Cardiorespiratory and anesthetic effects of combined alfaxalone, butorphanol, and medetomidine in Thoroughbred horses

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This study evaluated induction of anesthesia and cardiorespiratory and anesthetic effects during maintained anesthesia with the combination of alfaxalone, medetomidine, and butorphanol. Alfaxalone (1.0 mg/kg) was administered to induce anesthesia after premedication with medetomidine (7.0 µg/kg), butorphanol (25 µg/kg), and midazolam (50 µg/kg) in six Thoroughbred horses. Intravenous general anesthesia was maintained with alfaxalone (2.0 mg/(kg·hr)), medetomidine (5.0 µg/(kg·hr)), and butorphanol (30 µg/(kg·hr)) for 60 min. Electrical stimulation of the upper oral mucosa was used to assess anesthetic depth at 10 min intervals during anesthesia. Heart rate (HR), respiratory rate (RR), and mean arterial pressure (MAP) were measured. All horses became recumbent within 1 min after alfaxalone administration. Induction scores were 5 (best) in five horses and 4 in one horse. During the 60-min anesthesia, average HR, RR, and MAP were 35.8 ± 2.6 beat/min, 4.7 ± 0.6 breath/min, and 129 ± 3 mmHg, respectively. No horse moved with electrical stimulation; however, two horses experienced apnea (no respiration for 1 to 3 min). Recovery scores were 5 (best) in two horses and 3 in four horses. These results suggest that alfaxalone is effective for induction and maintenance of anesthesia and analgesia when combined with butorphanol and medetomidine for 60 min in Thoroughbreds. However, respiratory depression might require support.

Key words: *alfaxalone, butorphanol, medetomidine, TIVA, Thoroughbred*

Alfaxalone is a neuroactive steroid that has anesthetic effect by augmenting the inhibitory effects of gamma-amino butyric acid (GABA) on the $GABA_A$ receptor. Alfaxalone has become a commonly used anesthetic agent for induction and maintenance of anesthesia in dogs and cats [9, 10, 12, 13]. In horses, there are a few reports describing alfaxalone use for induction of anesthesia with different premedications, and for maintenance of anesthesia with different combinations of agents [1–5]. Keates *et al.* compared ketamine and alfaxalone for induction of anesthesia in horses following administration of xylazine and guaifenesin [4]. Goodwin *et al.* reported that alfaxalone and medetomidine in combination successfully maintained anesthesia in a Thoroughbred cross and 10 Australian stock horses undergoing field castration [1]. Klöppel *et al.* compared ketamine and alfaxalone for induction and maintenance of anesthesia in ponies undergoing castration [5]. Overall, there is relatively little information available regarding use of alfaxalone in Thoroughbreds and its effectiveness against painful stimulation other than the reports regarding castration (although that procedure is occasionally performed using only sedation and local anesthesia).

This study was conducted to evaluate induction of anesthesia with combined alfaxalone, medetomidine, and butorphanol and to monitor the cardiorespiratory and anesthetic effects during maintenance of anesthesia with this same cocktail combination in Thoroughbred horses. During the recovery from this totally intravenous anesthesia, measurements were made of the plasma concentrations of alfaxalone, medetomidine, and butorphanol in order to

better understand the changes in cardiorespiratory variables that were observed during a 60-min period of anesthesia.

Materials and Methods

A protocol for the study (No. 2014-2) was reviewed and approved by the Animal Use and Care Committee and the Animal Welfare and Ethics Committee of the Japan Racing Association's Equine Research Institute where the study was conducted.

Horses

Six healthy Thoroughbreds (one male, one gelding and four females, average age 5.0 ± 0.9 (SD) years, average weight 489 ± 44 kg) were studied. Food was withheld for about 12 hr before induction of anesthesia. Two 14-ga Teflon® catheters were placed into the left and right jugular veins following injection of a local anesthetic agent.

Anesthesia

Anesthesia was induced with alfaxalone (1.0 mg/kg; Meiji Seika Pharm Co., Ltd., Tokyo, Japan) 10–15 min following premedication with medetomidine (7.0 μg/kg; Meiji Seika Pharm), butorphanol (25 μg/kg; Meiji Seika Pharm) and midazolam (50 μg/kg; Astellas Pharma Inc., Tokyo, Japan). After induction, the horses were placed in lateral recumbency and intubated endotracheally. General anesthesia was maintained with a combination of intravenous alfaxalone (2.0 mg/(kg·hr)), medetomidine (5.0 μg/(kg·hr)), and butorphanol (30 μg/(kg·hr)) for 60 min. Alfaxalone was administered via the catheter in the left jugular vein. Medetomidine and butorphanol were mixed into 500 m*l* of saline and also administered via the catheter in the left jugular vein. The horses breathed spontaneously without mechanical ventilation. A 20-ga Teflon® catheter was placed into the transverse facial artery for measuring blood pressure (BP) and for arterial blood sample collection. The electrocardiogram was recorded with a base-apex lead.

Measurements

Measurements were made starting 10 min after induction and at subsequent 10 min intervals until 60 min had elapsed. Heart rate (HR), respiratory rate (RR), and BP were measured with a bedside monitor (BSM-2301, NIHON KOHDEN, Tokyo, Japan). Arterial blood samples were drawn from the catheter placed into the transverse facial artery, and arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$), and oxygen saturation (SaO_2) were measured with a blood gas/hemoximeter analyzer (ABL 800FLEX, Radiometer, Copenhagen, Denmark). Following those measurements, samples of arterial blood were centrifuged (12,000 × *g*; KH120A, Kubota, Tokyo,

Japan) to measure packed cell volume (PCV). Venous blood samples for measuring the plasma concentrations of alfaxalone, medetomidine, and butorphanol were drawn from the catheter placed into the right jugular vein. Electrical stimuli to confirm sufficiency of anesthetic depth were applied to the upper oral mucosa every 10 min during anesthesia with electrical current (50 V at 5 Hz for 10 ms for 1 min) using an electrical stimulator (SEN-5201, NIHON KOHDEN). The induction and recovery scores were quantified according to the description by Mama *et al.* [6, 7]. The recovery time was measured from cessation of the maintenance infusion of the combined drugs alfaxalone, medetomidine, and butorphanol until the horse was standing.

Analysis of drug concentrations

Alfaxalone in plasma samples were recovered by liquid-liquid extraction using methyl tert-butyl ether with 11-hydroxy progesterone as the internal standard. Butorphanol and medetomidine in plasma samples were extracted using Waters Oasis MCX solid phase extraction cartridges (3 cc 60 mg; Waters Co., Milford, MA, U.S.A.) with 4'-(Imidazol-1-yl) acetophenone as the internal standard. The extracted substances were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS; AB Sciex., Framingham, MA, U.S.A.) by electrospray ionization in positive ion mode. The calibration was carried out using linear standard curves in the ranges of 10 to 500 ng/m*l* for alfaxalone, 0.2 to 10 ng/m*l* for butorphanol, and 5.0 to 100 ng/m*l* for medetomidine. The lower limits of quantitation (LLOQ) of the assays for alfaxalone, butorphanol, and medetomidine were 10 ng/m*l*, 0.2 ng/m*l*, and 5.0 ng/m*l*, respectively. The recovery and repeatability were 94.0% and 4.80% for alfaxalone, 76.5% and 3.74% for butorphanol and 75.1% and 1.96% for medetomidine, respectively.

Results

All horses were induced smoothly and became recumbent within 1 min after alfaxalone administration. The scores for induction in five horses (with the best possible score being 5) were 5 and for one horse was 4. Five horses induced smoothly and collapsed into a recumbent position (Score 5). One horse also induced smoothly and collapsed into a recumbent position, but a slight movement of a forelimb was observed after it was recumbent, lowering its score to 4, although this movement was acceptable.

No horses responded by moving in response to the electrical stimulation, although a palpebral reflex and intermittent nystagmus were observed throughout anesthesia regardless of the simulation.

During the 60-min anesthesia, mean HR, RR, PaO_2, $PaCO_2$, PCV, and SaO_2 were 35.8 ± 2.6 beat/min, 4.7 ±

Table 1. Time course of changes in cardiorespiratory variables and plasma concentrations of alfaxalone, medetomidine, and butorphanol during 60-min of anesthesia following premedication with medetomidine, butorphanol, and midazolam

	Time	10	20	30	40	50	60
Heart rate	(beat/min)	33.2 ± 10.8	38.3 ± 10.8	34.3 ± 9.4	33.5 ± 10.8	36.3 ± 10.1	39.3 ± 14.0
Respiratory rate	(breath/min)	4.2 ± 2.9	4.0 ± 3.7	5.2 ± 2.6	4.5 ± 3.1	5.2 ± 3.5	5.3 ± 2.7
Systolic blood pressure	(mmHg)	169 ± 8	171 ± 15	171 ± 22	164 ± 17	168 ± 24	170 ± 30
Mean blood pressure	(mmHg)	129 ± 5	132 ± 7	129 ± 14	124 ± 12	128 ± 15	130 ± 19
Diastolic blood pressure	(mmHg)	107 ± 1	108 ± 8	109 ± 13	104 ± 12	106 ± 14	107 ± 15
$PaCO_2$	(mmHg)	44.4 ± 5.6	46.3 ± 5.7	47.2 ± 4.4	50.5 ± 4.9	49.3 ± 8.0	45.1 ± 3.3
PaO_2	(mmHg)	55.2 ± 11.6	46.8 ± 19.8	50.8 ± 4.2	47.3 ± 13.8	49.4 ± 14.2	55.3 ± 10.3
Packed cell volume	(%)	38.2 ± 3.4	37.1 ± 3.2	36.5 ± 3.3	37.4 ± 5.3	35.7 ± 3.1	35.3 ± 2.7
Oxygen saturation	(%)	88.5 ± 7.3	83.9 ± 15.7	88.0 ± 2.6	74.1 ± 21.8	81.8 ± 18.7	89.6 ± 3.8
Alfaxalone	(μg/ml)	2.28 ± 0.56	2.14 ± 0.33	2.17 ± 0.26	2.30 ± 0.26	2.33 ± 0.38	2.23 ± 0.31
Medetomidine	(ng/ml)	2.90 ± 0.76	2.82 ± 0.57	2.88 ± 0.45	2.99 ± 0.55	3.02 ± 0.49	2.84 ± 0.72
Butorphanol	(ng/ml)	41.1 ± 8.7	41.8 ± 6.8	41.6 ± 6.9	45.1 ± 6.3	45.7 ± 6.2	41.1 ± 9.7

Mean values (± SD) for six Thoroughbred horses after administration of alfaxalone.

0.6 breath/min, 50.8 ± 3.7 mmHg, 47.1 ± 2.4 mmHg, 36.7 ± 1.1% and 84.3 ± 5.8%, respectively. Systolic, mean, and diastolic blood pressures were 169 ± 2 mmHg, 129 ± 3 mmHg, and 107 ± 2 mmHg, respectively. Table 1 displays the time course of changes in cardiorespiratory variables and plasma concentrations of alfaxalone, medetomidine, and butorphanol during maintenance of anesthesia. Plasma concentrations of the three agents were stable and none of the drugs accumulated in concentration during the 60-min interval.

Two horses experienced apnea (no respiration for 1–3 min). The other four horses maintained RR of more than 2 breath/min. The recovery scores for two horses were 5 (with the best possible score being 5) and for four horses were 3. The mean time from cessation of infusion of maintenance drugs to standing was 68.5 ± 21.5 min (range 47 to 102 min).

Discussion

This study was conducted to evaluate the induction of anesthesia and the cardiorespiratory and anesthetic effects of maintaining anesthesia with the combination of the drugs alfaxalone, medetomidine, and butorphanol. The induction scores for alfaxalone were high and horses so induced tolerated anesthesia safely. During the 60-min anesthesia, although BP tended to be relatively high, it was not clinically hypertensive and average HR and mean BP were stable. None of the horses moved in response to electrical stimulation. These results suggest that alfaxalone is effective at inducing and maintaining anesthesia for 60 min in Thoroughbred horses when combined in a cocktail with butorphanol and medetomidine. The infusion rate of alfaxalone, medetomidine, and butorphanol effectively maintained depth of anesthesia as there were no observa-

tions of movement with electrical stimulation. The plasma concentrations of alfaxalone, medetomidine, and butorphanol were maintained well by the infusion rate used during the 60-min period. However, two horses experienced periods of apnea lasting from 1 to 3 min. Ensuring the availability of respiratory support in the form of a demand valve connected to an oxygen tank may be prudent when using this cocktail in the event that horses experience respiratory depression during maintained anesthesia.

During the maintenance of anesthesia, no movement was observed in response to electrical stimulation, suggesting that the horses experienced sufficient anesthetic depth with the infusion rate used of the three drugs. The mean times from the end of infusion of maintenance drugs to standing was 68.5 ± 21.5 min (range 47–102 min). This recovery time was longer than has been reported in foals, ponies, and Australian stock horses [1, 2, 5]. However, two horses stood in recovery with a single attempt with minimal ataxia (Score 5), and the other four horses stood with two attempts also with minimal ataxia (Score 3). All were acceptable recoveries, although the recovery times were long. None of the horses in this study were ataxic nor demonstrated any muscle abnormalities after recovery. We speculate that high BP during anesthesia may have contributed to the good recoveries.

Mean blood pressure was about 20 mmHg higher than the reported BP in Australian stock horses that were maintained with alfaxalone (2.0 mg/(kg·hr)) and medetomidine (5.0 μg/ (kg·hr)) [1]. Body weights of the Australian stock horses were 309 ± 42.6 kg or about 60% of those of the Thoroughbreds in this study. The difference in body weight may affect the rates of metabolism of the drugs. In this study, butorphanol (30 μg/(kg·hr)) was also included in the cocktail for maintaining anesthesia. It is unlikely that this was the cause of the higher BP observed during anesthesia. Electrical

stimulation did not affect BP; we did not detect any change in BP before or just after the stimulus was applied. It has been reported that the combination of tiletamine, ketamine, and detomidine might produce increased BP in horses [8]. The increase in BP elicited by the combination of tiletamine, ketamine, and detomidine upon simulation of the central nervous system is likely caused by an alternate mechanism from that of alfaxalone. Medetomidine, however, is an alpha-2-adrenoreceptor agonist that causes an increase in systemic vascular resistance. It was reported that mean arterial blood pressure increased when medetomidine was administered by itself to healthy beagle dogs [11]. Therefore, medetomidine may have contributed to the higher BP observed in this study, though further studies are needed to clarify this issue.

Respiratory depression was observed during maintained anesthesia. Respiratory rates in this study were lower than those reported for Australian stock horses [1]. It appears that respiratory depression likely caused the observed low PaO_2 and high $PaCO_2$. Two horses did not ventilate for periods of 1–3 min, which occurred during two separate 10-min measurement intervals. Apnea was not observed to occur with a tendency toward any particular time during anesthesia. The other four horses maintained RR of more than 2 breath/min. The infusion rate for the two horses that experienced bouts of apnea might have been elevated relative to their need for maintaining ventilation and hence caused their apnea. Plasma concentrations of alfaxalone, medetomidine, and butorphanol were constant and none of the drugs appeared to accumulate in concentration during the course of the 60-min anesthetic episode. Mechanical ventilation or oxygen supplementation might have been useful to support oxygenation of these two horses during the anesthetic episode.

In our preliminary study, it was found that induction of anesthesia with alfaxalone (1.0 mg/kg) after premedication with medetomidine (5.0 μg/kg) and butorphanol (25 μg/kg) and maintenance of anesthesia with alfaxalone (2.0 mg/(kg·hr)) and medetomidine (3.0 μg/(kg·hr)) and butorphanol (30 μg/(kg·hr)) did not provide sufficiently good induction or deep anesthesia to prevent movement following electrical stimulus. Therefore, midazolam was added for induction and the medetomidine dose was increased for both premedication and maintenance of anesthesia to obtain a more analgesic effect in this study. The doses used in this study may have had a respiratory depressant effect and elicited a prolonged recovery time. One castration was performed using the same anesthetic protocol as was used for this study, and another castration was performed using the same induction method but maintaining anesthesia with 75% of the dose of alfaxalone that we investigated (1.5 mg/(kg·hr)) but with the same doses of medetomidine and butorphanol

as in this study. Both anesthesias were sufficiently deep for castration and recovery times were 80 min (alfaxalone: 2.0 mg/(kg·hr)) and 46 min (alfaxalone: 1.5 mg/(kg·hr)), respectively. This observation suggests that reducing the infusion rate of alfaxalone for maintenance of anesthesia might result in shorter recovery times.

Acknowledgment

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