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Should refinement ‘mess with success’?

Dr. Ted Clark studied dietary cholesterol metabolism and drugs for controlling serum cholesterol levels. The animal model he used was the owl monkey (*Aotus nancymaae*) and a key part of most of his protocols included a cholecystectomy, the surgical removal of the animal’s gallbladder. A new laboratory animal veterinarian at Great Eastern University was pre-reviewing Clark’s most recent protocol. At first, she wondered if the cholecystectomy was really needed but Clark described the rationale in sufficient detail to convince the veterinarian that the surgery was required. However, the veterinarian questioned why Clark continued to use a relatively large laparotomy incision to remove the gallbladder, while research and

clinical experience suggested that one or two small incisions, just large enough for a laparoscope, could be used to achieve a cholecystectomy with less postoperative pain to the animal. Clark responded that he had nearly 20 years of experience with the open abdomen technique but none with laparoscopy. He never lost an animal, he always provided the post-operative analgesia and general care that was approved by the IACUC, and he had a solid record of publications that had never questioned his methodology. He did not understand why now, out of the blue, his methodology was being questioned, and he was being asked to spend a significant amount of research funds to purchase equipment and be trained in a technique that would lead

to research results that were no better than those he was already getting.

The IACUC office explained to Clark that the veterinarian was suggesting, not demanding, that a potentially less painful procedure be considered. This did not mollify Clark who saw the suggestion as an intrusion on his academic freedom and as second guessing of both a Principal Investigator and the decision of a NIH study section to fund his research.

What do you think? Should the veterinarian or the IACUC require a better explanation from Clark, or has his success over the years been sufficient proof that his methodology is appropriate for his research? Are there any other avenues for the IACUC to consider?

RESPONSE

Refinement, a part of a three-legged stool

Dinesh K. Hireallur-S., DVM, MVSc, PhD & Jennifer McElroy, RVT, LATG, CPIA

Since the origin of Russell and Burch’s concept of the 3Rs, replacement, reduction and refinement¹, investigators and institutions have been following these principles to reduce use and to minimize the distress and discomfort of laboratory animals². In fact, current guidelines such as the *Guide for the Care and Use of Laboratory Animals*³, the PHS policy⁴ and legislative documents including the Animal Welfare Act⁵ in the United States, and Directive 2010/63/EU⁶ in Europe encourage scientists and institutions to recognize and apply the 3Rs not only during studies but also during the planning stages of studies, when the animal use protocol is being developed. Of the 3Rs, refinement is the one that has the greatest

effect of minimizing pain and distress in laboratory animals used for studies.

According to the Animal Welfare Act, the IACUC should determine that the principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animal (§2.31(d); ref. 5). Therefore, in our opinion, the new veterinarian at Great Eastern University was justified both in questioning Clark’s invasive approach for cholecystectomy and in suggesting the novel, alternative laparoscopy approach, which would be less invasive. The concept of refinement is a dynamic process that constantly evolves as new technologies and inventions become available. On the other hand, it is reasonable to expect resistance from an investigator when suggesting refined methods if his methods are already working well. The burden often lies with the IACUC and its institution to convince Principal Investigators and implement refinement approaches to minimize distress and discomfort in laboratory animal procedures.

In this particular scenario, the IACUC committee should recommend that Clark

consult with the institution’s veterinarian to weigh the pros and cons of the suggested laparoscopic cholecystectomy approach. Based on available literature, the veterinarian should provide to Clark objective evidence of the advantages of the laparoscopic approach, such as less postoperative pain, faster recovery and less experimental variability. The veterinarian should also provide a scientific explanation as to why the newer approach would not affect the scientific goal of Clark’s study.

Similarly, the veterinarian and IACUC should provide an opportunity for Clark to express his concerns, and Clark should give a scientific rationale to support his views. Further, we don’t think that Clark’s explanation regarding the success of his past surgical approach provides sufficient justification to disregard the veterinarian’s suggestion to use a new, less invasive approach that will minimize animal discomfort and pain. Also, the monetary cost does not constitute a scientific justification for not adapting a suggested refinement technique. If Clark is still concerned about his scientific outcome, the IACUC should

recommend that he consider carrying out a pilot study to allow him to evaluate the feasibility of this newer approach.

If Clark agrees to consider the veterinarian's suggestion to include a laparoscopic approach for the cholecystectomy, the institution and researcher should work together to implement this new technique and the institution should provide training to Clark's group.

1. Russell, W.M.S. & Burch, R.L. *The Principles of Humane Experimental Technique* (Methuen, London, 1959).
2. Tannenbaum, J. & Bennett, B.T. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. *J. Am. Assoc. Lab. Anim. Sci.* **54**, 120–132 (2015).
3. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
4. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* (US Department of Health and Human Services, Washington, DC, 1986; amended 2002).
5. Animal Welfare Act regulations. 9 CFR. Chapter I, Subchapter A, Part 2, Subpart C.
6. European Union. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* **L276**, 33–79 (2010).

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RESPONSE

A necessary consideration

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Assuming that the laboratory animal veterinarian's questions were diplomatic, Clark's reaction was unjustified. The veterinarian was acting as a representative of the IACUC, which has the duty to oversee and evaluate all aspects of the animal care and use program, including application of the '3Rs' for humane animal research¹. A laparoscopic cholecystectomy, which produces only one or two small incisions rather than one large incision, is a prime example of a technical refinement that can reduce the invasiveness of the surgical approach, thereby decreasing postoperative pain and recovery time for the patient. In a study of human patients with acute cholecystitis,

those that received laparoscopic cholecystectomies had shorter surgical times and hospital stays, as well as fewer postoperative complications, than those that received open cholecystectomies².

We expect that Clark did not adequately address the regulatory requirement to consider alternatives. During pre-review the veterinarian appropriately raised this issue of a potentially beneficial alternative, but the IACUC must ensure that there was a "reasonable and good faith effort... to determine the availability of alternatives" and to justify why this alternative was not used³. Great Eastern University might have procedures in place to address unresolved concerns that arise in the pre-review process; if not, the protocol should be called for a full committee review.

Clark's years of experience, surgical survival rate and publication record are not adequate reasons for refusing to consider alternatives that could refine his procedures. Clark may have perfected the open abdomen technique, but it remains unknown whether the monkeys in his studies experience fewer postoperative complications than they would with a laparoscopic approach. His reluctance is most likely due to a lack of familiarity and skill with the suggested technique. However, it is ultimately the responsibility of the institution, in conjunction with the IACUC and attending veterinarian, to ensure that research personnel are appropriately qualified and trained in the procedures used at that institution. This might require a consultant to be involved in pre-surgical planning and training^{4,5}. Although a laparoscopic cholecystectomy might be more technically demanding than the open approach, with training the technique might have substantial advantages over open cholecystectomy.

Clark doubts whether the refined approach will lead to better research. To examine potential benefits of the laparoscopic technique, the institution should support a pilot study to compare postoperative pain scores, time to recovery and histopathology results for the two surgical approaches. Additionally, the IACUC can ask to review past postoperative records to verify the well-being of Clark's monkeys, or seek consultation from surgeons who have utilized both techniques.

Positive relationships built on open dialogue between the IACUC and principal investigators allow for better science and research. If concerns arise, intolerance and aversion to change create unwanted tension between parties. Working with animals is a privilege not a right, therefore a deep respect for animal welfare is paramount to the future success of our field.

1. Russell, W.M.S. & Burch, R.L. *The Principles of Humane Experimental Technique* (Methuen, London, 1959).
2. Lujan, J.A. et al. Laparoscopic cholecystectomy vs open cholecystectomy in the treatment of acute cholecystitis: a prospective study. *Arch. Surg.* **133**, 173–175 (1998).
3. US Department of Agriculture. *Animal Care Policy Manual Policy # 12 Consideration of Alternatives to Painful/Distressful Procedures* (USDA, Riverdale, MD, 2014).
4. Anderson, L.C. Institutional and IACUC Responsibilities for Animal Care and Use Education and Training Programs. *ILAR J* **48**, 90–95 (2007).
5. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).

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RESPONSE

A mutual understanding can promote progress

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Refinement of the cholecystectomy technique in Dr. Clark's laboratory is appropriate, but the approach of the veterinarian and IACUC also needs refinement. Inquiries from the IACUC or veterinarians can often be received and permanently viewed as directives, regardless of assurances to the contrary. Initial misunderstandings greatly affect the success of efforts to implement the 3Rs.

From the IACUC's perspective this refinement is a simple matter of amending Clark's protocol, training his staff and purchasing new equipment. On the other hand, from the perspective of the principal investigator (PI), there are considerable costs associated with this refinement.

A word from USDA and OLAW

In response to the questions posed in this scenario, the United States Department of Agriculture, Animal and Plant Health Inspection Service, Animal Care (USDA, APHIS, AC) and the Office of Laboratory Animal Welfare (OLAW) offer the following guidance:

This column presents the reader with the following questions: Should the IACUC require an explanation for the use of an invasive surgical method when an alternative might minimize pain? What other avenues should the IACUC consider in addressing the concerns raised by the veterinarian?

The Institutional Animal Care and Use Committee (IACUC) is justified under the Animal Welfare Act regulations to request additional information from the Principal Investigator (PI) regarding alternatives to the proposed surgical procedure. It is the IACUC's duty to review proposed animal activities and determine whether the PI has considered alternatives to procedures that might cause more than momentary or slight pain or distress (§2.31(d)(1)(ii); ref. 1). As a result, the PI is required to provide a written narrative description of methods and resources used to determine that an alternative was not available. Policy 12 of the Animal Care Policy Manual (ACPM) provides guidance on the methodology to consider alternatives to painful procedure and identifies the Animal Welfare Information Center as a valuable resource for developing an acceptable search strategy². In addition to alternative considerations, the regulations also require consultation with the attending veterinarian regarding painful procedures (§2.31(d)(1)(iv), §2.33(b)(4); ref. 1). Policy 11 of the ACPM provides guidance on determining painful procedures².

It is the Institution's responsibility however to ensure that all persons involved in animal care and use are qualified to perform their duties (§2.32(a); ref. 1). In the event it is determined that the less invasive procedure should be implemented, investigator training to develop proficiency would be required for compliance with the Animal Welfare Act regulations.

The PHS Policy explicitly states that the IACUC's review of animal activities requires the committee to determine that "procedures with animals will avoid or minimize discomfort, distress, and pain to the animals"³. Likewise, the Guide considers "the availability and appropriateness of less invasive procedures" a topic that, in this instance, requires the IACUC to engage the investigator and discuss the refinement⁴. The Guide also specifies that the veterinarian and the IACUC should be involved in determining if a surgical procedure is major or minor⁴. The IACUC is therefore justified in requesting an expanded explanation from the investigator. The IACUC must always proceed with the intention to reduce harm to the animal while meeting scientific objectives. It is appropriate for the IACUC to require refinements to methodology that improve animal wellbeing during their review of previously approved protocols³.

The IACUC may further address the concerns of the veterinarian by obtaining institutional support for a pilot study, identifying experienced consultants to assist in training in the minimally invasive method, and determining the availability of appropriate laparoscopic equipment within the institution. Additionally, the institution must support the decisions of the IACUC and inform NIH of the change in methodology⁵. Prior approval by the NIH grants management officer is not required to make such changes unless purchase of new equipment is necessary⁶.

1. Animal Welfare Act regulations. 9 CFR. Chapter I, Subchapter A, Part 2, Subpart C.
2. US Department of Agriculture. *Animal Care Policy Manual* (USDA, Riverdale, MD, 2015).
3. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* (US Department of Health and Human Services, Washington, DC, 1986; amended 2002).
4. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
5. National Institutes of Health. Clarification on the Roles of NIH Scientific Review Groups (SRG) and Institutional Animal Care and Use Committees (IACUC) in Review of Vertebrate Animal Research. Notice NOT-OD-10-128. (National Institutes of Health, Washington, DC, September 2010).
6. National Institutes of Health. NIH Grants Policy Statement. (National Institutes of Health, Bethesda, MD, 2015).

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While everyone agrees that animal welfare is the most important priority, and while both perspectives are correct, the potential negative cost to the PI's research and laboratory is daunting.

The principles of the 3Rs, as reflected in the *Guide for the Care and Use of Laboratory Animals*, require that researchers and IACUCs consider the "availability or appropriateness of the use of less-invasive procedures" and "unnecessary duplication of experiments" when reviewing a protocol¹. Addressing these principles can be a

quick, obvious resolution, or it can be more complicated and require upfront investment by the IACUC, as is the case with Clark. With changes of this magnitude, the IACUC should first address appropriateness and, here, the effect such changes could have on research that is funded by an institution like the NIH. Significant changes in methodology should not occur in the middle of a grant; instead, the suggested change should be thoroughly investigated and implemented at an appropriate time. This approach is especially appropriate in

Clark's scenario, considering that there is no immediate concern for the welfare of animals in Clark's current research: the IACUC approved the protocol and any pain and distress is addressed and relieved.

The veterinarian, IACUC and PI should work collaboratively and consider taking a few subsequent steps. Firstly, they can carry out one or more pilot studies and evaluate the experimental context of these techniques, such as time from surgery to experimental endpoint or next surgical procedure. Secondly, they can assess the

effect of these techniques on animal welfare and on Clark's research, determining, for example, how pain and distress are measured; to what degree pain and distress are attenuated; and whether laparoscopy's effects on post-operative recovery could affect the dependent variables of Clark's studies. Thirdly, they can consider the logistics of this new technique, determining whether experts are available to provide training; whether there is sufficient time for staff to become proficient; and whether funding is available to support the equipment and training needed to implement this new technique—and if Clark does

not have sufficient funds, whether Great Eastern University can provide the necessary funding and support for his research. Finally, they can determine the number of additional USDA-regulated animals that will be required for training and for any pilot studies, and then weigh this cost against the value of the refinement.

The bottom line is that Clark should be receptive to the veterinarian's suggestion and should be proactive in finding a resolution; at the same time, the IACUC, the veterinarian and Great Eastern University should be sensitive to the significant regulatory burden that is placed on PIs,

and they should assist Clark in finding resources to support this endeavor to address the 3Rs. Any change that is enacted must be a joint effort between the PI and the university, and must minimize any loss in study time or conflict with historical data. A collegial effort is fundamental to the continued success of an institution's animal care and use program.

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1. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).

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