ABSTRACT

Objective: To improve the standards of animal welfare, as well as scientific quality, good education and training is necessary for implementing reductions, refinements and replacement alternatives (3Rs). Ethical justification of laboratory animal use by our institutional committee has served to achieve a greater acceptance of the concept of humane experimental techniques.

Methods: In this retrospective study, a five year follow-up of data collected within Institutional Animal Use and Care Committee was evaluated.

Results: Our results show that reviewing the protocols and giving feedback to researchers has an educational value in improving the skills and attitude of persons involved in the design and performance of animal experiments.

Conclusion: Therefore, while giving education or training, the use of laboratory animals should be reviewed thoroughly, not only for the application of the 3Rs but also for further education of those involved in or responsible for animal use.

Key Words: Animal welfare, Pain, 3Rs, Animal ethics

INTRODUCTION

Concern for the well-being of animals used in research, testing and education encouraged scientists to develop comprehensive standards to assure the most humane treatment of animals possible without compromising scientific validity (1, 2). According to the guiding principles developed by the New York Academy of Sciences, each organization which uses animals should establish an Institutional Animal Care and Use Committee to review all proposals related to the use of animals. Based on these guidelines, Marmara University School of Medicine Animal Care and Use Committee (MUSM-ACUC) was established following its approval by the Faculty Executive Committee on June 6, 1996. Since then, the committee is responsible for reviewing the proposed uses of animals in research and education. MUSM-ACUC asks for the revisions of the protocols, unless the committee is convinced that the proposed protocol is essential for achieving research objectives.

The Council members must be convinced that no alternatives to avoid the use of animals exist and the most appropriate, simple species in minimum numbers will be used at procedures with minimum severity and pain. To ascertain the use of animals within ethical standards, researchers are asked to apply to the MUSM-ACUC before starting their experiments. Since December
2000, a two-day institutional training course has been organized for research assistants who are planning to perform experiments using laboratory animals during their training programs or in their future academic activities. A total of 6 certificated courses were given to a total of 72 trainees. In this course, lectures about the biology, housing and husbandry of rodents used in experimental studies, handling, methods of drug administration, procedures of collecting blood samples and body fluids, basic concepts of anesthesia and choice of anesthetic agents, general rules of laboratory work, including the disposal of waste products, are given. Guided practices are performed on trained rats to acquire skills on the methods of handling, drug administration and collection of blood samples. Methods of euthanasia and various experimental surgical procedures are demonstrated using video-recordings.

The aim of our study was to evaluate the application forms given over a 5 year period to demonstrate the animal use profile of Marmara University School of Medicine and to look for the impact of the course given to scientists before their applications, on the use of animals.

MATERIALS AND METHODS

Study
This is a retrospective study performed by analyzing the data collected from the application forms given to the Marmara University School of Medicine ACUC over 5 years (1997 to 2001).

Animal Care and Use Committee
The MUSM-ACUC is composed of a pharmacologist, a physiologist, a veterinary physician, a medical deontologist and a general surgeon. The first three members are also in the organization committee of the Animal House. The first two members of the committee have previously received personal Home Office licenses in the United Kingdom (1995, Liverpool and 1996, Birmingham) and the veterinary physician has received a certificate at a course organized in Turkey by the Home Office (May 1998).

The committee meets at least twice a year or upon specific issues that require discussion. The committee secretary collects the evaluation forms filled by the members to give an approval letter, to ask for revisions or to reject the proposed study.

Collection of Data
At the Marmara University School of Medicine, researchers who are willing to perform experimental studies using laboratory animals should apply to Marmara University School of Medicine Animal House for their animal requests; but if the Animal House cannot supply the requested species or if the number of requested animals are beyond the present stocks of animals, the researchers can obtain the animals from other sources to be kept in the Animal House. In either case, the researchers are asked to supply a letter of approval from the MUSM-ACUC. Moreover, if an approval letter is not included, the study may not be published as a thesis or it may not be presented at an international congress.

The researchers should fill the standard application forms, which ask for information about the species, strain, sex, weight, and number of the requested animals together with the experimental protocols and the details of all experimental procedures. Each application form must be accompanied by the Turkish translation of the guiding principles developed by The New York Academy of Sciences, signed by all researchers who accept that the principles are understood, and the committee must be informed of any change in the experimental protocol.

The forms of all applications, making a total of 183 research projects over a 5 year period, were examined and the data were analysed. The species, strain, sex, weight and number of the animals stated in the application forms were compared with the recordings of the Animal House, in order to analyse the actual data of used animals. Moreover, the revised forms of protocols that were changed upon the suggestions of the ACUC members were taken into consideration.

Statistical Analysis
The data are expressed as percentages and the statistical analysis was performed using $\chi^2$ test, where $P<0.05$ was accepted as significant.
RESULTS

The analysis of the animal species preferred by the researchers in respect to years showed that rats were the most commonly preferred laboratory animals, used at a ratio of 67-92%. Rabbits, guinea-pigs, mice, dogs and pigs in total were used at 8 to 33% (Table I).

Sixty per cent of 183 studies used animals of both sexes, while 17 and 23 per cent of the remainder used females and males, respectively (Fig. 1). The researchers preferred mature rabbits, mice and guinea-pigs, but requested rats with variable body weights. Sixty-three per cent of the projects performed within 5 years used rats between 200-300 g of body-weight, while 21 per cent of the studies requested rats <200g and 16 per cent used rats >300g of body-weight.

The number of experimental animals used per study between years 1997 and 2001 are summarized in Table II. The comparison of groups that used animals below 60/ study and above 60/ study did not produce a significant difference ($\chi^2 = 0.9; P = 0.2$). The comparison of the studies before and after the establishment of the institutional atelier training did not show any statistically significant difference, where there was at least one researcher in the group having a certificate of training ($\chi^2 = 1.2; P = 0.1$) (Fig. 2).

In the present studies, ketamine and chlorpromazine mixture was the most commonly used agent for anesthesia (44%). Brief ether anesthesia was the second most commonly preferred anesthesia (20%). The percentage of the use of xylazine, pentobarbital, urethane and other miscellaneous agents were 18%, 8%, 9% and 5%, respectively. The anesthetic choice in the studies performed in the years before and after the atelier training did not differ significantly among the anesthetic agents ($\chi^2$ and $P$ values = 0.2 and 0.3; 0.2 and 0.3; 0.4 and 0.3; 0.3 and 0.4; 0.7 and 0.2; 0.5 and 0.2 for ketamine and chlorpromazine mixture, ether, pentobarbital, urethane, xylazine and miscellaneous agents, respectively).

Methods of drug administration were analyzed and intraperitoneal injection was found to be the most commonly preferred method (54%). Fifty five percent of the intraperitoneal injections were made for the administration of anesthetic agents, while the remaining were to give the test compounds. The percentage of intravenous, subcutaneous, intramuscular, intracerebroventricular injections were as 14%, 7%, 11% and 5%, respectively. In 9% of the studies other methods of administration (e.g. orogastric) were used.

Experimental procedures that could potentially cause distress in the animals were given specific consideration in order to reduce unnecessary pain or distress. In 183 studies, 10 studies had experimental designs that were expected to cause more than minimal pain. Burn injuries (8 in 183) or bone fractures (3 in 183) were produced on the anesthetized animals. Five studies were designed with experimental procedures to induce psychological stress.

<table>
<thead>
<tr>
<th>Species</th>
<th>1997 n %</th>
<th>1998 n %</th>
<th>1999 n %</th>
<th>2000 n %</th>
<th>2001 n %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat1</td>
<td>21 67 %</td>
<td>43 92 %</td>
<td>24 73 %</td>
<td>29 77 %</td>
<td>25 73 %</td>
</tr>
<tr>
<td>- Sprague-Dawley</td>
<td>9 29 %</td>
<td>21 45 %</td>
<td>13 34 %</td>
<td>15 40 %</td>
<td>8 23 %</td>
</tr>
<tr>
<td>- Wistar albino</td>
<td>12 38 %</td>
<td>22 47 %</td>
<td>11 39 %</td>
<td>14 37 %</td>
<td>17 50 %</td>
</tr>
<tr>
<td>Rabbit</td>
<td>6 19 %</td>
<td>1 2 %</td>
<td>4 12 %</td>
<td>1 2 %</td>
<td>3 9 %</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>2 7 %</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mice</td>
<td>2 7 %</td>
<td>2 4 %</td>
<td>3 9 %</td>
<td>7 18 %</td>
<td>6 18 %</td>
</tr>
<tr>
<td>Other2</td>
<td>-</td>
<td>1 2 %</td>
<td>2 6 %</td>
<td>1 3 %</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>31 100 %</td>
<td>47 100 %</td>
<td>33 100 %</td>
<td>38 100 %</td>
<td>34 100 %</td>
</tr>
</tbody>
</table>

1 Two different inbred strains are available at the Animal House. Numbers and percentages are expressed as totals.

2 Mongrel dogs, pigs.
Fig. 1: The distribution of gender preference of all listed species in 183 studies performed within 5 years at MUSM.

Table II: Numbers and the percentages of animals used in 183 studies performed at Marmara University School of Medicine in the years between 1997 and 2001.

<table>
<thead>
<tr>
<th>Number of Animals used per Study</th>
<th>Years</th>
<th>1997</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>Total</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>n</td>
<td>15</td>
<td>10</td>
<td>9</td>
<td>12</td>
<td>14</td>
<td>60</td>
<td>33</td>
</tr>
<tr>
<td>30-60</td>
<td>n</td>
<td>12</td>
<td>25</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>60-100</td>
<td>n</td>
<td>3</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>3</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>&gt;100</td>
<td>n</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

1 Independent of the species and/or strain.

Fig. 2: The percentages of the studies performed before and after the establishment of the atelier study. The ranks are defined according to the number of animals used per study.

Table III: Numbers of studies with different recovery periods following surgical procedures between 1997 and 2001.

<table>
<thead>
<tr>
<th>Recovery Period (days)</th>
<th>Years</th>
<th>1997 - 1999</th>
<th>2000 - 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>1-7</td>
<td>27</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>&gt;15</td>
<td>55</td>
<td>67</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>100</td>
<td>40</td>
</tr>
</tbody>
</table>

In the rat studies where surgical intervention was planned, deprivation of food before the surgery decreased from 26% to 19% after the atelier training was put into practice. No statistically significant difference was found between the studies performed before and after the training. Post-operative care was given to the animals in 38% of the studies performed before the atelier education, where the percent of the post-operative care decreased to 17% after the atelier education. The comparison of studies before and after the atelier education showed a statistical significance ($\chi^2 = 6.016; P = 0.0142; df = 1$).

A wide spectrum of surgical procedures was described in the studies. Forty-seven percent of the studies used abdominal surgery. Thoracic surgical interventions comprised 22% of the studies involving surgical applications. Stereotaxic methods were performed in 18% of the studies. Extremity surgery was planned in 8% of the protocols and skin incision was used only in 5% of the studies. The number of days required for the recovery of the animals following surgery are listed in Table III.

As euthanasia was concerned, 63% of the studies used high doses of chemical anesthetic agents to sacrifice animals and 35% used cervical dislocation or decapitation. In two percent of the studies, anesthetized animals were sacrificed by other means such as bleeding from the heart or large vessels.

**DISCUSSION**

The results of the present study show that mostly rats are used in experiments. This is in accordance with the statistics that mice and rats account for about 80-90% of the animals used for testing and research (3, 4). It is one of the principles outlined by the New York Academy of
Training and animal use

Sciences (1) that use of the least complex species must be encouraged. Moreover, Animal House does not supply primates, but gives care to pigs, rabbits and dogs if purchased by the scientists. Since not easily available, these species are not preferred.

Much has been achieved since the publication of the 3Rs (5) and they have been widely adopted as the principle guide for the use of animals in experiments. As stated in the guidelines, consideration should be given to equal use of females and males (1, 6). In the present study, it is also evident that both sexes are used in most of the studies, due to the guidance of the ACUC. Moreover, young adults are preferred by the scientists. Another rule, which received specific consideration from the ACUC, is to reduce the use of animals to a minimum as appropriate. Our results show that the institutional training course organized for the research assistants does not appear to have any significant effect on the number of animals used per study. Less than 1/4 of all studies used above 60 animals per study either before or after the training. This may be explained by the fact that before the course the protocols were already guided by the ACUC in order reduce the animal use. However, the protocols of scientists, who have taken the course before, required less revision than those non-trained researchers. Similarly, one of the major issues of concern when reviewing experimental schedules was to prevent pain and distress. In the 5 year follow-up period, it is documented that a few studies had protocols causing stress or pain, where the maximum degree and duration of distress were clearly specified. In order to control pain and distress, it is advised to use appropriate anesthetic, analgesic and pain assessment regimens (7). In the present research, mostly ketamine-chlorpromazine mixture was preferred. This injectable surgical anesthesia is a useful technique providing a rapid recovery from anesthesia, which improves the animals post operative body temperature, food and fluid intake more quickly (7). Ether anesthesia was permitted for only short procedures such as orogastric or intravenous drug administration, but not for surgical anesthesia. The atelier training did not have a significant effect on the choice of anesthetic drugs, since the choices were evaluated by the ACUC throughout the 5 year study period.

Our results demonstrate that nearly half of the studies involve abdominal surgery. This explains why there is a high percentage (19-26%) of food deprivation before surgery, while mostly rodents are used. No long-term food deprivation was proposed in any of the studies. The percentage of animals, which received post-operative care, was significantly reduced with the implementation of the atelier education. This correlates with the reduction in the percentage of long term studies in the favor of studies to be completed in a week. Most of these short-term studies were completed promptly in the first 24 hours. Method for euthanizing an animal was also given specific consideration by the ACUC, in order to assure minimal physical and psychological suffering with a minimum duration. Most of the animals were killed while under anesthesia, while in 35% prompt killing by trained researchers was performed.

It is well known that to ensure the high standards of animal welfare, as well as scientific quality, the significance of the 3Rs needs greater recognition (8). Furthermore, good education and training for those involved in or responsible for animal use is a vital issue (9). In conclusion, ethical justification of laboratory animal use by our institutional committee has served to achieve a greater acceptance of the concept of humane experimental techniques. Therefore, while giving education or training, all use of laboratory animals should be reviewed thoroughly for implementing reductions, refinements and replacement alternatives. Reviewing the protocols and giving feedback to researchers has an educational value in improving the skills and attitude of persons involved in the design and performance of animal experiments.

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