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Vital signs monitoring during injectable and inhalant anesthesia in mice

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Abstract: Selecting the appropriate anesthetic protocol for the individual animal is an essential part of laboratory animal experimentation. The present study compared the characteristics of four anesthetic protocols in mice, focusing on the vital signs. Thirty-two male ddY mice were divided into four groups and administered anesthesia as follows: pentobarbital sodium monoanaesthesia; ketamine and xylazine combined (K/X); medetomidine, midazolam, and butorphanol combined (M/M/B); and isoflurane. In each group, rectal temperature, heart rate, respiratory rate, and O₂ saturation (SPO₂) were measured, and the changes over time and instability in these signs were compared. The anesthetic depth was also evaluated in each mouse, and the percentage of mice achieving surgical anesthesia was calculated. K/X anesthesia caused remarkable bradycardia, while the respiratory rate and SPO₂ were higher than with the others, suggesting a relatively strong cardiac influence and less respiratory depression. The M/M/B group showed a relatively lower heart rate and SPO₂, but these abnormalities were rapidly reversed by atipamezole administration. The pentobarbital group showed a lower SPO₂, and 62.5% of mice did not reach a surgical anesthetic depth. The isoflurane group showed a marked decrease in respiratory rate compared with the injectable anesthetic groups. However, it had the most stable SPO₂ among the groups, suggesting a higher tidal volume. The isoflurane group also showed the highest heart rate during anesthesia. In conclusion, the present study showed the cardiorespiratory characteristics of various anesthetic protocols, providing basic information for selecting an appropriate anesthetic for individual animals during experimentation.

Key words: isoflurane, ketamine, M/M/B (medetomidine, midazolam, and butorphanol combined), pentobarbital, rodent

Introduction

When following the basic principles of laboratory animal welfare, the selection of an appropriate and effective anesthetic protocol for each individual animal is an essential part of laboratory animal experimentation. Laboratory mice are anesthetized by either inhalation of

volatile anesthetics or injection of drugs. Injectable anesthesia is often administered in rodents through the subcutaneous or intraperitoneal route. Injectable anesthesia during rodent surgery can be achieved using a mixture of ketamine and sedatives or using medetomidine-based balanced anesthesia [2, 16, 17]. Inhalant anesthetics available for use in laboratory animals in-

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clude halothane, isoflurane, sevoflurane, and desflurane [1, 10, 12]. Among them, isoflurane is most the common inhalant anesthetic used in rodents [22]. Inhalant anesthesia is highly demanded in rodents because the anesthetic depth can be easily controlled.

Along with compliance with the principles of laboratory animal welfare, it is also important to choose an appropriate anesthesia for experimental intervention because it can influence the experimental data [10]. One factor in selecting an anesthetic is the required level of anesthetic depth, that is, muscle relaxation, analgesia, and hypnotic action, which varies depending on the surgical procedure. Another important factor is the potential for adverse reactions. Administration of anesthetic agents induces systemic effects on the neuronal, cardiorespiratory, metabolic, and immune systems [4, 19, 29]. Among them, cardiovascular and respiratory depression is a major adverse reaction. The particular cardiovascular abnormalities vary among anesthetic protocols [4, 10]. Therefore, understanding the effect of anesthesia on cardiorespiratory adverse reactions is important when selecting the anesthetic for laboratory animal experimentation. Cardiovascular and respiratory function can be assessed by monitoring vital signs, which include the heart rate, respiratory rate, blood pressure, and O₂ saturation (SPO₂).

Although mice have often been used in basic anesthesiology studies, the safety of mouse anesthesia for experimental use has not been fully evaluated. The main purpose of the present study was to compare the features of injectable and inhalant anesthetics in mice, focusing on vital signs. Cardiorespiratory influence was evaluated for four anesthetic protocols, including ketamine xylazine combined, medetomidine-based balanced anesthesia, pentobarbital sodium monoanesthesia, and isoflurane. The variance in anesthetic depth for each medication was also evaluated. The aim of the present study was to better understand murine anesthesia.

Materials and Methods

Animals

Thirty-two male ddY mice aged 7 weeks and weighing 33.4–36.0 g were obtained from a commercial vendor (SLC:ddY, Japan SLC Inc., Shizuoka, Japan). We selected ddY mice in the present study, since they have been used in animal experimentation, including pharmacological and toxicological trials. The animals were ac-

climated to the facility for 1 week before experimentation and kept in polycarbonate cages (CL-0106–1; 310 mm × 360 mm × 175 mm; CLEA Japan Inc., Tokyo, Japan) with wood shavings. The animals were kept in a room equipped with a barrier system at the Research Institute of Biosciences, Azabu University. The room was air-conditioned at a temperature of 22 ± 1°C and a humidity of 55 ± 5% and was lit 14 h each day from 06:00 to 20:00. Mice were fed a pelleted mouse diet (mouse and rat chow; MC-2, CLEA Japan, Tokyo, Japan) *ad libitum* and had unrestricted access to sterilized drinking water provided in a water bottle. All animals were examined and deemed clinically healthy before use in the study. All experiments were conducted when the mice were 8 weeks of age. To minimize the influence of the circadian rhythm, experiments and weighing procedures were performed between 10:00 and 13:00. At the study conclusion, the subjects were euthanized by cervical dislocation after intraperitoneal administration of pentobarbital sodium (Somnopentyl, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan) at 100 mg/kg. All procedures in the present study were in accordance with the guidelines approved by the Animal Research Committee of Azabu University.

Anesthesia

The anesthetic, sedative, and analgesic agents used in the present study were as follows: ketamine hydrochloride (Ketalar, Sankyo Lifetech Co., Ltd., Tokyo, Japan), xylazine (Celactar, Bayer Yakuhin Ltd., Tokyo, Japan), pentobarbital sodium (Somnopentyl, Kyoritsu Seiyaku Co., Ltd.), medetomidine hydrochloride (Domitol, Meiji Seika Pharma Co., Ltd., Tokyo, Japan), midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan), butorphanol (Vetorphale, Meiji Seika Pharma Co., Ltd.), and isoflurane (Isoflu, DS Pharma Animal Health Co., Ltd., Osaka, Japan). All agents were kept at room temperature before use. Animals were divided into four groups corresponding to each anesthetic protocol as follows: ketamine hydrochloride and xylazine combined (K/X; ketamine hydrochloride 100 mg/kg and xylazine 10 mg/kg); pentobarbital monoanesthesia (50 mg/kg); medetomidine, midazolam, and butorphanol combined (M/M/B; medetomidine 0.3 mg/kg, midazolam 4 mg/kg, and butorphanol 5 mg/kg); and inhalant anesthesia using isoflurane (5% for induction and 2% for maintenance). In the M/M/B group, mice were administered atipamezole (Antisedan, Zoetis Japan Inc., Tokyo, Japan) at a dose



Fig. 1. Vital signs monitoring during isoflurane anesthesia in mice. The rectal probe and pulse oximeter are located at the colorectum and tail base, respectively.

of 0.3 mg/kg 30 min after the administration of M/M/B. All injectable anesthetics were administered intraperitoneally. The dose and concentration of each agent were as reported previously in mice [4, 5, 17]. Before administration, the concentration of M/M/B, K/X, and pentobarbital sodium was adjusted to 6 ml/kg by diluting with saline. In the M/M/B anesthetic group, a mixture of medetomidine, midazolam, and butorphanol with saline was prepared and then concurrently administered. Similarly, the mixture of ketamine hydrochloride and xylazine was adjusted with saline before concurrent administration.

Isoflurane anesthesia was administered using a commercially available rodent inhalant anesthesia apparatus (SomnoSuite Small Animal Anesthesia System, Kent Scientific Corporation), which has a digital vaporizer and internal air-flow pump. The vaporized anesthetic gas was introduced into the induction chamber and nose mask (Kent Scientific Corporation) at a flow rate of 32 ml/min. The nose mask was covered with a latex membrane that had a hole in the center to fit closely around the nose. Initially, mice were induced with isoflurane at a 5% concentration. Once loss of the postural reaction and righting reflex was confirmed, the mice were rapidly transferred to the nose mask, and anesthesia was maintained with 2% isoflurane (Fig. 1).

Vital signs monitoring

Vital signs were measured before anesthesia and every 5 min during a 40 min period. Rectal temperature, heart rate, respiratory rate, and SPO₂ were evaluated in each animal. Rectal temperature (°C) was measured using a

commercial rectal temperature sensor (Right Temp, Kent Scientific Corporation) inserted into the colorectum. The heart rate (beats/min) and SPO₂ (%) were assessed using a rodent pulse oximeter and heart rate monitor (Mous-eSTAT, Kent Scientific Corporation), with the pulse oximeter sensor attached to the tail base (Fig. 1). The respiratory rate (breaths/min) was assessed by counting the number of thoracic movements per min. To measure the baseline values, the mice were introduced into animal holders. After beginning anesthesia, the mice were positioned on a nylon pad to maintain a consistent surface temperature underneath them.

Following vital signs monitoring, the trend in vital signs over time was compared between each group. The instability of vital signs was analyzed using the coefficient of variance (CV) in each individual mouse and the calculated mean CV per parameter in each group.

Assessment of anesthetic depth

Anesthetic depth in each group was assessed with noxious stimuli as previously reported in mice [17]. Four reflexes were evaluated, the pedal withdrawal reflex in the forelimbs and hind limbs, the tail pinch reflex, and the eyelid reflex. Noxious stimuli were performed using atraumatic forceps. The anesthetic depth score was determined based on the number of reflex reactions; if no reflexes to stimuli were observed, the score was 4. Anesthetic depth was measured every 10 min for 30 min after administering the agents. Scores of 3 and 4 were defined as indicating a surgical anesthetic depth.

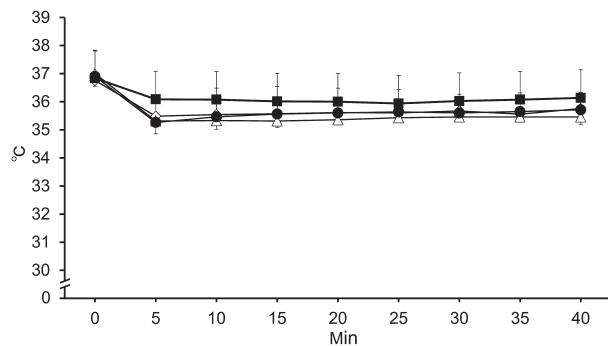


Fig. 2. Measured core body temperature over time during each anesthetic protocol.

△: Ketamine and xylazine combined anesthesia (K/X, 80/8 mg/kg). ◇: Medetomidine, midazolam, and butorphanol combined anesthesia with atipamezole (M/M/B, 0.3/4.0/5.0 mg/kg; atipamezole, 0.3 mg/kg). ■: Pentobarbital sodium (50 mg/kg). ●: Isoflurane (induction, 5%, maintenance, 2%). Data are represented as means \pm SD of 8 mice. There were no significant differences between the groups.

Data analyses

A repeated measures ANOVA was used to analyze each vital sign value. When data were significant, Dunnett's multiple comparison method was performed to compare each parameter between the baseline and other time points. The differences between the treatments at each time point were analyzed using Tukey's test. Instability of each vital sign in each group was compared with ANOVA, followed by Tukey's test. The rate mice reached a surgical anesthetic depth in each group was compared using Fisher's exact test. Data were expressed as the mean \pm SD. A P -value < 0.05 was considered significant. All analyses were performed using commercially available software (StatMate IV; ATMS Co., Ltd., Tokyo, Japan).

Results

During the study period, there were no fatal events, and anesthesia was induced in all animals within 5 min; thus vital signs were measured beginning 5 min after drug administration. Baseline differences between the groups in each parameter were not statistically significant.

The trend in rectal temperature over time is shown in Fig. 2. All groups showed a significant decrease in rectal temperature during the entire anesthetic period. There was no significant difference between the groups. Next, pulse rate was evaluated in each group. The results

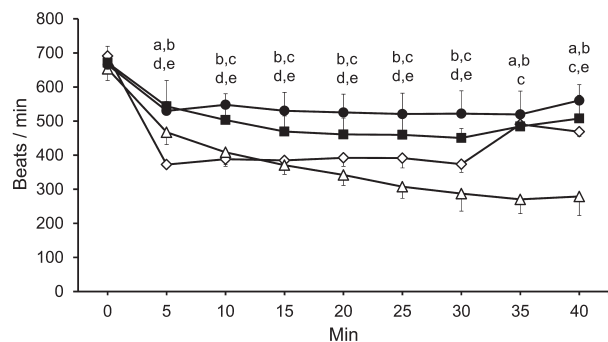


Fig. 3. Measured heart rate (beats/min) over time.

△: Ketamine and xylazine combined anesthesia (K/X, 80/8 mg/kg). ◇: Medetomidine, midazolam, and butorphanol combined anesthesia with atipamezole (M/M/B, 0.3/4.0/5.0 mg/kg; atipamezole, 0.3 mg/kg). ■: Pentobarbital sodium (50 mg/kg). ●: Isoflurane (induction, 5%; maintenance, 2%). Data are represented as means \pm SD of 8 mice. There were significant differences between the groups as follows: a, K/X and M/M/B groups; b, K/X and pentobarbital groups; c, K/X and isoflurane groups; d, M/M/B and pentobarbital groups; and e, M/M/B and isoflurane groups.

showed that a decreased heart rate was observed beginning 5 min after drug administration in all groups (Fig. 3). When compared to the isoflurane and pentobarbital groups, the K/X and M/M/B groups had significantly lower heart rates. In the M/M/B group, the heart rate was significantly increased 5 min after administering atipamezole (373 ± 24 beats/min at 30 min, 491 ± 17 beats/min at 35 min). The heart rate decrease in the K/X group was time dependent, and the heart rate did not recover during the study period.

To assess the influence of respiratory function, respiratory rate and SPO_2 were compared in each group. A decreased respiratory rate was observed in all groups during the anesthetic period (Fig. 4). The decrease was marked in the isoflurane group, and the difference was significant at 5–35 min compared with the injectable anesthesia groups. However, the respiratory rate recovered in the isoflurane group 5 min after discontinuing inhalation (78 ± 19 breaths/min at 35 min, 150 ± 49 breaths/min at 40 min, $P < 0.05$). The course of SPO_2 over time in each group is shown in Fig. 5. Each anesthetic group showed a significant decrease in SPO_2 after initiation of anesthesia. In the M/M/B, pentobarbital, and isoflurane groups, the SPO_2 was significantly decreased at 5–35 min compared with baseline. In the M/M/B group, a significant increase of SPO_2 value was observed 10 min after the administration of atipamezole ($87.8 \pm 1.6\%$ at 30 min, $95.7 \pm 1.2\%$ at 40 min, $P < 0.05$). The

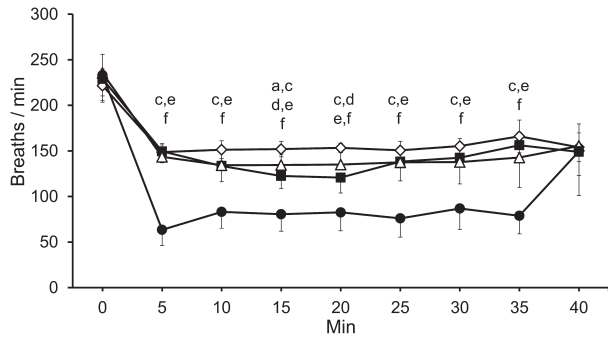


Fig. 4. Measured respiratory rate (breaths/min) over time. Δ : Ketamine and xylazine combined anesthesia (K/X: 80/8 mg/kg). \diamond : Medetomidine, midazolam, and butorphanol combined anesthesia with atipamezole (M/M/B: 0.3/4.0/5.0 mg/kg, atipamezole: 0.3 mg/kg). \blacksquare : Pentobarbital sodium (50 mg/kg). \bullet : Isoflurane (induction, 5%; maintenance, 2%). Data are represented as means \pm SD of 8 mice. There were significant differences between the groups as follows: a, K/X and M/M/B groups; c, K/X and isoflurane groups; d, M/M/B and pentobarbital groups; and e, M/M/B and isoflurane groups; and f, pentobarbital and isoflurane groups.

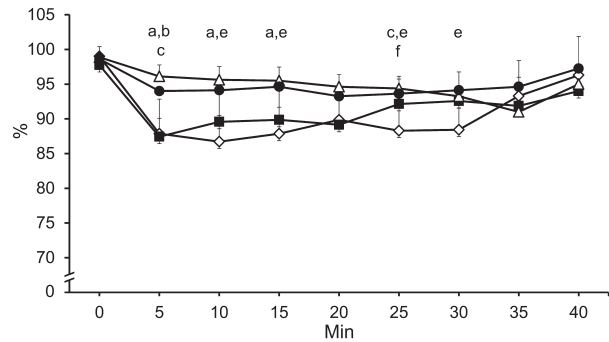


Fig. 5. Measured SPO₂ percentage over time. Δ : Ketamine and xylazine combined anesthesia (K/X, 80/8 mg/kg). \diamond : Medetomidine, midazolam, and butorphanol combined anesthesia with atipamezole (M/M/B, 0.3/4.0/5.0 mg/kg, atipamezole, 0.3 mg/kg). \blacksquare : Pentobarbital sodium (50 mg/kg). \bullet : Isoflurane (induction, 5%; maintenance, 2%). Data are represented as means \pm SD of 8 mice. There were significant differences between the groups as follows: a, K/X and M/M/B groups; b, K/X and pentobarbital groups; and e, M/M/B and isoflurane groups.

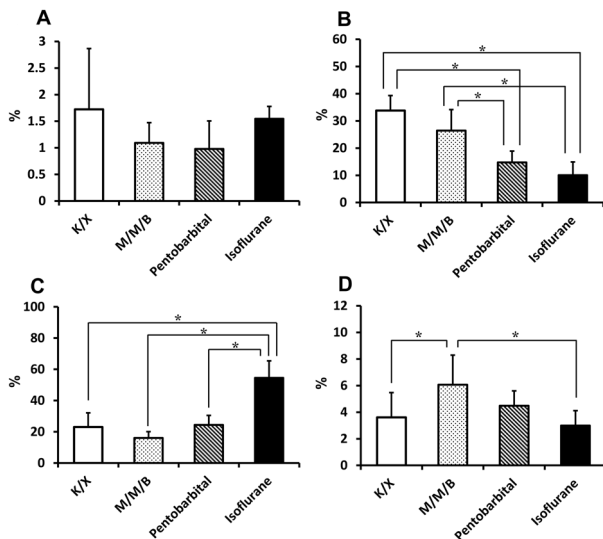


Fig. 6. Instability in vital signs observed in each anesthetic group. A: Rectal temperature. B: Heart rate. C: Respiratory rate. D: SPO₂. Data are represented as means \pm SD of 8 mice.

K/X group showed a significant SPO₂ decrease only at 35–40 min after anesthesia (98.9% \pm 0.1% at baseline, 91.0% \pm 1.6% at 35 min, 95% \pm 1.3% at 40 min, $P < 0.05$). Compared with the other injectable anesthetic groups, the K/X group showed significantly high SPO₂ values. The M/M/B group showed lower SPO₂ values compared with those of the isoflurane group.

The mean \pm SD of the CVs of each vital sign in each anesthetic group are shown in Fig. 6. Regarding the CV

Table 1. Rate of achieving surgical anesthetic depth (%) for the four anesthetic protocols performed on mice

Anesthetic protocol	Mice achieving surgical anesthesia (%)
K/X	100
M/M/B	100
Pentobarbital	37.5
Isoflurane	100

for rectal temperature, no significant differences were observed among the groups (Fig. 6A). The K/X and M/M/B groups showed higher variances in heart rate compared with the pentobarbital and isoflurane groups (Fig. 6B). The CV of the respiratory rate was significantly higher in the isoflurane group compared with other groups (Fig. 6C). In the MM/B group, the CV was significantly higher than those of the K/X and isoflurane groups (Fig. 6D).

Finally, we evaluated the rates of mice achieving a surgical anesthetic depth in each treatment group (Table 1). When anesthetized with pentobarbital, 62.5% of mice did not reach a surgical anesthetic depth. In contrast, the remaining anesthetic protocols achieved a surgical anesthetic depth at least once during the study period. The rate of mice reaching a surgical anesthetic depth in the pentobarbital group was significantly lower than that in the other 3 groups ($P < 0.05$).

Discussion

The present study demonstrated the characteristics of three injectable anesthetics and one inhalant anesthetic in mice, specifically the changes in vital signs. Each anesthetic protocol showed different effects on cardiovascular and respiratory parameters.

The vital signs were first evaluated in mice administered a mixture of ketamine and xylazine. For surgical anesthesia in rodents, ketamine should be coadministered with a sedative such as xylazine, acepromazine, or diazepam [4, 23]. Among these options, a combination of ketamine and xylazine was selected because it is one of the most frequently used anesthetic combinations in rodents [2, 4, 16]. Compared with other agents, K/X provides a relatively stable respiratory rate and SPO₂, suggesting lessened respiratory depression. In contrast, the heart rate was relatively unstable compared with the rates obtained with other injectable anesthetics such as pentobarbital and M/M/B. The heart rate decrease was time dependent, and no recovery was observed during the study period. A previous study demonstrated that the combination of ketamine and xylazine mediates remarkable hypotension in mice [4]. Taken together, cardiovascular abnormality, rather than respiratory depression, represents the major adverse reaction to K/X in mice. In the present study, the doses of ketamine and xylazine were set to 100 and 10 mg/kg, respectively. Although the cardiac influence was relatively strong, K/X at the present dose results in lower respiratory depression and a sufficient anesthetic depth. The reported doses of K/X in mice range from 60 to 150 mg/kg ketamine and 4.1 to 20 mg/kg xylazine [4]. As the required dose depends on the experimental conditions, monitoring of vital signs under other doses may be important, along with evaluation of anesthetic depth.

The combination of midazolam, butorphanol, and medetomidine in rodent experimental anesthesia is comparatively new [17, 18]. The efficacy of this anesthetic combination has also been reported in dogs, sea lions, and monkeys [15, 25, 28]. Anesthesia with this combination is reversed with the α 2-adrenergic antagonist atipamezole, which antagonizes the medetomidine [28]. The present study demonstrated the effect of M/M/B on the vital signs, as well as the reversal effect of atipamezole. M/M/B anesthesia resulted in a marked decrease in heart rate. In dogs, the heart rate markedly decreases when M/M/B is administered, while the blood pressure in-

creases [14]. A previous report in monkeys demonstrated that M/M/B administration decreases the SPO₂. Although the respiratory rate variance was relatively stable in the present study, the SPO₂ decrease was marked, suggesting a lower tidal volume during M/M/B anesthesia. However, these abnormal vital signs rapidly recovered following atipamezole administration. Therefore, the cardiac and respiratory depression observed in mice seems to be mainly associated with the action of medetomidine. Use of atipamezole will ensure the safety of M/M/B anesthesia in mice. We clarified the features of M/M/B and the reversal action of atipamezole in mice, especially its cardiorespiratory influence. Based on the present findings, comparison of M/M/B with representative anesthetic protocols in other rodents may be warranted.

It is said that pentobarbital monoanesthesia causes severe adverse cardiorespiratory reactions and has poor analgesic action [9]. In the present study, we adjusted the dosage of pentobarbital sodium to 50 mg/kg, which is a relatively low dose in mice [10]. As a result, the heart rate during pentobarbital anesthesia was higher than that during K/X. Stringent comparison of the cardiovascular impacts during pentobarbital anesthesia and other injectable anesthetics may be achieved by blood pressure evaluation. Regarding the respiratory rate, there was no significant difference between K/X and pentobarbital monoanesthesia. However, the SPO₂ during pentobarbital monoanesthesia was lower than that during K/X, indicating a lower tidal volume. Therefore, pentobarbital monoanaesthesia at this dose resulted in higher respiratory depression than K/X. In the present study, 62.5% of mice experienced an inadequate anesthetic depth under pentobarbital. Similarly, in previous reports, administration of pentobarbital at 50 mg/kg did not induce an adequate anesthetic depth in rodents [17, 23].

Isoflurane is one of the most commonly used volatile anesthetics in laboratory rodents [6]. The heart rate decrease with isoflurane was lower than that observed in the injectable anesthetics, suggesting that isoflurane produces the smallest cardiac influence. A recent study in rats also indicated that the pulse rate under isoflurane anesthesia was higher than that under pentobarbital anesthesia [20]. However, compared with the injectable anesthetics, isoflurane administration showed a prominent decrease in respiratory rate. Respiratory depression is a major adverse effect of isoflurane [10]. Monitoring the respiratory rate is important when adjusting the gas

concentration to regulate the anesthetic depth. Notably, the SPO₂ remained relatively stable under isoflurane anesthesia. This result indicates that the blood O₂ concentration is maintained by a high tidal volume during isoflurane anesthesia. A previous study also found that the hypercapnia associated with isoflurane was less intense than that observed with the injectable anesthetic pentobarbital [26]. Additionally, isoflurane anesthesia showed a higher SPO₂ level than pentobarbital monoanesthesia in rats [20]. In the present study, the isoflurane concentration was set at 2% for maintenance of anesthesia. The minimal alveolar concentration (MAC) is the concentration that prevents reaction to a standard surgical stimulus in 50% of animals and is an index for determining the inhalant anesthetic concentration [21]. Previous findings indicate that the MAC of isoflurane in many mouse strains is approximately 1.3–1.4% [24]. Administering inhalant anesthesia at 1.5 times the MAC is sufficient for surgical tolerance [6, 8]. Therefore, the isoflurane concentration investigated in this study is assumed for surgical anesthesia in mice. Further studies are warranted to determine the influence of isoflurane on vital signs across several concentrations and flow rates and for a longer anesthetic period.

In the present study, the closed colony strain ddY was used. Several factors, including strain, sex, circadian rhythm, and metabolism can affect cardiovascular function under anesthesia [7, 13, 27]. Evaluation of the vital signs during various anesthetic protocols according to strain, age, and sex may be a concern in the future. As operative stress alters cardiorespiratory function, assessment of vital signs during surgical procedures with each anesthesia may also be required. A rodent pulse oximeter was used to assess SPO₂ as an indicator of respiratory function. The pulse oximeter enables replicate measurement of the SPO₂ over time during anesthesia in mice without the need for any invasive procedures, while blood gas evaluation can only assess the O₂ concentration at a single point. The SPO₂ is routinely used to assess vital signs in human and veterinary medicine [3, 11]. Monitoring the SPO₂ is a valuable means of ensuring safe anesthesia in mice.

Anesthetic agents affect systemic adverse reactions, which may affect experimental data. Based on the present and previous studies, the combination of ketamine and xylazine has a greater cardiac influence than other anesthetic agents [4]. However, the respiratory depression with this combination is less than that observed with

other protocols, suggesting that this combination is suitable for respiratory experiments in mice. Among the four anesthetic protocols presently investigated, isoflurane anesthesia may be preferable for hemodynamic analysis or anesthesia in a cardiac disorder model. Although M/M/B anesthesia did influence cardiac and respiratory function, the use of atipamezole ensures its safety. Along with isoflurane, M/M/B is appropriate in cases requiring regulation of the duration of anesthesia. To definitively determine the cardiorespiratory influence of these agents, assessment of other parameters such as blood pressure, ETCO₂, and electrocardiogram during anesthesia is warranted.

In conclusion, we described the influence of several anesthetics on vital signs. The findings of the present study provide basic information for achieving appropriate anesthesia in mice and should contribute to improvement of laboratory animal welfare.

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