



Refining Animal Models to Enhance Animal Welfare

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【Abstract】 The use of animals in research will be necessary for scientific advances in the basic and biomedical sciences for the foreseeable future. As we learn more about the ability of animals to experience pain, suffering, and distress, and particularly for mammals, it becomes the responsibility of scientists, institutions, animal caregivers, and veterinarians to seek ways to improve the lives of research animals and refine their care and use. Refinement is one of the three R's emphasized by Russell and Burch, and refers to modification of procedures to minimize the potential for pain, suffering and distress. It may also refer to procedures used to enhance animal comfort. This paper summarizes considerations for refinements in research animal.

【Key words】 Animal welfare; Humane endpoints; Pilot studies; Analgesics care and use

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Introduction

Global attention to animal welfare is increasing rapidly, with agencies such as the World Animal Health Organization (OIE) and U. N. Food Agricultural Organization (FAO) taking a leading role creating international standards and capacity building. The OIE, of which China is a member country, first included animal welfare in their strategic plan in 2001–2005, indicating that it is “a complex international public policy issue, with important scientific, ethical, economic, cultural, religious and political^d dimensions that also impact international trade and policy considerations” (OIE, 2002). In 2010, the OIE introduced a chapter in their terrestrial code specifically dealing with the welfare of research animals, drawing public attention to this issue and placing a further burden of responsibility on member countries to ensure research animal well-being (OIE, 2010). While great strides have been made in increasing the quality of laboratory animals by standardizing housing and diet, optimizing genetics, and reducing opportunistic disease within colonies and troops, an increasing emphasis is being placed on scientists, institutions, and animal ethics committees to

consider the three R's whenever research animals are used.

The three R's refer to principles first proposed by Russell and Burch (1958) in reference to laboratory animal care and use. Specifically, the three R's refer to replacement, reduction and refinement of animal use. The meaning of the first two R's is self-evident; replacement refers to the use of nonanimal models or cells or tissues to replace whole or live animal use, while reduction refers to planning experiments carefully to reduce animal wastage such that only the correct number actually needed for any experiment are used. Refinement refers to modifications of any procedures to minimize pain and distress experienced by the animal and to enhance well-being and comfort. This is typically achieved through modifications to: technique, selection of appropriate model, alterations to animal environment such as the use of enrichment, use of anesthesia and analgesia, as appropriate, defining humane endpoints, and using pilot studies when experimental outcomes are unknown. These concepts will be explored further in subsequent sections of this paper.

In addition to being critical for improving animal welfare, introduction of refinements into experiments is

thought to improve the validity and therefore, the quality, of the scientific results. This is because the experience of pain and distress may lead to physiologic changes in animals, which may increase the variability of results. This makes sense in that we know that the neuronal, immune, and endocrine systems are inextricably intertwined, with signalling occurring between systems via various peptides, hormones, and neurotransmitters, such that alterations in homeostasis and activity in one system will induce shifts in the other two systems (Chapman et al, 2008).

Examples of Refinements for Laboratory Animals

Techniques: There are many examples of using more refined techniques to minimize animal pain and distress. Blood collection and administration of substances to animals are common procedures. Some extra thought and planning prior to study initiation may enhance animal comfort and even improve sample quality (see Turner et al, 2011 a & b). For example, when substances must be administered chronically to animals or when repeated blood samples must be withdrawn over an extended period, the use of indwelling intravenous catheters or vascular access ports may help to minimize animal handling and restraint, as well as repeated venipunctures for substance administration or blood collection, reducing animal pain.

Environmental Improvements: The routine use of environmental improvements has been demonstrated to enhance animal well-being. Environmental improvements (sometimes also called environmental enrichment) refer to the addition of biologically relevant features to the cage environment to facilitate or allow performance of natural motivated behaviors. Examples include the use of nesting material and hiding tubes for group-housed mice, provision of straw to pigs for rooting, or the use of elevated resting boards for dogs. In addition to increasing animal comfort and often reducing interanimal aggression, the use of nesting material gives mice and other animals an activity (eg, digging, burrowing) to do in an otherwise relatively barren environment.

Improving Animal Health Status: Improving the health status of animals by reducing intercurrent disease and opportunistic infections not only enhances animal well-being but also may improve the validity and thus quality of the animal model. Animals suffering from overt, subclinical or opportunistic infections do not make good research subjects as alterations in immune status, homeostasis or other body systems caused by infection may interfere with the experimental manipulation, increasing variability. Recent examples of results errors occurring from the use of animals with subclinical or overt infections have been published (Schoeb, et al, 2009). Maintenance of high health status animals occurs through implementation of a suitable institutional quality assurance program, generally with monitoring of sentinel animals at regular intervals, with the program overseen by an appropriately trained facility manager or laboratory animal veterinarian.

Use of Imaging: The use of recent advanced imaging techniques, for example, magnetic resonance imaging, computed tomography, positron emission tomography and ultrasound permits researcher to follow or track a disease or other condition in animals as small as mice. This may also lead to a reduction in animal use, since the same animal can be used for repeated data points. Great care must be taken to manage these animals carefully, as repeated use may adversely impact a single animal if care is not taken. Examples in trained animals for restraint if they cannot be sedated or anesthetised during the imaging, minimising the imaging time to reduce animal restraint and positional discomfort, etc. Imaging may also be used to determine when predetermined endpoints have been reached, before obvious adverse clinical signs become apparent. For example, ultrasound may be used to monitor renal blood flow to anticipate graft rejections in recipients of kidney transplants before animals become nephrotic. This may also remove the need for repeated renal biopsies, which can be an invasive procedure.

Training for Cooperation: Many species can be trained to cooperate with human caregivers for various

procedures. Examples include training nonhuman primates to voluntarily cooperate for oral and intravenous dosing , use of a sling for electrocardiography recordings in dogs and sheep , acclimating rodents to gentle handling for oral dosing studies , and acclimating cows to wearing activity monitors. In each of these examples , the consistent use of gentle , positive reinforcement training can be used to reduce animal stress. Many human caregivers using these techniques also report increased satisfaction in that there is less resistance to procedures , which may also improve their safety.

Use of Analgesics for Painful Procedures: The routine use of pain-relieving therapeutics for painful procedures is viewed as a very important refinement that must be considered by Animal Ethics Committees whenever painful procedures involving research animals are proposed. Animals that receive adequate analgesia return to normal body functions earlier than animals that do not receive analgesics. Procedures that are considered painful in humans should be considered painful in animals and analgesics should be used , whenever possible. A follow-up examination should be conducted after use to ensure that animals are comfortable as there can be individual variation in response to analgesic drugs in animals , as in humans. Combinations of drugs with synergistic effects but differing mechanisms of action can be used to lower the overall dose of any one drug and reduce the likelihood of toxicity occurring. Examples include the use of opioid drugs with nonsteroidal anti-inflammatory drugs. Topical products may also be used to desensitize skin to painful injections.

Humane Endpoints: The humane endpoint in a study is the point at which an experimental animal's pain and/or distress is terminated , minimized or decreased by killing the animal humanely , terminating a painful procedure (or withdrawing adverse drug or treatment) or giving the animal a treatment to relieve pain and/or distress. The use of humane endpoints provides an alternative to the use of more severe signs of animal pain and distress , including death , and contributes to refinement. Many Animal Ethics Committees require

researchers to include an assessment sheet for invasive procedures and to outline in detail what the expected adverse effects of the study will be. The earliest possible endpoint should always be selected and it is rarely necessary to permit animals to become moribund or to die during an experiment. Biomarkers can sometimes be used as surrogate endpoints for studies. An excellent description of endpoint determination can be found on the Canadian Council On Animal Care website: http://www.ccaac.ca/Documents/Standards/Guidelines/Appropriate_endpoint.pdf

Pilot Studies: Finally , the use of pilot studies is often recommended to researchers when the outcome of a study is unknown. For example , if a new infectious disease agent is being investigated or a new medicine is being studied for which no toxicity data exists. In these cases , the Animal Ethics Committee may recommend that the scientist use a small number of animals (typically five or less) to investigate the expected clinical outcome. This permits the scientist to better define what the endpoints will be for the definitive study.

Resistance to Implementing Refinements

Although these principles for refinement of laboratory animal care and use may seem straightforward , there can be resistance to their implementation during research projects. Procedures may take additional time to conduct properly and with care and technical staff , supervisors and scientists may feel that they are 'too busy' to comply. Some refinements , such as environmental improvements through the use of nesting for mice or food foraging items for nonhuman primates will introduce additional costs , which researchers or institutions may not be prepared to expend. In industry , there is sometimes conservatism and fear about using refinements as Study Directors may be concerned about the validity of the results if refinements are introduced or about comparisons with historical controls. Sometimes scientists or animal caregivers have not received sufficient training in this area or may be unaware of developments that may be used to improve research

animal well-being. Finally, there may be a lack of assistance or support within an institution to introduce refinements, for example, an insufficient number of trained veterinarians specialising in laboratory animal medicine. In all of these cases, it becomes an ethical responsibility of the institutional Animal Ethics or Research Oversight Committees to ensure that there is adequate training of personnel (scientists, animal caregivers, and veterinarians), as well as sufficient resources to implement the necessary refinements that will enhance animal comfort and decrease pain and distress. Data collected while an animal is stressed may not be valid, for reasons mentioned previously, which may make historical control data worthless for comparisons.

Conclusion

In conclusion, better planning of studies and the requirement by institutions and Animal Ethics Committees to consider refinements has led to improved welfare of research animals. Typically, humane science is equated with quality science, as animals are not experiencing pain or distress and may even be voluntarily cooperating with the researchers. Scientists need to remain open to the idea of modifying their research models as knowledge grows about different refinements, as the status quo is no longer considered acceptable by the public or by the international scientific community. Animal Ethics Committee and laboratory animal veterinarians have a significant role to play in implementing refinements by suggesting

refinements for researchers using for invasive animal models, as well as by seeking ways to improve the day-to-day care and management of research animals.

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