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Effects of an anesthetic mixture of medetomidine, midazolam, and butorphanol and antagonism by atipamezole in rabbits

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Abstract: Medetomidine (MED), midazolam (MID), and butorphanol (BUT) mixed anesthetic (MMB) has been used in laboratory animals since ketamine (KET) was designated as a narcotic in Japan in 2007. We previously reported that MMB produced anesthetic effects in mice and rats. We also demonstrated the efficacy of atipamezole (ATI), an antagonist of MED produced a quick recovery from anesthesia. Anesthetics have various anesthetic effects among different animal species. However, there is little information regarding its effects in rabbits. In the present study, we examined anesthetic effects of MMB compared to KET and xylazine mixed anesthetic (KX). We examined the antagonistic effects of ATI by intramuscular (IM) or intravenous (IV) injection in rabbits. We used the anesthetic score to measure surgical anesthetic duration and recovery time from anesthesia. During the experiments, we measured heart rate, respiratory rate, O_2-saturation, and blood pressure. We found there were no significant differences in anesthetic duration and recovery time between MMB and KX. There were no significant differences in heart rate after administration of MMB or KX. Systolic blood pressure at 10 min after administration of MMB was higher than that of KX. The antagonistic effect of ATI by IV injection worked faster than that by IM injection. Overall, MMB is a useful drug that can induce similar anesthetic effects to KX and has an antagonist of ATI that makes rabbits quickly recover from anesthesia. These results may contribute to the welfare of laboratory animals, especially rabbits.
Key words: anesthetic mixture, antagonist, blood pressure, ketamine, rabbits

Introduction

After ketamine (KET) was designated as a narcotic drug due to an abuse problem in Japan in 2007, medetomidine (MED), midazolam (MID), and butorphanol (BUT) mixed anesthetic (MMB) was introduced for anesthesia in mice in 2011 [12]. We reported that MMB produced closely similar anesthetic effects in both male and female BALB/c and C57BL/6J strain mice [14]. We also demonstrated that there were no significant differences in anesthetic duration among three different injection routes in mice [16]. Our study using rats indicated that MMB induced similar anesthetic effects in three different rat strains [15]. Another several studies of MMB in mice and rats were published [22, 23, 26, 27]. Not only mice and rats, the anesthetic effects of MMB for

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other laboratory animals such as monkeys [21], cotton rats [18], and hamsters [19] were reported. However, there is little information regarding its effects in rabbits. Konno *et al.* reported that MMB (MED: 0.5 mg + MID: 2.0 mg + BUT: 0.5 mg/kg) produced deep anesthetic effects for 90 min and did not awake for more than 120 min in rabbits [17]. However, this dose including 0.5 mg of MED caused cardiovascular impairment. MED is a specific alpha$_2$-adrenergic receptor agonist and a high dose of it produces cardiovascular impairment [5]. A high dose of MID induces strong sedation in rabbits [6]. Then we reduced the dose of MED and MID, increasing the dose of BUT for analgesia. Therefore in this study, we used the dose of MMB at MED: 0.15 mg + MID: 1.0 mg + BUT: 1.5 mg/kg.

At first, we administered MMB to rabbits and measured the surgical anesthetic duration along with heart rate, respiratory rate, and O_2-saturation compared to the non-anesthetized condition. Second, we used the dose of MMB described above and compared it to KET and xylazine (XYL) mixed anesthetic (KX) which has been a common and popular anesthetic used on rabbits [1, 8, 9, 29].

Atipamezole (ATI) is a synthetic alpha$_2$-adrenergic receptor antagonist which can antagonize an alpha$_2$-adrenergic receptor agonist such as MED and XYL [5]. However, MED is a more specific alpha$_2$-adrenergic agonist to be strongly reversed by ATI compared to XYL [28].

After administration of MMB, injection of ATI caused a rapid recovery from anesthesia. We reported the efficacy of ATI with a suitable dosage and timing in mice and rats [15, 16]. However, neither the appropriate dosage nor the optimum injection route of ATI after administration of MMB is clear in rabbits. Therefore in the third experiment, we examined antagonistic effects of ATI by intramuscular (IM) or intravenous (IV) injection in rabbits.

In this study, we used the anesthetic score to assess the anesthetic effects of MMB and KX administered to rabbits. During the experiments, we measured vital signs just before and after administration of anesthetics because parameters such as heart rate, respiratory rate, O_2-saturation, and blood pressure were related to the anesthetic condition of rabbits under anesthesia [4].

Materials and Methods

Animals and housing conditions

Animal care and experimental procedures were approved by the Animal Research Committee of Shimane University and conducted according to the Regulations for Animal Experimentation at Shimane University.

We used 12 male Japanese White strain rabbits in the experiments after allowing them to rest for at least 2 days after each drug administration. The rabbits were purchased at 11 weeks of age from a commercial supplier (Biotek Co., Ltd., Saga, Japan) and habituated for at least a week in the animal room before starting the experiment. The rabbits were 12 to 16 weeks of age during the experiment and their weights ranged from 2.36–3.12 kg (average 2.69 kg).

Rabbits were housed individually in a metal cage (Specific, W 480 × L 525 × H 380 mm, Natsume Seisakusho, Co., Ltd., Tokyo, Japan) under a strict light cycle (light on at 7:00 and off at 19:00).

The animal room for rabbits was maintained at a constant temperature (20 ± 2°C) and humidity (55 ± 10%). The rabbits were given 150 g of standard diet (LRC4®, Oriental Yeast Co., Ltd., Tokyo, Japan)/day and filtered tap water by an automatic water supply system.

Experimental procedure

The experiment was conducted in the animal room during daytime (13:00–17:00). The rabbits were weighed at first before starting the experiment and also just before the drug administration.

In the first experiment, we used 6 rabbits. The anesthetic mixture of MMB was administered by IM injection at the femoral region. Non-anesthetized rabbits were kept in a rabbit holder. We used same rabbits for the MMB administration group and for the non-anesthetized group. The experimental groups were named as the MMB group and the Non-anesthetized group. We measured heart rate, respiratory rate, and O_2-saturation of the rabbits before and every 5 min after administration of MMB until the rabbit completely recovered from anesthesia. Non-anesthetized rabbits were measured every 5 min for 90 min.

In the second experiment, we used another 6 rabbits. The anesthetic mixture of MMB or KX was administered by IM injection. The experimental groups were named as the MMB group and the KX group. Blood pressure and heart rate were measured before and then after at 5

min, and then every 10 min after administration of anesthetics until the rabbit completely recovered from anesthesia.

In the first and second experiments, the anesthetic score for each rabbit was measured every 5 min after administration of anesthetics until the rabbit completely recovered from anesthesia.

In the third experiment, we used the same 6 rabbits examined in the second experiment. At 30 min after IM administration of MMB, rabbits were administered ATI by IM (at the opposite femoral region of MMB administration) or IV injection (via pinna vein). The experimental groups were named as the ATI-IM group or the ATI-IV group. After administration of MMB, the anesthetic score was measured every 5 min up until 30 min. After injection of ATI, the anesthetic score was measured every 1 min until the rabbit completely recovered from anesthesia.

After administration of anesthetics, rabbits were kept on a heater mat (Hot carpet PC-41, Matsushita Electric Industrial Co., Ltd., Osaka, Japan) maintained at approximately 37°C.

The rabbits were euthanatized by IV injection of sodium pentobarbital (100 mg/kg) (Somnopentyl®, Kyoritsu Seiyaku Corp., Tokyo, Japan) after completion of the experiment.

Measurement of anesthetic scores

The method of measuring the anesthetic scores was based on 6 reflexes. The first was a body righting reflex: when a rabbit was put on its back, it was given a score of 1 if it did not get up and a score 0 if it did. The second was a corneal reflex: when a rabbit's eyes were stimulated by air using a Pasteur pipette with a silicone nipple 1 cm from its eyes, it was given a score of 1 if it did not move its eyelids and a score of 0 if it did. The third and the fourth were front legs reflexes: when a rabbit's front paws were pinched by hooked forceps, it was given a score of 1 on each right and left front leg if it did not move, it was given a score of 0 if it did. The fifth and the sixth were hind legs reflexes: when a rabbit's hind paws were pinched by hooked forceps, it was given a score of 1 on each right and left hind leg if it did not move, it was given a score of 0 if it did. The total anesthetic score was graded from 0 to 6. A rabbit which counted a score of 0 to 5 was not considered to be anesthetized. We measured the time when a rabbit started to lose its body righting reflex and then labeled the lost time for righting reflex. When the time reached a score of 6, we then labeled the starting time for surgical anesthesia. The surgical anesthetic duration was determined by adding up all the consecutive time periods with a score of 6. The time required for the anesthetic score returned to 0 after administration of anesthetics determined the recovery time from anesthesia.

Measurement of heart rate, respiratory rate, and O_2-saturation in the first experiment

We put a sensor clip along the ear artery of each rabbit and the instrument (Vital sign monitor, PhysioSuite®, Kent Scientific Corp., Torrington, CT, USA) was used to measure heart rate, respiratory rate, and O_2-saturation. We also measured such parameters in non-anesthetized rabbits kept in a rabbit holder.

Measurement of blood pressure and heart rate in the second experiment

An instrument (Animal blood pressure manometer, BP100D®, Fukuda M・E Kogyo, Co., Ltd., Tokyo, Japan) was used to measure systolic, diastolic, mean blood pressure, and heart rate of rabbits during the experiment. Two days before the experiment, fur on an upper part of the right front leg of each rabbit was removed using an electric shaver under sedation by IM injection of MED.

A cuff of the blood pressure manometer was banded around an upper part of a right front leg of a rabbit. Before and after administration of the anesthetics, we recorded the blood pressure and heart rate until the rabbit completely recovered from anesthesia.

Drug preparation

The anesthetic mixture of MMB was prepared as a mixture of three drugs: MED (Domitor®, Nippon Zenyaku Kogyo Co., Ltd., Tokyo, Japan), MID (Dormicum®, Astellas Pharma Inc., Tokyo, Japan), and BUT (Vetorphale®, Meiji Seika Pharma Co., Ltd., Tokyo, Japan). The anesthetic mixture of KX was prepared as a mixture of two drugs: KET (Ketalar®, Daiichi Sankyo Co., Ltd., Tokyo, Japan) and XYL (2% Celactar®, Bayer Yakuhin, Ltd., Tokyo, Japan).

To make MMB, we mixed MED: 0.15 mg/kg, MID: 1 mg/kg, and BUT: 1.5 mg/kg. For example, 3 ml of Domitor, 4 ml of Dormicum, and 6 ml of Vetorphale were mixed to make 13 ml of MMB. Administrative volume of MMB was 0.65 ml/kg. To make KX, we mixed KET: 35 mg/kg and XYL: 5 mg/kg. For example, 7 ml

of Ketalar and 2.5 ml of Celactar were added to 0.5 ml of sterilized saline (Otsuka Normal Saline®, Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) to make 10 ml of KX. Administrative volume of KX was 1.0 ml/kg. For sedation in order to shave the rabbit's fur, MED: 0.15 mg/0.15 ml/kg was given to the rabbits.

The anesthetic mixtures were prepared on the day before the experiment and kept in a refrigerator. The mixed drugs were allowed to be used for up to 1 week after being mixed.

In the third experiment, we used 0.75 mg/kg of ATI (Antisedan®, Nippon Zenyaku Kogyo Co., Ltd.) and administrative volume was 0.15 ml/kg.

Drug preparation was conducted at a clean bench in a sterile manner. Before administration, the drugs were warmed in an incubator set at a temperature of 37°C.

Statistical analysis

Statistical analysis was conducted using the Stat View software (Hulinks Inc., Tokyo, Japan). Graph data were presented as means ± SD. Differences between each experimental group were analyzed using unpaired Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant.

Results

All rabbits used in this experiment recovered from anesthesia.

First experiment

Body weight: The body weights (mean ± SD) of the MMB group (n=6) and the Non-anesthetized group (n=6) were 2.68 ± 0.06 and 2.99 ± 0.11 kg, respectively. The same rabbits were used in each group. There were significant differences between the two groups because after the rabbits administered MMB finished the experiments, we then measured the vital signs of the same rabbits as non-anesthetized rabbits 2 days later.

Measurements of lost time for righting reflex, starting time for surgical anesthesia, surgical anesthetic duration, and recovery time from anesthesia: After administration of MMB, the lost time for righting reflex was 2.4 ± 0.9 min, the starting time for surgical anesthesia was 11.7 ± 2.6 min, the surgical anesthetic duration was 25.9 ± 6.7 min, and the recovery time from anesthesia was 110.8 ± 13.2 min (Table 1).

Table 1. Measurements after administration of medetomidine, midazolam, and butorphanol mixed anesthetic

Measurement (n=6)	min (Mean ± SD)
Lost time for righting reflex	2.4 ± 0.9
Starting time for surgical anesthesia	11.7 ± 2.6
Surgical anesthetic duration	25.9 ± 6.7
Recovery time from anesthesia	110.8 ± 13.2

Lost time for righting reflex, starting time for surgical anesthesia, surgical anesthetic duration, and recovery time from anesthesia.

Measurements of heart rate, respiratory rate, and O_2-saturation: The heart rate and the respiratory rate of the MMB group were significantly lower than the Non-anesthetized group from 5 to 90 min after the administration of MMB. However values for the heart rate and the respiratory rate were stable during the experiment. (Figs. 1A and B) The O_2-saturation of the MMB group was significantly lower than the Non-anesthetized group from 5 to 70 min after administration of MMB (excluding those at 10, 40, and 50 min) (Fig. 1C).

Second experiment

Body weight: The body weights (mean ± SD) of the MMB group (n=6) and the KX group (n=6) were 2.50 ± 0.10 and 2.58 ± 0.13 kg, respectively. There were no significant differences between two groups (Table 2).

Anesthetic score: There were no significant differences in anesthetic scores between the MMB group and the KX group during anesthesia but at 105 min after drug administration, the anesthetic score of the MMB group was significantly higher than that of the KX group (1.33 ± 1.03 vs. 0.0 ± 0.0) (Fig. 2).

Lost time for righting reflex: The lost times for righting reflex of the MMB group and the KX group were 2.8 ± 0.6 and 1.8 ± 0.5 min, respectively. The lost time for righting reflex of the MMB group was significantly later than that of the KX group (Table 2).

Starting time for surgical anesthesia: The starting times for surgical anesthesia of the MMB group and the KX group were 11.7 ± 2.6 and 8.3 ± 2.6 min, respectively. The starting time for surgical anesthesia of the MMB group was significantly later than that of the KX group (Table 2).

Surgical anesthetic duration: The surgical anesthetic durations of the MMB group and the KX group were 30.0 ± 7.1 and 32.5 ± 6.9 min, respectively. There were no significant differences between the two groups. The shortest surgical anesthetic durations of two groups were

Table 2. Body weight, lost time for righting reflex, starting time for surgical anesthesia, surgical anesthetic duration, and recovery time from anesthesia of the medetomidine, midazolam, and butorphanol mixed anesthetic (MMB) and the ketamine and xylazine mixed anesthetic (KX) groups

Group	n	Body weight (kg)	Lost time for righting reflex (min)	Starting time for surgical anesthesia (min)	Surgical anesthetic duration (min)			Recovery time from anesthesia (min)		
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Shortest	Longest	Mean ± SD	Shortest	Longest
MMB	6	2.50 ± 0.10	2.8 ± 0.6*	11.7 ± 2.6*	30.0 ± 7.1	20	40	105.8 ± 17.4	80	125
KX	6	2.58 ± 0.13	1.8 ± 0.5	8.3 ± 2.6	32.5 ± 6.9	20	40	91.7 ± 9.8	80	105

Differences between two groups were analyzed using unpaired Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant. **P*<0.05 compared with the KX group.

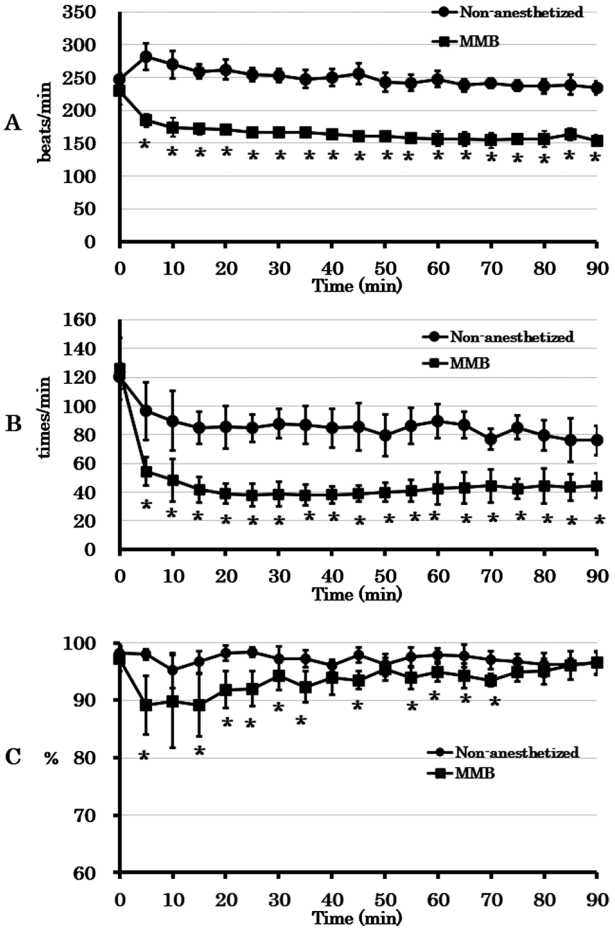

Fig. 1. Time courses of heart rate (A), respiratory rate (B), and O_2-saturation (C) of the medetomidine, midazolam, and butorphanol mixed anesthetic (MMB) and the Non-anesthetized groups. Differences between two groups were analyzed using unpaired Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant. **P*<0.05 compared with the Non-anesthetized group.

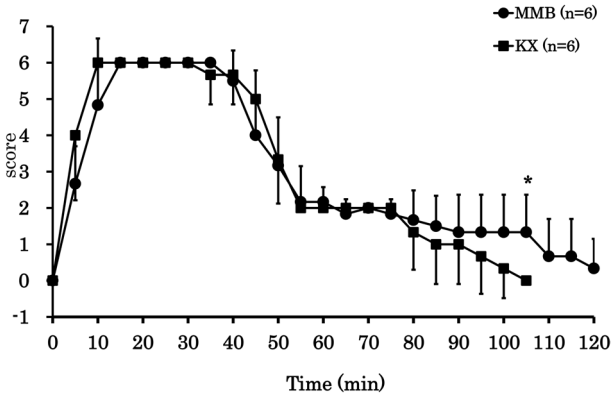

Fig. 2. Time courses of the anesthetic score of the medetomidine, midazolam, and butorphanol mixed anesthetic (MMB) and the ketamine and xylazine mixed anesthetic (KX) groups. Data are presented as means ± SD. Differences between two groups were analyzed using unpaired Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant. **P*<0.05 compared with the KX group.

20 min. The longest surgical anesthetic durations of both groups were 40 min (Table 2).

Recovery time from anesthesia: The recovery times from anesthesia of the MMB group and the KX group were 105.8 ± 17.4 and 91.7 ± 9.8 min, respectively. There were no significant differences between the two groups. The shortest recovery times from anesthesia of two groups were 80 min. The longest recovery times from anesthesia of the MMB group and the KX group were125 and 105 min, respectively (Table 2).

Measurements of blood pressure and heart rate:

1) Systolic blood pressure: There were no significant differences in systolic blood pressure between the MMB group and the KX group before drug administration. After administration of the anesthetic mixtures, the systolic blood pressures of the MMB group and the KX

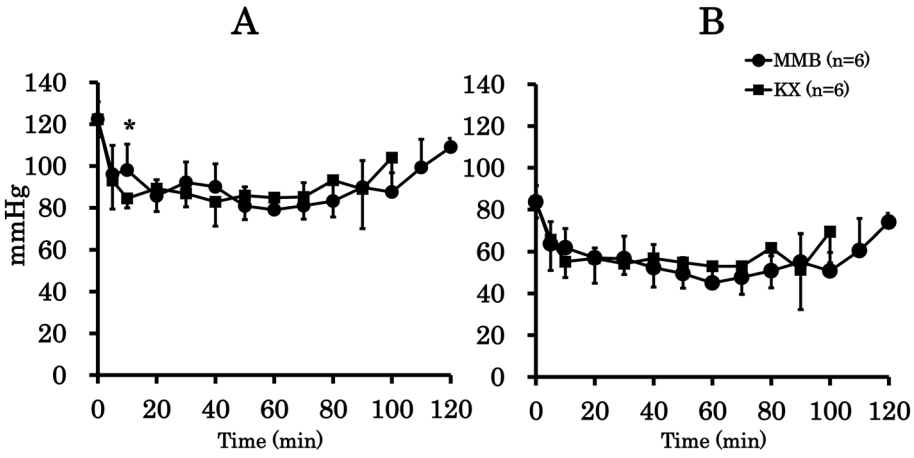

Fig. 3. Time courses of systolic blood pressure (A) and diastolic blood pressure (B) of the medetomidine, midazolam, and butorphanol mixed anesthetic (MMB) and the ketamine and xylazine mixed anesthetic (KX) groups. Data are presented as means ± SD. Differences between two groups were analyzed using unpaired Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant. **P*<0.05 compared with the KX group.

group decreased until 20 min and were stable during anesthesia. The systolic blood pressure of the MMB group at 10 min was significantly higher than that of the KX group (Fig. 3A).

2) Diastolic blood pressure: There were no significant differences in diastolic blood pressure between the MMB group and the KX group before drug administration. After administration of the anesthetic mixtures, the diastolic blood pressures of the MMB group and the KX group decreased until 20 min and were stable during anesthesia. There were no significant differences of the diastolic blood pressure between two groups during anesthesia (Fig. 3B).

3) Mean blood pressure: There were no significant differences in mean blood pressure between the MMB group and the KX group before and after administration of the anesthetic mixture. This showed the same tendency as the results of the diastolic blood pressure (data not shown).

4) Heart rate: There were no significant differences in heart rate between the MMB group and the KX group before drug administration. The heart rates of the MMB group and KX group decreased until 20 min after administration of the anesthetic mixtures and were stable during anesthesia. There were no significant differences of the heart rate between two groups during anesthesia (Fig. 4).

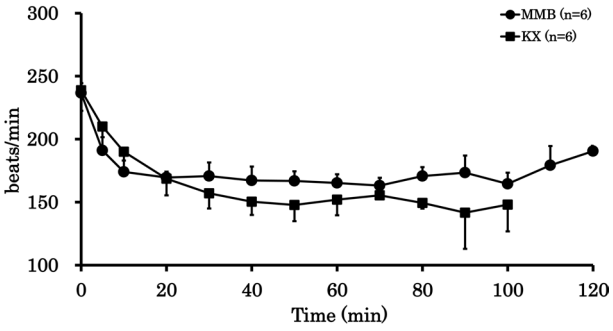

Fig. 4. Time courses of heart rate of the medetomidine, midazolam, and butorphanol mixed anesthetic (MMB) and the ketamine and xylazine mixed anesthetic (KX) groups. Data are presented as means ± SD. Differences between two groups were analyzed using unpaired Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant. There were no significant differences between two groups.

Third experiment

Body weight: The body weights (mean ± SD) of the ATI-IM group (n=6) and the ATI-IV (n=6) group were 2.71 ± 0.20 and 2.70 ± 0.19 kg, respectively. There were no significant differences between two groups (Table 3).

Anesthetic score: There were no significant differences in anesthetic scores between the ATI-IM group and the ATI-IV group during 30 min after administration of MMB. After injection of ATI, the anesthetic scores of the ATI-IV group were significantly lower than those of ATI-IM group until 12 min (excluding those at 7 and 8

Table 3. Body weight, injection route of the medetomidine, midazolam, and butorphanol mixed anesthetic and the atipamezole groups, concentration of atipamezole, and recovery time from anesthesia of two groups

Group	n	Body weight (kg)	MMB	ATI		Recovery time from anesthesia (min)
			Route	Route	Concentration	
ATI-IM	6	2.71 ± 0.20	IM	IM	0.75 mg/kg	17.5 ± 7.8*
ATI-IV	6	2.70 ± 0.19	IM	IV	0.75 mg/kg	7.8 ± 4.6

Data are presented as means ± SD. Differences between two groups were analyzed using unpaired Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant. **P*<0.05 compared with the atipamezole (ATI)-intravenous (IV) group. MMB, medetomidine, midazolam, and butorphanol mixed anesthetic; IM, intramuscular.

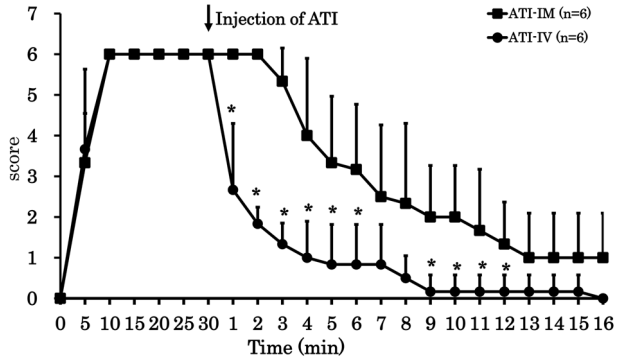

Fig. 5. Time courses of the anesthetic score of the atipamezole (ATI)-intramuscular (IM) and the ATI-intravenous (IV) groups. Data are presented as means ± SD. Differences between two groups were analyzed using unpaired Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant. **P*<0.05 compared with the ATI-IM group.

min) (Fig. 5).

Recovery time from anesthesia: After injection of ATI, the recovery times from anesthesia of the ATI-IM group and the ATI-IV group were 17.5 ± 7.8 and 7.8 ± 4.6 min, respectively. The recovery time of the ATI-IM group was significantly longer than that of the ATI-IV group (Table 3).

Discussion

There is little information regarding anesthetic effects of MMB in rabbits. Konno *et al.* reported that MMB of MED: 0.5 mg + MID: 2.0 mg + BUT: 0.5 mg/kg by IM administration produced anesthetic effects for 90 min in rabbits causing cardiovascular impairments such as an arrhythmia or an atrioventricular block [17]. MED is a specific alpha$_2$-adrenergic receptor agonist and a high dose of it produces cardiovascular impairment [5]. The anesthetic duration of 90 min seems to be a long time period for injectable anesthesia compared to our data. Santangelo *et al.* reported that the transnasally administered anesthetic mixture of dexmedetomidine (DXM): 0.1 mg + MID: 2 mg + BUT: 0.4 mg/kg caused deep sedation and analgesia suitable for minor surgical procedures in rabbits [24]. DXM is an alpha$_2$-adrenergic receptor agonist and has a double potency of an equal dose of MED [5]. A high dose of MID induces strong sedation [6]. Then we reduced the doses of MED and MID, while increasing the dose of BUT in order to increase analgesia. Therefore in this study, we used the dose of MMB of MED: 0.15 mg + MID: 1.0 mg + BUT: 1.5 mg/kg. In the first experiment, We introduced a surgical anesthetic duration of approximately 26 min and reduced but stabilized the heart rate, respiratory rate, and O_2-saturation after the administration of MMB at MED: 0.15 mg + MID: 1.0 mg + BUT: 1.5 mg/kg.

In the second experiment, we decided to compare the anesthetic effects between MMB and KX which has been a common and popular anesthetic used on rabbits [1, 8, 9, 29]. We used the dose of KX at KET: 35 mg + XYL 5 mg/kg. Flecknell published that this dose by IM administration produced 20–40 min of surgical anesthesia in rabbits [7]. Then we used the dose of KX described by Flecknell.

The second experiment indicated the surgical anesthetic durations of the MMB group and the KX group were 30.0 ± 7.1 and 32.5 ± 6.9 min, respectively (Table 2). There were no significant differences between the two groups. In addition, there were no significant differences in the recovery time from anesthesia between the two groups. However, the MMB group needed more time to lose the righting reflex and then start surgical anesthesia. These characteristics of MMB might be misunderstood by researchers that MMB had weaker anes-

thetic effects compared to KX.

After the administration of MMB or KX, blood pressure decreased until 20 min and was stable during anesthesia (Fig. 3). MED and XYL are both alpha$_2$-adrenergic receptor agonists to decrease blood pressure [3]. However, the systolic blood pressure at 10 min after the administration of the MMB group was significantly higher than that of the KX group (Fig. 3A). MED is a more specific alpha$_2$-adrenergic receptor agonist compared to XYL [5]. Therefore the increasing of blood pressure at 10 min after administration of MMB seemed to indicate that MED acted at an alpha$_{2B}$ receptor which was an alpha$_2$ subtype receptor and caused a contraction of peripheral vessels temporarily [20, 25]. Also Baumgartner *et al.* reported that the anesthetic mixture of MED, MID, and fentanyl induced an initial significant increase of blood pressure in rabbits due to initial peripheral vaso-constrictive properties of MED [2]. This temporary increased blood pressure after MMB administration was reported in dogs [10] but not in monkeys [11]. We previously found that the temporary increased blood pressure at 10 min after MMB administration caused in rats but not in mice (unpublished data). There might be different sensitivities of the actions of MED to an alpha$_{2B}$ receptor in different animal species.

After the administration of MMB or KX, heart rate decreased from about 240 beats/min to about 170 beats/min until 20 min and was stable, then it gradually increased after finishing the duration of surgical anesthesia (Fig. 4). There were no significant differences between the two groups. It was suggested that the effects to heart function from the use of MMB and KX in this experiment were almost equivalent.

In this experiment, we used only one dose of MMB at MED: 0.15 mg + MID: 1.0 mg + BUT: 1.5 mg/kg. The dosage of MMB and KX used in the experiment produced a similar anesthetic duration and recovery time from anesthesia. Therefore the MMB containing MED: 0.15 mg + MID: 1.0 mg + BUT: 1.5 mg/kg might be a strong candidate for a replacement of KX. However, in order to decide the most appropriate dosage of MMB, more experiments using a variety of dosages of MMB are needed. On this point, our experiment is limited.

ATI is a synthetic alpha$_2$-adrenergic receptor antagonist that can antagonize an alpha$_2$-adrenergic receptor agonist, MED or XYL [5]. We reported the efficacy of ATI with a suitable dosage and timing after administration of MMB in mice and rats [15, 16]. However, neither the appropriate dosage nor the optimum injection route of ATI after administration of MMB was clear in rabbits. In the present study, we used 0.75 mg/kg of ATI, a 5-times higher dose than that of MED 0.15 mg/kg at 30 min after administration of MMB. After injection of ATI, the recovery times from anesthesia by IM and IV routes were 17.5 ± 7.8 and 7.8 ± 4.6 min, respectively (Table 3). The recovery time from anesthesia by IV injection was significantly faster than that by IM injection. Konno *et al.* reported that IM injection of ATI 1.5 mg/kg, a 3-times higher dose than that of MED 0.5 mg/kg caused a rapid recovery from anesthesia at 120 min after administration of MMB [17]. Kim *et al.* reported that IV injection of ATI 0.35 mg/kg or ATI 0.7 mg /kg caused the rapid recovery from anesthesia at 35 min after IV administration of MED 0.35 mg/kg followed by KET 5 mg/kg in rabbits [13].

Our previous studies showed that recovery times by intraperitoneal injection of a 5-times higher dose of ATI than that of MED at 30 min after administration of MMB, was about 2.5 ± 0.6 and 4.0 ± 1.1 min in mice and rats, respectively [15, 16]. When compared to the results in mice and rats, rabbits needed more time to recover from anesthesia by injection of ATI. Therefore a 5-times higher dosage of ATI 0.75 mg/kg by IV injection seems to be more suitable in rabbits.

In summary, the present study indicated that MMB produced a similar surgical anesthetic duration with KX. Therefore the dose of MMB used in the present study could be considered as a strong candidate as a substitute for KX anesthesia. However, in order to decide the most appropriate dosage of MMB, more experiments using a variety of dosages of MMB are called for. The anesthetic mixture of MMB is a useful anesthetic that can be antagonized with ATI to help rabbits quickly and safely recover from anesthesia. These results may contribute to the welfare of laboratory animals, especially rabbits.

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References

1. Baumgartner, C., Bollerhey, M., Ebner, J., Laacke-Singer, L., Schuster, T. and Erhardt, W. 2010. Effects of ketamine-xylazine intravenous bolus injection on cardiovascular function in rabbits. *Can. J. Vet. Res.* 74: 200–208. [Medline]
2. Baumgartner, C., Bollerhey, M., Ebner, J., Schuster, T., Henke, J. and Erhardt, W. 2010. Effects of medetomidine-midazolam-fentanyl IV bolus injections and its reversal by specific antagonists on cardiovascular function in rabbits. *Can. J. Vet. Res.* 74: 286–298. [Medline]
3. Feldman, J., Fellmann, L. and Bousquet, P. 2008. The central hypotensive effect induced by alpha 2-adrenergic receptor stimulation is dependent on endothelial nitric oxide synthase. *J. Hypertens.* 26: 1033–1036. [Medline] [CrossRef]
4. Flecknell, P.A. 2016. Chapter 2, Managing and Monitering Anaesthesia, p. 89–91. In: Laboratory Animal Anaesthesia. 4th ed. ELSEVIER, Oxford.
5. Flecknell, P.A. 2016. Chapter 1, Basic Principle of Anaesthesia, p. 51–52. In: Laboratory Animal Anaesthesia. 4th ed. ELSEVIER, Oxford.
6. Flecknell, P.A. 2016. Chapter 1, Basic Principle of Anaesthesia, p. 51. In: Laboratory Animal Anaesthesia. 4th ed. ELSEVIER, Oxford.
7. Flecknell, P.A. 2016. Chapter 5, Analgesia of Common Labotayoty Species, p. 221. In: Laboratory Aninal Anaesthesia. 4th ed. ELSEVIER, Oxford.
8. Green, C.J., Knight, J., Precious, S. and Simpkin, S. 1981. Ketamine alone and combined with diazepam or xylazine in laboratory animals: a 10 year experience. *Lab. Anim.* 15: 163–170. [Medline] [CrossRef]
9. Henke, J., Astner, S., Brill, T., Eissner, B., Busch, R. and Erhardt, W. 2005. Comparative study of three intramuscular anaesthetic combinations (medetomidine/ketamine, medetomidine/fentanyl/midazolam and xylazine/ketamine) in rabbits. *Vet. Anaesth. Analg.* 32: 261–270. [Medline] [CrossRef]
10. Itamoto, K., Hikasa, Y., Sakonjyu, I., Itoh, H., Kakuta, T. and Takase, K. 2000. Anaesthetic and cardiopulmonary effects of balanced anaesthesia with medetomidine-midazolam and butorphanol in dogs. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* 47: 411–420. [Medline] [CrossRef]
11. Kalema-Zikusoka, G., Horne, W.A., Levine, J. and Loomis, M.R. 2003. Comparison of the cardiorespiratory effects of medetomidine-butorphanol-ketamine and medetomidine-butorphanol-midazolam in patas monkeys (Erythrocebus patas). *J. Zoo Wildl. Med.* 34: 47–52. [Medline] [CrossRef]
12. Kawai, S., Takagi, Y., Kaneko, S. and Kurosawa, T. 2011. Effect of three types of mixed anesthetic agents alternate to ketamine in mice. *Exp. Anim.* 60: 481–487. [Medline] [CrossRef]
13. Kim, M.S., Jeong, S.M., Park, J.H., Nam, T.C. and Seo, K.M. 2004. Reversal of medetomidine-ketamine combination anesthesia in rabbits by atipamezole. *Exp. Anim.* 53: 423–428. [Medline] [CrossRef]
14. Kirihara, Y., Takechi, M., Kurosaki, K., Kobayashi, Y. and Kurosawa, T. 2013. Anesthetic effects of a mixture of medetomidine, midazolam and butorphanol in two strains of mice. *Exp. Anim.* 62: 173–180. [Medline] [CrossRef]
15. Kirihara, Y., Takechi, M., Kurosaki, K., Kobayashi, Y., Saito, Y. and Takeuchi, T. 2016. Effects of an anesthetic mixture of medetomidine, midazolam, and butorphanol in rats-strain difference and antagonism by atipamezole. *Exp. Anim.* 65: 27–36. [Medline] [CrossRef]
16. Kirihara, Y., Takechi, M., Kurosaki, K., Kobayashi, Y., Saito, Y. and Takeuchi, T. 2015. Anesthetic effects of a three-drugs mixture--comparison of administrative routes and antagonistic effects of atipamezole in mice. *Exp. Anim.* 64: 39–47. [Medline] [CrossRef]
17. Konno, K., Horiuchi, S., Isoe, K., Matsuda, H., Fujiwara, H., Furuya, M. and Takashima, H. 2012. Evaluation of the combination of three anesthetics in rats and rabbits (Japanese). *Anuual Rep. Hatano Res. Inst.* 35: 53–59.
18. Nakamura, T., Ichii, O., Irie, T., Hosotani, M., Dantsuka, A., Nakamura, S., Sato, S., Sotozaki, K., Kouguchi, H., Yoshiyasu, T., Nagasaki, K.I. and Kon, Y. 2016. Usefulness of an anesthetic mixture of medetomidine, midazolam, and butorphanol in cotton rats (Sigmodn hispidus). *Jpn. J. Vet. Res.* 64: 273–276. [Medline]
19. Nakamura, T., Karakida, N., Dantsuka, A., Ichii, O., Elewa, Y.H.A., Kon, Y., Nagasaki, K.I., Hattori, H. and Yoshiyasu, T. 2017. Effects of a mixture of medetomidine, midazolam and butorphanol on anesthesia and blood biochemistry and the antagonizing action of atipamezole in hamsters. *J. Vet. Med. Sci.* 79: 1230–1235. [Medline] [CrossRef]
20. Nichols, A.J., Hieble, J.P. and Ruffolo, R.R. Jr. 1988. The pharmacology of peripheral alpha 1- and alpha 2-adrenoceptors. *Rev. Clin. Basic Pharm.* 7: 129–205. [Medline]
21. Ochi, T., Nishiura, I., Tatsumi, M., Hirano, Y., Yahagi, K., Sakurai, Y., Matsuyama-Fujiwara, K., Sudo, Y., Nishina, N. and Koyama, H. 2014. Anesthetic effect of a combination of medetomidine-midazolam-butorphanol in cynomolgus monkeys (Macaca fascicularis). *J. Vet. Med. Sci.* 76: 917–921. [Medline] [CrossRef]
22. Ochiai, Y., Iwano, H., Sakamoto, T., Hirabayashi, M., Kaneko, E., Watanabe, T., Yamashita, K. and Yokota, H. 2016. Blood biochemical changes in mice after administration of a mixture of three anesthetic agents. *J. Vet. Med. Sci.* 78: 951–956. [Medline] [CrossRef]
23. Osanai, H., and Tateno, T. 2016. Neural response differences in the rat primary auditory cortex under anesthesia with ketamine versus the mixture of medetomidine, midazolam and butorphanol. *Hear. Res.* 339: 69–79. [Medline] [CrossRef]
24. Santangelo, B., Micieli, F., Marino, F., Reynaud, F., Cassandro, P., Carfora, A., Petrella, R., Borriello, R., Cataldi, M. and Vesce, G. 2016. Plasma concentrations and sedative effects of a dexmedetomidine, midazolam, and butorphanol combination after transnasal administration in healthy rabbits. *J. Vet. Pharmacol. Ther.* 39: 408–411. [Medline] [CrossRef]
25. Takamatsu, I. 2011. Dexmedetomidine-α2-Adrenoceptors and Imidazoline Receptors (Japanese). *Anesthesia 21 Century* 13: 37–44.
26. Tsubokura, Y., Kobayashi, T., Oshima, Y., Hashizume, N., Nakai, M., Ajimi, S. and Imatanaka, N. 2016. Effects of pentobarbital, isoflurane, or medetomidine-midazolam-butorph-

anol anesthesia on bronchoalveolar lavage fluid and blood chemistry in rats. *J. Toxicol. Sci.* 41: 595–604. [Medline] [CrossRef]
27. Tsukamoto, A., Serizawa, K., Sato, R., Yamazaki, J. and Inomata, T. 2015. Vital signs monitoring during injectable and inhalant anesthesia in mice. *Exp. Anim.* 64: 57–64. [Medline] [CrossRef]
28. Virtanen, R. 1989. Pharmacological profiles of medetomidine and its antagonist, atipamezole. *Acta Vet. Scand. Suppl.* 85: 29–37. [Medline]
29. Yershov, A.L., Jordan, B.S., Fudge, J.M. and Dubick, M.A. 2007. Influence of the mode of ventilation on ketamine/xylazine requirements in rabbits. *Vet. Anaesth. Analg.* 34: 157–163. [Medline] [CrossRef]