Investigation of the effects of vatinoxan on somatic and visceral antinociceptive efficacy of medetomidine in dogs

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OBJECTIVE
To determine whether concurrent vatinoxan administration affects the antinociceptive efficacy of medetomidine in dogs at doses that provide circulating dexmedetomidine concentrations similar to those produced by medetomidine alone.

ANIMALS
8 healthy Beagles.

PROCEDURES
Dogs received 3 IV treatments in a randomized crossover-design trial with a 2-week washout period between experiments (medetomidine [20 µg/kg], medetomidine [20 µg/kg] and vatinoxan [400 µg/kg], and medetomidine [40 µg/kg] and vatinoxan [800 µg/kg]; M20, M20V400, and M40V800, respectively). Sedation, visceral and somatic nociception, and plasma drug concentrations were assessed. Somatic and visceral nociception measurements and sedation scores were compared among treatments and over time. Sedation, visceral antinociception, and somatic antinociception effects of M20V400 and M40V800 were analyzed for noninferiority to effects of M20, and plasma drug concentration data were assessed for equivalence between treatments.

RESULTS
Plasma dexmedetomidine concentrations after administration of M20 and M40V800 were equivalent. Sedation scores, visceral nociception measurements, and somatic nociception measurements did not differ significantly among treatments within time points. Overall sedative effects of M20V400 and M40V800 and visceral antinociceptive effects of M40V800 were noninferior to those produced by M20. Somatic antinociception effects of M20V400 at 10 minutes and M40V800 at 10 and 55 minutes after injection were noninferior to those produced by M20.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested coadministration with vatinoxan did not substantially diminish visceral antinociceptive effects of medetomidine when plasma dexmedetomidine concentrations were equivalent to those produced by medetomidine alone. For somatic antinociception, noninferiority of treatments was detected at some time points. (*Am J Vet Res* 2020;81:299–308)

M edetomidine, a racemic (50:50) mixture of dexmedetomidine and levomedetomidine, is a potent and widely used veterinary sedative with some antinociceptive activity.[1] The clinical use of medetomidine is currently limited to healthy animals because of undesirable cardiovascular effects that could be harmful in some patients. After administration of racemic medetomidine, exposure to dexmedetomidine creates both the desired and undesired drug effects, as levomedetomidine is pharmacologically inert.[2] Medetomidine produces sedation by activating α_2-adrenoceptors located in the locus coeruleus in the brainstem; the same mechanism also inhibits norepinephrine release from centrally and peripherally located noradrenergic nerve endings.[3] The antinociceptive effects of this drug are believed to be mediated by spinal α_2-adrenoceptors located in the dorsal horn of the spinal cord and supraspinally by the locus coeruleus.[4,5] Medetomidine also has important direct effects on the cardiovascular system.[6,7] When administered IV, medetomidine increases arte-

ABBREVIATIONS
AUC_{10-60}	Area under the plasma concentration-versus-time curve from 10 to 60 minutes after drug administration
CI	Confidence interval
CVP	Central venous blood pressure
HR	Heart rate
M20	Medetomidine (20 µg/kg)
M20V400	Medetomidine (20 µg/kg) and vatinoxan (400 µg/kg)
M40V800	Medetomidine (40 µg/kg) and vatinoxan (800 µg/kg)

rial blood pressure[6,8] through vasoconstriction mediated by activation of postsynaptic α_{2B}-adrenoceptors located on vascular smooth muscle cells,[9,10] leading to increased systemic vascular resistance.[7,11-13] The increase in arterial blood pressure partly explains the bradycardia and bradyarrhythmias associated with α_2-adrenoceptor agonist administration; inhibition of sympathetic nervous system activity also contributes to these findings.[6,8,12,14] Increased systemic vascular resistance and the marked decrease in HR that accompanies it are in turn responsible for the observed reduction in cardiac output.[7,12] These negative cardiovascular effects are evident at low doses.[7] The magnitude and duration of the sedation and antinociception provided by medetomidine are also dose dependent, and the adverse effects increase with dose.[7,14,15]

Vatinoxan, previously known as MK-467 and L-659′066, is an α_2-adrenoceptor antagonist that poorly penetrates the blood-brain barrier, at least in rats and marmosets, allowing selective blocking of α_2-adrenoceptors in peripheral tissues.[16] The peripheral selectivity of vatinoxan has also been recently found in dogs.[17] Vatinoxan has been used experimentally and clinically to prevent or attenuate the undesired peripheral effects of medetomidine and dexmedetomidine in dogs,[18-22] and reductions in the unwanted effects of the latter drugs are dose dependent.[18,21] Centrally mediated dexmedetomidine-induced sedation is not substantially reversed by the administration of vatinoxan in dogs,[19,23] although the duration of sedation is slightly shortened.[24] The modest reduction in the duration of sedation observed after vatinoxan administration probably results from decreased plasma dexmedetomidine concentrations[24,25] due to increased cardiac output, resulting in higher clearance and increased volume of distribution of dexmedetomidine.[25] Vatinoxan has also been shown to reduce the somatic antinociceptive potency of IV administered medetomidine in dogs, presumably through the same mechanism.[24] It has been proposed[24] that the reduction in sedative and antinociceptive effects should be eliminated by increasing the IV dose of medetomidine when vatinoxan is administered to achieve plasma concentrations of dexmedetomidine sufficient to produce sedation and analgesia. To the authors' knowledge, no studies have been performed to evaluate somatic antinociception in dogs that have equal plasma dexmedetomidine concentrations with or without vatinoxan, and although results of 1 study[26] revealed no evidence that vatinoxan abolishes the antinociceptive effect of dexmedetomidine after IV administration in rats used to study visceral pain, the possible impact of vatinoxan on medetomidine-induced visceral antinociception in dogs has not been assessed.

Preventing the peripherally mediated adverse effects of α_2-adrenoceptor agonists with vatinoxan would be useful only if it did not diminish the sedation and antinociception provided by the agonist drug. The purpose of the study reported here was to determine whether concurrent vatinoxan administration influences the antinociceptive efficacy of medetomidine in dogs when administered at doses that provide circulating dexmedetomidine concentrations similar to those produced by medetomidine alone. We aimed to compare the magnitude of medetomidine's sedative, somatic antinociceptive, and visceral antinociceptive effects with and without concomitant vatinoxan administration. On the basis of previous results,[24,25] we anticipated that plasma dexmedetomidine concentrations resulting from a given IV dose of medetomidine could be achieved when vatinoxan was coadministered IV with a higher dose of medetomidine. Our hypothesis was that medetomidine plus vatinoxan would have an antinociceptive and sedative efficacy similar to that achieved with medetomidine alone when plasma concentrations of dexmedetomidine were equal or nearly equal to those resulting from the latter treatment.

Materials and Methods

Dogs

Eight healthy purpose-bred Beagles approximately 1.5 years of age (2 females and 6 males; all neutered a few months prior to enrollment) were used in the study. Mean ± SD body weight of the dogs was 13.4 ± 0.9 kg. All animals were considered healthy on the basis of physical examination findings by a veterinarian and results of a CBC and routine serum biochemical analysis.

Dogs were housed in 1 group and fed commercially available dog food twice daily with fresh water freely available. On each study day, food was withheld for ≥ 8 hours prior to instrumentation and drug administration, and dogs were fed at the end of each experiment. All dogs were retired from research and adopted out after the study. The study protocol was approved by the Finnish National Animal Ethics Committee (license No. ESAVI/7187/04.10.03/2012).

Drugs and experimental design

The study was designed as a randomized 3 X 3 crossover trial. The order of treatments was randomized by use of numbered pieces of paper; the Williams design was used for the first 6 dogs, and treatment order for the remaining 2 dogs was randomly picked from the same design. Each dog received 3 treatments of medetomidine hydrochloride[a] alone or with vatinoxan hydrochloride[b] (M20, M20V400, and M40V800), with intervals of ≥ 2 weeks between treatments. The amount of vatinoxan was adjusted when the dose of medetomidine was increased to keep the medetomidine-to-vatinoxan concentration ratio consistent between the latter 2 treatments. The 2 drugs were mixed in the same syringe immediately prior to use. The drug combination was diluted with sterile saline (0.9% NaCl) solution to achieve a total volume of 10 mL.

At the start of each experimental session, a 20-gauge catheter[c] was inserted into the left or right

cephalic vein, and dogs were preoxygenated (100 mL/kg/min for ≥ 3 minutes) via a tight-fitting face mask. For instrumentation, general anesthesia was induced by IV administration of propofol[d] to effect; the mean ± SD dose required for tracheal intubation was 6.9 ± 1.1 mg/kg. Anesthesia was maintained with isoflurane[e] in oxygen administered through a circle breathing system, and dogs were allowed to breathe spontaneously. During instrumentation, acetated Ringer solution was administered IV at a rate of 4 mL/kg/h. Three ECG electrodes were placed, and dogs were covered with a blanket; rectal temperature was maintained between 36°C and 38°C with an electric heating pad. Following percutaneous infiltration of approximately 1 mL of 2% lidocaine hydrochloride,[f] dogs were instrumented with a central venous catheter[g] placed via the left or right jugular vein. The catheter was measured so that the tip extended to the second rib, and correct positioning of the distal port was confirmed by observation of a typical CVP waveform. After instrumentation, isoflurane administration and IV fluid administration were discontinued, and dogs were monitored through extubation and recovery. A ≥ 60-minute recovery interval was used to ensure appropriate recovery before recording of baseline assessments (immediately prior to study treatment administration), which included measurement of CVP and HR, sedation scoring, and measurement of somatic and visceral pressures used to evaluate nociception as subsequently described. After baseline data were obtained, the assigned study treatment was injected via the cephalic venous catheter over a 10-second period, dogs were placed in lateral recumbency on the electric heating pad, and the infusion of acetated Ringer solution was reinitiated (4 mL/kg/h, IV).

The central venous catheter was used for blood sample collection and for CVP measurements by use of a precalibrated pressure transducer[h] and a multiparameter monitor.[i] The sternum was used as the zero reference point for CVP measurements, and HR was recorded from the ECG results. The CVP and HR measurements were obtained before performing other evaluations at baseline and 5, 10, 15, 25, 30, 35, 50, 55, and 60 minutes after study drug injection.

Sedative and analgesic effects of the study treatments were determined by 1 researcher who was unaware of treatment assignment (VH). Sedation scoring was performed at baseline and repeated 5, 25, and 50 minutes after injection of the study treatment, with a composite sedation score determined as described in a previous study[23]; scores ranged from a minimum of 0 (no sedation) to a maximum of 16 (no palpebral reflex, tongue relaxed, and no reaction to surroundings).

Somatic nociception measurement was performed at baseline and 10, 30, and 55 minutes after study drug injection by the application of a standardized nociceptive force stimulus to the nail bed of a digit on the nondependent hind limb with an electronic algesimeter[j] (ie, toe pinch), with gradually in-creasing force as previously described.[27,28] Visceral nociception measurement was completed at baseline and 15, 35, and 60 minutes after study drug injection, as previously described[29,30]; briefly, an anorectal balloon catheter[k] was gently inserted into the rectum to a depth of approximately 5 to 8 cm, the balloon was gradually inflated, and the pressure exerted by the balloon on the visceral mucosa was measured with a mercury manometer.[l] During nociception testing, the dogs' responses to the stimuli (limb withdrawal, head lift, vocalization, tensing of abdominal muscles, or > 10% increase in HR) were closely monitored. Following a response to the stimulus, the test was immediately discontinued (ie, the toe pinch was withdrawn or the balloon was deflated), and the force or pressure readings were recorded. To prevent tissue damage, stimulation was also discontinued if a predetermined maximum pressure reading was reached and the dog did not respond. The cutoff force for the toe pinch was 800 g/mm^2 (7.845 N/mm^2) as recommended by the manufacturer, and the maximum inflation pressure applied with the rectal balloon was 1.5 times the baseline response pressure measured at the beginning of each experiment.

Blood samples (5 mL) were collected through the central venous catheter prior to each evaluation (ie, at baseline and 5, 10, 15, 25, 30, 35, 50, 55, and 60 minutes after injection of study drugs). The blood samples were centrifuged at 3,000 X g for 15 minutes, and collected plasma was frozen at –20°C until measurement of dexmedetomidine and vatinoxan concentrations.

At the end of each experiment, all catheters were removed. The dogs received a single 0.2-mg/kg dose of meloxicam,[m] SC, and the effects of medetomidine were reversed by IM administration of atipamezole hydrochloride,[n] (given according to the dose of medetomidine administered; 0.1 mg/kg for dogs that had received M20 or M20V400 and 0.2 mg/kg for dogs that had received M40V800).

Plasma drug concentration analysis

Concentrations of dexmedetomidine and vatinoxan in canine plasma were analyzed with high-performance liquid chromatography–tandem mass spectrometry as previously described.[24] The analytical methods were validated for range, precision, accuracy, carryover, interference of analytes and internal standards, matrix effects, and analyte stability to comply with regulatory guidance.[31] Reference standards were used for dexmedetomidine,[o] and the linear concentration range for each enantiomer of medetomidine was from 0.075 to 10 ng/mL. The interassay accuracy of the quality control samples (at concentrations of 0.15, 1.0, and 8 ng/mL) ranged from 99.4% to 103.0% for dexmedetomidine. Reference standards for vatinoxan were used,[p] and the linear range of the assay was from 10 to 900 ng/mL. The interassay accuracy of the quality control samples (at concentrations of 30, 400, and 740 ng/mL) ranged from 97.4%

to 109.1%. Intraassay coefficients of variation were < 8% for both analytes at all 3 concentrations, and < 15% at the lower limit of quantitation (0.075 ng/mL for dexmedetomidine and 10 ng/mL for vatinoxan).

Statistical analysis

Normality assumptions were checked for all response variables (HR, CVP, and AUC_{10-60} values for dexmedetomidine concentration, and areas under the measurement-versus-time curve over the same interval for sedation scores and visceral and somatic pressure measurements) with Kolmogorov-Smirnov tests.[q] Only CVP measurements were normally distributed. The visceral and somatic nociception measurements and sedation scores obtained after injection of study drugs were compared with the corresponding baseline measurements and among the 3 treatments within time points; this analysis was performed for related samples with the Friedman 2-way ANOVA by ranks, followed by Bonferroni post hoc corrections when appropriate.[q]

The sedation scores and baseline-adjusted visceral pressure measurements (calculated because the maximum balloon pressure applied depended on the baseline measurement) were further analyzed to assess noninferiority of medetomidine plus vatinoxan at each dose combination, compared with medetomidine alone for eliciting desired effects, and plasma drug concentration data were analyzed for equivalence calculation and comparisons of area under the curve between treatments.[r] Only time points ≥ 10 minutes after study drug administration were included in the analyses to ensure adequate drug distribution. The trapezoidal method was used to calculate the AUC_{10-60} for dexmedetomidine concentration and areas under the curve for sedation score and visceral pressures for each dog individually. Logarithmic (base 10) transformation was applied to area-under-the-curve values to normalize their distributions (confirmed with a Kolmogorov-Smirnov test). Treatment differences and 95% CIs were transformed back to the original scale and reported as the geometric mean ratio. For intertreatment comparison of plasma dexmedetomidine concentrations, the reference limits for equivalence were set at 0.8 and 1.25, similar to procedures used in bioequivalence studies.[32] For intertreatment comparison of sedation scores and visceral pressures, the reference limits for noninferiority were also set at 0.8 and 1.25.[32] Equivalence of drug concentrations or noninferiority of treatments was accepted when the 95% CI of the geometric mean ratio was within this reference range.

Because the maximum value for somatic nociceptive force measurements could not be defined for each dog (owing to use of a maximum cutoff force of 800 g/mm²), the area-under-the-curve method could not be reliably used for tolerance of somatic nociception, and nonparametric 95% CIs were calculated for median differences between the treatments on the basis of the Wilcoxon 1-sample test statistic.[r] The

noninferiority margin was set at –20% of the median value for dogs that received M20 (700 g/mm²), which was considered a clinically acceptable difference.

The differences in changes of HR and CVP from baseline (0 minutes = immediately prior to injection of study drugs) among treatments were evaluated with repeated-measures ANCOVA models.[r] Each model included the main effects of treatment and time point, 2-way interactions of treatment by time point, and a baseline covariate as fixed effects and included the main effect of dog and the 2-way interaction of dog by time point as random effects. For HR, square root transformation was used to normalize the distribution. Treatment differences and within-treatment changes with 95% CI were calculated from the same models with contrasts.

All analyses were performed with commercially available software.[q,r] For all comparisons, values of $P < 0.05$ were considered significant.

Results

One dog removed its central venous catheter after instrumentation and did not receive the M40V800 treatment. Another dog became slightly agitated and defecated loose feces together with a plastic foreign body 30 minutes after treatment administration of M20V400, and visceral nociceptive testing was discontinued for the animal at that time.

The log-transformed mean ± SD plasma dexmedetomidine and vatinoxan concentrations are depicted (**Figure 1**). The mean resulting plasma dexmedetomidine concentrations after M20 administration ranged from 9.31 to 2.73 ng/mL, those after M20V400 administration ranged from 4.62 to 1.45 ng/mL, and those after M40V800 ranged from 9.23 to 2.78 ng/mL; the highest values were measured at 10 minutes, and the lowest values were measured at 60 minutes (the 5-minute values were not included in the statistical analysis). The dexmedetomidine concentrations were equivalent between M40V800 and M20, as confirmed with the AUC_{10-60} comparisons (geometric mean ratio, 0.92 [95% CI, 0.82 to 1.04]). After M20V400 administration, plasma dexmedetomidine concentrations were approximately halved, compared with those after M20 administration, at all analyzed time points, and the plasma concentrations were not equivalent between M20V400 and M20 (geometric mean ratio, 0.46 [95% CI, 0.40 to 0.51]). The concentrations of vatinoxan in plasma were linearly proportional to the administered doses.

Median and range maximum rectal balloon inflation pressures (visceral nociception measurements) and the proportion of dogs that did not react at the maximum cutoff pressure after each treatment are shown (**Table 1**). The M20 and M40V800 treatments were each associated with significantly increased visceral pressure tolerance, compared with that at baseline in the same experiment, 15 and 35 minutes after treatment. No significant differences from baseline measurements were found after administration

of M20V400. When maximum visceral pressure measurements within time points were compared among the 3 treatments, no significant difference was found. Comparisons of areas under the measurement-versus-time curve from 10 to 60 minutes after study drug administration confirmed that visceral antinociception with M40V800 was noninferior to that produced by M20 administration (geometric mean ratio, 0.96 [95% CI, 0.84 to 1.1]). Noninferiority of visceral antinociception with M20V400 could not be shown (geometric mean ratio, 0.90 [95% CI, 0.79 to 1.0]).

Median and range maximum force applied to the nail bed (somatic nociception measurements) and the number of animals that did not react to the predetermined cutoff force are reported **(Table 2)**. Somatic nociceptive force tolerance was significantly in-creased at 10 and 30 minutes (but not at 55 minutes) after all treatments, compared with the respective baseline measurements. When somatic nociception measurements within time points were compared among the 3 treatments, no significant difference was found. With the noninferiority margin set at –20%, somatic antinociception with M20V400 was noninferior to that achieved with M20 10 minutes after treatment administration, and somatic antinociception with M40V800 was noninferior to that resulting from M20 at 10 and 55 minutes (but not 30 minutes) after treatment administration.

The median and range sedation scores are summarized **(Table 3)**. Compared with the respective baseline values, the scores obtained after all treatments were significantly higher (indicating signifi-
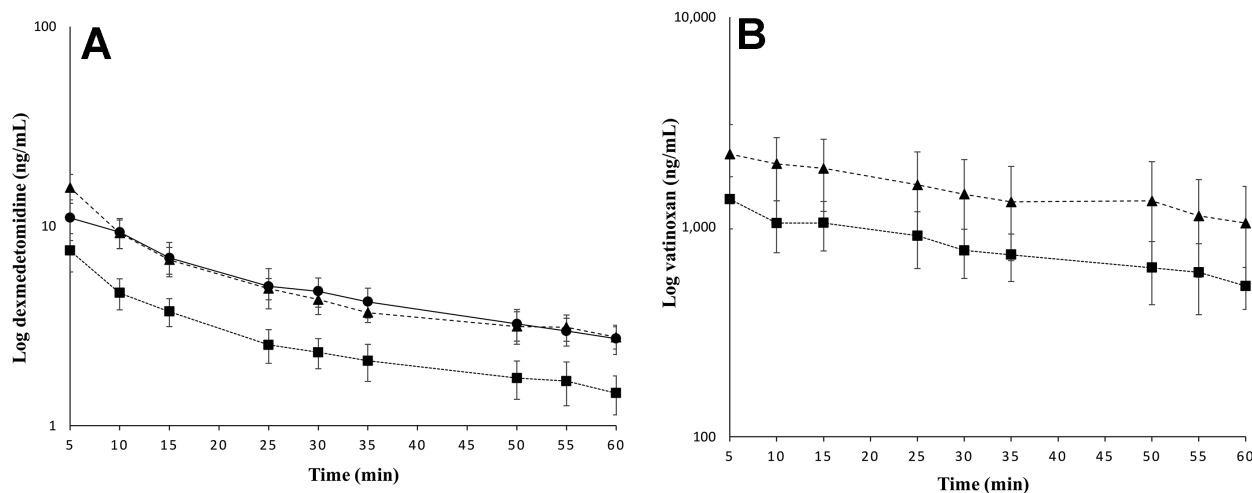

Figure I—Semilogarithmic plots of the mean ± SD plasma concentrations of dexmedetomidine (A) and vatinoxan (B) versus time in 8 healthy Beagles from 5 to 60 minutes after IV administration (time 0) of M20 (circles), M20V400 (squares), or M40V800 (triangles) sedative treatments in a randomized 3 X 3 crossover-design study. Combinations of medetomidine and vatinoxan were mixed in the same syringe; all study drugs were diluted with sterile saline (0.9% NaCl) solution to a total volume of 10 mL and injected IV over 10 seconds. There was a washout period of ≥ 14 days between treatments. Data for M40V800 represent results for 7 dogs (1 dog did not receive the treatment).

Table I—Median (range) maximum visceral pressure measurements in 8 healthy Beagles that received M20, M20V400, or M40V800 treatment in a randomized 3 X 3 crossover-design study and the proportion of dogs that did not react to the stimulus up to and including the maximum cutoff pressure applied (1.5 times the baseline pressure for the same dog during each experiment).

	M20		M20V400		M40V800	
Time (min)	Measurement (mm Hg)	Proportion with no reaction	Measurement (mm Hg)	Proportion with no reaction	Measurement (mm Hg)	Proportion with no reaction
0 (baseline)	126 (96–180)	—	128 (110–174)	—	128 (115–172)	—
15	187 (134–250)*	2/8	175 (152–242)	2/8	180 (140–250)*	4/7
35	189 (144–260)*	4/8	165 (120–248)	1/7	173 (156–207)*	2/7
60	171 (144–230)	2/8	164 (120–212)	2/7	165 (138–174)	0/7

Baseline values were obtained immediately prior to injection of study drugs. Measurements were obtained by use of an anorectal balloon catheter placed in the rectum; the balloon was gradually inflated until a reaction to the stimulus (limb withdrawal, head lift, vocalization, tensing of abdominal muscles, or > 10% increase in HR) was observed by an individual blinded to the treatment administered or until the predetermined cutoff pressure was reached, at which time the balloon was immediately deflated and the last inflation pressure was recorded. One dog was excluded from testing because it did not receive M40V800 treatment, and 1 dog had visceral nociception testing discontinued 30 minutes after M20V400 administration because it developed loose feces with evidence of prior foreign body consumption. Values of *P* < 0.05 were considered significant.

*Value is significantly different, compared with that at baseline for the same treatment.

— = Not applicable.

Table 2—Median (range) maximum somatic nociceptive force measurements for the dogs in Table 1 and the proportion of dogs that did not react to the stimulus up to and including the maximum cutoff force applied (800 g/mm^2).

	M20		M20V400		M40V800	
Time (min)	Measurement (g/mm^2)	Proportion with no reaction	Measurement (g/mm^2)	Proportion with no reaction	Measurement (g/mm^2)	Proportion with no reaction
0 (baseline)	371 (202–598)	0/8	321 (175–566)	0/8	440 (290–704)	0/7
10	800 (456–800)*	5/8	800 (756–800)*	6/8	800 (588–800)*	4/7
30	800 (574–800)*	5/8	725 (572–800)*	2/8	789 (506–800)*	3/7
55	604 (322–800)	2/8	520 (430–800)	1/8	601 (344–716)	0/7

Measurements were obtained by application of gradually increasing force (delivered by an electronic algesimeter) to the nail bed of a digit on the nondependent hind limb of dogs in lateral recumbency. The test was immediately discontinued when a reaction to the stimulus was observed by an individual blinded to the treatment administered or when the maximum cutoff force was reached and the last force applied was recorded.

See Table 1 for remainder of key.

Table 3—Median (range) sedation scores for the dogs in Table 1.

Time (min)	M20 (n = 8)	M20V400 (n = 8)	M40V800 (n = 7)
0 (baseline)	0 (0–0)	0 (0–0)	0 (0–0)
5	14 (8–16)*	12 (9–14)*	13 (8–15)*
25	13.5 (11–15)*	14 (11–15)*	14 (10–16)*
50	8.5 (8–10)*	7.5 (6–12)*	9 (8–12)*

Sedation was scored by 1 investigator who was blinded to the treatment administered; the scoring method was adapted from another source,[23] and possible scores ranged from 0 (no sedation) to 16 (maximum sedation; no palpebral reflex, tongue relaxed, no reaction to surroundings).

See Table 1 for remainder of key.

cantly greater sedation) at all evaluated time points. When sedation scores were compared among the 3 treatments at each of the assessment time points, no significant differences were detected. Noninferiority of M40V800 and of M20V400 relative to M20 for sedative effects was confirmed by comparison of areas under the measurement-versus-time curves from 10 to 60 minutes after the study drug injection: for M40V800, the geometric mean ratio was 1.01 (95% CI, 0.88 to 1.17), and for M20V400, the geometric mean ratio was 0.97 (95% CI, 0.84 to 1.12).

Measurements of HR were significantly higher and measurements of CVP were significantly lower at each time point after administration of M20V400 or M40V800, compared with the results for M20 treatment **(Table 4)**. The HR measurements were significantly lower and CVP measurements were significantly higher, compared with the respective baseline values at all postinjection time points for M20 treatment. No significant differences in HR or CVP were detected at any time point between M20V400 and M40V800.

Discussion

In the present study, we compared sedative and antinociceptive effects of 2 dose combinations of medetomidine and vatinoxan with those of medetomidine alone. After adequate drug distribution, which appeared to require > 5 minutes on the basis of the plasma concentration curve and data from previous studies, including a study[25] of dogs, M40V800 produced plasma dexmedetomidine concentrations equivalent to those resulting from M20 administration, and plasma concentrations remained similar between these 2 treatments throughout the investigation (from 10 to 60 minutes). When M20V400 was administered, the resulting plasma dexmedetomidine concentrations were considerably lower at approximately half those of M20 throughout the evaluation period, probably owing to the vatinoxan-induced reduction in initial vasoconstriction and preserved cardiac output (which were indirectly suggested by lower CVP and higher HR), leading to higher clearance from increased perfusion of elimination organs and a larger volume of dexmedetomidine distribution.[24,25] As expected, once plasma concentrations of dexmedetomidine were similar between the 2 treatments, the sedation and visceral antinociception produced by M40V800 was noninferior to that produced by M20 at all evaluated time points, with similar results for somatic antinociception at 10 and 55 minutes. The sedation achieved with M20V400 was also noninferior to that resulting from M20, despite the lower plasma dexmedetomidine concentrations. Unimpaired sedative effects were probably seen because even the reduced dexmedetomidine plasma concentrations after M20V400 injection were high enough to induce maximal sedative effects; therefore, the increased medetomidine dose (and plasma dexmedetomidine concentration) did not further augment the effect.

We assessed somatic antinociception effects of the treatments by applying a standardized nociceptive force to the nail bed, which was stopped when a predetermined maximum force was reached or when the dog withdrew the limb or showed any signs of sympathetic stimulation or discomfort. Limb withdrawal is a spinally mediated response to nociception,[4] and the antinociceptive effect of dexmedetomidine is believed to be mediated mainly by activation of α_2-adrenoceptors in the spinal cord.[33] In another study,[24] when 10 μg of medetomidine/kg was administered with 250 μg of vatinoxan/kg IV to dogs, the anticipated plasma dexmedetomidine concentration was halved. The resulting plasma dexmedetomidine concentration did not exceed 1.9 ng/mL,[24] which is

Table 4—Median (range) HR and CVP measurements for the dogs in Table 1.

	M20 (n = 8)		M20V400 (n = 8)		M40V800 (n = 7)	
Time (min)	HR (beats/min)	CVP (mm Hg)	HR (beats/min)	CVP (mm Hg)	HR (beats/min)	CVP (mm Hg)
0 (baseline)	86 (74 to 119)	1.5 (−1 to 3)	94 (72 to 127)	2.0 (0 to 5)	102 (76 to 153)	2.0 (0 to 3)
5	34.5 (28 to 44)*	8.5 (5 to 11)*	67 (44 to 88)†	3.5 (−1 to 7)†	72 (60 to 100)†	3.0 (1 to 5)†
10	32 (24 to 44)*	7.5 (5 to 11)*	76 (25 to 108)†	4.0 (−3 to 6)†	76 (61 to 92)†	2.0 (1 to 4)†
15	38 (30 to 48)*	7.0 (4 to 10)*	69 (55 to 110)†	3.0 (−3 to 6)†	75 (63 to 88)†	3.0 (1 to 4)†
25	36 (28 to 48)*	5.5 (3 to 10)*	70 (62 to 112)†	3.0 (−2 to 6)†	75 (60 to 88)†	2.0 (1 to 3)†
30	36 (36 to 48)*	5.5 (2 to 9)*	68 (57 to 92)†	2.5 (−3 to 6)†	72 (59 to 84)†	1.0 (0 to 3)†
35	37 (24 to 44)*	5.5 (1 to 9)*	66 (56 to 84)†	2.5 (−2 to 6)†	72 (60 to 76)†	2.0 (0 to 3)†
50	34 (32 to 44)*	5.0 (2 to 9)*	62 (44 to 79)†	2.5 (−3 to 5)†	64 (56 to 76)†	2.0 (−1 to 3)†
55	40 (28 to 42)*	4.0 (2 to 9)*	59 (56 to 80)†	2.0 (−2 to 6)†	65 (52 to 72)†	1.0 (−2 to 3)†
60	40 (24 to 44)*	4.5 (2 to 8)*	64 (52 to 138)†	2.0 (−4 to 4)†	64 (52 to 72)†	2.0 (−1 to 3)†

†Within a timepoint, value is significantly different from that for the same measurement after M20 administration.
See Table 1 for remainder of key.

the mean concentration considered to be required for antinociception in dogs.[34] This led to a significant reduction in medetomidine-induced somatic antinociception (measured by squeezing the nail bed with hemostats), compared with results when the same dose of medetomidine was given alone, and sedation was achieved but shortened relative to that from medetomidine alone.[24] Those findings corresponded with previous findings that higher plasma dexmedetomidine concentrations are required for antinociception than for sedation.[14,34] The mean plasma dexmedetomidine concentration associated with sedation in dogs is ≥ 0.533 ng/mL.[34] In our study, plasma dexmedetomidine concentrations persisted above the previously reported analgesic concentration (1.9 ng/mL)[34] in all dogs throughout the investigation period after the M20 or M40V800 treatment. After M20V400 administration, the mean plasma dexmedetomidine concentrations exceeded 1.9 ng/mL for the first 35 minutes, after which they decreased gradually but remained well above the concentration needed for sedation[34] until the end of our study.

Visceral antinociception was assessed by inflating a purpose-made anorectal balloon catheter inside the rectum and measuring pressures inside the balloon. We chose rectal distension for assessment of visceral pain because the rectal catheter is easy to insert, the results are quantifiable, and the method is minimally invasive; the procedure is commonly used to assess visceral nociception in rats, dogs, horses, and people.[26,29,30,35-38] We measured the pressure within the rectal balloon because in human subjects, visceral pain sensation is reportedly related to pressure rather than volume of distension.[39] We found that M40V800 was noninferior to M20 in the provision of visceral antinociception. Due to variations in baseline measurements even within the same dog, we decided to end testing at a maximum inflation pressure of 1.5 times the baseline pressure (or less, if the dog responded earlier). Although it would have been simpler to use a fixed maximum inflation pressure, we made this choice to avoid tissue damage. In addition to the ethical consideration to do no harm,

any degree of tissue injury could have interfered with subsequent measurements because the dogs served as their own controls in the crossover-design study. Other researchers have used a similar approach: results of another visceral analgesia study[29] in dogs revealed that rectal balloon inflation pressures of 120 to 190 mm Hg were required to elicit a response in awake dogs (pretreatment control values). After butorphanol administration, antinociception was assumed to be present if the pressure required to elicit a response was higher than the pretreatment values. The investigators of that study[29] expressed the inflation pressures as percentage increases from the control pressures; exact numeric values were not reported, but the highest pressures used were roughly 40% higher than the control pressures.[29] The reported control pressures and the percentage change in pressure tolerance after butorphanol administration[29] were similar to those measured in our study.

Overall, our results supported the assumption[24] that the previously described decrease in antinociceptive efficacy and the shorter duration of sedation in dogs that received vatinoxan in addition to medetomidine were more likely caused by lower plasma dexmedetomidine concentrations than the ability of vatinoxan to permeate the canine blood-brain barrier to any clinically relevant extent. It has been shown that vatinoxan injection in rats and marmosets produces minimal brain concentrations of the drug,[16] and recent results from our study group suggest that the same is true for dogs.[17] In dogs, however, vatinoxan has been shown to increase the minimum alveolar concentration of sevoflurane.[40] The spinal cord is considered important in suppressing movement in response to noxious stimulation under inhalation anesthesia and therefore in the measurement of minimum alveolar concentration[41-43]; if vatinoxan increases the minimum alveolar concentration of sevoflurane, it is possible that some spinal cord penetration occurs.[40] In the study reported here, no significant differences in spinally mediated somatic antinociception were found among the 3 treatments, and somatic antinociception resulting from M40V800 administration was not inferior to that from M20 at

10 and 55 minutes after administration. In addition, vatinoxan administration produces low spinal cord-to-blood concentration ratios of the drug in dogs, indicating that only a very small fraction of vatinoxan is able to permeate into the CNS.[17] However, the brain and spinal cord concentrations of vatinoxan sufficient to cause measurable CNS effects are not known.

Although the extents of sedation and antinociception provided by medetomidine are generally considered to be dose dependent and increasing plasma dexmedetomidine concentrations will initially result in more pronounced effects, a so-called ceiling effect at a certain concentration is reached in dogs, after which increasing the dose no longer increases the magnitude of sedation but increases the duration of this effect.[14,34] However, both sedation[44] and antinociception[44,45] in cats are intensified with increasing plasma dexmedetomidine concentrations, suggesting that higher concentrations may be required to reach such a ceiling effect in cats than in dogs.

Clinically relevant and statistically significant decreases in HR and increases in CVP were detected after M20 administration in the present study. As previously described,[18-21,46] vatinoxan administration abolished these hemodynamic changes. Both tested drug doses were sufficient to reverse the unwanted effects of medetomidine on HR and CVP.

One dog had loose feces and defecated a plastic foreign body 30 minutes after vatinoxan administration, after which the dog was excluded from further assessments of visceral antinociception. It was likely that the foreign body had irritated the bowel; however, it was also possible that vatinoxan alleviated medetomidine-induced intestinal relaxation and increased gut motility, thus leading to loosened feces. In people and horses, α_2-adrenoceptor agonists have been shown to inhibit gastric emptying and reduce gut motility.[47-49] Conversely, vatinoxan has been shown to antagonize detomidine-induced and romifidine-induced intestinal hypomotility in horses,[48,49] and results of 1 study[49] indicate abdominal discomfort, evidenced by restlessness and kicking, in 3 of 7 horses after administration of vatinoxan alone. Furthermore, loose feces were detected in one-third of client-owned dogs (9/27) that had been sedated with a medetomidine-butorphanol-vatinoxan combination for diagnostic imaging; however, in the same study,[22] several dogs that received atipamezole after being sedated with a medetomidine-butorphanol combination without vatinoxan also developed loose feces. In our study, only 1 dog was found to have loose feces, and no definitive conclusions could be drawn regarding the rate of occurrence of this possible adverse effect.

Our study had several limitations. The main limitation was that our study may have lacked statistical power to detect a significant difference in sedation scores among treatments, even when the dexmedetomidine plasma concentration was halved by co-administration of vatinoxan with the medetomidine.

A probable contributing factor was that the dexmedetomidine plasma concentration still remained high enough to induce (nearly) maximal sedation. It was also possible that our sedation scoring system, despite its frequent use in similar studies, was not sensitive enough to detect differences among the treatments. In ideal conditions, the postinjection observation period would have been longer, and the study would have included a treatment that consisted of vatinoxan alone. Also, considering that the aim of a noninferiority trial is to show that the experimental treatment is not less effective than the active control by more than the noninferiority margin, the noninferiority margin chosen should be the largest difference that can be considered clinically acceptable; however, this is a subjective judgement, and our choice of this margin was therefore a possible limitation.

As previously described, higher plasma dexmedetomidine concentrations were required for analgesia than for sedation in the present study. The addition of vatinoxan did not considerably interfere with the analgesia provided by medetomidine as long as the plasma dexmedetomidine concentrations remained greater than amounts required for antinociception. Vatinoxan also provided better cardiovascular stability (suggested by the absence of marked bradycardia and lower CVP), compared with the administration of medetomidine alone. Additional studies of vatinoxan-medetomidine combinations as a part of a balanced anesthesia protocol are warranted to verify the suitability of vatinoxan in clinical veterinary use in dogs.

Acknowledgments

Footnotes

a. Dorbene, Vetcare Ltd, Mäntsälä, Finland.
b. Vetcare Ltd, Mäntsälä, Finland.
c. Terumo Europe NV, Leuven, Belgium.
d. Vetofol 1%, Norbrook Laboratories, Newry, Northern Ireland.
e. Isoflo, Orion Pharma Ltd, Espoo, Finland.
f. Orion Oyj, Turku, Finland.
g. CV-12702, Arrow International, Reading, Pa.
h. Gabarith PMSET, Becton Dickinson, Sandy, Utah.
i. S/5 Anesthesia Monitor, GE Healthcare, Helsinki, Finland.
j. Randall-Selitto, IITC 2500 Digital Paw Pressure Meter, IITC Life Science Inc, Woodland Hills, Calif.
k. SR1B Single-use anorectal balloon/expulsion catheter, Mui Scientific, Mississauga, ON, Canada.
l. Welch Allyn UK Ltd, Buckinghamshire, England.
m. Metacam 5 mg/mL, Boehringer Ingelheim Vetmedica, Ingelheim, Germany.
n. Antisedan 5 mg/mL, Orion Pharma Ltd, Espoo, Finland.
o. Toronto Research Chemicals, Toronto, ON, Canada.

p. PCAS Finland Oy, Turku, Finland.
q. SPSS statistics for Mac, version 24.0, IBM Corp, Armonk, NY.
r. SAS for Windows, version 9.3, SAS Institute Inc, Cary, NC.

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