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Sedative and physiological effects of low-dose intramuscular alfaxalone in rabbits

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ABSTRACT. To evaluate sedative and physiological effects of low dose intramuscular (IM) alfaxalone, six healthy rabbits were administered single IM doses of alfaxalone at 1mg/kg (IM1), 2.5 mg/kg (IM2.5), or 5 mg/kg (IM5) with a minimum of 7-day washout period. Sedative effects were subjectively evaluated using a composite measure scoring system (maximum sedation score of 16) and pulse rate, respiratory rate, non-invasive blood pressure, and percutaneous oxygen-hemoglobin saturation were measured before and after IM alfaxalone. Loss of righting reflex (LRR) was achieved in all rabbits after IM2.5 and IM5 treatments but in only three rabbits after IM1 treatment. Median (interquartile range) times to LRR were 16 min (15–17), 6 min (6–6), and 4 min (4–4), and median durations of LRR were 0.5 min (0–7), 22.5 min (19–27), and 53 min (48–58) after IM1, IM2.5, and IM5 treatments, respectively. The duration of LRR after IM5 treatment was significantly longer than those after IM1and IM2.5 treatments ($P<0.01$). Median value of total sedation scores peaked at 10 min [score 3.5 (3–4)], from 10 min [score 13.5 (12–14)] to 15 min [score 13.5 (12–14)], and from 10 min [score 15 (12–15)] to 15 min [score 15 (14–15)] after IM1, IM2.5, and IM5 treatments, respectively. No rabbit showed circulatory depression and apnea although respiratory rate decreased after IM 2.5 and IM5 treatments. In conclusion, alfaxalone produced a dose-dependent sedative effect and a deep sedation was achieved by alfaxalone at 2.5 mg/kg IM in rabbits.

KEY WORDS: alfaxalone, intramuscular administration, rabbit, sedation

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Alfaxalone (3-alpha-hydroxy-5-alpha-pregnane-11, 20-dione) is a synthetic neuroactive steroid molecule which modulates the gamma-aminobutyric acid A ($GABA_A$) receptor-causing neuro-depression and muscular relaxation [1, 8]. Lower concentrations of alfaxalone facilitate the open state of the $GABA_A$ receptor channel, similar to that produced by benzodiazepine. On the other hand, alfaxalone at higher concentrations could directly activate the $GABA_A$ receptor channel as an agonist, similar to that produced by propofol or barbiturates [8]. Alfaxalone produces a fast induction of anesthesia, a smooth recovery with minimal cardiovascular effect in dogs and cats [10–13, 17]. In the last decade, alfaxalone formulated with 2-hydroxypropyl-beta-cyclodextrin (alfaxalone-HPCD) has been approved as an intravenous anesthetic induction agent for dogs and cats.

Recently, it was reported that a single intramuscular (IM) administration of alfaxalone-HPCD produces a dose-dependent sedative effect in dogs and cats [14–16]. Alfaxalone-HPCD has been also used successfully with IM route in a wide variety of exotic animal species including rabbits [3, 5, 7, 9]. In rabbits, Huynh *et al.* [6] reported that a single IM doses of alfaxalone-HPCD provided a dose-dependent sedation and recommended the doses of 4 to 6 mg/kg with oxygen support.

Rabbit medicine is a rapidly expanding area of veterinary practice and rabbits are now the third most popular mammalian pets in some countries including Japan. In clinical practice as well as research fields, a short-lived moderate sedation is often required to facilitate diagnostic or therapeutic procedures such as X-ray examination, venous cannulation, blood sampling, and dental treatments in rabbits. It is expected that such short-lived moderate sedation can be produced by a single IM dose of alfaxalone-HPCD that are lower than the doses recommended by Huynh *et al.* [6]. To the author's knowledge, however, the sedative effects of a single IM alfaxalone-HPCD alone at doses lower than 4 mg/kg have not been evaluated in rabbits.

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Table 1. Composite measure scoring system for evaluating sedative effect in rabbits

Spontaneous posture	Score	*Jaw relaxation*	Score
Standing	0	Poor	0
Tired and standing	1	Slight	1
Lying but can rise	2	Good	2
Lying with difficulty rising	3		
Unable to rise	4	*General attitude*	Score
		Excitable	0
Placement on side	Score	Awake and normal	1
Resists strongly	0	Tranquil	2
Modest resistance	1	Stuporous	3
Slight resistance	2		
No resistance	3		
		*Total sedation score**	0–16
Response to noise	Score		
Jump	0		
Hears and moves	1		
Hears and twitches ear	2		
Barely perceives	3		
No response	4		

Sedative effects were subjectively evaluated using a composite measure scoring system previously used in dogs [16] and cats [15]. This scoring system was consisted 5 categories (spontaneous posture, placement on side, response to noise, jaw relaxation and general attitude). These categories were rated in score 0 to 2, 0 to 3, or 0 to 4 based on responsiveness expressed by the rabbits. *Total sedation score was calculated as a sum of scores for the 5 categories: spontaneous posture, placement on side, response to noise, jaw relaxation and general attitude.

The present study aimed to preliminary investigate the sedative effect of a single IM alfaxalone-HPCD at lower doses of 1. 2.5, and 5 mg/kg in rabbits. We hypothesized that these lower single IM alfaxalone-HPCD doses would cause a dose-dependent mild to moderate sedation in rabbits.

MATERIALS AND METHODS

Experiment animals

Six healthy female Japanese White Rabbits (9 to 11 months old, 3.4 to 4.5 kg body weight) were used for the present study. The rabbits were judged to be in good to excellent health based upon a physical examination. The rabbits were cared for according to the principles of the "Guide for the Care and Use of Laboratory animals" prepared by Rakuno Gakuen University. The Animal Care and Use Committee of Rakuno Gakuen University approved the present study (Approval No. VH17A21).

Alfaxalone-HPCD administration

The present study used a randomized crossover design. The rabbits were assigned by a computer-generated randomization table to be administered one of three IM treatments on three different occasions with a washout period of 7-day washout period between treatments. The rabbits received three different IM doses of alfaxalone-HPCD (Alfaxan, Meiji Seika Pharma. Ltd., Tokyo, Japan): 1 mg/kg (IM1), 2.5 mg/kg (IM 2.5), and 5 mg/kg (IM 5). The total volumes of alfaxalone-HPCD administered to the rabbits were adjusted to 0.5 m*l*/kg with saline (Otsuka Normal Saline, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The IM doses were injected to the dorsal lumbar muscle of the rabbits by using a 24-gauge, 1-inch needle (TOP injection needle, TOP Co., Ltd., Tokyo, Japan). The rabbits were allowed to breathe room air spontaneously throughout the experiment. Sedative effects and cardio-respiratory valuables were evaluated in the rabbits before (baseline) and at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90 and 120 min after the treatments.

Evaluation of sedative effect

Sedative effects were subjectively evaluated by a composite measure scoring system used in dogs [16] and cats [15]. An observer (Y. I.) was blinded about the treatments. The scoring system consisted of 5 categories: spontaneous posture, placement on side, response to noise, jaw relaxation and general attitude. These categories were rated with a score of 0 to 2 for jaw relaxation, 0 to 3 for placement on side and general attitude, and 0 to 4 for spontaneous posture and response to noise based on the responsiveness expressed by the rabbits (Table 1). Total sedation score was calculated as the sum of scores in the 5 categories (a maximum of 16).

In addition, time to loss of the righting reflex (LRR) after the treatments (time to LRR) and periods between LRR and reappearance of the righting reflex (duration of LRR) were recorded. The righting reflex was evaluated by the presence or absence of resistance when the rabbit were forced to lie on lateral recumbent. The righting reflex was evaluated every 2 min until LRR was

Table 2. Times related to sedative effect and total sedation score at 10 min after intramuscular (IM) administration of alfaxalone-HPCD in rabbits

	Alfaxalone-HPCD administration		
	1 mg/kg IM	2.5 mg/kg IM	5 mg/kg IM
Number of rabbit positioned lateral recumbency with loss of the righting reflex (LRR)	3	6	6
Time to LRR (min)	16 (15–17)	6 (6–6)[a)]	4 (4–4)[a)]
Duration of LRR (min)	0.5 (0–7)	22.5 (19–27)[a)]	53 (48–58)[a,b)]
Total sedation score at 10 min	3.5 (3–4)	13.5 (12–14)[a)]	15 (12–15)[a)]

Data are expressed as median (interquartile range) from 6 rabbits except for time to LRR after IM alfaxalone-HPCD at 1 mg/kg. The time to LRR after the IM alfaxalone-HPCD at 1 mg/kg was obtained from 3 rabbits that lay down. Time to LRR: a time to LRR after the treatments. Duration of LRR: a period between LRR and reappearance of the righting reflex. a) Significant difference from IM alfaxalone-HPCD at 1 mg/kg (P<0.05). b) Significant difference from IM alfaxalone-HPCD at 2.5 mg/kg (P<0.05).

achieved after the treatments. The reappearance of the righting reflex was defined as a recovery of the resistance to lying on lateral recumbency when the rabbits did head up and returned to sternal recumbency.

Measurements of cardio-respiratory valuables

Pulse rate (PR; beats/min), respiratory rate (RR; breath/min), non-invasive mean arterial blood pressure (NMABP; mmHg) and percutaneous oxygen saturation of hemoglobin (SpO_2; %) were recorded as cardio-respiratory valuables. The PR was measured by calculation from the number of pulses over 10 sec that were manually counted or by using a pulse oximeter (Radical-7, Masimo Japan Corp., Tokyo, Japan). The PR and SpO_2 were measured by a pulse oximeter probe (RD rainbow SET-2 Neo, Masimo Corp.) on the left forelimb and connected to the pulse oximeter. The RR was counted by observing thoracic movements. The NMABP was measured by an oscillometric electronic sphygmomanometer (Pet MAP, Ramsey Medical, Inc., Hudson, OH, U.S.A.) with a blood pressure cuff (Size 2.0 cm; Critter Cuff, Ramsey Medical, Inc.) that was placed on the hair clipped right thoracic limb area above the carpus. The arterial blood pressure was measured two times at each assessment and their average of NMABP was recorded.

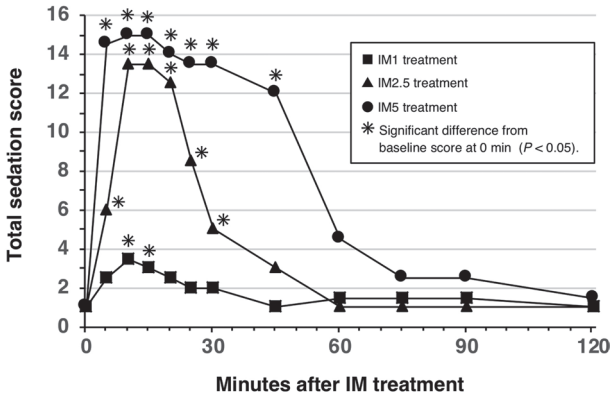

Fig. 1. Total sedation score-time profiles after the intramuscular administration of alfaxalone-HPCD in rabbits. Each plot represented a median value of total sedation score from 6 rabbits. Total sedation score was calculated as a sum of scores in 5 categories shown in Table 1. *Significant difference (P<0.05) from baseline score recorded before the treatments (0 min).

Statistical analysis

EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analyses. Data were expressed as median (interquartile range). The baseline values of each score of 5 categories, total sedation score, and cardio-respiratory valuables, peak values of each score of 5 categories and total sedation scores, times to LRR, and durations of LRR were analyzed using Kruskal-Wallis tests and multiple comparisons using Steel-Dwass test between treatments. The incidences of adverse events were compared using χ^2 test between treatments. In addition, each score of 5 categories, total sedative scores and cardio-pulmonary valuables after the treatments were compared with their correspondent baseline values using Kruskal-Wallis tests and multiple comparisons using Steel test. The level of significance was set at P<0.05.

RESULTS

All rabbits showed discomfort during the IM injection and a short period of excitement were observed in 5 rabbits (83%), 4 rabbits (67%), and 3 rabbits (50%) after IM1, IM2.5, and IM5 treatments, respectively, but there was no significant difference between treatments. A deep level of sedation that made the rabbits lay down with LRR was achieved in 3 rabbits after IM1 treatment and all rabbits after IM2.5 and IM5 treatments. The times to LRR after IM2.5 and IM5 treatments were significantly shorter than that after IM1 treatment (P<0.05, Table 2). The duration of LRR was significantly prolonged in a dose-dependent manner of alfaxalone-HPCD (P<0.05, Table 2).

Figure 1 and Table 3 showed total sedation score-time profiles and the details of each category of sedation score after each treatment. The total sedation scores increased significantly from 10 to 15 min, from 5 to 30 min, and from 5 to 45 min after IM1, IM2.5, and IM5 treatments, respectively (P<0.05). Median value of total sedation scores peaked at 10 min [score 3.5 (3–4)], from

10 min [score 13.5 (12–14)] to 15 min [score 13.5 (12–14)], and from 10 min [score 15 (12–15)] to 15 min [score 15 (14–15)] after IM1, IM2.5, and IM5 treatments, respectively. The total sedation scores at 10 min after IM2.5 and IM5 treatments were significantly higher than that after IM1 treatment (Table 2).

Table 4 showed changes in cardio-respiratory valuables after each treatment. There was no significant change in PR and NMABP although PR seemed to increase transiently after the treatments. On the other hand, RR decreased significantly from 10 to 25 min and from 5 to 30 min after IM2.5 and IM5 treatments, respectively. The median value of RR decreased to minimum values of 48 breaths/min from 15 to 20 min after IM2.5 treatment ($P<0.05$) and 42 breaths/min from 15 to 30 min after IM5 treatment ($P<0.05$). Corresponding with the decrease in RR, SpO_2 values lower than 95% were detected in a rabbit (SpO_2 93%) after IM2.5 treatment and 3 rabbits (SpO_2 89, 91, and 93%) after IM5 treatment. There were some lacks of SpO_2 data after the IM alfaxalone-HPCD treatments.

During recovery period, ataxia was observed in a rabbit that received IM1 treatment, 2 rabbits received IM2.5 treatment and 3 rabbits received IM5 treatment. Nystagmus was observed and remained until the rabbit obtained sternal recumbency in 2 rabbits received IM5 treatment. Trembling and swing were also observed in a rabbit received IM5 treatment. No rabbits showed discomfort (excessive grooming at injection site or avoiding touching the injection site), and swelling, redness and/or other inflammatory changes around the IM injection site after each experiment.

DISCUSSION

As we hypothesized, a single IM alfaxalone-HPCD at a low-dose (1 to 5 mg/kg) provided a dose-dependent sedation in rabbits. Beyond our expectations, a single IM alfaxalone-HPCD at 2.5 mg/kg produced a deep level of sedation that the rabbits lay down with LRR. On the other hand, a single IM alfaxalone-HPCD at 5 mg/kg produced a longer lasting deep sedation in the rabbits. It is considered that a single IM alfaxalone-HPCD at 2.5 mg/kg may be optimal to produce a deep sedation in healthy rabbits.

Alfaxalone produces neuro-depression and muscular relaxation through the modulations of $GABA_A$ receptor-causing neuro-depression and muscular relaxation but does not have analgesic property [1, 8]. Huynh *et al.* [6] reported that the hindlimb withdrawal reflex elicited by a hard pinch with two fingernails on a digit of the hindlimb was present at all time in all rabbits receiving an IM alfaxalone-HPCD alone at higher doses (4 to 8 mg/kg). The objective of the present study was to investigate the sedative effect of a lower single IM alfaxalone-HPCD alone in rabbits. Therefore, we adopted the experimental design that had the least amount of nociceptive stimulation (i.e. manipulations) during the assessment of sedative effect and the cardio-respiratory variables.

Huynh *et al.* [6] reported that the length of sedation defined as the period of time between the disappearance and reappearance of the righting reflex was dose-dependently prolonged and the mean lengths of sedation were 36.9, 51.8, and 58.4 min in the rabbits receiving a single IM alfaxalone-HPCD alone at the doses of 4, 6, and 8 mg/kg, respectively. In the present study, the durations of LRR were also dose-dependently prolonged and the median durations of LRR were 0.5, 22.5, and 53 min in the rabbits receiving the IM alfaxolone-HPCD treatments at doses of 1, 2.5, and 5 mg/kg, respectively. The results of present study and the previous study complement each other to demonstrate a dose-dependent sedative effect of IM alfaxalone-HPCD treatments in rabbits.

All rabbits showed lateral recumbency with LRR after the IM alfaxalone-HPCD treatments at 2.5 and 5 mg/kg, respectively. On the other hand, the IM alfaxalone-HPCD treatment at 1 mg/kg produced lateral recumbency with LRR in only 3 rabbits. These findings indicated that a single IM alfaxalone-HPCD treatment at 2.5 mg/kg is enough to produce deep sedation. The previous studies [14, 15] reported that a single IM alfaxalone-HPCD at 2.5 mg/kg produced a deep level of sedation in dogs and cats. In particular, the changes in the total sedative score after the IM alfaxalone-HPCD treatments in the rabbits were quite similar to those in cats that their levels of sedation were evaluated using the same composite measure scoring system [15]. It seems that species differences in sedative effect produced by a single IM alfaxalone-HPCD treatments may be minimum among dogs, cats, and rabbits.

A single IM volume considered as good practice is up to 0.25 m*l*/kg and the maximal dose volume is 0.5 m*l*/kg in the guidelines by the European Federation of Pharmaceutical Industries Associations (EFPIA) and the European Centre for the Validation of Alternative Methods (ECVAM) [4]. In the present study, all the rabbits showed discomfort during the IM injection and a short period of excitement was observed in many rabbits. Previous reports [15, 16] showed that adverse effect, such as vocalization struggling during the IM administration, was observed in dogs and cats. Michou *et al.* [11] reported that the intravenous use of alfaxalone-HPCD was reported to be less painful than lipid-free propofol because of its neutral pH. Huynh *et al.* [6] reported that an IM alfaxalone-HPCD (dose volumes of 0.4–0.8 m*l*/kg) was well tolerated, but many rabbits reacted during the IM injection. In the present study, it was assumed that a relatively large dose volume (0.5 m*l*/kg) was responsible for the discomfort and excitement after the IM injection.

There was no significant change in PR and NMABP after the IM alfaxalone-HPCD treatments in the rabbits, although PR seemed to increase transiently after the treatments. Huynh *et al.* [6] reported that a similar change in PR was observed following the reaction to the IM injection of alfaxalone-HPCD in rabbits. In the present study, all rabbits showed discomfort during the IM injection and a short period of excitement was also observed in some rabbits. Therefore, the transient increase in PR might be caused by the discomfort during the IM injection. Although minimum cardiovascular depression was observed in the rabbits receiving the lower doses of IM alfaxalone-HPCD, sophisticated cardiovascular measurements such as cardiac output were not employed and arterial blood pressure was measured by non-invasive oscillometric method that had a gap to direct arterial blood pressure [2]. Therefore, further studies will be necessary to confirm the cardiovascular effects of IM alfaxalone-HPCD treatments in rabbits.

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Table 3. Changes in each category of sedation score before and after intramuscular (IM) administration of alfaxalone-HPCD in rabbits

		Minutes after the administration of alfaxalone											
		Baseline	5	10	15	20	25	30	45	60	75	90	120
Spontaneous posture	1 mg/kg IM	0 (0–0)	0 (0–0)	0.5 (0–1)	0.5 (0–1)	0.5 (0–2.5)	0 (0–2.3)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	2.5 mg/kg IM	0 (0–0)	1 (1–2.5)	4 (4–4)	4 (4–4)	4 (4–4)	3 (3–3.8)	2.5 (1–4)	0 (0–2.3)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 mg/kg IM	0 (0–0)	4 (4–4)	4 (4–4)	4 (4–4)	4 (4–4)	4 (4–4)	4 (4–4)	4 (2.5–4)	1 (0.3–2.5)	0 (0–0)	0 (0–0)	0 (0–0)
Placement on side	1 mg/kg IM	0 (0–0)	1 (1–1)	1 (1–1)	1 (1–1.8)	1 (0.3–2.5)	0.5 (0–1.8)	0.5 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	2.5 mg/kg IM	0 (0–0)	1.5 (1–2)	3 (3–3)	3 (3–3)	3 (3–3)	2.5 (1.3–3)	1 (1–2.5)	0.5 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 mg/kg IM	0 (0–0)	3 (3–3)	3 (3–3)	3 (3–3)	3 (3–3)	3 (3–3)	3 (3–3)	3 (2.3–3)	1 (1–1)	0 (0–0)	0 (0–0)	0 (0–0)
response to noise	1 mg/kg IM	1 (1–1)	1.5 (1–2)	1 (1–1)	1 (1–1)	1 (1–1.8)	1.5 (1–2)	1 (1–1.8)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)
	2.5 mg/kg IM	1 (1–1)	2 (1.3–2)	3.5 (3–4)	3.5 (1.5–4)	3 (1.5–3.8)	1.5 (1–2)	2 (1.3–2.8)	1 (1–1.8)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)
	5 mg/kg IM	1 (1–1)	4 (1.8–4)	4 (4–4)	4 (4–4)	4 (3.3–4)	4 (1.8–4)	4 (3.3–4)	2 (1–3.8)	1 (1–1.8)	1.5 (1–2)	1.5 (1–2)	1 (1–1.8)
Jaw relaxation	1 mg/kg IM	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	2.5 mg/kg IM	0 (0–0)	0 (0–0)	1 (1–1)	1 (0.3–1)	1 (0.3–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 mg/kg IM	0 (0–0)	1 (1–1)	1 (1–1)	1 (1–1.8)	1 (1–1)	1 (1–1)	0.5 (0–1)	0 (0–0)	1 (0–0)	2 (0–0)	3 (0–0)	4 (0–0)
General attitude	1 mg/kg IM	0 (0–0)	0 (0–0)	1 (0.3–1)	1 (0.3–1.8)	0 (0–1.5)	0 (0–1.5)	0 (0–0.8)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	2.5 mg/kg IM	0 (0–0)	1.5 (1–2)	2 (2–2)	2.5 (2–3)	2 (2–2)	1.5 (0.3–2)	1 (1–1.8)	1 (0.3–1)	0 (0–0.8)	0 (0–0.8)	0 (0–0.8)	0 (0–0)
	5 mg/kg IM	0 (0–0)	2.5 (2–3)	3 (2.3–3)	3 (2.3–3)	2.5 (2–3)	2 (2–2.8)	2 (2–2.8)	2 (2–2)	1 (1–1)	1 (0.3–1)	0.5 (0–1)	0 (0–0)

Data are expressed as median (interquartile range).

Table 4. Changes in cardio-respiratory valuables before and after intramuscular (IM) administration of alfaxalone-HPCD in rabbits

		Minutes after the administration of alfaxalone											
		Baseline	5	10	15	20	25	30	45	60	75	90	120
PR (beats/min)	1 mg/kg IM	234 (6: 217–245)	249 (6: 235–266)	243 (6: 236–253)	233 (6: 217–239)	255 (6: 247–264)	231 (6: 213–252)	223 (6: 212–232)	240 (6: 236–242)	247 (6: 226–272)	224 (6: 212–244)	230 (6: 220–260)	223 (6: 207–236)
	2.5 mg/kg IM	201 (6: 185–240)	288 (6: 261–300)	285 (6: 248–309)	237 (6: 221–254)	230 (6: 218–238)	235 (6: 227–257)	237 (6: 222–238)	227 (6: 212–240)	223 (6: 217–231)	229 (6: 211–240)	233 (6: 229–266)	223 (6: 211–232)
	5 mg/kg IM	219 (6: 199–240)	269 (6: 247–302)	256 (6: 244–271)	242 (6: 240–253)	244 (6: 225–268)	260 (6: 235–285)	267 (6: 233–275)	233 (6: 221–276)	232 (6: 218–245)	229 (6: 214–234)	232 (6: 215–259)	231 (6: 213–258)
RR (breaths/min)	1 mg/kg IM	186 (6: 180–192)	156 (6: 141–171)	144 (6: 123–171)	161 (6: 135–167)	139 (6: 122–164)	156 (6: 93–228)	126 (6: 120–159)	195 (6: 188–230)	225 (6: 201–257)	183 (6: 180–200)	186 (6: 171–213)	212 (6: 186–228)
	2.5 mg/kg IM	192 (6: 168–210)	116 (6: 100–129)	66 (6: 51–81)[a)]	48 (6: 42–57)[a)]	48 (6: 39–66)[a)]	96 (6: 57–117)[a)]	72 (6: 48–105)	117 (6: 99–149)	162 (6: 147–177)	150 (6: 132–186)	174 (6: 168–213)	174 (6: 147–206)
	5 mg/kg IM	162 (152–200)	54 (6: 39–87)[a)]	48 (6: 39–57)[a)]	42 (6: 32–48)[a)]	42 (6: 32–48)[a)]	42 (6: 36–48)[a)]	42 (6: 32–48)[a)]	45 (6: 38–48)	63 (6: 51–80)	96 (6: 78–105)	126 (6: 111–141)	155 (6: 147–174)
NMABP (mmHg)	1 mg/kg IM	133 (6: 127–137)	143 (6: 114–149)	110 (6: 99–138)	125 (6: 110–129)	118 (6: 104–142)	107 (6: 98–111)	108 (6: 102–109)	114 (6: 107–119)	114 (6: 97–123)	128 (6: 117–133)	108 (6: 104–121)	120 (6: 111–130)
	2.5 mg/kg IM	145 (6: 130–157)	129 (6: 118–143)	117 (6: 110–129)	114 (6: 101–123)	115 (6: 106–126)	116 (6: 104–141)	113 (6: 110–122)	134 (6: 123–141)	139 (6: 120–140)	151 (6: 124–159)	128 (6: 118–131)	145 (6: 135–158)
	5 mg/kg IM	132 (6: 126–153)	115 (6: 106–119)	110 (6: 108–119)	117 (6: 107–129)	112 (6: 112–116)	117 (6: 108–122)	124 (6: 111–135)	133 (6: 117–156)	121 (6: 117–131)	145 (6: 122–159)	128 (6: 122–159)	150 (6: 117–164)
SpO_2 (%)	1 mg/kg IM	97 (6: 95–98)	100 (4: 100–100)	98 (5: 98–99)	98 (5: 97–100)	97 (5: 96–100)	100 (5: 98–100)	100 (5: 98–100)	100 (6: 98–100)	99 (6: 97–100)	99 (6: 98–100)	100 (6: 99–100)	98 (6: 97–99)
	2.5 mg/kg IM	100 (6: 97–100)	97 (4: 97–98)	96 (4: 96–97)	96 (4: 96–97)	100 (5: 98–100)	96 (5: 96–100)	99 (5: 95–100)	100 (6: 97–100)	99 (6: 96–100)	99 (6: 98–100)	100 (6: 98–100)	99 (6: 97–100)
	5 mg/kg IM	98 (6: 97–100)	95 (4: 93–97)	98 (4: 95–98)	95 (3: 92–95)	95 (3: 94–95)	92 (2: 92–93)	95 (3: 93–98)	96 (4: 95–98)	99 (5: 97–100)	100 (5: 100–100)	98 (5: 97–100)	100 (6: 98–100)

Data are expressed as median (number of collected data: interquartile range). There were some lacks of SpO_2 data from 5 to 90 min after the IM alfaxalone-HPCD treatments. PR: pulse rate, RR: respiratory rate, NMABP: non-invasive mean arterial blood pressure, SpO_2: percutaneous oxygen saturation. a) Significant difference from baseline value ($P<0.05$).

In the present study, RR decreased significantly after the IM alfaxalone-HPCD treatments in a dose-dependent manner. Some rabbits showed a moderate hypoxemia corresponding with the decrease in RR. It was reported that spontaneous breathing was maintained but a dose-dependent decrease in RR was observed and clinically relevant hypoxemia developed in dogs and cats receiving an IM alfaxalone-HPCD [15, 16]. Huynh *et al.* [9] reported that apnea was not observed in the rabbits receiving an IM alfaxalone-HPCD at 4 and 6 mg/kg, however, one rabbit that received an IM dose of 8 mg/kg had an episode of apnea and died. In the present study, SpO_2 were measured by using a pulse oximeter that would be sometimes incapable of measuring SpO_2 correctly when PR was too fast or there was insufficient blood flow at the measuring site. Thus, there were some lacks of SpO_2 data during present study. But the low values of SpO_2 indicating hypoxemia were recorded in the rabbits after an IM alfaxalone-HPCD at 2.5 and 5 mg/kg. Further studies will be necessary to confirm the extent of respiratory depression after the IM administration of low-dose alfaxalone-HPCD in rabbits.

During the early stage of recovery period, undesirable effects, such as ataxia, a transient muscular tremor and pisthotonus-like posture, pronounced limb extension and a transient paddling, were observed quite often in dogs and cats receiving an IM alfaxalone-HPCD treatment [15, 16]. On the other hand, such undesirable events were not observed in rabbits during the recovery period of IM alfaxalone-HPCD treatment, except for one rabbit that died 10 min after an IM alfaxalone-HPCD at 8 mg/kg [6]. In the present study, tremors and nystagmus were observed in only one rabbit receiving an IM alfaxalone-HPCD at 5 mg/kg. Further investigation using large population of rabbits will be necessary to confirm the adverse effects of IM alfaxalone-HPCD treatments in rabbits.

In conclusion, a single IM alfaxalone-HPCD alone at a low-dose range of 1 to 5 mg/kg provided a dose-dependent sedation in rabbits with mild adverse effects. In particular, a single IM alfaxalone-HPCD at 2.5 mg/kg produced a deep sedation with LRR lasting for about 20 min.

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