

Survey of the Review Process for Approved Animal Protocols and Feedback to Education and Training

Shintaro Matsuba ^{1,2)}, Ryoya Yamamoto ³⁾, Yasuhito Ishigaki ¹⁾, Hirofumi Nishizono ^{1)*}

¹⁾ *Research Support Center, Medical Research Institute, Kanazawa Medical University*

²⁾ *Pharmacy Division, Kanazawa Medical University Hospital*

³⁾ *Research Promotion Division, Kanazawa Medical University*

Abstract: Background: Laboratory animals are widely used in life sciences, research on the causes of diseases and their remedies, and to confirm their safety. Many countries have adopted laws and guidelines to ensure the ethical appropriateness of animal experiments. The Institutional Animal Care and Use Committee (IACUC) has been established at each university and research institute to monitor and manage the humane use of animal experiments. The Kanazawa Medical University (KMU) - IACUC plays a crucial role in monitoring and managing the humane use of animals in research across the university. KMU-IACUC is responsible for both animal protocol review and education and is also required to update the contents of its education and training to keep up with the latest research.

Methods: We investigated the reviewer's comments on animal experiment protocols in the KMU review process over the past three years and identified common issues in many applications by recounting the review comments of animal protocols.

Results and Conclusion: There was a trend toward more reviewer comments in the following categories: euthanasia, anesthesia, and pain control. Taken together, we have identified that new educational resources need to be developed for euthanasia, anesthesia, and pain relief. Our approach can help improve the education and training related to animal experimentation.

Key Words: animal protocols, education and training, animal experiments, institutional animal care and use committee

Introduction

Laboratory animals are widely used in life sciences, research on the causes of diseases and their remedies, and to confirm their safety. Although the use of alternative methods is increasing owing to growing public concern, it is not easy to completely replace animal experiments, and the use of laboratory animals will continue to be essential. Many countries have adopted laws and guidelines to ensure ethically appropriate animal experiments. The Institutional Animal Care and Use Committee (IACUC) has been established in each university and research institute to monitor and manage the humane use of animal experiments (1-7).

In Japan, the need for appropriate animal care and management and experimental methods in animal experiments is stated in the “Act on Welfare and Management of Animals,” the “Standards relating to the Care and Keeping and Reducing the Pain of Laboratory Animals,” and the “Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions” (8-10). In particular, the fundamental guidelines clearly state that when conducting animal experiments using experimental animals for education or research, the animal protocols must be prepared by principal investigators (PIs) and reviewed and approved by the IACUC. Animal experiments conducted at Kanazawa Medical University (KMU) are also required to comply with the law and guidelines and the PIs must submit animal protocols and must be approved by the IACUC of KMU (KMU-IACUC) before conducting the animal experiments (11).

KMU-IACUC plays a crucial role in monitoring and managing the humane use of animals in research across

* Research Support Center, Medical Research Institute, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Kahoku, Ishikawa 920-0293, Japan

Email: hirofumi@kanazawa-med.ac.jp

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the university. It was established in 1993 and advocates implementing the 3Rs that take initials of replacement, reduction, and refinement concerning animal research (12, 13), when PIs propose experiments. KMU-IACUC is organized by the president and consists of an animal facility director, qualified individuals with experience in animal experiments, talented individuals with expertise in laboratory animals and animal ethics, and other qualified individuals with academic knowledge. The KMU-IACUC reviews more than 100 animal protocols annually. In addition, it is stipulated that KMU-IACUC provides education and training for animal experiments and that PIs and other animal experimenters must obtain the latest knowledge and experience to conduct animal experiments (11). Thus, KMU-IACUC is responsible for both animal protocol review and education and is also required to update the contents of its education and training to keep up with the latest research and other developments.

This report summarizes the activities of the KMU-IACUC over the past three years. As a result of scrutinizing the reviewer comments of the members in the review process of animal protocols, it became clear that there were some tendencies.

Materials and Methods

The data were used as animal protocols submitted to Kanazawa Medical University and reviewed by the IACUC from 2019 to 2021. All experiments in the experimental protocol used in this study were based on institutional rules and regulations of the Guide for the Care and Use of Laboratory Animals (<https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>). This study was conducted following the Code of Ethics for Life Science and Medical Research Involving Human Subjects at Kanazawa Medical University.

Counting of review comments.

The number of reviewer comments was collected from the review process of approved animal protocols over the past three years and categorized by issue or keyword. The issues, including experiment protocol, reduction of pain, methods of euthanasia, species and number of animals used, distress assessment, humane endpoints, animal feeding, storage facility, mating, experimenter, animal laboratory, the purpose of animal experiments, classification of experiments, disposal of animal carcasses, and others, were selected from the animal protocol format in Kanazawa Medical University (11). For example, the comments regarding anesthesia and analgesics were included in the category of “reduction of pain.” The percentages were calculated as follows: the number of reviewer comments on the indicated issue or keywords/numbers of the total reviewer comments.

Statistics.

All statistical analyses were performed using GraphPad Prism software version 8.4.3 (GraphPad, San Diego, CA, USA).

Results

The Institutional Animal Care and Use Committee identified several issues with each animal protocol at Kanazawa Medical University.

We collected data on the review process of animal protocols conducted by the IACUC to improve the training of researchers in animal experimentation. The average number of animal protocol applications and reviews at Kanazawa Medical University was 120.3 ± 8.7 per year. During the study period, 13 protocols were withdrawn: 6 in 2019, four in 2020, and three in 2021. The reasons for the withdrawals included the closure of laboratories due to COVID-19 and changes to other animal protocols during the proposal review process. Twelve to fourteen members of the IACUC reviewed these animal protocols. The committee’s composition during this period was as follows: one director of an animal facility, 6-8 qualified individuals with experience in animal experiments, three experts in laboratory animals and animal ethics, and two other knowledgeable individuals. Experts in laboratory animals were individuals who had experience in animal experiments and specialized in veterinary medicine or laboratory animal ethics. The review process is illustrated in Figure 1. First, the protocol of the PI is

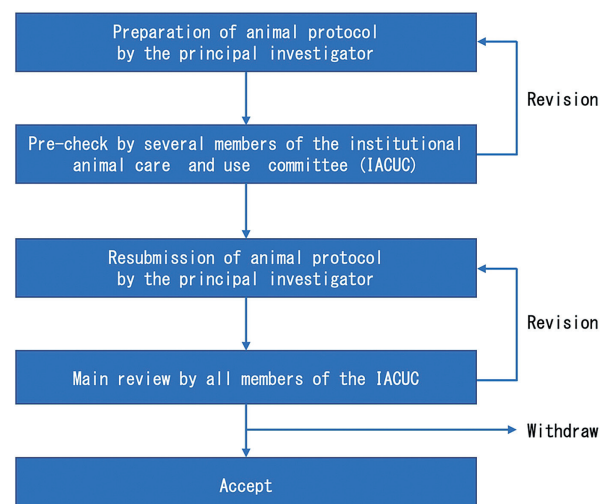


Figure 1. Review process of the animal protocol.

The animal protocols are reviewed by the Institutional Animal Care and Use Committee (IACUC). First, the protocols are pre-checked by several members of the IACUC, and if necessary, revisions are requested. Second, the resubmitted protocols are reviewed by all members of the IACUC, and only protocols that meet the criteria are approved.

reviewed by several members of the IACUC. This process is called the pre-check process. The IACUC will request a revision by the PI if problems are found during the pre-check. Then, all IACUC members review the animal protocol which passes the pre-check (called the main review). An average of 7.9 ± 5.1 revisions were noted by the IACUC per animal protocol (Fig. 2). The average number was not significantly different from year to year ($F = 2.662, P = 0.0745$, one-way ANOVA). In addition, the required for the review were 30.3 ± 38.5

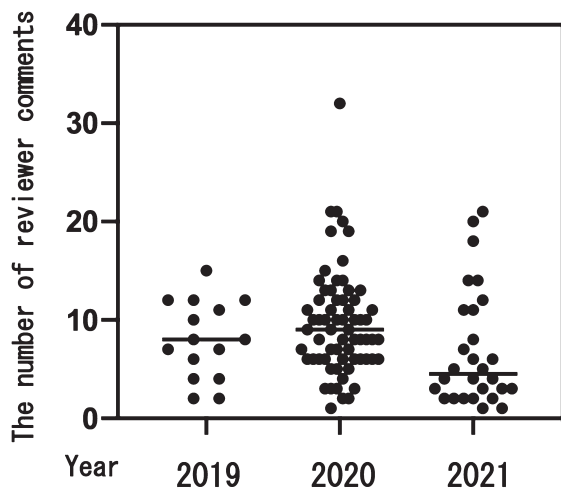


Figure 2. Total number of reviewer comments per protocol in 2019-2021. The number of reviewer comments per approved animal protocol was counted during the review process and plotted over the past three years. Circles indicate the number of comments per protocol. Horizontal bars represented the mean. The number of comments was the total number of pre-checks and main reviews. Total number of protocols was 14 in 2019, 66 in 2020, and 28 in 2021.

Table 1. Species of used experimental animals in 2019-2021.

Species	Year		
	2019	2020	2021
Mouse	10,531	10,637	9,749
Rat	1,682	1,443	908
Hamster	29	15	0
Mongolian gerbil	11	37	48
Rabbit	200	224	218
Gunia pig	29	11	46
Dog	3	0	0
Total	12,485	12,367	11,940

The number of experimental animals used, sorted by species, during the past three years, has been shown.

days in 2019, 34.3 ± 14.7 days in 2020, 65.6 ± 35.8 days in 2021. Furthermore, the number of experimental animals used annually was 11940 ± 843 during the past three years at Kanazawa Medical University (Table 1); the average number was not significantly different from year to year. To identify which parts of the training content were deficient before the animal experiments, we analyzed the major revisions pointed out by the IACUC. Although the numbers varied over the three years, the most frequent comments were about experimental methods and reducing animal pain (Fig. 3). Although the percentages ranged somewhat over the three years, the comments on experimental protocols, methods of reducing pain, and euthanasia methods accounted for 50% of the total comments in all years. Interestingly, while the number of animals used decreased yearly (Table 1), the percentage of comments on the number of animals used in the planning phase increased. The number of comments on humane endpoints also increased from year to year.

Discussion

We investigated the animal protocols at Kanazawa Medical University for the past three years and identified common issues in many applications by recounting the review process of animal protocols. In the Figure 3, the reviewer’s comments were more frequent for euthanasia, anesthesia, and pain control, indicating that the PI’s knowledge of these issues is not at the level required by the IACUC, or that the explanation in the protocol

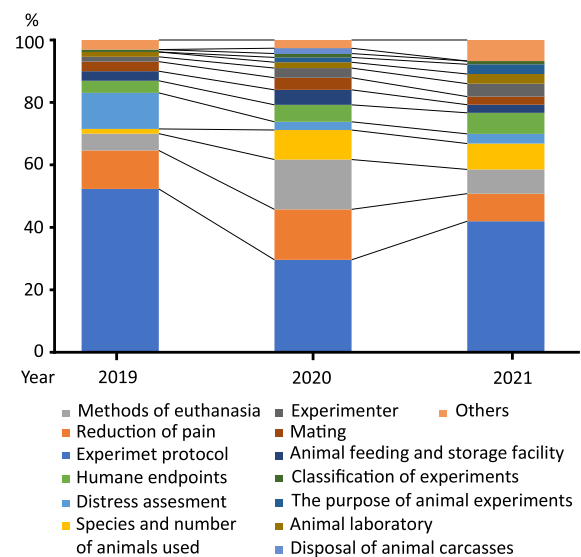


Figure 3. Stacked bar chart by category of reviewer comments in 2019-2021. Reviewer comments in the review process were sorted by category, and the percentages are shown for the indicated year. Categories were selected based on issues in the animal protocol.

is insufficient. These categories of comments relate to the refinement of 3R principles (12, 13). Pre-animal experiment education at Kanazawa Medical University is standardized by the Japanese Association of Laboratory Animal Facilities of National Corporations and the Japanese Association of Laboratory Animal Facilities of Public and Private Universities. However, our results suggest that these standard educational programs require further updates to refine individual animal experiments. These issues can be used as areas for improvement of educational processes. As in other countries where animal experimentation is reviewed based on the 3Rs principle (4-7), Japanese law also requires that animal experimentation be reviewed based on this principle⁹. The committee of Kanazawa Medical University made the IACUC review mandatory as soon as the law on animal experiments started in Japan. On the other hand, the laws related to animal testing are revised approximately every five years. When the law is changed, scientists, legislators, citizens, and others provide their opinions, and the law is revised based on these opinions. Three years have passed since our university changed its old review system following the latest legal update. We conducted this survey to understand the current problems and found scope for some improvements that could be made in the education and training of PIs who prepare animal experimental plans. These improvements are mainly related to education on refinement, which we believe can be solved by providing the IACUC with information on anesthesia and euthanasia methods, the latest research on experimental methods, etc.

Our study was limited to scrutinizing the animal protocol review process at Kanazawa Medical University during the past three years. We must carefully consider whether these trends are similar in other universities. However, verification of applications can be conducted by different institutes and universities. Thus, these findings could help improve the education and training related to animal experimentation.

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Conflict of Interest

All authors declare no COI for disclosure.

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