Use of Alpha$_2$-Agonist / Antagonist in the Sedation of Swine

Alpha$_2$受容体作動薬・拮抗薬の応用による豚の鎮静法の確立

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Preface

In recent years, pigs have been recognized as valuable and useful laboratory animals for many types of biomedical researches [43]. It is mainly due to the similarities in structure and function between pigs and human beings. The pig is similar to humans in size, feeding patterns, dental characteristics, renal morphology and physiology, eye structure and visual acuity, skin morphology and physiology, coronary artery distribution, pulmonary vascular bed structure, respiratory rate, tidal volume, and digestive anatomy and physiology. Pigs have been used successfully as models to evaluate alcoholism, diabetes, absorption, digestion, total parenteral nutrition, organ transplantation, atherosclerosis, exercise, hypertension, hemorrhagic hypotension, melanoma, gingivitis, osteochondrosis, dermal healing and septic shock [63]. In addition, the pigs make an ideal animal model for immunological studies [43]. The placenta of the pig allows almost no antibody transfer from the maternal circulation to the developing fetus so that the neonate is essentially devoid of immune antibodies before ingesting colostrum. Recently pigs have also been used to replace dogs for many purposes. Dogs have been most commonly used as laboratory animals, but the use of these species in biomedical research has become increasingly difficult due to their restricted availability, high cost , and social pressure [39].

However, pigs are so shy, excitable and hysteric that sedation is widely used to facilitate all handling and minor procedures. In addition, pigs have fewer accessible superficial veins, particularly in small swine, causing intravenous administration of drugs to be more difficult. Preanesthetic intramuscular administration of a sedative is urgently required for restraint prior to general anesthesia.

A number of agents including short-acting barbiturates, phenothiazines, butyrophenones, benzodiazepines and dissociative anesthetics have been used for this purpose. Among these agents, azaperone which is one of the butyrophenones is probably the most widely used drug for swine sedation. Sedation induced by azaperone increases with dose rate, and its higher doses produce deeper sedation with sternal recumbency, which may be used for premedication preventing of rejection of piglets by sows and suppression of fighting when pigs are mixed [33]. However, in order to ensure prompt onset of action and full response, pigs should be handled as gently as possible during the injection procedure. Pigs should also be left undisturbed for 20 to 30 min before attempting diagnostic or other manipulative procedures or until the desired clinical response is achieved [64]. As with other tranquilizers, when pigs are subjected to rough handling, being kept in noisy surroundings, or otherwise situations with frequent stimuli before full onset of azaperon's action, they may become agitated instead of tranquilized. Excitement may occur during this induction phase even in the absence of stimulation, although it is usually mild and rarely clinically significant. Moreover, azaperone does not induce significant analgesia and painful stimuli can cause in sudden awake in an agitated state [22].

α_2-Agonists such as xylazine have been very widely used as a sedative in many species [21]. These drugs are also used for premedication because they greatly reduce the dose requirements and undesirable effects of inhalation anesthetics [68] or parenteral anesthetics such as ketamine or pentobarbital [27, 43]. Pharmacologically, it is very interesting that the interaction between α_2-agonists and these anesthetics seems to be rather synergistic than simple additive [22]. α_2-Agonists activate the presynaptic α_2-receptor that inhibits release of noradrenalin which probably constitutes an important

negative feed back mechanism. They exert their potent sedative effects by inhibiting the locus coeruleus, which contains dominant α_2-adrenoceptors and pathways involved in the maintenance of vigilance [2,61]. α_2-Agonists have been also reported to activate postsynaptic α_2-adrenoceptors in the brain [34], and both pre- and post-synaptic mechanisms of α_2-agonists may induce their extraordinary potent ability. Practically, α_2-agonists are more valuable and attractive than other sedatives since they also produce analgesic effects through both spinal and central action and muscle relaxant effects through central action [21]. Moreover, their effects can be reversed by α_2-antagonists quickly [10]. Their potency and characters seem to be very attractive as sedatives, however, for reasons as yet unknown, xylazine is ineffective as a sedative in pigs when used alone [22].

Recently, a new drug of the α_2-agonists, medetomidine was developed. Medetomidine differs from other α_2-agonists in several respects. It is more lipophilic, is more selective, and has more potency and efficacy at α_2-adrenoceptors [66]. Medetomidine has an α_2/α_1 receptor selectivity binding ratio of 1620 compared with 260, 220 and 160 for detomidine, clonidine, and xylazine, respectively [79]. Medetomidine produces a reliable degree of sedation, muscle relaxation and analgesia suitable for use in small animals [71] and its sedative effect can be reversed by atipamezole [79], which is a newly developed potent and highly selective and specific α_2- antagonist. In both dogs and cats, medetomidine induces dose-dependent sedative actions (10 to 80 μg/kg for dogs and 50 to 150 μg/kg for cats) [74]. In dogs given 30 μg/kg i.m., medetomidine is equivalent to a recommended (2.2 mg/kg i.m.) dose of xylazine and higher dose of medetomidine exerts a much more potent effect [66]. Therefore, it is worth to evaluate the efficacy of medetomidine as a sedative in laboratory pigs.

Salonen et al. reported the synergistic interaction between an α_2-agonist and midazolam which is a sedative classified to benzodiazepine [49]. Clinical effects achieved with this combination in human patients were greater than that expected from a simple additive response [56]. Although the administration of midazolam alone did not induce effective sedation in animals [22], midazolam greatly enhanced the effect of α_2-agonist in dogs [67]. If medetomidine can produce a satisfactory sedative effect, a combination of medetomidine and midazolam may also induce a very potent sedation in pigs.

The major focus of this study is to establish a potent and widely available sedative combination in laboratory pigs. The final goal of the most suitable sedative combination in pigs is as follows; the combination includes only non-controlled drugs which can be administered intramuscularly with small amount of injection volume, produces deep sedation which is potent enough to depress or abolish the arousal reaction induced by sensory stimuli with less side effect, induces sedation quickly and smoothly, can be combined with other anesthetics effectively and safely, and can be reversed by a specific antagonist even if combined with other anesthetics.

This thesis consists of three parts. Part 1 deals with the fundamental aspects of an α_2-agonist medetomidine in pigs. In this part the sedative effect and optimal dose of medetomidine were investigated in pigs and its sedative effect was compared with other major sedatives. In addition, antagonistic ability of α_2-antagonist atipamezole against medetomidine induced sedation was evaluated. In this part the pharmacokinetic of each drug was also determined. Part 2 deals with the establishment of a potent and satisfactory sedative combination using medetomidine and midazolam. The antagonistic effect of atipamezole on this sedative combination was also evaluated. Moreover, the effect of this combination on cardiopulmonary system and blood glucose were determined. Finaly,

Part 3 deals with the efficacy of this combination as a preanesthetic before parenteral (ketamine) and inhalation (isoflurane) anesthesia in pigs. In this part a combination of medetomidine and midazolam was used with ketamine or ketamine and butorphanol and its anesthetic efficacy and cardiopulmonary effects were evaluated. In addition, the antagonistic effect of atipamezole on this anesthetic combination and its cardiopulmonary effect were evaluated. Furthermore, the effect of preanesthetic administration of medetomidine-midazolam on mask induction of isoflurane anesthesia and its isoflurane-sparing and cardiopulmonary effects were evaluated.

Part 1- Fundamental aspects of an α_2-agonist medetomidine in pigs

Part 1-1

Sedative Effects of Medetomidine in Pigs

As described in preface, xylazine, an α_2-adrenoceptor agonist, has been widely used as a novel sedative in many species [18]. Although the combination of xylazine and ketamine [6] or xylazine, butorphanol and ketamine [42] provides satisfactory anesthesia, it is ineffective as a sole agent in pigs [8]. Recently, medetomidine, a selective and specific α_2-adrenoceptor agonist, has been developed and reported as a more potent and valuable sedative than xylazine in dogs, cats and many other species [29, 71].

The purpose of this experiment was to evaluate the sedative property of medetomidine comparing with xylazine, and to investigate its optimal dosage in laboratory pigs.

MATERIALS AND METHODS

Animals:

Eleven castrated mixed breed pigs of specific pathogen-free (SUMICHIKU Co. Ltd., Japan) with the age ranging from 9 to 14 (mean 11.3) weeks old and weight ranging from 15.0 to 23.0 (mean 18.3) kg were used in this study. The pigs were kept in the conventional environment, fed diet (NS; Nisseiken Co. Ltd., Japan) once a day, and allowed free access to water. The food was withheld at least for 12 hours prior to the experiments. Those pigs were used one to four times in the following six regimens and each regimen was applied to 5 different pigs. The interval of the experiments for the same animal was at least 7 days.

Drugs:

The regimens used were as follows: 1. xylazine (Celactal; Bayer Japan Ltd., Japan) 2 mg/kg of body weight (Xy), 2. medetomidine (Domitor; Farmos Ltd., Finland) 30 μg/kg (Me30), 3. medetomidine 50 μg/kg (Me50), 4. medetomidine 80 μg/kg (Me80),

medetomidine 100 μg/kg (Me100) and 6. medetomidine 150 μg/kg (Me150). All drugs were administered intramuscularly into the cervical muscle combined with 25 μg/kg of atropine in the same syringe.

Experimental design:

The experiments were performed in a quiet room with a controlled temperature at 24.0±1.5°C and humidity at 50±15 %. The pigs were administered one of the drugs tested, and were kept in solitary cages to keep the animals from disturbing each other. Sedative effects were repeatedly assessed before injections of the drugs, and 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min after dosing. Sedative effect was assessed by posture score, as lateral recumbency with no spontaneous movement (score=3), lateral recumbency with spontaneous movement (score=2), ventral recumbency or sitting on their hind legs (score=1), and standing or walking (score=0). Furthermore, walk time (time from lateral recumbency to walking) was also recorded in the pigs showing lateral recumbency at least for 10 min. Muscle relaxation was observed and recorded in the muscles of the jaw, the leg, the abdomen and the rectal sphincter. When the complete relaxation was observed in one or more muscles, duration of the effect was recorded. When pigs lost spontaneous movement, analgesic effect was tested by clamping the ear, nose and tail. Recovery condition and undesirable side effects were also observed throughout the experiment.

Statistical analyses:

Posture score, walk time and the duration of muscle relaxation were compared between the regimens using Kruskal-Wallis's non-parametric method. Values of $p<0.05$ were considered significant.

RESULTS

Sedative effects:

Smooth onset of sedative effect without excitation was observed within 10 min post-injection in pigs given medetomidine at any doses, whereas pigs given xylazine showed only poor sedative effect. Depth of sedation assessed by posture score and its statistical analysis between the regimens are shown in Fig. 1 and Table 1, respectively. From 30 to 90 min post-administration, the depth of sedation produced by medetomidine increased up to 80 mg/kg, at which dose the depth was significantly higher than that produced by Xy or Me30. At doses of 100 and 150 μg/kg, its potency was not increased, although the duration of sedative effect was longer than that at lower doses. All pigs in Me50 or Me80 showed continuous lateral recumbency, whereas one pig in Me30 and Me150, and two pigs in Xy and Me100 did not. Walk time tended to be prolonged according to an increase in doses of medetomidine (Table 2). However, there were no significant dose dependency because the results of walk time were variable even in the pigs received the same regimen.

Muscle relaxant and analgesic effects:

As shown in Table 2, all pigs administered xylazine did not show apparent muscle relaxation, however 4 of 5 pigs (Me30 and Me50) or all 5 pigs (Me80, Me100 and Me150) administered medetomidine showed moderate muscle relaxation. The degree of the relaxation seemed to be dose dependent within the range from 30 to 80 μg/kg. The duration of the relaxation was prolonged according to an increase in the dose of medetomidine up to 150 μg/kg. The pigs given xylazine hardly showed analgesia, however those given medetomidine showed mild analgesia, though it was not enough for the painful manipulation.

During recovery period, the pigs in all regimens showed no excitation, but appeared to be slightly sensitive to sound and manipulation. Sedative effects were continued approximately for 1 to 1.5 hr in Xy, 1 to 2.5 hr in Me30, 1.5 to 3 hr in Me50, 1.5 to 4 hr in Me80 and Me100, and 2 to 5 hr in Me150, respectively. After walking pigs often returned to sleep particularly in the regimens with higher doses of medetomidine. Excessive salivation and vomiting were not observed in all regimens.

DISCUSSION

Both xylazine and medetomidine possess an affinity to α-adrenoceptors, especially to α_2-subtype of the receptors, which are located pre- and post-synaptically in the central nervous system [79]. Their activation results in sedation, analgesia and other complex effects. The α_2/α_1 selectivity ratio of medetomidine is about ten times higher than that of xylazine [79], therefore medetomidine is considered to have higher potency of sedation, muscle relaxation and analgesia than xylazine. In the present study, the administration of xylazine at a dose of 2 mg/kg, which is the recommended dose of xylazine when combined with other anesthetics in pigs [18], produced poor sedation with poor muscle relaxation as previously reported. On the contrary, Me80 produced significantly deeper sedation expressed by posture score with moderate muscle relaxation than xylazine, and even the lower doses (Me30 and Me50) seemed to produce deeper sedation than xylazine. The depth of the sedation produced by medetomidine showed significant dose dependency within the range from 30 to 80 mg/kg, and the duration of the sedative effect increased at higher doses of medetomidine without further deepening of sedation. Since two pigs in Me100 and a pig in Me150 did not show continuous lateral recumbency, it seemed that sedative effect of Me100 and Me150 might be unstable as compared with

Me80. Although medetomidine has high α_2/α_1 selectivity ratio [79], it is considered to have some effect mediated by α_1-adrenoceptor. This might be one of the causes of unstable sedation produced by medetomidine at higher doses, however precise etiology of this inconsistent results is unclear. But there was no excitation or other undesirable effect in pigs of these regimens, therefore both Me100 and Me150 might not be an overdose in pigs.

In conclusion, medetomidine induced significant (at 80 μg/kg) and dose-dependent (from 30 to 80 μg/kg) sedative effects which were much more potent than those of xylazine in pigs. These results also suggested that 80 μg/kg of medetomidine is suitable for sedation with lateral recumbency and moderate muscle relaxation without notable side effects.

SUMMARY

Sedative effects of medetomidine were evaluated in atropinized pigs using 5 different doses (30, 50, 80, 100 and 150 μg/kg of body weight) and compared with those of xylazine (2 mg/kg). All drugs were administered intramuscularly. Medetomidine at a dosage of 30 μg/kg produced more potent sedation than xylazine. The depth of sedation induced by medetomidine was dose dependent within the range from 30 to 80 μg/kg. At 100 or 150 μg/kg, the depth of sedation was mostly the similar level to that at 80 μg/kg but the duration was prolonged. The degree of muscle relaxation produced by medetomidine also seemed to be dose dependent from 30 to 80 μg/kg and was stronger than that produced by xylazine. An increase in the duration of muscle relaxation was dose dependent up to 150 μg/kg. No analgesic effect was produced by xylazine, however moderate analgesia was obtained by medetomidine. From these results, medetomidine has a significant and dose-dependent sedative effects which are much more potent than those of xylazine, and 80 μg/kg of medetomidine is suitable for sedation with lateral recumbency and moderate muscle relaxation without notable side effects in pigs.

Table 1. Statistical analysis of the posture score between each regimen[a)]

Drug	Dose	0 to 30	30 to 60	60 to 90	90 to 120	0 to 120 (min)
Xylazine	2 mg/kg	A	A	A	A	A
Medetomidine	30 μg/kg	A	A	AB	A	AB
	50 μg/kg	A	AB	BC	AB	BC
	80 μg/kg	A	B	C	ABC	CD
	100 μg/kg	A	B	C	BC	D
	150 μg/kg	A	B	C	C	D

a)Data are compared at 30 min interval and full term (from 0 to 120 min post-injection) using Kruskal-Wallis's non-parametric method.
a-d: Different characters show that significant difference ($P<0.05$) exists between the regimens.

Table 2. Effects of xylazine and medetomidine on walk time and muscle relaxation in 5 pigs of each regimen[a)]

Drug	Dose	Walk time		Muscle relaxation	
		Number of pigs[b)]	Time(min)[c)]	Number of pigs[d)]	Duration(min)
Xylazine	2 mg/kg	3	11.8±10.9[A]	0[e)]	-
Medetomidine	30 μg/kg	4	25.0±19.2[AB]	4	28±18[A]
	50 μg/kg	5	37.2±16.8[AB]	4	42±25[AB]
	80 μg/kg	5	56.4±11.7 [B]	5	60±12[AB]
	100 μg/kg	3	47.6±47.9[AB]	5	82±23 [B]
	150 μg/kg	4	67.0±70.5[AB]	5	88±41 [B]

a)Data are expressed as mean±SD.
b)The number of pigs showing lateral recumbency (more than 10 min) after drug administration in each regimen.
c)Time from continuous lateral recumbency to being able to walk.
d)The number of pigs observed complete muscle relaxation in the muscle of the jaw the leg, the abdomen or the rectal sphincter after drug administration in each regimen.
e)No or poor muscle relaxation was observed in all pigs.
AB:Results with different superscripts are significantly different ($p<0.05$).

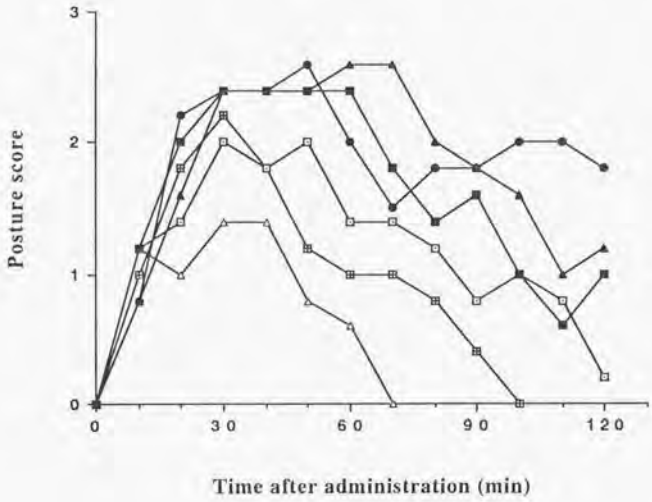


Fig. 1. Effects of 2 mg/kg of xylazine (-△-), 30 μg/kg of medetomidine (-⊞-), 50 μg/kg of medetomidine (-□-), 80 μg/kg of medetomidine (-■-), 100 μg/kg of medetomidine (-▲-) and 150 μg/kg of medetomidine (-●-) on posture score in pigs. Each symbol represents the mean value of the posture score in five pigs.

Part 1-2

Comparison of sedative effects induced by medetomidine, acepromazine, azaperone, droperidol and midazolam in pigs

As described in Part 1-1 medetomidine induced the sedation accompanied with lateral recumbency and mild analgesia in pigs. On the basis of chemical structure, sedatives are divided into four major groups which are butyrophenones, phenothiazines, benzodiazepines and thiazines. Several drugs of these groups have been reported to be available for swine sedation [33]. Azaperone, a butyrophenone derivative, has been most widely used, and acepromazine (a phenothiazine derivative), droperidol (a butyrophenone derivative) and diazepam (a benzodiazepine derivative) have been reported to be available for sedation in pigs.

The purpose of this experiment was to compare qualitatively and quantitatively the sedative effect of medetomidine with those of the sedatives described above or its derivative (azaperone, acepromazine, droperidol and midazolam). Special attention was paid to evaluate the depressive abilities of the arousal reaction induced by sensory stimuli of these drugs.

MATERIALS and METHODS

Animals:

Six castrated mixed breed pigs were repeatedly used at a weekly interval in this study. Their mean age was 12.2 weeks (range 9 to 15 weeks) and mean body weight was 20.8 kg (range 15.5 to 26.0 kg). During the period of stabilization (for more than a week), the pigs were fed a commercial ration once a day and given water *ad libitum*. The pigs were fasted for more than 12 hr before the experiments, and each animal was exposed to different drugs in a randomized block design.

Drugs:

The drugs and their dosages used in this study were as follows: acepromazine maleate (Acepromazine maleate injection, TechAmerica Veterinary Products, U.S.A.) at a dose of 2 mg/kg of body weight, azaperone (Stresnil,Sankyo Co.Ltd.,Japan) at 8 mg/kg, droperidol (Droleptan, Sankyo Co. Ltd., Japan) at 2 mg/kg, midazolam (Dormicum, Yamanouchi Pharmaceutical Co., Japan) at 2 mg/kg and medetomidine (Farmos Group Ltd., Finland) at 80 μg/kg. The dose of each drug was determined from the recommended or previously reported dose in pigs [22, 64] or the results in Part 1-1. All drugs were injected intramuscularly into the cervical muscle.

Experimental design:

Experimental conditions including room temperature, humidity and the caging system were the same as those in Part 1-1. Sedative effects were repeatedly assessed before injections of the drugs, and 10, 20, 30, 40, 60, 90, 120, 180, 240, 360 and 480 min after dosing and/or until the complete recovery from sedation.

Sedative effect of each drug was totally assessed by ataractic character and analgesic/anesthetic character and by induction time (time from injection of the drug until the animal became ventral recumbency), standing time (time from injection of the drug until the animal could stand) and total recovery time (time from injection of the drug until the animal could not be distinguished from untreated animals) [24]. Ataractic character of each drug was evaluated from posture, response to noise, resistance to restraint and resistance to mouth open and to pull tongue outwards. Analgesic/anesthetic character was evaluated from response to nose-pinching and toe-pinch withdrawal response.

The criteria for posture score used in this experiment were modified from that in Part 1-1: score 0: normal; score 1: being able to stand or sit on their hind legs; score 2: keeping the position of ventral recumbency; score 3: lateral recumbency with apparent

spontaneous movement (head lifting or limb struggling); score 4: lateral recumbency with subtle spontaneous movement (ear and nose twitching or blink); score 5: lateral recumbency without any spontaneous movement. Response to noise was assessed as follows: score 0: normal response; score 1: hears and moves; score 2: hears and twitches ear; scores 3: barely perceives; scores 4: no perception. Resistance of the pig against the restraint was scored as follows: score 0: strong resistance against being laid laterally recumbent; score 1: moderate resistance against being laid laterally recumbent; score 2: slight resistance against being laid laterally recumbent; score 3: no resistance against being laid laterally recumbent but moderate resistance against dorsally recumbent; score 4: no resistance against being laid both laterally and dorsally recumbent. Resistance against mouth open and pulling the tongue outwards was evaluated as follows: score 0: normal tonus; score 1: slightly weakened; score 2: moderately weakened; score 3: markedly weak; score 4: no resistance.

Response to nose (nasal septum) -pinching was assessed as follows: score 0: total body movement; score 1: raising the head; score 2: slight movement of the head; score 3: no response. Toe-pinch withdrawal response was scored as follows; score 0: normal response; score 1: weakened; score 2: only be induced by an increased stimulus; score 3: no response. The results of the individual parameters described above were summed up to form the each character.

Recovery condition and undesirable side effects were also observed throughout the experiment.

Statistical analysis:

Differences in sedative effects between drugs or dosages at corresponding time were assessed by use of Kruskal-Wallis test and Williams Wilcoxon multiple comparison

procedure. The data of induction time, standing time and total recovery time were analyzed by one-way analysis of variance and Duncan's multiple comparison procedure. In all analyses, values were considered to be statistically significant when $P<0.05$.

RESULTS

Following the intramuscular injection of medetomidine, pigs were smoothly induced to sedation and became ataxic and drowsy within a few min. The animals consistently became ventral recumbency in less than 15 min (Table 1), then became lateral recumbency and maintained this position for approximately 60 min. During being in lateral recumbency, these pigs were in deep sedation with losing consciousness. They did not respond to most of sensory stimuli and were not aroused when an observer approached to the animals, clapped the hands, touched the body, pulled the extremity, pinched the nose or toe and restrained in lateral recumbency, however they resisted against restraint in dorsal recumbency. In addition, these pigs showed apparent muscle relaxation during this period and hardly resisted against mouth open. Mean total score used for evaluating ataractic character of medetomidine maintained much higher values than those of other sedatives from 20 to 60 min after administration (Fig. 1). There were significant differences in the scores between pigs given medetomidine and all other sedatives at 30 and 40 min after injections of drugs (Table 2). There were also significant differences in the scores between medetomidine and azaperone, acepromazine or droperidol at 20 min after injection, and between medetomidine and acepromazine, droperidol or midazolam at 60 min after injection (Table 2). In addition to the ataractic character, medetomidine produced weak analgesic/anesthetic effects for a short duration (Fig.1). On the contrary, other sedatives produced no analgesic/anesthetic effects and

there were significant differences in a total score used to monitor analgesic/anesthetic effects between medetomidine and other sedatives at 20, 30 and 40 min after administration.

The pigs given azaperone were also smoothly induced to sedation and became ataxic and drowsy within a few min, but only 3 of the 6 pigs became lateral recumbency and the others maintained ventral recumbency. In these pigs, it took a longer time to reach maximum effects and similar sedative condition was maintained for longer duration. The mean total score used to ataractic character in these pigs marked higher values than those in other sedatives at 90 min after injection and thereafter (Fig. 1). There were significant differences in those scores between azaperone and all other sedatives at 180 min after injections and thereafter (Table 2). However, even at the maximum action, azaperone did not have an apparent muscle relaxant effect and did not depress the reaction to tactile and painful stimuli, though it moderately depressed the reaction to visual and auditory stimuli.

Induction of sedation by acepromazine and droperidol was not so smooth and quick as medetomidine and azaperone (Table 1). One of the 6 pigs in these groups did not become ventral recumbency and maintained standing position throughout the observation period. Other pigs repeated being ventral or sitting position and/or standing position until being in continuous ventral recumbency. Acepromazine and droperidol produced light sedation and the mean totals score used for evaluation of ataractic character in these pigs were lower than that of azaperone throughout the observation period. These sedatives exerted mainly quietening and calming effects, and the pigs became lateral recumbency for short duration or did not and stood up in about 90 (acepromazine) or 120 min (droperidol) after administration in an average (Table 1). Furthermore, the animals showed no apparent

muscle relaxation and responded to sensory stimuli normally or even in an exaggerated manner.

Administration of midazolam resulted in marked ataxia with struggling in their cages for 5 to 15 min. After that all the animals calmed down and 5 of 6 pigs became ventral recumbency and then lateral recumbency. However, the sedative effect of midazolam continued for only a short duration and the pigs stood up within 40 min after administration in an average (Table 1).

Recovery from sedation in pigs given medetomidine was quick and smooth without excitement and other unpleasant effects. The mean standing time and the mean total recovery time in these pigs were significantly and much shorter than those in azaperone. It took more than 12 hr for total reconvert in 2 of the 6 pigs given azaperone. During the recovery phase, all the pigs given azaperone, 3 pigs given acepromazine and 2 pigs given droperidol continued to lick and gnaw the inside of their cages until they recovered completely, among which the pigs given azaperone showed severer behavior which caused in much salivation and bleeding from their oral cavity.

DISCUSSION

For the sedation of pigs as experimental animals, losing consciousness and arousal reaction against sensory stimuli is essential even for minor handling. And it is also desired to induce the animals to sedation quickly and smoothly as well as the rapid recovery after completion of the procedures or examinations.

In this study administration of medetomidine induced a satisfactory and the most profound degree of sedation with greater drowsiness than was achieved with other sedatives tested. One of the most apparent differences between medetomidine and other

sedatives was the effect on arousal reaction induced by sensory stimulation such as visual, auditory, tactile or painful stimuli. This reaction was depressed in pigs given medetomidine, while those in pigs given other sedatives were not influenced so much. It has been known that sensory stimuli activate the locus coeruleus in the pons of the upper brain stem [31]. The sedative action of medetomidine is generally ascribed to inhibition of locus coeruleus, which contains pathways involved in the maintenance of vigilance, mediated through presynaptic α_2-adrenoceptors [2, 61]. This mechanism might also reduce the reactivity to sensory stimuli [13]. In addition, medetomidine has been reported to activate postsynaptic α_2-adrenoceptors in the brain and both pre- and postsynaptic mechanisms induce an extraordinary potent ability of medetomidine. Although this depressant effect may not be strong enough to avoid any arousal reaction by manipulation and restraint, medetomidine has the most suitable sedative effect as a chemical restraint agent for pigs among the drugs tested in this study.

In addition, medetomidine was thought to be a suitable sedative as a chemical restraint agent for laboratory pigs because it produced apparent muscle relaxation and weak analgesia which were not observed apparently in other sedatives. Furthrmore, the onset and recovery from sedation of medetomidine was quick and smooth as compared with other sedatives. The pigs given medetomidine were induced to sedation without any apparent adverse effects and totally recovered from sedation much earlier than pigs given azaperone. This is why medetomidine has a high affinity and selectivity at α_2-adrenoceptors and has a short elimination half-time [50]. The duration of valuable sedative action of medetomidine was shorter than that in azaperone, however, that might be long enough for most of practical uses.

Pigs given azaperone, acepromazine and droperidol showed the strange behavior such as persistent licking or gnawing during recovery phase. It has been known that these drugs produce their sedative effects by antagonism of dopamine as a neurotransmitter which also causes extrapyramidal side effects characterized by abnormal motions [60]. This abnormal behavior observed in this study may be related to this neurological side effect.

Although diazepam has been reported to produce sedation in pigs, we selected midazolam instead of diazepam in this study. Recently diazepam has been widely replaced by midazolam in human medicine, since midazolam has a greater advantage to diazepam; potent effects, short half-life and water-solubility [60]. However, administration of midazolam in this study resulted in marked ataxia with struggling for a certain time and the duration of desirable sedation was too short. Furthermore, injection volume of midazolam for sedation was relatively large (8 ml for the pig of 20 kg of body weight) with a higher cost and its administration caused pain and head shaking. Thus, midazolam might find little clinical support as a sole agent for sedation or chemical restraint. However, benzodiazepines are more important or valuable as adjuncts to other anesthetics or opioids in veterinary practice [64]. Furthermore, it has been reported that the combination of midazolam and metoclopramide or droperidol showed synergistic effects and produced good sedation in pigs [22]. Further investigation for the combined use of midazolam at a lower dose with other sedatives such as medetomidine was thought to be valuable for development of more suitable sedation in pigs.

In conclusion, among the sedatives tested in this study, medetomidine produces the most potent and satisfactory sedative effects in pigs, and its sedative character is the most

suitable for a chemical restraint agent as compared with those of azaperone, acepromazine, droperidol and midazolam.

SUMMARY

The sedative effects of medetomidine, acepromazine, azaperone, droperidol and midazolam were compared in pigs. In these sedatives, medetomidine produced the most profound degree of sedation with greater drowsiness than was achieved by other sedatives tested. One of the most apparent differences between medetomidine and other sedatives was the effect on arousal reaction induced by sensory stimulation such as visual, auditory, tactile or painful stimuli. This reaction was depressed in pigs given medetomidine, while those in pigs given other sedatives were not influenced so much. Although this depressive effect may not be strong enough to avoid any arousal reaction by manipulation and restraint, medetomidine has the most suitable sedative effect as a chemical restraint agent for pigs among the drugs tested in this study.

In addition, medetomidine was thought to be a suitable sedative as a chemical restraint agent for laboratory pigs because it produced apparent muscle relaxation and weak analgesia which were not observed apparently in other sedatives. Furthrmore, the onset and recovery from sedation of medetomidine were quick and smooth as compared with other sedatives. The sedative character of medetomidine seemed to be the most suitable as a chemical restraint agent among the sedatives tested in pigs.

Table 1. Induction time, standing time and total recovery time in pigs given medetomidine, azaperone, acepromazine, droperidol and midazolam[a)]

sedative	mean induction time (min)	mean standing time (min)	mean total recovery time (min)
azaperone	13.2±13.3	177.3±82.8 [A]	648.0±107.3 [A]
droperidol	16.0±3.0	121.0±86.4 [AB]	288.0± 55.4 [BC]
acepromazine	24.6±11.6	88.7±51.0 [BC]	355.0±105.7 [B]
medetomidine	12.5±5.7	79.2±16.4 [BC]	227.2± 36.0 [CD]
midazolam	7.8±4.8	39.3±26.0 [C]	185.8± 78.4 [D]

a) Data were expressed as mean ± standard deviation.

A,B,C: Mean values with same superscripts are not significantly different (P>0.05)

Table 2. Statistical analysis of the total scores used for evaluating ataractic character[a)]

	time after administration (min)													
sedative	0	10	20	30	40	60	90	120	180	240	300	360	420	480
medetomidine	A	A	A	A	A	A	AB	AB	A	A	A	A	A	A
azaperone	A	A	AB	B	B	AB	C	A	B	B	B	B	B	B
midazolam	A	AB	B	B	B	C	A	C	C	A	A	A	A	A
droperidol	A	C	BC	BC	B	BC	BC	BC	A	A	A	A	A	A
acepromazine	A	BC	C	C	B	C	AB	BC	A	A	A	A	A	A

a)Same alphabet (A,B,C) means that there is no significant difference in posture score between each group (P>0.05)

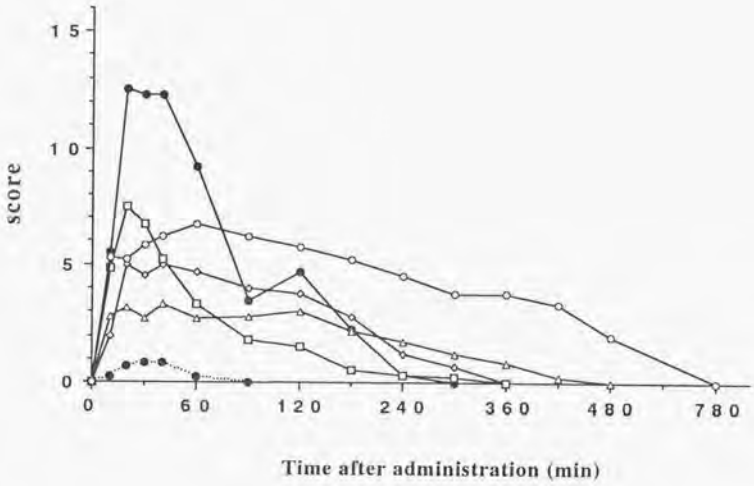


Fig. 1. Effects of medetomidine (●), azaperone (○), acepromazine (◇), droperidol (□) and midazolam (△) on ataractic variables (—; full marks=16) and analgesic/anesthetic (---; full marks=6)variables. Each symbol represents the mean value of the total scores used for evaluating ataractic character or analgesic/anesthetic character.

Part 1-3

Antagonism of medetomidine sedation by atipamezole in pigs

As described in Part 1-1 and 1-2, medetomidine smoothly and quickly induced sedation with weak analgesia and muscle relaxation and depressed arousal reaction deeply as compared with other sedatives, and its sedative character seemed to be the most suitable as a chemical restraint agent among the sedatives tested in pigs.

Recent researches have shown that atipamezole, a highly selective adrenergic antagonist, is effective for reversal of sedation and analgesia caused by medetomidine in dogs and cats [73, 70]. An antidotal procedure for the reversal of medetomidine is also useful in shortening the recovery period or controlling the excessive or prolonged sedative condition in pigs. The purpose of this study was to evaluate the reversal effect of atipamezole on medetomidine-induced sedation in pigs. This study was also designed to evaluate the effects of medetomidine and atipamezole on heart rate and body temperature.

MATERIALS AND METHODS

Animals:

Six castrated mixed breed pigs of specific pathogen-free were repeatedly used at a weekly interval in this study. Their mean age was 16.3 weeks (range 14 to 18 weeks) and mean body weight was 25.4 kg (range 22.5 to 29.0 kg). During a 7-day period of conditioning, the pigs were fed a commercial ration once a day and given water *ad libitum*. The pigs were fasted for 12 to 16 hr before the experiments, and each animal was exposed to 5 different combinations of the drugs in a randomized block design.

Experimental design:

The experiments were performed in a quiet room with a controlled temperature at 24.0±1.5 °C and humidity at 50±15 %. The pigs were injected medetomidine intramuscularly at a dose of 80 μg/kg of body weight, which was the optimal dose for sedation in pigs. Then the animals were kept in solitary cages to keep the animals from disturbing each other. Thirty min after administration of medetomidine, the pigs were given saline solution as a control or atipamezole (Farmos Group Ltd., Finland) at doses of 80, 160, 320 and 480 μg/kg, which were one, two, four and six times higher than the dose of preceding medetomidine. Saline solution was administered in amount of 0.096 ml/kg which was the same volume as atipamezole at the dose of 480 μg/kg. All injections were made intramuscularly into the neck.

Antagonistic effects of atipamezole were evaluated by the observation of posture, standing time (time from injection of atipamezole or saline solution until the animal could stand) and total recovery time (time from injection of atipamezole or saline solution until the animal could not be distinguished from untreated animals). Posture was scored by the same criteria in Part 1-2. Posture was repeatedly scored before injections of medetomidine and atipamezole, and then 10, 20, 40, 60, 90, 120, 180, 240, 300 and 360 min after injection of atipamezole.

Heart rate and rectal temperature were measured before injection of medetomidine as base-line values, during which time the pigs were kept on a canvas sling. After drug administration, those measurements were repeated at the same interval as the posture scoring at their own cages. Residual sedation/ataxia, behavioral phenomena, relapse phenomena and adverse effects were also recorded.

Statistical analyses of the results of the posture score and various sedative times were also performed using the same method described in Part 1-2. The data of heart rate and

rectal temperature were analyzed by one-way analysis of variance and Duncan's multiple comparison procedure, and by paired-*t* test. In all analyses, values were considered to be statistical significant when $P<0.05$.

RESULTS AND DISCUSSION

Following the intramuscular injection of medetomidine, the pigs were smoothly induced to sedation and became ataxic and drowsy within a few min. All the animals in each group consistently became ventral recumbency, and then lateral recumbency in averages of 15.5 to 20.2 min (Table 1).

Atipamezole injections at doses of 160, 320 and 480 μg/kg quickly and effectively reversed the medetomidine-induced sedation (Fig. 1). At these doses, the first signs of arousal (starting to follow the environment with the eye, lifting the head) were seen 2 to 7 min after atipamezole injections, and all the pigs rose to standing position with least ataxia within 12 min. Ten min after injection, posture scores in pigs given atipamezole at 320 and 480 μg/kg significantly decreased compared to those in a control group. Twenty min after injection, the score in pigs given even twofold of atipamezole was also significantly different from that in a control group (Table 2). The mean standing time and total recovery time at these doses (160, 320 and 480 μg/kg) were significantly shortened to one- to two-tenth of the values without atipamezole. Among these groups, posture score, mean standing time and mean total recovery time were not significantly different from each other (Tables 1 and 2).

Recovery from sedation in pigs given atipamezole at 160 and 320 μg/kg was smooth with minimal adverse effects and with no relapses into sedation, although one of the pigs given atipamezole at 320 μg/kg appeared mildly hyperactive for short duration. Four of

the 6 pigs given atipamezole at 480 μg/kg appeared mildly hyperactive or mildly excited, three manifested grunting and chewing and one had mild muscular tremors during the recovery phase. These undesirable effects presented in these pigs were probably caused by the excitatory effects of α_2-adrenergic antagonist on central nervous system [83]. However, these signs of increased activity were not serious and all pigs appeared totally normal within 10 to 40 min after atipamezole injection. Similar results have recently been reported in cats, in which the most suitable dosage of atipamezole was two to four times the preceding dosage of medetomidine (100 μg/kg), and atipamezole at higher dosages caused alertness and panting in some cases [70].

On the contrary, atipamezole at the same dose as the preceding medetomidine dose had an insufficient effect. Although the mean standing time and mean total recovery time in this dose group were significantly shorter than those in a control group, mean total recovery time was significantly longer than those in groups with higher atipamezole doses (Table 1). In addition, relapse to ventral or lateral recumbency and unconsciousness occurred 20 to 40 min after the injection of atipamezole in four of 6 pigs. Mean posture score in this dose group was not significantly different from that in a control group until 90 min after injection of atipamezole (Table 2).

Table 3 shows the effects of atipamezole on heart rate and body temperature in pigs sedated by medetomidine. Thirty min after administration of medetomidine, heart rate mildly decreased to 75 to 90% of the base-line values. Although mean heart rate in a control group continued to fall gradually to the lowest value of 67 beats /min, these changes were not so severe as those reported in dogs and cats [73, 70]. Atipamezole reversed these medetomidine-induced changes in heart rate within 10 min after its injection, and the increasing effect on heart rate was dose-dependent. The lowest dose of

atipamezole (80 μg/kg) brought heart rate to approximately base-line levels. At the highest dose of atipamezole (480 μg/kg), heart rate significantly increased from the base-line value and caused moderate tachycardia (up to 180 beats/min) in three of the 6 pigs for 20 to 40 min. Tachycardia was probably caused by a central stimulant effect of atipamezole, and by a peripheral effect on vascular bed, which induced transient vasodilative hypotention and reflex increase in heart rate [20].

Body temperature gradually decreased after injection of medetomidine in each group. In a control group, mean body temperature decreased to 35.6°C at 120 min after medetomidine injection, and it took more than 240 min to recover to the base-line level. Atipamezole at any doses (80, 160, 320 and 480 μg/kg) effectively reversed or blocked medetomidine-induced hypothermia. In these atipamezole treated groups, the values did not significantly differ from each other on and after 40 min after injection of atipamezole.

In conclusion, the intramuscular injection of atipamezole effectively reversed medetomidine (80 μg/kg, intramuscularly)-induced sedation in pigs. The optimal action was seen at doses of 160 and 320 μg/kg. Recovery from sedation was quick and smooth, and minimal adverse effects were seen with either dose.

SUMMARY

The efficacy of atipamezole as a medetomidine antagonist was evaluated in pigs. The atipamezole doses (intramuscularly) were 80, 160, 320 and 480 μg/kg of body weight, which were one, two, four and six times higher than the preceding medetomidine dose (80 μg/kg, intramuscularly). Atipamezole effectively reversed medetomidine-induced sedation, and the optimal action was seen at doses of 160 and 320 μg/kg. Recovery from sedation was quick and smooth, and adverse effects such as hyperactivity or tachycardia were minimal with either dose.

Table 1. Recumbency time, standing time and total recovery time in pigs given medetomidine and atipamezole or saline solution[a)]

atipamezole dose	mean recumbency time (min)	mean standing time (min)	mean total recovery time (min)
saline solution	15.5± 9.3[¶]	68.3± 20.4[¶]	261.2± 38.7[¶]
80μ g/kg	18.7± 8.6[¶]	10.0± 5.7[§]	70.7± 47.2[§]
160μ g/kg	18.0± 6.3[¶]	7.2± 3.3[§]	29.3± 20.9[#]
320μ g/kg	15.0± 7.7[¶]	5.2± 1.3[§]	12.3± 3.4[#]
480μ g/kg	20.2± 7.7[¶]	4.7± 2.7[§]	19.2± 13.2[#]

a)Atipamezole or saline solution were administered intramuscularly 30 min after the injection of medetomidine. Data were expressed as mean± standard deviation.
¶§#:Mean values with same superscripts are not significantly different(P>0.05).

Table 2. Statistical analysis of the posture score between each atipamezole dose group[a)]

atipamezole dose	time after injection of atipamezole (min)										
	-30	0	10	20	40	60	90	120	180	240	300
saline solution	A[b)]	A	A	A	A	A	A				A
80μ g/kg	A	A	AB	AB	AB	AB	AB	A	A	A	A
160μ g/kg	A	A	AB	B	B	B	B	A	A	A	A
320μ g/kg	A	A	B	B	B	B	B	A	A	A	A
480μ g/kg	A	A	B	B	B	B	B	A	A	A	A

a)Atipamezole or saline solution were administered intramuscularly 30 min after the injection of medetomidine.

b)Same alphabet(A,B) means that there is no significant difference in posture score between each dose group($p>0.05$).

Table 3. Effects of atipamezole on heart rate and body temperature in pigs sedated by medetomidine[a)]

	time after injection of atipamezole(min)											
atipamezole dose	-30[b)]	0	10	20	40	60	90	120	180	240	300	360
Heart rate(beats/min)												
Saline solution	102.0± 8.5¶	79.0± 8.8¶*	78.0± 7.6¶*	73.3± 6.7¶*	69.0± 6.3¶*	78.0±21.5¶*	67.0±24.1¶*	77.0±16.7*	83.3±21.0	84.5±15.8	104.3± 9.5	102.3± 6.3
80μg/kg	101.0± 9.6¶	92.0±10.5¶*	103.0±20.9¶§	100.0±20.0¶§	92.0±16.8¶§	91.3±10.8¶§	91.3±10.8§					
160μg/kg	108.3± 7.3¶	92.3±12.2¶*	116.0±30.0§#	107.0±16.7§	101.0±23.2§	101.7±20.0§	96.3±16.3§					
320μg/kg	103.3±15.8¶	83.5±19.0¶*	129.0±20.3§#	104.0±11.8§	105.0±12.4	102.0±12.0§	103.3± 7.0§					
480μg/kg	99.0± 7.4¶	76.0±15.0¶*	145.0±38.6#	128.0±40.5§	118.0±32.6§	102.0±20.8§	104.7± 8.1§					
Body temperature(℃)												
Saline solution	38.3± 0.2¶	38.1± 0.5¶	37.7± 0.6¶*	37.2± 0.5¶*	36.8± 0.6¶*	36.3± 0.7¶*	35.8± 0.5¶*	35.6± 0.7*	35.7± 0.9*	36.9± 1.0#	37.8± 0.8	38.3± 0.4
80μg/kg	38.5± 0.2¶	38.7± 0.6¶	38.3± 0.9¶	38.2± 0.6§	38.4± 0.8§	38.6± 0.5§	38.5± 0.2§					
160μg/kg	38.6± 0.3¶	38.1± 0.5¶	37.8± 0.4¶*	37.8± 0.2¶§*	38.2± 0.4§	38.3± 0.3§	38.4± 0.4§					
320μg/kg	38.6± 0.2¶	38.5± 0.9¶	38.1± 0.7¶*	38.2± 0.6§	38.4± 0.5§	38.5± 0.3§	38.6± 0.3§					
480μg/kg	38.6± 0.5¶	38.1± 0.7¶	37.7± 0.9¶*	38.0± 0.7§	38.4± 0.6§	38.5± 0.6§	38.8± 0.5§					

a)Atipamezole or saline solution were administered intramuscularly 30 min after the injection of medetomidine. Data are expressed as mean± standard deviation.

b)Before the injection of medetomidine heart rate and body temperature were measured as the baseline-value.

¶§# :Mean values with same superscripts are not significantly different (P>0.05) from each other.

*:Significantly different (P<0.05) from the base-line value.

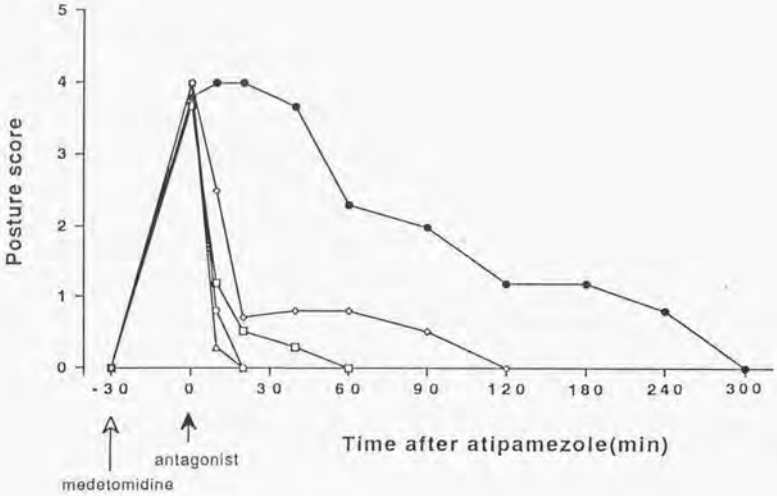


Fig. 1 Effects of atipamezole on posture score in pigs sedated by medetomidine. Medetomidine (80μ g/kg of body weight) was injected intramuscularly (↑) 30 min before injection of atipamezole. The pigs were given atipamezole intramuscularly (↑) in amounts one(80μ g/kg, ◇-◇), two(160μ g/kg, □-□), four(320 μ g/kg, △-△), and six(480μ g/kg, ○-○) times higher than the dose of medetomidine or saline solution as a control(●-●). Each symbol represents the mean value of the posture score in six pigs.

Part 1-4

Pharmacokinetics of medetomidine, midazolam and atipamezole in pigs

As described in Part 1-1 and 1-2, medetomidine produced a potent and satisfactory sedation as compared with azaperone, acepromazine, droperidol and midazolam, and its effect was quickly and smoothly antagonized by atipamezole. However, the pharmacokinetics such as clearance and elimination half-lives of medetomidine and atipamezole in pigs have not yet been investigated. Understanding the pharmacokinetics of injectable drugs is essential for their sound practical use. Pharmacokinetics allows predictions about drug concentrations in the body as related to dosage, time, and physiological and pathological alternation in biological functions [17]. In addition, when using antagonists in the clinical practice, it is necessary to consider the pharmacokinetics of the drugs involved, because if the antagonist is eliminated faster than the antagonist, resedation will occur [22].

High performance liquid chromatography with UV or fluorescence detection is one of the most widely used methods for the determination of drugs in biological samples. However, medetomidine has no significant absorption at wavelengths longer than 220 nm and lacks native fluorescence [76], which makes it difficult to develop a chromatographic assay method. Recently, Kanazawa et al. reported the liquid chromatography-atmospheric pressure chemical ionization mass spectrometric (LC-APCI-MS) assay method for the determination of the sedatives and anesthetics in plasma [30]. This assay method enabled the simultaneous determination of many species of sedatives including medetomidine and anesthetics in plasma.

The purpose of this experiment was to investigate the pharmacokinetics of medetomidine, atipamezole in pigs. This study was also designed to evaluate the

pharmacokinetics of midazolam which was used in Part 2 and Part 3 and the pharmacokinetic interactions among these drugs when used simultaneously.

MATERIALS and METHODS

Animals and animal preparation :

Six mixed breed pigs in good health were repeatedly used at a weekly interval in this study. Their mean age was 12.5 weeks (range 10 to 15 weeks) and mean body weight was 25.5 kg (range 18.5 to 30 kg). Management for these pigs were the same as those in Part 1-2. At least 7 days before the experiments, the pigs were implanted 14G heparin-coated polyvinyl chloride catheters (Toray Medical Co., Anthron) into the right lateral jugular vein under isoflurane anesthesia. The pigs were fasted for approximately 12 hr before the experiments, and each animal was exposed to 5 different regimens in a randomized block design.

Experimental protocol :

After collecting blood samples as a control (9 ml each) from the pig keeping in a cloth sling, 40 μg/kg of medetomidine, 0.2 mg/kg of midazolam (Dormicum, Yamanouchi Pharmaceutical Co., Japan), 160 μg/kg of atipamezole, medetomidine-midazolam or medetomidine- midazolam-atipamezole at the above doses were administered into the neck muscle. Nine ml of venous blood samples for determination of plasma drugs levels were then collected into heparinized glass tubes via the implanted catheter 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240 and 360 min after administration of the drug. The blood was centrifuged, and the plasma was collected and frozen at -80°C until assayed.

Assays :

The plasma concentrations of medetomidine, atipamezole and midazolam were measured by an original LC-APCI-MS method as previously reported [30]. The main steps of the procedure were as follows: After addition of 50 ng of the internal standard/ml plasma (detomidine: Farmos Group Ltd. Finland, 1 μg of detomidine in 1 ml of distilled water), 5 ml volume of plasma samples were applied to a octadecylsilane bonded-phase packings (Sep-Pak C_{18}, Waters Associates, Inc., U.S.A.) pretreated with water, methanol, and 0.1 M ammonium acetate. After washing the column with 0.1 M ammonium acetate, the sample was eluted with methanol and eluate was evaporated to dryness under reduced pressure. The residue was dissolved in 200 μl of the eluent and 50 μl of the solution was injected into LC-APCI-MS system (Hitachi L-6200 HPLC instrument with a Rheodyne Model 7125 valve, connected to a Hitachi M1000 mass spectrometer-computer system through APCI interface). The nebulizer and vaporizer temperatures were 260 and 399°C, respectively. The chromatographic separation was carried out on a column of octadecylsilysilica (Hitachi gel 3056, 150 mm × 4.6 mm I.D.) using methanol- 0.1M ammonium acetate (65: 35) as the eluent (1 ml/min) at room temperature.

Standard curves for medetomidine, midazolam and atipamezole were prepared by plotting the peak area ratio (the area under each drug peak divided by the area under the internal standard peak) versus the concentration of each drug in spiked plasma standards.

Pharmacokinetics analysis and statistical analysis :

Linear one- or two-compartment open models with first-order output were used for pharmacokinetic calculations. Non-linear least squares fits of the data were performed with the computer program MULTI [82], which incorporates Hartley's modification of the Gauss-Newton method. The pharmacokinetic parameters defined and calculated were

as follows : C_{max} = the observed peak plasma concentrations, t_{max} = the time from dosage to the peak concentration, K_{el} = first-order elimination rate constant, $t_{1/2}$ (elimination half-life) = $\ln 2/K_{el}$, CL = clearance and V_d (distribution volume during terminal phase) = CL/K_{el} assuming a 100% absorbed fraction [50] .

RESULTS

Assay:

Mass chromatograms and mass spectra of each drug spiked in plasma is represented in Fig. 1. The well resolved chromatogram were obtained within 10 min and quasi-molecular ions $(M+H)^+$ were observed as base peaks. The standard curve of each drug was linear and reproducible. The correlation coefficients between the peak area ratios of medetomidine, midazolam and atipamezole to the internal standard and their concentration were 0.9993, 0.9997 and 0.9997, respectively. The lower limit of a quantitative detectability in each drug was equivalent to a plasma concentration of 1 to 2 ng/ml. The recovery rates of medetomidine, midazolam, atipamezole and detomidine from plasma were 98.2±7.0 (SD), 93.0±3.9, 96.2±5.7 and 100.6±6.4% (n=6), respectively.

Pharmacokinetics of medetomidine, midazolam and atipamezole:

Figures 2 and 3 show the changes in plasma medetomidine and atipamezole concentrations after intramuscular administration (semi-logarithmic plots), respectively. The data of these agents were well fitted by a one-compartment open model with a transient absorption phase followed by an elimination phase. Absorption of these drugs occurred rapidly and peak concentrations in plasma were obtained within 10 min of the dosing. Disposition also occurred rapidly and elimination half-time was relatively short in both drugs. The mean pharmacokinetic parameters for each drug are listed in Table 1.

Although most of the pharmacokinetic parameters of atipamezole were similar to those of medetomidine, its maximal plasma concentration was much higher than that of medetomidine and maintained the higher level throughout the experimental period (Fig. 4). These parameters of medetomidine and atipamezole were not affected when they administered in conjunction with other drugs (medetomidine-midazolam and medetomidine-midazolam-atipamezole).

Changes in midazolam concentrations after administration were fitted by a two-compartment open model with a transient absorption phase followed by an elimination phase (Fig. 5). Absorption of midazolam alone occurred rapidly and the peak concentration in plasma was obtained within 5 min of the dosing. On the contrary, absorption of midazolam was delayed when it was administered with medetomidine (medetomidine-midazolam and medetomidine-midazolam-atipamezole) (Fig. 3). The C_{max} and t_{max} for midazolam alone were higher than those for medetomidine-midazolam or medetomidine-midazolam-atipamezole, however, other parameters revealed similar values (Table 1).

DISCUSSION

As expected from a lipophilic compound [51], medetomidine reached its maximal plasma level very quickly after intramuscular administration, which was corresponding to the quick onset of clinical sign observed in Part 1-2. In the same way, duration of sedation after administration (approximately 80 min) observed in Part 1-2 is comparable with the half-time of plasma concentration (approximately 60 min). However, the time necessary to obtain the maximal plasma concentration slightly preceded the onset time of maximal sedative effect. The sedation induced by medetomidine was evident by 10 min

and reached its maximal level by 20-30 min despite the plasma concentration reached its maximal level at 10 min after dosing. This time-lag might be induced by the delay of the penetration of medetomidine into the central nervous system, which is mainly attributed to low free fraction (0.15) of medetomidine in plasma and equilibration across the blood-brain barrier with the low free drug concentration [50]. It took 15 to 20 min for the drug to reach its maximum concentration in rat brains, although high plasma levels of medetomidine were presented already at 5 min after dosing [50].

Although the most of the pharmacokinetic parameters of atipamezole were similar to those of medetomidine, its maximal plasma concentration was much higher than that of medetomidine and the higher level was maintained throughout the experimental period. In addition, elimination half-time of atipamezole was slightly longer than that of medetomidine. When using antagonists in the clinical practice, antagonists must maintain the proper and higher plasma level than that of the agonist and they should not be eliminated faster than the agonist to reverse cardiopulmonary effects and to avoid re-sedation [22].

As expected from the high lipophilicity of midazolam at physiologic pH [46], midazolam was also absorbed and distributed very quickly after intramuscular administration. In experimental models, the drug rapidly enters the cerebrospinal fluid, and equilibration between plasma and the central nervous system generally occurs within a few min after intravenous administration. In the present study, the pharmacokinetic profile of midazolam was altered when it was administered with medetomidine (medetomidine-midazolam and medetomidine-midazolam-atipamezole). The maximal plasma midazolam concentration decreased greatly and the time necessary to obtain the maximal concentration was delayed when it was administered with medetomidine. This

depressed absorption of midazolam was mainly attributed to the peripheral vasoconstriction induced by medetomidine [52].

SUMMARY

The pharmacokinetics of medetomidine, atipamezole and midazolam and their pharmacokinetics interactions were evaluated in pigs. The data of medetomidine and atipamezole were well fitted by a one-compartment open model with a transient absorption phase followed by an elimination phase. Absorption of these drugs occurred rapidly and the peak concentrations in plasma were obtained within 10 min of the dosing. Disposition also occurred rapidly and the elimination half-time was relatively short in both drugs (approximately 50 to 70 min). Although the most of the pharmacokinetic parameters of atipamezole were similar to those of medetomidine, its plasma concentration was much higher than that of medetomidine when used simultaneously. These parameters of medetomidine and atipamezole were not affected when they administered in conjunction with other drugs (medetomidine-midazolam and medetomidine-midazolam-atipamezole). The data of midazolam were fitted by a two-compartment open model with a transient absorption phase followed by an elimination phase. Absorption of midazolam alone was rapid and peak concentration in plasma was obtained within 5 min of the dosing. Disposition also occurred rapidly and elimination half-time was relatively short (approximately 100 to 120 min), though it was longer than those of medetomidine and atipamezole. However, absorption of midazolam was delayed when it was administered with medetomidine (medetomidine-midazolam and medetomidine-midazolam-atipamezole) through the peripheral vasoconstriction induced by medetomidine. The peak plasma concentrations (C_{max}) and the time from dosage to the peak concentration (t_{max}) for midazolam when used with medetomidine were lower than those for medetomidine-midazolam or medetomidine-midazolam-atipamezole, however other parameters revealed similar values.

Table 1. Summary of pharmacokinetic parameters of medetomidine, atipamezole and midazolam in pigs[a)]

drug	C_{max} (ng/ml)	t_{max} (min)	CL (ml/min•kg)	$t_{1/2}$ (min)	V_d (l/kg)
medetomidine					
med[b)]	23.4	9.2	22.0	57.3	1.82
med-mid[c)]	27.7	10.0	18.2	57.8	1.52
med-mid-atip[d)]	25.2	9.0	21.8	49.5	1.56
atipamezole					
atip[e)]	88.9	10.0	20.4	61.9	1.82
med-mid-atip	101.1	10.0	20.2	69.3	1.80
midazolam					
mid[f)]	500.7	5.0	12.1	97.6[g)]	1.70[h)]
med-mid	119.4	19.0	15.1	114.6[g)]	2.50[h)]
med-mid-atip	127.4	11.3	16.0	111.4[g)]	2.57[h)]

a) Data are represents as mean value (n=6 each).

b) Pigs were given medetomidine (med) at a dose of 40 μg/kg.

c) Pigs were given medetomidine (med) at a dose of 40 μg/kg and midazolam (mid) at a dose of 0.2 mg/kg.

d) Pigs were given atipamezole (atip) at a dose of 160 μg/kg after administration of med-mid.

e) Pigs were given atipamezole (atip) at a dose of 160 μg/kg.

f) Pigs were given midazolam (mid) at a dose of 0.2 mg/kg.

g) $t_{1/2}$ in the β−phase

h) V_d in the β−phase

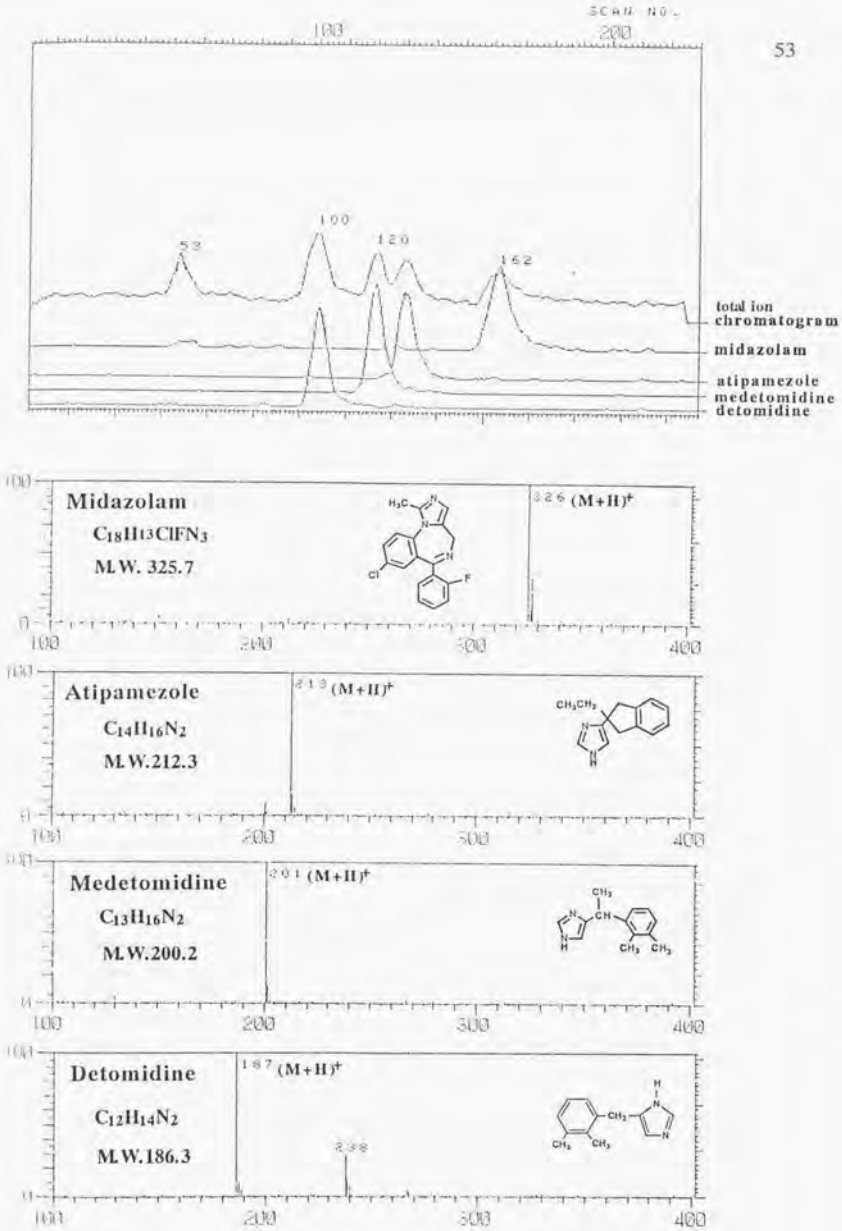


Fig. 1. Mass chromatograms (upper) and mass spectra scanned at the peak tops of the mass chromatograms of swine plasma spiked with midazolam, atipamezole, medetomidine and detomidine (lower)

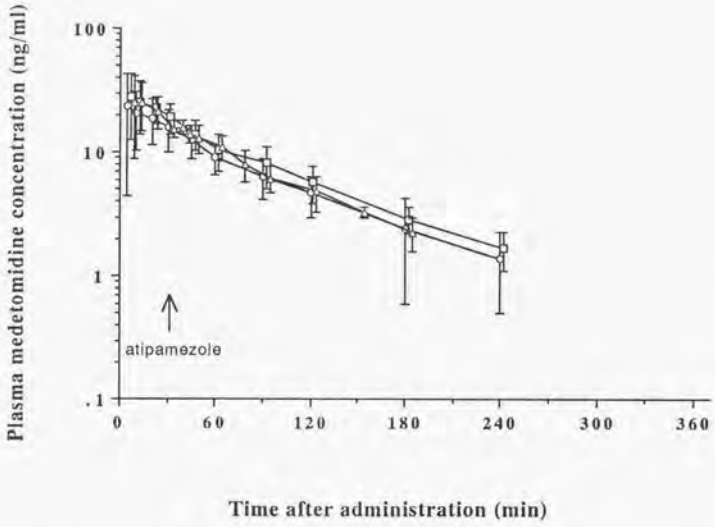


Fig. 2. Plasma medetomidine concentration following intramuscular administration of medetomidine alone (○), medetomidine- midazolam (□) and medetomidine-midazolam atipamezole (△) in pigs. Mean values from six pigs with standard deviations are given.

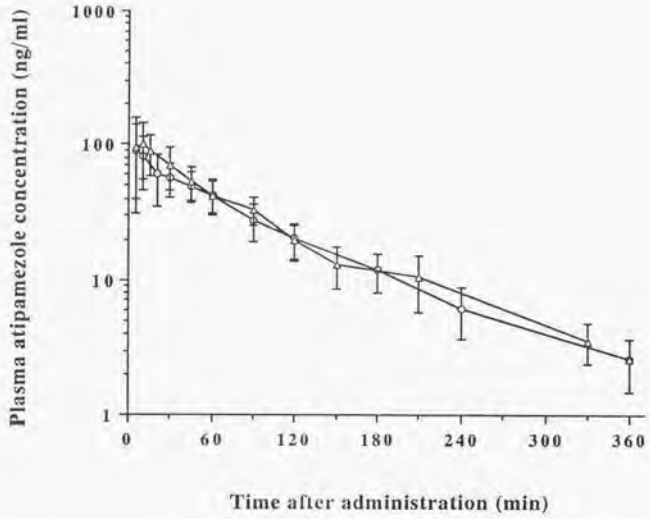


Fig. 3. Plasma atipamezole concentration following intramuscular administration of atipamezole alone (○) and medetomidine-midazolam- atipamezole (△). Mean values from six pigs with standard deviations are given.

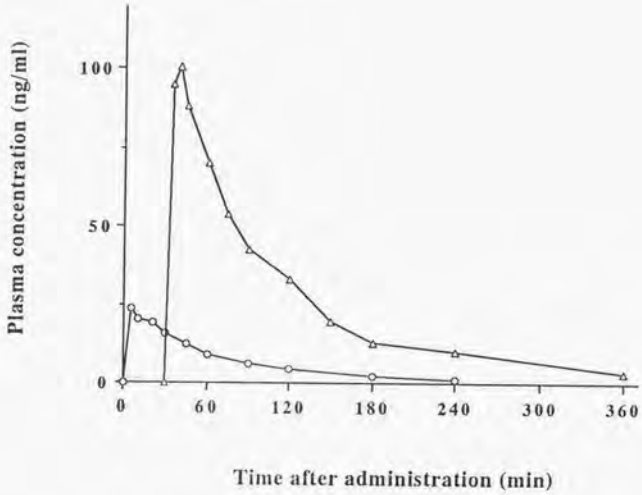


Fig. 4.Plasma concentration of medetomidine (○) and atipamezole (△)in pigs given medetomidine- midazolam- atipamezole. Mean values from six pigs with standard deviations are given.

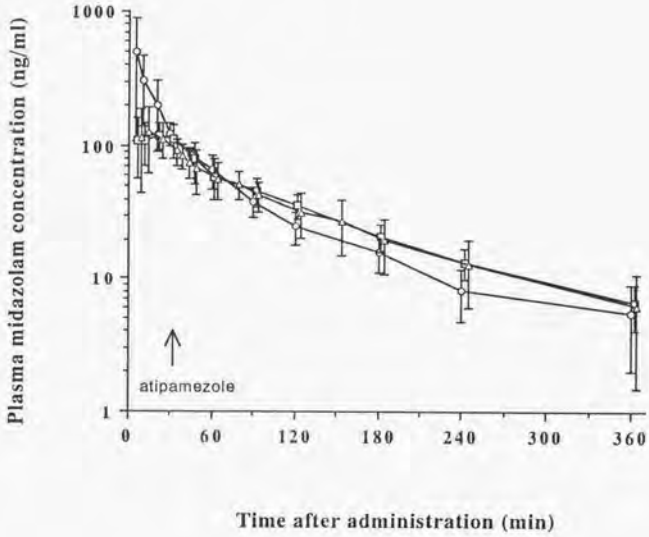


Fig. 5 Plasma midazolam concentration following intramuscular administration of midazolam alone (○), medetomidine-midazolam (□) and medetomidine-midazolam-atipamezole (△) in pigs. Mean values from six pigs with standard deviations are given

Part 2- Establishment of a potent and satisfactory sedation in pigs

Part 2-1

Sedative effect induced by a combination of medetomidine and midazolam in Pigs

As described in Part 1, medetomidine induced a satisfactory sedation and had a more preferable sedative character than azaperone, acepromazine, droperidol and midazolam in pigs. Moreover, medetomidine has a great advantage in its effect being quickly and smoothly antagonized by atipamezole. However, its effect was not potent enough to restrain pigs in dorsal recumbency without resistance. In addition, the animals should be left undisturbed for 20 to 30 min to be induced sedation successfully. When pigs are sedated for experimental purposes, it is to be desired that the sedation should be deep enough to lose arousal reaction on most sensory stimuli and that the induction should not be disturbed or delayed by the stimulation.

Recently, the synergistic interaction between an α_2-agonist and a benzodiazepine has been reported in various animals including humans [22, 49, 56, 67], and the similar synergistic effects by this combination can be expected in pigs.

The major side effect of medetomidine is on the cardiovascular system. In dogs, medetomidine induces dose-dependent depressant effects such as bradycardia, atrioventricular blocks, changes in blood pressure, and a decrease in cardiac output [52]. On the contrary, midazolam has minimum cardiopulmonary effects in a clinical dose. The combination of medetomidine and midazolam would lower the requiring dose of medetomidine with their synergistic effect and cause less cardiovascular effects.

The purpose of this experiment was to evaluate the sedative effect of a combination of medetomidine and midazolam in pigs.

MATERIALS and METHODS

Animals:

Six castrated mixed breed pigs in good health were repeatedly used at a week interval in this study. Their mean age was 11.9 weeks (range 9 to 15 weeks) and mean body weight was 19.2 kg (range 15.5 to 24 kg). Management for these pigs before and during the experiment were the same as the previous experiments.

Experimental design:

The pigs were administered medetomidine at a dose of 80 μg/kg of body weight and 0.2mg/kg of midazolam (med_{80}-mid), 40 μg/kg of medetomidine and 0.2 mg/kg of midazolam (med_{40}-mid), 40 μg/kg of medetomidine and 0.2 mg/kg of midazolam with continuous stimulation by strong pressing of heads and backs during the induction phase for 20 min (med_{40}-mid+stim), 80 μg/kg of medetomidine alone (med_{80}), 40 μg/kg of medetomidine alone (med_{40}) or 0.2 mg/kg of midazolam alone (mid). All drugs were injected intramuscularly into the cervical muscle.

After administration of the drugs the animals were kept in solitary cages to keep the animals from disturbing each other. The sedative effects were repeatedly assessed before injections of the drugs, and 5,10, 20, 30, 40, 50, 60, 80, 100, 120, 180, 240 and 300 min after dosing and/or until the animals totally recovered.

Heart rate and rectal temperature were measured before injection of medetomidine and /or midazolam as base-line values, during which time each pigs was kept on a canvas sling. After drug administration, those measurements were repeated 10, 20, 30, 40, 60, 80, 120 and 180 min after dosing in their own cages.

Assessment of sedative effect:

Sedative effect of each regimen was totally assessed by ataractic character and analgesic/anesthetic character and by recumbency time, standing time and total recovery time as described in Part 1-2. Briefly, ataractic character was assessed from the total score of posture, response to noise, resistance to restraint and resistance to mouth open and to pull tongue outwards; and analgesic/anesthetic character was assessed from total score of response to nose (nasal septum) -pinching and toe-pinch withdrawal response.

Recovery condition and undesirable side effects were also observed throughout the experiment.

Statistical analyses:

Differences of sedative effects and antagonistic effects at corresponding time and the data of recumbency time, arousal time, standing time and total recovery time were statistically analyzed using the same method described in Part 1-2. The data of heart rate and rectal temperature were also analyzed using the same method described in Part 1-2. In all analyses, values were considered to be statistical significant when $P<0.05$.

RESULTS

Following the intramuscular injection of medetomidine and midazolam in either medetomidine dose, the pigs were smoothly and quickly induced to sedation and became ataxic and drowsy within a few min. The mean induction time of med_{80}-mid was 3.8 min and that of med_{40}-mid was 6.3 min, both of which were significantly shorter than that of med_{80} (Table 1).

After being in lateral recumbency, the sedative condition in pigs given either med_{80}-mid and med_{40}-mid deepened further and reached to maximal effects according to the similar time course (Fig. 1), and there were no significant differences in the total score

used for evaluating ataractic character between med_{80}-mid and med_{40}-mid until 100 min after administration (Table 2). The sedation in med_{80}-mid and med_{40}-mid assessed by the total scores for ataractic character reached to the maximal level within 20 min after administrations, and maintained significantly higher level than that in pigs given med_{80} until 50 min after administrations (Fig. 1, Table 2). In addition, the maximal effects in med_{80}-mid and med_{40}-mid were maintained for 30 to 40 min, which was also longer than in med_{80}.

During being in deep sedation accompanied by apparent muscle relaxation, the pigs given med_{80}-mid or med_{40}-mid lost consciousness and the animals did not respond to most of sensory stimuli. These animals did not resist even if they were restrained in dorsal recumbency, and swallowing and laryngeal reflexes were deeply depressed. On the contrary, pigs given med_{80} resisted and struggled when they were placed in dorsal position and pulled their tongue outwards.

Med_{80}-mid and med_{40}-mid exerted the moderate analgesic/anesthetic effect in contrast to the minimal effect in med_{80} (Fig. 2). Although the duration of maximal analgesic/anesthetic effect was relatively short in med_{80}-mid or med_{40}-mid, during which time, pigs reacted only to strong pain induced by nose-pinching. There were no significant differences in the scores for analgesic/anesthetic character between med_{80}-mid and med_{40}- mid, and those scores were significantly higher than that in med_{80} (Table 3).

As shown in Figs. 1 and 2 and Tables 1 to 3, continuos stimulation during induction phase in pigs given med_{80}-mid (med_{80}-mid+stim) did not affect the induction manner, the maximal degree of sedation achieved, the duration of sedation and recovery condition.

Sedation induced by med_{40} alone was light (Fig. 1) and only one of the 6 animals in this group became lateral recumbency for a short duration, while others maintained dorsal position for 27 to 99 min. These pigs were aroused easily and occasionally stood up even by a mild or slight sensory stimulation. Midazolam at a dose of 0.2 mg/kg alone (mid) produced a minimum sedative effect and pigs became slightly ataxic for a short duration. Both regimens exerted no significant analgesic/anesthetic effect.

Pigs given med_{80}-mid and med_{40}-mid were aroused in approximately 90 and 70 min in averages after administration, respectively (Table 1). All the animals recovered smoothly without exciting or other apparent undesirable effects, however the animals were slightly ataxic until total recovery. As compared with med_{40}-mid, it took longer time in med_{80}-mid until they could stood up and recovered completely (Table 1).

Table 4 shows the effect of each regimen on heart rate and body temperature. Heart rate tended to decrease after administration of either dose of medetomidine alone, conversely that slightly increased in mid. Changes in heart rate in med_{80}-mid and med_{40}-mid were similar; slight increasing just after administration of sedatives and then gradual decreasing.

Body temperature gradually decreased after injection of either dose of medetomidine and med-mid and marked significantly lower values than base-line values after 40 or 50 min after administrations. Mild hypothermia sustained for a longer time in pigs given higher dose of medetomidine. On the contrary, body temperature in pigs given mid unchanged throughout the observation period.

DISCUSSION

As mentioned in preface, medetomidine is a highly selective and specific α_2- agonist, and its potent effect associated with muscle relaxation and analgesia is based on activation of pre- and postsynaptic α_2- adrenoceptors in the central nervous system [2,34,61,79].

Midazolam, which is one of the benzodiazepine compounds, exert their main effects through depression of the limbic system. This action is thought to be through stimulation of specific benzodiazepine receptors, which potentiates the effect of inhibitory transmitter γ- aminobutyric acid (GABA). GABA acts on the chloride channel, increasing the flow of chloride ions into the cell, causing hyperpolarization and therefore making the cell more refractory to other stimuli [22].

In this study, a combination of medetomidine and midazolam produced a much more potent effect than an optimal dose of medetomidine alone, while the dose of medetomidine was reduced to one-half. This potent sedative effect seemed to be induced by a synergistic interaction of these sedatives rather than an additive interaction, because either of 40 μg/kg of medetomidine and 0.2 mg/kg of midazolam exerted only a light or minimum sedative effect when used alone and sedative effects achieved with this combination were greater than those which could be expected from a simple additive response.

It has been reported that medetomidine and midazolam showed a significant synergism in rats and the pharmacodynamic mechanism between these drugs was proposed [49]. In this report, an α_2- antagonist atipamezole did not block hypnotic response to midazolam, and conversely, a benzodiazepine antagonist flumazenil did not block the hypnotic response to medetomidine in rats. These results indicate that pharmacodynamic interaction dose not include the drugs' receptor binding sites. In addition, no cross-

displacement by the agonists for the alternative receptor was showed in the radiolabeled ligand binding studies [49], which means there are no possible lack of selectivity of either medetomidine at the benzodiazepine binding site or midazolam at the α_2- adrenergic binding site.

Regarding the molecular mechanism o f α_2- adrenoceptor agonist, it has been demonstrated that the receptors activated by α_2- agonists activate intracellular G proteins which transduce a signal to potassium channels, resulting in increased potassium conductance and an eventual hyperpolarization of the cell membrane [12]. As mentioned above, administration of midazolam results in hyperpolarization of the cell membrane induced by increased flow of chloride ion into the cell. Based on these mechanisms, it has been proposed that a synergistic effect on membrane hyperpolarization and resulting synergistic central nervous system depression could be exerted when administered simultaneously [67].

In the present study, this combination considerably depressed the arousal reaction induced by sensory stimuli. During being sedated the pigs never responded to visual, auditory and tactile stimuli. The pigs could be placed in dorsal recumbency without any resistance. Moreover, this combination depressed swallowing and laryngeal reflexes deeply enough for oral manipulation, though the reflex enough for the prevention of aspiration to lungs was remained.

This combination also exerted a moderate analgesic/anesthetic effect. In the present study, an analgesic effect was assessed by pinching the nasal septum where pigs are quite sensitive to a painful stimulus. Pigs given this combination were not aroused even by this painful stimulus, though the animals sharked their heads to be freed from such pain. In addition to these preferable characters as a chemical restraint agent, this combination

smoothly and consistently induced sedation even under the continuous stimulation. Generally, the animal must be left undisturbed for 20 to 30 min after administration of a sedative, as interference before this time may cause poor sedative effect or provoke an excitement reaction [33]. With this combination pigs may be induced to sedation rapidly and smoothly even if the animal is quite nervous or is kept in noisy surroundings.

Mild but significant decreases in body temperature seemed to be one of the disadvantages of this combination. This decrease was considered to be mainly caused by depression of thermoregulation with medetomidine. Thermoregulation of pigs appears to be deeply influenced by medetomidine as compared with other species because they have inefficient thermoregulatory mechanism and have less body hair [56].

In conclusion, a combination of medetomidine (40 μg/kg) and midazolam (0.2 mg/kg) exerted a very potent sedative effect in pigs and was practical and valuable as a chemical restraint agent for most procedures even if accompanied by light pain.

SUMMARY

Sedative effect induced by a combination of medetomidine and midazolam was evaluated in pigs. This combination exerted a much more potent sedative effect than that induced by a medetomidine alone, even if the dose of medetomidine was reduced to one half of its optimal dose. Pigs given this combination were induced to sedation smoothly and very quickly, even if the pigs were stimulated continuously during the induction phase. During being sedated, the arousal reaction induced by sensory stimuli was depressed profoundly and pigs could be placed in dorsal recumbency without any resistance. In addition, this combination produced moderate analgesic effect and apparent muscle relaxation. This potent effect induced by this combination seemed to be induced by a synergistic interaction of these drugs because the sedative effect achieved with this combination was much greater than expected from a simple additive response of both sedatives. This sedative combination may be practical and valuable as a chemical restraint agent in pigs.

Table 1. Recumbency time, arousal time, standing time and total recovery time in pigs given medetomidine$_{80}$-midazolam (med$_{80}$- mid), medetomidine$_{40}$-midazolam (med$_{40}$- mid), medetomidine$_{40}$-midazolam with stimulation (med$_{40}$- mid+stim) and medetomidine$_{80}$ alone (med$_{80}$), medetomidine$_{40}$ alone (med$_{40}$) and midazolam alone (mid)[a)]

sedative(s)	mean recumbency time (min)	mean arousal time (min)	mean standing time (min)	mean total recovery time (min)
med$_{80}$- mid	3.8± 0.8 [A]	89.0±30.5	98.3±30.1	246.7±64.7 [A]
med$_{40}$- mid	6.3± 1.8 [A]	67.8±13.3	69.2±12.8	179.0±31.3 [C]
med$_{40}$- mid+stim	ND[b)]	68.2±17.7	69.7±19.0	200.0±20.9 [BC]
med$_{80}$	15.0± 7.2 [B]	62.7±20.9	79.2±20.9	227.2±31.0 [AB]
med$_{40}$	ND	ND	68.0±25.7	220.0±21.9 [ABC]
mid	ND	ND	ND	97.2±21.6 [D]

a) Pigs were administered medetomidine at 80 μg/kg and midazolam at 0.2 mg/kg (medetomidine$_{80}$- midazolam), medetomidine at 40 μg/kg and midazolam at 0.2 mg/kg (medetomidine$_{40}$- midazolam), medetomidine at 80 μg/kg alone (medetomidine$_{80}$). medetomidine at 40 μg/kg alone (medetomidine$_{40}$) and midazolam at 0.2 mg/kg alone (mid). Data were expressed as mean ± standard deviation (n=6).

b)not detected

A.B.C.D: Mean values with same superscripts are not significantly different (P>0.05).

Table 2. Statistical analysis of the total scores used for evaluating ataractic character in pigs given medetomidine$_{80}$-midazolam (med$_{80}$- mid), medetomidine$_{40}$- midazolam (med$_{40}$- mid), medetomidine$_{40}$- midazolam with stimulation (med$_{40}$- mid+stim) and medetomidine$_{80}$ alone (med$_{80}$), medetomidine$_{40}$ alone (med$_{40}$) and midazolam alone (mid)[a)]

	time after administration (min)												
sedative	0	5	10	20	30	40	50	60	80	100	120	180	240
med$_{80}$- mid	A[b)]	A	A	A	A	A	A	A	A	A	AB	A	A
med$_{40}$- mid	A	AB	A	AB	A	A	A	A	A	B	A	B	BC
med$_{40}$- mid+stim	A	ND[c)]	ND	AB	A	A	A	A	A	AB	A C	ABC	B
med$_{80}$	A	B	B	B	B	B	B	AB	A	AB	BC	A C	A C
med$_{40}$	A	B	BC	C	BC	BC	BC	B	A	B	A	A C	B
mid	A	C	C	C	C	C	C	B	B	C	D	B	BC

a) Pigs were administered medetomidine at 80 μg/kg and midazolam at 0.2 mg/kg (medetomidine$_{80}$- midazolam), medetomidine at 40 μg/kg and midazolam at 0.2 mg/kg (medetomidine$_{40}$- midazolam), medetomidine at 80 μg/kg alone (medetomidine$_{80}$), medetomidine at 40 μg/kg alone (medetomidine$_{40}$) and midazolam at 0.2 mg/kg alone (mid).

b)Same alphabet (A,B,C,D) means that there is no significant difference in posture score between each group (P>0.05).

c)not detected

Table 3. Statistical analysis of the total scores used for evaluating analgesic/anesthetic character in pigs given medetomidine$_{80}$- midazolam (med$_{80}$- mid), medetomidine$_{40}$- midazolam (med$_{40}$- mid), medetomidine$_{40}$- midazolam with stimulation (med$_{40}$- mid+stim) and medetomidine$_{80}$ alone (med$_{80}$), medetomidine$_{40}$ alone (med$_{40}$) and midazolam alone (mid)[a)]

	time after administration (min)									
sedative	0	10	20	30	40	50	60	80	100	120
med$_{80}$- mid	A[b)]	A	A	A	A	A	A	A	A	A
med$_{40}$- mid	A	A	AB	AB	AB	A	A	A	A	A
med$_{40}$- mid+stim	A	A	A	A	AB	AB	A	A	A	A
med$_{80}$	A	A	BC	BC	BC	BC	A	A	A	A
med$_{40}$	A	A	C	C	C	C	A	A	A	A
mid	A	A	C	C	C	C	A	A	A	A

a) Pigs were administered medetomidine at 80 μg/kg and midazolam at 0.2 mg/kg (medetomidine$_{80}$- midazolam), medetomidine at 40 μg/kg and midazolam at 0.2 mg/kg (medetomidine$_{40}$- midazolam), medetomidine at 80 μg/kg alone (medetomidine$_{80}$), medetomidine at 40 μg/kg alone (medetomidine$_{40}$) and midazolam at 0.2 mg/kg alone (mid).

b)Same alphabet (A,B,C,D) means that there is no significant difference in posture score between each group (P>0.05).

Table 4. Effects of medetomidine-midazolam, medetomidine alone and midazolam alone on heart rate and body temperature[a)]

sedatives	time after administration (min)								
	0	10	20	30	40	60	80	120	180
Heart rate(beats/min)									
med_{80}-mid	104.3+ 8.1^{A}	118.7+27.7^{AB}	111.7+21.8^{AB}	97.7+13.9^{AB}	87.7+12.0^{AB}	93.5+15.9^{AB}	90.0+23.3^{AB}	91.0+26.4^{AB}	ND
med_{40}-mid	104.7+ 6.5^{A}	117.7+30.3^{A}	108.3+27.5^{AB}	108.0+20.4^{A}	97.0+20.9^{AB*}	90.0+12.6^{AB}	93.0+ 8.3^{A}	92.0+13.0^{A}	ND
med_{40}-mid+stim	99.0+ 8.2^{A}	ND[b)]	121.0+26.4^{A}	108.0+20.4^{A}	97.0+20.9^{A}	90.0+12.6^{AB}	93.0+ 8.3^{A}	92.0+13.0^{AB}	ND
med_{80}	107.7+18.3^{A}	102+13.0^{AB}	93.6+16.2^{AB}	89.3+14.8^{B}	83.7+14.7^{B}	87.0+18.1^{AB}	80.4+20.6^{AB}	75.0+20.5^{A}	ND
med_{40}	93.0+11.2^{A}	84.0+21.6^{B}	84.0+21.6^{B}	87.0+ 9.9^{B}	79.7+15.7^{B}	72.0+13.7^{B}	72.0+18.6^{B}	75.0+11.2^{A}	ND
mid	90.0+ 8.5^{A}	115.0+11.6^{AB*}	110.4+10.5^{A*}	103.0+11.0^{AB*}	98.0+9.8^{AB}	97.0+12.2^{A}	96.0+ 3.8^{A}	99.0+ 4.2^{B}	ND
Body temperature(°C)									
med_{80}-mid	38.5+0.6^{A}	ND	38.6+0.6^{A}	ND	37.7+0.6^{A*}	36.8+0.7^{A*}	35.9+1.4^{A*}	35.9+0.8^{AB*}	35.6+1.3^{A*}
med_{40}-mid	38.6+0.4^{A}	ND	38.4+0.4^{A}	ND	37.4+0.4^{A*}	36.9+0.4^{A*}	36.5+0.5^{A*}	36.5+0.4^{AB*}	36.8+0.6^{AB*}
med_{40}-mid+stim	38.5+0.4^{A}	ND	38.7+0.8^{A}	ND	37.9+0.8^{A}	37.1+0.7^{A*}	36.6+0.7^{A*}	36.5+0.7^{AB*}	37.1+0.8^{AB*}
med_{80}	38.5+0.5^{A}	ND	38.1+0.8^{A}	ND	37.6+0.7^{A*}	36.9+0.9^{A*}	36.4+1.0^{A*}	35.6+0.3^{B*}	37.0+1.0^{AB}
med_{40}	38.5+0.3^{A}	ND	38.2+0.7^{A}	ND	37.5+0.7^{A*}	37.0+0.6^{A*}	36.7+0.7^{A*}	36.7+0.8^{A*}	37.0+1.0^{B*}
mid	38.6+0.5^{A}	ND	38.5+0.6^{A}	ND	38.6+0.4^{B}	38.6+0.5^{B}	38.6+0.5^{B}	38.6+0.5^{C}	ND

a) Pigs were administered medetomidine at 80 μg/kg and midazolam at 0.2 mg/kg (med_{80}- mid), medetomidine at 40 μg/kg and midazolam at 0.2 mg/kg (med_{40}- mid), medetomidine at 80 μg/kg alone (med_{80}), medetomidine at 40 μg/kg alone (med_{40}) and midazolam at 0.2 mg/kg alone (mid). Data were expressed as mean ± standard deviation (n=6).

b)not detected

A,B,C: Mean values with same superscripts are not significantly different (P>0.05)

* : significantly different from pre-value (P>0.05)

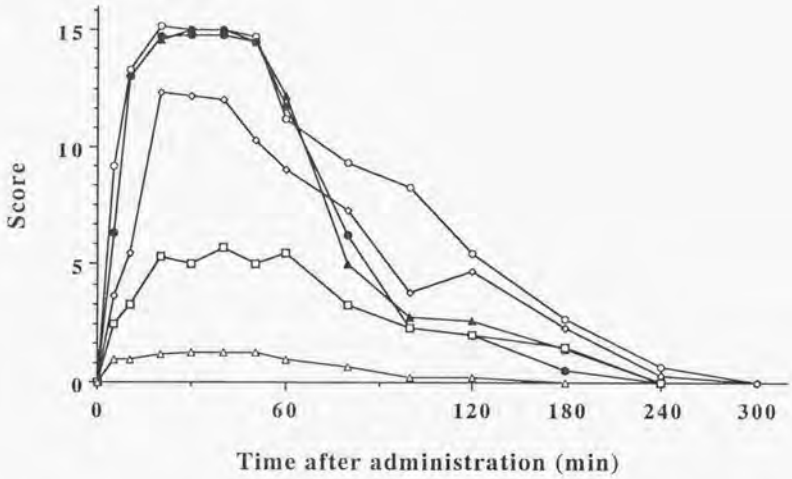


Fig. 1. Effects of 80 μg/kg of medetomidine and midazolam (○), 40 μg/kg of medetomidine and midazolam (●), 40 μg/kg of medetomidine and midazolam with continuous stimulation during induction phase (▲), 80 μg/kg of medetomidine alone (◇), 40 μg/kg medetomidine alone (□), and midazolam alone (△) on ataractic variables (full marks = 16). Each symbol represents the mean value of the total score of posture, response to noise, resistance to restraint and resistance to mouth open and to pull tongue outwards.

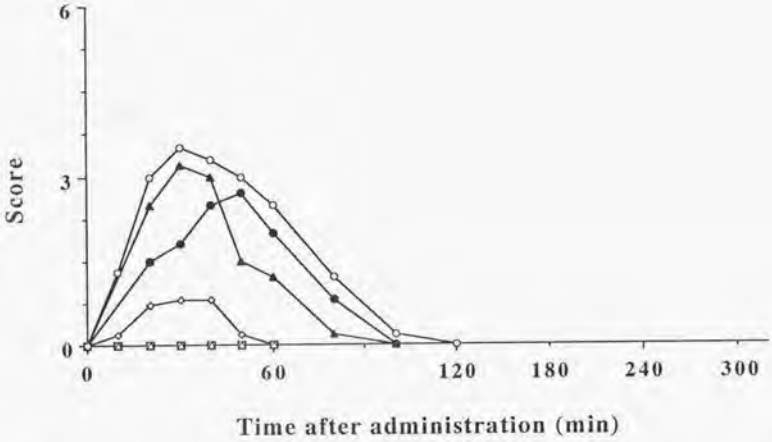


Fig. 2. Effects of 80 μg/kg of medetomidine and midazolam (○), 40 μg/kg of medetomidine and midazolam (●), 40 μg/kg of medetomidine and midazolam with continuous stimulation during induction phase (▲), 80 μg/kg of medetomidine alone (◇), 40 μg/kg medetomidine alone (□), and midazolam alone (△) on analgesic/anesthetic variables (full marks = 6). Each symbol represents the mean value of the total score of response to nose- pinching and toe- pinch withdrawal response.

Part 2-2

Antagonistic effects of atipamezole and flumazenil on medetomidine-midazolam induced sedation in pigs

As described in Part 2-1, a combination of medetomidine-midazolam exerted a much more potent sedative effect than that induced by a medetomidine alone with good muscle relaxation and moderate analgesia, even under the various stimulation during the induction phase.

Atipamezole is a highly selective and specific α_2- antagonist and has been reported to have the ability to reverse the effects of medetomidine alone or combined with other drugs [78]. Flumazenil is a potent and specific benzodiazepine antagonist and is now being widely employed to reverse effects of midazolam in medical practice [22].

If the sedative condition induced by medetomidine-midazolam can be reversed quickly and smoothly by these antagonists, this combination should be more valuable and more widely available in pigs because of the easiness in a management of sedation.

The purpose of this experiment was to evaluate and compare the antagonistic effects of atipamezole and flumazenil on the sedative effect induced by medetomidine-midazolam in pigs and to determine the optimal dose of antagonists on this combination.

MATERIALS and METHODS

Animals:

Six castrated mixed breed pigs in good health were used repeatedly at a week interval in this study. Their mean age was 11.5 weeks (range 9 to 14 weeks) and mean body weight was 17.9 kg (range 15 to 23 kg). Management of the pigs before and during the experiments were the same as the former ones.

Drugs:

The drugs used in this study were medetomidine, midazolam, atipamezole and flumazenil (Hoffman-La Roche, U.S.A.). Flumazenil was dissolved in acidic aqueous solution at the concentration of 0.1 mg/ml. Medetomidine, midazolam and atipamezole were injected intramuscularly into the cervical muscle and flumazenil was injected intravenously into the ear vein.

Experimental design:

The pigs were administered 40 μg/kg of medetomidine and 0.2 mg/kg of midazolam, and were kept in solitary cages to keep the animals from disturbing each other. Thirty min after dosing, these animals were given saline solution as a control, atipamezole at doses of 80, 160 and 240 μg/kg, which were two, four and six times higher than the dose of preceding medetomidine, 100 μg/kg of flumazenil or a combination of 80 μg/kg of atipamezole and 100 μg/kg of flumazenil. The dose of atipamezole was chosen from its optimal dose against the medetomidine- induced sedation based upon the results in Part 1-2. The antagonistic effect of 100 μg/kg of flumazenil on 0.2mg/kg of midazolam has been certified in a preliminary study using 6 pigs. Saline solution was administered in amount of 0.048 ml/kg which was the same volume as atipamezole at the dose of 240 μg/kg. Antagonistic effects were repeatedly assessed 5,10, 20, 30, 40, 60, 80, 120, 180, 240 and 300 min after dosing and/or until the animals totally recovered.

Heart rate and rectal temperature were measured before injection of medetomidine-midazolam as base-line values, during which time each pig was kept on a canvas sling. After drugs administration, those measurements were repeated 10, 20, 30, 40, 60, 80 and 120 min after dosing at their own cages.

Assessment of antagonistic effect of atipamezole and flumazenil :

Antagonistic effects of atipamezole were evaluated by the total score for evaluating the ataractic character and by arousal time (time from injection of antagonists until the animal raises the head when stimulated), standing time (time from injection of atipamezole or saline solution until the animal stands) and total recovery time (time from injection of atipamezole or saline solution until the animal can not be distinguished from untreated animals). Ataractic character was assessed by the total score of posture, response to noise, resistance to restraint and resistance to mouth open and to pull tongue outwards as described in Part 1-2. Recovery condition and undesirable side effects were also recorded throughout the experiment.

Statistical analyses:

Differences of antagonistic effects at corresponding time were statistically analyzed using the same method described in Part 1-3. The data of heart rate and rectal temperature were also analyzed by the same method described in Part 1-3. In all analyses, values were considered to be statistical significant when $P<0.05$.

RESULTS

Following the intramuscular injection of medetomidine-midazolam, any of the pigs were quickly and smoothly induced to sedation. The animals became lateral recumbency in 4 to 11 min (Table 1), and the total score for ataractic character reached to the maximal level soon after the lateral recumbency. During being in lateral recumbency, these pigs lost consciousness and the animals were not aroused even by restraining in dorsal recumbency.

Atipamezole injections at any doses (80, 160 and 240 μg/kg) effectively reversed the medetomidine-midazolam induced sedation (Fig. 1). Pigs were aroused 2 to 7 min after

atipamezole injections, and all the pigs rose to standing position within 12 min. The total score for evaluating ataractic character in pigs given 160 and 240 μg/kg of atipamezole decreased significantly at the first 5 min- observation time as compared with those in control pigs. Twenty min after injection, the scores in pigs given 80 μg/kg of atipamezole were also significantly decreased (Table 2).

The mean arousal time and the mean standing time in pigs given atipamezole at either dose (80, 160 and 240 μg/kg) were significantly shortened to one-fourth to one-seventh of the values in control pigs. These values were shortened in proportion to the increase in atipamezole dose, however, there were no significant differences between each dose group (Table 1). On the contrary, the mean total recovery time in pigs given 80 μg/kg of atipamezole was significantly longer than that in pigs given the higher dose of medetomidine.

Recovery from sedation in pigs given atipamezole at 80 and 160 μg/kg was smooth with minimal adverse effects. Relapses into sedation were not seen, however, some of the animals became slightly ataxic for 20 to 30 min after administration of atipamezole. Three of the 6 pigs given atipamezole at 240 μg/kg appeared mildly hyperactive and had mild muscular tremors for a short duration.

Intravenous administration of flumazenil at a dose of 100 μg/kg also drastically reversed the medetomidine-midazolam induced sedation. These animals were aroused during or just after injection of the drug and 5 of the 6 pigs stood up soon after the arousal. The mean arousal time was significantly shorter than those in pigs given saline solution or 80 μg/kg of atipamezole. However, all the animals returned to moderate sedation 5 to 20 min after standing and became dorsal recumbency again. The ataractic character score rebounded until 30 min after administration of flumazenil and stood up

again approximately 40 min after administration (Table 1). Although the mean total recovery time was slightly shortened by flumazenil from the control value, that the value was significantly longer than those in pigs given other antagonists.

Simultaneous administration of 160 μg/kg of atipamezole and 100 μg/kg of flumazenil quickly and effectively reversed the sedation induced by a combination of medetomidine and midazolam. Ataractic character score significantly decreased just after administration in this group. Recovery from sedation was smooth and the mean arousal time, mean standing time and mean total recovery time were significantly shortened comparing to control values. However these data were not significantly different from those in pigs given 160 and 240 μg/kg of atipamezole.

Table 3 shows the effects of atipamezole and/or flumazenil on heart rate and body temperature in pigs sedated by a combination of medetomidine and midazolam. Thirty min after administration of medetomidine-midazolam (just before administration of antagonists), heart rate of any groups were lower than their base-line values. Atipamezole or both of atipamezole and flumazenil reversed this medetomidine-midazolam induced decreases in heart rate within 10 min after the injection. The increasing effect of atipamezole on heart rate was dose-dependent. At the highest dose of atipamezole (240 μg/kg), heart rate significantly increased from the base-line value and moderate tachycardia (up to 180 beats/min) was observed in two of the six pigs for less than 10 min.

Body temperature significantly decreased after administration of medetomidine and midazolam in each group. In pigs given saline solution or flumazenil alone, body temperature continued to decrease until 40 to 80 min after injections of antagonists and reached to approximately 36°C. On the contrary, body temperature in pigs given

atipamezole or both of atipamezole and flumazenil was reversed after administrations of antagonists. Between these atipamezole or atipamezole and flumazenil treated groups, the values did not significantly differ from each other .

DISCUSSION

Administration of atipamezole quickly and smoothly reversed the effects induced by a combination of medetomidine and midazolam, and the arousal time, standing time and total recovery time were significantly reduced as compared with control pigs. Atipamezole is a potent and highly selective and specific α_2- antagonist which produces a potent antagonism on the effects of medetomidine in dogs, cats, zoo animals and pigs [11, 29, 70]. It has been also reported that this antagonistic effect was obtained when medetomidine was used with ketamine and butorphanol [78]. When ketamine is used with medetomidine, its dose can be reduced to one half to one fourth of its anesthetic dose, and butorphanol itself has a very weak sedative effect, thus antagonism of medetomidine by atipamezole could reverse their anesthetic/sedative effects. As described in Part 2-1, 0.2mg/kg of midazolam alone exerted minimum sedative effect for a short duration. Therefore the pigs might be hardly influenced by residual midazolam when the atipamezole was injected (30 min after administration of medetomidine-midazolam) and the effect of medetomidine was abolished.

The optimal dose of atipamezole alone against medetomidine-midazolam induced sedation was 160 μg/kg which was four times higher than the preceding medetomidine dose. The antagonistic effect was more potent in proportion to the increase in atipamezole dose administered, and the mean total recovery time in pigs given 160 μg/kg and 240 μg/kg of atipamezole were significantly shorter than that in pigs given 80 μg/kg

of atipamezole. However, there were no significant differences in its antagonistic effect between 160 μg/kg of atipamezole and 240 μg/kg of atipamezole.

Some of the pigs given 240 μg/kg of atipamezole appeared mildly hyperactive during the recovery phase. This undesirable excessive effect, which was also observed when a higher dose of atipamezole was administered to the pigs given medetomidine alone (Part 1-3), was probably caused by the excitatory effects of α_2-adrenergic antagonist on the central nervous system [83]. In addition, this highest dose of atipamezole caused an increase in heart rate which was also partly caused by a central stimulant effect of atipamezole [20]. Those results may indicate that 240 μg/kg of atipamezole is slightly excessive for antagonism of medetomidine-midazolam induced sedation.

The pigs given flumazenil at a dose of 100 μg/kg were aroused very quickly and most of the pigs stood up soon after arousal. However, flumazenil was not valuable as a sole antagonistic agent as compared with atipamezole, because all the animals returned to moderate sedation after standing. This relapse was apparently induced by residual medetomidine because the total score used for evaluating the ataractic character after administration of flumazenil was similar to that in pigs given 40 μg/kg of medetomidine alone (Part 2-1). Flumazenil is a specific and exclusive benzodiazepine antagonist with a high affinity for benzodiazepine receptors, where it exerts minimal agonist activity [60]. As a competitive antagonist, flumazenil reverses all the agonist effects of benzodiazepine quickly when used intravenously [22] but dose not block the hypnotic response to medetomidine [49].

The sedative effect induced by medetomidine and midazolam was most effectively reversed by a combination of atipamezole and flumazenil in pigs even if the lower dose of atipamezole was used. However, there were no significant differences in the mean

arousal time, mean standing time and mean total recovery time between atipamezole -flumazenil and 160 or 240 μg/kg of atipamezole. As it is difficult to concentrate flumazenil solution because of its water insolubility, more injection volume of flumazenil with a higher cost is needed. Practically, atipamezole alone is thought to be potent enough to antagonize the sedation induced by a combination of medetomidine-midazolam.

In conclusion, the sedative effect and the decease in body temperature induced by a combination of medetomidine and midazolam could be reversed quickly and smoothly by atipamezole alone. The optimal action was seen at a dose of 160 μg/kg, which was the four times higher than the preceding medetomidine dose. The possible use of a antagonist may enhance the value and availability of medetomidine-midazolam as a chemical restraint agent in pigs.

SUMMARY

Antagonistic effects of atipamezole (80, 160 and 240 μg/kg, im) and flumazenil (100 μg/kg, iv) or simultaneous use of atipamezole (80 μg/kg) and flumazenil (100 μg/kg) on medetomidine-midazolam induced sedation were evaluated in pigs. Atipamezole at each dose effectively reversed sedation, and the arousal time, standing time and total recovery time were significantly shortened. The optimal action was seen at a dose of 160 μg/kg. At this dose recovery from the sedation was quick and smooth, and adverse effects such as hyperactivity or tachycardia were minimal. Flumazenil reversed sedation temporary, but the pigs returned to moderate sedation soon after arousal. The combination of atipamezole and flumazenil most effectively reversed the sedation, however atipamezole (160 μg/kg) alone was thought to be practically potent enough to antagonize sedation induced by medetomidine-midazolam in pigs.

Table 1. Recumbency time, arousal time, standing time and total recovery time in pigs given medetomidine-midazolam and atipamezole or/and flumazenil or saline solution[a)]

antagonist(s)	mean recumbency time (min)	mean arousal time (min)	mean standing time (min)	mean total recovery time (min)
saline solution	5.3± 1.0	41.2±13.7 A	62.7±41.5 A	224.4±32.3 A
atipamezole 80μg/kg	6.8± 2.1	10.5± 4.5 B	14.7±6.5 B	124.8±27.9 B
atipamezole 160μg/kg	7.2± 2.1	7.8± 2.5 BC	10.3±3.2 B	68.3±33.7 C
atipamezole 240μg/kg	6.2± 1.2	5.7± 2.0 BC	9.2±4.4 B	67.3±19.0 C
flumazenil 100μg/kg	6.7± 1.0	1.0± 2.1 C	9.0± 17.0 B (38.0± 9.9) (C)b)	173.3±24.7 D
atipamezole 80μg/kg +flumazenil 100μg/kg	7.0± 4.0	1.2± 0.4 C	2.0± 1.5 B	42.3±15.0 C

a) Atipamezole ,flumazenil, atipamezole and flumazenil or sanine solution were administered intramuscularly 30 min after the injection of medetomidine at 40 μg/kg and midazolam at 0.2 mg/kg.

Data were expressed as mean ± standard deviation (n=6).

b) Data of the time when the pigs stood up again.

A,B,C,D: Mean values with same superscripts are not significantly different (P>0.05).

Table 2. Statistical analysis of the total scores used for evaluating ataractic character in pigs given medetomidine-midazolam and atipamezole, flumazenil, atipamezole and flumazenil or saline solution[a)]

	time after administration (min)														
antagonist(s)	-30	0	5	10	20	30	40	50	60	80	100	120	160	200	240
saline solution	A[b)]	A	A	A	A	A	A	A	A	A	A	A	A	A	A
atipamezole 80μg/kg	A	A	AB	AB	BC	B	B	BC	BC	B	BC	B	B	B	A
atipamezole160μg/kg	A	A	B	BC	D	B	C	B	B D	BC	B D	C	B	B	A
atipamezole240μg/kg	A	A	BC	CD	B D	B	C	B	B D	BC	D	C	B	B	A
flumazenil	A	A	C	BC	A C	A	AB	A C	A C	A	A C	AB	B	B	A
atipamezole 80μg/kg+ flumazenil	A	A	C	D	D	B	C	B	CD	C	D	C	B	B	A

a) Atipamezole ,flumazenil, atipamezole and flumazenil or sanine solution were administered intramuscularly 30 min after the injection of medetomidine at 40 μg/kg and midazolam at 0.2 mg/kg.

b)Same alphabet (A,B,C,D) means that there is no significant difference in posture score between each group (P>0.05).

Table 3.Effects of atipamezole, flumazenil or atipamezole and flumazenil on the changes of heart rate and body temperature induced by medetomidine-midazolam in pigs[a)]

antagonist(s)	time after antagonists administration (min)								
	-30	0	10	20	30	40	60	80	120
Heart rate (beats/min)									
med-mid-ps	104.0± 12.4^{A}	87.2± 21.0^{A}	87.0± 15.1^{A}	86.0± 20.3^{A}	82.0± 20.7^{A*}	81.0± 19.6^{A*}	81.6±19.6^{A}	86.4± 23.1^{AB}	93.1± 20.5^{A}
med-mid-A_{80}	100.0± 13.5^{A}	95.0± 23.2^{A}	99.7± 16.9^{A}	104.0± 18.5^{AB}	95.0±19.1^{AB}	95.0±19.1^{A}	92.0±11.8^{AB}	90.0± 10.0^{AB}	95.0± 16.3^{A}
med-mid-A_{160}	97.0± 8.8^{A}	82.0± 10.5^{A*}	105.0± 23.3^{AB}	103.0± 10.3^{AB}	104.0± 11.8^{B}	99.0±11.8^{A}	86.4±16.2^{AB}	93.6± 6.8^{AB}	95.0± 1.4^{A}
med-mid-A_{240}	98.0± 11.2^{AB}	87.0± 7.3^{A}	129.0± 35.5^{B*}	120.0± 16.5^{B*}	100.0±14.5^{AB}	99.0±10.6^{A}	104.0± 6.2^{B}	102.7± 6.1^{A}	106.0± 2.8^{A}
med-mid-F	100.0± 9.8^{A}	93.0± 15.1^{A}	91.0± 14.9^{A}	97.3± 18.0^{A}	88.0±17.7^{AB}	ND[b)]	82.0±15.5^{A*}	83.0± 13.9^{B*}	84.0± 17.0^{A}
med-mid-F/A_{80}	98.0± 9.0^{A}	86.0± 7.3^{A*}	97.2± 6.6^{A}	96.0± 7.3^{A}	93.6± 3.3^{AB}	88.5± 9.0^{A}	90.0± 10.4^{AB}	96.0± 0.0^{AB}	ND
Body temperature(°C)									
med-mid-ps	38.7± 1.0	37.8±1.2	37.3±1.1	36.9±1.1*	ND	36.3±1.1^{A*}	36.3±1.1^{A*}	36.2±1.0^{A*}	36.6± 0.7*
med-mid-A_{80}	38.6± 0.6	38.1±0.7	37.2±0.8	37.1±1.0*	ND	37.3±1.0^{AB}	37.4±0.8^{BC*}	37.9±0.8^{B}	38.5±1.3
med-mid-A_{160}	38.5± 0.4	37.6±0.6*	36.9±0.5*	36.9±0.4*	ND	37.4±0.5^{BC}	37.7±0.6^{C*}	37.8±0.5^{B*}	38.3±0.4
med-mid-A_{240}	38.5± 0.3	37.7±0.6*	37.1±0.6*	37.3±0.5	ND	37.8±0.6^{C}	38.1±0.7^{C}	38.1±0.5^{B}	ND
med-mid-F	38.8± 0.4	38.1±0.7*	37.4±0.7*	37.1±0.9*	ND	36.4±0.9^{AB*}	36.6±0.9^{AB*}	36.5±0.8^{A*}	37.7±1.1*
med-mid-F/A_{80}	38.5± 0.3	37.6±0.3*	37.3±0.5*	37.2±0.4*	ND	37.5±0.3^{C*}	37.7±0.4^{C*}	38.0±0.6^{B}	ND

a) Pigs were administered saline solution (med-mid-ps), aipamezole at 80 μg/kg (med-mid-A_{80}), 160 μg/kg (med-mid-A_{160}), 240μg/kg (med-mid-A_{240}), flumazenil at 100 μg/kg (med-mid-F) or atipamezole 80 μg/kg and flumazenil at 100 μg/kg (med-mid-F/A_{80}) thirty min after injection ofmedetomidine at 40 μg/kg and midazolam at 0.2 mg/kg.

Data were expressed as mean ± standard deviation (n=6).

b)not detected

A,B,C: Mean values with same superscripts are not significantly different (P>0.05).

* : significantly different from pre-value (P>0.05).

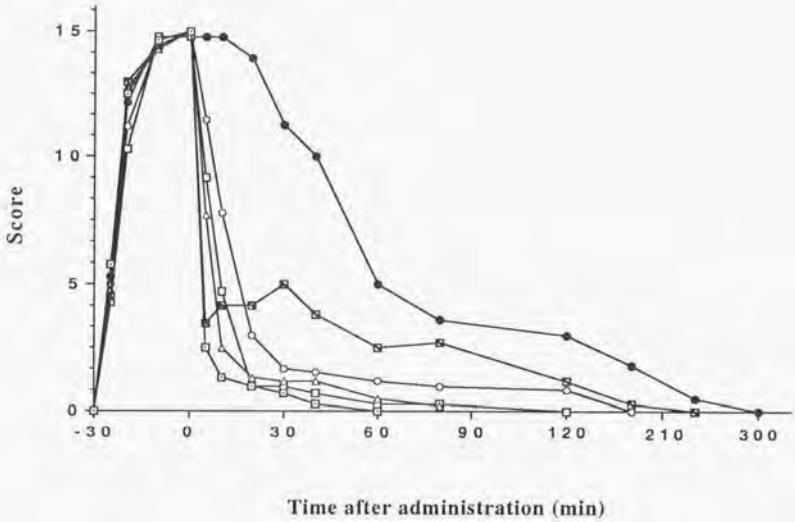


Fig. 1. Effects of atipamezole and flumazenil on ataractic variables (full marks = 16) in pigs sedated by medetomidine- midazolam. Medetomidine and midazolam were injected intramuscularly 30 min before injection of antagonist(s). The pigs were given 80 μg/kg of atipamezole (○), 160 μg/kg of atipamezole (□), 240 μg/kg of atipamezole (△), 100 μg/kg of flumazenil (⊠), 80 μg/kg of medetomidine and 100 μg/kg of flumazenil (⊡) and saline solution (●). Each symbol represents the mean value of the total score of posture, response to noise, resistance to restraint and resistance to mouth open and to pull tongue outwards.

Part 2-3

Cardiopulmonary effects of medetomidine-midazolam and medetomidine-midazolam-atipamezole in pigs

As described in Part 2-1 and Part 2-2, a combination of medetomidine and midazolam produced a deep sedation which was effectively antagonized without undesirable side effects by atipamezole quickly and smoothly in pigs.

However, α_2- agonists has been known to affect the cardiovascular functions [21]. In dogs, medetomidine induces dose-dependent depressant effects such as bradycardia, atrioventricular blocks, changes in blood pressure, and a decrease in cardiac output mediated through central and peripheral actions [52]. On the contrary, midazolam has minimum cardiopulmonary effects at clinical doses [46].

The purpose of this experiment was to evaluate the effect of a combination of medetomidine and midazolam on the cardiopulmonary system and to compare with that of medetomidine alone. This study was also designed to evaluate the effect of atipamezole on the medetomidine-midazolam induced changes in cardiopulmonary system.

MATERIALS and METHODS

Animals:

Eighteen castrated mixed breed pigs in good health were used in this study. After a 1 week period of stabilization the pigs were randomly assigned to 3 groups of 6 pigs each. Their mean age was 10.9 weeks (range 10 to 12 weeks) and mean body weight was 20.5 kg (range 18.5 to 24 kg). Management for these pigs were the same as those in other experiments.

Drugs:

The drugs used were medetomidine, midazolam and atipamezole. Those drugs were injected intramuscularly into the cervical muscle.

Animal preparation:

At least 5 days before the experiment, a 14G heparin-coated polyvinyl chloride catheter (Toray Medical Co., Anthron) was implanted into the left common carotid artery and a 6-French 10 cm introducer (SI-5600, Arrow International Inc., U.S.A.) into the right lateral jugular vein under isoflurane anesthesia.

Experimental design:

Five to seven days after surgery, each conscious pig was placed in the sling and a 5-French Swan-Gantz catheter (Baxter Healthcare Corporation, model 93-132-5F) was inserted through the placed introducer and advanced into the pulmonary artery, through monitoring intravascular pressures. The Swan-Gantz catheter was then positioned with the proximal port in the right atrium. After swine condition were stabilized, all base-line values of cardiopulmonary measurements were obtained. Following the measurements in the conscious state, each animal was given 40 μg/kg of medetomidine and 0.2 mg/kg of midazolam (12 pigs; 2 groups) or 80 μg/kg of medetomidine (6 pigs; one group). Thirty min after administration of medetomidine-midazolam, 6 pigs were administered 160 μg/kg of atipamezole. All the cardiopulmonary measurements were repeated 10, 20, 30, 40, 60, 80 and 120 min after administration of medetomidine-midazolam or medetomidine alone. In pigs given atipamezole, these measurements were repeated and 5, 10, 20, 30, 40 and 60 min after administration of atipamezole.

Determination of cardiopulmonary effects:

Cardiopulmonary measurements were made as follows: Heart rate (HR) was recorded on a multi-function monitor (BSM-8301, Nihon Kohden, Japan). Arterial (through a

arterial catheter) (AP), right atrial (RAP) and pulmonary arterial (PAP) phasic and mean blood pressures and pulmonary arterial wedge pressure (PAWP, through a Swan-Gantz catheter) were measured using a calibrated pressure transducer (PR-AS123S, Terumo Co. Ltd., Japan) connected to a multi-function monitor. Cardiac output (CO) was determined by thermodilution technique using a multi-function monitor, by an injection of 3 ml 0°C saline solution into the right atrium during end-expiration. Cardiac output determinations were performed in triplicate and the mean of the 3 determinations was taken as the correct value. Arterial and pulmonary arterial (mixed venous) blood were sampled for measurements of pHa, PaO_2 and $PaCO_2$ (IL-1303, Instrumentation Laboratory Ltd., U.S.A.). Blood gas and pH values were corrected to the animal's temperature and base excess (BE) and bicarbonate concentration ($[HCO_3^-]a$) were calculated from these measurements.

From above values, following cardiovascular parameters were calculated. Cardiac index (CI)=1000·CO / body weight (ml / min·kg), stroke volume (SV)=CI / HR (ml / kg), systemic vascular resistance (SVR) = (APm-RAP) / C I (mmHg·kg·min / ml), pulmonary vascular resistance (PVR) = (PAPm-PAPW) / C I (mmHg·kg·min / ml), rate pressure product (RPP) = BPs·HR (mmHg / min).

Following respiratory parameters were also calculated. Arterial O_2 saturation (SaO_2) (%), mixed venous O_2 saturation (SvO_2) (%), arterial oxygen content (CaO_2) =1.39·Hb·SaO_2 / 100 + 0.0031·PaO_2 (ml / blood 100 ml), mixed venous O_2 content (CvO_2) =1.39·Hb·SvO_2/ 100 + 0.0031·PvO_2 (ml / blood 100 ml), delivery O_2 (DO_2) =CaO_2·CI/100 (ml / min·kg), oxygen consumption (VO_2) =(CaO_2-CvO_2)·CI / 100 (ml / min·kg), oxygen utilization ratio (UO_2) = DO_2 / VO_2.

Statistical analyses:

The values of cardiopulmonary parameters after drug administration were compared with base-line values using paired-*t* test. Differences in the values between medetomidine-midazolam and medetomidine alone or medetomidine-midazolam and medetomidine-midazolam-atipamezole at corresponding time were analyzed by nonpaired - *t* test. In all analyses, values were considered to be statistically significant when $P<0.05$.

RESULTS

Cardiovascular effects of medetomidine-midazolam (med-mid) and medetomidine at 80 μg/kg (med_{80}) :

The effects of med-mid and med_{80} on cardiovascular system are summarized in Tables 1 to 4. Heart rate in med-mid remained stable and maintained around the baseline-value throughout the observation period. The baseline-value of HR in med_{80} was significantly higher than that in med-mid, however, it rapidly fell to the level seen in pigs given med-mid just after administration of medetomidine and maintained similar values (Fig. 1).

AP and PAP moderately but significantly increased from base-line values 5 min after administration of either med-mid or med_{80}, then gradually decreased (Fig. 2). PAWP and RAP also increased after administration of the sedative. There were no significant differences in those values between med-mid and med_{80}.

CI decreased slightly but significantly from the base-line value in both regimens (Fig. 3) after administration of the drug, then gradually increased or maintained those values. These changes were accompanied by a decrease in the similar extent in SV and moderate increases in SVR and PVR (Fig. 3).

RPP in pigs given med-mid increased just after administration of drugs and gradually decreased to around the base-line values. RPP in med_{80} was significantly higher than those in med-mid, but its fluctuation pattern was similar to that in med-mid.

Effects of atipamezole on medetomidine-midazolam induced changes in cardiovascular system:

Tables 1 to 4 show the effects of atipamezole on med-mid-induced changes in cardiovascular system. HR (Fig. 1), CI (Fig. 3) and RPP increased just after administration of atipamezole then decreased rapidly to base-line levels. Conversely, AP (Fig. 2), PAP (Fig. 2), RAP, PAWP and SVR (Fig. 3) and PVR decreased and returned to base-line levels.

Respiratory effects of medetomidine-midazolam and medetomidine-midazolam-atipamezole:

The changes in P_aO_2 , P_aCO_2 , pH_a and $[HCO_3^-]_a$ were minimal in any of the pigs given med-mid, med_{80} and med-mid-ati (Tables 5 to 8). DO_2 in either regimen decreased because of the decrease in CI and UO_2 increased due to the decrease in VO_2.

DISCUSSION

Medetomidine-midazolam and medetomidine alone caused a similar mild pressor response, characterized by mild but rapid increase in AP and PAP after administration, then those pressures gradually decreased soon after those reached the peak level and maintained the higher values than base-line.

These pressor effects were thought to be mainly induced by medetomidine, because midazolam has been demonstrated to have a minimum cardiovascular effect and medetomidine alone exerted the similar but more profound changes. Those changes were

not accompanied by an increase in cardiac output and correlated well to the changes in systemic or pulmonary vascular resistance, thus they may be mediated mainly through systemic or pulmonary vasoconstriction. It has been known that α_2-agonists produce their sedative action through activation of pre- and postsynaptic α_2-adrenoceptors in the central nervous system [21], and peripherally they also activate the vascular postsynaptic α_2-adrenoceptors and consequently induce vasoconstriction [28].

α_2-Agonists generally cause marked bradycardia and biphasic pressor-depressor response, characterized by an early hypertensive phase and a late hypotensive phase. The bradycardia and hypotension following hypertensive phase have been thought to be mediated through vagus nerves [4] and through activation of postsynaptic α_2-adrenoceptors at bulbar vasomotor and cardiac center [54].

However in the present study, despite the initial phase response after administration of medetomidine-midazolam or medetomidine alone subsequent hypotension and bradycardia were not observed in pigs. Recently, it has been proposed that imidazoline-sensitive and catecholamine-insensitive membrane receptors located in the ventrolateral area of the medulla are important sites in the mediation of hemodynamic actions of centrally acting antihypertensive agents such as clonidine and rimenidine, because these α_2- agonists exert antihypertensive action depending on a selectivity for these imidazoline receptors [9, 15]. Medetomidine may have a less effect on these receptors which mediate bradycardia and hypotension in contrast to a potent effect on α_2-receptors which mediate sedation centrally and hypertension peripherally in pigs.

As the results of increased arterial blood pressure and unchanged heart rate, rate pressure product which represents the oxygen consumption in the myocardium increased mildly and temporarily but those values were within physiological level.

Cardiac output decreased mildly in pigs given either medetomidine-midazolam or medetomidine alone. These changes were mainly attributed to the mild decrease in stroke volume because heart rate did not change throughout the observation period. Medetomidine has been reported to have little direct action on the myocardium [22]. As the preload, represented by right atrial pressure and pulmonary arterial wedge pressure, increased after administration of drugs, the decrease in stroke volume was thought to be induced by increase in the after load which was represented by an increase in vascular resistance.

The effects of medetomidine-midazolam or medetomidine alone on respiratory system were small. Although the oxygen delivery decreased mildly due to a slight decrease in cardiac output, the oxygen utility ratio increased slightly. This increase in utility ratio was attributed to decrease in oxygen consumption. The decrease in oxygen consumption is most likely due to sedation, continued suppression of norepinephrine release, and perhaps direct action on brain stem regulation centers induced by medetomidine [37].

In conclusion, a combination of medetomidine and midazolam exerted less cardiovascular effects than medetomidine alone and caused moderate vasoconstriction accompanied by the mild increase in blood pressure and the slight decrease in cardiac output caused by the increase in afterload in pigs. Administration of atipamezole resulted in a transient marked decrease in vascular resistance, a decrease in blood pressure and increases in cardiac output and heart rate. However, these changes were relatively small and sustained for a short duration. Thus, the combination of medetomidine and midazolam and its antagonist can be used as a highly safe regimen in pigs.

SUMMARY

The cardiovascular effects of medetomidine (80 μg/kg) alone, medetomidine (40 μg/kg)- midazolam (0.2 mg/kg) and medetomidine (40 μg/kg)- midazolam (0.2 mg/kg)-atipamezole (160 μg/kg) were evaluated in pigs. The intramuscular administrations of medetomidine alone and medetomidine-midazolam caused a similar pressor response, characterized by mild but rapid increase in arterial and pulmonary arterial pressure mediated mainly through systemic and pulmonary vasoconstriction. These pressures gradually decreased soon after showing the peak level 5 to 10 min after administrations of sedatives, but maintained the slightly higher values than the base-line. Cardiac output decreased mildly after administration of either medetomidine alone or medetomidine-midazolam. This change was mainly attributed to a mild increase in the afterload of the heart which was represented by the increase in vascular resistance. All these changes in pigs given medetomidine-midazolam were smaller than those in pigs given medetomidine alone and within physiological fluctuation. In addition, medetomidine-midazolam did not induce bradycardia and subsequent hypotension which were generally observed by α_2-agonists and caused less changes in the respiratory system. Administration of atipamezole resulted in a transient marked decrease in vascular resistance, and it caused a decrease in blood pressure and increases in cardiac output and heart rate. However, these changes were relatively small and sustained for a short duration. Thus the combination and its antagonist can be used as a highly safe regimen in pigs.

Table 1. Changes in heart rate (HR), systolic arterial pressure (APs), mean arterial pressure (APm) and diastolic arterial pressure (APd) in pigs given medetomidine-midazolam (M-M), medetomidine-midazolam-atipamezole (M-M-A) and medetomidine alone (Me80)

		min	0	5	10	15	20	30	35	40	60	80	100	120
HR	MM	Mean	99	98	100	96	95	92	ND	89	91	92	90	95
(beats/min)		±SD	15	15	14	13	12	12		10	11	10	13	9
		p	–	NS	NS	NS	NS	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	96	93	99	92	90	84	137	111	100	ND	ND	ND
		±SD	9	15	16	16	15	13	23	23	11			
		p	–/–	NS/–	NS/–	NS/–	NS/–	NS/–	↑/↑	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	121	94	97	99	95	92	ND	85	86	84	ND	ND
		±SD	11	13	13	14	9	8		10	8	9		
		p	–/↑	↓/NS	↓/NS	↓/NS	↓/NS	↓/NS	–/–	↓/NS	↓/NS	↓/–	–/–	–/–
APs	MM	Mean	123	151	153	149	147	143	ND	143	136	137	143	142
(mmHg)		±SD	8	13	16	14	13	13		10	14	9	10	6
		p	–	↑	↑	↑	↑	↑	–	↑	NS	↑		
	MMA	Mean	121	150	156	152	150	146	109	128	137	ND	ND	ND
		±SD	6	12	10	8	11	12	18	9	15			
		p	–/–	↑/–	↑/–	↑/–	↑/–	↑/–	NS/↓	NS/↓	NS/NS	–/–	–/–	–/–
	Me80	Mean	142	169	164	155	158	154	ND	150	152	148	ND	ND
		±SD	7	27	26	19	17	14		15	11	9		
		p	–/↑	↑/NS	NS/NS	NS/NS	↑/NS	↑/NS	–/–	NS/NS	↑/NS	↑/NS	–/–	–/–
APm	MM	Mean	102	130	130	126	124	121	ND	121	114	115	119	118
(mmHg)		±SD	8	12	12	12	12	10		8	11	9	8	4
		p	–	↑	↑	↑	↑	↑	–	↑	↑	↑	↑	↑
	MMA	Mean	96	124	130	126	124	119	88	108	113	ND	ND	ND
		±SD	6	9	6	5	6	8	18	7	12			
		p	–/–	↑/–	↑/–	↑/–	↑/–	↑/–	NS/↓	NS/NS	↑/NS	–/–	–/–	–/–
	Me80	Mean	115	138	133	129	129	126	ND	122	124	116	ND	ND
		±SD	6	16	14	14	10	10		11	7	6		
		p	–/↑	↑/NS	↑/NS	NS/NS	↑/NS	↑/NS	–/–	NS/NS	↑/NS	NS/NS	–/–	–/–
APd	MM	Mean	79	110	110	103	100	96	ND	97	91	91	94	95
(mmHg)		±SD	8	11	11	11	10	8		6	9	7	7	4
		p	–	↑	↑	↑	↑	↑	–	↑	↑	↑	↑	↑
	MMA	Mean	66	101	108	101	99	93	69	87	88	ND	ND	ND
		±SD	6	9	5	6	7	6	16	7	12			
		p	–/–	↑/–	↑/–	↑/–	↑/–	↑/–	NS/↓	↑/NS	↑/NS	–/–	–/–	–/–
	Me80	Mean	90	113	110	107	106	102	ND	98	100	95	ND	ND
		±SD	6	15	11	12	7	7		9	6	5		
		p	–/↑	↑/NS	↑/NS	NS/NS	↑/NS	↑/NS	–/–	NS/NS	↑/NS	NS/NS	–/–	–/–

↑↓ : Significantly different from the base-line value (MM), from the base-line value/ pre atipamezole-administration value (MMA) and from base-line value/ the value in MM (Me80)

NS: not significant

Table 2. Changes in right atrial pressure (RAP), systolic pulmonary arterial pressure (PAPs), mean pulmonary arterial pressure (PAPm) and diastolic pulmonary arterial pressure (PAPd) in pigs given medetomidine-midazolam (M-M), medetomidine-midazolam-atipamezole (M-M-A) and medetomidine alone (Me80)

		min	0	5	10	15	20	30	35	40	60	80	100	120
RAP	MM	Mean	2	4	3	2	2	2	ND	2	2	1	1	1
(mmHg)		±SD	1	2	1	2	2	2		2	1	1	1	1
		p	–	↑	NS	NS	NS	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	0	2	2	2	2	1	-2	1	1	ND	ND	ND
		±SD	0	0	1	1	1	1	2	1	1			
		p	–/–	↑/–	NS/–	NS/–	NS/–	NS/–	NS/NS	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	-1	4	2	2	2	2	ND	1	1	0	ND	ND
		±SD	1	2	1	1	1	1		1	1	1		
		p	–/NS	↑/NS	↑/NS	↑/NS	↑/NS	NS/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	–/–
PAPs	MM	Mean	28	41	38	35	32	31	ND	34	40	33	34	36
(mmHg)		±SD	6	7	5	5	4	3		8	10	3	2	11
		p	–	↑	↑	NS	NS	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	27	37	36	32	31	29	27	28	34	ND	ND	ND
		±SD	2	6	5	3	3	4	2	5	5			
		p	–/–	↑/–	↑/–	↑/–	↑/–	NS/–	NS/NS	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	37	42	43	45	40	37	ND	36	33	32	ND	ND
		±SD	2	9	10	11	9	6		5	3	7		
		p	–/↑	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	–/–
PAPm	MM	Mean	19	29	27	24	23	22	ND	23	28	23	23	24
(mmHg)		±SD	5	5	4	5	4	3		4	6	2	3	7
		p	–	↑	↑	↑	NS	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	17	25	23	21	20	18	18	19	21	ND	ND	ND
		±SD	2	4	2	2	2	2	1	3	3			
		p	–/–	↑/–	↑/–	↑/–	NS/–	NS/–	NS/NS	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	22	28	28	29	27	24	ND	23	24	20	ND	ND
		±SD	3	7	8	10	8	6		5	6	5		
		p	–/NS	↑/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	–/–
PAPd	MM	Mean	11	18	17	15	13	14	ND	14	18	14	13	14
(mmHg)		±SD	4	5	4	4	5	4		4	5	3	5	7
		p	–	↑	↑	↑	NS	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	7	15	12	11	11	10	7	9	9	ND	ND	ND
		±SD	2	5	1	2	2	2	3	2	1			
		p	–/–	↑/–	↑/–	↑/–	↑/–	↑/–	NS/NS	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	9	17	17	18	16	12	ND	11	11	9	ND	ND
		±SD	3	8	9	11	9	6		6	5	6		
		p	–/NS	↑/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	–/–

↑↓ : Significantly different from the base-line value (MM), from the base-line value/ pre atipamezole-administration value (MMA) and from base-line value/ the value in MM (Me80)

NS: not significant

Table 3. Changes in pulmonary arterial wedge pressure (PAWP), systemic vascular resistance (SVR), pulminary vascular resistance (PVR) and cardiac index (CI) in pigs given medetomidine-midazolam (M-M), medetomidine-midazolam (M-M-A) and medetomidine alone (Me80)

		min	0	5	10	15	20	30	35	40	60	80	100	120
PAWP	MM	Mean	7	7	6	6	6	6	ND	6	7	7	7	7
(mmHg)		±SD	2	1	1	1	1	1		2	2	1	1	1
		p	–	NS	NS	NS	NS	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	5	6	7	7	7	5	4	4	6	ND	ND	ND
		±SD	1	2	1	1	2	2	2	1	1			
		p	–/–	NS/–	NS/–	NS/–	NS/–	NS/–	NS/NS	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	7	8	10	8	9	8	ND	7	8	7	ND	ND
		±SD	1	2	1	1	3	3		2	2	3		
		p	–/NS	NS/NS	↑/↑	NS/↑	NS/↑	NS/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	–/–
SVR	MM	Mean	775	ND	1160	ND	1052	1020	ND	1036	1029	1022	1042	1131
(mmHg·kg·min/ml)		±SD	160		331		264	266		233	271	269	222	189
		p	–	–	↑	–	↑	↑	–	↑	NS	↑	↑	↑
	MMA	Mean	674	ND	1082	ND	1087	1014	577	843	891	ND	ND	ND
		±SD	49		156		181	110	133	117	184			
		p	–/–	–/–	↑/–	–/–	↑/–	↑/–	NS/↓	↑/↓	NS/NS	–/–	–/–	–/–
	Me80	Mean	865	ND	1380	ND	1171	1156	ND	1131	1082	1069	ND	ND
		±SD	115		468		321	224		214	139	119		
		p	–/NS	–/–	NS/NS	–/–	↑/NS	NS/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	–/–
PVR	MM	Mean	95	ND	184	ND	144	133	ND	146	189	142	142	168
(mmHg·kg·min/ml)		±SD	30		49		28	22		17	40	27	30	91
		p	–	–	↑	–	↑	↑	–	↑	↑	↑	NS	NS
	MMA	Mean	80	ND	139	ND	117	108	92	115	127	ND	ND	ND
		±SD	16		28		29	28	21	38	34			
		p	–/–	–/–	↑/–	–/–	NS/–	NS/–	NS/NS	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	110	ND	184	ND	160	141	ND	144	145	121	ND	ND
		±SD	15		76		55	38		32	39	34		
		p	–/NS	–/–	NS/NS	–/–	NS/NS	NS/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	–/–
CI	MM	Mean	134	ND	116	ND	121	123	ND	119	116	118	117	106
(ml/kg/min)		±SD	21		24		22	25		23	26	26	20	14
		p	–	–	NS	–	NS	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	142	ND	121	ND	115	118	155	128	128	ND	ND	ND
		±SD	9		16		17	15	24	10	11			
		p	–/–	–/–	↓/–	–/–	↓/–	↓/–	NS/↑	↓/↑	NS/NS	–/–	–/–	–/–
	Me80	Mean	135	ND	102	ND	113	110	ND	110	112	109	ND	ND
		±SD	12		22		21	15		15	15	12		
		p	–/NS	–/–	↓/NS	–/–	↓/NS	↓/NS	–/–	↓/NS	↓/NS	↓/NS	–/–	–/–

↑↓ : Significantly different from the base-line value (MM), from the base-line value/ pre atipamezole-administration value (MMA) and from base-line value/ the value in MM (Me80)

NS: not significant

Table 4. Changes in stroke volume (SV) and rate pressure product (RPP) in pigs given medetomidine-midazolam (M-M) and medetomidine-midazolam (M-M-A)

		min	0	5	10	15	20	30	35	40	60	80	100	120
SV	MM	Mean	1.37	ND	1.17	ND	1.27	1.34	ND	1.33	1.27	1.28	1.31	1.12
(ml/kg)		±SD	0.23		0.21		0.15	0.21		0.16	0.22	0.24	0.25	0.09
		p	–	–	NS	–	NS	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	1.49	ND	1.24	ND	1.28	1.4	1.07	1.2	1.31	ND	ND	ND
		±SD	0.06		0.19		0.13	0.12	0.2	0.21	0.22			
		p	–/–	–/–	NS/–	–/–	NS/–	NS/–	NS/↓	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	1.12	ND	1.05	ND	1.19	1.2	ND	1.29	1.31	1.31	ND	ND
		±SD	0.11		0.17		0.16	0.13		0.13	0.09	0.16		
		p	–/↓	–/–	NS/NS	–/–	NS/NS	NS/NS	–/–	NS/NS	↑/NS	NS/NS	–/–	–/–
RPP	MM	Mean	12154	14709	15158	14342	13942	13021	ND	12769	12270	12602	12801	13367
(mmHg/min)		±SD	2316	2363	2139	2047	1812	1532		1480	907	1225	1857	784
		p	–	↑	↑	↑	↑	NS	–	NS	NS	NS	NS	NS
	MMA	Mean	11624	14012	15383	14027	13442	12287	14638	14214	13775	ND	ND	ND
		±SD	1116	2877	2418	2304	2204	1919	2889	3508	2753			
		p	–/–	NS/–	↑/–	↑/–	NS/–	NS/–	NS/↑	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	17101	15579	15564	15121	14876	14140	ND	12711	12566	11632	ND	ND
		±SD	1342	963	1407	1385	884	1691		1429	1512	1155		
		p	–/↑	NS/NS	NS/NS	NS/NS	↓/NS	↓/NS	–/–	↓/NS	↓/NS	↓/NS	–/–	–/–

↑↓ : Significantly different from the base-line value (MM), from the base-line value/ pre atipamezole-administration value (MMA) and from base-line value/ the value in MM (Me80)

NS: not significant

Table 5. Changes in PaO_2, $PaCO_2$ and pHa in pigs given medetomidine-midazolam (M-M), medetomidine-midazolam-atipamezole (M-M-A) and medetomidine alone (Me80)

		min	0	5	10	20	30	35	40	50	60	80	100	120
PaO2	MM	Mean	104.7	96.0	98.6	93.6	96.8	ND	101.4	ND	92.9	97.3	93.5	96.0
(mmHg)		±SD	11.8	11.4	13.3	9.6	10.2		9.7		8.1	8.4	5.3	7.1
		p	–	↓	↓	NS	↓	–	NS	–	↓	NS	NS	NS
	MMA	Mean	99.3	95.5	97.1	93.8	95.4	99.9	96.2	103.5	102.0	ND	ND	ND
		±SD	7.5	8.6	7.2	5.0	6.0	11.6	2.7	11.5	6.1			
		p	–/–	NS/–	NS/–	↓/–	↓/–	NS/NS	NS/NS	NS/NS	↑/↑	–/–	–/–	–/–
	Me80	Mean	102.5	100.4	104.6	100.7	98.6	ND	99.7	ND	99.7	99.3	ND	ND
		±SD	10.1	9.3	12.4	5.7	5.9		6.1		7.1	4.8		
		p	–/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–	NS/NS	–/–	NS/NS	NS/NS	–/–	–/–
PaCO2	MM	Mean	38.9	39.0	39.8	38.9	39.2	ND	39.0	ND	38.9	38.3	38.5	38.3
(mmHg)		±SD	2.6	2.9	2.6	2.5	3.9		3.1		4.6	2.9	3.0	3.5
		p	–	NS	NS	NS	NS	–	NS	–	NS	NS	NS	NS
	MMA	Mean	38.0	37.9	39.3	39.1	38.3	36.4	37.4	38.0	35.2	ND	ND	ND
		±SD	4.5	5.3	4.9	4.6	4.2	2.5	3.3	5.2	2.5			
		p	–/–	NS/–	NS/–	NS/–	NS/–	NS/NS	NS/NS	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	44.8	43.6	42.8	43.1	42.9	ND	42.3	ND	43.2	43.0	ND	ND
		±SD	2.6	2.5	2.6	2.6	1.6		1.5		1.5	2.6		
		p	–/↑	NS/↑	NS/NS	NS/↑	NS/NS	–/–	NS/NS	–/–	NS/NS	NS/↑	–/–	–/–
pHa	MM	Mean	7.43	7.42	7.41	7.42	7.43	ND	7.43	ND	7.42	7.44	7.44	7.44
		±SD	0.02	0.02	0.01	0.01	0.02		0.03		0.02	0.01	0.02	0.02
		p	–	↓	↓	NS	NS	–	NS	–	NS	NS	NS	NS
	MMA	Mean	7.43	7.42	7.41	7.42	7.43	7.44	7.42	7.40	7.41	ND	ND	ND
		±SD	0.01	0.01	0.01	0.02	0.02	0.03	0.04	0.03	0.04			
		p	–/–	NS/–	↓/–	NS/–	NS/–	NS/NS	NS/NS	NS/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	7.40	7.40	7.40	7.41	7.42	ND	7.42	ND	7.42	7.43	ND	ND
		±SD	0.02	0.01	0.04	0.02	0.01		0.01		0.01	0.01		
		p	–/↓	NS/NS	NS/NS	NS/NS	NS/NS	–/–	NS/NS	–/–	NS/NS	NS/NS	–/–	–/–

↑↓ : Significantly different from the base-line value (MM), from the base-line value/ pre atipamezole-administration value (MMA) and from base-line value/ the value in MM (Me80)

NS: not significant

Table 6. Changes in [HCO_3^-]a, arterial base excess (BEa) and arterial hemoglobin oxygen saturation (SaO_2) in pigs given medetomidine-midazolam (M-M), medetomidine-midazolam-atipamezole (M-M-A) and medetomidine alone (Me80)

		min	0	5	10	20	30	35	40	50	60	80	100	120
[HCO3⁻]a	MM	Mean	25.6	24.7	25.0	25.0	25.3	ND	25.5	ND	25.3	25.7	25.4	25.7
(mmol/l)		±SD	1.2	1.5	1.3	1.6	2.0		2.3		3.2	2.1	2.1	1.2
		p	–	NS	NS	NS	NS	–	NS	–	NS	NS	NS	NS
	MMA	Mean	25.2	24.3	24.9	25.5	25.2	25.0	24.3	23.4	22.4	ND	ND	ND
		±SD	2.4	3.1	2.9	2.5	2.3	2.2	3.4	3.0	1.9			
		p	–/–	NS/–	NS/–	NS/–	NS/–	NS/NS	NS/NS	↓/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	27.3	25.6	26.2	26.8	27.4	ND	27.1	ND	28.0	28.2	ND	ND
		±SD	2.7	3.5	3.4	2.2	1.1		1.0		1.3	1.3		
		p	–/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–	NS/NS	–/–	NS/NS	NS/↑	–/–	–/–
BEa	MM	Mean	1.6	0.5	0.7	0.8	1.1	ND	1.3	ND	0.9	1.4	1.2	1.6
(mmol/l)		±SD	1.2	1.4	1.1	1.6	2.0		2.4		3.1	2.1	2.1	0.9
		p	–	NS	↓	NS	NS	–	NS	–	NS	NS	NS	↓
	MMA	Mean	1.3	0.1	0.5	1.2	0.9	0.9	-0.2	-1.4	-2.0	ND	ND	ND
		±SD	2.3	2.9	2.8	2.4	2.3	2.4	3.8	3.0	2.4			
		p	–/–	NS/–	NS/–	NS/–	NS/–	NS/NS	NS/NS	↓/NS	NS/NS	–/–	–/–	–/–
	Me80	Mean	3.0	1.1	1.8	2.4	3.1	ND	2.8	ND	3.7	3.8	ND	ND
		±SD	3.0	4.3	4.0	2.5	1.2		1.1		1.5	1.3		
		p	–/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–	NS/NS	–/–	NS/NS	NS/NS	–/–	–/–
SaO2	MM	Mean	96.9	95.9	96.1	95.8	96.1	ND	96.7	ND	95.8	96.4	96.0	96.3
(%)		±SD	0.8	1.3	1.4	1.2	1.0		0.8		0.8	0.7	0.4	0.6
		p	–	NS	NS	NS	NS	–	NS	–	↓	NS	NS	NS
	MMA	Mean	96.6	96.0	96.2	96.0	96.2	96.7	96.2	96.5	96.7	ND	ND	ND
		±SD	0.5	1.1	0.7	0.5	0.6	0.8	0.5	1.0	0.5			
		p	–/–	NS/–	NS/–	↓/–	NS/–	NS/NS	NS/NS	NS/NS	NS/↑	–/–	–/–	–/–
	Me80	Mean	96.5	96.3	97.2	96.5	96.4	ND	96.5	ND	96.5	96.6	ND	ND
		±SD	0.7	0.6	1.4	0.4	0.5		0.5		0.7	0.5		
		p	–/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–	NS/NS	–/–	NS/NS	NS/NS	–/–	–/–

↑↓ : Significantly different from the base-line value (MM), from the base-line value/ pre atipamezole-administration value (MMA) and from base-line value/ the value in MM (Me80)

NS: not significant

Table 7. Changes in delivery O_2 (DO_2), oxygen consumption (VO_2), oxygen utilization ratio (UO_2)= DO_2 / VO_2 and body temperature at pulmonary artery (BT(PA)) in pigs given medetomidine-midazolam(M-M), medetomidine-midazolam-atipamezole (M-M-A) and medetomidine alone (Me80)

		min	0	5	10	20	30	35	40	50	60	80	100	120
DO2	MM	Mean	434	ND	355.2	393.2	376.8	ND	369.1	ND	361.5	378.4	383.5	347.4
(l/kg/min)		±SD	109.4		86.2	63.9	89		92.8		63.5	77.2	75.9	69
		p	–	–	NS	NS	NS	–	NS	–	NS	NS	NS	NS
	MMA	Mean	425.2	ND	336.7	339.7	339.7	ND	461.4	351.9	403.8	ND	ND	ND
		±SD	87.9		93.3	113.6	88.2		107.9	40.3	90.7			
		p	–/–	–/–	↓/–	↓/–	↓/–	–/–	NS/NS	NS/NS	↓/NS	–/–	–/–	–/–
	Me80	Mean	402	ND	299.9	334.3	323.2	ND	326.3	ND	331.5	321.2	ND	ND
		±SD	52.5		48.6	53.7	36.7		49.9		44	33.6		
		p	–/NS	–/–	↓/NS	↓/NS	↓/NS	–/–	↓/NS	–/–	↓/NS	↓/NS	–/–	–/–
VO2	MM	Mean	113	ND	94.4	102.6	95.2	ND	112.7	ND	129.3	137	148.6	106.5
(l/kg/min)		±SD	29.8		22.9	17.7	22.7		34.6		51.1	14.5	4.3	34.1
		p	–	–	NS	NS	↓	–	NS	–	NS	NS	NS	NS
	MMA	Mean	136.1	ND	88.2	90.1	85.2	ND	154.9	136.1	164.1	ND	ND	ND
		±SD	56.7		13.6	25.7	16		29.8	32.5	23.2			
		p	–/–	–/–	NS/–	NS/–	NS/–	–/–	NS/↑	NS/NS	NS/↑	–/–	–/–	–/–
	Me80	Mean	113.9	ND	97.8	90.2	97.7	ND	84.5	ND	101.3	93.4	ND	ND
		±SD	28.7		29.8	10.9	17.1		12.4		27.6	3.8		
		p	–/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	NS/NS	–/–	NS/NS	NS/↓	–/–	–/–
UO2	MM	Mean	3.86	ND	3.92	3.95	4.02	ND	3.5	ND	3.02	2.79	2.59	3.15
		±SD	0.43		1.02	0.94	0.74		0.94		0.8	0.59	0.54	0.51
		p	–	–	NS	NS	NS	–	NS	–	NS	↓	↓	NS
	MMA	Mean	3.36	ND	3.79	3.73	3.74	ND	3.03	2.76	2.53	ND	ND	ND
		±SD	0.86		0.59	0.25	0.38		0.4	0.79	0.68			
		p	–/–	–/–	NS/–	NS/–	NS/–	–/–	NS/↓	NS/NS	NS/↓	–/–	–/–	–/–
	Me80	Mean	3.66	ND	3.21	3.69	3.36	ND	3.88	ND	3.45	3.55	ND	ND
		±SD	0.59		0.57	0.31	0.39		0.43		0.78	0.36		
		p	–/NS	–/–	NS/NS	NS/NS	NS/NS	–/–	NS/NS	–/–	NS/NS	NS/↓	–/–	–/–

↑↓ : Significantly different from the base-line value (MM), from the base-line value/ pre atipamezole-administration value (MMA) and from base-line value/ the value in MM (Me80)

NS: not significant

Table 8. Changes in pulmonary arterial blood temperature (BT(PA)) in pigs given medetomidine-midazolam(M-M), medetomidine-midazolam-atipamezole (M-M-A) and medetomidine alone (Me80)

		min	0	5	10	20	30	35	40	50	60	80	100	120
BT(PA)	MM	Mean	39.2	39.2	39	38.7	38.4	ND	38.2	ND	38	37.9	38	37.9
(℃)		±SD	0.5	0.5	0.6	0.7	0.8		0.9		1	0.9	0.9	0.9
		p	–	NS	↓	↓	↓	–	↓	–	↓	↓	↓	↓
	MMA	Mean	39.2	39	38.9	38.6	38.3	37.9	38.1	38.3	38.4	ND	ND	ND
		±SD	0.3	0.3	0.3	0.3	0.2	0.4	0.2	0.3	0.4			
		p	–/–	NS/–	↓/–	↓/–	↓/–	↓/↓	↓/NS	↓/NS	↓/NS	–/–	–/–	–/–
	Me80	Mean	40.1	40	39.7	39.2	38.9	ND	38.6	ND	38.2	37.8	ND	ND
		±SD	0.3	0.4	0.4	0.3	0.3		0.3		0.3	0.4		
		p	–/↑	NS/↑	↓/NS	↓/NS	↓/NS	–/–	↓/NS	–/–	↓/NS	↓/NS	–/–	–/–

↑↓ : Significantly different from the base-line value (MM), from the base-line value/ pre atipamezole-administration value (MMA) and from base-line value/ the value in MM (Me80)

NS: not significant

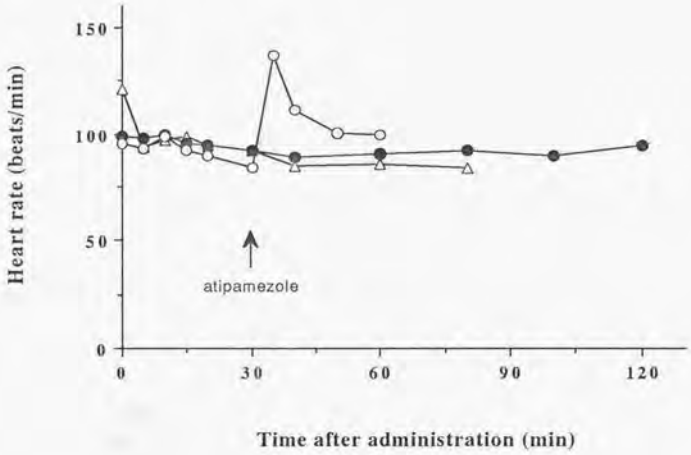
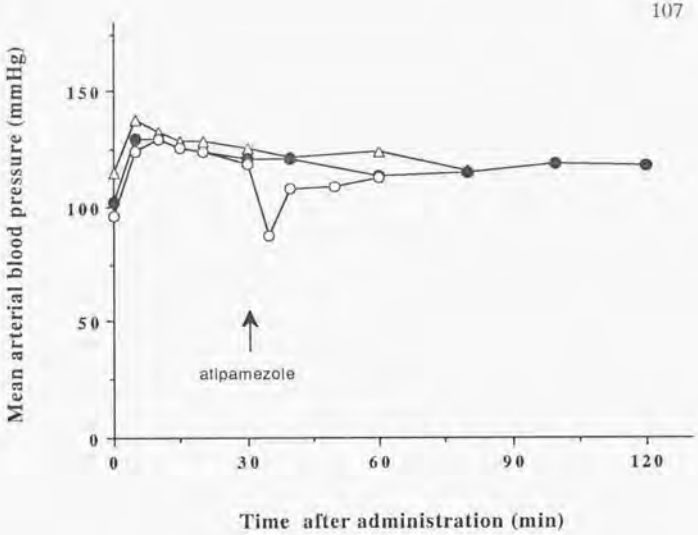


Fig. 1. Changes in heart rate in pigs given medetomidine (40 μg/kg)- midazolam (0.2mg/kg) (●), medetomidine (40 μg/kg)- midazolam (0.2mg/kg)- atipamezole (160 μg/kg) (○) and medetomidine (80 μg/kg) alone (△). Each symbol represents the mean value in each group (n= 6).



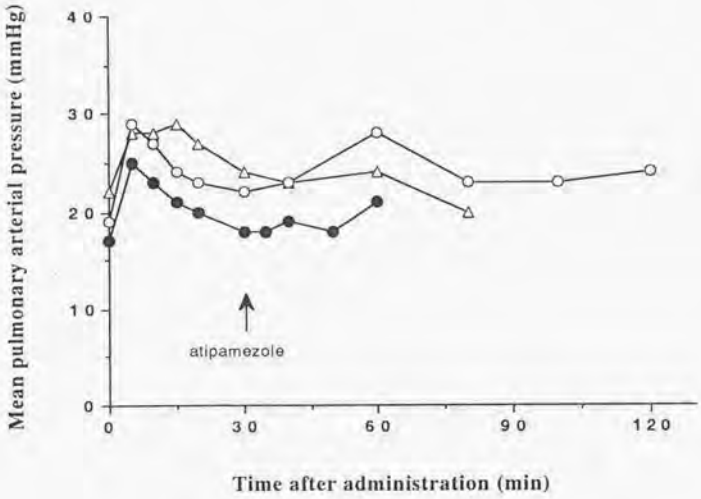


Fig. 2.Changes in mean arterial blood pressure (upper) and mean pulmonary arterial pressure (lower) in pigs given medetomidine (40 μg/kg)- midazolam (0.2mg/kg) (●), medetomidine (40 μg/kg)- midazolam (0.2mg/kg)- atipamezole (160 μg/kg) (○) and medetomidine (80 μg/kg) alone (△). Each symbol represents the mean value in each group (n= 6).

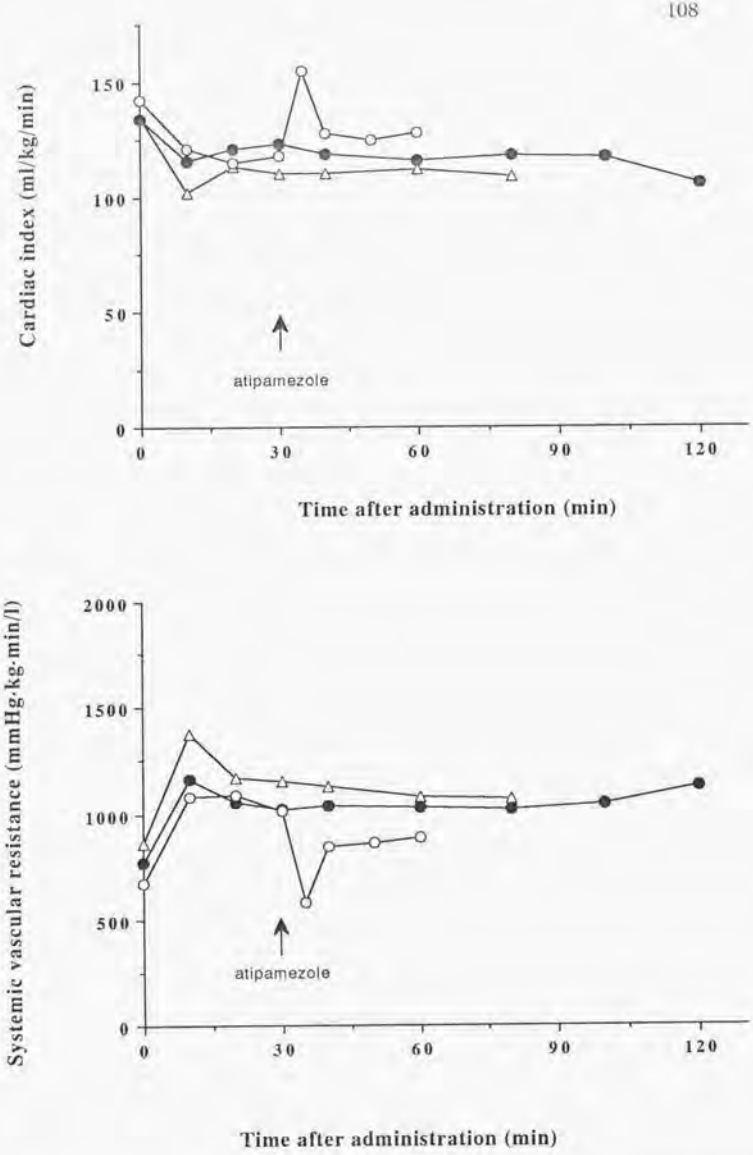


Fig. 3. Changes in cardiac index (upper) and systemic vascular resistance (lower) in pigs given medetomidine (40 μg/kg)- midazolam (0.2mg/kg) (●), medetomidine (40 μg/kg)- midazolam (0.2mg/kg)- atipamezole (160 μg/kg) (○) and medetomidine (80 μg/kg) alone (△). Each symbol represents the mean value in each group (n= 6).

Part 2-4

The Effect of Medetomidine- Midazolam on Plasma Glucose Concentration in Pigs

α_2-Agonists exert deep sedation, muscle relaxation and analgesia through activation of α_2-adrenoceptors in the central nervous system [2, 34, 61]. However, α_2-adrenoceptors are widely distributed throughout the body, not always having an inhibitory action and concurrent stimulation of these cause the wide variety of undesirable effects [32]. The most clinically apparent and easily assessable sign of peripheral effects of α_2- agonists is hyperglycemia [19, 65] which is results from its action on the pancreas [16].

The purpose of this experiment was to evaluate the peripheral effects of medetomidine-midazolam assessed by the changes in plasma glucose and insulin concentrations and to compare with a high dose of medetomidine alone. This study was also designed to evaluate the effects of both central and peripheral actiong or peripheral acting α_2-antagonists on those changes.

MATERIALS and METHODS

Animals and animal preparation :

Six mixed breed pigs in good health were repeatedly used at a weekly interval in this study. Their mean age was 10.2 weeks (range 9 to 12 weeks) and mean body weight was 20.0 kg (range 18.5 to 22 kg). Management for these pigs were the same as those in Part 1-4. At least 7 days before the experiments, the pigs were implanted 14G heparin-coated polyvinyl chloride catheters (Toray Medical Co., Anthron) into the right lateral jugular vein under isoflurane anesthesia. The pigs were fasted for approximately 12 hr before the experiments, and each animal was exposed to 7 different regimens in a

randomized block design. Management for these pigs were the same as those in other experiments.

Drugs:

The drugs used in this study were medetomidine, midazolam, atipamezole and L659,066 (Merck & Co., Inc. U.S.A.) which does not cross the blood-brain barrier. L659,066 was dissolved in distilled water at the concentration of 1 mg/ml. Medetomidine, midazolam and atipamezole were injected intramuscularly into the cervical muscle and L659,066 was injected intravenously into the ear vein.

Experimental design:

More than 7 days after implanting the catheter, each conscious pig in good health was placed in the sling. The animals were administered 40 or 80 μg/kg of medetomidine, 0.2 mg/kg of midazolam, 40 μg/kg of medetomidine and 0.2 mg/kg of midazolam (med-mid), med-mid and 160 μg/kg of atipamezole, 80 μg/kg of medetomidine and 320 μg/kg of atipamezole and 80 μg/kg of medetomidine and 100 μg/kg of L659,066. Atipamezole and L659,066 were injected 30 min after administration of medetomidine or med-mid. Two ml of venous blood samples were then collected into glass tubes containing sodium fluoride via the implanted catheter 0, 30, 60, 90, 120, 150, 180, 240 and 360 min after dosing for determination of plasma glucose. Another 2 ml of blood samples were collected 0, 30, 60 and 120 min after dosing into a glass tube containing EDTA-Na for determination of plasma insulin concentrations. After centrifugation, plasma was collected and frozen at -80°C until assayed.

Analytical materials and procedures :

Plasma glucose concentration was determined by glucose oxidase method (Glucose B-TEST Wako, Wako Pure Chemical Industries, Ltd. Japan). Plasma insulin concentration

was measured by a double-antibody enzyme-linked immunosorbent assay using a commercial kit (Glazyme Insulin-EIA TEST, Wako Pure Chemical Industries, Ltd. Japan). Since the commercial kit used in this study was developed to determined human insulin concentrations, some preliminary work was performed to determine the cross-reactivity of swine insulin. A solution of swine insulin (Sigma Chemical Company, U.S.A.) was prepared and standard dilutions (0, 5, 10, 125 and 250 μU of swine insulin /ml) were made with bovine serum albumin which was the matrix used in preparation of the assay kit standards to establish a standard curve. Although the reactivity was low in swine insulin, the standard curve generated by swine standards showed the comparable linearity with that by kit standards (Fig. 1). The lower limit in this assy system for swine insulin was 5 μU/ml. All samples were analyzed in duplicate.

The plasma glucose concentrations after drug administration were compared with base-line values using paired-*t* test.

RESULTS and DISCUSSION

Figure 2 shows the changes in plasma glucose concentrations in pigs given med-mid, med-mid-atipamezole, 40 μg/kg of medetomidine alone and midazolam alone. The administration of med-mid induced a gradual increase in blood glucose after administration, which was the similar pattern in pigs given 40 μg/kg of medetomidine alone. However, its change was relatively small and there were no significant differences between pre- and post-administration values. Since midazolam did not induce the increase in blood glucose level, it was thought that the increase in blood glucose in med-mid was to be mainly attributed to the action of medetomidine. The stimulating effect of medetomidine on plasma glucose concentration was also evident because this weak

hyperglycemic effect of med-mid was completely antagonized by the administration of atipamezole.

On the contrary, the administration of 80 μg/kg of medetomidine produced moderate hyperglycemia, which reached maximal levels (more than 200 mg/dl) after approximately 90 min (Fig.3). This hyperglycemic effect was completely blocked by atipamezole (Fig. 3). The administration of L659,066 which inhibits only peripheral α_2-adrenoreceptors also effectively blocked the change induced by 80 μg/kg of medetomidine (Fig. 3).

Although the variations among the individuals were relatively large, plasma concentrations of insulin decreased greatly in pigs given 80 μg/kg of medetomidine (Fig. 5). These changes were relatively small in pigs given med-mid, med-mid-atip, 40 μg/kg of medetomidine alone and midazolam alone (Fig. 4). The decreased insulin level generated by 80 μg/kg of medetomidine was completely reversed by the administration of atipamezole. In addition, plasma concentration of insulin was recovered soon after administration of L659,066 (Fig. 5).

It has been reported that α_2-agonists induce hyperglycemia through the peripheral effect on α_2- adrenoceptor in the pancreatic β-cells which are linked to the inhibition of insulin release in rats [3]. The data obtained here confirmed that the increase in plasma glucose concentration in pigs was induced through activation of peripheral α_2-adrenoceptors by medetomidine.

In conclusion, the hyperglycemic effect of medetomidine-midazolam via the peripheral α_2-adrenoceptors in pancreatic β-cells was relatively small and did not lead to any clinical problems in pigs. It may indicate that the peripheral effect of medetomidine-midazolam generally small and this combination have less undesirable effect than medetomidine alone.

SUMMARY

The peripheral effects of medetomidine (40 μg/kg) -midazolam (0.2 mg/kg) and medetomidine (80 μg/kg) alone assessed by the changes in plasma glucose and insulin concentrations were evaluated in pigs. The intramuscular administration of med-mid induced a gradual hyperglycemic response which was much smaller than those in pigs given 80 μg/kg of medetomidine alone. This mild increase in plasma glucose concentration was thought to be mainly attributed to the effect of medetomidine on peripheral α_2-adrenoreceptors. However its change was relatively small and did not lead to any clinical problems in pigs. It may indicate that the peripheral effect of medetomidine-midazolam generally small and this sedative combination have less undesirable effect than medetomidine alone.

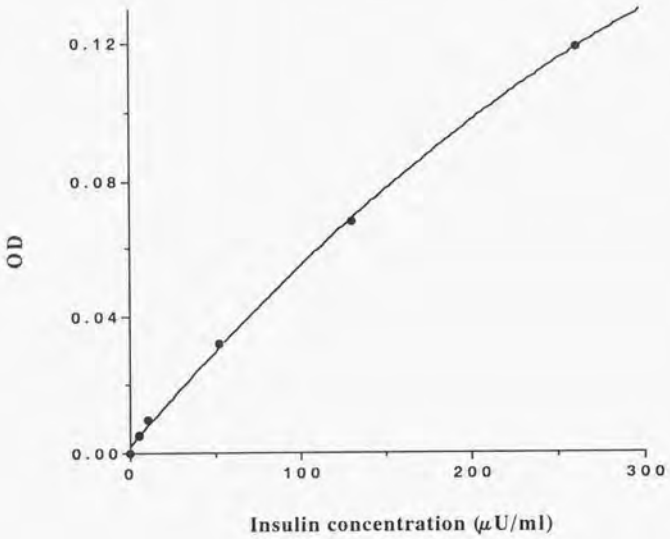


Fig. 1. The standard curve for swine insulin using ELISA method

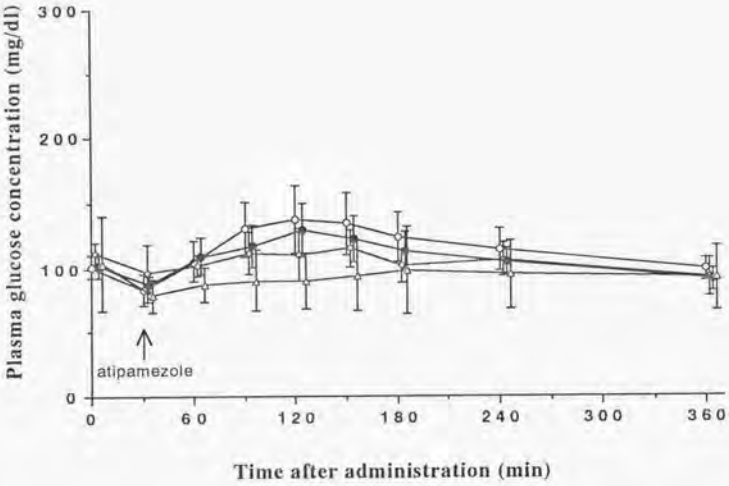


Fig. 2. Changes in plasma glucose concentration in pigs given medetomidine-midazolam (○), medetomidine-midazolam-atipamezole (◇)40 μg/kg of medetomidine alone (●), 0.2 mg/kg of midazolam alone (△). Each value represents the mean ± SD in each group (n= 6).

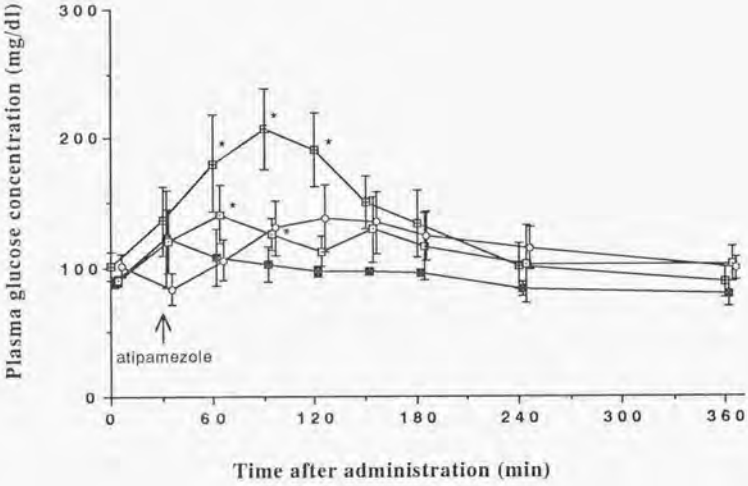


Fig. 3. Changes in plasma glucose concentration in pigs given 80 μg/kg of medetomidine alone (⊞), 80 μg/kg of medetomidine and atipamezole (⊠), 80 μg/kg of medetomidine and L659,066 (⊡) and medetomidine-midazolam (○). Each value represents the mean ± SD in each group (n= 6). *P<0.05 when compared with the base-line value.

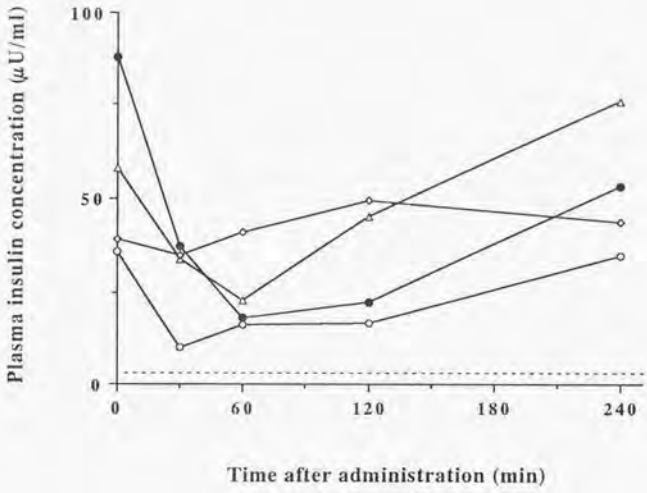


Fig. 4. Changes in plasma insulin concentration (--- lower limit: 5 μU/ml) in pigs given medetomidine-midazolam (○), medetomidine-midazolam-atipamezole (◇), 40 μg/kg of medetomidine alone (●), 0.2 mg/kg of midazolam alone (△). Each value represents the mean in each group (n= 6).

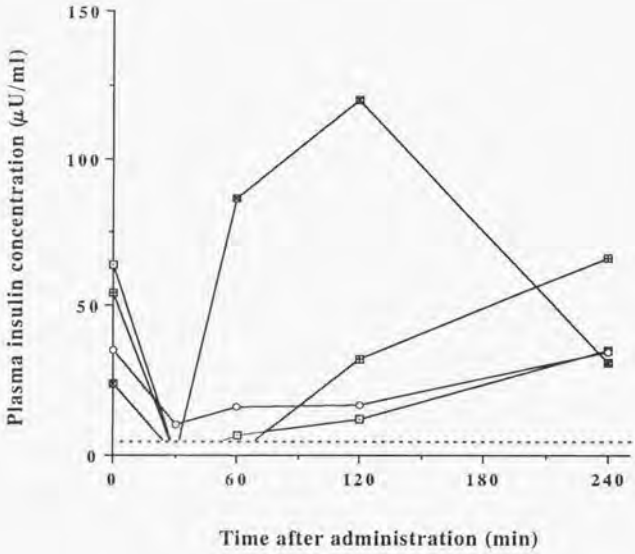


Fig. 5. Changes in plasma insulin concentration (--- lower limit: 5 μU/ml) in pigs given 80 μg/kg of medetomidine alone (⊞), 80 μg/kg of medetomidine and atipamezole (⊠), 80 μg/kg of medetomidine and L659,066 (⊡) and medetomidine-midazolam (○). Each value represents the mean in each group (n= 6).

Part3- Efficacy of medetomidine-midazolam as a preanesthetic before ketamine and isoflurane anesthesia in pigs

Part 3-1

A balanced anesthesia with a combination of medetomidine, midazolam, ketamine and butorphanol and its antagonism by atipamezole in pigs

There are equally many situations where general anesthesia is needed in laboratory pigs. For minor surgery or various minor procedures accompanied by pain, it is to be desired practically that the anesthetic procedures employed is simple and needs no special apparatus. Ketamine has been used as a major component of many anesthetic regimens in pigs, since it can be given by an intramuscular route and induces rapid onset of anesthesia with profound somatic analgesia [64]. However when given alone, ketamine induces poor muscle relaxation and poor visceral analgesia and is frequently accompanied by emergence delirium during the recovery phase.

An α_2-agonist and a benzodiazepine are useful agents to improve these undesirable effects and a combination of xylazine or diazepam and ketamine is commonly used in cats, dogs, horses, ruminants, zoo animals and other species [58, 81]. A combination of medetomidine-midazolam and ketamine is also expected to exert a satisfactory anesthesia in pigs. In addition, butorphanol which is a non-controlled synthetic analgesic, has been reported to enhance the effect of xylazine and the combination of xylazine-ketamine-butorphanol exerted the more potent and well balanced anesthesia as compared with xylazine-ketamine, while reducing the amount of ketamine to one third [42].

The purpose of this study was to evaluate and compare the anesthetic effects of medetomidine-midazolam-ketamine (M-M-K) and medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) (experiment 1), and to evaluate the antagonistic effect of atipamezole on M-M-K-B induced anesthesia in pigs (experiment 2). The present study was also designed to evaluate the cardiopulmonary effects of M-M-K-B and medetomidine-midazolam-ketamine-atipamezole (M-M-K-B-A) (experiment 3).

MATERIALS AND METHODS

Animals:

Eighteen castrated mixed breed pigs in good health were used in this study. After a week period of stabilization the pigs were randomly assigned to 2 groups of 6 (for experiments 1 and 2) and 12 pigs (for experiment 3). Their mean age was 12.0 weeks (range 9 to 15 weeks) and mean body weight was 20.0 kg (range 18.0 to 24.0 kg). Management for these pigs before and during the experiment were the same as the previous experiments.

Drugs:

The drugs used in this study were medetomidine at a dose of 40 μg/kg of body weight, midazolam at a dose of 0.2 mg/kg, ketamine hydrochloride (Ketaral, Sankyo Co., Japan) at a dose of 10 or 5 mg/kg, butorhanol tartrate (Torbugesic, Bristol Veterinary Products, U.S.A.) at a dose of 0.2 mg/kg and atipamezole at a dose of 160 μg/kg. All the drugs were injected intramuscularly into the cervical muscle.

Experimental design:

Experiment 1- Anesthetic effects of M-M-K and M-M-K-B in pigs

Six pigs were repeatedly used at a weekly interval. Twenty min after administration of medetomidine and midazolam the pigs were given 10 mg/kg of ketamine or 5 mg/kg of ketamine and 0.2 mg/kg of butorphanol.

After administration of ketamine or ketamine and butorphanol, reaction to nose (nasal septum) -pinching, hindlimb pedal reflex and laryngeal reflex were tested every 10th min. The degree of muscle relaxation was also recorded. In addition, induction time of anesthesia (time from the administration of ketamine or ketamine and butorphanol to loss of the reaction to nose-pinching), arousal time (time from administration of ketamine or

ketamine and butorphanol to a return of motor activity and righting reflex, as indicated by ability of the pig to raise the head when stimulated), surgical anesthesia time (duration of loss of the reaction to nose-pinching), standing time (time from administration of ketamine or ketamine and butorphanol to ability to stand when stimulated) and total recovery time (time from administration of ketamine or ketamine and butorphanol until the animal can not be distinguished from untreated animals) were measured.

Experiment 2- Antagonistic effect of atipamezole on M-M-K-B induced anesthesia

The same 6 pigs used in experiment 1 were repeatedly used at weekly interval to evaluate the antagonistic effect of atipamezole on M-M-K-B induced anesthesia. All the pigs received the drugs as the same manner as in experiment 1. Thirty min after administration of ketamine-butorphanol, 160 μg/kg of atipamezole, which was the optimal dose against the sedation induced by medetomidine-midazolam, was administered. Antagonistic effects were repeatedly assessed 5, 10, 20, 30, 40, 60, 80, 120, 180, 240 and 300 min after administration of atipamezole and/or until the animals totally recovered, and arousal time (time from administration of atipamezole to a return of motor activity and righting reflex), standing time (time from administration of atipamezole to ability to walk when stimulated) and total recovery time (time from administration of ketamine or ketamine and butorphanol until the animal can not be distinguished from untreated animals) were recorded.

Experiment 3- Cardiopulmonary effects of M-M-K-B and M-M-K-B-A

Twelve pigs were used to evaluate the cardiopulmonary effects of these two regimens (six pigs each). Animal preparations and the methods for cardiopulmonary measurements

were the same as described in Part 2-3. Following the measurements of base-line values in the conscious state, each animal was given M-M-K-B in the same manner as in experiment 1. Thirty min after administration of ketamine-butorphanol, 6 pigs were injected 160 mg/kg of atipamezole. All the cardiopulmonary measurements were repeated 10 and 20 min after administration of medetomidine-midazolam and 5, 10, 20, 30, 40, 60 and 80 min after administration of ketamine-butorphanol. In pigs given atipamezole, these measurements were repeated 5, 10, 20, 30 and 40 min after administration of atipamezole.

Statistical analyses:

The data of induction time, surgical anesthesia time, duration of loss of pedal reflex, arousal time, standing time and total recovery time in M-M-K and M-M-K-B were statistically analyzed using the same methods described in Part 1-2. The values of cardiopulmonary parameters in M-M-K-B and M-M-K-B-A were analyzed using the same methods described in Part 2-3. In all analyses, values were considered to be statistical significant when $P<0.05$.

RESULTS

Anesthetic effects of M-M-K and M-M-K-B:

An intramuscular injection of medetomidine-midazolam induced deep sedation in the same manner as in previous experiments. Minimum pain or irritation was observed at the site of the intramuscular injection of ketamine or ketamine-butorphanol. All pigs of both groups were smoothly induced to anesthesia soon after the administration of ketamine or

ketamine-butorphanol. Mean induction time in both groups did not differ significantly (Table 1).

During the anesthesia, excellent muscle relaxation was constantly evident in pigs given M-M-K-B, while satisfactory muscle relaxation for a shorter term was produced in pigs given M-M-K. Involuntary muscle movement, excitability, profuse salivation and convulsion were not observed in any of the experimental pigs. Both combinations exerted satisfactory analgesia, however the duration of surgical anesthesia in M-M-K-B (longer than 60 min) was significantly longer than that in M-M-K (shorter than 30 min). Pedal reflex was lost in all pigs of both groups, however the mean duration of absence of pedal reflex in M-M-K-B was significantly longer than that in M-M-K (Table 1). Laryngeal reflex was also lost in all pigs of both groups and their duration was similar to that of surgical anesthesia time.

Recovery from anesthesia was invariably smooth with no emergence delirium or aberrant behaviors in pigs given M-M-K-B, while it was not so smooth in pigs given M-M-K, in which the pigs manifested grunting and hyperpnea and had prolonged hindlimb weakness and ataxia. Although the mean arousal time and mean standing time in M-M-K-B were significantly longer than those in M-M-K, the total recovery time was not significantly different from each other (Table 1). After standing, the pigs given M-M-K-B showed mild residual sedation, however they colud walk upon manipulation and did not return to sleep.

Antagonistic effect of atipamezole on M-M-K-B induced anesthesia :

An intramuscular injection of atipamezole at a dose of 160 μg/kg caused rapid arousal from M-M-K-B induced anesthesia. Pigs given atipamezole were aroused approximately

10 min after its injection and stood up soon after that. Relapse to recumbency or unconsciousness did not occur in any pigs after arousal and the pigs totally recovered in approximately 120 min. Recovery from anesthesia was almost smooth except for mild muscle tremors and slight hyperactivity which disappeared in 30 to 40 min after atipamezole injection.

Cardiopulmonary effects of M-M-K-B and M-M-K-B-A

Tables 2 to 4 show the cardiovascular changes in both groups. HR decreased slightly after administration of medetomidine-midazolam and increased slightly after administration of ketamine-butorphanol (Fig. 1), however those were not significant changes. AP and PAP moderately but significantly increased from base-line values 10 min after administration of medetomidine-midazolam, thereafter it gradually decreased (Fig. 2). PAP increased again after administration of ketamine-butorphanol, but gradually decreased again. PAWP and right atrial pressure also increased mildly after administration of medetomidine-midazolam and then gradually decreased .

CI decreased slightly but significantly from the base-line value after administration of medetomidine-midazolam (Fig. 3), however it increased after administration of ketamine-butorphanol and returned to the base-line value. These changes were accompanied by similar changes in SV and by reverse changes in SVR (Fig. 3).

RPP increased just after administration of medetomidine-midazolam and then gradually decreased.

Tables 2 to 4 show the effects of atipamezole on M-M-K-B induced changes in the cardiovascular system. HR, CI and RPP increased just after administration of atipamezole and maintained those values (Figs. 1 and 3). Conversely, AP, PAP (Fig. 2),

RAP, and PAWP and SVR (Fig. 3) and PVR decreased and returned to the base-line levels .

Respiratory effects of M-M-K-B and M-M-K-B-A

Although the administration of M-M-K-B caused a mild decrease in PaO_2 and a mild increase in $PaCO_2$, those changes were relatively small. pHa, BEa and $[HCO_3^-]$a maintained the values near the base-line level. DO_2 in M-M-B-K decreased because of the decrease in CI, however UO_2 increased due to the decrease in VO_2. Those values did not evidently change after administration of atipamezole.

DISCUSSION

An intramuscular injection of ketamine frequently causes signs of severe pain at the site of injection and induces excitement. In the present study, a prior administration of medetomidine-midazolam effectively protected these undesirable effects with smooth induction of anesthesia.

Butorphanol is a non-controlled synthetic analgesic which has a greater potency than morphine or pentazocine, but has weak or no sedative effect. Butorphanol has been used as a preoperative or preanesthetic medication and as a supplement in balanced anesthesia in human patients, and has been reported to increase the level of sedation when combined with an α_2-agonist [53].

The principal advantage of the addition of butorphanol on medetomidine-midazolam-ketamine in pigs was an enhancement of anesthetic condition. The addition of butorphanol significantly prolonged the duration of analgesia, satisfactory muscle relaxation, and the loss of laryngeal reflex, while reducing the amount of ketamine to one

half in M-M-K. In this study, response to pinching the nasal septum, which is thought to be one the most sensitive site to a painful stimulus, was used as an indicator of surgical anesthesia. Therefore the results of this response suggested that M-M-K-B can be used for minor surgery or various procedures with pain for approximately 1 hr.

The absence of laryngeal reflex enabled the endotracheal intubation easily. Although the breathing of pigs given M-M-K-B was not depressed, and the values of pHa, PaO_2, $PaCO_2$ and bicarbonate concentration were almost within physiological ranges, it may be safer to use an endotracheal tube, because respiratory depression caused by limited expansion of the chest wall due to body fat or abnormal body positioning is frequently associated with general anesthesia in pigs [38].

These enhancing effects of butorphanol on the anesthetic condition were considered to be mainly mediated by enhancement of sedative and analgesic properties of medetomidine and partially of midazolam. Although butorphanol has minimum sedative properties when used alone, a combination with medetomidine is believed to act in a synergetic manner and induces a profound sedation and analgesia [47]. A combination of midazolam and butorphanol has also been used for preanesthetic mild sedation in poor risk patients [7].

Another advantage of using M-M-K-B in pigs over M-M-K is a reduction of the undesirable effects. Ketamine, when used alone, frequently produces rough recovery which is speculated to be mediated by its central nervous system stimulating effect through acting at opioid receptors [60]. Although this undesirable effect can be controlled by an α_2-agonist or a benzodiazepine, its effect may disappear before disappearance of undesirable effects of ketamine when a larger dose of ketamine is used. Those may cause

that post-anesthetic recovery from M-M-K was accompanied by mild hyperactivity and ataxia, while M-M-K-B induced a smoother recovery.

In addition, the pigs given M-M-K-B were in a preferable sedative condition after arousal. This may be caused by the residuary effect of midazolam and butorphanol, which is reported to be effective for sedation in the dog and cat with cardiomyopathy [7]. This residuary effect may be very important for the management of postsurgical animals as well as from a humane point of view, and especially in the light of the increasing concern of society for animal rights.

Atipamezole was quite useful in reversing M-M-K-B anesthesia, and this result must enhance the use of this anesthetic combination. Rapid reversal of M-M-K-B anesthesia by atipamezole was mainly due to its antagonism of medetomidine's effects. Atipamezole competes medetomidine at α_2- adrenergic receptors [79]. Moreover, an α_2-antagonist has been reported to influence other receptors or biochemical process and to partially antagonize the effects of ketamine [26].

The intramuscular administration of medetomidine-midazolam resulted in similar changes in the cardiovascular system as observed in Part 2-3. Ketamine and butorphanol had relatively small effects on the cardiovascular changes induced by medetomidine-midazolam. After administration of ketamine and butorphanol, CI increased mildly and returned to the base-line value. PAP also increased, but gradually decreased. These changes were accompanied by similar but slight changes in HR and SV and by a reverse change in SVR. The mild increase in CI after administration of ketamine-butorphanol was attributed to the slightly increased HR and CI. This increased HR was thought to be attributed to a centrally mediated and generalized increase in sympathetic tone caused by ketamine [5], although this effect might be greatly masked by depressing effect of

medetomidine on sympathetic tone [77]. Slightly increased SV was primarily thought to be due to the mild decrease in SVR which meant the decrease in cardiac afterload. These vasodilative effects were mainly induced by butorphanol [44], because ketamine has weak or no direct effects on vascular bed [69]. On the contrary, ketamine increases the pulmonary vascular resistance [59] which caused the increase in PAP after administration of ketamine-butorphanol in this study. The increase in SV might be partially attributed to the effects of ketamine. Ketamine has been reported to increase the cardiac contractility through the activation of sympathetic tone, although it has the direct myocardial depressant effects [55]. The mild increase in CI and unchanged HR produced the mild increase in RPP which represented the oxygen consumption in myocardium, however that change was within physiological level.

In the present study, atipamezole caused mild tachycardia with an increase in CI and a transient fall in AP accompanied by the decrease in the SVR. Tachycardia was probably caused by promotion of the synthetic outflow from the central nervous system and inhibition of baroreceptor activities by atipamezole [79]. Transient hypotension was caused by vasodilation mediated by α_2-adrenoceptor blocking activity of atipamezole [72].

M-M-B-K produced the corresponding increase in $PaCO_2$ and decrease in PaO_2 after administration of ketamine-butorphanol. However those decreased values were not significantly different from base-line values and were thought not to be clinically important. The decrease in PaO_2 was led partially by hypoventilation and partially by a decreased efficiency of blood oxygenation which might be mainly induced by ketamine [25], because butorphanol has been reported to induce minimum respiratory depression even when combined with an α_2-agonist [47]. Although the DO_2 decreased due to a

slight decrease in cardiac output, the UO_2 was not changed or even increased until aroused because of decreased VO_2. The decrease in VO_2 is most likely due to sedation, continued suppression of norepinephrine release, and perhaps direct action on brain stem regulation centers induced by medetomidine [37].

Body temperature decreased mildly after administration of medetomidine-midazolam. These depressions were mainly induced by depression of thermoregulation by medetomidine. Thermoregulation of pigs appears to be deeply influenced by an α_2-agonist compared with other species because the pigs have inefficient thermoregulatory mechanism [38] and less body hair.

In conclusion, the combination of medetomidine, midazolam, ketamine and butorphanol appears to be a relatively safe and widely available anesthesia for the minor surgery or various procedures with pain for the period within one hour in pigs.

SUMMARY

The anesthetic effects of medetomidine-midazolam-ketamine (M-M-K) and medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) and the antagonistic effect of atipamezole on M-M-K-B induced anesthesia were evaluated in pigs. Both combinations induced the anesthesia soon after administration of ketamine or ketamine -butorphanol, however M-M-K-B induced the more potent and well balanced anesthesia as compared with M-M-K, while the amount of ketamine was reduced to one half in M-M-K. The duration of analgesia, an indicator of surgical anesthesia, in M-M-K-B (65.2±15.8min) was significantly ($p<0.05$) longer than in M-M-K (29.0±4.9min). In addition, M-M-K-B was accompanied by loss of laryngeal reflex for longer time. Recovery from anesthesia in M-M-K-B was smoother than in M-M-K, and the administration of atipamezole (160 μg/kg) could rapidly and smoothly reverse the anesthesia induced by M-M-K-B, although it was accompanied by a transient fall in blood pressure and tachycardia. The combination of medetomidine-midazolam-ketamine-butorphanol appears to be a relatively safe and widely available anesthesia in pigs for the minor surgery or various procedures with pain for the period within one hour.

Table 1. Induction time, duration of anlgesia, duration of loss of pedal reflex, arousaltime, standing time, and total recovery time induced by medetomidine-midazolam-ketamine (M-M-K), medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (M-M-K-B-A)

Regimen	Induction time (min)	Surgical anesthesia time (min)	Duration of loss of pedal reflex (min)	Arousal time (min)	Standing time (min)	Total recovery time (min)
M-M-K	8.3± 2.6[a)]	29.0± 4.9	32.3± 5.4	53.5± 7.7	95.2±31.5	268.0± 46.0
M-M-K-B	5.8± 2.0	65.2± 15.8*	85.8± 9.1*	104.8± 3.9*	133.7± 20.8*	252.0± 26.8
M-M-K-B-A	ND[b)]	ND	ND	10.8± 4.4[c)]	15.2± 5.3[c)]	122.8± 26.3[c)]

a) Data are expressed as the mean±SD. n=6.

b) Not detected

c) Time from administration of atipamezole

*= Significantly different from the means in M-M-K

Table 2. Changes in heart rate (HR), systolic arterial pressure (APs), mean arterial pressure (APm), diastolic arterial pressure (APd) and right atrial pressure (RAP) in pigs given medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (M-M-K-B-A)

		min	0	5	10	15	20	25	30	40	50	55	60	70	80	90	100
HR	MMKB	Mean	105	94	95	92	90	101	109	97	98	ND	102	ND	96	ND	97
(mmHg)		±SD	18	13	11	9	10	17	20	15	14		16		9		13
		p	—	NS	NS	NS	NS	NS	NS	NS	NS	—	NS	—	NS	—	NS
	MMKBA	Mean	117	112	110	111	109	113	111	111	117	133	156	132	138	149	ND
		±SD	13	19	19	25	25	22	18	23	32	31	41	10	24	21	
		p	—/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	—/—
APs	MMKB	Mean	118	151	151	149	146	140	138	134	129	ND	130	ND	133	ND	137
(mmHg)		±SD	7	7	11	12	11	11	11	9	11		20		13		6
		p	—	↑	↑	↑	↑	↑	↑	NS	NS	—	NS	—	NS	—	↑
	MMKBA	Mean	133	157	154	149	148	142	142	133	127	93	113	137	139	137	ND
		±SD	8	26	18	16	12	12	11	11	12	7	15	11	11	9	
		p	—/—	NS/—	↑/—	↑/—	↑/—	↑/—	↑/—	NS/—	NS/—	↓/↓	NS/NS	NS/NS	NS/↑	NS/NS	—/—
APm	MMKB	Mean	93	125	125	123	120	117	116	112	108	ND	108	ND	109	ND	113
(mmHg)		±SD	5	4	8	7	7	7	8	7	8		14		10		5
		p	—	↑	↑	↑	↑	↑	↑	↑	↑	—	NS	—	↑	—	↑
	MMKBA	Mean	108	131	130	124	124	120	120	113	109	78	92	111	111	111	ND
		±SD	7	17	13	13	9	9	8	10	10	6	11	7	6	6	
		p	—/—	↑/—	↑/—	↑/—	↑/—	↑/—	↑/—	NS/—	NS/—	↓/↓	NS/↓	NS/NS	NS/NS	NS/NS	—/—
APd	MMKB	Mean	65	97	102	97	94	92	93	92	87	ND	87	ND	89	ND	86
(mmHg)		±SD	7	9	7	6	4	5	5	7	5		8		11		11
		p	—	↑	↑	↑	↑	↑	↑	↑	↑	—	↑	—	↑	—	↑
	MMKBA	Mean	84	111	110	103	102	100	100	92	89	61	66	84	82	85	ND
		±SD	4	13	10	10	6	7	8	8	8	5	6	3	6	7	
		p	—/—	↑/—	↑/—	↑/—	↑/—	↑/—	↑/—	NS/—	NS/—	↓/↓	↓/↓	NS/NS	NS/NS	NS/NS	—/—
RAP	MMKB	Mean	2	6	5	5	4	4	3	2	2	ND	2	ND	3	ND	2
(mmHg)		±SD	1	3	2	2	2	2	2	3	2		2		2		4
		p	—	↑	↑	↑	NS	NS	NS	NS	NS	—	NS	—	NS	—	NS
	MMKBA	Mean	2	4	4	3	3	2	1	0	0	-2	-1	-1	0	-1	ND
		±SD	3	2	2	2	1	2	1	2	2	3	3	3	2	0	
		p	—/—	↑/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	—/—

↑↓ : Significantly different from the base-line value (MMKB), from the base-line value/ pre atipamezole-administration value (MMKBA)

NS: not significant

Table 3. Changes in systolic pulmonary arterial pressure (PAPs), mean pulmonary arterial pressure (PAPm), diastolic pulmonary arterial pressure (PAPd) and pulmonary arterial wedge pressure (PAWP) and systemic vascular resistance (SVR) in pigs given medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (M-M-K-B-A)

		min	0	5	10	15	20	25	30	40	50	55	60	70	80	90	100
PAPs	MMKB	Mean	35	47	45	37	38	48	49	41	35	ND	36	ND	36	ND	36
(mmHg)		±SD	7	9	10	5	6	5	4	1	2		3		6		7
		p	—	↑	NS	NS	NS	NS	↑	NS	NS	—	NS	—	NS	—	NS
	MMKBA	Mean	36	45	42	41	40	46	45	43	38	32	42	43	40	42	ND
		±SD	9	8	12	13	15	13	11	12	10	7	15	12	10	8	
		p	—/—	↑/—	↑/—	NS/—	NS/—	↑/—	↑/—	↑/—	NS/—	NS/↓	NS/NS	NS/NS	NS/NS	NS/NS	—/—
PAPm	MMKB	Mean	24	32	32	27	27	35	36	31	24	ND	25	ND	24	ND	22
(mmHg)		±SD	4	6	7	4	4	6	5	2	2		4		4		5
		p	—	↑	↑	NS	NS	↑	↑	↑	NS	—	NS	—	NS	—	NS
	MMKBA	Mean	25	32	30	29	29	34	32	30	27	23	27	29	26	28	ND
		±SD	7	6	9	10	11	10	9	9	8	6	9	8	7	6	
		p	—/—	↑/—	↑/—	NS/—	NS/—	↑/—	↑/—	↑/—	NS/—	↓/↓	NS/NS	NS/NS	NS/NS	NS/NS	—/—
PAPd	MMKB	Mean	13	17	19	15	16	20	29	17	15	ND	11	ND	11	ND	10
(mmHg)		±SD	4	5	6	5	5	9	9	6	2		4		3		4
		p	—	NS	↑	NS	NS	NS	↑	NS	NS	—	NS	—	NS	—	NS
	MMKBA	Mean	14	21	20	20	18	23	22	20	18	13	13	17	16	17	ND
		±SD	7	5	9	10	10	10	9	9	8	5	6	7	6	7	
		p	—/—	↑/—	↑/—	↑/—	↑/—	↑/—	↑/—	↑/—	↑/—	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	—/—
PAWP	MMKB	Mean	9	10	11	9	8	7	8	7	6	ND	5	ND	5	ND	6
(mmHg)		±SD	3	1	3	1	1	2	2	2	2		1		1		3
		p	—	NS	NS	NS	NS	NS	NS	NS	NS	—	NS	—	↓	—	NS
	MMKBA	Mean	8	8	7	7	7	6	6	5	5	4	6	5	5	5	ND
		±SD	3	2	1	2	2	2	3	2	2	2	2	3	2	2	
		p	—/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	—/—
SVR	MMKB	Mean	717	ND	1214	ND	1038	812	796	842	827	ND	855	ND	967	ND	866
(mmHg·kg·min/ml)		±SD	105		254		152	140	123	39	58		101		53		36
		p	—	—	↑	—	↑	NS	NS	NS	NS	—	NS	—	NS	—	NS
	MMKBA	Mean	701	ND	1078	ND	978	812	913	786	823	577	527	651	672	621	ND
		±SD	75		201		224	99	178	111	132	145	153	168	239	126	
		p	—/—	—	↑/—	—	↑/—	↑/—	↑/—	NS/—	NS/—	NS/↓	NS/↓	NS/↓	NS/NS	NS/NS	—/—

↑↓ : Significantly different from the base-line value (MMKB), from the base-line value/ pre atipamezole-administration value (MMKBA)

NS: not significant

Table 4. Changes in pulmonary vascular resistance (PVR), cardiac index (CI), stroke volume (SV) and rate pressure product (RPP) in pigs given medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (M-M-K-B-A)

		min	0	5	10	15	20	25	30	40	50	55	60	70	80	90	100
PVR	MMKB	Mean	126	ND	183	ND	131	170	170	149	123	ND	158	ND	108	ND	131
(mmHg·kg·min/ml)		±SD	20		114		62	63	57	52	28		67		34		35
		p	–	–	NS	–	NS	NS	NS	NS	NS	–	NS	–	NS	–	NS
	MMKBA	Mean	115	ND	203	ND	189	164	212	149	177	133	121	133	136	126	ND
		±SD	55		105		131	43	97	25	77	43	52	29	55	40	
		p	–/–	–	↑/–	–	NS/–	↑/–	↑/–	↑/–	↑/–	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–
CI	MMKB	Mean	133	ND	109	ND	119	142	144	132	128	ND	136	ND	126	ND	130
(ml/kg/min)		±SD	36		19		14	22	23	12	6		15		21		9
		p	–	–	NS	–	NS	NS	NS	NS	NS	–	NS	–	NS	–	NS
	MMKBA	Mean	153	ND	121	ND	130	149	135	145	136	145	189	181	181	188	ND
		±SD	16		25		29	22	23	17	22	23	54	39	56	43	
		p	–/–	–	↓/–	–	↓/–	NS/–	↓/–	NS/–	↓/–	NS/↑	NS/NS	NS/NS	NS/NS	NS/NS	–/–
SV	MMKB	Mean	1.27	ND	1.15	ND	1.33	1.41	1.34	1.37	1.33	ND	1.36	ND	1.33	ND	1.37
(ml/kg)		±SD	0.21		0.11		0.1	0.04	0.1	0.09	0.16		0.22		0.25		0.2
		p	–	–	NS	–	NS	NS	NS	NS	NS	–	NS	–	NS	–	NS
	MMKBA	Mean	1.33	ND	1.14	ND	1.28	1.45	1.25	1.43	1.24	1.15	1.26	1.37	1.19	1.25	ND
		±SD	0.26		0.29		0.39	0.18	0.28	0.04	0.33	0.29	0.33	0.24	0.25	0.16	
		p	–/–	–	↓/–	–	NS/–	NS/–	NS/–	NS/–	NS/–	NS/NS	NS/↑	NS/NS	NS/NS	NS/NS	–/–
RPP	MMKB	Mean	12394	14133	14265	13639	13000	14155	14918	13004	12543	ND	12782	ND	11989	ND	13235
(mmHg/min)		±SD	2536	1395	1555	1181	1431	2488	2892	2409	2084		2025		1363		1864
		p	–	NS	NS	NS	NS	NS	↑	NS	NS	–	NS	–	NS	–	NS
	MMKBA	Mean	15504	17151	16646	16227	15927	15915	15737	14763	14750	12237	17734	18014	19068	20545	ND
		±SD	1428	2260	2307	2441	3069	2744	2551	3139	3704	2947	5938	1250	3045	4007	
		p	–/–	NS/–	NS/–	NS/–	NS/–	NS/–	NS/–	NS/–	NS/–	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	–/–

↑↓ : Significantly different from the base-line value (MMKB), from the base-line value/ pre atipamezole-administration value (MMKBA)

NS: not significant

Table 5. Changes in PaO_2, $PaCO_2$ and pHa in pigs given medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (M-M-K-B-A)

		min	0	10	20	30	40	50	55	60	70	80	90	100
PaO2	MMBK	Mean	97.5	93.6	82.3	80.6	83.4	86.2	ND	84.0	ND	82.2	ND	82.8
(mmHg)		±SD	9.3	12.9	10.2	5.7	5.9	4.9		5.3		11.9		11.0
		p	–	NS	NS	NS	NS	NS	–	NS	–	↓	–	NS
	MMBKA	Mean	97.1	96.4	95.0	79.6	82.5	86.8	84.8	80.8	84.6	81.0	86.5	ND
		±SD	6.0	10.3	11.9	9.2	10.3	9.6	12.1	11.6	10.1	11.2	12.8	
		p	–/–	NS/–	NS/–	↓/–	↓/–	NS/–	NS/NS	↓/NS	NS/NS	↓/NS	NS/NS	–/–
PaCO2	MMBK	Mean	37.4	36.3	38.2	39.4	38.2	42.5	ND	38.7	ND	36.9	ND	36.8
(mmHg)		±SD	3.0	2.1	3.6	2.3	1.7	8.2		5.2		0.3		3.1
		p	–	NS	NS	↑	NS	NS	–	NS	–	NS	–	NS
	MMBKA	Mean	43.4	41.2	40.3	46.2	45.2	44.5	41.4	44.5	43.0	43.4	43.8	ND
		±SD	6.2	5.4	5.5	6.2	6.5	6.4	6.9	7.1	6.2	5.5	6.0	
		p	–/–	NS/–	NS/–	NS/–	NS/–	NS/–	NS/↓	NS/NS	NS/NS	NS/NS	NS/NS	–/–
pHa	MMBK	Mean	7.42	7.42	7.40	7.37	7.39	7.40	ND	7.42	ND	7.43	ND	7.43
(mmHg)		±SD	0.01	0.01	0.03	0.02	0.01	0.02		0.03		0.02		0.02
		p	–	NS	NS	↓	↓	NS	–	NS	–	NS	–	NS
	MMBKA	Mean	7.41	7.42	7.42	7.38	7.39	7.40	7.43	7.39	7.40	7.40	7.40	ND
		±SD	0.01	0.02	0.01	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.01	
		p	–/–	NS/–	NS/–	↓/–	NS/–	NS/–	NS/↑	NS/NS	NS/NS	NS/NS	NS/NS	–/–

↑↓ : Significantly different from the base-line value (MMKB), from the base-line value/ pre atipamezole-administration value (MMKBA)

NS: not significant

Table 6. Changes in [HCO_3^-]a, arterial base excess (BEa) and arterial hemoglobin oxygen saturation (SaO_2) in pigs given medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (M-M-K-B-A)

		min	0	10	20	30	40	50	55	60	70	80	90	100
[HCO3⁻]a	MMBK	Mean	24.0	23.1	23.7	23.0	23.3	26.4	ND	25.0	ND	26.2	ND	24.5
(mmol/l)		±SD	2.1	1.3	2.0	0.8	0.7	4.9		3.5		3.8		1.7
		p	—	NS	NS	NS	NS	NS	—	NS	—	NS	—	NS
	MMBKA	Mean	26.8	26.3	26.1	27.0	27.1	27.6	27.2	26.6	26.0	26.1	26.2	ND
		±SD	3.4	3.3	3.2	2.8	3.2	3.2	3.3	3.6	3.1	2.7	3.4	
		p	—/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	—/—
BEa	MMBK	Mean	-0.1	-1.2	-0.9	-2.2	-1.7	1.5	ND	0.3	ND	0.0	ND	0.0
(mmol/l)		±SD	2.2	1.2	2.1	0.7	0.8	4.9		3.6		1.5		1.6
		p	—	NS	NS	NS	NS	NS	—	NS	—	NS	—	NS
	MMBKA	Mean	2.5	2.1	1.8	1.9	2.1	2.8	2.7	1.6	1.4	1.8	2.0	ND
		±SD	3.5	3.3	3.1	2.7	3.1	3.0	3.0	3.4	3.1	2.5	3.3	
		p	—/—	NS/—	NS/—	NS/—	NS/—	NS/—	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	—/—
SaO2	MMBK	Mean	96.2	95.5	93.3	93.0	94.0	94.7	ND	94.6	ND	93.8	ND	93.9
(%)		±SD	0.8	1.5	2.6	1.4	1.1	0.8		0.9		2.6		2.6
		p	—	NS	NS	↓	↓	NS	—	NS	—	NS	—	NS
	MMBKA	Mean	96.1	96.0	95.8	92.4	93.3	94.4	94.3	92.9	93.8	93.0	94.0	ND
		±SD	0.7	1.3	1.5	2.5	2.3	1.6	1.9	2.5	2.2	2.7	2.8	
		p	—/—	NS/—	NS/—	↓/—	NS/—	NS/—	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	—/—

↑↓ : Significantly different from the base-line value (MMKB), from the base-line value/ pre atipamezole-administration value (MMKBA)

NS: not significant

Table 7. Changes in delivery O_2 (DO_2), oxygen consumption (VO_2), oxygen utilization ratio (UO_2)= DO_2 / VO_2 and pulmonary arterial blood pressure (BT(PA)) in pigs given medetomidine-midazolam-ketamine-butorphanol (M-M-K-B) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (M-M-K-B-A)

		min	0	10	20	30	40	50	55	60	70	80	90	100
DO2	MMBK	Mean	373.2	229.9	276	329.4	306.2	295.9	ND	312.3	ND	288.9	ND	339.2
(l/kg/min)		±SD	92.5	38.6	51.7	102.9	73.8	73.9		84.7		73.3		45.2
		p	—	↓	NS	NS	NS	NS	—	NS	—	NS	—	NS
	MMBKA	Mean	448.1	349.6	375.7	374.6	405.3	387.6	432.3	538.9	538.5	511	519.6	ND
		±SD	60.3	56.1	63.7	50.3	30.4	59.3	63.8	109.7	95.9	114.3	73.4	
		p	—/—	↓/—	NS/—	NS/—	NS/—	↓/—	NS/↑	NS/↑	NS/↑	NS/NS	NS/↑	—/—
VO2	MMBK	Mean	127.2	82.7	96	89.8	103.1	97.3	ND	117.6	ND	145	ND	179.6
(l/kg/min)		±SD	56.8	10.2	26.3	28.4	21	23.5		35.3		36.2		61.2
		p	—	NS	NS	NS	NS	NS	—	NS	—	NS	—	NS
	MMBKA	Mean	131.2	99.2	98.9	91.4	101.6	103.8	ND	314.2	309.2	301.6	317.4	ND
		±SD	29.1	21.3	17.3	10.6	19.1	25.7		58.6	64.7	69.5	38.2	
		p	—/—	↓/—	↓/—	↓/—	NS/—	NS/—	—/—	↑/↑	↑/↑	↑/↑	↑/↑	—/—
UO2	MMBK	Mean	3.22	2.78	3.04	3.51	3.1	3.5	ND	2.83	ND	2.12	ND	2.08
		±SD	0.75	0.28	0.69	0.48	0.63	0.4		0.79		0.75		0.68
		p	—	NS	NS	NS	NS	NS	—	NS	—	NS	—	NS
	MMBKA	Mean	3.51	3.65	3.87	4.15	4.1	4.08	ND	1.71	1.76	1.7	1.78	ND
		±SD	0.56	0.86	0.79	0.75	0.68	0.68		0.04	0.14	0.11	0.17	
		p	—/—	NS/—	NS/—	↑/—	NS/—	NS/—	—/—	↓/↓	↓/↓	↓/↓	↓/↓	—/—
BT(PA)	MMBK	Mean	39.2	38.9	38.5	38	37.6	37.3	ND	37.1	ND	37.2	ND	37.3
(℃)		±SD	0.4	0.5	0.5	0.5	0.5	0.6		0.7		0.7		1.1
		p	—	NS	↓	↓	↓	↓	—	↓	—	↓	—	↓
	MMBKA	Mean	39.6	39.1	38.6	38.1	37.8	37.6	37.4	37.9	38.9	39.7	40.7	ND
		±SD	0.5	0.6	0.6	0.7	0.6	0.6	0.5	0.7	0.7	0.8	0.5	—
		p	—/—	↓/—	↓/—	↓/—	↓/—	↓/—	↓/—	↓/↑	NS/↑	NS/↑	NS/↑	—/—

↑↓ : Significantly different from the base-line value (MMKB), from the base-line value/ pre atipamezole-administration value (MMKBA)

NS: not significant

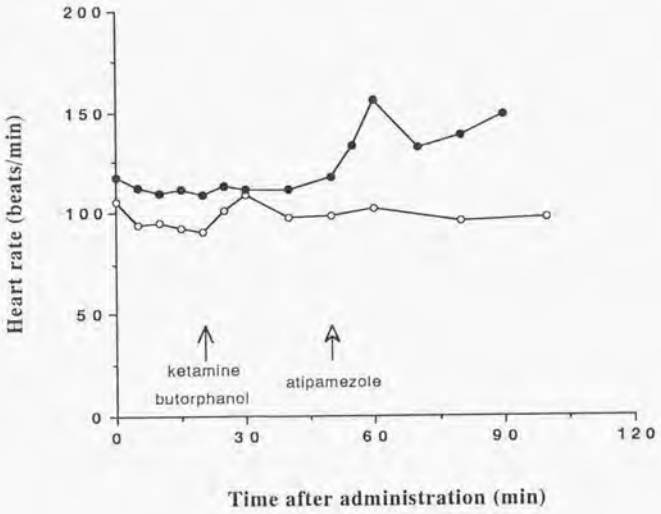
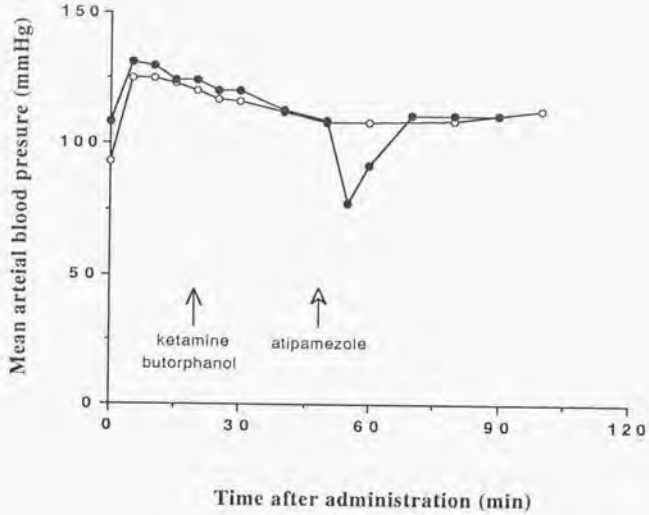


Fig. 1. Changes in heart rate in pigs given medetomidine-midazolam-ketamine-butorphanol (○) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (●). Each symbol represents the mean value in each group.



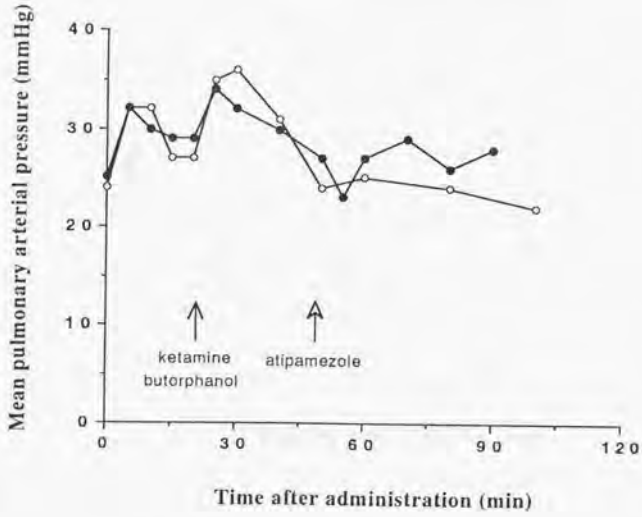


Fig. 2. Changes in mean arterial (upper) and pulmonary arterial pressure (lower) in pigs given medetomidine-midazolam-ketamine-butorphanol (○) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (●). Each symbol represents the mean value in each group.

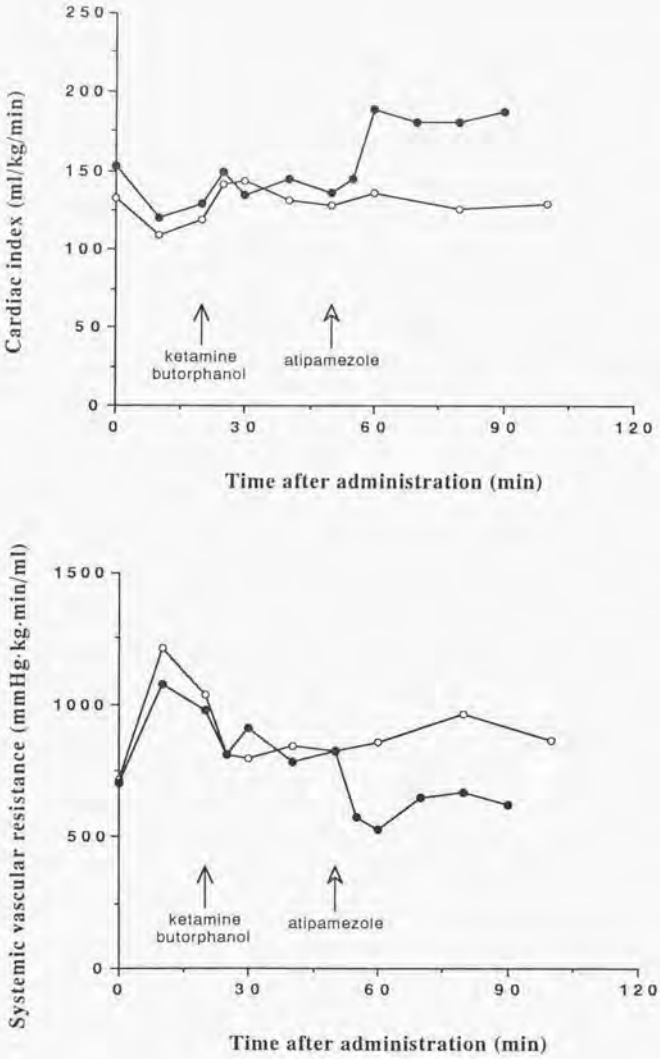


Fig. 3. Changes in systemic vascular resistance (upper) and cardiac index (lower) in pigs given medetomidine-midazolam-ketamine-butorphanol (○) and medetomidine-midazolam-ketamine-butorphanol-atipamezole (●). Each symbol represents the mean value in each group.

Part 3-2

Medetomidine-midazolam as a preanesthetic prior to isoflurane anesthesia in pigs

Many protocols in biomedical researches using pigs require anesthesia for instrumentation and other surgical procedures, some of which may be complex and time consuming [14]. In these situations, inhalation anesthesia offers advantages over injectable anesthesia. As mentioned before, the pig's reaction to restraint is unpleasant for all concerned and, therefore, preanesthetic intramuscular administration of a sedative is urgently required for restraint prior to general anesthesia.

A combination of medetomidine and midazolam has been shown to exert a potent sedation without apparent cardiopulmonary changes in pigs in Part 2. The induction of inhalation anesthesia would be greatly smoothed when this sedative combination is used as a preanesthetic medication in pigs.

Medetomidine and midazolam have been shown to effectively reduce the minimum alveolar concentration (MAC) of volatile anesthetic in experimental animals [1, 23, 41, 45, 57]. Isoflurane is a highly safe inhalation anesthetic in swine [64], however its cost is extremely high. Administration of medetomidine-midazolam prior to isoflurane anesthesia may also be useful for reduction of its cost in great degree.

The purpose of this study was to evaluate the effect of preanesthetic administration of medetomidine-midazolam on mask induction of isoflurane anesthesia (experiment 1). This study was also designed to investigate the anesthetic-reducing and hemodynamic effects of medetomidine-midazolam as preanesthetic medication in pigs receiving isoflurane anesthesia (experiment 2).

MATERIALS and METHODS

Animals:

Eighteen castrated mixed breed pigs in good health were used in this study. After a week period of stabilization the pigs were randomly assigned to 2 groups of 12 (experiment 1) and 6 pigs (experiment 2). Their mean age was 10.1 weeks (range 9 to 11 weeks) and mean body weight was 19.8 kg (range 18.5 to 21.5 kg). Management for these pigs were the same as those in other experiments.

Experimental design:

Experiment 1- Effects of the pretreatment with medetomidine-midazolam on mask induction of isoflurane in pigs

Six pigs given 40 mg/kg of medetomidine and 0.2 mg/kg of midazolam and other untreated 6 pigs were used. Twenty min after administration of these agents, those animals were induced to anesthesia with 5.0% isoflurane delivered in 100% oxygen by face mask, and induction condition and induction time (time from start of inhalation until the ability being intubated) were compared.

Experiment 2- Isoflurane sparing effect of medetomidine-midazolam and cardiovascular effect of medetomidine-midazolam-isoflurane in pigs

Animal preparations and the methods for cardiopulmonary measurements were the same as described in Part 2-3. Following the measurements of base-line values in the conscious state, 6 pigs were induced to anesthesia with 5% isoflurane delivered in 100% oxygen by face mask and orotrancheally intubated. The lungs were mechanically ventilated to maintain end-tidal PCO_2 at 35±5 mmHg. Lactated Ringer's solution was infused at a rate of 10 $ml \cdot kg^{-1} \cdot hr^{-1}$ into the right atrium through Swan-Gantz catheter. Body temperature (pulmonary artery) was maintained at 38.2±0.3°C using a warming

blanket. End-tidal isoflurane concentration and $PaCO_2$ were measured by an infrared analyzer (AGM-103, Datex, Finland).

Following 1-hr stabilization of anesthesia maintained at approximately 2.0% (end tidal) of isoflurane delivered in 100% oxygen, minimum alveolar concentration (MAC) of isoflurane (control MAC) was determined by the tail-clamping method [36]. Then the measurements of MAC were repeated at 3 different plasma levels of medetomidine and midazolam to assess the isoflurane sparing effect of these agents. Namely, each animal received medetomidine and midazolam at three incremental infusion rates (Table 1) through the venous catheter indwelled bilaterally into the ear vein. The approach to stable drug concentrations in blood was hastened by a proportional priming infusion over 20 min [80]. The infusion rate was determined to achieve the plasma concentration at 30, 60 and 120 min after intramuscular administration of medetomidine-midazolam in pigs which were observed in Part 1-4. The determination of MAC was repeated at each infusion rate following a 1-hr stabilization period. After determination of MAC, the pigs were maintained anesthesia at that concentration of isoflurane for more than 20 min and all the cardiopulmonary measurements were repeated. Arterial blood samples (9 ml) for measurement of plasma medetomidine and midazolam concentrations were obtained just after determinations of MAC. The medetomidine and midazolam concentrations in plasma were determined by LC-API-MS system as described in Part 1-4.

Statistical analyses:

The degree of MAC reduction produced by three infusion rates were compared with a control value or each other using paired-*t* test. The values of cardiopulmonary parameters after drug administration were compared with base-line values using paired-*t* test. In all analyses, values were considered to be statistically significant when $P<0.05$.

RESULTS

Effects of the pretreatment with medetomidine-midazolam on mask induction by isoflurane in pigs:

Pre-administration of medetomidine-midazolam induced deep sedation in all pigs as described elsewhere without resistance to face mask for induction of anesthesia. All the pigs were smoothly intubated to the trachea after 2.9±0.2 min of inhalation of isoflurane. On the contrary, control pigs without sedation resisted to face mask and it took longer time (6.6±1.1 min) till the tracheal intubation. In addition, those animals resisted to mouth open because anesthesia induced by isoflurane alone accompanied by poor muscle relaxation during induction phase.

Isoflurane-sparing effect of medetomidine-midazolam in pigs:

Incremental increases in the infusion rate of medetomidine and midazolam produced proportional increases in plasma medetomidine (r^2=0.991) and midazolam concentrations (r^2=0.989) (Figs. 1 and 2, Table 2). Mean plasma concentrations of 4.6±0.5 ng/ml in medetomidine and 14.8±1.4 ng/ml in midazolam, which correspond to those at 120 min after intramuscular administration of medetomidine-midazolam, produced a 21.2±8.2% reduction of MAC. Higher infusion rates produced significantly larger reductions of isoflurane MAC and the highest concentration of medetomidine and midazolam, which corresponds to that at 30 min after administration of medetomidine-midazolam, reduced isoflurane MAC by more than 50%. It was shown that the reduction in isoflurane MAC was well correlated to the concentrations of either medetomidine and/or midazolam (Figs. 3, 4 and 5).

Cardiovascular effects of medetomidine-midazolam-isoflurane anesthesia :

Isoflurane anesthesia caused mild and not significant increases in HR and CI, and mild but significant decreases in AP, PAP, SVR and PVR compared with base-line values (Table 3 and Figs. 6, 7 and 8). Conversely, administration of medetomidine and midazolam at constant rates under isoflurane anesthesia induced dose-dependent increases in AP, PAP, SVR and PVR and dose-dependent decreases in HR, CI (Table 3 and Figs. 6, 7 and 8). These changes were accompanied with a decrease in DO_2, but VO_2 decreased (Table 4). However, these changes were relatively small and were within physiological ranges. Changes in cardiovascular parameters at the lowest infusion rate of medetomidine-midazolam were minimum because the effect of isoflurane and that of medetomidine-midazolam offset each other.

DISCUSSION

Premedication with medetomidine and midazolam contributed to a smoother induction of isoflurane anesthesia by allowing much easier handling of the pigs. It also reduced the induction time (until endotracheal intubation) and made the endotracheal intubation much easier because of apparent jaw muscle relaxation.

Three incremental doses of medetomidine-midazolam resulted in the plasma concentrations of these drugs which corresponded to those at 30, 60 and 120 min after intramuscular administration of this combination. The present study demonstrated that a combination of medetomidine and midazolam at these plasma concentrations reduced the isoflurane MAC to a considerably great extent, which enable the considerable reduction in the required amount of isoflurane, then its cost.

Takeuchi *et al.* examined the anesthetic efficacy of medetomidine alone or midazolam alone in terms of its ability to reduce isoflurane MAC in dogs [62]. Medetomidine

produced a more potent isoflurane sparing effect than midazolam and produced 20 to 64 % of reduction in isoflurane MAC over an infusion rate of 33 to 266 ng/kg/min. On the contrary, midazolam produced 22 to 43% of MAC reduction over an infusion rate of 2400 to 19200 ng/kg/min. In the present study, a regression curve representing the relationships between plasma midazolam concentrations and the percent MAC reduction revealed a shallower slope and high value of y intercept than that in medetomidine. This may indicate that medetomidine contributes to isoflurane MAC reduction more potently than midazolam, although its isoflurane sparing effect is not as potent as in dogs [62].

It has been speculated that medetomidine's action in decreasing anesthetic requirements relates to its attenuating effect on central noradrenergic neurotransmission [35] which is hypothesized to modulate the depth of the anesthetic response [40]. However, additional factors such as postsynaptic action must also be operating [57] because the higher dose of medetomidine produced reduction in MAC to a extent of more than 90% in rats and dogs [45, 57] in spite of only 30 to 40% of MAC reduction when noradrenergic neurotransmission is totally abolished [48]. Although the mechanism of an inhalant sparing effect in midazolam has been still unclear, depression of sympathetic neurotransmission through the stimulation of specific benzodiazepine receptors which then potentiates the effect of inhibitory transmitter γ− aminobutyric acid (GABA) may be mainly attributed to this effect.

The cardiovascular effects of medetomidine-midazolam under isoflurane anesthesia in pigs were similar to those in conscious ones. The administration of medetomidine-midazolam induced dose-dependent increases in AP, PAP, SVR and PVR and dose-dependent decreases in HR and CI. However, those changes were relatively small even

at the higher infusion rate, because those effects might be offset to some degree by isoflurane which has the converse effects on the cardiovascular system.

In conclusion, pre-anesthetic administration of medetomidine-midazolam smoothed the induction of isoflurane anesthesia and greatly reduced the isoflurane MAC for 1 to 2 hr without apparent cardiovascular changes in pigs. This sedative combination may be very useful as a preanesthetic to isoflurane anesthesia in pigs.

SUMMARY

The effect of preanesthetic administration of medetomidine-midazolam on inhalation (isoflurane) anesthesia was evaluated in pigs. Premedication with medetomidine and midazolam contributed to a much smoother induction of isoflurane anesthesia by allowing the easier handling and apparent jaw muscle relaxation in the pigs.

Three incremental constant infusion rates of medetomidine-midazolam, which resulted in the plasma concentrations corresponded to those at 30, 60 and 120 min after intramuscular administration of this combination, produced a 54.5±5.4, 36.2±6.1 and 21.2±8.2% of minimum alveolar concentration (MAC) reduction, respectively. Although the administration of medetomidine-midazolam induced dose-dependent increases in arterial and pulmonary arterial pressures and systemic and pulmonary vascular resistances and dose-dependent decrease in heart rate, cardiac index, those changes were relatively small even at the high infusion rate because those effects might be offset to some degree by isoflurane which has the converse effects on the cardiovascular system.

Those results indicate that medetomidine-midazolam may be quite useful as a preanesthetic to isoflurane anesthesia in pigs.

Table 1. Sequence of medetomidine and midazolam infusions[a)]

	medetomidine		midazolam	
rate	Priming infusion rate (ng/kg/min)	Maintenance infusion rate (ng/kg/min)	Priming infusion rate (ng/kg/min)	Maintenance infusion rate (ng/kg/min)
low	580	125	1820	320
middle	580	250	2730	800
high	580	375	9110	2400

a) The priming infusion rate was constant over 20 min. The maintenance infusion rate was constant from the beginning to the end of each dose period.

Table 2. Isoflurane MAC reduction and plasma concentrations produced by incremental increase in medetomidine and midazolam infusion rate[a)]

Maintenance infusion rate	Medetomidine (ng/ml plasma)	Midazolam (ng/ml plasma)	MAC (%)	% Reduction
-	0	0	1.67±0.16	-
Low	4.6±0.5	14.8±1.4	1.32±0.19*	21.2±8.2
Middle	11.6±1.9	46.6±9.9	1.06±0.13*¶	36.2±6.1
High	16.6±2.0	111.8±18.5	0.76±0.07*¶§	54.5±5.4

a)Data are expressed as the mean ±SD. n=6.
* Significantly different from control value (P<0.05)
¶ Significantly different from the value at low infusion rate (P<0.05)
§ Significantly different from the value at middle infusion rate (P<0.05)

Table 3. Cardiovascular effects of isoflurane alone (iso), isoflurane and medetomidine-midazolam at the low maintenance infusion rate (mmL), isoflurane and medetomidine-midazolam at the middle maintenance infusion rate (mmM), isoflurane and medetomidine-midazolam at the high maintenance infusion rate (mmL) (n= 6).

		base-line	iso	mmL	mmM	mmH
HR	Mean	107	122	102	91	81
(beats/min)	±SD	11	16	6	10	5
	p		NS	NS	NS	↓
APs	Mean	128	104	116	142	157
(mmHg)	±SD	10	10	14	13	14
	p		↓	NS	↑	↑
APm	Mean	100	84	94	113	124
(mmHg)	±SD	7	13	14	13	13
	p		↓	NS	↑	↑
APd	Mean	69	65	73	87	94
(mmHg)	±SD	5	15	13	14	14
	p		NS	NS	↑	↑
RAP	Mean	2.5	4.5	4.0	3.7	3.8
(mmHg)	±SD	2.3	1.5	1.0	0.9	1.1
	p		NS	NS	NS	NS
PAPs	Mean	35	29	28	33	36
(mmHg)	±SD	5	3	2	2	5
	p		↓	↓	NS	NS
PAPm	Mean	23	18	19	21	23
(mmHg)	±SD	3	1	1	2	2
	p		↓	↓	NS	NS
PAPd	Mean	12	9	10	10	11
(mmHg)	±SD	2	2	2	3	2
	p		NS	NS	NS	NS
PAWP	Mean	7	8	7	7	6
(mmHg)	±SD	3	1	2	2	1
	p		NS	NS	NS	NS
SVR	Mean	687	517	709	1081	1292
(mmHg·kg·min/	±SD	55	86	180	206	366
ml)	p		↓	NS	↑	↑
PVR	Mean	109	67	93	140	184
(mmHg·kg·min/	±SD	15	11	21	34	35
ml)	p		↓	↓	↑	↑
CI	Mean	142	155	129	103	98
(ml/kg/min)	±SD	8	16	11	11	16
	p		NS	NS	↓	↓
SV	Mean	1.35	1.28	1.27	1.15	1.20
(ml/kg)	±SD	0.21	0.14	0.07	0.15	0.19
	p		NS	NS	NS	NS
RPP	Mean	13620	12791	11693	12884	12643
(mmHg/min)	±SD	1322	2682	958	1733	1589
	p		NS	↓	NS	NS

↑↓ : Significantly different from the base-line value
NS: Not significant

Table 4. Respiratory effects of isoflurane alone (iso), isoflurane and medetomidine-midazolam at the low maintenance infusion rate (mmL), isoflurane and medetomidine-midazolam at the middle maintenance infusion rate (mmM), isoflurane and medetomidine-midazolam at the high maintenance infusion rate (mmL) (n= 6).

		base-line	iso	mmL	mmM	mmH
PaO2	Mean	103.6	481.3	475.4	475.4	484.9
(mmHg)	±SD	2.9	37.8	33.4	46.0	29.8
	p		↑	↑	↑	↑
PaCO2	Mean	35.8	33.7	35.7	32.2	35.9
(mmHg)	±SD	4.1	2.0	3.0	1.2	3.4
	p		NS	NS	NS	NS
pHa	Mean	7.43	7.49	7.49	7.48	7.45
	±SD	0.01	0.02	0.02	0.01	0.03
	p		↑	↑	↑	NS
$[HCO_3^-]a$	Mean	23.6	25.5	27.4	24.2	24.9
(mmol/l)	±SD	2.7	1.2	2.0	0.7	2.2
	p		NS	NS	NS	NS
BEa	Mean	-0.2	2.2	4.2	0.9	1.1
(mmol/l)	±SD	2.8	1.2	2.1	0.8	2.4
	p		NS	↑	NS	NS
SaO2	Mean	97.0	99.9	99.9	99.9	99.9
(%)	±SD	0.2	0.0	0.0	0.0	0.0
	p		NS	NS	NS	NS
DO2	Mean	429.9	518.2	383.6	338.6	263.7
(l/kg/min)	±SD	39.9	58.0	30.3	16.3	123.4
	p		↑	NS	↓	↓
VO2	Mean	115.1	117.8	90.7	89.0	66.9
(l/kg/min)	±SD	23.9	15.2	5.8	8.3	32.8
	p		NS	↓	↓	↓
UO2	Mean	3.81	4.44	4.23	3.83	4.02
	±SD	0.42	0.51	0.22	0.30	0.60
	p		NS	↑	NS	NS
BT(PA)	Mean	39.6	38.1	38.2	38.3	38.3
(℃)	±SD	0.3	0.1	0.1	0.2	0.1
	p		↓	↓	↓	↓

↑↓ : Significantly different from the base-line value
NS: Not significant

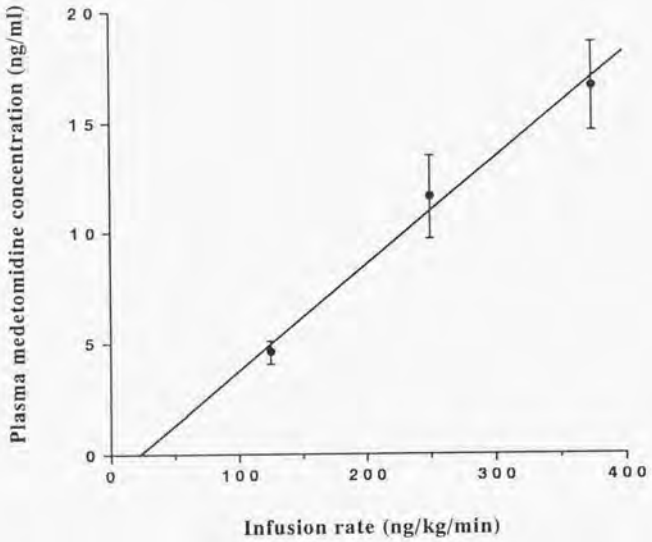


Fig. 1. Medetomidine concentration in plasma *versus* maintenance infusion rate. Each point represents the mean (±SD) concentrations of medetomidine. Regression analysis yields the line $y=0.048\ x -1.1$, $r^2=0.991$.

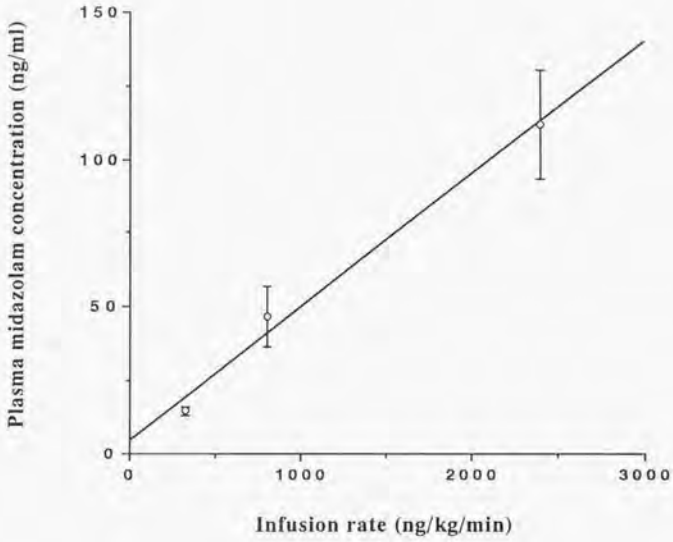


Fig. 2. Midazolam concentration in plasma *versus* maintenance infusion rate. Each point represents the mean (±SD) concentrations of midazolam. Regression analysis yields the line $y=0.045\ x+4.8$, $r^2=0.989$.

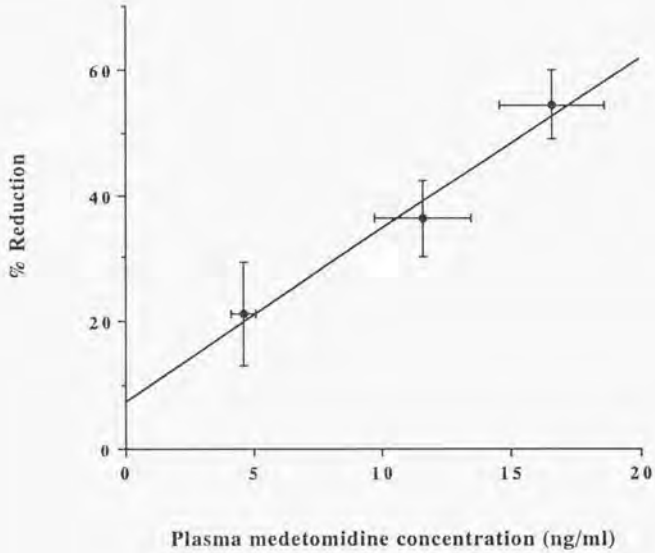


Fig. 3. Plasma medetomidine concentration (ng/ml) *versus* percent reduction of isoflurane MAC. Each point represents the mean (±SD) percent reduction of isoflurane MAC and mean (±SD) concentration of medetomidine at each infusion rate. Regression analysis yields the line $y=2.7x+7.4$, $r^2=0.977$.

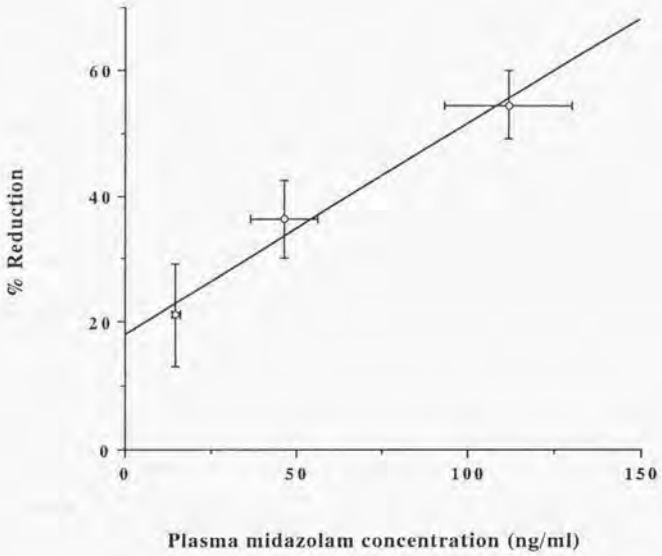


Fig. 4. Plasma midazolam concentration (ng/ml) *versus* percent reduction of isoflurane MAC. Each point represents the mean (±SD) percent reduction of isoflurane MAC and mean (±SD) concentration of midazolam at each infusion rate. Regression analysis yields the line $y=0.33x+18.0$, $r^2=0.981$.

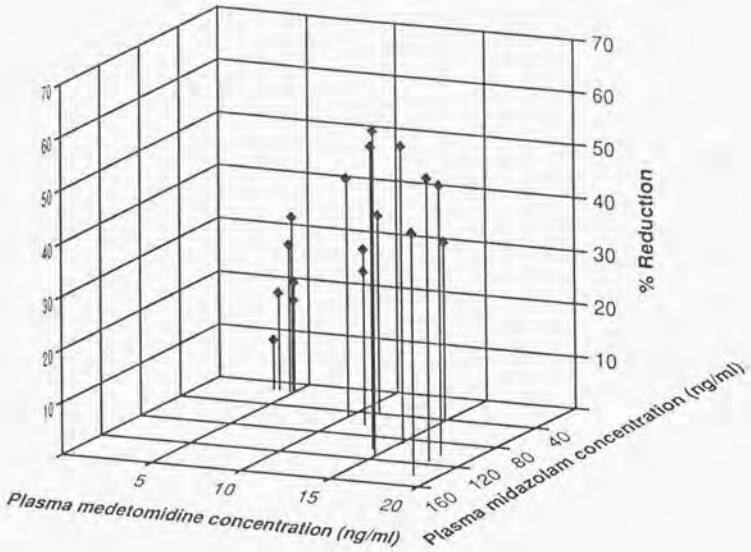


Fig. 5. Plasma medetomidine and midazolam concentration (ng/ml) *versus* percent reduction of isoflurane MAC. Each point represents the mean percent reduction of isoflurane MAC.

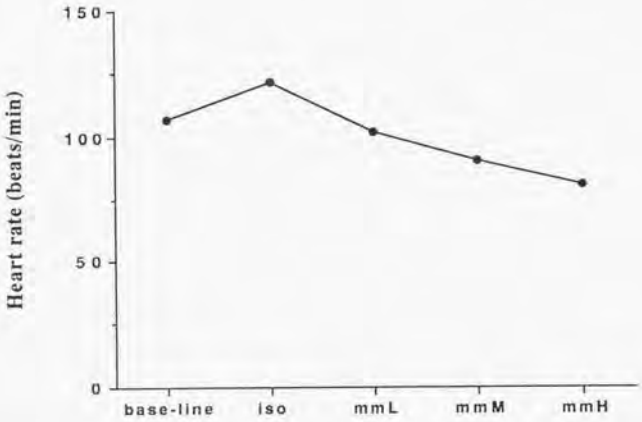


Fig. 6.Changes in heart rate in pigs given isoflurane with or without medetomidine-midazolam at incremental infusion rates. Each symbol represents the mean value of base-line, isoflurane alone (iso), isoflurane and medetomidine-midazolam at the low maintenance infusion rate (mmL), isoflurane and medetomidine-midazolam at the middle maintenance infusion rate (mmM), isoflurane and medetomidine-midazolam at the high maintenance infusion rate (mmL) (n= 6).

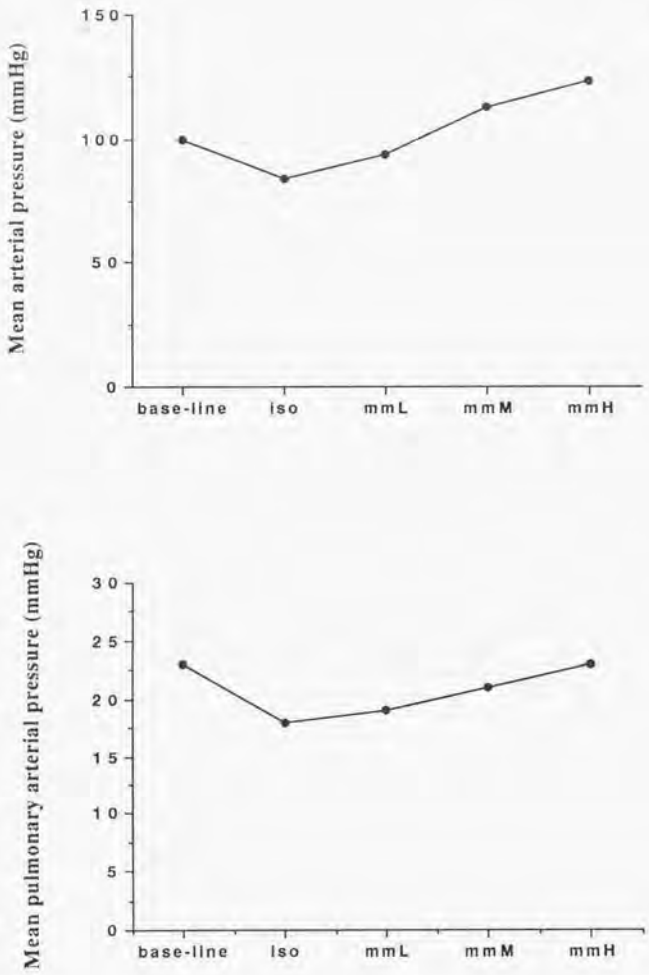


Fig. 7.Changes in mean arterial blood pressure (upper) and mean pulmonary arterial pressure (lower) in pigs given isoflurane with or without medetomidine-midazolam at incremental infusion rates. Each symbol represents the mean value of base-line, isoflurane alone (iso), isoflurane and medetomidine-midazolam at the low maintenance infusion rate (mmL), isoflurane and medetomidine-midazolam at the middle maintenance infusion rate (mmM), isoflurane and medetomidine-midazolam at the high maintenance infusion rate (mmL) (n= 6).

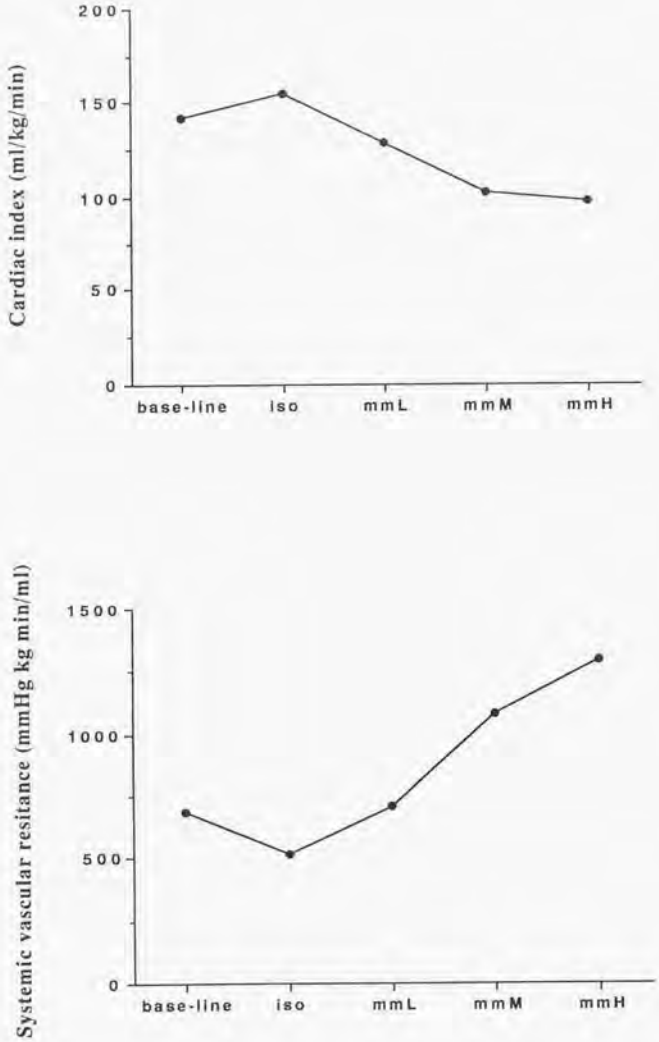


Fig. 8.Changes in cardiac index (upper) and systemic vascular resistance (lower) in pigs given isoflurane with or without medetomidine-midazolam at incremental infusion rates. Each symbol represents the mean value of base-line, isoflurane alone (iso), isoflurane and medetomidine-midazolam at the low maintenance infusion rate (mmL), isoflurane and medetomidine-midazolam at the middle maintenance infusion rate (mmM), isoflurane and medetomidine-midazolam at the high maintenance infusion rate (mmL) (n= 6).

Conclusion

In recent years, pigs have been recognized as valuable and useful laboratory animals for many types of biomedical researches. However, pigs are so shy, excitable and hysteric that sedation is urgently required to facilitate all handling and minor procedures and for restraint prior to general anesthesia. The major focus of this study was to establish a potent, widely available and safe sedative combination using an α_2-agonist medetomidine in laboratory pigs.

In Part 1, the fundamental aspects of medetomidine was investigated in pigs.

1. Medetomidine induced a satisfactory sedation accompanied with muscle relaxation and weak analgesia which was much more potent than that of xylazine. The depth of sedation induced by medetomidine was dose dependent within the range from 30 to 80 μg/kg. At 100 or 150 μg/kg, the depth of sedation was mostly the similar level to that at 80 μg/kg but the duration was prolonged. The optimal dose of medetomidine in pigs was suggested to be 80 μg/kg for sedation with lateral recumbency and moderate muscle relaxation without notable side effects.

2. Medetomidine produced the most profound degree of sedation with greater drowsiness than was achieved by other major sedatives; acepromazine, azaperone, droperidol and midazolam. One of the most apparent differences between medetomidine and other sedatives was the depressant effect on arousal reaction induced by sensory stimulation such as visual, auditory, tactile or painful stimuli, although its effect may not be strong enough to avoid any arousal reaction by manipulation and restraint. In addition, muscle relaxant and weak analgesic effects were not observed apparently in other sedatives. Furthrmore, the onset and recovery from sedation of medetomidine was quick and smooth as compared with other sedatives. The sedative character of medetomidine

seemed to be the most suitable as a chemical restraint agent for pigs among the sedatives tested in this study.

3. An α_2-agonist atipamezole effectively reversed 80 μg/kg of medetomidine-induced sedation, and the optimal action was seen at doses of 160 and 320 μg/kg. Recovery from sedation was quick and smooth, and adverse effects such as hyperactivity or tachycardia were minimal with either dose. Clinically, atipamezole is the considerably effective antagonist against the sedation induced by medetomidine.

4. The pharmacokinetic data of medetomidine and atipamezole were well fitted by a one-compartment open model with a transient absorption phase followed by an elimination phase. Absorption of these drugs occurred rapidly and the peak concentrations in plasma were obtained within 10 min of the dosing. Disposition also occurred rapidly and the elimination half-time was relatively short in both drugs (approximately 50 to 70 min). The data of midazolam were fitted by a two-compartment open model with a transient absorption phase followed by an elimination phase. Absorption of midazolam alone was rapid and peak concentration in plasma was obtained within 5 min of the dosing. Disposition also occurred rapidly and elimination half-time was relatively short (approximately 100 to 120 min), though it was longer than those of medetomidine and atipamezole. However, absorption of midazolam was delayed when it was administered with medetomidine through the peripheral vasoconstriction induced by medetomidine.

In Part 2, a potent and satisfactory sedative combination using medetomidine and midazolam was established and the antagonistic effect of atipamezole on this sedative combination was investigated. In addition, the effect of this combination on cardiopulmonary system and blood glucose were evaluated.

1. The combination of medetomidine and midazolam exerted a much more potent sedative effect than that induced by medetomidine alone, while the dose of medetomidine was reduced to one half of its optimal dose. Pigs given this combination were induced to sedation smoothly and very quickly, even if the pigs were stimulated strongly and continuously during the induction phase. During being sedated, the arousal reaction induced by sensory stimuli was depressed profoundly and pigs could be placed in dorsal recumbency without any resistance. In addition, this combination produced moderate analgesic effect and apparent muscle relaxation. This potent effect induced by this combination seemed to be induced by a synergistic interaction of these drugs because the sedative effect achieved with this combination was much greater than expected from a simple additive response of both sedatives. This sedative combination may be practical and valuable as a chemical restraint agent in pigs.

2. Atipamezole effectively reversed sedation induced by medetomidine-midazolam smoothly and quickly, and the arousal time, standing time and total recovery time were significantly shortened. The optimal action was seen with 160 μg/kg of atipamezole. At this dose, adverse effects such as hyperactivity or tachycardia were minimal. Flumazenil which was a specific antagonist to midazolam reversed sedation temporary, but the pigs returned to moderate sedation soon after arousal. The combination of atipamezole and flumazenil revealed the most effective antagonism, however atipamezole alone was thought to be practically potent enough to antagonize sedation induced by medetomidine-midazolam in pigs. The possible use of an antagonist may enhance the value and availability of medetomidine-midazolam as a chemical restraint agent in pigs.

3. The intramuscular administrations of medetomidine alone and medetomidine-midazolam caused a similar pressor response, characterized by mild but rapid increases

in arterial and pulmonary arterial pressure mediated mainly through systemic and pulmonary vasoconstriction caused by medetomidine. These pressures gradually decreased soon after showing the peak level 5 to 10 min after administrations of sedatives, but maintained the slightly higher values than the base-line level. Cardiac output decreased mildly after administration of either medetomidine alone or medetomidine-midazolam. This change was mainly attributed to a mild increase in the afterload of the heart which was represented by the increase in vascular resistance. All these changes in pigs given medetomidine-midazolam were smaller than those in pigs given medetomidine alone and within physiological fluctuation. In addition, medetomidine-midazolam did not induce bradycardia and subsequent hypotension which were generally observed by α_2-agonists and caused less changes in the respiratory system. Administration of atipamezole resulted in a transient marked decrease in vascular resistance, and it caused a decrease in blood pressure and increases in cardiac output and heart rate. However, these changes were relatively small and sustained for a short duration. Thus the combination and its antagonist can be used as a highly safe regimen in pigs.

4. The intramuscular administration of medetomidine-midazolam induced a gradual hyperglycemic response which was thought to be mainly attributed to the effect of medetomidine on peripheral α_2-adrenoreceptors. However, its change was much smaller than that in pigs given 80 μg/kg of medetomidine alone and did not lead to any clinical problems in pigs. It may indicate that the peripheral effect of medetomidine-midazolam generally small and this sedative combination have less undesirable effect than medetomidine alone.

In Part 3, the efficacy of medetomidine-midazolam as a preanesthetic before parenteral (ketamine) and inhalation (isoflurane) anesthesia in pigs were evaluated.

1. The combinations of medetomidine, midazolam and ketamine (M-M-K) or medetomidine, midazolam, ketamine and butorphanol (M-M-K-B) induced the anesthesia soon after administration of ketamine or ketamine-butorphanol. In these combinations, M-M-K-B induced the more potent and well balanced anesthesia as compared with M-M-K, while the amount of ketamine was reduced to one half in M-M-K. The duration of analgesia, an indicator of surgical anesthesia, in M-M-K-B (65.2±15.8 min) was significantly ($p<0.05$) longer than in M-M-K (29.0±4.9 min). In addition, M-M-K-B was accompanied by loss of laryngeal reflex for longer time. Recovery from anesthesia in M-M-K-B was smoother than in M-M-K. The administration of atipamezole (160 mg/kg) could rapidly and smoothly reverse the anesthesia induced by M-M-K-B, although it was accompanied by a transient fall in blood pressure and tachycardia. The combination of medetomidine-midazolam-ketamine-butorphanol appears to be a relatively safe and widely available anesthesia in pigs for the minor surgery or various procedures with pain fot the period within one hour.

2. Premedication with medetomidine and midazolam contributed to a much smoother induction of isoflurane anesthesia by allowing the easier handling and apparent jaw muscle relaxation in the pigs. In addition, three incremental constant infusion rates of medetomidine-midazolam, which resulted in the plasma concentrations corresponded to those at 30, 60 and 120 min after intramuscular administration of this combination, produced a 54.5±5.4, 36.2±6.1 and 21.2±8.2% of minimum alveolar concentration (MAC) reduction, respectively. Although the administration of medetomidine-midazolam induced dose-dependent increases in arterial and pulmonary arterial pressures and

systemic and pulmonary vascular resistances and dose-dependent decrease in heart rate and cardiac index, those changes were relatively small even at the high infusion rate because those effects might be offset to some degree by isoflurane which has the converse effects on the cardiovascular system. Thus medetomidine-midazolam may be quite useful as a preanesthetic to isoflurane anesthesia in pigs.

In conclusion, medetomidine has the most suitable sedative character as a chemical restraint agent among the major sedatives. The combination of medetomidine and midazolam is safe, valuable and practical for sedation in laboratory pigs. In addition, this sedative combination may be quite useful as an adjunct or a preanesthetic to parenteral and inhalation anesthesia.

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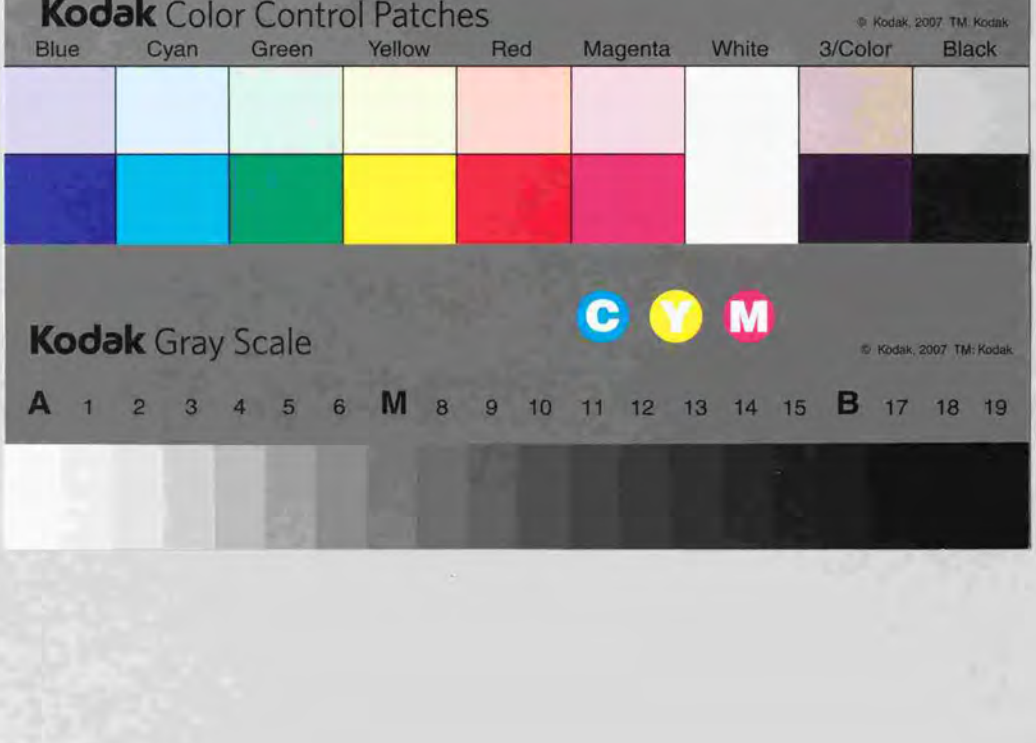
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