animal experimental procedures.

Examples of refinement by introducing more humane endpoints earlier

It is important to emphasize that the majority of animals used in biomedical research never experience severe stress, suffering and pain. They may be deprived of stimuli and live in fairly sterile and barren environments, but the importance of the need for enrichment and, particularly, for a suitable social environment is gaining general recognition in the biomedical community (Anonymous 1993). It is quite obvious from the current scientific literature on laboratory animal husbandry that improvements in the cage environment and housing in appropriate social groups are being introduced into European animal facilities (Blom *et al*. 1993, Jacobs *et al*. 1995, Baumans & van de Weerd 1996, Saibaba *et al*. 1996, Augustsson & Hau 1999).

Refinement with respect to the alleviation of fear, pain and distress in animals during experiments is also taking place in experimental laboratories by the use of improved methods such as the increased use of imaging and telemetric techniques, and by changing experimental designs based on animal welfare considerations. It is, nevertheless, a fact that many experiments of longer duration in e.g. toxicity and carcinogenicity studies, as

well as studies of autoimmunity and infectious diseases, often have death as a result of the treatment as the ultimate endpoint. This endpoint may also be a requirement of the regulatory authorities according to the *European Pharmacopoeia*.

This should not, however, discourage scientists from attempting to introduce earlier endpoints and, as a minimum, euthanasia of moribund animals. Study directors and principal investigators should, as a general rule, be asked to justify the endpoint of death following a period of suffering as a necessary component of the experiment. The following is an example of how earlier endpoints can be introduced and accepted by the authorities. In an application to our regional Ethics Committee a group of researchers wanted permission to use a mouse model of swine erysipelas, infecting mice with *Erysepelothrix rhusiopathiae* and using death as the endpoint, as stipulated in the *European Pharmacopoeia*. The Ethics Committee questioned the need to use death instead of signs of clinical disease, and the scientists successfully convinced the *European Pharmacopoeia* to allow the euthanasia of the animals once behavioural and clinical signs had been observed, indicating that the animals were moribund.

It may also be possible to introduce earlier endpoints by refining the animal model system. I can give an example of this from our own laboratory in which we developed refined mouse models for the study of mycotic infections. Traditionally mice have been used for screening the virulence of fungi and the efficacy of antimycotic drugs by the systemic injection of large doses of fungi using death as the endpoint. We developed a model of localized candidiasis by inducing mycotic mastitis in lactating female mice. Hence the infection remains localized and there are no macroscopic clinical signs of disease (Guhad *et al*. 1995). The model is sensitive and can be used to distinguish the virulence of different *Candida* strains (Guhad *et al*. 1998). There are no signs of stress or suffering in the infected animals prior to euthanasia.

Mellor and Morton (1997) have addressed the need for improving the understanding of the pathophysiological processes underlying the effects of an experiment. They correctly argue that the introduction of earlier endpoints may be less difficult to achieve in experiments where the biological mechanisms are better known (e.g. vaccine trials) than in those where the toxicity of new chemical compounds is being investigated.

The importance of teaching and training for the refinement and introduction of earlier endpoints

The most important means for improving the welfare of the animals used in biomedical research is education—not just of laboratory animal technicians, scientists and laboratory animal specialists, but also of the general public and special interest groups—about the true needs of animals. The aim is to provide the animals with a low stress environment in which pain and suffering are reduced to an absolute minimum. The Euro-pean requirement for competence in all staff categories associated with laboratory animal care and experimentation, and the resulting establishment of mandatory education in European countries is thus to be welcomed as a most important contribution to laboratory animal welfare.

Detailed curricula for courses in four different categories (A–D) for technicians, scientists and laboratory animal specialists have been established by FELASA working groups. These may set appropriate standards for future European courses in laboratory animal science and welfare. An important component of the mandatory competence courses is ethics and the increasing awareness of the necessity to ensure that animals are treated with care and responsibility based on an understanding and knowledge of the biology and behaviour of the relevant species. This is perhaps particularly important for the short (80 h) FELASA category C courses for scientists, because the use of animals in research is a completely novel subject to many young biomedical scientists. Of major importance for ensuring the humane treatment of animals is a thorough introduction to the biology, and true behavioural needs, of the species most commonly used as laboratory

animals. It is vital that all staff are taught that the perceptual world of an animal is different from that of humans and that important behavioural characteristics vary between and within species depending on age, gender, strain and position in the animal hierarchy. A thorough introduction to animal welfare and how to minimize stress and reduce fear by conditioning as well as pain assessment, and ways to eliminate pain perception in animal experimentation, are also important theoretical components of the education of scientists and laboratory animal technicians. Compassion for the animals in experiments must be recognized as essential for the improvements of animal welfare. Education will ensure that the negative impact of experiments on animals can be assessed as objectively as possible, and anthropomorphic subjective views on how animals perceive experimental treatment can be avoided.

Practical training in the correct handling, sampling and administration of substances must be considered essential components of any competence course in laboratory animal science. The use of animals in the development of manual skills has been the subject of debate in Europe. In certain countries such as the UK, it is not permitted to train staff using animals. As an exception, the use of rats is accepted in microsurgery training programmes, but a number of British surgeons have had to learn minimal access surgical techniques abroad because of this strict legislation.

The numbers of animals used in training programmes are miniscule compared with research use and it must be considered essential, from an animal welfare point of view, for technicians and scientists to be taught how to restrain animals without causing fear and stress, as well as how to anaesthetize them. Once the animals are anaesthetized, the course participants can learn all the relevant sampling techniques as well as the administration of substances, after which the animal is killed and dissected for comparative anatomy. Although it is often claimed that there is strong opposition to the use of animals in training programmes, this is not supported by the feedback from course evaluation forms. I have taught and trained technical and scientific staff at all levels in universities in Denmark, the UK and Sweden for more than 20 years, and the vast majority of students are extremely positive about the use of animals in training programmes. It goes without saying that it is important for the trainers to show respect for the animals used and to demonstrate thoroughly how to pick up, restrain and anaesthetize the animals before the students do it themselves.

An important feature of training courses is the instruction and training in the methods of humane killing. All animals should be guaranteed a humane killing either as the endpoint of an experiment or immediately after the experiment is finished. Several guidelines on acceptable euthanasia methods have been issued, following extensive consultation of laboratory animal science experts, which ensure that all laboratory animals can be humanely killed at the end of an experiment. Some euthanasia techniques require more skill than others, and in particular physical methods like cervical dislocation or killing conscious animals by shooting with either a free bullet or captive bolt, should only be attempted by experienced personnel who have received sufficient training to allow them to use these methods confidently and effectively.

Conclusion

Knowledge and competence obtained through education and training in all staff categories are essential for the continuous refinement of animal experimentation. The search for early endpoints in projects and tests where death is used as the endpoint must be given a high priority. However, in addition to competence, the way forward also requires commitment and collaboration.

Commitment in both scientific and technical staff is needed to improve the welfare of animals in experiments and to search actively for ways and means of terminating experiments as early as possible. We should search actively for behavioural, clinical and physiological parameters that may be used as indices of the effect of treatment rather than

death or the obvious moribund state of an animal.

Collaboration through good communication between laboratory animal technicians, scientists, laboratory animal veterinarians, institutional certificate holders and the regulatory and inspection authorities is vital for progress in this area. Mistrust, particularly between scientists and ethics committees and license-issuing authorities, may hinder this development, and efforts should be made to establish frictionless contact and collaboration between all personnel involved in laboratory animal experimentation.

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