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Recommended doses of medetomidine-midazolam-butorphanol with atipamezole for preventing hypothermia in mice

Mizuho TASHIRO[1)] and Atsushi TOHEI[1)]*

[1)]Laboratory of Experimental Animal Science, Nippon Veterinary and Life Science University, Tokyo, Japan

ABSTRACT. A non-narcotic anesthetic combination (Me/Mi/Bu) of medetomidine (Me), midazolam (Mi), and butorphanol (Bu) has been recommended as the injectable anesthesia in mice. An original dose of Me/Mi/Bu (0.3/4.0/5.0 mg/kg) has provided sufficient anesthetic duration of 40–50 min in mice. In addition, atipamezole is available for reversal of Me/Mi/Bu anesthesia. As an adverse effect of Me/Mi/Bu anesthesia, however, severe hypothermia has been also observed in mice. In the present study, we investigated 1) the main agent in Me/Mi/Bu to cause of hypothermia, 2) the effects of the differential doses of atipamezole on hypothermia induced by Me/Mi/Bu anesthesia and on the plasma levels of creatinine phosphokinase and transaminases, and 3) those recommended doses for preventing hypothermia induced by Me/Mi/Bu anesthesia in mice. The results suggested that 1) the α_2-agonist medetomidine is most likely to induce hypothermia in mice under Me/Mi/Bu anesthesia, 2) the antagonism of atipamezole within proper dose range is effective in promoting the recovery from Me/Mi/Bu-induced hypothermia, and 3) Me/Mi/Bu at the recommended dose of 0.2/6.0/10.0 mg/kg enable to provide anesthetic effects for 40 min and is more considerable to prevent the hypothermia than that at the original dose of 0.3/4.0/5.0 mg/kg.

KEY WORDS: anesthesia, atipamezole, hypothermia, medetomidine, mice

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A three-anesthetic mixture of medetomidine, midazolam, and butorphanol (Me/Mi/Bu) serves as injectable anesthesia for rodents. In particular, the Me/Mi/Bu anesthesia has been recommended as an injectable anesthetic combination without narcotic drugs in Japan [16–18, 23–25, 28, 30, 31]. Several studies have reported that Me/Mi/Bu anesthesia provides sufficient anesthetic duration of 40–50 min in the strains of ICR, BALB/c, and C57BL/6 mice [16, 17]. Another study showed that Me/Mi/Bu mixture induced the same anesthetic effects by different administrative routes and atipamezole is available for reversal of these anesthetic effects [18]. To our knowledge, the reported dose ranges of Me/Mi/Bu were 0.3/4.0–6.0/5.0–7.5 mg/kg by intraperitoneal injection and 0.3–0.9/4.0–12.0/5.0–15.0 mg/kg by subcutaneous injection in mice [16–18, 23–25, 28, 30, 31]. In addition, a dose range of atipamezole was 0.3–1.5 mg/kg via intraperitoneal injection in mice anesthetized with Me/Mi/Bu at the original dose of 0.3/4.0/5.0 mg/kg reported previously [18]. However, our previous study showed that mice induced severe hypothermia after administration of Me/Mi/Bu 0.3/4.0/5.0 mg/kg following treatment of 0.3 mg/kg atipamezole even though the mice were treated with thermal support for 1–3 hr [28]. Mice are susceptible to decrease in body temperature during anesthesia because of their large surface-area/body-mass ratio [6]. The purposes of this study are 1) to determine the key component in Me/Mi/Bu to cause hypothermia, 2) to evaluate the dose-related effects and the effects of atipamezole, and 3) to propose the improved dose for prevention of hypothermia induced by Me/Mi/Bu anesthesia in mice.

MATERIALS AND METHODS

Ethical statement

All experiments were carried out following the provisions of the Nippon Veterinary and Life Science University (Approved No. 28S-62, 29K-25, 30K-26, and 2019K-14).

Animals and housing conditions

A total of 94 male Kwl:ICR mice at 8 weeks were purchased from a commercial breeder (Tokyo Laboratory Animal Science

*Correspondence to: Tohei, A.: tohei@nvlu.ac.jp, Laboratory of Experimental Animal Science, Nippon Veterinary and Life Science University, 1-7-1 Kyonan, Musashino, Tokyo 180-8602, Japan

Co., Ltd., Tokyo, Japan). These mice were acclimated for a week and were used from the age of 9 weeks in this study. The mice were housed 3–5 per cage on wood-shave bedding (Soft chip; Sankyo Labo Service Corp., Inc., Tokyo, Japan) in polycarbonate cages (CL-0104-2; 225 × 338 × 140 mm, CLEA Japan, Inc., Tokyo, Japan) and were received a commercial diet (EF; Oriental Yeast Co., Ltd., Tokyo, Japan) with water *ad libitum*. The housing environment was maintained under controlled conditions on 12:12-hr light/dark cycle (lights on/off at 7:00/19:00), an ambient temperature of 23–25°C, and 40–60% relative humidity. All experimental procedures were conducted between 12:00–17:00 in this study.

Drugs preparation

In the present study, we used pharmaceutical-grade products as follows: medetomidine hydrochloride (Domitor®; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), midazolam hydrochloride (Dormicum®; Astellas Pharma Inc., Tokyo, Japan), butorphanol tartrate (Vetorphale®; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) and atipamezole hydrochloride (Antisedan®; Nippon Zenyaku Kogyo Co., Ltd.). In addition, the doses of these agents used in this study were described as follows: medetomidine (0.1, 0.2 and 0.3 mg/kg), midazolam (4.0 mg/kg), butorphanol (5.0 mg/kg), the midazolam-butorphanol mixture (4.0/5.0 mg/kg), the three-anesthetic mixture (Me/Mi/Bu: 0.3/4.0/5.0, 0.3/6.0/7.5, 0.15/6.0/7.5, 0.15/6.0/10.0, 0.2/6.0/7.5 and 0.2/6.0/10.0 mg/kg) and atipamezole (Ati: 0.3, 0.6, 1.2, and 2.4 mg/kg). These drugs were diluted in sterile saline to 0.1 ml/10 g bodyweight of the animal and were administered by intraperitoneal injection (IP) in this study.

Body temperature measurement (nano tag® system)

To obtain mouse body temperature data, we used nano tag® system. This system consisted of the device (nano tag®; 15 × 14.2 × 7.1 mm, 2.5 g, KISSEI COMTEC Co., Ltd., Matsumoto, Japan) for measuring automatically body temperature in mouse, a radio-frequency-identification reader (PaSoRi; SONY Co., Ltd., Tokyo, Japan), and software (nanotag viewer®, KISSEI COMTEC Co., Ltd.). The nano tag® system allowed to reserve the sequential measurement of the body temperature and to record the body temperature data. In the present study, the body temperature in mice was measured every 5 min for 50 hr (from 6:00 a.m. on the previous day before the experiments to 8:00 a.m. on the next day after the experiments). After the end of measuring the body temperature, the recorded data were collected from the animals. In the acquired body temperature data, normal values of physiologic body temperature (T_{pre}) were determined as the lowest values of body temperature data in mice on a day before the experiment (between 7:00–7:00). The normal values of body temperature (T_{pre}) were compared with the lowest values of body temperature in mice within 7 hr after anesthetic injection (T_{post}) (in *Experiments 2-a, 3-a, and 3-c*).

Implantation surgery (nano tag® system)

For the implantation of nano tag® device, a total of 51 mice were anesthetized with Me/Mi/Bu 0.3/4.0/5.0 mg/kg IP. After confirming the loss of their righting reflex, the mice were placed on abdominal position on a heating pad (BWT-100A; Bio Research Center Co., Ltd., Nagoya, Japan) controlled at 37°C. All surgical instruments were disinfected with 75% ethanol. Briefly, a left side than midline abdominal incision was performed after the surgical site was cleaned by alcohol cotton, and the nano tag® device was implanted into the abdominal cavity. After the surgical sites were sutured, the animals were administered with Ati 0.3 mg/kg IP. The mice were returned to their home cages on a heating plate (HP-4530; AS ONE Co., Ltd., Osaka, Japan) maintained at 43°C and were spent overnight. The set temperature of 43°C lets the surface of wood-shave bedding maintain approximately 35°C. On the following day, the mice were housed individually in polycarbonate cages (CL-0103-2; CLEA Japan, Inc.). After the implantation surgery, the animals were provided with a postoperative recovery period of at least 2 weeks.

Experiments

1) Body temperature after administration of Me, Mi, Bu, and Mi/Bu: In this experiment, the body temperature in mice was measured by the nano tag® system as described above. To investigate the key component in Me/Mi/Bu to cause hypothermia, mice (n=25) implanted with the nano tag® device were randomly assigned to either medetomidine 0.3 mg/kg IP (Me group: n=6), midazolam 4.0 mg/kg IP (Mi group: n=7), butorphanol 5.0 mg/kg IP (Bu group: n=6) or midazolam/butorphanol 4.0/5.0 mg/kg IP (Mi/Bu group: n=6). After the intraperitoneal anesthetic injection, the mice were put into their home cages.

2) Effects of atipamezole on the hypothermia induced by Me/Mi/Bu and the blood biochemical parameters: 2-a) Antagonism of hypothermia induced by Me/Mi/Bu: The mice (n=10) implanted with the nano tag® device were used less than or equal to 3 times at random repeatedly after intervals of a week. The mice were randomly assigned to Me/Mi/Bu 0.3/4.0/5.0 mg/kg IP and atipamezole 0.3 mg/kg (Ati 0.3 group: n=6), 0.6 mg/kg (Ati 0.6 group: n=8), 1.2 mg/kg (Ati 1.2 group: n=6) or 2.4 mg/kg IP. (Ati 2.4 group: n=10). The mice were anesthetized with Me/Mi/Bu 0.3/4.0/5.0 mg/kg IP. After 40 min, the mice were injected with atipamezole. After administration of atipamezole, the mice were put into the polycarbonate cage with four compartments (KN-606; Natsume Seisakusho Co., Ltd., Tokyo, Japan). In addition, the mice in the cage were treated thermal support for 1 hr on the heating plate controlled at 46°C. The set temperature of 46°C lets the surface of wood-shave bedding maintains approximately 37°C in our previous study [28]. After the thermal support for 1 hr, the animals were returned to individual home cages. In this experiment, the time from injection of a reversal agent to return of righting reflex (RORR) was recorded when the mouse was returned from ventral to dorsal recumbency without assistance. Recovery time was defined as the time from administration of atipamezole at 40 min to recover the same or overvalues of T_{pre} of the mouse. The recovery time was defined as 0 min when the body temperature of the mouse from administration of atipamezole maintained a higher value than T_{pre}.

2-b) Plasma levels of creatinine phosphokinase and transaminases: Mice (n=26) were administered with saline IP (Saline group:

n=14) or atipamezole 2.4 mg/kg IP (Ati 2.4 group: *n*=12). Blood was collected at 1 (*n*=6–7) or 3 hr (*n*=6–7) after the injection. The total blood was collected approximately 0.8–1.2 ml by decapitation at each time point and was heparinized (25 µl, Heparin Sodium 10,000 IU/10 ml; Mitsubishi Tanabe Pharma Corporation, Osaka, Japan). The blood samples were centrifuged at 15,000 rpm ×20 min at 4°C. The obtained plasma samples were frozen at −70°C until analyzed within 1 week from blood sampling. The activity of creatinine phosphokinase (CPK), aspartate transaminase (AST), and alanine transaminase (ALT) in plasma were measured using a chemical analyzer (Fuji Dry-chem 3500s, Fuji Dry-chem slides; FUJIFILM Holdings Corp., Tokyo, Japan).

3) Dose determination for Me/Mi/Bu anesthesia for preventing hypothermia in mice: 3-a) Body temperature after administration of different doses of Me: In this experiment, the mice (*n*=6) implanted with the nano tag® device were used less than or equal to 3 times at random repeatedly after intervals of a week. The mice were randomly assigned to either medetomidine 0.1 mg/kg (Me 0.1 group: *n*=7), 0.2 mg/kg (Me 0.2 group: *n*=7) or 0.3 mg/kg IP (Me 0.3 group: *n*=4). After administration of medetomidine, mice were put on wood-chip bedding in a polycarbonate cage with four compartments. In addition, the mice in the cage were treated with thermal support for 2 hr on the heating plate as described previously. After the thermal support for 2 hr, the mice were returned to their home cages.

3-b) Anesthetic effects of the modified doses of Me/Mi/Bu anesthesia for preventing hypothermia in mice: In this experiment, mice (*n*=42) were used at random repeatedly after intervals of a week. The mice were anesthetized with Me/Mi/Bu at the doses of 0.3/4.0/5.0 mg/kg (*n*=7) (original), 0.3/6.0/7.5 mg/kg (*n*=7) (higher than original), 0.15/6.0/7.5 mg/kg (*n*=7), 0.15/6.0/10.0 mg/kg (*n*=7), 0.2/6.0/7.5 mg/kg (*n*=7) and 0.2/6.0/10.0 mg/kg IP (*n*=7) (4-modified doses). Regarding the level of anesthesia after injection of Me/Mi/Bu, anesthetic scoring was performed by a single operator and based on a presence: 1 or absence: 0 of each reflex in mice. The level of anesthesia in mice was measured every 5 min for 40 min by confirming the 5-point reflex 1) loss of the righting reflex, loss of the pedal withdrawal reflex in each of 2) forelimbs and 3) hindlimbs, 4) loss of the tail pinch reflex and 5) loss of the corneal reflex. The forelimb, hindlimb, and tail were pinched with noxious mechanical stimulation by forceps. For the corneal reflex, air stimulation to cornea was induced by a Pasteur pipette (IK-PAS-9P; AGC TECHNO GLASS Co., Ltd., Tokyo, Japan) with a silicone nipple. The obtained scores by confirming the 5-point reflex were summed and the score of 4 to 5 was defined as surgical anesthetic score. In case mice recovered from the loss of their righting reflex within 40 min, the total anesthetic score of 0 was defined. This measurement of the anesthetic scoring was based on a previous report [16].

3-c) Body temperature after administration of the improved doses of Me/Mi/Bu anesthesia for preventing hypothermia in mice: The body temperature in mice was measured by the nano tag® system as described previously. In this experiment, the mice (*n*=10) implanted with the nano tag® device were used less than or equal to 3 times at random repeatedly after intervals of a week. The mice were randomly assigned to either Me/Mi/Bu 0.3/4.0/5.0 mg/kg IP (*n*=19), with an original dose in laboratory mice [16–18, 30, 31], or 0.2/6.0/10.0 mg/kg IP (*n*=10), with the improved dose recommended in *Experiment 3-b*. The mice were put into the warming cage for 10 min after administration of Me/Mi/Bu, and then the mice were put on the heating pad at 37°C until the administration of atipamezole 0.3 mg/kg IP at 40 min. After the administration of atipamezole, the mice were put back into the warming cage until the end of thermal support for 2 hr, and the animals were returned to their home cages. In this experiment, the time from the injection of Me/Mi/Bu to loss of righting reflex (LORR) was recorded when the mouse was not returned from ventral to dorsal recumbency. Moreover, the time to RORR was recorded as described previously. Recovery time was defined and recorded similarly as described previously (in *Experiment 2-a*).

Statistical analysis

All data obtained in the present study were presented as mean ± SE. The experimental data were analyzed statistically using free available statistical packages (JSTAT ver. 10.0; http://toukeijstat.web.fc2.com/, MEPHAS; http://www.gen-info.osaka-u.ac.jp/MEPHAS/welcome.html). Difference among groups was statistically analyzed with one-way analysis of variance (ANOVA) followed by Tukey's test. For nonparametric datasets of more than 3 groups, Kruskal Wallis analysis of variance followed by Steel test was used. In a comparison of intragroup parametric datasets, Paired *t*-test was applied. For nonparametric datasets of both groups, Mann-Whitney *U* test was carried out. In addition, Student's *t*-test was used to analyze intergroup difference. Significant difference was defined as $P<0.05$ statistically.

RESULTS

1) Body temperature after administration of Me, Mi, Bu, and Mi/Bu

In each anesthetic group, the mean values for the lowest body temperature within 7 hr after anesthetic injection (T_{post}) are shown in Fig. 1. In comparison to anesthetic groups, the value of T_{post} in Me group was significantly lowest than those in other anesthetic

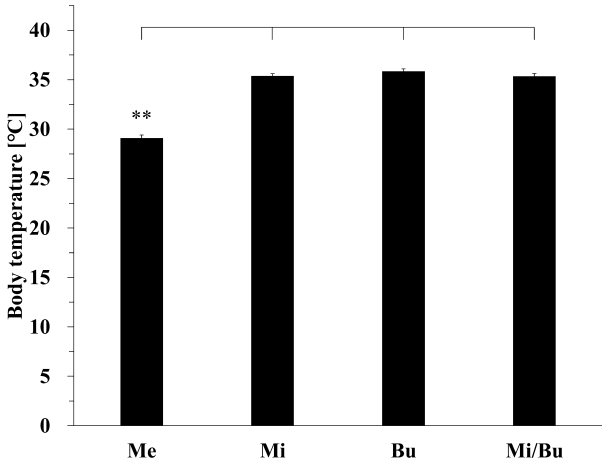

Fig. 1. Comparison of mean values of T_{post} in anesthetic groups. The value of T_{post} is defined as the lowest value of body temperature in mouse within 7 hr after anesthetic injection. Data are expressed as mean ± standard error in anesthetic groups (Me group: *n*=6, Mi group: *n*=7, Bu group: *n*=6 and Mi/Bu group: *n*=6). Statistically, a significant difference was presented as **: $P<0.01$.

groups (Me group: 29.1 ± 0.31°C, $P<0.01$). In contrast to the Me group, there was no significant difference of T_{post} in other anesthetic groups (Mi group: 35.38 ± 0.22°C, Bu group: 35.86 ± 0.22°C and Mi/Bu group: 35.36 ± 0.26°C).

2) Effects of atipamezole on the hypothermia induced by Me/Mi/Bu and the blood biochemical parameters

2-a) Antagonism of hypothermia induced by Me/Mi/Bu: In each Ati group, the mean values for T_{pre} and T_{post} are shown in Fig. 2(a). The administration of atipamezole 0.3, 0.6, 1.2, and 2.4 mg/kg IP dose-dependently prevented hypothermia in the mice anesthetized with Me/Mi/Bu (0.3/4.0/5.0 mg/kg IP). In the comparison between T_{pre} and T_{post}, T_{post} was significantly decreased compared with T_{pre} in Ati 0.3 (T_{pre}: 34.52 ± 0.08°C and T_{post}: 30.08 ± 0.71°C, $P<0.01$) and 0.6 groups (T_{pre}: 34.54 ± 0.07°C and T_{post}: 31.96 ± 0.71°C, $P<0.05$), but higher doses of atipamezole prevented hypothermia and there were no significant differences between T_{pre} and T_{post} in Ati 1.2 (T_{pre}: 34.83 ± 0.07°C and T_{post}: 33.72 ± 0.51°C) and 2.4 groups (T_{pre}: 35.17 ± 0.19°C and T_{post}: 34.66 ± 0.40°C). The recovery time from the injection of atipamezole to return of T_{pre} shortened in a dose-dependent manner with atipamezole (Fig. 2(b)). Particularly, the recovery time in Ati 0.3 group (249.17 ± 24.91 min) was significantly different compared with Ati 1.2 (129.17 ± 28.91 min, $P<0.05$) and 2.4 groups (82 ± 27.14 min, $P<0.05$). In addition, time to RORR was also shortened in a dose-dependent manner with atipamezole (Fig. 2(c)). Compared with Ati 0.3 group (11.46 ± 2.34 min), the time to RORR significantly decreased in Ati 1.2 (3.82 ± 0.37 min, $P<0.05$) and 2.4 groups (1.80 ± 0.18 min, $P<0.05$).

2-b) Plasma levels of creatinine phosphokinase and transaminases: The mean values of CPK, AST, and ALT at 1 and 3 hr after the injection of atipamezole are shown in Fig. 3(a–c). The plasma concentration of CPK significantly decreased in Ati 2.4 group at 3 hr compared with that in control group ($P<0.05$), however, there was no significant difference at 1 hr after the injection (Fig. 3(a)). There were no significant differences at 1 and 3 hr, although the level of AST in Ati 2.4 group at 3 hr tended to decrease ($P=0.064$) compared with that in control groups (Fig. 3(b)). The plasma concentration of ALT in Ati 2.4 group significantly decreased ($P<0.05$.) compared with control group (Fig. 3(c)).

3) Dose determination for Me/Mi/Bu anesthesia for preventing hypothermia in mice

3-a) Body temperature after administration of different doses of Me: In Me 0.1, 0.2, and 0.3 groups, the mean values of body temperature from 0 min (at the end of thermal support) to 180 min are shown in Fig. 4. The body temperature in Me 0.3 group significantly decreased between 20–180 min from the end of thermal support for 2 hr compared with those in Me 0.2 and 0.1 groups ($P<0.01$).

3-b) Anesthetic effects of the modified doses of Me/Mi/Bu anesthesia for preventing hypothermia in mice: In each mixture dose of Me/Mi/Bu, the anesthetic score was shown in Fig. 5. In the 4-modified doses of Me/Mi/Bu, the administration of Me/Mi/Bu 0.2/6.0/10 mg/kg (the improved dose) produced surgical anesthetic depth (the total scores of 4 and 5) between 10–40 min (Fig. 5(a)). This mixture dose of Me/Mi/Bu 0.2/6.0/10 mg/kg showed similar surgical anesthetic depth to the reported doses of Me/Mi/Bu 0.3/4.0/5.0 (original) and 0.3/6.0/7.5 mg/kg (Fig. 5(b)).

3-c) Body temperature after administration of the improved doses of Me/Mi/Bu anesthesia for preventing hypothermia in mice: In both groups of Me/Mi/Bu 0.3/4.0/5.0 (the original dose) and 0.2/6.0/10.0 mg/kg (the improved dose), the mean values of T_{pre} and T_{post} are shown in Fig. 6(a). In the comparison of T_{pre} and T_{post}, T_{post} were significantly decreased compared with T_{pre} in Me/Mi/Bu 0.3/4.0/5.0 mg/kg. In contrast, the significant difference between T_{pre} and T_{post} was not observed in Me/Mi/Bu 0.2/6.0/10 mg/kg. In each group, the recovery time to the return of T_{pre} from the administration of atipamezole is shown in Fig. 6(b). The recovery time were dramatically improved in Me/Mi/Bu 0.2/6.0/10.0 mg/kg (11.11 ± 4.55 min) compared with Me/Mi/Bu 0.3/4.0/5.0 mg/kg (64.21 ± 13.58 min, $P<0.01$). In addition, both times to LORR and RORR were shown in Fig. 6(c). Compared with Me/Mi/Bu 0.3/4.0/5.0 mg/kg IP (225 ± 12.06 sec), the time to LORR significantly were shortened after administration of Me/Mi/Bu 0.2/6.0/10.0 mg/kg IP (174.11 ± 8.98 sec, $P<0.05$). The time to RORR was not significantly different in both anesthetic groups.

DISCUSSION

In the present study, we have presented that 1) medetomidine is most likely to induce hypothermia in mice under Me/Mi/Bu anesthesia, 2) the treatment of atipamezole within the proper dose range shortens to recover from Me/Mi/Bu anesthesia, and 3) the Me/Mi/Bu anesthesia at the improved dose of 0.2/6.0/10.0 mg/kg induces the anesthetic effects for 40 min and the risk of the hypothermia in this dose is relatively low compared with it in the original dose of 0.3/4.0/5.0 mg/kg.

The Me/Mi/Bu anesthesia provides the anesthetic duration for surgical procedures at least 40 min in mice [16, 17], and the anesthetic effects have been induced at the original dose of 0.3/4.0/5.0 mg/kg [16]. In addition to the original dose, the other reported dose of 0.3/6.0/7.5 mg/kg used higher amounts of midazolam and butorphanol than the original also provides more sufficient anesthetic depth for several strains of mice [23]. In contrast to anesthetic main effects, the Me/Mi/Bu anesthesia induces the decreases of heart rate and SpO_2, intraocular hypertension, diuresis, hyperglycemia, and severe hypothermia as the adverse effects [24, 25, 28, 30]. In our previous study, we reported that mice caused severe hypothermia after intraperitoneal injection at the original dose of Me/Mi/Bu 0.3/4.0/5.0 mg/kg, and their hypothermia was certainly prevented by the treatment of thermal support over 5 hr after Me/Mi/Bu administration [28]. Hypothermia can rapidly develop during anesthesia in mice and delay the normal living activities of organisms [6]. To avoid hypothermia, it should be necessary to carry out the refinement strategies of the additional thermal support, optimal dosages of anesthetics [1], and administration of reversal agents.

The Me/Mi/Bu anesthesia has the advantage that atipamezole is available for the antagonist of medetomidine. The atipamezole is

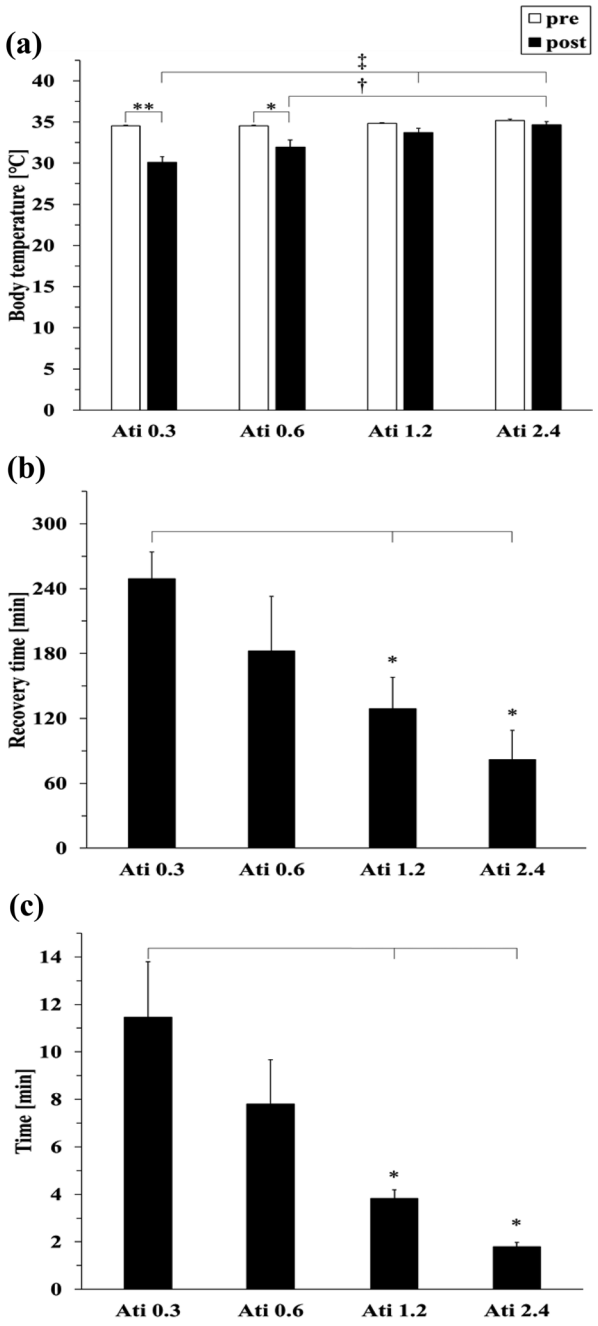

Fig. 2. The dose-dependent effects of atipamezole on the body temperature (a), the time for recovery of T_{pre} from the administration of atipamezole (b), and RORR (c). The value of T_{pre} is defined as the lowest value of body temperature in mice on the day before the experiment (between 7:00–7:00). (a) The value of T_{post} is defined as the lowest value of body temperature in mouse within 7 hr after anesthetic injection. (b) The recovery time was defined as the time from administration of atipamezole at 40 min to recover the same value or overvalue of T_{pre} of the mouse. The recovery time was defined as 0 min when the body temperature of the mouse from administration of atipamezole maintained higher value than T_{pre}. (c) The time from injection of atipamezole to return of righting reflex (RORR) was recorded when the mouse was returned from ventral to dorsal recumbency without assistance. Data are expressed as mean ± SE in atipamezole groups (Ati 0.3 group: n=6, Ati 0.6 group: n=8, Ati 1.2 group: n=6 and Ati 2.4 group: n=10). Statistically significant differences were presented as *, †: P<0.05 and **, ‡: P<0.01.

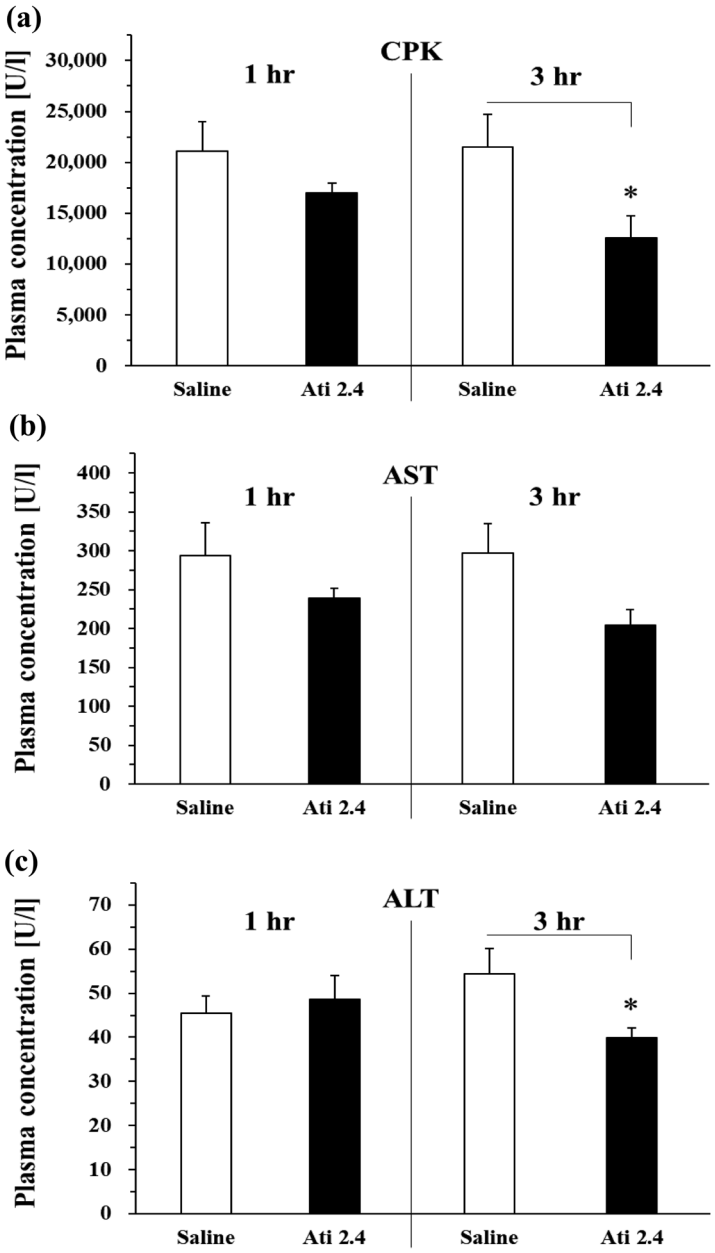

Fig. 3. Plasma concentrations of creatinine phosphokinase (CPK) (a), aspartate transaminase (AST) (b), and alanine transaminase (ALT) (c) at 1 and 3 hr from the administration of saline or atipamezole. Data are expressed as mean ± standard error in each Saline groups: at 1 (n=7) and 3 hr (n=7), and each Ati 2.4 groups: at 1 (n=6) and 3 hr (n=6). Statistically, a significant difference was presented as *: P<0.05.

an α_2-antagonist with 100 times higher affinity for α_2-adrenoceptors and an over 200–300 times higher selectivity for α_2/α_1 ratio than yohimbine as an antagonist of α_2-adrenoceptor [10, 14, 22, 35]. Atipamezole rapidly reverses the anesthetic effect of α_2-agonists such as xylazine, medetomidine, and its dextro-enantiomer dexmedetomidine [2, 3, 14, 18]. As a result, atipamezole is commonly used to facilitate the recovery from anesthesia induced by α_2-agonists in companion and laboratory animals [5, 36]. In the present study, our results showed that the administration of atipamezole restored hypothermia to normal body temperature (Fig. 2(a)), and shorten

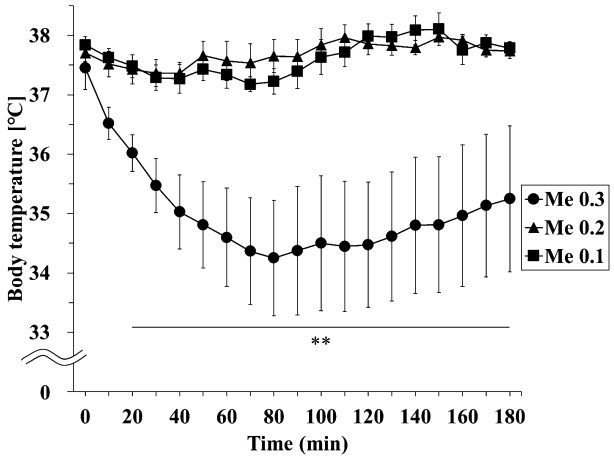

Fig. 4. Changes of body temperature from 0 min (at the end of thermal support for 2 hr) to 180 min in mice treated with-medetomidine alone. Data are expressed as mean ± standard error in Me 0.1 (closed square: *n*=7), Me 0.2 (closed triangle: *n*=7) and Me 0.3 groups (closed circle: *n*=7). Statistically, a significant difference was presented as **: $P<0.01$.

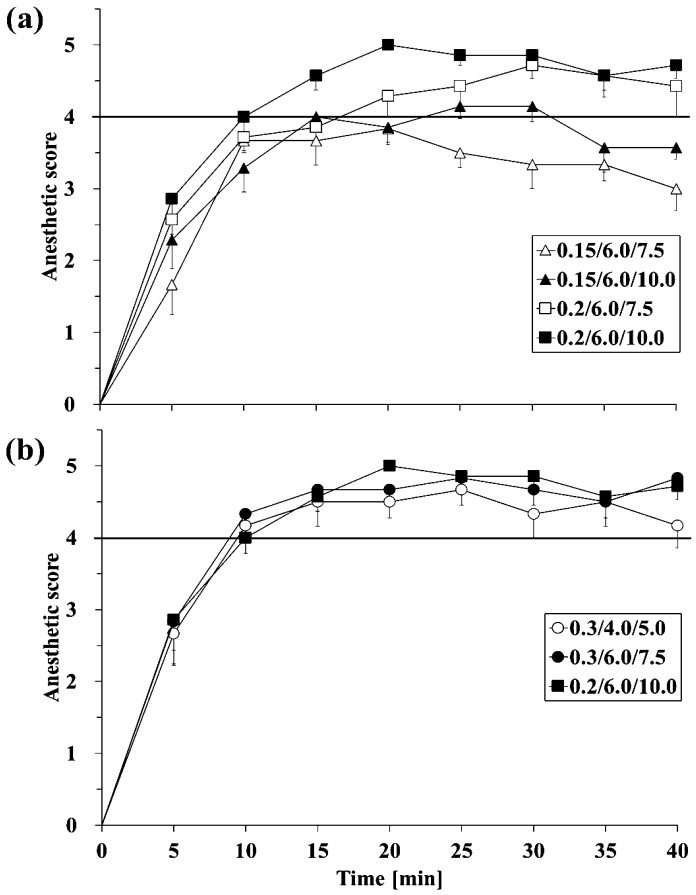

Fig. 5. Comparisons of the anesthetic score for 40 min after administration of medetomidine-midazolam-butorphanol (Me/Mi/Bu) at the 4-modified doses (a) and at the improved dose (our recommended in the modified doses) (b). Data are expressed as mean ± standard error in Me/Mi/Bu 0.15/6.0/7.5 (open triangle: *n*=6), 0.15/6.0/10.0 (closed triangle: *n*=7), 0.2/6.0/7.5 (open square: *n*=7), 0.2/6.0/10.0 mg/kg (closed square: *n*=7) (4-modified doses), 0.3/4.0/5.0 (original dose, open circle: *n*=6), 0.3/6.0/7.5 mg/kg (higher dose than original, closed circle: *n*=6) and 0.2/6.0/10.0 mg/kg (the improved dose with our recommended in the modified doses, closed square: *n*=7). Anesthetic scoring was based on a presence: 1 or absence: 0 of each reflex in mouse. The anesthetic score was measured every 5 min for 40 min by confirming the 5-point reflex 1) loss of the righting reflex, loss of the pedal withdrawal reflex in each of 2) forelimbs and 3) hindlimbs, 4) loss of the tail pinch reflex, and 5) loss of the corneal reflex. The total anesthetic score of 4 to 5 was defined as a surgical anesthetic score.

both times for the recovery of body temperature (Fig. 2(b)) and the time for the return of righting reflex (Fig. 2(c)) in a dose-dependent manner. One previous study has reported that atipamezole dose-dependently reverses the mydriasis, sedation, and hypothermia induced by the administration of medetomidine in rodents [10]. The previous results suggested that administration of atipamezole 1.0 mg/kg reversed mydriasis induced by medetomidine 0.3 mg/kg in rats, and sedation and hypothermia in mice. In dogs and cats, doses of atipamezole were required 2–4 times (in cats) and 4–6 times (in dogs) dose of medetomidine to completely reverse its effects [32, 33]. Although the optimal dosage of atipamezole can promote the recovery from hypothermia in mice, we also concluded that antagonization with overdose of atipamezole can add undesirable effects to mice. In laboratory rodents, the administration of atipamezole at LD_{50} (over 30 mg/kg) causes miserable death as a result of cardiac and pulmonary disturbances [26]. One previous study also concluded that the recommended dose range of atipamezole was 1.0–2.5 mg/kg by intraperitoneal injection in mice [14]. The vocalization might be induced by the effects of atipamezole on the responses of startle, anxiogenic, or excitatory [2, 14, 22, 34]. Generally, atipamezole can lead to an increase in pain-related response because it reverses the analgesic effect of α_2-agonists mediated by noradrenergic pathways in the presynaptic α_2 auto-receptors [22, 26]. In addition to noradrenergic pathways, administration of higher dose of atipamezole may attenuate the analgesic effect of butorphanol, which is a synthetic agonist (κ-opioid)-antagonist (μ-opioid) mediated by opioid receptors [4, 13, 15]. Butorphanol provides analgesic effect for relatively short duration (1–2 hr) in rodents [8, 21]. In rats, the administration of atipamezole 2.0 mg/kg attenuated the analgesic effects of butorphanol [13], though, atipamezole 1.0 mg/kg did not alter the antinociceptive effect of butorphanol in mice [12]. In general, tissue injury during anesthesia is associated with increased levels of CPK, AST, and ALT [29, 37]. Moreover, these physiologic and anatomic parameters serve as the early major signs of hepatic injury induced by stress on the anesthetic injection, surgical pain, and psychological depression [29, 37, 38]. In the present study, our results showed that the plasma concentration of CPK (Fig. 3(a)) and ALT (Fig. 3(c)) significantly decreased at 3 hr after administration of atipamezole 2.4 mg/kg in mice. The reason why these enzymes decreased after administration of atipamezole 2.4 mg/kg was unknown, generally, chemical substances increased these enzyme activities [29, 37]. However, these alterations in liver function may affect the major pathway of drug elimination. One previous study showed that prior administration of medetomidine reduced cardiac output and hepatic blow,

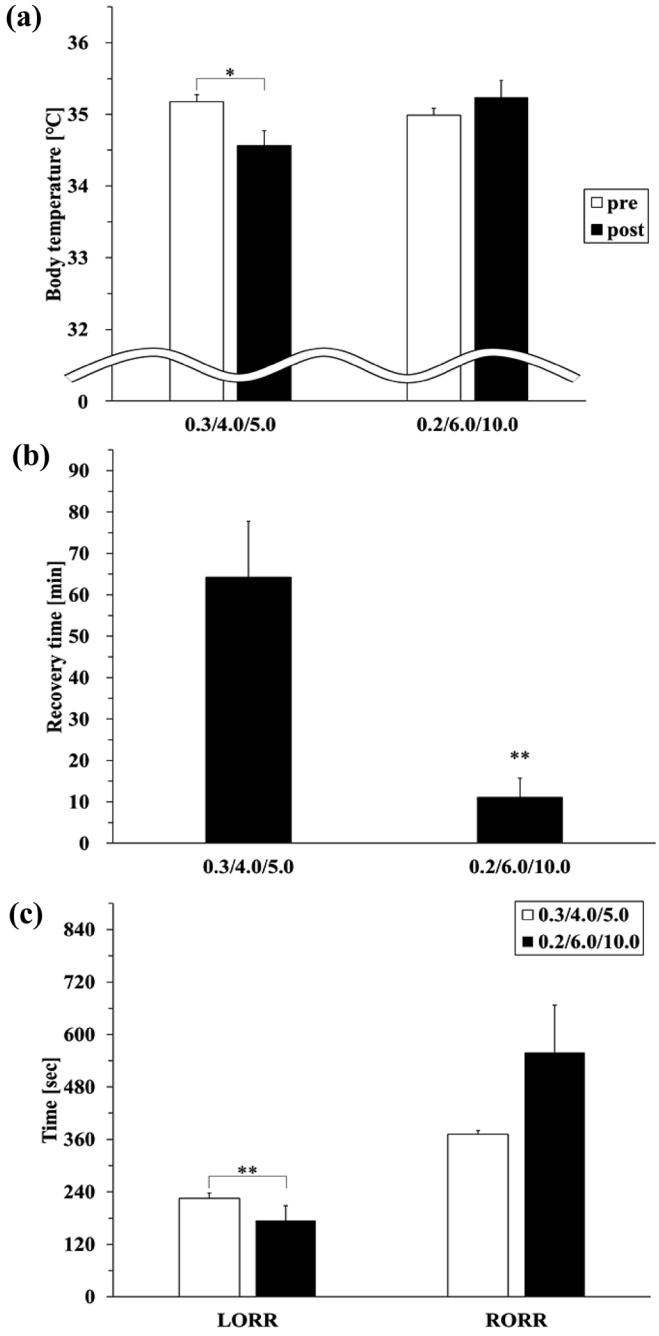

Fig. 6. Comparisons of the body temperature (a), the time for recovery of T_{pre} from the administration of atipamezole (b), and LORR and RORR (c) in Me/Mi/Bu 0.3/4.0/5.0 (the original dose) and 0.2/6.0/10 (the improved dose) groups. The value of T_{pre} is defined as the lowest value of body temperature in mice on the day before the experiment (between 7:00–7:00). (a) The value of T_{post} is defined as the lowest value of body temperature in mouse within 7 hr after anesthetic injection. (b) Recovery time was defined as the time from administration of atipamezole at 40 min to recover the same value or overvalues of T_{pre} of the mouse. The recovery time was defined as 0 min when the body temperature of the mouse from administration of atipamezole maintained higher value than T_{pre}. (c) The times from injection of Me/Mi/Bu to loss of righting reflex (LORR) and from atipamezole to return of righting reflex (RORR) were recorded. Data are expressed as mean ± standard error in Me/Mi/Bu groups (0.3/4.0/5.0 mg/kg: n=19 and 0.2/6.0/10.0 mg/kg: n=9). Statistically significant differences were presented as *: P<0.05 and **: P<0.01.

which resulted in the delay of absorption and metabolism of atipamezole in dogs [27]. Therefore, we recommended that administration of atipamezole should be minimized to avoid additional effects of its atipamezole and Me/Mi/Bu although optimal dosage of atipamezole is effective to reverse the adverse effects of Me/Mi/Bu anesthesia.

In the present study, our results showed that the administration of medetomidine 0.3 mg/kg is significantly decreased body temperature in mice (Fig. 4). In addition, the improved dose of Me/Mi/Bu 0.2/6.0/10.0 mg/kg provided a comparable anesthetic effect for 40 min (Fig. 5), and more rapidly induced the recovery from hypothermia (Fig. 6(b)) and LORR (Fig. 6(c)) compared with the original dose in mice. The duration showing over point 4 was almost the same (less than 60 min) between the original dose and the improved dose group. Medetomidine, which is a highly selective α_2-agonist, has been widely used as a sedative, analgesic, and muscle relaxant in small animals [5, 22, 36]. In addition, the administration of medetomidine has an anesthetic-sparing effect that decreases the requirements of other anesthetic agents in several species [5, 22]. Therefore, the medetomidine-based combinations of medetomidine-ketamine, medetomidine-midazolam-fentanyl, and Me/Mi/Bu were widely used as anesthesia in mice [3, 7, 16]. Alternatively, the α_2-agonist medetomidine has been known to induce the decrease of body temperature in mice [9–11, 20]. Administration of medetomidine did not induce hypothermia in the α_{2AC}-KO (knock out) mice [9], and the hypothermic effect of medetomidine could be accounted for by α_2-adrenergic receptor subtypes, which is the both of α_{2A}- and α_{2C}-adrenoceptors [9, 11, 19, 20]. Moreover, the previous study suggested that α_{2A}-receptor predominantly contributed to the decrease of body temperature of mice compared with α_{2C}-receptor [11]. To avoid the dose-dependent hypothermia under Me/Mi/Bu anesthesia in mice, the dose of medetomidine should be minimized, and we would recommend the improved dose of Me/Mi/Bu 0.2/6.0/10.0 mg/kg for anesthesia in mice. However, we did not investigate whether the improved dose of Me/Mi/Bu (0.2/6.0/10.0 mg/kg) affects several vital signs (blood pressure, heart rate, respiratory rate, and SpO_2) and blood biochemical parameters (glucose, insulin, electrolytes, transaminases and creatinine phosphokinase) of mice after the administration of anesthesia. Compared with the original dose of Me/Mi/Bu (0.3/4.0/5.0 mg/kg), the improved dose (0.2/6.0/10.0 mg/kg) consists of relatively high doses of midazolam and butorphanol respectively. To our knowledge, the maximal dose of Me/Mi/Bu not to induce anesthetic death was reported at the dose of 0.9/12.0/15.0 mg/kg [24]. The previous study reported that 1.5- and 3-times higher doses than original were increased the level of CPK although did not induce anesthetic death after the administration of Me/Mi/Bu. In addition, the level of the skeletal muscle type isoenzyme of CPK (CPK-MM) increased by administration of 12 mg/kg midazolam alone, and the level of brain type isoenzyme of CPK (CPK-BB) also increased by medetomidine 0.9 mg/kg. However, the administration of 15 mg/kg butorphanol alone did not change the levels of CPK isoenzymes [24]. These

results partly support the safety of Me/Mi/Bu anesthesia at the individual dose (0.2/6.0/10.0 mg/kg) in mice.

In conclusion, the present study has demonstrated that 1) the administration of α_2-agonist medetomidine induces the dose-dependent decrease of body temperature, 2) the optimal dose range of atipamezole is effective in the prevention of hypothermia induced by Me/Mi/Bu anesthesia, 3) the recommended dose of Me/Mi/Bu 0.2/6.0/10.0 mg/kg attenuates the development of hypothermia in mice. However, additional thermal support is essential for at least 2 hr to prevent hypothermia after anesthesia. Further studies are needed to understand the anesthetic adverse effects, refinement strategies (optimal doses of anesthetics and its reversals, thermal support, and veterinary care), and both the pharmacological and neurological mechanisms under anesthesia for laboratory animals.

POTENTIAL CONFLICTS OF INTEREST. The authors declare no conflicts of interest in the carrying out of this work.

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