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The impact of MK-467 on plasma drug concentrations, sedation and cardiopulmonary changes in sheep treated with intramuscular medetomidine and atipamezole for reversal

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The effect of MK-467, a peripheral α_2-adrenoceptor antagonist, on plasma drug concentrations, sedation and cardiopulmonary changes induced by intramuscular (IM) medetomidine was investigated in eight sheep. Additionally, the interactions with atipamezole (ATI) used for reversal were also evaluated. Each animal was treated four times in a randomized prospective crossover design with 2-week washout periods. Medetomidine (MED) 30 μg/kg alone or combined in the same syringe with MK-467 300 μg/kg (MMK) was injected intramuscular, followed by ATI 150 μg/kg (MED + ATI and MMK + ATI) or saline intramuscular 30 min later. Plasma was analysed for drug concentrations, and sedation was subjectively assessed with a visual analogue scale. Systemic haemodynamics and blood gases were measured before treatments and at intervals thereafter. With MK-467, medetomidine plasma concentrations were three-fold higher prior to ATI, which was associated with more profound sedation and shorter onset. No significant differences were observed in early cardiopulmonary changes between treatments. Atipamezole reversed the medetomidine-related cardiopulmonary changes after both treatments. Sedation scores decreased more rapidly when MK-467 was included. In this study, MK-467 appeared to have a pronounced effect on the plasma concentration and central effects of medetomidine, with minor cardiopulmonary improvement.

1 | INTRODUCTION

Alpha$_2$-adrenocepor agonists are used extensively as sedatives, analgesics and premedication in both human medicine and animal medicine. Medetomidine is a highly selective and potent α_2-adrenoceptor agonist with a 1,620:1 affinity ratio for α_2- vs. α_1-adrenoceptors (Virtanen, Savola, Saano, & Nyman, 1988). The use of medetomidine, as well as other α_2-agonists, in sheep is accompanied with a number of adverse effects, most notably hypoxaemia (Celly, McDonell, & Black, 1999; Celly, McDonell, Young, & Black, 1997; Kästner, Ohlerth, Pospischil, Boller, & Huhtinen, 2007; Kästner et al., 2003), bradycardia and reduction in cardiac output (Bryant, Clarke, & Thompson, 1996; Bryant, Thompson, & Clarke, 1998; Raekallio, Honkavaara, & Vainio, 2010; Talke, Traber, Richardson, Harper, & Traber, 2000). Activation of peripherally located, vascular α_2-adrenoceptors is considered to be the primary cause of these effects (Bloor et al., 1992; Kästner et al., 2007).

MK-467 is a peripherally acting α_2-adrenoceptor antagonist that poorly penetrates mammalian central nervous tissues; its α_2- vs. -α_1 selectivity ratio is approximately 105:1 (Clineschmidt et al., 1988). The ability of MK-467 to attenuate the adverse haemodynamic influences attributed to α_2-agonists has been reported in several species, such as dogs (Enouri, Kerr, McDonell, O'Sullivan, & Neto, 2008; Honkavaara, Restitutti, Raekallio, Kuusela, & Vainio, 2011; Pagel et al., 1998; Rolfe, Kerr, & McDonell, 2012), horses (Bryant

et al., 1998; Vainionpää et al., 2013; de Vries et al., 2016), cats (Honkavaara, Pypendop, & Ilkiw, 2017; Pypendop, Honkavaara, & Ilkiw, 2017a) and sheep (Bryant et al., 1998; Raekallio et al., 2010). Furthermore, MK-467 had no substantial effect on the intensity of sedation induced by medetomidine or dexmedetomidine (Restitutti, Honkavaara, Raekallio, Kuusela, & Vainio, 2011; Rolfe et al., 2012). Moreover, MK-467 increased the absorption rate and early-stage plasma concentrations of α_2-agonists drugs when coadministered intramuscularly in cats (Pypendop, Honkavaara, & Ilkiw, 2017b) and dogs (Restitutti et al., 2017), resulting in faster induction of sedation (Restitutti et al., 2017).

Atipamezole (ATI) is a specific α_2-adrenoceptor antagonist with an α_2- vs. α_1 selectivity ratio of 8,500:1 (Virtanen, 1989). It is effective in reversing medetomidine-induced sedation in sheep (Ko & McGrath, 1995; Ranheim, Arnemo, Stuen, & Horsberg, 2000). However, relapses into sedation (i.e. resedation after an initial recovery) have been reported in some ruminant species (Ranheim, Arnemo, Ryeng, Soli, & Horsberg, 1999; Ranheim, Soli, Ryeng, Arnemo, & Horsberg, 1998; Ranheim et al., 1997). In addition, ATI did not completely reverse medetomidine-induced hypoxaemia in sheep (Ko & McGrath, 1995; Talke et al., 2000).

There are no previous reports on the concurrent intramuscular use of medetomidine with MK-467 followed by ATI in sheep. Therefore, we aimed to evaluate the effects of MK-467 and ATI on plasma drug concentrations, sedation and cardiopulmonary function in sheep receiving intramuscular medetomidine. We hypothesized that MK-467 would increase the absorption of medetomidine when given simultaneously via the intramuscular route. Moreover, we speculated that MK-467 might potentiate the effects of ATI on haemodynamics when the sheep were exposed to both of these antagonists.

2 | MATERIALS AND METHODS

2.1 | Animals

Eight adult, healthy, nonpregnant female sheep (five crossbreed and three Texel) aged 1–5 years and weighing 44–78 kg were used in this study. The sheep were presumed healthy based on physical examination, complete blood counts and serum chemistry analysis. The animals were kept in one group and fed hay and concentrate with free access to water. Six months before the start of the study, the right carotid arteries of the study animals were surgically elevated into a subcutaneous position under general anaesthesia using a technique modified from Tapio, Arguelles, Gracia-Calvo, and Raekallio (2017). The study was approved by the National Animal Experiment Board of Finland, licence number: ESAVI/9394/04.10.07/2015. The license fulfils the requirements of the European Union (EU) legislation.

2.2 | Treatment groups

Each sheep received the following four treatments in a prospective, randomized, blinded, four-period crossover study with a minimum of 14 days between consecutive treatments:

a) Medetomidine HCl (Dorbene 1 mg/ml; Syva Laboratories S.A., Spain) 30 μg/kg (Kästner et al., 2003) alone (MED) followed by saline 30 min later
b) MED + MK-467 HCl (Vetcare Ltd., Finland) 300 μg/kg (MMK) in the same syringe followed by saline 30 min later
c) MED followed by ATI HCl (Alzane 5 mg/ml; Syva Laboratories) 150 μg/kg 30 min later (MED + ATI)
d) MMK followed by ATI HCl 150 μg/kg 30 min later (MMK + ATI).

All treatments were administered intramuscular into the semimembranosus muscle; the injection site (right and left) was alternated every other treatment. ATI or the corresponding volume of saline was administered in the contralateral muscle for the medetomidine-containing treatment. The MK-467 dose was derived from our previous study (Adam et al., 2016), whereas ATI dosing and timing of administration were according to the manufacturer's recommendations (five times the dose of medetomidine after 30 min).

2.3 | Instrumentation

On the morning of the experiment day, the animals were weighed and kept upright in a padded sling such that their hooves did not touch the floor. The catheter insertion sites were aseptically prepared. Five mg of lidocaine (20 mg/ml; Orion, Finland) was infiltrated under the skin at each catheter site. First, a single-lumen polyethane central venous catheter (Cavafix Certo; B. Braun Melsungen, Germany) was inserted via the left jugular vein into the thoracic vena cava for measuring the central venous pressure (CVP) and collecting blood samples. Correct placement was confirmed by the shape of pressure waves. An 18-gauge venous cannula (Terumo Europe, Leuven, Belgium) was then placed in the right jugular vein for lithium injection. Finally, a 20-gauge arterial catheter (Becton Dickinson, Sandy, Utah, USA) was inserted in the elevated carotid artery. All catheters and cannulas were secured in position with sutures and surgical glue. The central venous and arterial catheters were connected to pressure transducers (Gabarith PMSET; Becton Dickinson, Sandy, Utah, USA), which were calibrated with a mercury manometer and zeroed to atmospheric pressure at the level of the manubrium.

2.4 | Study protocol

Heart rate (HR), CVP, systolic, diastolic and mean arterial pressures (SAP, DAP and MAP) were monitored (S/5 Compact Critical Care Monitor, Datex-Ohmeda; GE Healthcare, Finland) at baseline and continuously for up to 120 min after treatment. Respiratory rate (RR) was measured by observing chest movement at baseline and every 10 min after treatment. Cardiac output (CO) was measured at baseline and at 20, 40, 50, 60, 75 and 90 min after treatment with the lithium indicator dilution method (LidCO plus Hemodynamic Monitor; LidCO Ltd, Cambridge, UK) as previously described by Raekallio et al. (2010). Standard values of 10 g/dl haemoglobin and 140 mM of sodium were used and later corrected with measured values obtained from simultaneously taken arterial blood samples. These samples were

anaerobically obtained (Pico50; Radiometer, Copenhagen, Denmark) *via* the arterial catheter at baseline and 10, 20, 40, 50, 60, 75 and 90 min and from the CVP catheter at baseline, 20, 40, 60, 75 and 90 min after treatment. The samples were placed in ice water for no longer than 10 min until analysed with a blood gas analyser (GEM Premier 4000, Bedford, Massachusetts, USA). Measurements were corrected to rectal temperature. Standard formulas (Boyd, McDonell, & Valliant, 1991) were used to calculate the following cardiopulmonary variables: cardiac index (CI), oxygen delivery (DO_2), arterial oxygen content (CaO_2) and systemic vascular resistance (SVR). A formula specific to sheep (Maginniss, Olszowka, & Reeves, 1986) was used for calculating arterial (SaO_2) and venous oxygen saturation (SvO_2).

Sedation was assessed using a visual scale from 0 to 10 (0 means no sedation; 10 means the sheep is deeply sedated and unresponsive to manipulation and hand clapping). Assessments were performed before drug administration and at intervals for up to 120 min thereafter by an investigator blinded to the treatments (MA). Resedation was defined as reduction in alertness and activity after the initial recovery.

For plasma drug concentrations, 6 ml of blood was collected from the arterial catheter at 20, 40, 50, 60, 75 and 90 min after treatment, kept in ice water and centrifuged at 3,000 *g* for 15 min. Plasma was stored at −20°C. At the end of each trial, all catheters were removed and meloxicam 0.5 mg/kg (Metacam 20 mg/ml; Boehringer Ingelheim, Germany) was administered intravenous.

2.5 | Drug analyses

The concentrations of dex- and levomedetomidine (reference standard: racemic medetomidine, TRC, Toronto, Ontario, Canada) in EDTA plasma were determined with high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) after solid-phase extraction (Sep-Pak®tC18 96-well extraction plates, Waters Corporation, Milford, MA, USA) with racemic d3-medetomidine as internal standard. After chiral separation (Chiralpak® AGP column, 4 × 150 mm, 5 μm; Chiral Technologies Europe, Illkirch, France) with 10 mM ammonium acetate (pH 4.5) and acetonitrile containing 0.1% formic acid as solvents, quantitative detection was performed in multireaction monitoring mode (MRM) with a triple quadrupole mass spectrometer (triple quadrupole mass spectrometer, 4000QTrap, MDS Sciex, Concord, Ontario, Canada). The respective precursor ions (m/z) were 201.2 (dexmedetomidine [DEX] and levomedetomidine [LEV]) and 204.2 (d3-medetomidine). The fragment ions (m/z) monitored and used for quantitation were 95.1 (DEX and LEV) and 98.05 (internal standard). The chromatograms were processed with industry-standard software (Applied Biosystems/MDS Sciex software, Analyst version 1.6.1). The linear concentration range was from 0.10 to 10.0 ng/ml. The inter-assay accuracy of the quality control (QC) samples (at 0.225, 1.0, 8.0 and 50 ng/ml) ranged from 94.4% to 99.8% (DEX) and 92.5% to 99.2% (LEV).

Sample preparation for the analysis of MK-467 and ATI was as follows: 50 μl plasma aliquots were treated with in-well protein precipitation on a Sirocco plate (Sirocco plate; Waters Co.) with 250 μl of internal standard solution in acetonitrile (ACN) (containing 100 ng/ml propranolol and 20 ng/ml chlorpromazine). After vigorous mixing for 5 min, the samples were centrifuged for 20 min at 4,000 RPM. 50 μl aliquots of the sample supernatants were transferred onto UPLC 96-well plates, diluted with 450 μl of 20% acetonitrile in water and analysed. Reference samples were prepared in drug-free sheep plasma at analyte concentrations of 0.02–20,000 ng/ml. Quality control samples were analysed at concentrations of 0.2, 2, 20, 200 and 2,000 ng/ml. Analyses were performed with HPLC-MS/MS (Waters Acquity UPLC with Waters TQ-S triple quadrupole MS; Waters Co.) employing a reversed-phase C18 (2.1 × 50 mm, 1.7 μm) column (Waters Acquity BEH C18; Waters Co.). The following conditions were applied: column temperature 40°C, autosampler temperature 10°C and injection volume 4 μl. The aqueous eluent (A) was 0.5% formic acid in water; acetonitrile served as the organic eluent (B). The eluent flow rate was 0.5 ml/min. The proportions of B in the employed gradient elution were 2%, 2%, 50%, 90%, 90% and 2% and were applied from 0, 0.5, 2, 2.5, 3 and 4 min. Nitrogen was used for desolvation and cone gas with flow rates of 900 and 150 L/hr, respectively. The desolvation temperature was 650°C and the source temperature was 150°C. The capillary voltage was 1,000 V and the polarity was set as positive ionization. MRM transitions, cone voltages (V), collision energies (eV) and retention times (min) were as follows: 419 > 200, 25, 20 and 1.55 (MK-467) and 213 > 145, 40, 20 and 1.86 (ATI). The corresponding values for the internal standards were as follows: 260 > 116, 28, 18 and 1.9 (propranolol) and 319 > 58, 15–22 and 2.26 (chlorpromazine). The quantitation range (ng/mL) for MK-467 and ATI was 1–10 000.

2.6 | Statistical analysis

On the basis of previous studies in dogs (Honkavaara et al., 2011) and sheep (Raekallio et al., 2010), power analysis indicated that eight animals would detect the difference between treatments in the primary cardiopulmonary outcome variables and provide a power of 80% with an alpha level of 0.05 as follows (±*SD*): HR: 11 (10) beats/min, CO: 1.4 (1.2) L/min, MAP: 17 (15) mmHg, SVR: 915 (600) dynes s/cm^5 and PaO_2: 17 (15) mmHg. The normality assumption was tested for parametric data with Shapiro–Wilk's test. A generalized linear mixed model with time, treatment and their interaction was used for the repeated continuous cardiopulmonary variables. Holm–Bonferroni corrections were used as appropriate for multiple comparisons. Sedation scores (discrete variable) were compared with the following non-parametric tests: Friedman's test was used for the time effect within each treatment while Kruskal–Wallis and Mann–Whitney *U* tests were used where appropriate for comparisons between treatments. Plasma drug concentrations were compared between treatments using one-way ANOVA followed by post hoc Tukey's test (DEX and LEV) and Student's *t*-test (MK-467 and ATI). Parametric data are expressed as mean ± *SD*, whereas non-parametric data are expressed as median (range). $p < .05$ was considered statistically significant. All analyses were computed using IBM SPSS Statistics version 24.0 for Windows (IBM Corp., Armonk, NY, USA).

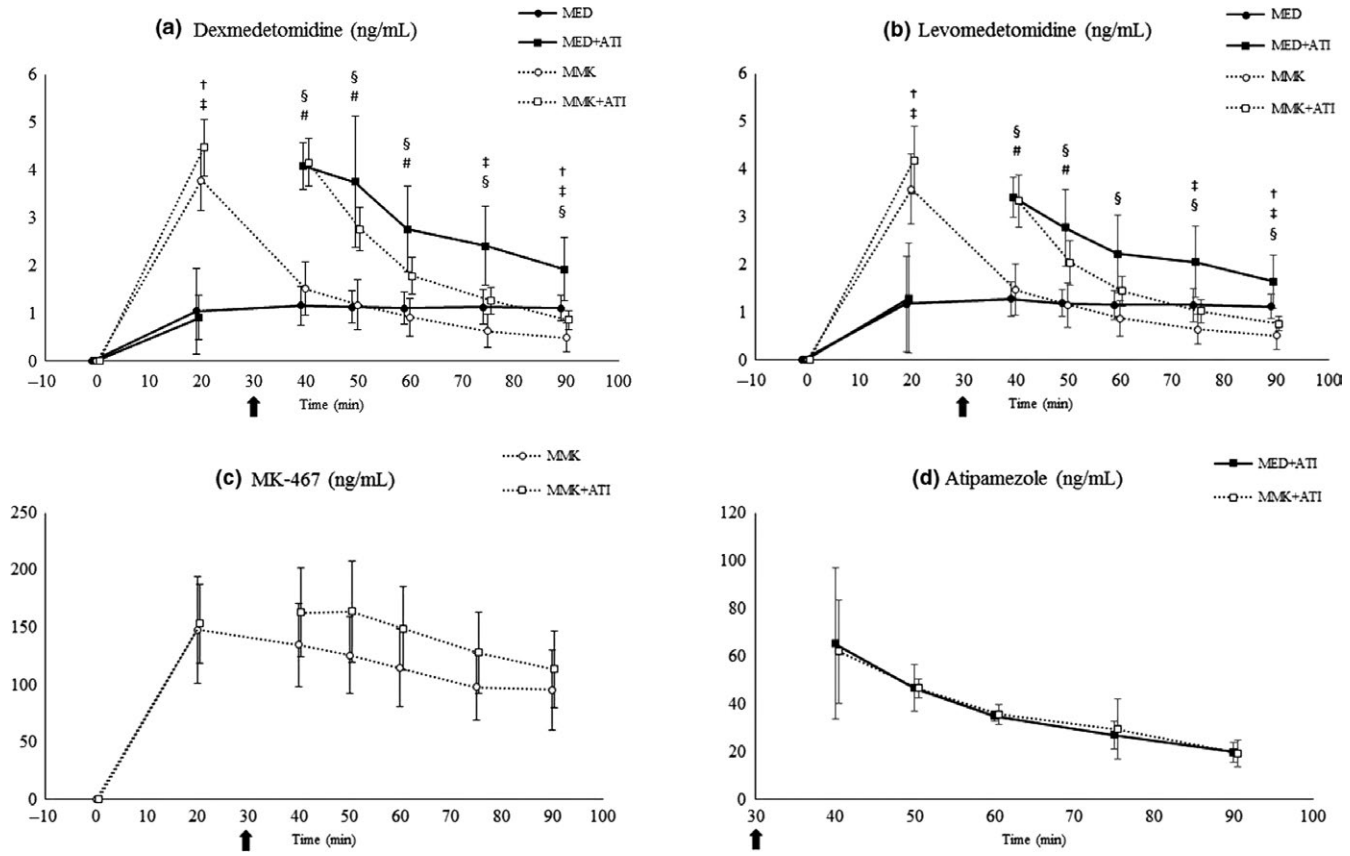

FIGURE 1 Mean ± *SD* of (a) dexmedetomidine, (b) levomedetomidine, (c) MK-467 and (d) atipamezole (ATI) concentrations in plasma of eight sheep treated with intramuscular medetomidine (30 μg/kg) alone (MED, closed circles), or combined with MK-467 300 μg/kg (MMK, open circles) in the same syringe, followed by intramuscular atipamezole 150 μg/kg (MED + ATI, MMK + ATI, closed and open squares, respectively) or an equal volume of saline 30 min later. The black arrow indicates the time of atipamezole or saline administration. Statistically significant differences between treatments ($p < .05$) are as follows: †Significant difference between MED and MMK. ‡Significant difference between MED + ATI and MMK + ATI. §Significant difference between MED and MED + ATI. #Significant difference between MMK and MMK + ATI

TABLE 1 Visual analogue sedation scores in sheep administered intramuscular medetomidine HCl (30 μg/kg, MED) alone and combined with MK-467 HCl (300 μg/kg, MMK) in the same syringe, followed by intramuscular atipamezole (150 μg/kg, MED + ATI, MMK + ATI) or an equal volume of saline 30 min later. Values are presented as median (range)

	Time (min)								
	BL	10	20	40	50	60	75	90	120
MED	0 (0–0)	4 (2–6)*,†	7 (3–8)*,†	6 (4–8)*	6 (4–8)*	5.5 (4–7)*	5.5 (3–7)*	5.5 (3–6)*	3.5 (2–4)*,†
MED + ATI	0 (0–0)	4.5 (2–7)*,‡	7 (4–9)*,‡	2.5 (0–7)*	2 (0–7)*,‡	0.5 (0–7)*,‡	0.5 (0–5)	2.5 (0–5)*	1 (0–3)
MMK	0 (0–0)	5 (5–8)*	8 (7–10)*	8 (6–10)	6.5 (5–8)*	5 (4–7)*	5 (4–7)*	3.5 (2–7)*	1.5 (0–3)
MMK + ATI	0 (0–1)	5 (5–8)*	8 (7–9)*	0.5 (0–5)	0 (0–1)	0 (0–1)	0 (0–2)	0 (0–4)	0 (0–3)

Significant difference from baseline (BL) is indicated by *, while † and ‡ denote significant differences between MED and MMK, and MED + ATI and MMK + ATI, respectively.

3 | RESULTS

Plasma concentrations of DEX and LEV at 20 min were threefold higher with MMK than with MED alone. When MED was administered alone, administration of ATI increased DEX and LEV plasma concentrations (Figure 1a–d). On the other hand, the exposure to MK-467 (or DEX and LEV) was not significantly different between MMK and MMK + ATI.

The sedation scores are presented as median (range) in Table 1. They were initially significantly higher with MMK than with MED. After 90 min, the scores were higher with MED than with MMK. After ATI, the scores decreased significantly in both treatments, but with MMK + ATI they were significantly lower than with MED + ATI. Resedation was observed in one animal after MMK + ATI and in four animals after MED + ATI.

Cardiopulmonary variables are presented as mean ± *SD* in Figure 2a–e and Tables 2 and 3. No significant differences were

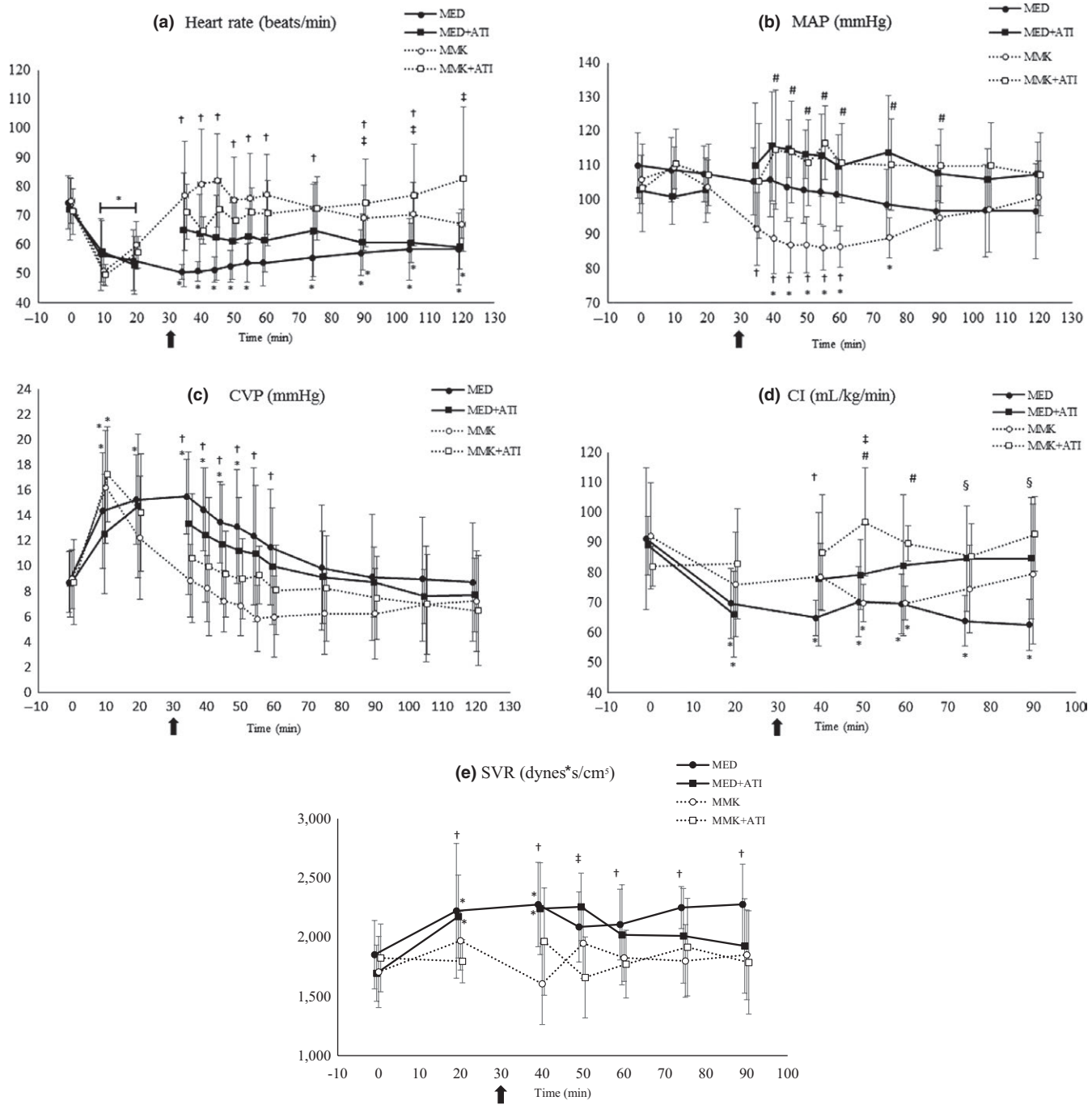

FIGURE 2 Mean ± *SD* of (a) heart rate, (b) mean arterial pressure (MAP), (c) central venous pressure (CVP), (d) cardiac index (CI) and (e) systemic vascular resistance (SVR) in eight sheep treated with intramuscular medetomidine 30 μg/kg (MED, closed circles) or combined with MK-467 300 μg/kg (MMK, open circles) in the same syringe, followed by intramuscular atipamezole 150 μg/kg (MED + ATI, MMK + ATI, closed and open squares, respectively) or an equal volume of saline 30 min later. The black arrow indicates the time of atipamezole (ATI) or saline administration. Statistically significant differences within and between treatments ($p < .05$) are as follows: *Significant difference from the baseline within the same treatment. †Significant difference between MED and MMK. ‡Significant difference between MED + ATI and MMK + ATI. §Significant difference between MED and MED + ATI. #Significant difference between MMK and MMK + ATI

detected between treatments at the baseline measurements. HR decreased after MED and, even after ATI, remained below the baseline values. With MMK, HR initially decreased, but from 40 min onwards, HR recovered to values insignificantly different from baseline, even without ATI (Figure 2a). MAP remained unchanged after MED, whereas with MMK there was a significant decrease from baseline over time (Figure 2b). CVP initially increased and after treatment with MED, CVP remained elevated until 60 min (Figure 2c).

CI decreased after MED alone and remained below baseline values throughout the observation period. ATI reversed this

TABLE 2 Mean ± *SD* of blood gas analyses, rectal temperature (Temp) and haemoglobin (Hb) at baseline (BL) and at various time points in eight sheep after intramuscular administration of medetomidine HCl (30 μg/kg, MED) alone and combined with MK-467 HCl (300 μg/kg, MMK) in the same syringe, followed by intramuscular atipamezole (150 μg/kg, MED + ATI, MMK + ATI) or an equal volume of saline 30 min later

		Time (min)					
	Treatment	BL	20	40	60	75	90
PaO_2 (mmHg)	MED	102 ± 7.3	90 ± 5.7*	89 ± 3.5*	89 ± 7.9*	84 ± 5.1*	88 ± 6.1*
	MED + ATI	104 ± 4.5	89 ± 8.4*	90 ± 11.4*	99 ± 12.2	102 ± 9.9§	102 ± 5.6§
	MMK	96 ± 10.1	87 ± 14	85 ± 10.6	89 ± 16	92 ± 7.9	91 ± 7.4
	MMK + ATI	101 ± 4.3	90 ± 11.4*	96 ± 9.7	100 ± 6.6	99 ± 8.2	106 ± 9.8#
$PaCO_2$ (mmHg)	MED	38.3 ± 2.9	43.3 ± 4.1	44.7 ± 3.2*	45.4 ± 1.9*	46.7 ± 4.4*	44.9 ± 3.5*
	MED + ATI	38.6 ± 2.6	45.2 ± 3.0*	41.8 ± 2.8*	41.9 ± 2.9§	41.3 ± 2.6§	42.1 ± 2.7
	MMK	40.1 ± 2.1	47.0 ± 2.4*	46.0 ± 2.1*	45.4 ± 2.9*	44.7 ± 3.9	45.1 ± 3.4*
	MMK + ATI	39.3 ± 3.1	47.5 ± 2.9*	44.9 ± 8.6	41.0 ± 1.3#	41.5 ± 1.7	40.9 ± 4.2
PvO_2 (mmHg)	MED	46.3 ± 8.0	42.2 ± 7.8	40.9 ± 5.3*	43.4 ± 6.1	40.8 ± 6.4*	41.8 ± 5.3*
	MED + ATI	49.9 ± 4.9	44.1 ± 6.2	46.4 ± 6.8	46.0 ± 5.2	46.7 ± 7.3	46.5 ± 3.9
	MMK	46.5 ± 7.9	44.1 ± 7.7	44.3 ± 8.6	42.0 ± 5.9	43.1 ± 5.8	40.1 ± 5.9*
	MMK + ATI	46.4 ± 8.6	45.3 ± 9.5	42.4 ± 6.1	47.8 ± 3.5	47.7 ± 5.4	47.4 ± 8.3
Temp (°C)	MED	39.3 ± 0.22	39.3 ± 0.17	38.9 ± 0.45	38.7 ± 0.55	38.4 ± 0.60*	38.3 ± 0.75*
	MED + ATI	39.4 ± 0.23	39.4 ± 0.29	39.3 ± 0.47	39.2 ± 0.48	39.1 ± 0.45	39.1 ± 0.37§
	MMK	39.3 ± 0.24	39.2 ± 0.17	38.7 ± 0.32	38.4 ± 0.47*	38.3 ± 0.52*	38.4 ± 0.57*
	MMK + ATI	39.3 ± 0.33	39.4 ± 0.38	38.9 ± 0.52	39.0 ± 0.57	38.9 ± 0.65	39.0 ± 0.66
Hb (g/L)	MED	87.9 ± 8.9	77.8 ± 9.2	73.1 ± 5.5*	72.0 ± 5.0*	72.8 ± 5.6*	73.5 ± 5.5*
	MED + ATI	82.6 ± 9.8	72.6 ± 5.8	82.6 ± 10.8	80.4 ± 10.0	84.9 ± 14.3	86.6 ± 20.9
	MMK	84.6 ± 9.7	75.6 ± 5.2	70.7 ± 5.2*	69.9 ± 5.6*	69.8 ± 4.7*	70.4 ± 4.5*
	MMK + ATI	83.6 ± 6.5	74.5 ± 5.7	83.4 ± 7.6#	92.7 ± 15.6#	93.4 ± 14.8#	90.9 ± 15.6#

Statistically significant differences within and between treatments ($p < .05$) are as follows: *Significant difference from the baseline (BL) within the same treatment, †Significant difference between MED and MMK, ‡Significant difference between MED + ATI and MMK + ATI, §Significant difference between MED and MED + ATI, and #Significant difference between MMK and MMK + ATI.

medetomidine-induced decrease (Figure 2d). SVR increased after MED alone; SVR remained significantly elevated even after ATI. No significant changes were detected with MMK regardless of ATI (Figure 2e).

PaO_2 initially decreased after all treatments. After MED alone, PaO_2 remained below baseline until the end of follow-up (Table 2). However, there were no differences between the treatments. Moreover, RR (data not shown) did not differ significantly between treatments. DO_2 decreased significantly from baseline after both MED and MMK and remained reduced for the entire observation period. This reduction was reversed with ATI (Table 3).

4 | DISCUSSION

MK-467 increased the early plasma concentrations of both of medetomidine's stereoisomers (DEX and LEV) when coadministered intramuscular in the same syringe. This finding is consistent with recent reports in dogs (Restitutti et al., 2017) and cats (Pypendop et al., 2017b). In sheep, we detected an almost threefold increase in DEX concentration in plasma by MK-467. Similarly, MK-467 after intramuscular administration approximately doubled the C_{max} of DEX and reduced its T_{max} in dogs (Restitutti et al., 2017) and cats (Pypendop et al., 2017b). We did not take frequent enough samples to determine T_{max}, as this was not the primary aim of our study. This accelerated absorption of medetomidine probably resulted from reversion of the medetomidine-induced local vasoconstriction by MK-467 when both drugs were mixed in the same syringe and thus administered to the same site, as suggested by Restitutti et al. (2017). In the present study, the plasma concentrations of medetomidine's enantiomers were nearly identical. This is in contrast to the findings in dogs (Bennett, Salla, Raekallio, Scheinin, & Vainio, 2017; Bennett et al., 2016), where DEX concentrations were significantly higher than those of LEV. In dogs, the clearance of LEV was significantly higher than that of DEX after administration of racemic medetomidine (Bennett et al., 2016), whereas in sheep, the clearance of the enantiomers may be less different.

Reversal with ATI resulted in a remarkable increase in plasma DEX concentrations. For example, with MED, DEX plasma concentrations were increased threefold 10 min after ATI. However, in the presence of MK-467 (MMK + ATI), ATI did not increase plasma DEX concentrations. This difference between treatments could be attributed to the earlier achievement of C_{max} with MMK as previously suggested.

TABLE 3 Mean ± *SD* values for calculated cardiopulmonary variables in eight sheep at baseline (BL) and various time points after intramuscular administration of medetomidine HCl (30 μg/kg, MED) alone and combined with MK-467 HCl (300 μg/kg, MMK) in the same syringe, followed by IM atipamezole (150 μg/kg, MED + ATI, MMK + ATI) or an equal volume of saline 30 min later

		Time (min)					
	Treatment	BL	20	40	60	75	90
SaO_2 (%)	MED	95.8 ± 0.8	94.1 ± 1.2*	93.9 ± 0.6*	93.8 ± 1.6	92.8 ± 1.3*	93.6 ± 1.1*
	MED + ATI	96.1 ± 0.4	93.7 ± 2.0*	93.6 ± 2.4	95.2 ± 1.7	95.7 ± 1.4§	95.9 ± 0.6§
	MMK	94.9 ± 2.0	92.9 ± 3.1	92.6 ± 3.0	93.0 ± 3.6	94.3 ± 1.7	94.1 ± 1.5
	MMK + ATI	95.8 ± 0.5	93.6 ± 2.4	95.0 ± 1.4	95.6 ± 0.7	95.5 ± 0.8	96.3 ± 0.9#
SvO_2 (%)	MED	66.8 ± 11.7	60.6 ± 13.2	59.2 ± 10.1	63.2 ± 10.6	58.9 ± 11.8	60.7 ± 8.8
	MED + ATI	72.5 ± 6.3	64.0 ± 9.7	67.4 ± 8.9	67.2 ± 7.5	67.6 ± 11.5	68.3 ± 5.4
	MMK	65.7 ± 11.8	63.7 ± 10.9	63.6 ± 12.8	60.9 ± 10.3	62.8 ± 9.6	57.7 ± 10.1
	MMK + ATI	66.7 ± 11.7	64.6 ± 13.4	61.5 ± 10.3	70.1 ± 4.6	69.6 ± 7.3	68.2 ± 12.8
CaO_2 (mL/dL)	MED	12.0 ± 1.3	10.4 ± 1.2	9.8 ± 0.7*	9.7 ± 0.8*	9.7 ± 0.7*	9.8 ± 0.7*
	MED + ATI	11.4 ± 1.3	9.7 ± 0.7*	10.8 ± 1.6	10.9 ± 1.2§	11.6 ± 1.8§	11.9 ± 2.8
	MMK	11.4 ± 1.0	9.9 ± 0.8*	9.3 ± 0.7*	9.3 ± 0.8*	9.4 ± 0.6*	9.5 ± 0.6*
	MMK + ATI	11.5 ± 0.8	10.0 ± 0.7*	11.5 ± 0.9#	12.4 ± 2.1#	12.7 ± 2.0#	12.5 ± 2.1#
DO_2 (mL/min/kg)	MED	11.1 ± 3.6	6.7 ± 0.8*	6.4 ± 0.5*	6.7 ± 0.7*	6.2 ± 0.8*	6.2 ± 0.9*
	MED + ATI	10.1 ± 1.8	6.5 ± 1.7*	8.7 ± 3.0§	9.0 ± 3.1	9.9 ± 3.0§	10.0 ± 2.9§
	MMK	10.4 ± 1.4	7.8 ± 2.1	7.4 ± 1.5*	6.5 ± 0.9*	7.0 ± 1.3*	7.5 ± 1.9*
	MMK + ATI	9.4 ± 1.0	8.2 ± 1.6	10.5 ± 2.3#	11.4 ± 2.4#	10.9 ± 2.8#	11.7 ± 3.3#

*Significant difference from the baseline (BL) within the same treatment, §significant difference between MED and MED + ATI, and #significant difference between MMK and MMK + ATI.

However, the increase in plasma medetomidine concentration after ATI administration is a well-documented phenomenon in ruminants and was also reported after intravenous administration of the agonist (Ranheim et al., 1997, 1998, 1999, 2000). For example, in sheep receiving intravenous ATI (200 μg/kg) 60 min after intravenous medetomidine (40 μg/kg), the maximum increase (approximately twofold to threefold) was detected 5 min after ATI administration; 25–45 min were necessary to regain the pre-ATI plasma medetomidine concentrations (Ranheim et al., 2000). This phenomenon was not observed in other animals such as dogs (Salonen, Vuorilehto, Vainio, & Anttila, 1995), horses (Knych, Steffey, & Stanley, 2012), ponies (Dyer, Hsu, & Lloyd, 1987) or humans (Scheinin et al., 1998). Ranheim et al. (2000) suggested that ATI might have displaced medetomidine from the highly perfused organs such as kidneys, lungs and liver, thus increasing its plasma concentration. The findings in the present study support this displacement hypothesis. In our study, ATI might have also increased the absorption of medetomidine from its injection site (due to improvement of global cardiovascular function), as after MED alone, plasma concentrations of DEX were stable throughout the follow-up period (suggesting equal absorption and elimination rates or a flip-flop phenomenon).

Sedation was induced more rapidly, and early sedation appeared to be more profound with treatments that included MK-467. On the other hand, from 90 min onwards, the level of sedation was higher with MED than MMK. These findings seemed to directly reflect the DEX plasma concentrations. Our results support the speculation that in sheep, there was no or limited receptor hysteresis and the degree of sedation directly represented (dex)medetomidine concentrations in plasma (Kästner et al., 2003; Muge, Chambers, & Livingston, 1996; Ranheim et al., 2000), at least with the concentrations detected in our study. In some other ruminants, such as cattle and reindeer, plasma medetomidine concentrations appear to correlate less directly with its sedative effects (Ranheim et al., 1997, 1998, 1999).

The sedation scores also declined faster after MMK + ATI than with MED + ATI. None of the sheep exhibited signs of overalertness after ATI. This is in contrast to a report by Ranheim et al. (2000), where sheep that received a similar dose ratio of ATI: medetomidine (5:1) became excited and exhibited muscle tremors. This could be due to the later administration of ATI (60 min) in that study, after considerable decay of the effects of medetomidine.

As our primary goal in this study was to evaluate ATI effects when used for reversal, we did not focus on monitoring the early period after treatment administration. However, we observed that MK-467 did not immediately antagonize the early cardiovascular impact of medetomidine. In our first sample taken at 20 min, the DEX:MK-467 plasma concentration ratio was approximately 1:38, which appeared insufficient to completely reverse the cardiovascular effects of MED. For example, in isoflurane-anaesthetized dogs receiving medetomidine and MK-467 as an intravenous step infusion, the cardiovascular effects of MED were significantly attenuated at a plasma concentration ratio of 1:50 (Kaartinen et al., 2014). In our study, when the proportion of MK-467 gradually increased resulting in plasma drug concentration ratios of approximately 1:90 (DEX:MK-467) at 40 min, HR, CVP and CI were no longer significantly different from their

baseline values. However, MAP was significantly decreased and was also lower than with MED. This could be partially attributed to the central sympatholytic effects of MED, as MK-467 alone did not significantly alter MAP in dogs (Honkavaara et al., 2011) or cats (Honkavaara et al., 2017; Pypendop et al., 2017a). Nevertheless, there was no hypotension (MAP < 60 mmHg) in the present study as no values less than 80 mmHg were observed. In contrast to MED, SVR remained unchanged from its baseline values with MMK, suggesting that MK-467 alleviated MED-induced vasoconstriction. It is worth noting, however, that our baseline values probably did not represent a resting state, because the animals seemed agitated when placed in the sling before treatment administration.

After reversal with ATI, HR initially returned towards baseline values with MED + ATI but decreased again from 90 min onwards. This is consistent with findings in goats receiving ATI (100 μg/kg intravenous) 25 min after MED (20 μg/kg) (Carroll et al., 2005). With MMK + ATI, HR increased and no later decrease was observed. Regardless, ATI restored CI in both treatments, which would be explained by the larger stroke volumes in the MED + ATI treatment after reversal. SVR remained elevated after MED + ATI, while with MMK + ATI SVR remained unchanged throughout the monitoring period. ATI increased MAP after MMK, but no significant difference was detected between MED and MED + ATI. Previously, ATI was shown to increase MAP in calves (Rioja, Kerr, Enouri, & McDonell, 2008) but not in sheep (Talke et al., 2000) and goats (Carroll et al., 2005) treated with medetomidine.

Arterial hypoxaemia is a common adverse effect associated with use of all α_2-agonists in sheep. The dose, route of administration and individual sensitivity appear to determine the degree of effect on PaO_2 (Bryant et al., 1996; Dyck, Maze, Haack, Vuorilehto, & Shafer, 1993; Kästner et al., 2007; Talke et al., 2000). In our study, PaO_2 initially (at 10 min) decreased after all treatments, although none of the sheep became hypoxaemic (PaO_2 < 60 mmHg). That MK-467 did not seem to significantly improve PaO_2 may be related to its slow absorption or may be this population of sheep was not sensitive to medetomidine-induced hypoxaemia, at least after intramuscular administration. DO_2 is a product of CaO_2 and CO. In our study, the sustained decrease in DO_2 with MMK was probably attributed more to the decrease in CaO_2, which reflects changes both in Hb concentration and PaO_2. Thus, as Hb concentrations (and PaO_2) were restored after ATI, both CaO_2 and DO_2 increased. Nevertheless, at no time point did MED or MED + ATI perform better than the treatments that included MK-467.

In the current study, medetomidine concentrations in blood were much below those reported to induce relevant bias with LiDCO sensors in vitro (Ambrisko, Kabes, & Moens, 2013). On the other hand, ATI does not markedly interfere with the performance of LiDCO electrodes (Ambrisko et al., 2013). Currently, there are no available data about the possible effect of MK-467 on LiDCO sensor voltage, and it is unclear whether it affected the accuracy of the readings. In general, we believe that our CO measurements and consequently CI were minimally affected by the used drugs. Last, all of the animals in the present study were intact females. As there is no published information on possible gender effects on the responses to α_2-adrenoceptor agonists and antagonists in animals, further investigation characterising the impact of sex and/or hormonal status may be warranted.

In conclusion, intramuscular coadministration of the peripheral α_2-adrenoceptor antagonist MK-467 with medetomidine in sheep substantially increased the plasma concentrations of DEX, which was reflected by rapid induction and deeper early-stage sedation. The high DEX concentrations in plasma with MK-467 may have overridden some of the early cardiovascular α_2-adrenoceptor antagonist effects of MK-467. However, MK-467 at the given dose appeared to alleviate some of the later medetomidine-induced cardiopulmonary adverse effects. No deleterious effects were observed when ATI was used as a reversal agent after sedation with medetomidine combined with MK-467. MK-467 thus seemed to be a beneficial adjunct to α_2-adrenergic agonists in sheep when the sedation was reversed with ATI. However, the pharmacokinetic-pharmacodynamic interactions of this combination are complex and require further investigation.

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AUTHOR CONTRIBUTIONS

All authors have read and approved the final manuscript. M.A., M.R. and T.K. designed the study, collected and analysed the data, and wrote the article. J.H. designed the study and wrote the article. M.S. analysed plasma drug concentrations and wrote the article. M.K. analysed plasma drug concentrations and wrote the article. S.M. prepared the animals and wrote the article. O.V. designed, supervised the study and wrote the article.

CONFLICT OF INTEREST

The laboratory of M. Scheinin has carried out contract research for Vetcare Ltd. The sponsor did not affect the design of the study protocol, collection and analysis of data, statistics, writing the manuscript, and drawing the conclusions. Therefore, the other authors declare no conflict of interest.

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