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RESEARCH PAPER

Cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in dogs

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Abstract

Objective To determine the cardiorespiratory and anesthetic effects of 2, 6, and 20 mg kg^{-1} IV alfaxalone in hydroxypropyl beta cyclodextrin (Alfaxan) in dogs.

Study design Blinded four-way crossover randomized by dose.

Animals Eight healthy adult purpose-bred mixed breed dogs (four male, four female) weighing between 12 and 28 kg.

Methods Four (0, 2, 6, 20 mg kg^{-1}) IV treatments of alfaxalone were administered to each dog with a 3-hour washout period between doses. Measurements of heart rate, aortic systolic, mean, and diastolic blood pressures, pulmonary arterial and right atrial mean pressures, cardiac output, respiratory rate, tidal and minute volumes, and arterial blood pH, blood gases (PaO_2, $PaCO_2$) were performed prior to and at predetermined intervals after drug administration. Systemic vascular resistance and rate pressure product were calculated. The quality of induction, maintenance, and recovery from anesthesia were categorically scored as was the response to noxious stimulation.

Results The administration of alfaxalone resulted in dose-dependent changes in cardiovascular and respiratory parameters. Decreases in arterial blood pressure and increases in heart rate occurred at higher doses with most variables returning to baseline in 15–30 minutes. Respiratory rate, minute volume, and PaO_2 decreased and apnea was the most common side effect. The duration of anesthesia increased with dose, and induction, maintenance, and recovery were judged to be good to excellent with all doses studied.

Conclusions and clinical relevance Alfaxalone produced good to excellent short-term anesthesia in unpremedicated dogs. Cardiorespiratory effects were minimal at lower doses. Anesthesia was judged to be good to excellent and associated with unresponsiveness to noxious stimulation for the majority of anesthesia. Hypoventilation and apnea were the most prominent and dose-dependent effects.

Keywords alfaxalone, anesthesia, cardiorespiratory.

Introduction

The central depressant and hypnotic effects of progesterone-related steroids were first identified by

Selye (1941). His studies identified that the metabolites of progesterone, pregnanolone, and pregnanedione possessed potent anesthetic, muscle relaxant, and analgesic properties (Selye 1941; Sear 1996). These actions have subsequently been attributed to selective modulation of γ-aminobutyric acid (GABA) type A receptors (Albertson et al. 1992; Franks & Lieb 1994; Goodchild et al. 2000). The most potent anesthetic steroid, pregnanedione, was favorably devoid of endocrine effects but insoluble in water, leading to the synthesis of a water-soluble ester, hydroxydione (Sear 1996). Hydroxydione possessed good to excellent anesthetic qualities in humans and animals, but induction of anesthesia was prolonged, large doses were required, and intravenous administration was hampered by pain on injection and thrombophlebitis (Child et al. 1971; Sear 1996).

Two rapidly acting but hydrophobic steroid anesthetics, alfaxalone (3-hydroxy-5-pregnane-11,20-dione) and alfadolone acetate (acetoxy-3-hydroxy-5-pregnane-11,20 dione), were subsequently developed and solubilized as a three to one mixture (CT1341) in 20% polyethoxylated castor oil (Cremophor-EL) surfactant (Child et al. 1971; Sear 1996). Alfaxalone has almost twice the anesthetic potency of alfadolone (Goodchild et al. 2000; Nadeson & Goodchild 2000). Alfadolone was included in the formulation because it supposedly increased the solubility of alfaxalone in the vehicle and because it possessed some anesthetic and antinociceptive effects (Child et al. 1971; Nadeson & Goodchild 2000). The CT1341 was popularized as an anesthetic for use in humans (Althesin) and animals (Saffan) in the UK and elsewhere, but was never marketed for use in the United States of America.

The cardiorespiratory effects following both single and incremental doses of CT1341 to humans and animals are comparable, but were less pronounced than thiopental, methohexital, ketamine, or propofol, suggesting superior safety (Child et al. 1972; Haskins et al. 1975; Blake & Korner 1981). Despite the apparent safety of CT1341, hypersensitivity reactions related to the diluent (Cremophor-EL) were noted in cats, dogs, and humans (Prys-Roberts & Sear 1980). In cats, Saffan produces hyperemia of the forepaws or pinnae in approximately 70% of animals injected (Haskins et al. 1975; Middleton et al. 1982). In humans and dogs, histamine release attributed to Cremophor-EL resulted in anaphylactoid reactions with hypotension, urticaria, and cutaneous erythema, ultimately leading to the withdrawal of Althesin from human clinical practice (Prys-Roberts & Sear 1980; Mehta 1981) and Saffan not receiving registration for use in dogs.

Both alfaxalone and alfadolone are metabolized by the human liver and the major metabolites are excreted in the urine leading to a dose-dependent duration of action and relatively rapid recovery from anesthesia (Child et al. 1971). Alfaxalone has been reformulated as a clear colorless sterile 1% (w/v) solution in 2-hydroxypropyl-β-cyclodextrin (HPCD). Cyclodextrins are cyclic amylase-derived oligomers produced by bacterial degradation of starch, creating a cone-shaped molecule with a hydrophobic center and hydrophilic exterior (Brewster & Bodor 1990). The use of HPCD increases the solubility of alfaxalone by 375 times and has a single lethal dose in dogs of over 5000 mg kg^{-1} (Brewster & Bodor 1990; Coussement et al. 1990). The formulation of alfaxalone in HPCD is completely devoid of histamine release and is marketed as an injectable anesthetic (Alfaxan) for use in dogs and cats in Australia and New Zealand (Pearson et al. 2003) and more recently in South Africa and the United Kingdom. Experimental studies investigating the cardiorespiratory and anesthetic effects of alfaxalone in dogs and cats suggest minimal cardiorespiratory depression, an excellent safety margin and dose-dependent anesthetic properties (Pearson et al. 2003). The plasma pharmacokinetics of alfaxalone after intravenous bolus administration have also been evaluated (Ferre et al. 2006). Our study was designed to determine the cardiorespiratory and anesthetic effects of clinically relevant doses of alfaxalone to better define the dose required to produce induction and maintenance of short-term anesthesia in dogs.

Materials and methods

Eight, adult, purpose-bred, mixed breed dogs (four male, four female) were used for this study. The dogs had a body mass of 15.4 ± 2.2 kg (mean ± SD) (range 12–28 kg), were 8 months to 10 years of age, not pregnant, and judged to be healthy based upon physical examination, an electrocardiogram, serum chemistry, and hematologic analyses performed prior to anesthesia. Chemistry analysis included assessment of total protein, albumin, sodium, potassium, chloride, alkaline phosphatases, alanine aminotransferase, aspartate aminotransferase, creatin kinase, creatinine, and blood urea nitrogen. Dogs were acclimated for 7 days prior to anesthesia, during which time no medications or vaccinations were administered. The experimental

protocol was approved by the University Institutional Laboratory Animal Care and Use Committee.

Experimental preparation and procedures

The day before the administration of the test solution (day −1), all dogs were anesthetized and surgically prepared for placement of right carotid artery and jugular vein catheter introducers (Arrow International, Inc., Reading, PA, USA). Anesthesia was induced with propofol (6 mg kg^{-1} IV once; PropoFlo; Abbott Laboratories, North Chicago, IL, USA) and maintained with isoflurane (2–3%; IsoFlo; Abbott Laboratories) in oxygen. The catheter introducers were flushed with heparinized saline, subcutaneously tunneled to exit the side of the neck, sutured in place with 1-0 silk, and bandaged. Local anesthetic (Ropivacaine HCl, Naropin; AstraZeneca Pharmaceuticals, Wilmington, DE, USA) was administered at the surgical site to reduce pain. An Elizabethan collar was placed on each dog prior to returning the dogs to their cages.

On the day of the experiment (day 0), dogs were brought to the laboratory and their bandages removed. A 7F Swan-Ganz thermodilution catheter (American Edwards Laboratories, Irvine, CA, USA) was advanced into the right jugular vein through the catheter introducer and positioned such that its distal tip was 1–2 cm distal to the pulmonary valve. A fluid-filled catheter was advanced into the right carotid artery through the introducer and positioned so that its distal tip was in the ascending aorta. Both catheters were electronically connected to P23 pressure transducers, calibrated with a mercury manometer, and connected to the data acquisition system (PO-NE-MAH Data Acquisition Computer; Gould Instruments Systems Inc., Valley View, OH, USA) to allow continuous analog display and digital recording of data. The level of the right atrium was considered the zero pressure point. Lead II electrocardiogram (PO-NE-MAH) and body temperature (Cardiac Output Computer; American Edwards Laboratories) were continuously recorded for each dog. A hand-held volumeter (Medishield; Fraser Harlake, Orchard Park, NY, USA) was attached to a tight-fitting face mask and placed over each dog's muzzle to measure respiratory rate and tidal volume. The same instrument was attached to the end of an endotracheal tube for continuous measurement of respiratory rate and tidal volume following administration of the test anesthetic. Heparinized anaerobic blood samples were collected from the arterial catheter for arterial blood pH and blood gas analysis, including PaO_2 and $PaCO_2$ (Model ABL 500; Radiometer America, Inc., Westlake, OH, USA). Heart rate (beats $minute^{-1}$) and rhythm, left ventricular end-diastolic pressure (mmHg), aortic systolic, diastolic, and mean blood pressures (mmHg), pulmonary artery and right atrial mean blood pressures (mmHg), cardiac output (mL kg^{-1} $minute^{-1}$; thermodilution), respiratory rate (breaths $minute^{-1}$), tidal volume (mL), minute volume (mL $minute^{-1}$), core body temperature (°C), arterial pH, blood gases ($PaCO_2$, PaO_2; mmHg) and standard base excess (mmol L^{-1}) were recorded before and at predetermined times after test article administration. Systemic vascular resistance (SVR; dynes second cm^{-5}) and rate pressure product (bpm mmHg) were calculated prior to and following drug administration.

The quality of anesthetic induction, maintenance, and recovery, and the response to noxious stimulation were graded according to categorized criteria. The times to first head lift, removal of the endotracheal tube, and return to sternal recumbency (minutes) were recorded. Two noxious stimuli were used to determine depth of anesthesia. First, a toe pinch using a 10-cm Rochester-Pean hemostat was applied to the second digit (middle phalanx) of the left rear leg for 30 seconds or until limb withdrawal. In addition, two needle electrodes (Grass Medical Instruments, Quincy, MA, USA) were placed 1 cm apart in the buccal mucosa of the dog's mouth. The wire leads from the electrodes were attached to a stimulator preset to deliver a series of 10 mseconds, 5 Hz, 50 V electrical pulses for 30 seconds (Grass SD-9 Stimulator; Grass Medical Instruments). Each dog's response to toe pinch and buccal mucosal stimulation was categorically rated as: no response = 0; minimal movement = 1; limb withdrawal = 2; limb withdrawal and lifting of the head = 3. Induction to anesthesia was scored as follows: no outward sign of excitement, rapidly assumes lateral recumbency, good muscular relaxation, easily intubated within 60 seconds of finishing dosing = 1; mild signs of excitement, some struggling, may or may not be intubated within 60 seconds of finishing dosing = 2; hyperkinesis, obvious signs of excitement, vocalization, defecation or urination, cannot be intubated = 3. Maintenance of anesthesia was scored as follows: no tongue flicking and head shaking, maintains lateral recumbency and immobilization, minimal muscle tremors or twitching, no response to

noise = 1; occasional tongue flicking and head shaking, frequent movement, short duration of lateral recumbency and numerous attempts to rise immediately after assuming lateral recumbency, some muscle tremors and twitching = 2; constant tongue flicking and head shaking, does not become laterally recumbent or assumes lateral recumbency briefly, muscle rigidity accompanied with twitching, vocalization, defecation and responds to noise = 3. Recovery from anesthesia was scored as follows: assumes sternal recumbency with little or no struggling, and attempts to stand and walk with little or no difficulty = 1; some struggling, requires assistance to sternal recumbency or standing, responsive to external stimuli, becomes quiet in sternal recumbency = 2; prolonged struggling, unable to assume sternal recumbency or difficulty in maintaining sternal or standing position, becomes hyperkinetic when assisted, prolonged paddling and swimming motion = 3. The duration of apnea was calculated as the difference in minutes and seconds between the onset of lateral recumbency and the first inspiratory effort. If positive pressure ventilation was used (PaO_2 < 60 mmHg), the duration of apnea was calculated as the difference in minutes and seconds between the onset of lateral recumbency and the onset of positive pressure ventilation. Positive pressure ventilation was instituted with 100% oxygen at six breaths minute^{-1} if PaO_2 decreased below 60 mmHg and continued until spontaneous breathing supported a PaO_2 > 80 mmHg.

Experimental plan

The study was conducted as a blinded four-way crossover randomized by dose. Three intravenous doses (2, 6, and 20 mg kg^{-1}) of alfaxalone in HPBC (Alfaxan; Jurox Pty Ltd, Rutherford, NSW, Australia) or a control (0.0 mg kg^{-1}; 0.09% NaCl; Baxter Healthcare Corporation, Deerfield, IL, USA) were administered in random order. Each dose was separated by a 3-hour washout period. The doses were chosen based on the selection of 2 mg kg^{-1} IV Alfaxan as the labeled dose in the dog, with the 6 and 20 mg kg^{-1} doses equivalent to 3 and 10 times the anticipated clinical dose, respectively. Each dose was infused at a constant rate over 60 seconds. The washout interval was chosen as approximately five to six times the terminal elimination half-life (Ferre et al. 2006). The tracheas of all dogs were intubated and the animals were allowed to breathe room air. The time of dosing was recorded as the time at which the administration of each dose was finished. All data were collected 1 hour (−60 minutes) and 5 minutes (−5 minutes) before dosing, and at 1, 5, 10, 15, and 30 minutes after dosing. Thereafter, data were collected at 10 minute intervals until the dog was responsive to noxious stimuli (i.e., score >2). Arterial pH and blood gas values were recorded at −5, 1, 5, 15, and 30 minutes after dosing, and repeated at 10 minute intervals until the dog responded to a noxious stimulus.

Statistical analysis

Numerical variables were analyzed separately using analysis of variance for repeated measures, with dose as the independent variable. If differences were found, *post-hoc* analyses (Dunett's, Tukey's) were performed to identify differences compared to baseline values and among dose groups (Systat Software, Inc., San Jose, CA, USA). Categorical data were analyzed using nonparametric procedures. A $p < 0.05$ was considered significant.

Results

All dogs finished all phases of the study and were judged to be in excellent health based on the results of a physical examination, ECG and complete blood count the day before and day after the experimental day. There were no differences in any cardiovascular, respiratory, pH and blood gas (PaO_2, $PaCO_2$) value at baseline, −60, and −5 minutes (Tables 1 & 2). The administration of alfaxalone produced dose-dependent changes in cardiovascular, respiratory, pH, and blood gas (PaO_2, $PaCO_2$) values (Tables 1 & 2). The duration of anesthesia increased with increasing doses of alfaxalone (Table 3). Induction, maintenance, and recovery from anesthesia were judged to be good to excellent and uneventful (Table 3).

The administration of 6 and 20 mg kg^{-1} alfaxalone increased heart rate and produced dose-dependent decreases in arterial (systolic, diastolic, mean) blood pressure, and mean pulmonary arterial pressure. Cardiac output and mean right atrial pressure (data not reported) did not change. Systemic vascular resistance and the rate pressure product did not change after the administration of 2 mg kg^{-1} IV alfaxalone. Changes in these hemodynamic variables were numerically greater after the administration of 6 and 20 mg kg^{-1} IV alfaxalone but there were no significant changes (Table 1). The mean pulmonary arterial pressure

Table 1 Hemodynamic averages and standard deviation values (n = 8 dogs unless specified)

	Time (minutes)							
Variable dose (mg kg^{-1})	**−60**	**−5**	**1**	**5**	**10**	**15**	**30**	**60**
HR (bpm)								
0	146 ± 25	124 ± 21						
2	126 ± 13	120 ± 21	155 ± 18	143 ± 17**				
6	124 ± 26	112 ± 28	158 ± 33*	150 ± 20*	144 ± 18*	137 ± 22*	128 ± 38**	
20	127 ± 28	128 ± 33	162 ± 16*	131 ± 9	139 ± 19	134 ± 12	128 ± 15	128 ± 28
SAP (mmHg)								
0	152 ± 14	145 ± 19						
2	140 ± 11	143 ± 14	139 ± 24‡	133 ± 13‡**				
6	152 ± 11	130 ± 14	145 ± 26§	122 ± 23§	125 ± 22§	125 ± 16	124 ± 15**	
20	145 ± 11	147 ± 15	118 ± 34*‡§	79 ± 12*‡§	93 ± 27*§	116 ± 19*	121 ± 19*	131 ± 22*
DAP (mmHg)								
0	109 ± 21	102 ± 15						
2	99 ± 12	100 ± 13	99 ± 15	98 ± 8‡**				
6	112 ± 14	100 ± 12	108 ± 30§	87 ± 18§	93 ± 16§	94 ± 12	92 ± 6**	
20	101 ± 14	103 ± 15	77 ± 28*‡§	56 ± 14*‡§	67 ± 25*§	83 ± 12*	87 ± 14	97 ± 11
MAP (mmHg)								
0	126 ± 15	120 ± 12						
2	117 ± 9	120 ± 12	115 ± 18	114 ± 9‡**				
6	128 ± 10	113 ± 12	122 ± 28§	101 ± 18*§	107 ± 17§	107 ± 12	105 ± 8* **	
20	121 ± 11	123 ± 13	94 ± 29*§	65 ± 13*‡§	77 ± 25*§	97 ± 13*	102 ± 15*	112 ± 13
PAP (mmHg)								
0	20 ± 5	19 ± 7						
2	18 ± 3	17 ± 5	16 ± 6	15 ± 4**				
6	16 ± 5	21 ± 4	15 ± 4*	14 ± 5*	13 ± 3*	13 ± 4*	18 ± 3* **	
20	15 ± 4	18 ± 4	19 ± 6	12 ± 5*	13 ± 5*	13 ± 6	13 ± 5*	12 ± 4*
CO (mL kg^{-1} minute^{-1})								
0	240 ± 53	246 ± 48						
2	247 ± 46	229 ± 60	245 ± 53	231 ± 77**				
6	240 ± 44	218 ± 62	238 ± 80*	222 ± 71	222 ± 66	247 ± 51	215 ± 78**	
20	267 ± 28	246 ± 71	221 ± 60*	190 ± 64*	222 ± 56*	236 ± 4	228 ± 45	196 ± 46

Table 1 (*Continued*)

	Time (minutes)							
Variable dose (mg kg^{-1})	**−60**	**−5**	**1**	**5**	**10**	**15**	**30**	**60**
SVR (dynes second cm^{-5})								
0	2808 ± 881	2556 ± 602						
2	2465 ± 580	2865 ± 1207	2537 ± 980	2906 ± 1739**				
6	2777 ± 579	2830 ± 905	2803 ± 1057	2441 ± 1004	2666 ± 1058	2230 ± 386	2709 ± 945**	
20	2337 ± 397	2657 ± 654	2180 ± 664	1781 ± 350	1735 ± 359	2118 ± 446	2294 ± 374	2949 ± 435
RPP (bpm mmHg)								
0	18 404 ± 4526	14 920 ± 3317						
2	14 789 ± 1936	14 184 ± 2067	17 835 ± 3403	16 203 ± 2282**				
6	15 679 ± 2668	12 855 ± 4290	19 119 ± 5808	14 964 ± 2544	15 349 ± 2041	14 676 ± 3141	13 358 ± 3674**	
20	15 334 ± 3713	15 734 ± 4522	15 107 ± 4863	8594 ± 1805	10 777 ± 4158	13 036 ± 2262	13 040 ± 2835	14 487 ± 4424

HR: heart rate; SAP: systolic arterial pressure; DAP: diastolic artery pressure; MAP: mean arterial pressure; PAP: pulmonary artery pressure; CO: cardiac output; SVR: systemic vascular resistance; RPP: rate pressure product.

*Significant difference from minute −5 during a dose; ‡significant difference between 2 and 20 mg kg^{-1} doses for a reading; §significant difference between 6 and 20 mg kg^{-1} doses for a reading; **n = 6 dogs.

Table 2 Respiratory and blood gas averages and standard deviation values (n = 8 dogs unless specified)

Variable dose (mg kg^{-1})	Time (minutes)							
	−60	−5	1	5	10	15	30	60
RR (bpm)								
0	35 ± 35	22 ± 8						
2	33 ± 36	32 ± 36	25 ± 14	20 ± 10§§				
6	17 ± 7	17 ± 6	9 ± 9*¶	13 ± 8*¶	16 ± 9	20 ± 12	30 ± 26*§§	
20	18 ± 9	31 ± 37	6 ± 4*¶	9 ± 5*§	11 ± 9*	12 ± 8*	15 ± 11*	19 ± 10‡‡
Tidal volume (mL)								
0	204 ± 72	217 ± 55						
2	228 ± 102	212 ± 46	186 ± 117	210 ± 119§§				
6	223 ± 55	234 ± 78	198 ± 92	216 ± 139	254 ± 161	238 ± 101	208 ± 93§§	
20	274 ± 69	230 ± 68	179 ± 71*	179 ± 99*	254 ± 218	296 ± 205	311 ± 178	246 ± 128‡‡
Minute volume (mL)								
0	8355 ± 11353	4740 ± 2191						
2	8083 ± 11382	5890 ± 3106	3739 ± 1778‡§	3280 ± 732*‡§ §§				
6	3918 ± 2063	3789 ± 1668	1526 ± 986*¶	2318 ± 1049*‡¶	3095 ± 1268¶	3790 ± 1049¶	4586 ± 2086§§	
20	5665 ± 3155	6008 ± 6186	1136 ± 759*§	1330 ± 594*§¶	1953 ± 1451*¶	2520 ± 746*¶	3363 ± 1517*	3820 ± 886*‡‡
pH								
0	7.38 ± 0.02	NA			NA			
2	7.39 ± 0.02	NA	7.36 ± 0.04	7.38 ± 0.03§§	NA			
6	7.39 ± 0.02	NA	7.35 ± 0.01	7.33 ± 0.03	NA	7.36 ± 0.02	7.38 ± 0.05§§	
20	7.39 ± 0.02	NA	7.35 ± 0.02	7.29 ± 0.05	NA	7.29 ± 0.04	7.34 ± 0.03	7.39 ± 0.01‡‡
PCO_2 (mmHg) [kPa]								
0	32 ± 2 [4.3 ± 0.3]	NA			NA			
2	32 ± 2 [4.3 ± 0.3]	NA	34 ± 3 [4.5 ± 0.4]	33 ± 3 [4.4 ± 0.4]§§	NA			
6	32 ± 3 [4.3 ± 0.4]	NA	35 ± 7 [4.7 ± 0.9]	39 ± 4 [5.2 ± 0.5]†	NA	36 ± 2 [4.8 ± 0.3]	33 ± 3 [4.4 ± 0.4]§§	
20	31 ± 3 [4.1 ± 0.4]	NA	39 ± 2 [5.2 ± 0.3]	43 ± 5 [5.7 ± 0.7]†	NA	43 ± 7 [5.7 ± 0.9]†	36 ± 4 [4.8 ± 0.5]	32 ± 2 [4.3 ± 0.3]‡‡
PO_2 (mmHg) [kPa]**								
0	100 ± 4 [13.3 ± 0.5]	NA			NA			
2	109 ± 18 [14.5 ± 2.4]	NA	79 ± 10 [10.5 ± 1.3]†‡§	87 ± 15 [11.6 ± 2.0]§§	NA			
6	97 ± 9 [12.9 ± 1.2]	NA	58 ± 13 [7.7 ± 1.7]†‡¶	73 ± 10 [9.7 ± 1.3]†	NA	89 ± 9 [11.9 ± 1.2]	94 ± 9 [12.5 ± 1.2]§§	
20	99 ± 4 [13.2 ± 0.5]	NA	43 ± 17 [5.7 ± 2.3]§¶	352 ± 151 [46.9 ± 20.1]¶¶	NA	87 ± 27 [11.6 ± 3.6]††	101 ± 20 [13.5 ± 2.7]	107 ± 21 [14.3 ± 2.8]‡‡

Table 2 (*Continued*)

Variable dose (mg kg^{-1})	Time (minutes)							
	−60	−5	1	5	10	15	30	60
SBE (mmol L^{-1})								
0	−5.6 ± 1.4	NA			NA			
2	−5.5 ± 1.6	NA	−5.6 ± 1.7	−5.5 ± 1.3§§	NA			
6	−5.2 ± 1.8	NA	−5.8 ± 3.8	−5.1 ± 1.2	NA	−4.9 ± 0.9	−5.4 ± 1.3§§	
20	−5.5 ± 1.8	NA	−4.2 ± 1.4	−5.4 ± 1.6	NA	−5.8 ± 1.1	−5.5 ± 1.7	−5.2 ± 1.6‡‡
Temperature (°C)								
0	38.7 ± 0.3	38.7 ± 0.4						
2	38.9 ± 0.4	38.8 ± 0.4	38.2 ± 0.3	38.2 ± 0.3§§				
6	38.6 ± 0.4	38.7 ± 0.4	38.0 ± 0.5	38.0 ± 0.4	37.8 ± 0.4	37.6 ± 0.4		
20	38.8 ± 0.4	38.9 ± 0.5	38.2 ± 0.4	38.1 ± 0.6	37.9 ± 0.5	37.8 ± 0.5	37.4 ± 0.6§§	37.0 ± 0.7‡‡

RR: respiratory rate; SBE: standard base excess.

*Significant difference from minute −5 during a dose; †significant difference from minute −60 during a dose; ‡significant difference between 2 and 6 mg kg^{-1} doses for a reading; §significant difference between 2 and 20 mg kg^{-1} doses for a reading; ¶significant difference between 6 and 20 mg kg^{-1} doses for a reading; **calculations based on dogs breathing room air; ††all dogs at this time point were breathing room air; ‡‡*n* = 7 dogs; §§*n* = 6 dogs; ¶¶three dogs supplemented with 100% oxygen.

Table 3 Anesthesia data (see the text for the scales used for the scored variables)

Dose	2 mg kg^{-1} (n = 8)	6 mg kg^{-1} (n = 8)	20 mg kg^{-1} (n = 8)
Parameter	**Average ± standard deviation**		
Time until lateral (minutes)*	0.9 ± 0.3	0.5 ± 0.1	0.5 ± 0.4
Time until intubation (minutes)†	0.7 ± 1.1	0.4 ± 0.4	0.6 ± 1.0
Duration of nonresponsiveness to noxious stimulus (minutes)‡	9.3 ± 2.9	32.0 ± 7.1	69.7 ± 23.5
Duration of anesthesia (minutes)§	9.8 ± 2.4	31.4 ± 6.9	75.1 ± 18.9
Duration of apnea (minutes)	0.5 ± 0	0.7 ± 0.3	1.0 ± 0.7
Quality of induction¶	1.3 ± 0.5	1.0 ± 0.0	1.0 ± 0.0
Quality of anesthesia¶	2.0 ± 0.5	1.4 ± 0.5	1.0 ± 0.0
Quality of recovery¶	1.4 ± 0.5	1.6 ± 0.7	1.3 ± 0.5
Overall anesthesia score	4.6 ± 1.2	4.0 ± 0.5	3.3 ± 0.5

*From start of injection; †from end of injection; ‡from induction; §from induction to extubation; ¶scored from 1 to 3 (description in text).

remained decreased for over 60 minutes in dogs administered with 20 mg kg^{-1} IV alfaxalone. The 20 mg kg^{-1} IV dose of alfaxalone produced consistent decreases in all hemodynamic variables except heart rate. Several cardiovascular variables increased above baseline values during recovery from anesthesia (Table 1).

The administration of alfaxalone produced dose-dependent decreases in respiratory rate, minute volume, and PaO_2 (Table 2). This effect was most pronounced after the administration of 20 mg kg^{-1} IV dose and persisted for approximately 15 minutes. Tidal volume remained relatively unchanged from baseline (−5-minute) values (Table 2). Three dogs administered with 6 and 20 mg kg^{-1} IV alfaxalone had variable periods of apnea lasting 1–3 minutes (Table 3). The duration of apnea was directly related to the dose of alfaxalone and was more frequent in dogs administered with 6 and 20 mg kg^{-1} (Table 3) *versus* the 2 mg kg^{-1} dose. The $PaCO_2$ did not change after the administration of 2 mg kg^{-1} IV alfaxalone but increased after the administration of 6 and 20 mg kg^{-1} (Table 2). The administration of alfaxalone produced insignificant dose-dependent decreases in arterial pH (Table 2). This decrease in pH was greatest between 5 and 15 minutes after the administration of the 20 mg kg^{-1} IV alfaxalone and returned to baseline values by 60 minutes. The base excess did not change at any time or following any dose of alfaxalone (Table 2).

The quality of induction, maintenance, and recovery from anesthesia were judged to be good to excellent following the administration of alfaxalone (Table 3). Transition to lateral recumbency and recovery from anesthesia were smooth, excitement free, and uneventful. Each of the three doses of alfaxalone was administered at a constant rate over 1 minute, resulting in the time to lateral recumbency being inversely proportional to the dose administered. Induction to anesthesia was characterized by quiet, uneventful relaxation to sternal, and lateral recumbency in all but one dog. The average times to lateral recumbency for 2, 6, and 20 mg kg^{-1} were less than 1 minute from the beginning of drug injection (Table 3). One dog experienced a longer time to lateral recumbency (1.6 minutes) after the administration of 20 mg kg^{-1} IV. The range of induction times in the remaining dogs was 0.3–0.5 minutes. There were no significant differences among the three drug doses for time to endotracheal intubation. One dog had a longer time to orotracheal intubation when it received 2 and 20 mg kg^{-1} doses of alfaxalone. This dog also displayed a brief period (<10 seconds) of head shaking immediately after the 2 mg kg^{-1} IV dose of alfaxalone. The trachea of the dog was easily orotracheally intubated and the anesthetic period proceeded uneventfully from that point forward.

The maintenance phase of anesthesia was characterized by good muscle relaxation and little or no response to noxious stimuli (Table 3). The anesthetic maintenance score, like the induction score, improved with increases in drug dose (Table 3). Recovery scores were not different from each other (Table 3).

Discussion

Our study showed that alfaxalone produced good to excellent short-term anesthesia in unpremedicated dogs and had few adverse effects across a relatively wide dose range. Anesthetic induction was good to excellent in all dogs. The maintenance of anesthesia was characterized by excellent muscle relaxation and minimal or no response to noxious stimulation. Cardiorespiratory effects were dose dependent, with larger doses producing increased cardiopulmonary depression. The most important and clinically relevant adverse effect noted was dose-dependent respiratory depression. Most hemodynamic, respiratory, acid-base, and blood gas ($PaCO_2$) data, except PaO_2, were within normal limits for dogs administered with 2 and 6 mg kg^{-1} doses of alfaxalone.

Early studies evaluating the anesthetic and cardiorespiratory effects of neurosteroid anesthetics (alfaxalone, hydroxydione) in animals and humans determined that they produced minimal cardiorespiratory depression, good muscle relaxation, pleasant, uneventful recovery, and possessed a high therapeutic index (Campbell et al. 1971; Child et al. 1971, 1972; Savege et al. 1972; Haskins et al. 1975; Prys-Roberts & Sear 1980). Adverse effects were uncommon other than pain upon injection (hydroxydione), a variable duration (minutes) to maximal drug effect, and, rarely, vomiting during recovery (Campbell et al. 1971). More focused studies designed to define the anesthetic and cardiorespiratory effects of the neurosteroid drug combination alfaxalone–alfadolone in dogs and cats demonstrated rapid induction to anesthesia, minimal to mild respiratory depression, rapid recovery from anesthesia with few adverse side effects (reddening of the ears, edema of the paws and face due to the formulation vehicle, Cremophor-EL), and a wide safety margin (Child et al. 1971; Haskins et al. 1975). One study suggested that the anesthetic and cardiorespiratory effects of the alfaxalone–alfadolone drug combination in cats were superior to thiopental, methohexital, pentobarbital, propanidid, and ketamine (Child et al. 1972). This conclusion was based upon comparisons of therapeutic dose range and assessment of anesthetic quality and cardiovascular effects. Transient tachycardia and hypotensive effects have been noted immediately after drug administration when larger doses (19.2 mg kg^{-1}, IV) were administered (Child et al. 1972; Dyson et al. 1987). Favorably, CT1341 produced less respiratory depression at equianesthetic doses than the other drugs evaluated (Child et al. 1972). Subsequent studies have demonstrated that pain on injection, reddening of the ears, and edema of the paws were attributable to the formulation diluent (Cremophor-EL) and were not effects caused by either of the drugs (Middleton et al. 1982; Sear 1996).

The effects of anesthetic drugs on cardiovascular function are frequently assessed by evaluation of heart rate and rhythm, arterial blood pressure, and cardiac output. Cardiac output is dependent upon heart rate, venous return (preload), vascular impedance (resistance + reactance; afterload), and cardiac contractile force. Increases in heart rate, preload, and cardiac contractile force and decreases in afterload all result in increased cardiac output. Our study suggests that cardiac output does not change or increases minimally after the IV administration of 2 and 6 mg kg^{-1} of alfaxalone. This effect was most likely due to a transient increase in heart rate and small decreases in peripheral vascular resistance (decreased afterload) secondary to peripheral vasodilation (Blake & Korner 1981). We did not assess cardiac contractility and are thus unable to determine its contribution to changes in cardiac output. We suspect that, if affected, contractility was minimally changed at the 2 and 6 mg kg^{-1} doses because changes in cardiac output were small.

Hemodynamic changes were more pronounced after the administration of 20 mg kg^{-1} alfaxalone. For example, the average systolic arterial blood pressure decreased below 80 mmHg (79 ± 12) at the 5 minute recording, a value reported to be representative of hypotension in anesthetized dogs (Haskins et al. 2005). Qualitatively similar results have been reported in cats when doses of Saffan greater than 12 mg kg^{-1} IV were administered (Dyson et al. 1987). Heart rate increased initially while cardiac output and SVR remained unchanged during this same time period in our study, suggesting that cardiac contractile force might have decreased. The rate pressure product also decreased due to decreased arterial blood pressure, suggesting a decrease in myocardial oxygen consumption. Most of the hemodynamic changes did not return to baseline values within 60 minutes after the administration of the 20 mg kg^{-1} IV dose of alfaxalone, suggesting that peripheral vasodilation with a possible decrease in cardiac contractile force were the primary causes for hypotension.

Respiratory depression and apnea are common following the administration of most intravenous anesthetics (thiopental, propofol, ketamine) to cats and dogs (Muir et al. 2007). The slope of the minute volume-CO_2 curve is shifted down and to the right and inspiratory occlusion pressure, a measure of central nervous system respiratory drive, is decreased (Lerche & Muir 2004). Respiratory depression has been linked to anesthetic depression of the cortex and brainstem, increased GABA concentration, and decreased quantities of excitatory glutamate neurotransmitters in the central nervous system (Lambert et al. 2003). Our studies showed that 2 mg kg^{-1} IV alfaxalone produced minimal changes in respiratory rate, variable effects upon tidal volume, and decreases in minute volume. Minute volume remained within normal values for conscious dogs (Gaudy et al. 1984, 1986; Suria et al. 1988). Apnea did not occur at this dose and $PaCO_2$ did not change, although PaO_2 decreased to values that would be considered hypoxemic (79 ± 10) at the 1-minute recording period. The administration of 6 and 20 mg kg^{-1} IV alfaxalone did produce respiratory depression, with early decreases in respiratory rate and minute volume and an increased incidence of apnea. In addition, the increase in $PaCO_2$ and decrease in PaO_2 were apparent after the 6 and especially the 20 mg kg^{-1} IV doses. Similar respiratory responses have been observed in normal dogs administered with an anesthetic 'induction dose' of 6 mg kg^{-1} IV thiopental or propofol (Muir & Gadawski 1998). We did not compare the anesthetic potency of alfaxalone to other injectable anesthetics, but think that alfaxalone produced less respiratory depression than thiopental and propofol based upon the results of this and earlier studies (Child et al. 1971). Clinical trials will need to be completed to further evaluate this issue.

Definitions of anesthesia generally incorporate the production of unconsciousness, from which the animal cannot be physically aroused, muscle relaxation, and analgesia. Furthermore, the rapid attainment of an anesthetic state, uneventful maintenance period, and rapid recovery from general anesthesia are considered important qualities. Alfaxalone produced rapid and excitement free induction to anesthesia, uneventful maintenance, good to excellent muscle relaxation, and stress free recovery from anesthesia. Only one dog administered with 6 mg kg^{-1} IV alfaxalone demonstrated a temporary period of head shaking that did not interfere with orotracheal intubation. The average time to sternal recovery was relatively rapid, ranging from approximately 20 to 80 minutes following the 2 and 20 mg kg^{-1} doses, respectively. These qualitative and quantitative anesthetic effects are comparable to those reported for CT1341 in cats (Evans et al. 1972) and a safety trial investigating the effects of 9, 16 and 30 mg kg^{-1} IV alfaxalone in cyclodextrin in dogs (Pearson et al. 2003). One study investigating the anesthetic, cardiovascular, respiratory, and adverse effects of CT1341, thiopental, methohexital, pentobarbital, propanidid, and ketamine suggested that neurosteroid anesthesia produced better quality anesthesia with fewer adverse effects (Child et al. 1972). We did not compare the anesthetic, cardiovascular, respiratory, and adverse effects of the IV doses of alfaxalone to any of the aforementioned drugs but based upon our results and clinical experience were impressed by the quality of anesthesia and relative absence of adverse effects following the 2 mg kg^{-1} IV dose.

In conclusion, our data suggest that alfaxalone produced effective anesthesia in dogs. Induction of anesthesia was rapid and uneventful. The maintenance and recovery periods were characterized by good to excellent muscle relaxation. Cardiovascular status was well maintained when a dose of 6 mg kg^{-1} IV was administered. Respiratory depression and apnea were the only notable disadvantages when a larger dose (>6 mg kg^{-1} IV) was administered. Alfaxalone provides an alternative to currently available injectable anesthetics when administered for short surgical procedures.

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