Effects of the α_2-adrenoceptor agonist medetomidine on the distribution and clearance of alfaxalone during coadministration by constant rate infusion in dogs

Rachel C. Bennett VetMB

Kati M. Salla DVM

Marja R. Raekallio DVM PhD

Mika Scheinin MD, PhD

Outi M. Vainio DVM, PhD

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From the Departments of Equine and Small Animal Medicine, University of Helsinki, 00014 Helsinki, Finland (Bennett, Salla, Raekallio, Vainio); School of Veterinary Medicine, University College Dublin, Dublin 4, Ireland (Bennett); Department of Pharmacology, Drug Development and Therapeutics, University of Turku, 20014 Turku, Finland (Scheinin); and Unit of Clinical Pharmacology, Turku University Hospital, 20521 Turku, Finland (Scheinin). Ms. Bennett's present address is Anderson Moores, The Granary, Bunstead Barns, Poles Ln, Hursley, Winchester, SO21 2LL Hampshire, England.

Address correspondence to Ms. Bennett (rachel.bennett@helsinki.fi).

OBJECTIVE
To assess the possible impact of medetomidine on concentrations of alfaxalone in plasma, when coadministered as a constant rate infusion (CRI) to dogs, and to determine the possible impact of medetomidine on the cardiopulmonary effects of alfaxalone during CRI.

ANIMALS
8 healthy adult Beagles.

PROCEDURES
3 treatments were administered in a randomized crossover design as follows: 1= saline (0.9% NaCl) solution injection, followed in 10 minutes by induction of anesthesia with alfaxalone (loading dose, 2.4 mg/kg; CRI, 3.6 mg/kg/h, for 60 minutes); 2 = medetomidine premedication (loading dose, 4.0 µg/kg; CRI, 4.0 µg/kg/h), followed by alfaxalone (as in treatment 1); and, 3 = medetomidine (as in treatment 2) and MK-467 (loading dose, 150 µg/kg; CRI, 120 µg/kg/h), followed by alfaxalone (as in treatment 1). The peripherally acting α_2-adrenoceptor antagonist MK-467 was used to distinguish between the peripheral and central effects of medetomidine. Drugs were administered IV via cephalic catheters, and there was a minimum of 14 days between treatments. Cardiopulmonary parameters were measured for 70 minutes, and jugular venous blood samples were collected until 130 minutes after premedication. Drug concentrations in plasma were analyzed with liquid chromatography–tandem mass spectrometry.

RESULTS
The characteristic cardiovascular effects of medetomidine, such as bradycardia, hypertension, and reduction in cardiac index, were obtunded by MK-467. The concentrations of alfaxalone in plasma were significantly increased in the presence of medetomidine, indicative of impaired drug distribution and clearance. This was counteracted by MK-467.

CONCLUSIONS AND CLINICAL RELEVANCE
The alteration in alfaxalone clearance when coadministered with medetomidine may be attributed to the systemic vasoconstrictive and bradycardic effects of the α_2-adrenoceptor agonist. This could be clinically important because the use of α_2-adrenoceptor agonists may increase the risk of adverse effects if standard doses of alfaxalone are used. (*Am J Vet Res* 2017;78:956–964)

Alfaxalone (3α-hydroxy-5α-pregnane-11, 20-dione) is a synthetic neurosteroid drug, which interacts with γ-aminobutyric acid A receptors to induce anesthesia and muscle relaxation.[1,2] Alfaxalone in combination with alfadolone was formerly marketed for veterinary use. However, this preparation has a number of well-recognized adverse effects in dogs and cats, largely related to the use of polyethoxylated castor oil as an excipient.[3,4] In recent years, a new preparation of alfaxalone, now solubilized in 2-hydroxypropyl-beta-cyclodextrin,[a] has been marketed for use in dogs. This new formulation is considered to have fewer adverse effects and therefore has rekindled interest in the use and potential applications of alfaxalone in clinical veterinary medicine.

The pharmacokinetic characteristics of alfaxalone indicate that it would be suitable for administration not only as a bolus injection,[5,6,b] but also as a CRI. Other investigators previously compared the pharmacodynamic effects of alfaxalone with those of propofol during TIVA.[7,8] Results of these studies sug-

ABBREVIATIONS

AUC_{last}	Area under the curve to the last sampling point
CI	Cardiac index
CL_{ss}	Steady-state clearance
CRI	Constant rate infusion
f_R	Respiratory frequency
MAP	Mean arterial blood pressure
SVRI	Systemic vascular resistance index
$T_{1/2}$	Elimination half-life
TIVA	Total IV anaesthesia
Vd_{ss}	Steady-state volume of distribution

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gested that the 2 drugs are comparable in terms of efficacy and safety and that therefore alfaxalone may be a suitable alternative to propofol for the maintenance of anesthesia in dogs.[7,8]

A clinical study[9] performed in dogs compared the effects of premedication with dexmedetomidine versus those of acepromazine prior to TIVA with alfaxalone. The animals receiving dexmedetomidine were more sedated and required a lower alfaxalone infusion rate than those given acepromazine preoperatively. The use of the α_2-adrenoceptor agonist medetomidine, or its pharmacologically active enantiomer dexmedetomidine, may thus offer some potential advantages over acepromazine when used prior to alfaxalone. However, it is also well known that α_2-adrenoceptor agonists have substantial cardiovascular effects. A recently published study[10] comparing the cardiovascular effects of dexmedetomidine CRI combined with alfaxalone CRI again confirmed these pharmacodynamic effects. Specifically, dexmedetomidine was associated with reductions in heart rate, CI, and f_R, whereas arterial blood pressure and systemic vascular resistance were markedly increased.

MK-467 is a peripherally acting α_2-adrenoceptor antagonist, which does not markedly cross the blood-brain barrier.[11] Therefore, it counteracts the cardiovascular and endocrine effects of dexmedetomidine and medetomidine in dogs, without substantial alteration in the degree of sedation.[12–17] Previously, MK-467 has been shown to counteract the effects of medetomidine during inhalant general anesthesia.[18]

Although alfaxalone has been used for TIVA, no reports have so far documented the possible effects of medetomidine on the plasma concentrations of concomitantly administered alfaxalone. Neither has the impact of medetomidine administered as a CRI on the cardiopulmonary effects of alfaxalone been described. The aims of the study reported here were as follows: to assess the possible impact of medetomidine on concentrations of alfaxalone in plasma, when given as a CRI to dogs, and to determine the possible impact of medetomidine on the cardiopulmonary effects of alfaxalone during CRI. MK-467 was used to prevent the peripheral cardiovascular effects of medetomidine as a means of determining their role in the investigated drug-drug interaction.

Materials and Methods

Animals

This was a randomized crossover study. Randomization was performed by means of listing all the possible order of treatments on separate pieces of paper, placing these pieces of paper into a bag, and withdrawing them blindly from the bag (1 piece/dog). Because there were 6 possible treatments and 8 dogs, the papers were mixed again, and then a further 2 treatment orders were withdrawn for the remaining 2 dogs. The study protocol was approved by the National Animal Experiment Board of Finland (license No. ESAVI-2010-07734/Ym-23). Eight purpose-bred Beagles (2 females and 6 males [all neutered]; mean ± SD weight, 13.4 ± 2.1 kg; approx age, 4.5 years) participated in the study. They were considered healthy on the basis of physical examination findings and results of hematologic evaluation and blood biochemical analysis. The dogs were housed in groups and fed standard commercial dog food with water freely available. All procedures were performed during the day; on each study day, the animals were fed after the study procedures were completed. The dogs have since been rehomed.

Instrumentation

Prior to each experiment, dogs were instrumented while anesthetized with isoflurane. Anesthesia was induced by IV administration of propofol (maximum dose, 6 mg/kg), and dogs were allowed to recover from anesthesia for a minimum of 60 minutes or until normal locomotor activity had returned. During instrumentation, two 20-gauge cannulae were inserted into the cephalic veins following application of lidocaine gel. A 7F double-lumen catheter was introduced into a jugular vein following SC injection of 1 mL of 2% lidocaine solution, for the measurement of central venous pressure. The insertion site of the central venous catheter was premeasured from the cranial border of the second rib at the costochondral junction, and the position of the catheter tip was verified by assessment of the central venous pressure waveform. A 22-gauge arterial cannula was inserted into a dorsal pedal artery. Lactated Ringer solution was infused via the cephalic catheter at 10 mL/kg/h during instrumentation. Throughout the experimental period, the animals' rectal temperatures were monitored; each dog was placed on a heating pad. If its body temperature was < 37°C, the animal was covered with additional blankets to maintain normothermia.

Drugs

Each dog received 3 treatments separated by at least 14 days. All drugs were administered IV via a cephalic catheter. The 3 treatments were as follows: 1 = saline (0.9% NaCl) solution injection, followed in 10 minutes by induction of anesthesia with alfaxalone[c] (loading dose, 2.4 mg/kg; CRI, 3.6 mg/kg/h, for 60 minutes); 2 = medetomidine[d] premedication (loading dose, 4.0 µg/kg; CRI, 4.0 µg/kg/h), followed in 10 minutes by alfaxalone (as in treatment 1); and 3 = medetomidine (as in treatment 2) and MK-467[e] (loading dose, 150 µg/kg; CRI, 120 µg/kg/h), followed in 10 minutes by alfaxalone (as in treatment 1). The peripherally acting α_2-adrenoceptor antagonist MK-467 was used to distinguish between the peripheral and central effects of medetomidine.

MK-467 powder was dissolved in saline solution to a concentration of 10 mg/mL. For treatment 3, medetomidine and MK-467 were mixed in a single syringe before administration. All loading doses were administered over 60 seconds. The infusions of medetomidine with or without MK-467 were started at time 0, immediately after the loading doses. Fol-

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lowing the administration of the loading dose of alfaxalone, the CRI was commenced immediately. The airway of each dog was intubated, and oxygen was delivered throughout anesthesia at 2 L/min by use of a semiclosed rebreathing system.[f] Animals were allowed to breathe spontaneously. The total infusion rate of all solutions was 11 mL/kg/h, which included saline solution in treatments 1 and 2.

Measurements

Venous blood samples were collected into potassium-EDTA blood collection tubes from the jugular catheter at 15, 30, 45, 60, 70, 80, 90, 100, 110, and 130 minutes after the start of premedication. The samples were centrifuged at 3,000 X *g* for 15 minutes. The plasma was then divided into 3 tubes and frozen at –20°C or colder until analyzed. High-performance liquid chromatography–tandem mass spectrometry was used as the analytic method and was appropriately validated for range, precision, accuracy, carryover, interference of analytes, and internal standards and analyte stability.

The concentration of alfaxalone in canine plasma samples was analyzed with the study drug product as the reference substance.[a] Sample preparation involved protein precipitation with acetonitrile, and prednisolone was used as the internal standard. Reversed-phase separation was performed[g] with a gradient of 2 solvents. Solvent A was 5mM ammonium formate, and solvent B was 5mM ammonium formate in a mixture of acetonitrile and methanol (vol/vol, 1:1). Quantitative detection was performed in multiple-reaction mode by use of a triple quadrupole mass spectrometer.[h] Turbo ion spray was used for ionization. The scanned ion pairs (m/z) were 333.3 and 297.4 for alfaxalone and 361.2 and 343.4 for prednisolone. The linear concentration range for the determination of alfaxalone was from 100 to 4,000 ng/mL. Calibration standards with 8 nonzero concentrations and quality controls samples with 3 concentrations (low, medium, and high) were included in all assays. The chromatograms were analyzed and processed.[i] Linear regression was used for the calculations. The accuracy of the method ranged from 101% to 112%, and the intra-assay coefficient of variation ranged from 1.4% to 5.1% for quality control samples at the lower limit of quantitation (100 ng/mL) and at the 3 quality control sample concentrations (300, 1,625, and 3,250 ng/mL).

The concentrations of dexmedetomidine and levomedetomidine (reference standard, racemic medetomidine[j]) in plasma were determined after solid-phase extraction,[k] with diphenylimidazole as the internal standard. After chiral separation[l] by use of 10mM ammonium acetate and acetonitrile as solvents, quantitative detection was performed as previously described.[h] For dexmedetomidine or levomedetomidine and diphenylimidazole, the respective precursor ions (m/z) were 201.1 and 221.1. The fragment ions (m/z) monitored and used for quantitation were 95.1 for dexmedetomidine and levomedetomidine and 194.0 for diphenylimidazole. The chromatograms were processed.[i] The linear concentration range was from 0.01 to 5.0 ng/mL. The interassay accuracy of quality control samples (0.03, 0.45, and 4.0 ng/mL) ranged from 96% to 99% for dexmedetomidine and from 94% to 99% for levomedetomidine.

The concentration of MK-467 in plasma was determined after solid-phase extraction,[k] with RS-79948[m] as the internal standard. Reversed-phase separation was performed[n] with a gradient solvent system (0.1% formic acid in water and acetonitrile). Quantitative detection was performed as described.[h] For MK-467 and RS-79948, the respective precursor ions (m/z) scanned were 419.0 and 365.0. The fragment ions monitored and used for quantitation were 200.0 for MK-467 and 190.2 for RS-79948. The chromatograms were analyzed.[o] The linear assay range was from 1.00 to 500 ng/mL. The intra-assay accuracy of quality control samples (at 3, 45, and 450 ng/mL) was 98% to 112%.

Cardiac rate and rhythm were monitored continuously by ECG lead II. Heart rate was recorded either from the ECG or by auscultation of the thorax. Systolic arterial pressure, diastolic arterial pressure, MAP, central venous pressure, and the inspired fraction of oxygen were recorded by use of a multichannel monitor.[p] The pressure transducers[q] were zeroed to atmospheric pressure prior to each experiment. The dogs were positioned in either right or left lateral-most recumbency, with the arterial catheter positioned in the lower, hind limb, and the manubrium was used as 0 reference. The gas analyzer was calibrated before the experiments by use of the calibration gas supplied by the manufacturer.[r] Cardiac output was measured with the lithium indicator dilution method[s] as previously described[19] by use of a standard dose of 0.075 mmol of lithium chloride. Standard values of hemoglobin (10 g/L) and sodium (140 mmol/L) were used and later corrected with actual values obtained from simultaneously collected arterial blood gas samples. Arterial and central venous blood gas samples were collected anaerobically into heparinized syringes via the arterial and central venous catheters, stored in ice water for no longer than 15 minutes, and analyzed.[t] Oxygen and carbon dioxide partial pressures (Pa_{O_2} and Pa_{CO_2}) as well as arterial pH were recorded. Subsequently, CI and SVRI were calculated by use of standard equations.[20] Before the induction of anesthesia, f_R was measured by means of counting the chest movements over a minute and subsequently from the respiratory gas monitor.

A nociceptive pressure tester[u] was used to assess the responsiveness of the dogs during anesthesia. The tester applied an increasing pressure (mm Hg) at the tail base. Baseline measurements were obtained before drug administration, and assessments were repeated at each time point. The responses were graded into 1 of 3 categories: 1, no response; 2, change in f_R, heart rate, or blood pressure (10% to 20% from

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baseline); and 3, > 20% change in these parameters or substantial limb or body movement.

Sixty minutes after the alfaxalone loading dose was administered, all infusions were stopped, and the dogs were allowed to recover. After recovery, the catheters were removed, and the dogs received meloxicam (0.2 mg/kg, SC) to relieve any discomfort resulting from catheter placement.

The AUC_{last} was calculated for alfaxalone, dexmedetomidine, levomedetomidine, and MK-467, and $T_{1/2}$ of alfaxalone was calculated.[v] The following were applied: noncompartmental analysis, linear trapezoidal linear-log interpolation, best fit analysis (slope), and uniform weighting. Only results determined on the basis of concentration data sets where the elimination phase was well described by linear regression (correlation coefficient of regression line > 0.90) were reported and used in statistical analyses. Steady-state clearance of alfaxalone was calculated from the mean concentration of alfaxalone in plasma between 30 and 60 minutes for each dog and each treatment. These steady-state concentrations were then used to calculate clearances by use of the following formula: CL_{ss} = dose/steady-state drug concentration. The Vd_{ss} was calculated by use of the following formula: $T_{1/2} = \ln 2 \, (Vd_{ss}/CL_{ss})$.

Statistical analyses

Parametric data were reported as mean ± SD and nonparametric data as median (minimum and maximum). Pharmacokinetic data were reported are shown as geometric mean ± 95% confidence interval for plasma concentration data or geometric mean and range (minimum-maximum) where data numbers were limited. For cardiopulmonary data, the change from the baseline measurement was calculated for all the response variables, and the change was used as a response in the modeling. The differences between treatments were evaluated with repeated-measures ANCOVA models. The model included a baseline covariate, the main effects of treatment, time point and period (the order of treatments). and 2-way-interactions of treatment × time point and period × time point as fixed effects and included the main effect of subject and 2-way interactions of subject × time point and subject × period as random effects.

The estimates of treatment effects were calculated both over time and by time point from the fitted models. For the overall and time-specific treatment differences, 95% confidence intervals and *P* values were calculated.

Pharmacokinetic data were logarithmically transformed, and the derived parameters were analyzed by use of paired *t* tests. Nociceptive responses were analyzed by use of a Friedman 2-way ANOVA. Values of $P < 0.05$ were considered significant. Cardiopulmonary analyses[w] and other analyses[x] were performed by use of commercially available statistical software programs.

Results

Plasma alfaxalone concentrations were determined **(Figure 1)**, and alfaxalone CL_{ss}, Vd_{ss}, and $T_{1/2}$ values were summarized **(Table 1)**. The AUC_{last} of alfaxalone was more than doubled for the medetomidine-alfaxalone combination, compared with that of the other treatments. Alfaxalone clearance was reduced by more than 50% in the presence of medetomidine.

Mean plasma concentrations of dexmedetomidine and levomedetomidine were determined **(Figure 2)**. Geometric mean and range (minimum and maximum) of AUC_{last} for dexmedetomidine and dexmedetomidine with MK-467 were 182 ng•min/mL (150 to 233 ng•min/mL) and 86 ng•min/mL (67 to 116 ng•min/mL), respectively ($P < 0.05$). For levomedetomidine, AUC_{last} was 101 ng•min/mL (88 to 133 ng•min/mL), which was significantly greater than

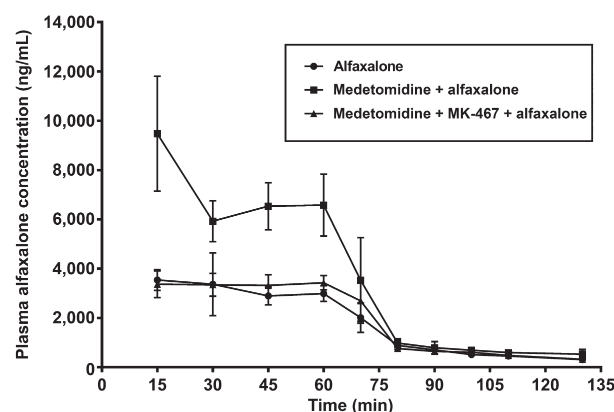

Figure 1—Plasma alfaxalone concentrations (geometric mean and 95% confidence intervals) in 8 Beagles during anesthesia with alfaxalone alone (loading dose, 2.4 mg/kg; CRI, 3.6 mg/kg/h); medetomidine (loading dose, 4.0 µg/kg; CRI, 4.0 µg/kg/h), followed by alfaxalone (as described); or medetomidine (as described) and MK-467 (loading dose, 150 µg/kg; CRI, 120 µg/kg/h), followed by alfaxalone (as described).

Table 1—Geometric mean and range (minimum and maximum) values of CL_{ss}, Vd_{ss}, and $T_{1/2}$ of alfaxalone for 3 treatments (ie, alfaxalone alone, medetomidine-alfaxalone combination, and medetomidine-alfaxalone combination plus MK-467) in 8 healthy adult Beagles.

Treatment	AUC_{last} (ng•min/mL)	CL_{ss} (mL/kg/min)	Vd_{ss} (L/kg)	$T_{1/2}$ (min)
Alfaxalone (n = 7)	220,000 (162,000–270,000)*	19.7 (14.3–27.0)*	1.1 (0.6–1.9)	39.3 (20.1–57.1)
Medetomidine-alfaxalone (n = 4)	450,000 (350,000–601,000)†	8.8 (8.0–10.2)†	0.70 (0.4–1.1)	54.4 (30.2–82.9)
Medetomidine-alfaxalone and MK-467 (n = 7)	240,000 (191,000–270,000)	18.4 (16.3–23.1)	0.93 (0.8–1.2)	35.6 (26.8–39.4)

*Significant ($P < 0.05$) difference between alfaxalone and medetomidine-alfaxalone treatments. †Significant ($P < 0.05$) difference between medetomidine-alfaxalone and medetomidine-alfaxalone and MK-467 treatments.

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the value of 49 ng•min/mL (38 to 69 ng•min/mL) in the presence of MK-467. Dexmedetomidine AUC_{last} values with and without MK-467 were significantly greater than those of levomedetomidine AUC_{last} with and without MK-467 (by 80% and 76%, respectively). Plasma MK-467 concentrations over time were graphed **(Figure 3)**.

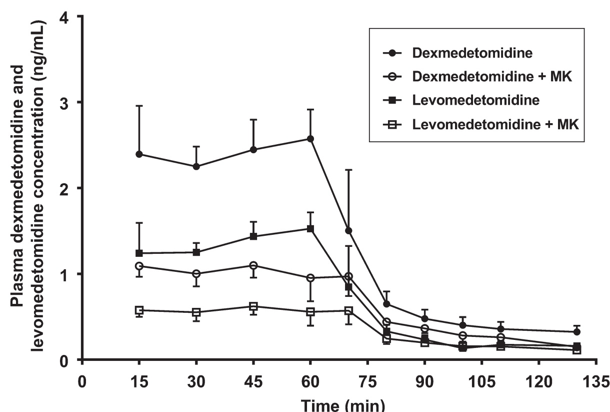

Figure 2—Plasma dexmedetomidine and levomedetomidine concentrations (geometric mean and 95% confidence intervals) in 8 Beagles during anesthesia with medetomidine, followed by alfaxalone, or medetomidine and MK-467, followed by alfaxalone. ***See*** Figure 1 for dosages of medetomidine, alfaxalone, and MK-467.

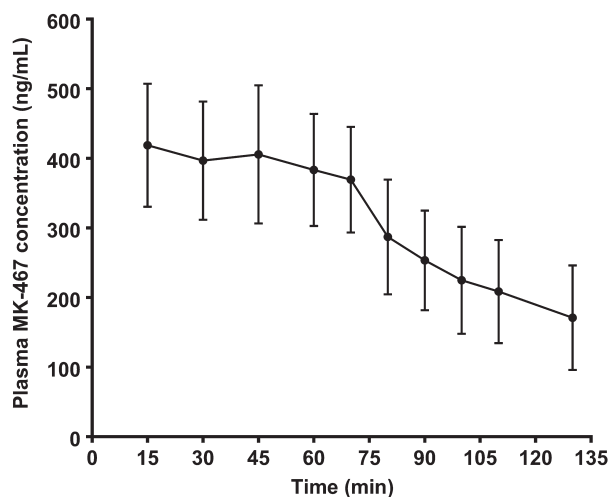

Figure 3—Plasma MK-467 concentrations (geometric mean and 95% confidence intervals) in 8 Beagles during anesthesia with medetomidine and MK-467, followed by alfaxalone. ***See*** Figure 1 for dosages of medetomidine, alfaxalone, and MK-467.

Between 15 and 30 minutes after premedication, no significant differences were detected in the nociceptive response scores between treatments **(Table 2)**. From 45 minutes onward, animals receiving alfaxalone CRI alone were more responsive, compared with other treatments. Nociceptive response scores were significantly higher with alfaxalone alone than with the other treatments at 45 minutes, when corresponding mean alfaxalone concentrations were 2,930 ± 480, 6,645 ± 129, and 3,365 ± 580 ng/mL for the alfaxalone alone, medetomidine-alfaxalone combination, and medetomidine-alfaxalone plus MK-467 treatments, respectively. At 45 minutes, the concentration of dexmedetomidine in plasma was 2.5 ± 0.5 ng/mL and 1.1 ± 0.2 ng/mL in the absence and presence, respectively, of MK-467.

Alfaxalone administration was associated with significant and sustained increases in heart rate **(Table 3)**. Coadministration of medetomidine resulted in marked decreases in heart rate and CI and increases in MAP and SVRI. MK-467 abolished these medetomidine-induced changes in cardiovascular function. The administration of medetomidine with alfaxalone led to a greater degree of hypoventilation and acidemia and relative hypoxemia (inspired fraction of oxygen > 0.94) following the induction of anesthesia, compared with alfaxalone administration alone. MK-467 administration diminished both the effect of medetomidine on ventilation and the extent of acidemia.

After the induction of anesthesia, body temperatures were significantly lower than baseline at each time point within each treatment. There were no significant differences among the treatments.

Discussion

To our knowledge, this was the first study in which plasma alfaxalone concentrations during CRI in dogs and the impact of medetomidine on the disposition of alfaxalone have been determined. In the present study, the larger AUC_{last} during the medetomidine-alfaxalone infusion was predominantly the result of the reduction in systemic clearance of alfaxalone. The reduction in clearance was likely caused by the reduction in CI induced by the active enantiomer

Table 2—Median nociceptive response scores (minimum and maximum) for 3 treatments (ie, alfaxalone alone, medetomidine-alfaxalone combination, and medetomidine-alfaxalone plus MK-467) in 8 healthy adult Beagles at baseline and 15, 30, 45, 60, and 70 minutes after premedication.

Treatment	Baseline	15 minutes	30 minutes	45 minutes	60 minutes	70 minutes
Alfaxalone	3 (2–3)	1 (1–2)	2 (1–2)	2*† (1–2)	2* (1–2)	2* (1–3)
Medetomidine-alfaxalone	3 (2–3)	1 (1–2)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)
Medetomidine-alfaxalone plus MK-467	3 (2–3)	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)

Nociceptive responses were graded as: 1 = no response; 2 = change in f_R, heart rate, or blood pressure (10% to 20% from baseline); and, 3 = a > 20% change in these parameters or substantial limb or body movement.*Significant (P < 0.05) difference between alfaxalone alone and medetomidine-alfaxalone combination. †Significant (P < 0.05) difference between alfaxalone alone and medetomidine-alfaxalone plus MK-467.

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Table 3—Mean ± SD of heart rate, MAP, CI, SVRI, f_R, Pa_{O_2}, and Pa_{CO_2} in dogs (n = 8) while under alfaxalone anesthesia (ALF). Premedication consisted of medetomidine (MA), medetomidine and MK-467 (MA and MK), or none (ALF).

Parameters	Treatment	Baseline	15 minutes	30 minutes	45 minutes	60 minutes	70 minutes
Heart rate (beats/min)	ALF	79 ± 16	129 ± 26*†‡	104 ± 27*†‡	95 ± 12*†‡	101 ± 30*†‡	84 ± 14*‡
	MA	79 ± 20	77 ± 17§	61 ± 10*	55 ± 15*§	52 ± 10*§	48 ± 10*§
	MA-MK	82 ± 16	105 ± 17*	74 ± 12	71 ± 10	68 ± 12*	70 ± 9
MAP (mm Hg)	ALF	105 ± 14	104 ± 16†‡	103 ± 12‡	110 ± 15‡	112 ± 13‡	106 ± 12‡
	MA	111 ± 14	130 ± 12*§	109 ± 13§	106 ± 12§	106 ± 12§	105 ± 11§
	MA-MK	104 ± 14	92 ± 12*	78 ± 9*	78 ± 8*	79 ± 8*	79 ± 8*
CI (L/min/m²)	ALF	3.71 ± 1.09	4.91 ± 1.07*†	4.05 ± 0.66*‡	3.99 ± 0.78*	3.89 ± 0.99*‡	4.06 ± 1.72*‡
	MA	3.99 ± 1.04	2.31 ± 0.73*§	1.84 ± 0.18*§	1.80 ± 0.34*§	1.85 ± 0.33*§	2.13 ± 0.34*§
	MA-MK	3.78 ± 0.71	4.45 ± 1.03*	3.40 ± 0.72	3.41 ± 0.74	3.19 ± 0.66*	3.19 ± 0.76*
SVRI (dyne•s/cm⁵/m²)	ALF	2,340 ± 669	1,820 ± 530*†	2,070 ± 291†	2,270 ± 594†	2,450 ± 749†	2,390 ± 988†
	MA	2,320 ± 701	4,530 ± 1,240*§	4,530 ± 870*§	4,620 ± 1,115*§	4,500 ± 1,278*§	3,860 ± 979*§
	MK-MA	2,220 ± 413	1,740 ± 670	1,900 ± 575	1,880 ± 558	2,040 ± 625	2,080 ± 628
f_R (breaths/min)	ALF	18 ± 6	8 ± 5*†	15 ± 6†	16 ± 7†‡	18 ± 11†‡	18 ± 11†‡
	MA	19 ± 7	3 ± 3*	7 ± 2*	7 ± 3*	7 ± 3*	7 ± 4*
	MA-MK	18 ± 5	4 ± 2*	10 ± 5*	10 ± 5*	11 ± 4*	11 ± 5*
Pa_{O_2} (mm Hg)	ALF	100 ± 5	455 ± 117*†‡	558 ± 36*	542 ± 55*	535 ± 76*	542 ± 79*
	MA	102 ± 3	220 ± 106*§	537 ± 56*	540 ± 54*	554 ± 43*	545 ± 56*
	MA-MK	101 ± 8	362 ± 124*	547 ± 24*	550 ± 28*	537 ± 57*	568 ± 23*
Pa_{CO_2} (mm Hg)	ALF	34 ± 2	43 ± 5*†‡	37 ± 2*†	36 ± 4†	36 ± 3†	35 ± 3†
	MA	34 ± 1	56 ± 5*	47 ± 6*§	46 ± 7*§	45 ± 3*§	48 ± 5*§
	MA-MK	33 ± 3	52 ± 5*	40 ± 3*	39 ± 3*	39 ± 3*	39 ± 3*
Arterial pH	ALF	7.40 ± 0.02	7.33 ± 0.04*†‡	7.37 ± 0.01*†	7.37 ± 0.04*†	7.36 ± 0.03*†	7.37 ± 0.03*†‡
	MA	7.39 ± 0.01	7.23 ± 0.03*	7.27 ± 0.04*§	7.28 ± 0.05*§	7.28 ± 0.03*§	7.27 ± 0.04*§
	MA-MK	7.40 ± 0.02	7.25 ± 0.03*	7.33 ± 0.02*	7.33 ± 0.02*	7.33 ± 0.02*	7.33 ± 0.02*

*Significant differences ($P < 0.05$) in values from baseline. Significant ($P < 0.05$) difference in values for †ALF versus MA, ‡MA versus MA-MK, and §ALF versus MA-MK.

of medetomidine, dexmedetomidine, as previously described.[21-24] However, in addition to the primary effect on clearance, dexmedetomidine may have reduced the initial volume of distribution of alfaxalone as the result of vasoconstriction. Thereby, the identical alfaxalone loading dose led to increased plasma alfaxalone concentration prior to reaching steady state with the medetomidine-alfaxalone treatment, compared with other treatments. We did not calculate the initial volume of distribution because early sample collection was not performed. However, Bührer et al[25] showed that dexmedetomidine reduced the dose requirement of thiopental in humans as a result of a reduction in both the distribution volume and distribution clearance of the barbiturate drug and not as a result of a centrally mediated additive or synergistic effect.

The lower alfaxalone clearance in the presence of medetomidine may result from a reduction in hepatic blood flow, an alteration in metabolism, or both. The proposed mechanism of alfaxalone biotransformation is via hepatic metabolism.[26] Medetomidine has previously been shown to inhibit the CYP2B11-mediated metabolism of some coadministered anesthetic drugs in vitro.[27] Although we made no attempt to measure hepatic perfusion, other authors[23,28,29] have suggested that both dexmedetomidine and medetomidine reduce their own rate of metabolism by reducing hepatic blood flow. This is more likely to be secondary to reductions in cardiac output rather than a direct effect on hepatic blood vessels.[30] Coadministration of atipamezole (a centrally and peripherally acting α_2-adrenoceptor antagonist) increased medetomidine clearance, presumably by counteracting vasoconstriction and the ensuing reduction in cardiac output and thereby maintaining hepatic perfusion.[23] Our results seemed to support this mechanism, as MK-467 antagonized the effect of medetomidine on alfaxalone clearance, most likely by maintaining cardiac output and thus perfusion at the site of alfaxalone metabolism. The systemic cardiovascular effects of medetomidine were clearly evident in the present study, where medetomidine administration was associated with a > 50% reduction in the CI and doubling of the SVRI. Concomitant administration of the antagonist MK-467 abolished these effects. In so doing, MK-467 reestablished alfaxalone clearance, probably by improving hepatic perfusion.

Several studies in dogs have investigated the impact of medetomidine on the dose requirements of injectable anesthetic agents; typically, reductions have been observed.[31-35] None of the aforementioned studies included pharmacokinetic analysis. We suggest that the observed reductions in the dosage of injectable anesthetic agents may be explained by alterations in drug disposition and thus drug concentrations in plasma (and further, in target tissues) rather than pharmacodynamic drug-drug interactions per se.

In the present study, greater nociceptive responsiveness was detected when alfaxalone was adminis-

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tered alone. The alfaxalone infusion rate we selected was lower than the dose rates reported by other authors.[7-9] However, we wanted to use the same alfaxalone infusion rate in the presence of medetomidine without the risk of excessively deep anesthesia. During the medetomidine and medetomidine-plus-MK-467 infusions, the nociceptive responses were diminished, compared with those of alfaxalone alone. This finding may in part be attributable to the sedative and analgesic effects of dexmedetomidine[36] because the medetomidine and MK-467 combination also suppressed the nociceptive responses without concomitant increases in the concentration of alfaxalone. The lowest response scores were recorded during the medetomidine-alfaxalone combination, when the observed plasma concentrations of both drugs and presumably also their anesthetic and analgesic effects were greatest. The plasma concentrations of dexmedetomidine exceeded the reported minimum amount associated with antinociception (2 ng/mL) for most of the anesthetic period.[36] Still, it was impossible to differentiate between the respective anesthetic and antinociceptive effects of the 2 individual drugs in the present study. The ideal plasma concentrations of dexmedetomidine and alfaxalone were not defined. However, we suggest that a minimum plasma dexmedetomidine concentration of 2 ng/mL should be targeted in combination with a minimum plasma alfaxalone concentration of 3,500 ng/mL. Further studies are required to confirm the optimal dexmedetomidine and alfaxalone concentrations in plasma for safe and efficacious anesthesia and analgesia.

In the present study, the administration of alfaxalone CRI alone resulted in significant increases in heart rate and CI, which may be secondary to a reduction in SVRI, as previously suggested by other authors.[6,37-39] Premedication with medetomidine resulted in bradycardia, a reduction in CI, and increases in MAP and SVRI. In the present study, coadministration of MK-467 efficiently obtunded the peripheral vasoconstrictive and bradycardic effects of medetomidine, as reported earlier.[12,13,18,40] Mean arterial blood pressure decreased from baseline with all treatments but always remained above 70 mm Hg, which is considered the minimum clinically acceptable value necessary to maintain renal perfusion and urine production in dogs. In the present study, lithium dilution was used for the measurement of CI. Assessments of cardiac output as determined by the lithium dilution method may be sensitive to drug-dependent bias because of interference of drugs with the lithium electrode.[41] Dexmedetomidine has been reported to not influence the electrode read-out at a plasma concentration of 10 ng/mL.[41] To our knowledge, however, no published information is available on the possible impact of MK-467 or alfaxalone on cardiac output measurements with the lithium dilution technique.

All treatments were associated with a reduction in f_R, increase in Pa_{CO_2}, and acidemia following the induction of anesthesia. Individually, both medetomidine and alfaxalone may cause respiratory depression,[7,42] and the level of hypoventilation was greatest during the administration of this combination. We believe this can be partially explained by the high plasma alfaxalone concentration with this drug combination, compared with that of the other treatments.[5] MK-467 ameliorated the respiratory depressant effects of the drug combination, probably as a result of its impact on the disposition of both alfaxalone and dexmedetomidine, rather than a direct central action per se. Initial mean Pa_{O_2} values were significantly lower with the medetomidine-alfaxalone combination than with the other treatments, which was probably the result of a combination of hypoventilation and pulmonary shunt. The administration of MK-467 effectively prevented these changes. Previously, MK-467 has inhibited medetomidine-induced increases in pulmonary arterial wedge pressure,[12,17] but there is little information describing its influence on the pulmonary vasculature. These results suggest that it may alter pulmonary vascular resistance and thereby diminish ventilation-perfusion mismatch in dogs. Hypoxemia following administration of dexmedetomidine and alfaxalone has been reported by other authors[10] and again illustrates the need to provide supplemental oxygen before and during the induction of anesthesia.

From a clinical standpoint, it might be argued that the use of MK-467 in combination with medetomidine-alfaxalone led to greater hemodynamic and ventilatory stability, compared with that of either alfaxalone alone or the combination of medetomidine with alfaxalone. This may be an important consideration in animals where dramatic alterations in heart rate, increases in SVRI, or decreases in arterial oxygenation should be avoided. Further studies may be required to determine the impact of MK-467 on cardiovascular function in systemically ill animals.

In conclusion, the distribution and clearance of alfaxalone were reduced by the coadministration of the α_2-adrenoceptor agonist medetomidine, but this effect was prevented by the addition of MK-467. Plasma alfaxalone concentrations were approximately doubled in the presence of medetomidine during CRI. Plasma dexmedetomidine concentrations were greater than plasma levomedetomidine concentrations, and both were reduced in the presence of MK-467. MK-467 ameliorated the peripheral cardiovascular effects of medetomidine, which probably resulted in lower alfaxalone and dexmedetomidine concentrations in plasma during combined infusion of all 3 drugs. Higher alfaxalone and dexmedetomidine concentrations in plasma were associated with lower nociceptive response scores. MK-467 diminished the combined cardiopulmonary effects of medetomidine and alfaxalone. It remains unclear whether the anesthetic-sparing effect of the α_2-adrenoceptor agonist is primarily related to its sedative and analgesic effects, its impact on the pharmacokinetics of

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coadministered drugs, or a combination of both. Further studies are required to differentiate between the pharmacokinetic and pharmacodynamic interactions of the α_2-adrenoceptor agonist with other drugs used for sedation and anesthesia.

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Footnotes

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b. Pasloske KS, Gazzard B, Perkins N, et al. A multicentre clinical trial evaluating the efficacy and safety of Alfaxan-CD RTU administered to dogs for induction and maintenance of anaesthesia (abstr), in *Proceedings*. 48th Annu Br Small Anim Vet Assoc Cong 2005;556.
c. Vetoquinol UK Ltd, Buckingham, Buckinghamshire, England.
d. Dorbene, Laboratories Syva sa, León, Spain.
e. Merck & Co Inc, Rahway, NJ.
f. Anesco Inc, Georgetown, Ky.
g. Gemini C_{18} column (2.0 × 150 mm; 5 µm), Phenomenex, Torrance, Calif.
h. API 4000, MDS Sciex, Concord, ON, Canada.
i. Analyst, version 1.4.1, SCIEX, Concord, ON, Canada.
j. Fermion Oy, Espoo, Finland.
k. Sep-PaktC_{18} 96-well extraction plates, Waters Corp, Milford, Mass.
l. Chiralpak AGP column (4 × 100 mm; 5 µm), Chiral Technologies Europe, Illkirch-Graffenstaden, France.
m. Tocris Bioscience, Bristol, England.
n. SunFir C_{18} column (2.1 × 150 mm; 3.5 µm), Waters Corp, Milford, Mass.
o. Analyst, version 1.4.2, SCIEX, Concord, ON, Canada.
p. S/5 Anesthesia Monitor, GE Healthcare, Helsinki, Finland.
q. Gabarith PMSET, Becton Dickinson, Sandy, Utah.
r. Quick Cal Calibration gas, GE Healthcare, Hatfield, Hertfordshire, England.
s. LiDCO Plus Hemodynamic Monitor, LiDCO Ltd, Cambridge, England.
t. ABL800, Radiometer, Copenhagen, Denmark.
u. Randall Selitto Paw Pressure Test Apparatus, IITC Life Science Inc, Woodland Hills, Calif.
v. WinNonLin, Phoenix 64, version 6.3, Certara USA Inc, Princeton, NJ.
w. SAS System for Windows, version 9.3, SAS Institute Inc, Cary, NC.
x. SPSS, version 22, IBM SPSS Inc, Chicago, Ill.

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