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Sedative and physiological effects of alfaxalone intramuscular administration in cynomolgus monkeys (*Macaca fascicularis*)

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ABSTRACT. To evaluate the sedative and physiological effects of alfaxalone intramuscular (IM) administration, 12 healthy cynomolgus monkeys were administered single IM doses of alfaxalone at 0.625 mg/kg (ALFX0.625), 1.25 mg/kg (ALFX1.25), 2.5 mg/kg (ALFX2.5), 5 mg/kg (ALFX5), 7.5 mg/kg (ALFX7.5), or 10 mg/kg (ALFX10); saline was used as the control (CONT). The sedative effects were subjectively evaluated using a composite measure scoring system in six animals. Changes in respiratory rate, pulse rate, non-invasive blood pressure, percutaneous oxygen-hemoglobin saturation (SpO_2), and rectal temperature were observed after IM treatments in the other six animals. All animals were allowed to lay down following the ALFX5, ALFX7.5, and ALFX10 treatments, whereas lateral recumbency was achieved in only two animals after ALFX2.5 treatment and none after the CONT, ALFX 0.625, and ALFX1.25 treatments. The median time (interquartile range) to lateral recumbency was 6.5 min (5.3–7.8), 4.0 min (4.0–4.0), and 3.0 min (3.0–3.8), and the duration of immobilization was 27.5 min (19.0–33.8), 56.0 min (42.3–60.8), and 74.5 min (62.8–78.0) after the ALFX5, ALFX7.5, and ALFX10 treatments, respectively. Endotracheal intubation was achieved in all six animals after the ALFX7.5 and ALFX10 treatments. Dose-dependent decreases in respiratory rate, non-invasive blood pressure, SpO_2, and rectal temperature were observed, and the quality of recovery was smooth in all animals after the ALFX5, ALFX7.5, and ALFX10 treatments. Thus, alfaxalone IM induced a dose-dependent sedative effect in cynomolgus monkeys, but at higher doses, hypotension, hypoxemia, and hypothermia could be induced.

KEY WORDS: alfaxalone, cynomolgus monkey, intramuscular administration, sedation

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Cynomolgus monkeys (*Macaca fascicularis*) are widely used in drug discovery research as they have numerous physiological features in common with human beings [14]. Because monkeys can sometimes display aggression towards their caregivers and/or researchers, it is often required to immobilize them to conduct experimental procedures or medical treatments. In addition, immobilization is necessary to reduce stress during these procedures and treatments. Ketamine is the most common anesthetic agent that is used for the chemical immobilization of monkeys due to its reliability when administered by intramuscular (IM) injection [16, 22, 27, 29, 30]. However, the IM administration of ketamine can cause problems, such as muscle tissue damage, decreased food intake, persistent antidepressant activity, salivation, and muscle rigidity [3, 6, 7, 21, 28]. In addition, ketamine has become inconvenient to use in animal experiments as it has been legally controlled since January 2007 in Japan. For these reasons, the development of another anesthesia protocol that can immobilize monkeys via a single IM administration is warranted.

Medetomidine is a highly selective alpha2-adrenoceptor agonist that produces deep sedation, muscle relaxation, and analgesia [20]. The combination of medetomidine, midazolam (a benzodiazepine derivative), and butorphanol (a synthetic opioid with κ-agonist and μ-antagonist properties) is widely used for sedation and immobilization in various animal species including cynomolgus monkeys [19]. However, medetomidine is known to produce a marked peripheral vasoconstriction and cardiovascular depression when administered at recommended doses [20]. Furthermore, Ochi *et al.* [19] reported that cardiorespiratory depression such as bradycardia, hypotension, and respiratory depression, and loss of thermoregulatory ability can occur in cynomolgus monkeys after the IM administration of medetomidine-midazolam-butorphanol.

Alfaxalone (3-alpha-hydroxy-5-alpha-pregnane-11,20-dione) is a synthetic neuroactive steroid, which modulates the gamma-aminobutyric acid A ($GABA_A$) receptor that can cause neuro-depression and muscle relaxation [1, 13]. In the past decade, its formulation with 2-hydroxypropyl-beta-cyclodextrin (alfaxalone-HPCD) has been approved in many countries, including Japan, as

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an intravenous anesthetic induction agent for dogs and cats. Recently, it was reported that IM administration of alfaxalone-HPCD can produce dose-dependent sedative effects with a relatively mild cardiorespiratory depression in cats [24], dogs [25], and rabbits [12, 13]. It was also reported that IM combinations of alfaxalone-HPCD and an alpha2-adrenergic receptor agonist can produce sedative effects in nonhuman primates [2, 4, 5, 26]. For example, Casoni *et al.* [5] reported that an IM combination of alfaxalone-HPCD (2 mg/kg) and medetomidine (15 μg/kg) produced sedation in cynomolgus monkeys, which allowed placement of an intravenous catheter with or without a mild reaction. However, to the best of our knowledge, there has been no report investigating the sedative and cardiorespiratory effects of IM alfaxalone-HPCD, specifically in cynomolgus monkeys. Thus, the aims of the present study are to determine the sedative and cardiorespiratory effects of IM alfaxalone-HPCD as the sole anesthetic in cynomolgus monkeys.

MATERIALS AND METHODS

Humane care and use of animals

Cynomolgus monkeys were housed in compliance with the Astellas Global Policy for the Care and Use of Laboratory Animals, the Guide for the Care, as well as the Use of Laboratory Animals guidelines. The present study was approved by the Institutional Animal Care and Use Committee (IACUC) of Astellas Pharma Inc., Tsukuba Research Center, which was accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The protocol numbers approved by the IACUC were C-T 18260 and C-T 18270.

Experimental subjects

Twelve clinically healthy adult male cynomolgus monkeys (4 to 7 years old, 3.8 to 6.2 kg body weight) were used for this study. Six animals were used for the evaluation of sedative effects, while the other six monkeys were used for the observation of changes in cardiorespiratory variables. All the animals were judged by a veterinarian (S.W.) to be in good health based on a physical examination (checking body condition, food consumption, body weight, and blood tests). Food was withheld from the animals for 10 hr before each experiment, while filtered water was available *ad libitum*. The animals were housed separately in stainless steel cages under a 12:12 hr light:dark cycle (light period: from 07:00 to 19:00) with controlled temperature (25 ± 2°C), humidity (55 ± 5%), and 15–20 air changes per hour. The animals were fed standard commercial primate food (approximately 100 g/monkey/day, PS-A; Oriental Yeast Co., Ltd., Tokyo, Japan). Under the environmental enrichment program, one stainless steel or plastic toy was kept in each cage, with small amounts of dried grapes and fresh apples, which were provided on a daily basis.

Study design

Seven different IM drug treatments, comprising six doses of alfaxalone-HPCD (Alfaxan 10 mg/m*l*; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) and the saline solution (Otsuka Normal Saline; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) were administered to the animals at random, with a minimum 4-day washout period between treatments. The IM doses of alfaxalone-HPCD were 0.625 mg/kg (ALFX0.625), 1.25 mg/kg (ALFX1.25), 2.5 mg/kg (ALFX2.5), 5 mg/kg (ALFX5), 7.5 mg/kg (ALFX7.5), and 10 mg/kg (ALFX10). Each total IM injection volume was 0.0625, 0.125, 0.25, 0.5, 0.75, and 1.0 m*l*/kg for the ALFX0.625, ALFX1.25, ALFX2.5, ALFX5, ALFX7.5, and ALFX10 groups, respectively. Saline solution (1.0 m*l*/kg) was administered as a negative control (CONT). One IM dose was injected into a monkey's dorsal lumbar muscle using a 23-gauge, 1-inch needle (Terumo injection needle, Terumo Co., Ltd., Tokyo, Japan) over the span of 1 min. The maximum volume of the IM injection was 0.5 m*l*/kg/site. Thus, for the CONT, ALFX7.5, and ALFX10 treatments, the dosing solution was injected into two separate sites on the dorsal lumbar muscle. All the animals were manually restrained during each IM administration before they were released into their cages. All the animals were allowed to breathe room air spontaneously throughout the experiments. The sedative effects were evaluated in six animals at 5-min intervals between 10 and 120 min after the IM treatment, while the cardiorespiratory variables were measured in the other six animals at 10, 20, 30, 45, 60, 90, and 120 min after the IM injection(s).

Evaluation of the sedative effects

An observer (S.W.), who was not blinded to the IM treatment allocations, evaluated the sedative effects in the animals throughout the study. The sedative effects were evaluated by the degree of neuro-depression, quality of anesthetic induction, ease of endotracheal intubation, and quality of recovery. The degree of neuro-depression was evaluated using a composite measure scoring system previously reported in rhesus and cynomolgus monkeys [15]. This scoring system consisted of five categories: spontaneous movement, pedal reflex, palpebral reflex, jaw tone, and limb manipulation (Table 1). The degree of spontaneous movement was scored from 1 to 5 according to the motion of each animal. The pedal reflex was scored from 1 to 5 according to the animal's response to toe-pinch with a hemostat. When the animal showed facial and/or limb movements, the pedal reflex was not evaluated and was scored as 0. The palpebral reflex was scored from 1 to 5 according to the animal's response when the observer applied light digital pressure to the medial canthus. When the animal showed facial and/or limb movements, the palpebral reflex was not evaluated and was scored as 0. The jaw tone was scored from 1 to 5 according to the rigidity of the animal's mouth when it was opened with the observer's finger. When the animal seemed to bite the observer, the jaw tone was not evaluated and was scored as 0. The limb manipulation was scored from 1 to 5 according to the animal's response when the observer lifted the animal's limb with one hand and let it fall into the other hand. If the limb could not be grasped, the limb manipulation was not evaluated and was scored as 0. Hence, the total sedation score was calculated as the sum of the scores in the five categories (maximum score of 25).

The quality of induction and recovery after the IM drug treatments was scored from 0 to 4 by using numerical scoring systems,

Table 1. Composite measure scoring system for evaluating neuro-depression in cynomolgus monkeys [15]

Spontaneous movement	Score
Whole-body movements	1
Hand or foot movements	2
Facial movements	3
Twitching of hands or feet	4
No movement	5

Pedal reflex	Score
Not evaluated	0
Strongly pulls away	1
Weakly pulls away (immediately)	2
Weakly pulls away (delayed)	3
Flexes or extends digits	4
No movement	5

Palpebral reflex	Score
Not evaluated	0
Blinking and other body movement	1
Normal blinking	2
Weak blinking (immediate)	3
Weak blinking (delayed)	4
No movement	5

Jaw tone	Score
Not evaluated	0
Increased tone	1
Normal tone	2
Decreased tone	3
Minimal tone	4
No tone	5

Limb manipulation	Score
Not evaluated	0
Movement with limb and other parts	1
Withdrawn promptly when handled	2
Weak withdrawal	3
Flexes or extends digits	4
No response	5

Total sedation score*	1–25

*The total sedation score was calculated as the sum of the scores in the five categories.

Table 2. Scoring system for evaluating the qualities of induction of anesthesia and recovery from anesthesia in cynomolgus monkeys

Categories	Conditions in cynomolgus monkeys
Induction score	
0 (impossible)	Impossible to intubate because of no or poor sedation.
1 (Poor)	Vocalization and physical movement during entire intubation period, additional induction agent might need for intubation.
2 (Moderately smooth)	Swallowing a lot, more than 3 attempts to intubate, coughing, vocalization and/or physical movement for more than half the induction time.
3 (Quite smooth)	Some swallowing, intubation after 2–3 attempts, no coughing, some physical movement, no vocalization.
4 (Very smooth)	No swallowing, intubation at first attempt, no coughing, no struggling, no vocalization.
Recovery score	
0 (no recovery)	Animal could not recover after extreme excitement, aggression, vocalization, violent movement or convulsions.
1 (Poor)	Extreme excitement observed, aggression, vocalization, violent movement or convulsions.
2 (Moderately smooth)	Moderate excitement. Some paddling, vocalization,trembling or vomiting. No convulsions.
3 (Quite smooth)	A little excitement. Some head movement, possibly some shivering but no paddling,vocalizing, trembling or vomiting. No convulsions.
4 (Very smooth)	No excitement. No paddling, vocalizing, trembling or vomiting. No convulsions.

The induction and recovery scores were modified from the scoring system used for dogs in previous reports [23, 25].

which were modified from scoring systems applied in dogs in previous reports [23, 25] (Table 2). Endotracheal intubation using an endotracheal tube (Tracheal tube cuffed; I.D. 4.5 mm; Covidien Japan Inc., Tokyo, Japan) was used when the animal showed either twitching of the limbs or no movement. In addition, the time required after the completion of the IM administration (time 0) to the onset of lateral recumbency (time to recumbency), to the first appearance of spontaneous movement, and to unaided sitting or standing were recorded. The durations from the onset of lateral recumbency to the first appearance of spontaneous movement (duration of immobilization), as well as from the first appearance of spontaneous movement to unaided sitting or standing (recovery time) were calculated.

As mentioned above, our work included a composite measure scoring system, which consisted of five categories, including the pedal reflex (animal response to toe-pinch using a hemostat), used for the evaluation of neuro-depression that could be produced by the IM drug treatments. The pinching of an animal's toe with a hemostat can act as a nociceptive stimulation that could affect cardiorespiratory variables, particularly the heart rate (HR) and noninvasive mean arterial blood pressure (NMABP). Furthermore, endotracheal intubation can affect cardiorespiratory variables. In order to eliminate these influences on the evaluation of neuro-depression and endotracheal intubation, the cardiorespiratory variables were evaluated in six additional animals. These six animals were not used for the evaluation of neuro-depression and acceptance of endotracheal intubation.

Measurement of cardiorespiratory variables

Respiratory rate (RR; breaths/min), HR (beats/min), NMABP (mmHg), percutaneous oxygen saturation of hemoglobin (SpO_2;%), and rectal temperature (RT;°C) were measured in the monkeys that were restrained in a monkey chair. The monkeys were relatively quiet during the measurements. The RR was assessed by observing thoracic movements. The HR, NMABP, SpO_2, and RT were measured using an animal monitoring system (BSM-3592 Life Scope VS; Nihon Kohden Corp., Tokyo, Japan). The HR was measured by the standard bipolar limb lead (Lead-II) using clip electrodes, while the NMABP was measured by an oscillometric method using a blood pressure cuff (Size 4.0 cm; Critter Cuff; Nihon Kohden Corp.) that was placed on the right thoracic limb. The SpO_2 was measured by using a pulse oximeter probe that was placed on the upper lip, while the RT was measured using a temperature probe placed in the rectum.

Statistical analysis

GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis. Data are expressed as medians (interquartile ranges), where the differences in the total sedation score and cardiorespiratory variables between the saline IM treatment (CONT) and the alfaxalone-HPCD IM treatments (ALFX0.625, ALFX1.25, ALFX2.5, ALFX5, ALFX7.5, and ALFX10) at each time point were compared using the Friedman test with Dunn's multiple comparisons test. Differences in total sedation score, qualities of anesthetic induction/recovery, time to recumbency, duration of immobilization, and recovery periods among the IM drug treatments were compared by the Friedman test with Dunn's multiple comparisons test. The level of significance was set at $P<0.05$.

RESULTS

Sedative effects

None of the monkeys displayed any pain-related behavior or discomfort, such as vocalizing or struggling during the IM injections. Furthermore, no abnormal behaviors, such as limping, were observed following the IM drug treatments. No sedative effect was observed in all animals receiving the CONT and ALFX0.625 treatments. However, all animals lay down within 9, 5, and 4 min after the ALFX5, ALFX7.5, and ALFX10 treatments, respectively. Immobilization was achieved in all animals following the ALFX5, ALFX7.5, and ALFX10 treatments. In addition, all animals accepted the endotracheal intubation after the ALFX7.5 and ALFX10 treatments.

The times and scores related to the sedative effects after the IM drug treatments are summarized in Table 3. The time to recumbency was significantly shorter in animals receiving the ALFX10 treatment than in those receiving the ALFX5 treatment ($P=0.007$). The duration of immobilization was prolonged with the dosage of alfaxalone-HPCD in a dose-dependent manner, which was significantly longer with the ALFX10 treatment than with the ALFX5 treatment ($P=0.005$). The quality of recovery was comparable among the ALFX2.5, ALFX5, ALFX7.5, and ALFX10 treatments. During recovery, tremor was observed in 1, 2, 3, and 4 animals receiving ALFX2.5, ALFX 5, ALFX 7.5, and ALFX 10 treatments, respectively.

Figure 1 illustrates the score-time profiles of the total sedation score and the scores of the five individual categories. No change in scores was observed after the CONT or ALFX0.625 treatments. Spontaneous movement disappeared in all animals (i.e., all six animals with a score of 5), which received the ALFX5, ALFX7.5, and ALFX10 treatments. Pedal reflex disappeared in all

Table 3. Times and scores related to sedative effect after intramuscular (IM) drug treatments in 6 cynomolgus monkeys

	IM drug treatments (n=6)						
	CONT	ALFX0.625	ALFX1.25	ALFX2.5	ALFX5	ALFX7.5	ALFX10
Number of animals became lateral recumbency	0	0	0	2	6	6	6
Number of animals accepted endotracheal intubation	0	0	0	0	3	6	6
Induction score	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1 and 1	1.5 (1.0–2.0)	3.5 (3.0–4.0)[a)]	4.0 (3.3–4.0)[a)]
Recovery score	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	3 and 4	4.0 (3.3–4.0)	3.5 (3.0–4.0)	3.0 (3.0–3.8)
Maximum total sedation score	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	2.0 (1.3–9.5)	16.0 (15.0–20.0)	24.0 (22.5–24.0)	25.0 (25.0–25.0)[a)]
Time to recumbency (min)	N.C.	N.C.	N.C.	5.0 and 8.0	6.5 (5.3–7.8)	4.0 (4.0–4.0)	3.0 (3.0–3.8)[a)]
Duration of immobilization (min)	N.C.	N.C.	N.C.	5.0 and 19.0	27.5 (19.0–33.8)	56.0 (42.3–60.8)	74.5 (62.8–78.0)[a)]
Recovery time (min)	N.C.	N.C.	N.C.	4.0 and 19.0	12.0 (8.0–14.5)	12.5 (11.3–13.0)	10.5 (7.8–14.0)

The animals were administered IM saline at 1.0 ml/kg (CONT) and IM alfaxalone-HPCD at 2.5 mg/kg (ALFX2.5), 5 mg/kg (ALFX5), 7.5 mg/kg (ALFX7.5), and 10 mg/kg (ALFX10) with a minimum 4-day washout period. Data are expressed as number of animals, value obtained, or median (interquartile range) obtained from six cynomolgus monkeys. N.C.: not calculated. Total sedation score (maximum score of 25) was calculated as the sum of scores for the five categories (maximum score of 5 each) shown in Table 1. Descriptions of induction and recovery scores are shown in Table 2. Time to recumbency: time from the completion of IM administration (time 0) to the onset of lateral recumbency. Duration of immobilization: duration from the onset of lateral recumbency to the first appearance of spontaneous movement. Recovery time: duration from the first appearance of spontaneous movement to unaided sitting or standing in animals showing lateral recumbency after IM drug administration. Statistical analysis was performed among the ALFX5, ALFX7.5, and ALFX10 treatments. a) Significant difference from 5 mg/kg IM ($P<0.05$). HPCD: 2-hydroxypropyl-beta-cyclodextrin.

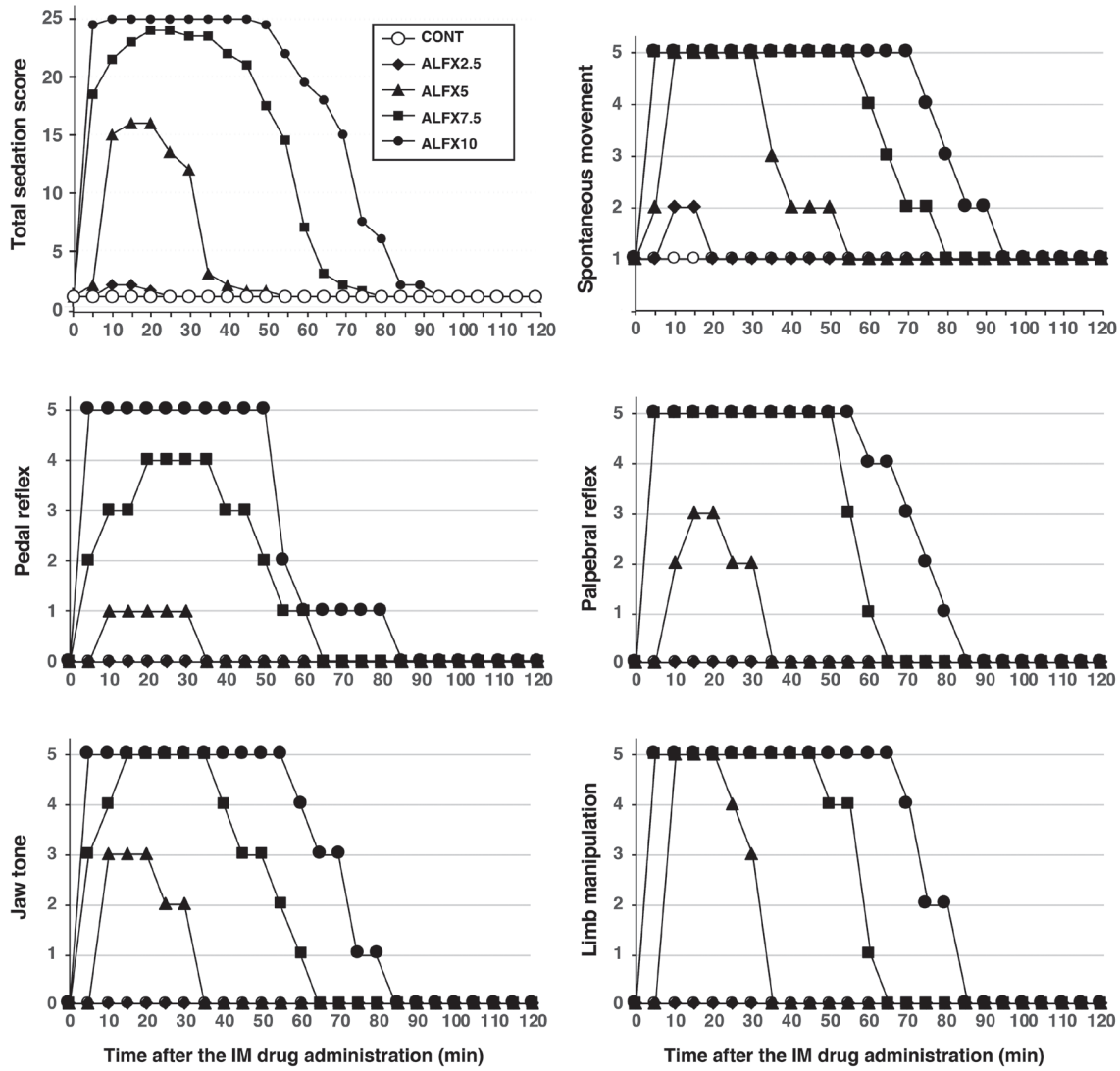

Fig. 1. Score-time profiles of the total sedation score in six cynomolgus monkeys across the five categories of spontaneous movement, pedal reflex, palpebral reflex, jaw tone, and limb manipulation, which were measured after intramuscular (IM) drug administration. The animals were administered IM saline at 1.0 ml/kg (CONT) and IM alfaxalone-HPCD at 2.5 mg/kg (ALFX2.5), 5 mg/kg (ALFX5), 7.5 mg/kg (ALFX7.5), and 10 mg/kg (ALFX10) after a minimum 4-day washout period. Each plotted point shows the median score of six animals. The total sedation score, with a maximum of 25, was calculated as the sum of the scores in the five categories, using a maximum score of 5 each, which is summarized in Table 1. HPCD: 2-hydroxypropyl-beta-cyclodextrin.

animals receiving the ALFX10 treatment, while the palpebral reflex disappeared in all animals receiving the ALFX7.5 or ALFX10 treatment. Loss of the palpebral reflex occurred more rapidly and was maintained much longer in the animals that received the ALFX10 treatment compared to those that received the ALFX7.5 treatment. Jaw tone disappeared in all animals receiving the ALFX10 treatment, while the response to limb manipulation disappeared in all animals receiving either the ALFX7.5 or ALFX10 treatment. Reflecting on these changes in the five categories, the total sedation scores significantly increased from 5 to 45 min and from 5 to 65 min following the ALFX7.5 and ALFX 10 treatments, respectively. The maximum total sedation score was significantly higher in the animals receiving the ALFX10 treatment (median: 25.0, interquartile range: 25.0–25.0) than in those receiving the CONT (median: 1.0, interquartile range: 1.0–1.0, P=0.002), ALFX0.625 (median: 1.0, interquartile range: 1.0–1.0, P=0.002), and ALFX1.25 (median: 1.0, interquartile range: 1.0–1.0, P=0.005) treatments.

Measurement of cardiorespiratory variables

Changes in the cardiorespiratory variables after the IM drug treatments are summarized in Table 4. Spontaneous breathing was maintained in all animals in each treatment, but the RR was found to decrease significantly after 10 to 20 min, 10 to 60 min, and 10 to 60 min following the ALFX5, ALFX7.5, and ALFX10 treatments, respectively. The NMABP was reduced significantly after 30 min and after 10 to 30 min following the ALFX7.5 and ALFX10 treatments, respectively. Hypotension (NMABP <60 mmHg)

Table 4. Change in cardiorespiratory variables after intramuscular (IM) drug treatments in six cynomolgus monkeys

Valuables	Treatments	Minutes after the IM drug administration						
		10	20	30	45	60	90	120
RR (breaths/min)	CONT	38 (36–40)	37 (36–40)	34 (34–40)	36 (36–36)	36 (35–38)	34 (34–37)	35 (34–41)
	ALFX0.625	36 (35–36)	33 (32–36)	35 (34–36)	36 (36–36)	38 (34–38)	36 (35–38)	35 (34–36)
	ALFX1.25	35 (34–39)	36 (35–36)	38 (35–40)	35 (34–38)	37 (35–38)	38 (36–42)	35 (34–36)
	ALFX2.5	35 (29–40)	35 (28–36)	36 (30–38)	38 (30–40)	39 (32–40)	39 (35–42)	39 (34–42)
	ALFX5	27 (23–30)[a)]	26 (23–31)[a)]	25 (21–29)	29 (25–32)	30 (29–35)	31 (30–37)	30 (30–38)
	ALFX7.5	28 (26–33)[a)]	25 (23–26)[a)]	22 (21–25)[a)]	23 (22–27)[a)]	23 (21–26)[a)]	29 (27–32)	33 (29–34)
	ALFX10	25 (21–30)[a)]	24 (19–28)[a)]	23 (21–26)[a)]	23 (20–28)[a)]	22 (20–29)[a)]	25 (23–31)	33 (26–37)
HR (beats/min)	CONT	237 (232–242)	224 (215–245)	224 (212–230)	227 (201–244)	233 (224–240)	225 (214–233)	219 (217–221)
	ALFX0.625	233 (226–238)	232 (228–235)	221 (218–235)	222 (213–236)	216 (201–225)	227 (219–232)	204 (198–212)
	ALFX1.25	238 (237–241)	230 (215–244)	233 (212–244)	232 (214–234)	227 (213–234)	224 (214–228)	217 (206–219)
	ALFX2.5	234 (233–235)	223 (219–225)	226 (221–225)	217 (215–227)	231 (223–234)	225 (220–231)	225 (217–234)
	ALFX5	232 (228–235)	223 (213–202)	209 (201–235)	205 (191–208)	200 (194–213)	201 (195–216)	207 (195–213)
	ALFX7.5	243 (236–246)	233 (230–198)	208 (206–236)	200 (191–203)	205 (192–215)	208 (197–213)	205 (191–211)
	ALFX10	246 (239–253)	241 (239–207)	211 (208–244)	194 (185–205)	192 (189–207)	202 (193–216)	199 (188–212)
NMABP (mmHg)	CONT	100 (93–107)	98 (89–106)	97 (90–98)	101 (95–104)	95 (92–100)	95 (89–101)	92 (89–95)
	ALFX0.625	97 (94–101)	101 (94–103)	98 (90–101)	99 (96–103)	103 (99–104)	103 (98–105)	98 (94–102)
	ALFX1.25	100 (95–103)	95 (89–106)	93 (91–103)	102 (95–111)	99 (92–104)	97 (89–103)	102 (101–105)
	ALFX2.5	99 (97–103)	90 (84–95)	86 (86–94)	91 (89–95)	106 (101–110)	103 (101–109)	104 (99–107)
	ALFX5	89 (82–99)	100 (86–103)	99 (95–101)	98 (94–104)	107 (102–108)	108 (105–112)	106 (97–112)
	ALFX7.5	82 (79–92)	82 (71–84)	76 (62–78)[a)]	78 (74–81)	93 (83–105)	99 (88–104)	103 (98–106)
	ALFX10	76 (70–79)[a)]	71 (68–78)[a)]	71 (64–84)[a)]	76 (70–80)	101 (92–103)	98 (92–101)	98 (92–106)
SpO_2 (%)	CONT	98 (97–99)	98 (97–98)	98 (98–98)	98 (97–98)	98 (97–98)	98 (98–98)	98 (97–98)
	ALFX0.625	98 (97–98)	98 (98–99)	97 (97–99)	98 (97–98)	98 (98–98)	98 (98–99)	98 (98–99)
	ALFX1.25	98 (97–99)	98 (97–98)	98 (97–98)	97 (97–97)	98 (97–98)	99 (98–99)	98 (97–99)
	ALFX2.5	97 (95–97)	97 (96–97)	97 (97–98)	97 (97–97)	98 (97–99)	98 (98–98)	98 (98–98)
	ALFX5	95 (93–96)	93 (92–94)	94 (93–96)	96 (95–97)	96 (96–97)	97 (94–98)	97 (96–99)
	ALFX7.5	93 (92–94)[a)]	92 (91–93)[a)]	91 (90–92)[a)]	93 (92–94)[a)]	94 (93–96)[a)]	96 (95–97)	98 (96–98)
	ALFX10	92 (91–93)[a)]	90 (89–92)[a)]	90 (89–91)[a)]	92 (91–93)[a)]	94 (93–95)[a)]	96 (94–97)	97 (96–98)
RT (°C)	CONT	38.5 (38.4–38.6)	38.6 (38.5–38.9)	38.7 (38.6–38.8)	38.7 (38.6–38.9)	38.5 (38.4–38.5)	38.5 (38.2–38.7)	38.6 (38.5–38.8)
	ALFX0.625	38.5 (38.4–38.6)	38.5 (38.4–38.6)	38.5 (38.3–38.7)	38.5 (38.4–38.5)	38.5 (38.5–38.6)	38.6 (38.6–38.7)	38.4 (38.4–38.6)
	ALFX1.25	38.4 (38.3–38.6)	38.5 (38.4–38.5)	38.5 (38.4–38.5)	38.6 (38.5–38.6)	38.6 (38.4–38.7)	38.4 (38.3–38.5)	38.5 (38.4–38.6)
	ALFX2.5	38.4 (38.3–38.7)	38.1 (38.1–38.3)	37.8 (37.4–38.1)	37.8 (37.7–38.0)	38.1 (38.0–38.2)	38.4 (38.2–38.6)	38.6 (38.5–38.7)
	ALFX5	38.0 (37.8–38.2)	37.2 (37.0–37.2)[a)]	36.9 (36.8–37.1)[a)]	36.8 (36.6–36.8)[a)]	36.6 (36.4–36.8)	37.3 (36.8–37.5)	37.7 (37.5–37.9)
	ALFX7.5	37.4 (37.3–37.6)[a)]	36.6 (36.3–36.8)[a)]	36.0 (35.9–36.2)[a)]	35.3 (35.1–35.3)[a)]	35.1 (35.0–35.3)[a)]	35.1 (35.0–35.5)[a)]	36.4 (35.8–36.7)[a)]
	ALFX10	37.5 (37.0–37.7)[a)]	36.3 (36.3–36.7)[a)]	35.9 (35.7–36.1)[a)]	35.4 (35.2–35.4)[a)]	34.8 (34.7–34.9)[a)]	34.8 (34.3–35.4)[a)]	35.8 (35.2–36.6)[a)]

Animals were administered IM saline at 1.0 ml/kg (CONT) and IM alfaxalone–HPCD at 2.5 mg/kg (ALFX2.5), 5 mg/kg (ALFX5), 7.5 mg/kg (ALFX7.5), and 10 mg/kg (ALFX10) with a minimum 4–day washout period. Data are expressed as medians (interquartile ranges) obtained from six cynomolgus monkeys. HR: heart rate, RR: respiratory rate, NMABP: noninvasive mean arterial blood pressure, SpO_2: percutaneous oxygen saturation of hemoglobin, RT: rectal temperature. a) Significant difference from CONT treatment at each time point ($P<0.05$). HPCD: 2–hydroxypropyl–beta–cyclodextrin.

was observed in two animals, which received the ALFX7.5 and ALFX10 treatments. The SpO_2 significantly decreased after 10 to 60 min following the ALFX7.5 and ALFX10 treatments. In addition, hypoxemia (SpO_2 <90%) was observed in 1, 3, and 5 animals receiving the ALFX5, ALFX7.5, and ALFX10 treatments, respectively. RT significantly decreased after 20 to 45 min, 10 to 120 min, and 10 to 120 min following the ALFX 5, ALFX7.5, and ALFX10 treatments, respectively. Furthermore, hypothermia (RT <35.9°C) was observed in one animal receiving the ALFX5 treatment and in all the animals that received either the ALFX7.5 or ALFX10 treatment.

DISCUSSION

In this study, the IM administration of alfaxalone-HPCD alone produced a dose-dependent sedative effect as well as cardiorespiratory depression in healthy cynomolgus monkeys. The IM doses of alfaxalone-HPCD of 7.5 and 10 mg/kg produced a relatively rapid onset of lateral recumbency with a deep level of sedation, where the monkeys were immobilized for over 40 min and accepted endotracheal intubation. However, clinically relevant hypotension, hypoxemia, and hypothermia were observed in a number of monkeys that varied across the different treatments. In addition, high incidences of shivering during the recovery period were observed following the ALFX7.5 and ALFX10 treatments. Furthermore, the IM dose of 5 mg/kg alfaxalone-HPCD produced a similar onset of recumbency as the IM doses of 7.5 and 10 mg/kg. This was achieved with a moderate to deep level of sedation,

where the monkeys did not seem to respond to limb manipulation for over 20 min, with signs of minimal cardiorespiratory depression. It can be concluded that an IM dose of 5 mg/kg alfaxalone-HPCD can cause a sedative effect that allows the completion of veterinary procedures, including an intravenous catheterization in healthy cynomolgus monkeys with only minimal cardiorespiratory depression.

It was reported that IM doses of alfaxalone-HPCD alone between 5 and 10 mg/kg produced a relatively rapid onset of sedation with a dose-dependent duration of immobilization in cats [24], dogs [25], and rabbits [12, 13]. In these previous reports, the median or mean times to recumbency that were produced by IM doses of alfaxalone-HPCD were 2.5 and 2.1 min using 5 and 10 mg/kg alfaxalone-HPCD, respectively, in cats [24]; 3.9, 3.8, and 2.8 min using 5, 7.5, and 10 mg/kg, respectively, in dogs [25]; 2.4 and 2.3 min using 6 and 8 mg/kg, respectively, in rabbits [12]; and 4.0 min using 5 mg/kg in rabbits [13]. Furthermore, the durations of immobilization or sedation were 35 and 72 min using 5 and 10 mg/kg alfaxalone-HPCD, respectively, in cats [24]; 36, 87, and 115 min using 5, 7.5, and 10 mg/kg, respectively, in dogs [25]; 51.8 and 58.4 min using 6 and 8 mg/kg, respectively, in rabbits [12]; and 53 min using 5 mg/kg in rabbits [13]. In addition, all dogs accepted endotracheal intubation following the administration of alfaxalone-HPCD at 7.5 and 10 mg/kg IM [25]. Hence, the IM doses of alfaxalone-HPCD alone between 5 and 10 mg/kg produced a dose-dependent sedative effect with a relatively rapid onset in cynomolgus monkeys, which was similar to that previously observed in cats, dogs, and rabbits.

Undesirable issues during recovery, such as nausea, vomiting, ataxia, muscle tremors, opisthotonus-like postures, pronounced limb extension, and paddling were reported to some extent in many animal species receiving intravenous (IV) or IM administration of alfaxalone-HPCD alone [8, 10, 12, 13, 18, 24, 25]. Tamura *et al.* [24] observed ataxia, tremors, and opisthotonus-like postures during the early recovery period after administration of IV alfaxalone-HPCD at 5 mg/kg, as well as after using IM alfaxalone-HPCD at 2.5, 5, and 10 mg/kg. Maney *et al.* [18] reported no significant differences in the quality of recovery between alfaxalone-HPCD and propofol in dogs, where tremors, vocalizing, and paddling were still observed during the recovery from IV administration of alfaxalone-HPCD alone. Tamura *et al.* [25] considered the qualities of recovery from IM alfaxalone-HPCD at 5, 7.5, and 10 mg/kg to be satisfactory in dogs, but all the dogs in this study still exhibited transient muscular tremors and staggering gaits. In addition, tremors, paddling, seizure-like activity, and thrashing were observed during the recovery period in alpacas [8] and calves [10], which were anesthetized with IV alfaxalone-HPCD alone. Huynh *et al.* [12] reported that the recovery from IM alfaxalone-HPCD at 4, 6, and 8 mg/kg was uneventful in rabbits except for one animal that died 10 min after an IM injection of 8 mg/kg. In addition, Ishikawa *et al.* [13] reported that ataxia, nystagmus, trembling, and swing were observed during the recovery period in rabbits receiving IM administrations of alfaxalone-HPCD alone. In the present study, tremors during the recovery period were observed, but the recovery quality was scored as 3 or 4, which was comparable with alfaxalone-HPCD IM doses of 5, 7.5, and 10 mg/kg. On the other hand, hypothermia was observed during the recovery period in all monkeys receiving alfaxalone-HPCD IM doses of 7.5 and 10 mg/kg. Hence, it is speculated that the tremors were caused by a decrease in body temperature (i.e., shivering), rather than neurological symptoms induced by alfaxalone-HPCD. Species specificity in the quality of recovery from anesthesia or sedation has been observed when using alfaxalone-HPCD alone.

In this study, IM administration of alfaxalone-HPCD at 7.5 to 10 mg/kg produced anesthetic effects, which allowed endotracheal intubation in cynomolgus monkeys. However, the unavoidably large IM injection volumes (0.75 to 1 m*l*/kg), owing to the concentration of alfaxalone-HPCD in the approved product (10 mg/m*l*), make their clinical applications more difficult. Furthermore, guidelines by the European Federation of Pharmaceutical Industries and Associations and the European Centre for the Validation of Alternative Methods provide recommendations for the IM dose volume (0.25 m*l*/kg) and maximum IM dose volume (0.5 m*l*/kg) to administer to monkeys [9]. Although all IM drug treatments were completed successfully without the monkeys showing signs of obvious pain-related behavior or discomfort during the IM injections in this study, it is preferable to reduce the IM drug volume to less than 0.5 m*l*/kg and preferably closer to the recommended IM dose volume of 0.25 m*l*/kg from the viewpoint of animal welfare.

It was reported that the IM drug combination of alfaxalone-HPCD with sedative and/or analgesic drugs successfully produced anesthetic effects using volumes that are close to the recommended values in cats [17], dogs [23], and rhesus monkeys [4]. Bertrand *et al.* [4] reported that a subcutaneous administration of a mixture of alfaxalone 2 mg/kg, medetomidine 20 μg/kg, and midazolam 0.3 mg/kg (0.28 m*l*/kg of total administration volume) provided a mean anesthesia time of 56 min in rhesus monkeys, with a complete absence of response to noxious stimuli for at least 20 min. The anesthetic effect produced by this subcutaneous drug mixture of alfaxalone, medetomidine, and midazolam seemed to be adequate for medical treatments. Thus, IM combinations of alfaxalone-HPCD with sedatives and/or analgesics may greatly reduce the required IM injection volume of the approved alfaxalone-HPCD product in cynomolgus monkeys.

In this study, in order to minimize nociceptive stimulation in each monkey, the oscillometric and pulse oximetry methods were employed to measure arterial blood pressure and blood oxygen levels, respectively. However, the oscillometric method was reported to result in a minor underestimation of the arterial blood pressure compared to that obtained using direct arterial blood pressure measurement in rhesus monkeys [11], and the pulse oximetry method was reported to generate a slightly higher blood oxygen level compared to the actual arterial oxygen saturation in cynomolgus monkeys [31]. Despite these minor limitations, these methods were found to be reliable for the evaluation of arterial blood pressure and blood oxygen levels in the present study.

In conclusion, the IM administration of alfaxalone-HPCD alone produced a dose-dependent sedative effect with cardiorespiratory depression in healthy cynomolgus monkeys. An IM dose of alfaxalone-HPCD of 5 mg/kg can cause a deep sedative effect for over 20 min.

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