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Evaluation of a Medetomidine–Midazolam Combination for Immobilizing and Sedating Japanese Monkeys (*Macaca fuscata*)

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We clinically and clinicopathologically investigated the immobilizing and sedative effects of a medetomidine–midazolam (MM) combination in Japanese monkeys (*Macaca fuscata*) and its antagonism with atipamezole. MM (medetomidine, 60 μg/kg; midazolam, 0.3 mg/kg) was administered intramuscularly to each monkey (n = 11). All animals were laterally recumbent within 13 ± 6 min after administration of MM. This combination induced deep sedation accompanied by analgesia, muscle relaxation, and markedly depressed arousal reactions to external stimuli. After administration of atipamezole (240 μg/kg intramuscularly), the animals recovered rapidly and smoothly to their normal postures within 10 ± 2 min. In this study, the hematologic and serum biochemical parameters of Japanese monkeys given MM did not differ significantly from those of Japanese monkeys under general anesthesia via ketamine. Salivary α-amylase activities (stress indexes) ranged from 4 to 99 kU/l in Japanese monkeys, similar to levels measured in humans. An important advantage of MM was that its effects were reversible with atipamezole. We have confirmed that MM is valuable as a chemical restraint agent in Japanese monkeys for various experimental procedures.

Abbreviations: Gal-G2-CNP, 2-chloro-4-nitrophenyl-4-O-β-D-galactopyranosylmaltoside; MM, medetomidine–midazolam

In Japan, ketamine hydrochloride has been classified as a narcotic. An increase in the number of reports of misuse of ketamine is one of the reasons for this revised classification in human and veterinary medicine. In addition, public concern regarding the use of ketamine in nonhuman primates is increasing. In experiments involving animals, researchers attempt to use alternative methods of anesthesia to minimize the use of ketamine.

Balanced anesthesia combining medetomidine and midazolam is an effective injectable anesthetic combination in dogs.[6,7,9,10,15,17] Medetomidine, a racemic mixture of dexmedetomidine and levomedetomidine, is a selective α_2-adrenocepter agonist and is widely used as tranquillizer or preanesthetic medication in veterinary medicine.[9,10] In nonhuman primates, medetomidine is used widely in combination with ketamine.[8] The combination of medetomidine and ketamine is the most common induction regime of nonhuman primates. In addition, a recent study reported that medetomidine–butorfanol could be combined with either ketamine or midazolam to safely and effectively immobilize captive patas monkeys (*Erythrocebus patas*) for routine medical procedures.[12] However, medetomidine alone (50, 100, 150, and 200 μg/kg) yielded an inconsistent anesthetic plane in rhesus macaques.[3]

Midazolam, a water-soluble benzodiazepine, produces anxiolytic and sedative–hypnotic effects through the activation of γ-aminobutyric acid ($GABA_A$) receptors, which play a major role in the anesthetic effects for injectable anesthetics.[4,22] Midazolam is a sedative in humans.[13] In small animal medicine, the combination of medetomidine–midazolam (MM) has been reported to reinforce the actions of each drug.[6] This combination allows the restraint of dogs during induction, minimizes stress and pain, and increases the threshold for induction-related complications.[18] Hayashi and colleagues[6] reported that the MM combination exerted a much more potent and preferable sedative effect than that from medetomidine alone. However, the effects of α_2-adrenocepter agonists in primates are different from what we might expect, on the basis of results in companion animals.[8] Although there are several preliminary studies using drug combinations such as medetomidine, butorphanol, zolazepam, and midazolam,[12,21] little information is available about the synergistic effects of these agents in nonhuman primates.

The purpose of this study was to investigate the clinical characteristics and clinicopathologic changes in MM-treated Japanese monkeys (*Macaca fuscata*) and to compare them with our reference values from the same species under general anesthesia via ketamine. In addition, we determined salivary α-amylase activities in monkeys after administration of MM to assess the degree of psychologic and physiologic stress associated with this anesthesia protocol. We also sought to evaluate the antagonistic effects of atipamezole on immobilization induced by the MM combination.

Materials and Methods

Animals. We used 11 Japanese monkeys (*Macaca fuscata*, 3 male and 8 female, 3.00 to 5.65 kg) in this study. The estimated age range of the monkeys was 3 to 6 y at the time of introduction and they were used for this study 2 mo after acclimatization. These animals were purchased from a commercial supplier (Hamri Co, Ibaraki, Japan). Before the experiment, the monkeys were determined to be healthy on the basis of general appearance, activity, and tuberculosis skin testing. All animals were seronegative for B virus by enzyme-linked immunosorbent assay. The status of simian retrovirus and simian T-cell lymphotrophic virus 1 were not examined in the monkeys used in this study.

The monkeys were housed individually in stainless steel

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cages (60 × 70 × 160 cm) in an animal room controlled at 25 ± 1 °C and 50% ± 10% relative humidity with 10 to 15 exchanges of 100% fresh air hourly and a 12:12-h light:dark cycle (lights on, 0600). They were fed a commercial primate food (PS, Oriental Yeast Co, Tokyo, Japan), provided ad libitum and supplemented with a variety of fresh fruit, vegetables, and other treats daily. Water was provided through an automatic watering system furnished to each cage.

All procedures involving animals were approved by the Animal Use Committee of National Institutes of Natural Sciences of Japan and followed institutional guidelines of animal care and experimentation.

Experimental protocol. In our preliminary test of MM administration (data not shown), we confirmed a ceiling effect of midazolam at 0.3 mg/kg. In addition, we noted that medetomidine (10 to 25 µg/kg) had no sedative effect on the monkeys. Drug doses for the combinations were tested in this preliminary examination to establish dose combinations for adequate sedation.

Experiments were performed in a quiet room. Medetomidine (60 µg/kg; Meiji Seika, Tokyo, Japan) and midazolam (0.3 mg/kg; Fuji Pharmaceutical, Tokyo, Japan) were mixed in the same syringe and injected intramuscularly in a hindlimb (the quadriceps muscle) of each animal. The monkeys underwent MM administration under restraint with the squeeze-back mechanism of a standard nonhuman primate cage. At 30 min after MM administration, atipamezole (240 µg/kg; Meiji Seika) was administrated intramuscularly into the quadriceps muscles of all monkeys.

Assessment of immobilizing effects. Immobilizing effects of the MM combination were assessed by observations of sedation, analgesia, and muscle relaxation. The level of sedation produced by the MM combination was evaluated from posture, response to sound, and depression of swallowing and pedal reflexes. Analgesia was evaluated by response to clamping of nose or tail, needle pricks, and toe pinches. Analgesia was determined by the degree of reflex response (normal, requiring an increased stimulus, and no response). Muscle relaxation was estimated by observing jaw tone and the resistance to movement of the tail and ear and of facial and leg muscles. The pattern and depth of respiration, color of mucous membranes, eyeball position, salivation, twitching, and spontaneous movement also were observed during immobilization, and side effects were recorded whenever they occurred.

The immobilizing effect of the MM combination was determined as the time to onset of deep sedation (time from MM administration to no response to external stimuli). The reversal effect of the antagonist was assessed as the time to complete recovery (time from atipamezole administration to total recovery from immobilization). During the immobilization period (20 min), test procedures were performed.

Blood sample collection. Blood samples were collected without anticoagulant from the cephalic vein of each animal. At 30 min after collection of blood samples, sera were separated by centrifugation at 1500 × *g* for 10 min for biochemical analysis. For hematologic samples, blood was collected into tubes containing potassium ethylenediamine tetraacetic acid. In addition, citrated blood samples were analyzed immediately for determination of coagulation parameters.

Hematology. The following parameters were examined by using an automated cell counter (pocH-iV, Sysmex, Kobe, Japan): red blood cell count, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white cell count, and platelet count.

Serum biochemistry. The following parameters were measured by using a blood chemistry analyzer (Dry Chem 3500, Fuji Film, Tokyo, Japan): total protein, albumin, albumin:globulin ratio, total bilirubin, urate, blood urea nitrogen, glucose, triglycerides, total cholesterol, asparate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatine kinase, amylase, electrolytes (K^+, Ca^{2+}, and Mg^{2+}), and inorganic phosphorus.

Salivary α-amylase activities. The system for monitoring salivary α-amylase activities (Cocoro Meter, Nipro, Osaka, Japan) consisted of a test strip (containing both a collection strip and a reagent strip), a saliva transfer device, and an calibrated analyzer. After immobilization of the monkey with MM, the test strip was positioned under its tongue and was left in place for 30 s to collect sufficient saliva. The saliva transfer device then was used to transfer the necessary quantity of saliva from the collection strip to the reagent strip. The reagent strip contains a new chromogen (2-chloro-4-nitrophenyl-4-O-β-D-galactopyranosylmaltoside [Gal-G2-CNP]) for measuring amylase activity; Gal-G2-CNP acts as a substrate of α-amylase. Salivary α-amylase reacted with Gal-G2-CNP (colorless) to yield CNP, which is yellow in color. The reagent strip was inserted into the calibrated analyzer, and the magnitude of the color change was assumed to parallel salivary α-amylase activity.

Statistical evaluation. Data are expressed as mean ± 1 standard deviation; statistical analysis was performed using the Student *t* test (Statcel, OMC Ltd, Tokorozawa, Japan). We compared the hematologic and serum biochemical results from the monkeys anesthetized with MM with institutional reference values from Japanese monkeys under general anesthesia via ketamine. The salivary α-amylase activities in the Japanese monkeys were compared with the criteria for psychologic stress in humans.[23,30]

Results

Clinical findings. The initial effects of MM (that is, incoordination, lethargy, and relaxation) occurred within 5 min after injection of the drugs. Induction of sedation was smooth and rapid. The onset of immobilization (sternal or lateral recumbency with inability to get up) was 13 ± 6 min after injection. The sedation induced by the MM combination was so profound that monkeys maintained sternal or lateral recumbency without spontaneous movement or blinking. The MM-treated monkeys failed to respond to sound and contact and showed moderately depressed swallowing and pedal reflexes and depressed response to nose clamping.

Although adverse effects such as mild bradycardia and hypothermia were observed more frequently in this study more than in ketamine-anesthetized monkeys, we did not observe vomiting and excitement after the MM administration. In addition, there were a slight decrease in the respiratory rate and mild arrhythmia.

Intramuscular administration of atipamezole at 240 µg/kg rapidly and effectively reversed the sedative effects of the MM combination without adverse effects such as vomiting, defecation, and diarrhea. Recovery from MM anesthesia after administration of atipamezole was smooth, with no signs of excitement or relapse. The monkeys given atipamezole showed arousal signs such as blinking, head lifting, limb twitching, and spontaneous movement within 10 min after its injection, and they had regained almost normal posture and normal response to sound within 13 min. Complete recovery from MM-induced immobilization was 10 ± 2 min after the administration of atipamezole. Atipamezole reversed the decrease in respiratory rate

Table 1. Hematologic findings after MM administration in Japanese monkeys

		Units	MM[a]	Reference[b]
Male				
	Red blood cell count	$\times 10^4/\mu l$	560 ± 7	507 ± 37
	Hb concentration	g/dl	13.6 ± 0.5	13.5 ± 1.1
	Packed cell volume	%	45.3 ± 1.8	40.8 ± 3.3
	Mean corpuscular volume	μm^3	80.9 ± 3.2	80.6 ± 3.8
	Mean corpuscular hemoglobin	pg	24.3 ± 0.9	26.7 ± 1.2
	Mean corpuscular hemoglobin concentration	%	30.0 ± 0.3	33.2 ± 1.4
	White blood cell count	$\times 10^3/\mu l$	9.9 ± 2.0	12.0 ± 3.8
	Platelets	$\times 10^3/\mu l$	327 ± 60	284 ± 75
Female				
	Red blood cell count	$\times 10^4/\mu l$	532 ± 43	502 ± 38
	Hb concentration	g/dl	13.3 ± 1.0	13.6 ± 1.2
	Packed cell volume	%	44.1 ± 3.0	41.1 ± 3.2
	Mean corpuscular volume	μm^3	83.0 ± 4.8	81.9 ± 4.3
	Mean corpuscular hemoglobin	pg	25.0 ± 1.9	27.1 ± 1.5
	Mean corpuscular hemoglobin concentration	%	30.1 ± 0.7	33.1 ± 1.5
	White blood cell count	$\times 10^3/\mu l$	11.8 ± 3.5	12.5 ± 3.8
	Platelets	$\times 10^3/\mu l$	337 ± 39	297 ± 81

MM, medetomidine–midazolam.
[a]Values for MM-treated animals are based on 3 male and 8 female Japanese monkeys.
[b]Reference values are based on 86 male and 75 female Japanese monkeys anesthetized with ketamine at the National Institutes of Natural Sciences.

to baseline within 10 min after its administration. In addition atipamezole effectively reversed MM-induced hypothermia, unaccompanied by hyperemia and pain at the injection site or a local reaction to its administration. Neither apparent discomfort nor behavioral abnormalities (escape, fear, threat and distrust) was noted after recovery from immobilization by the MM combination.

Hematologic findings. Hematologic parameters after MM administration in Japanese monkeys (Table 1) showed no significant differences compared with reference values from Japanese monkeys under general anesthesia with ketamine. All test results were within normal limits.

Serum biochemical findings. Serum biochemical parameters in Japanese monkeys (Table 2) revealed no significant differences between the monkeys treated with the MM combination and those that received ketamine. These data all were within normal ranges.

Salivary α-amylase activity. Salivary α-amylase activities during immobilization after administration of the MM combination (Figure 1, which also includes the criteria for diagnosis of the degree of stress) varied relatively widely (42 ± 36 kU/l). For example, 7 of the 11 immobilized monkeys showed salivary α-amylase activities consistent with minimal stress (45 kU/l or less). In contrast, the remaining 4 monkeys had increased salivary α-amylase levels indicative of marked physiologic or psychologic stress.

Discussion

Kojima and colleagues[15] reported considerable variation in the quality of induction among Beagles given medetomodine alone. These dogs showed subtle spontaneous movement and responded to sound even at the highest dose (80 μg/kg), and sound stimuli often caused temporary arousal. These dogs had minimal (if any) depression of reflexes and analgesia to nose clamping. Capuano and colleagues[3] reported that intravenous administration of medetomidine alone could be used safely for immobilizing rhesus macaques that were not chair-restrained. Although muscular relaxation in their monkeys was excellent, sedative and analgesic effects were inconsistent. In our preliminary test using 4 monkeys, the MM combination exerted more potent sedative and immobilizing effects than did any dose of medetomidine (30, 60, 90, and 120 μg/kg) alone. Even with a higher dose (120 μg/kg) of medetomidine, 3 of the 4 monkeys were immobilized insufficiently and were susceptible to arousal by an external stimulus.[14] Therefore, we could not examine salivary α-amylase activities or hematologic and serum biochemical values in the medetomidine-only monkeys.

In this study, we produced a favorable sedative condition in Japanese monkeys by using the MM combination. This combination synergistically exhibited several advantages, including rare induction of vomiting and enhanced sedative, analgesic, and muscle-relaxant effects. However, this combination also induced bradycardia and respiratory depression (as determined by auscultation); medetomidine is known to produce cardiovascular changes and decreases in respiratory rate.[5,9,15] In a study using rhesus macaques, Capuano and colleagues[3] found that intravenous administration of medetomidine caused a transient but significant increase in respiratory rate followed by a prolonged significant decrease. In addition, the authors noted that the monkeys developed bradycardia, hypotension, and loss of thermoregulatory ability that accompanied the biphasic respiratory response. Therefore, the bradycardia and respiratory depression we experienced in our monkeys likely were due to medetomidine. Future studies should systematically address the effects of MM on heart rate, respiratory rate, and body temperature. Midazolam seemed to contribute to respiratory depression. Although we did not monitor heart and respiratory rates during this examination, body temperature decreased from 36.7 to 36.0 °C.

All 11 Japanese monkeys treated with MM became immobilized with moderate muscle relaxation, depression of reflexes, and analgesia. This method invariably induced profound sedation and immobilization, and interindividual differences among

Table 2. Serum biochemical findings after MM administration in Japanese monkeys

		Units	MM[a]	Reference[b]
Male				
	Total protein	g/dl	6.6 ± 0.2	6.9 ± 0.6
	Albumin	g/dl	3.8 ± 0.2	4.3 ± 0.6
	Albumin:globulin ratio		1.3 ± 0.1	1.7 ± 0.4
	C-reactive protein	mg/dl	<0.3	0.3 ± 0.3
	Total bilirubin	mg/dl	0.2 ± 0	0.2 ± 0.1
	Blood urea nitrogen	mg/dl	20.6 ± 4.9	17.5 ± 5.5
	Creatinine	mg/dl	0.7 ± 0.3	0.9 ± 0.4
	Glucose	mg/dl	108 ± 10.6	104 ± 30
	Total cholesterol	mg/dl	152 ± 42	138 ± 29
	Triglycerides	mg/dl	90 ± 40	76 ± 44
	Aspartate aminotransferase	U/l	21 ± 1	32 ± 14
	Alanine aminotransferase	U/l	23 ± 1	28 ± 12
	Alkaline phosphatase	U/l	765 ± 91	635 ± 491
	Lactate dehydrogenase	U/l	200 ± 32	253 ± 92
	Amylase	U/l	192 ± 19	214 ± 82
	Creatine kinase	U/l	292 ± 297	208 ± 145
	Ca^{2+}	mg/dl	9.0 ± 0.2	9.4 ± 0.7
	Inorganic P	mg/dl	3.9 ± 0.4	4.9 ± 1.1
	Mg^{2+}	mg/dl	1.9 ± 0.05	1.7 ± 0.2
	Na^{+}	mEq/l	142 ± 5	144 ± 5
	K^{+}	mEq/l	3.8 ± 0.1	3.9 ± 0.3
	Cl^{-}	mEq/l	100 ± 5	102 ± 4
Female				
	Total protein	g/dl	6.6 ± 0.6	6.8 ± 0.6
	Albumin	g/dl	3.7 ± 0.1	4.2 ± 0.6
	Albumin:globulin ratio		1.3 ± 0.2	1.6 ± 0.4
	C-reactive protein	mg/dl	<0.3	0.3 ± 0.2
	Total bilirubin	mg/dl	0.2 ± 0.1	0.2 ± 0.1
	Blood urea nitrogen	mg/dl	15.3 ± 3.2	16.0 ± 4.7
	Creatinine	mg/dl	0.6 ± 0.1	0.8 ± 0.2
	Glucose	mg/dl	98 ± 39	100 ± 27
	Total cholesterol	mg/dl	133 ± 29	143 ± 27
	Triglycerides	mg/dl	80 ± 37	78 ± 50
	Aspartate aminotransferase	U/l	25 ± 8	27 ± 10
	Alanine aminotransferase	U/l	24 ± 8	27 ± 15
	Alkaline phosphatase	U/l	929 ± 422	514 ± 378
	Lactate dehydrogenase	U/l	235 ± 58	232 ± 88
	Amylase	U/l	215±49	206 ± 69
	Creatine kinase	U/l	140 ± 57	215 ± 279
	Ca^{2+}	mg/dl	9.0 ± 0.2	9.4 ± 0.6
	Inorganic P	mg/dl	4.3 ± 0.9	4.1 ± 1.0
	Mg^{2+}	mg/dl	2.1 ± 0.3	1.7 ± 0.2
	Na^{+}	mEq/l	141 ± 3	143 ± 5
	K^{+}	mEq/l	3.8 ± 0.5	3.9 ± 0.4
	Cl^{-}	mEq/l	101 ± 5	101 ± 4

MM, medetomidine–midazolam.
[a]Values for MM-treated animals are based on 3 male and 8 female Japanese monkeys.
[b]Reference values are based on 86 male and 75 female Japanese monkeys anesthetized with ketamine at the National Institutes of Natural Sciences.

the MM-treated animals were few. We believe that the MM combination is useful for various diagnostic procedures that require excellent immobilization or for therapeutic procedures associated with mild pain.

The onset time of immobilization after MM administration was 13 min longer in Japanese monkeys than in dogs.[6,16] This result shows that the response to MM differs among species. The ability of α_2-agonists to produce sedation and analgesia depends on their affinity and specificity for receptors in the central nervous system.[28]

Atipamezole is a highly selective and specific α_2-adrenoreceptor antagonist that effectively inhibits the action of

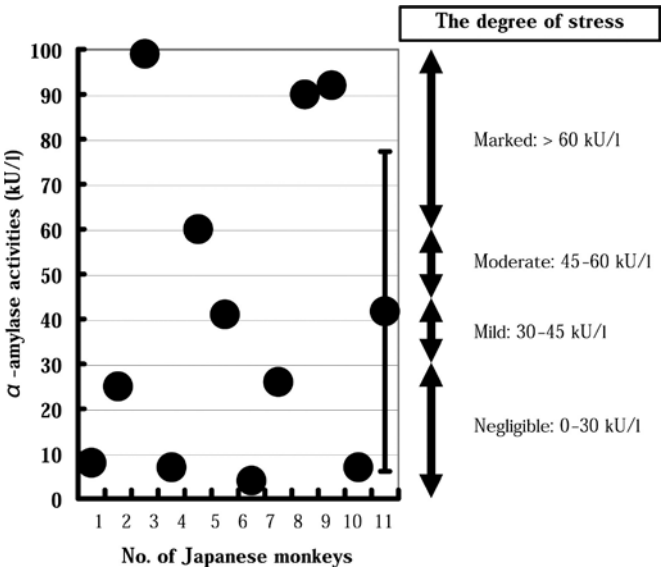

Figure 1. Salivary α-amylase activities after administration of the MM combination in Japanese monkeys. The mean value is indicated by the error bar, which represents 1 standard deviation.

medetomidine in dogs.[24,25] This antagonistic effect can be obtained even when medetomidine is used with other drugs (for example, butorphanol and midazolam).[27] In using the MM combination to induce immobilization, our results in Japanese monkeys agreed with clinical findings in dogs.[7] Our results further revealed that the sedative and immobilizing effects due to MM can be reversed quickly and smoothly by atipamezole. Specifically, 240 μg/kg of atipamezole (4 times the dose of medetomidine) was sufficient to antagonize the immobilization induced by combined treatment with 60 μg/kg of medetomidine and 0.3 mg/kg of midazolam. Our findings confirm that the atipamezole-induced reversibility of immobilization is a clear advantage to using MM instead of ketamine.

In human clinical medicine,[11] midazolam frequently is used in surgical anesthesia to reduce the anxiety that a patient may have prior to surgical procedures. From our observations of recovery from immobilization, we expect that this agent will produce temporary amnesia[2] for the undesirable experiences that subjects had during the experimental procedure.

Other anesthetics such as barbiturates, diethyl ether, and halothane cause alternations in several parameters.[1] In contrast, hematologic and serum biochemical measurements among Japanese monkeys treated with the MM combination did not often differ significantly from those in animals given ketamine. These findings revealed that medication with the MM combination had no deleterious effects on hematologic and serum biochemical values.

The release of salivary enzyme α-amylase reacts sensitively to physiologic and psychologic stressors.[18-20,26,29,30] Previous application of this method to laboratory animals has not been reported. Our results show that salivary α-amylase can be added to the noninvasive biomarkers that are available for use in monkeys. The salivary α-amylase activities of healthy, unanesthetized Japanese monkeys ranged from 4 to 99 kU/l (data not shown), and these levels are similar to those measured in human saliva.[30] Of the 11 monkeys we studied, the salivary α-amylase activities of 7 remained at control levels after anesthesia with the MM combination, whereas the remaining 4 animals showed increased activities. This increase in salivary α-amylase seemed to be associated with prolonged physical restraint via the squeeze-back mechanism.

In conclusion, the MM combination is suitable not only as a preanesthetic in healthy Japanese monkeys that are difficult to restrain but also for procedures requiring excellent immobilization. The MM combination is an effective alternative to ketamine.

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