RESEARCH PAPER

Effects of intramuscular vatinoxan (MK-467), co-administered with medetomidine and butorphanol, on cardiopulmonary and anaesthetic effects of intravenous ketamine in dogs

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Abstract

Objective To investigate the impact of intramuscular (IM) co-administration of the peripheral α_2-adrenoceptor agonist vatinoxan (MK-467) with medetomidine and butorphanol prior to intravenous (IV) ketamine on the cardiopulmonary and anaesthetic effects in dogs, followed by atipamezole reversal.

Study design Randomized, masked crossover study.

Animals A total of eight purpose-bred Beagle dogs aged 3 years.

Methods Each dog was instrumented and administered two treatments 2 weeks apart: medetomidine (20 μg kg^{-1}) and butorphanol (100 μg kg^{-1}) premedication with vatinoxan (500 μg kg^{-1}; treatment MVB) or without vatinoxan (treatment MB) IM 20 minutes before IV ketamine (4 mg kg^{-1}). Atipamezole (100 μg kg^{-1}) was administered IM 60 minutes after ketamine. Heart rate (HR), mean arterial (MAP) and central venous (CVP) pressures and cardiac output (CO) were measured; cardiac (CI) and systemic vascular resistance (SVRI) indices were calculated before and 10 minutes after MVB or MB, and 10, 25, 40, 55, 70 and 100 minutes after ketamine. Data were analysed with repeated measures analysis of covariance models. A p-value <0.05 was considered statistically significant. Sedation, induction, intubation and recovery scores were assessed.

Results At most time points, HR and CI were significantly higher, and SVRI and CVP significantly lower with MVB than with MB. With both treatments, SVRI and MAP decreased after ketamine, whereas HR and CI increased. MAP was significantly lower with MVB than with MB; mild hypotension (57–59 mmHg) was recorded in two dogs with MVB prior to atipamezole administration. Sedation, induction, intubation and recovery scores were not different between treatments, but intolerance to the endotracheal tube was observed earlier with MVB.

Conclusions and clinical relevance Haemodynamic performance was improved by vatinoxan co-administration with medetomidine–butorphanol, before and after ketamine administration. However, vatinoxan was associated with mild hypotension after ketamine with the dose used in this study. Vatinoxan shortened the duration of anaesthesia.

Keywords anaesthesia, butorphanol, cardiopulmonary, ketamine, medetomidine, vatinoxan.

Introduction

α_2-Adrenoceptor agonists, such as medetomidine or its pharmacologically active enantiomer dexmedetomidine, can be combined with butorphanol resulting in reliable sedation, muscle relaxation and analgesia for clinical procedures (Bartram et al. 1994; Ko et al. 2000; Kuo & Keegan 2004; Leppänen et al. 2006). Cardiovascular depression characterized by increased systemic vascular resistance index (SVRI) and decreased heart rate (HR) and cardiac index (CI), attributed to α_2-adrenoceptor agonist-induced peripheral vasoconstriction and decrease in sympathetic outflow, limit the usefulness of such combinations (Bartram et al. 1994; Pypendop & Verstegen 1998; Kuo & Keegan 2004).

To reduce these adverse cardiovascular effects in dogs, a peripheral α_2-adrenoceptor antagonist, vatinoxan (also known as MK-467 and L-659'066) has been tested for concomitant use with medetomidine. The direct influence of vatinoxan is restricted to peripheral tissues because of its limited penetration across the mammalian blood–brain barrier (Clineschmidt et al. 1988). Therefore, vatinoxan does not markedly counteract medetomidine-induced sedation (Honkavaara et al. 2008; Restitutti et al. 2011; Rolfe et al. 2012) but helps to maintain cardiac output (CO) by preventing the arterial vasoconstrictive effects of α_2-adrenoceptor agonists (Piascik et al. 1996; Pagel et al. 1998; Enouri et al. 2008; Honkavaara et al. 2011). Even if the initial haemodynamic effects of medetomidine are not entirely prevented by intramuscularly (IM) co-administered vatinoxan, their intensity and duration are reduced (Restitutti et al. 2017). Similarly, when IM medetomidine–vatinoxan is further combined with butorphanol, lesser reductions in HR (Salla et al. 2014; Kallio-Kujala et al. 2018a) and CI have been observed (Salla et al. 2014).

Ketamine, an antagonist of *N*-methyl-D-aspartate receptors, is a dissociative anaesthetic often used for induction of anaesthesia in dogs. The advantages of premedication with an α_2-adrenoceptor agonist prior to ketamine induction include a dose-sparing effect and predictable, rapid and smooth induction of anaesthesia followed by short-lasting surgical anaesthesia for mildly painful procedures (Hellebrekers & Sap 1997; Hellebrekers et al. 1998). Post-procedural recovery may be hastened with the use of an α_2-adrenoceptor antagonist, such as atipamezole (Ko et al. 2000). Anaesthetic doses of ketamine are often associated with transient respiratory depression that may be intensified by α_2-adrenoceptor agonists and opioids (Ko et al. 2001; Barletta et al. 2011; Krimins et al. 2012). Ketamine has sympathomimetic effects, stimulating cardiovascular function by increasing HR, CO and arterial blood pressure (Haskins et al. 1985). However, ketamine may also have direct negative inotropic effects (Pagel et al. 1992).

Ketamine has been investigated for intravenous (IV) anaesthetic induction in dogs sedated with medetomidine and vatinoxan (Salla et al. 2017), but the dose of ketamine administered was low (1 mg kg^{-1}) and no opioid agent was used. In the present study, the primary aim was to evaluate the influence of vatinoxan on cardiopulmonary function and anaesthetic effects in dogs administered medetomidine–butorphanol IM 20 minutes before induction of anaesthesia with a higher IV dose of ketamine (4 mg kg^{-1}). Subsequently, recovery characteristics before and after atipamezole administration were assessed. Atipamezole interacts favourably with vatinoxan when used to reverse medetomidine-induced sedation (Turunen et al. 2019). However, there are no reports of the interaction of atipamezole and vatinoxan with ketamine.

We hypothesized that: 1) vatinoxan would improve cardiovascular performance by decreasing SVRI and increasing HR and CI; 2) by preserving CO, vatinoxan would hasten the plasma clearance of ketamine and shorten the duration of anaesthesia; and 3) atipamezole administered 60 minutes after ketamine would shorten recovery without detrimental adverse effects. We did not expect any impact of vatinoxan on ventilation, clinical quality of induction or intubation between treatments.

Materials and methods

Animals

A total of eight purpose-bred Beagle dogs (six neutered males and two neutered females) approximate age 3 years, weighing 13.3 ± 1.7 kg [mean ± standard deviation (SD)] were studied. The dogs were considered healthy based on history and comprehensive clinical examinations including complete blood counts and routine serum chemistry. The dogs were housed in groups in a kennel, fed with commercial food with free access to water. Food was withheld for 12 hours prior to experiments. The study was approved by Finnish Animal Experimental Board (ESAVI/7187/04.10.03/2012). All dogs have been rehomed.

Treatments

Each dog was administered two treatments in a randomized (www.random.org) crossover design, separated by ≥2 weeks. Dogs in treatment MVB were premedicated IM with medetomidine hydrochloride (20 μg kg^{-1}; Dorbene, 1 mg mL^{-1}; Laboratories SYVA S.A.U., Spain), butorphanol tartrate (100 μg kg^{-1}; Torpudor vet, 10 mg mL^{-1}; Richter Pharma AG, Austria) and vatinoxan hydrochloride (500 μg kg^{-1}; Recipharm, Sweden). Dogs in treatment MB were premedicated IM with medetomidine and butorphanol at the same dose rates. Drugs were administered from one syringe 20 minutes before IV ketamine (4 mg kg^{-1}; Ketaminol, 50 mg mL^{-1}; Intervet International B.V., The Netherlands). Atipamezole hydrochloride (100 μg kg^{-1}; Alzane, 5 mg mL^{-1}; Laboratories SYVA S.A.U.) was administered IM 60 minutes after ketamine.

The medetomidine–vatinoxan solution was prepared immediately before use in a sterile vial containing 25 mg vatinoxan powder by adding 1 mL medetomidine and 1 mL physiological saline solution (Natriumklorid, 0.9%; B Braun Melsungen AG, Germany) and mixing the vial until the solution was clear by visual inspection. The final drug concentrations in the solution were 500 μg mL^{-1} of medetomidine and 12.5 mg mL^{-1} of vatinoxan (ratio, 1:25). The medetomidine solution was prepared similarly in an empty vial. An injection volume of 0.04 mL kg^{-1} was drawn from the vial and butorphanol was added to the same syringe.

Instrumentation

Prior to each experiment, each dog was instrumented under general anaesthesia. A cephalic vein was aseptically cannulated with a 22 gauge catheter for induction of anaesthesia using propofol (6.3−14.6 mg kg^{-1}; Vetofol, 10 mg mL^{-1}; Norbrook Laboratories, Ireland) administered IV to effect. The dog was then intubated orotracheally. Anaesthesia was maintained with isoflurane (Vetflurane 100%; Virbac S. A., France) in oxygen delivered via a circle breathing system (Anesco Inc., FL, USA) maintaining the end-tidal isoflurane concentration at 1.5%. Mechanical ventilation was applied to maintain the end-tidal carbon dioxide partial pressure at 35−45 mmHg (4.7−6.0 kPa). Blood pressure, electrocardiography and pulse oximetry were monitored with noninvasive methods. Acetated Ringer's solution (Ringer-Acetat Baxter Viaflo; Baxter Finland, Finland) was infused at 5 mL kg^{-1} $hour^{-1}$. A 20 gauge arterial catheter (Arteriofix V; B Braun Melsungen AG) was aseptically introduced into a femoral artery. A 7 Fr double-lumen central venous catheter (CV-12702; Arrow International Inc., PA, USA) was aseptically inserted into a jugular vein under local anaesthesia (0.25 mL; Lidocain, 20 mg mL^{-1}; Orion Pharma, Finland), introduced to a premeasured distance for the tip of the catheter to reach the cranial border of the second rib at the costochondral junction and secured with sutures and a light bandage. Next, delivery of isoflurane and IV fluid were discontinued, and the dog was allowed to recover from anaesthesia. At least 60 minutes elapsed after extubation prior to baseline measurements.

Measurements

The dog was minimally restrained in sternal recumbency on an examination table covered with an insulating foam pad and electrical heating pad. The arterial and central venous catheters were connected to pressure transducers (Gabarith PMSET; Becton Dickinson Medical, UT, USA) with saline-filled pressure tubing (Argon Medical Devices Netherlands B.V., Finland). The accuracy of the transducers was verified before each experiment at values higher and lower than the normal physiological range using a mercury manometer, and zeroed to atmospheric pressure at the level of the manubrium. Adhesive electrodes were placed on the shaved skin of both thoracic limbs and the left pelvic limb for electrocardiography. Invasive arterial and venous pressures and a continuous lead II electrocardiogram (ECG) were monitored (S/5 Anesthesia Monitor; GE Healthcare Finland Oy, Finland) throughout the session. Immediately after obtaining baseline values and blood samples, the assigned treatment was injected into the gluteal muscles of the right pelvic limb. At 20 minutes after premedication, ketamine was administered IV over 2 minutes. Orotracheal intubation was performed immediately after ketamine administration, and oxygen (1 L $minute^{-1}$) was delivered via a circle breathing system (Anesco Inc.). If apnoea (30 seconds without spontaneous ventilation) occurred, manual ventilation was applied, peak inspiratory pressure 10−15 cm H_2O and 2−3 breaths $minute^{-1}$, until spontaneous breathing resumed. Atipamezole was injected into the gluteal muscles of the left pelvic limb 60 minutes after ketamine. Negative aspiration for blood was performed to confirm extravascular drug administration for all IM injections.

The HR (recorded from ECG and confirmed by auscultation over 1 minute), respiratory rate (f_R; counting chest movements during 1 minute) and blood pressures were recorded first; at −20 (baseline), −10, 10, 15, 25, 40, 55, 70 and 100 (and 120 for HR) minutes from administration of ketamine. Arterial blood was anaerobically collected prior to CO measurements into heparinized syringes (Pico50; Radiometer Medical ApS, Denmark) and analysed immediately for pH, arterial partial pressures of carbon dioxide ($PaCO_2$) and oxygen (PaO_2), lactate, sodium and haemoglobin concentrations (ABL 855; Radiometer Medical ApS), corrected to individual rectal temperature. CO was measured with the lithium dilution method (LiDCO Plus haemodynamic monitor; LiDCO Ltd, UK) using a standard dose of 0.075 mM of lithium chloride (LiDCO Ltd) injected via the central venous catheter (Mason et al. 2001) at −20 (baseline), −10, 10, 25, 40, 55, 70 and 100 minutes from the administration of ketamine. Initial standard values of 10 g dL^{-1} for haemoglobin and 140 mmol L^{-1} for sodium were later corrected with actual values obtained from arterial blood. CI, stroke volume index (SVI), rate pressure product (RPP) and SVRI were calculated using standard equations (Haskins et al. 2005).

Venous blood (6 mL, tubes containing ethylenediaminetetraacetic acid) was collected from the central venous catheter at 10, 40, 55 and 70 minutes for plasma concentration analyses of studied drugs (dexmedetomidine, butorphanol, ketamine, vatinoxan, atipamezole). Samples were separated by refrigerated centrifugation (2520 ***g*** for 15 minutes at 4 °C), and plasma was kept frozen at −20 °C or colder until analysed. The purpose of drug concentration analysis was to detect the influence of vatinoxan on plasma concentrations of co-administered drugs, and comparison with clinical observations.

Composite sedation scores (ranging from 0, no sedation, to 20, deep sedation) were determined after recording HR. Induction scores (ideal, good, unsatisfactory, not reached), jaw tone (poor, slight, good, total) and intubation scores (smooth, mild coughing, pronounced coughing, swallowing or gagging, failed attempt) were recorded immediately after successful endotracheal intubation. The early recovery score (easy or fairly easy transition to alertness, restless, needs restraint) was assessed after extubation and the late recovery score (alert and responsive, slightly sedated, sedated, very sedated) 120 minutes after ketamine administration. An investigator (HT)

unaware of the treatment and the cardiovascular variables assigned all scores. Times from administration of premedication to sedation, defined as the head resting on the table, and to return of consciousness were recorded. The times when the dog coughed or swallowed around the endotracheal tube, resulting in extubation and any adverse clinical signs during recovery, such as nausea, were recorded. After recovery from anaesthesia, the catheters were removed and meloxicam (0.2 mg kg^{-1}; Metacam, 5 mg mL^{-1}; Boehringer Ingelheim Vetmedica GmbH, Germany) was administered subcutaneously. The dog was offered food prior to returning to its kennel.

Analytical methods

Concentrations of dexmedetomidine in canine plasma were determined with chiral high-performance liquid chromatography−mass spectrometry (HPLC−MS/MS, Sciex API 4000; AB Sciex Pte. Ltd, ON, Canada) as described previously (Adam et al. 2018). Reference samples were prepared in drug-free canine plasma. The precursor ion−fragment ion pairs monitored were m z^{-1} 201.2−95.1 for dexmedetomidine and m z^{-1} 204.2−98.1 for the internal standard, deuterated (D_3) medetomidine. The accuracy of the quality control samples (at concentrations of 0.225, 1.0 and 8.0 ng mL^{-1}) ranged from 91.3% to 99.1%, and intra-assay coefficient of variation at these concentrations ranged from 1.2% to 3.9% for dexmedetomidine.

The concentrations of butorphanol, ketamine, atipamezole and vatinoxan in plasma were analysed with HPLC−MS/MS (Waters Acquity UPLC + Waters TQ-S triple quadripole MS; Waters Corp., MA, USA) (Kallio-Kujala et al. 2018b). The selected reaction monitoring was m z^{-1} of 328 > 124 for butorphanol, m z^{-1} of 238 > 220 for ketamine, m z^{-1} of 213 > 117 for atipamezole, m z^{-1} of 419 > 200 for vatinoxan and m z^{-1} of 260 > 116 for the employed internal standard propranolol. Results of all quality control samples were within 85% to 115% of their nominal concentration.

Statistical methods

The sample size calculations were based on data (mean ± SD) from previous studies (Honkavaara et al. 2008, 2011; Restitutti et al. 2017). Differences (peak effects) between treatments (paired two-tailed test, α-level 0.05, power 80%) were detected using eight dogs: HR 11 beats $minute^{-1}$ (50 ± 10 *versus* 61 ± 10 beats $minute^{-1}$), CO 1.4 L $minute^{-1}$ (4.0 ± 1.0 *versus* 5.4 ± 1.2 L $minute^{-1}$), mean arterial pressure (MAP) 17 mmHg (110 ± 10 *versus* 93 ± 15 mmHg).

Differences in several responses within and between treatments were evaluated with repeated measures analysis of covariance models. For between-treatment comparisons, actual values were used as response in the model, and for within-treatment comparisons change from baseline was used. The models included the main effects of treatment and time point, two-way interactions of treatment and time point, and a baseline covariate as fixed effects, and the main effect of dog, the two-way interactions of dog and time point and dog and treatment as random effects. Additionally, the values after ketamine and atipamezole were evaluated including only time points after administration, and the values prior to ketamine and atipamezole administration were used as baseline covariates. With continuous variables measured only once, depending on their distribution, either paired *t* tests or Wilcoxon's signed rank-sum test were used. Differences in the occurrence of nausea (yes or no) were evaluated with McNemar's test. Normality assumptions were checked with Kolmogorov−Smirnov test. In case normality assumptions were not met, common transformations were used (logarithm, inverse). Estimates of treatment differences were calculated over time and by time point with contrasts from the fitted models. Similarly, within-group changes were calculated for each time point with contrasts from the fitted models. Differences in plasma drug concentrations and their areas under the curve (AUCs) were compared with paired, two-tailed *t* tests. For the group differences and within-group changes, 95% confidence intervals and *p* values were calculated. The *p* values by time point were adjusted using the Bonferroni correction within each model (and treatment) and *p* values <0.05 were considered statistically significant. SAS for Windows Version 9.3 (SAS Institute Inc.; NC, USA) was used for all statistical analyses.

Results

At 10 minutes after IM administration of premedication (MB or MVB), significant decreases in HR ($p < 0.001$) and CI ($p < 0.004$), and significant increases in CVP ($p < 0.001$) and SVRI ($p < 0.007$) from baseline were detected with both treatments (Fig. 1 & Table 1). Values were significantly lower for HR and CI, whereas significantly higher for CVP, MAP and SVRI ($p < 0.001$) with MB than with MVB. After ketamine, HR ($p < 0.001$) and CI ($p < 0.004$) increased significantly, and SVRI ($p < 0.001$) decreased significantly with both treatments when compared with values at −10 minutes. MAP was significantly lower in MVB than in MB after ketamine, and MAP 57−59 mmHg was observed in two dogs 40 and 55 minutes after ketamine ($p < 0.007$). SVRI decreased ($p < 0.001$) and CI increased ($p < 0.005$) in both treatments 10 minutes after atipamezole administration.

In both treatments, f_R decreased significantly from baseline at −10 minutes (Table 2). f_R stayed significantly lower than baseline for 55 minutes after ketamine administration in both treatments ($p < 0.001$). In MVB at −10 minutes, PaO_2 decreased (range, 69.1−95.9 mmHg; 10.8−13.2 kPa; $p = 0.046$) and $PaCO_2$ increased (37.5−43.6 mmHg; 5.0−5.8

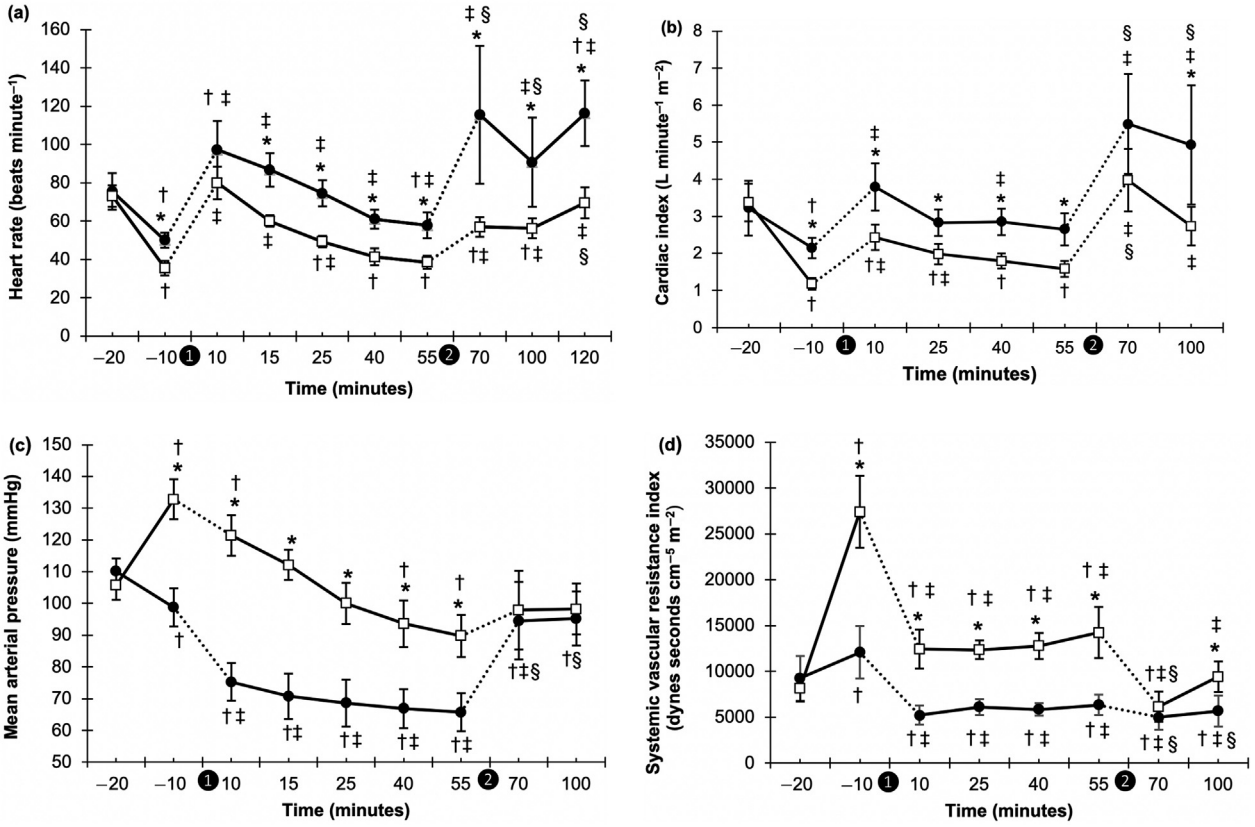

Figure 1 Mean ± standard deviation of (a) heart rate, (b) cardiac index, (c) mean arterial pressure and (d) systemic vascular resistance index of eight dogs administered intramuscular (IM) premedication with medetomidine (20 μg kg^{-1}) and butorphanol (100 μg kg^{-1}; treatment MB; open squares) or medetomidine and butorphanol at the same dose rates with vatinoxan (500 μg kg^{-1}; treatment MVB; solid circles) 20 minutes before intravenous ketamine (4 mg kg^{-1}; ❶) and 80 minutes before IM atipamezole (100 μg kg^{-1}; ❷). *Significant difference between treatments ($p < 0.05$). †Significantly different from −20 minutes within the treatment ($p < 0.05$). ‡Significantly different from −10 minutes within the treatment ($p < 0.05$). §Significantly different from 55 minutes within a treatment ($p < 0.05$).

kPa; $p = 0.013$). Arterial pH and lactate concentration were significantly lower in MVB than in MB at −10 minutes ($p = 0.016$ and $p < 0.001$, respectively; Table 2). Ventilation was assisted for 1−16 minutes to treat apnoea in six of the eight dogs in both treatments immediately after ketamine administration. $PaCO_2$ was higher at 10 minutes than at −10 minutes in both treatments ($p < 0.001$), and higher in MB than in MVB (51.8−70.1 mmHg; 6.9−9.3 kPa *versus* 48.2−59.2 mmHg; 6.4−7.9 kPa, respectively; $p = 0.06$).

Composite sedation scores indicated marked sedation after both premedications. There were no significant differences between the treatments in induction scores, jaw tone or intubation scores. Induction score was assessed ideal in all dogs with both treatments, and the mouth could be opened without resistance in six dogs in MB and seven dogs in MVB. Mild coughing, during or immediately after endotracheal intubation, was recorded in two dogs in MB and three dogs in MVB.

Composite sedation score was lower in MVB than in MB at 55 and 60 minutes after ketamine administration ($p < 0.001$). Extubation was performed at 52 ± 14 and 62 ± 14 minutes after ketamine administration in MVB and MB, respectively. Six dogs in MVB and one dog in MB were extubated before administration of atipamezole. After atipamezole was administered at 60 minutes, dogs were alert at 64 ± 11 and 68 ± 4 minutes in MVB and MB, respectively. With MVB, the transition to alertness was fairly easy in five dogs and easy in two dogs, but one dog was restless during early recovery. With MB, transition was easy in seven dogs and fairly easy in one dog. Late recovery scores at 120 minutes after ketamine administration did not differ between treatments; six dogs were alert and responsive and two dogs were slightly sedated or slow to react. Nausea, defined as drooling, swallowing or lack of appetite, was recorded in three dogs in MVB and two dogs in MB. Defaecation was observed in one dog in MB and tenesmus with mucous faeces in one dog in MVB.

Plasma concentrations of dexmedetomidine and butorphanol were higher at 90 and 30 minutes, respectively, after premedication in MVB than in MB (Fig. 2). Plasma ketamine

Table 1 Cardiovascular variables in eight dogs that were administered intramuscularly (IM) medetomidine (20 μg kg^{-1}) and butorphanol (100 μg kg^{-1}; treatment MB) or medetomidine (20 μg kg^{-1}), vatinoxan (500 μg kg^{-1}) and butorphanol (100 μg kg^{-1}; treatment MVB) immediately after baseline measurements at time −20. Intravenous injection of ketamine (4 mg kg^{-1}) was administered at time 0. Intramuscular injection of atipamezole (100 μg kg^{-1}) was administered at time 60. Values are mean ± standard deviation

Variable	Treatment	Time (minutes)								
		−20	−10	10	15	25	40	55	70	100
SAP	MB	163 ± 28	185 ± 20‡	159 ± 17	152 ± 12‡	145 ± 15†‡	139 ± 13†‡	134 ± 13†‡	143 ± 23‡	147 ± 15
(mmHg)	MVB	164 ± 14	154 ± 14*†	107 ± 16*†‡	107 ± 13*†‡	106 ± 12*†‡	105 ± 11*†‡	107 ± 9*†‡	148 ±23‡§	150 ±16‡§
DAP	MB	80 ± 7	108 ± 5†	106 ± 7†	93 ± 8	78 ± 10‡	73 ± 10‡	70 ± 8‡	75 ± 14‡	76 ± 10‡
(mmHg)	MVB	84 ± 9	91 ± 38*	59 ± 8*†‡	54 ± 9*†‡	52 ± 10*†‡	51 ± 8*†‡	49 ± 8*†‡	72 ± 14‡§	72 ± 11‡§
CVP	MB	1 ± 1	8 ± 1†	5 ± 1†	4 ± 1†‡	3 ± 1†‡	3 ± 1†‡	3 ± 1†‡	1 ± 1‡§	2 ± 1‡
(mmHg)	MVB	1 ± 2	4 ± 2*†	−1 ± 2*†‡	−1 ± 2*†‡	0 ± 2*‡	0 ± 2*‡	0 ± 2*‡	−1 ± 3*†‡	0 ± 2*‡
SVI	MB	2.0 ± 0.3	1.4 ± 0.2†	1.4 ± 0.5†	–	1.8 ± 0.4	1.9 ± 0.3‡	1.8 ± 0.6	2.7 ± 0.5†‡§	2.1 ± 0.5‡
mL kg^{-1}	MVB	1.8 ± 0.3	1.8 ± 0.3	1.7 ± 0.4	–	1.6 ± 0.3	2.0 ± 0.3	1.9 ± 0.4	2.0 ± 0.6*	2.2 ± 0.3
RPP	MB	7700 ± 644	4720 ± 877†	9690 ± 1570‡	6720 ± 546‡	4900 ± 414†	3880 ± 912†‡	3440 ± 478†‡	5620 ± 1400†	5570 ± 1210†
(mmHg minute^{-1})	MVB	8350 ± 1830	4930 ± 629†	7320 ± 1780‡	6100 ± 929	5100 ± 964†	4110 ± 905†	3830 ± 947†‡	11500 ± 5900*‡§	8970 ± 4490*‡§

CVP, central venous pressure; DAP, diastolic arterial pressure; RPP, rate pressure product; SAP, systolic arterial pressure; SVI, stroke volume index.
* Significantly different from MB at the same time ($p < 0.05$). † Significantly different from time −20 within the same treatment ($p < 0.05$). ‡ Significantly different from time −10 within the same treatment ($p < 0.05$). § Significantly different from time 55 within the same treatment ($p < 0.05$).

concentration was higher 10 minutes after IV administration in MB than in MVB. The AUC of ketamine at 10−70 minutes was significantly smaller in MVB (36,700 ± 5200 minute ng mL^{-1}) than in MB (63,600 ± 14,700 minute ng mL^{-1}).

Discussion

Overall, vatinoxan attenuated the medetomidine-evoked decreases in HR and CI by reducing SVRI but did not completely prevent the early cardiovascular changes. Full pharmacokinetic evaluation during the absorption phase of medetomidine, butorphanol and vatinoxan could not be performed in the present study. However, a probable explanation in previous studies is that vatinoxan accelerates the absorption and distribution of concomitantly IM administered drugs by reducing vasoconstriction systemically and at the injection site; meanwhile the absorption rate of vatinoxan is slower (Restitutti et al. 2017; Kallio-Kujala et al. 2018b). Furthermore, the central effects of medetomidine are unaffected by vatinoxan, resulting in decreased HR and CI.

Table 2 Mean ± standard deviation of respiratory rate (f_R), arterial partial pressure of carbon dioxide ($PaCO_2$), arterial pH (pHa), lactate concentration and rectal temperature (RT) in eight dogs. Variables were recorded before drug administration (time −20), 10 minutes after premedication (time −10) with medetomidine (20 μg kg^{-1}) and butorphanol (100 μg kg^{-1}; treatment MB) or medetomidine (20 μg kg^{-1}), vatinoxan (500 μg kg^{-1}) and butorphanol (100 μg kg^{-1}; treatment MVB) intramuscularly (IM), after intravenous ketamine (4 mg kg^{-1}) administered at time 0 and after IM atipamezole (100 μg kg^{-1}) at time 60

Variable	Treatment	Time (minutes)							
		−20	−10	10	25	40	55	70	100
f_R (breaths	MB	24 ± 6	13 ± 3†	12 ± 8†	17 ± 4	15 ± 8†	14 ± 5†	21 ± 11	22 ± 8‡
minute^{-1})	MVB	24 ± 6	13 ± 3†	12 ± 6†	13 ± 4†	13 ± 4†	12 ± 4†	26 ± 12*‡§	29 ± 16‡§
$PaCO_2$	MB	35.3 ± 3.0	35.3 ± 3.6	59.1 ± 6.5†‡	47.6 ± 4.6†‡	44.3 ± 4.5†‡	42.6 ± 4.2†‡	40.7 ± 6.1†	37.5 ± 2.9§
(mmHg)	MVB	36.4 ± 1.4	40.9 ± 2.4*†	54.2 ± 4.0*†‡	47.1 ± 3.6†‡	44.2 ± 3.3†	43.5 ± 1.5†	41.3 ± 2.2†	38.7 ± 3.7§
$PaCO_2$	MB	4.7 ± 0.4	4.7 ± 0.5	7.9 ± 0.9†‡	6.3 ± 0.6†‡	5.9 ± 0.6†‡	5.7 ± 0.6†‡	5.4 ± 0.8†	5.0 ± 0.4§
(kPa)	MVB	4.8 ± 0.2	5.4 ± 0.3*†	7.2 ± 0.5*†‡	6.2 ± 0.5†‡	5.9 ± 0.4†	5.8 ± 0.2†	5.5 ± 0.3†	5.2 ± 0.5§
pHa	MB	7.38 ± 0.02	7.36 ± 0.03	7.23 ± 0.03†‡	7.28 ± 0.03†‡	7.31 ± 0.03†‡	7.32 ± 0.02†‡	7.35 ± 0.03†§	7.37 ± 0.03§
	MVB	7.38 ± 0.02	7.34 ± 0.02*†	7.25 ± 0.03*†‡	7.29 ± 0.04†‡	7.31 ± 0.03†	7.32 ± 0.02†	7.35 ± 0.02†§	7.35 ± 0.04†§
Lactate	MB	0.54 ± 0.42	0.76 ± 0.47†	0.84 ± 0.28†‡	0.78 ± 0.31†	0.81 ± 0.34†‡	0.78 ± 0.34†	0.76 ± 0.37†	0.91 ± 1.03†
(mmol L^{-1})	MVB	0.43 ± 0.19	0.42 ± 0.30*	0.56 ± 0.24‡	0.60 ± 0.32‡	0.64 ± 0.29‡	0.55 ± 0.26*‡	0.60 ± 0.28‡	1.54 ±1.56†‡§
RT (°C)	MB	37.9 ± 0.5	37.8 ± 0.6	37.9 ± 0.7	37.6 ± 0.6	37.5 ± 0.8	37.5 ± 0.7	37.4 ± 0.5	37.6 ± 0.3§
	MVB	37.6 ± 0.2	37.4 ± 0.4	37.0 ± 0.6*†	36.9 ± 0.5*†‡	36.9 ± 0.6*†‡	36.9 ± 0.6*†‡	37.0 ± 0.5†‡	37.6 ± 0.3§

* Significantly different from MB at the same time ($p < 0.05$); † Significantly different from time −20 within the treatment ($p < 0.05$); ‡ Significantly different from time −10 within the treatment ($p < 0.05$); § Significantly different from time 55 within the treatment ($p < 0.05$).

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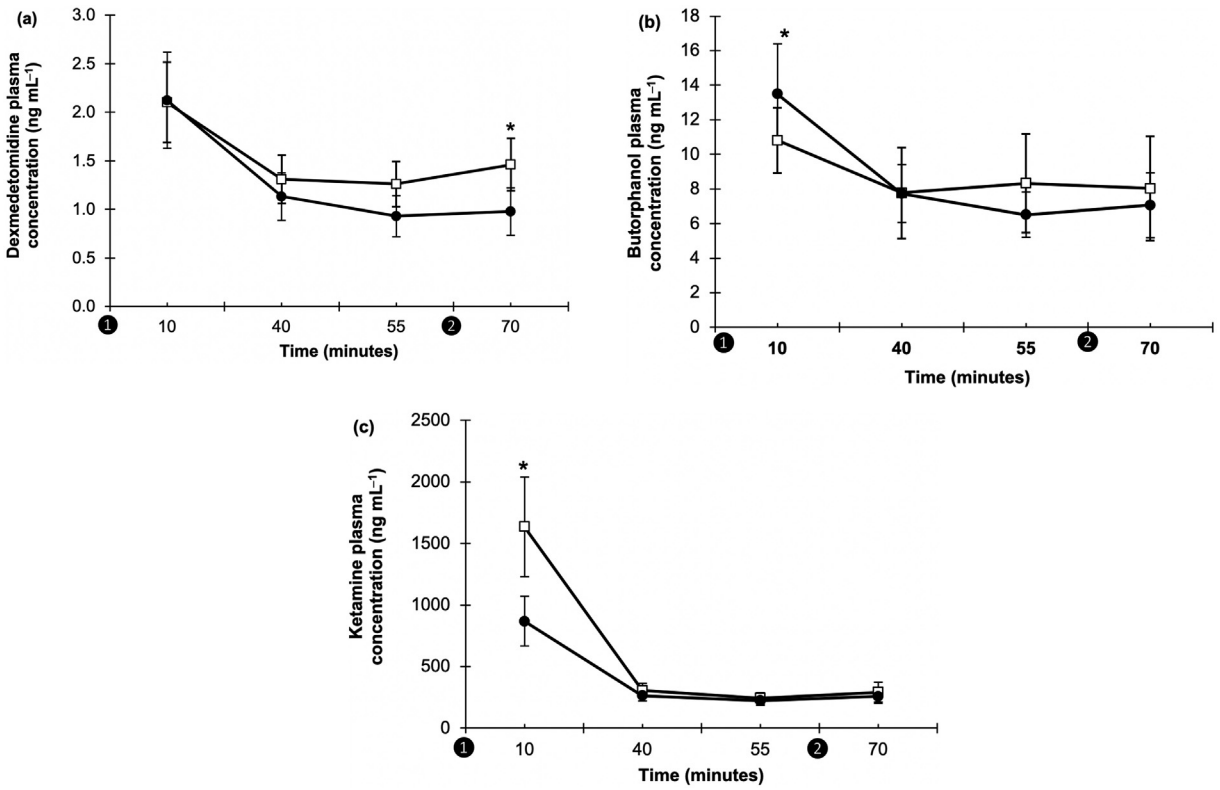

Figure 2 Mean ± standard deviation plasma concentrations of (a) dexmedetomidine, (b) butorphanol and (c) ketamine in eight dogs administered intramuscular (IM) premedication with medetomidine (20 μg kg^{-1}) and butorphanol (100 μg kg^{-1}; treatment MB; open squares) or medetomidine and butorphanol at the same dose rates with vatinoxan (500 μg kg^{-1}; treatment MVB; solid circles) 20 minutes before intravenous ketamine (4 mg kg^{-1}; ❶) and 80 minutes before IM atipamezole (100 μg kg^{-1}; ❷). *Significant difference between treatments ($p < 0.05$).

In both treatments, SVRI and MAP decreased and CI and HR increased after ketamine administration. These results are similar to those of a previous study where ketamine administered IV approximately 5 minutes after IV xylazine induced moderate haemodynamic changes with a decrease in SVR and increases in both HR and CO (Haskins et al. 1986). In the present study, the increase in CI was attributed mainly to an increase in HR because SVI was not increased. The significant decrease in CVP in MVB may have resulted from decreased preload by less central shifting of peripheral venous blood or lower SVRI and higher CI. The present study demonstrated that once the initial vasoconstrictive effect of medetomidine waned or was prevented by co-administration of vatinoxan, ketamine reversed the bradycardia and improved CI. However, because the ketamine effects were transient, they were probably superseded by the sympatholytic effects of medetomidine. Hence, a sustained effect may be achieved by repeated or continuous ketamine administration.

Bloor et al. (1992) reported that dexmedetomidine decreased plasma noradrenaline and adrenaline concentrations below those that impact the cardiovascular system, and that after administration of atipamezole, noradrenaline and adrenaline concentrations returned to baseline. Ketamine has direct cardiovascular depressant effects that are usually masked by the ensuing sympathetic stimulation opposing its direct vasodilatory and myocardial depressant effects (Diaz et al. 1976). The positive inotropic effects of ketamine are indirect and attributed to increased sympathetic nervous system activity (White et al. 1982). Medetomidine decreases sympathetic outflow from the central nervous system with decreased catecholamine concentrations and may blunt ketamine inotropic effects. Although plasma catecholamine concentrations were not measured, the results of the current study indicate that vatinoxan did not antagonize the medetomidine-induced inhibition of noradrenaline and adrenaline release, an effect that was presumably reversed by atipamezole. The results obtained indicate that the central cardiovascular depressor effects of medetomidine probably contributed to the decreased HR and CI following initial effects of ketamine.

Overall, MAP remained higher in MB because of increased SVRI. Hypotension was recorded in two dogs in MVB before atipamezole administration. Nonetheless, tissue perfusion may have been better maintained in MVB because CI was significantly higher and SVRI lower. No differences in RPP were

detected between treatments, probably because the differences in MAP were opposed by those in HR. Medetomidine administered alone induces a biphasic blood pressure response with an initial increase in MAP resulting from peripheral vasoconstriction (Schmeling et al. 1991; Bloor et al. 1992; Flacke et al. 1993). Then MAP decreases via a central sympatholytic effect, becoming more apparent as vasoconstriction wanes (Schmeling et al. 1991; Bloor et al. 1992; Flacke et al. 1993). Vatinoxan ameliorates the early hypertensive effect of α_2-adrenoceptor agonists by blocking vascular α_2-adrenoceptors (Pagel et al. 1998), and in the absence of the characteristic α_2-adrenoceptor agonist-induced vasoconstriction, the late depressor effects may result in decreased MAP. IV administration of vatinoxan combined with dexmedetomidine at the same dose ratio of 1:25 as in the present study resulted in no significant reductions in MAP in conscious dogs (Honkavaara et al. 2011). Furthermore, no hypotension developed when vatinoxan was combined with medetomidine and butorphanol (Salla et al. 2014). Therefore, cardiovascular actions of ketamine probably influenced the temporal effects in the present study.

Notably, except for endotracheal intubation, the dogs in the present study were not stimulated. In clinical practice, any surgical or other procedural stimuli may modify the cardiovascular function, and combining drugs with vasodilatory, sympatholytic, negative inotropic or chronotropic effects may exacerbate the decrease in MAP when administered with vatinoxan. A limitation of the current study was that there were no treatments without ketamine.

In both treatments, f_R decreased significantly after premedication. Assisted ventilation was required in 75% of the dogs after ketamine injection and subsequently $PaCO_2$ increased significantly in both treatments. Similarly, a previous study reported apnoea in 50% of the dogs administered medetomidine (10 μg kg^{-1}) and ketamine (4 mg kg^{-1}) (Ko et al. 2001). A high dose of ketamine (4 mg kg^{-1}) was selected for the present study to ensure appropriate conditions for tracheal intubation (Ko et al. 2001) and to induce significant cardiovascular effects (Pagel et al. 1992). The dose of ketamine induced apnoea in some dogs, and the incidence was not influenced by vatinoxan. When using these premedication combinations with ketamine, preoxygenation is recommended and a method to support ventilation should be available.

The quality of anaesthesia induction and conditions for endotracheal intubation were not different between the pretreatments, even though inclusion of vatinoxan decreased the plasma concentration of ketamine. Ketamine concentrations were probably decreased in MVB compared with MB because hepatic blood flow was better maintained. Overall smaller AUC of ketamine manifested clinically as shorter duration of anaesthesia in MVB than in MB.

The quality of recovery after atipamezole administration in the present study was not optimal as some dogs exhibited nausea and/or defaecation. Ketamine causes salivation and defaecation in dogs (Haskins et al. 1985; Jacobson & Hartsfield 1993). Atipamezole induces defaecation in dogs by abolishing the inhibitory effect of medetomidine on colonic motility mainly via activation of peripheral α_2-adrenoceptors (Maugeri et al. 1994). Vatinoxan may be expected to increase colonic smooth muscle tone and restore motility. Vomiting and defaecation have been reported in dogs recovering from sevoflurane anaesthesia and vatinoxan administration (Hector et al. 2017). It is probable that the nausea and defaecation observed in approximately 30% of the dogs in the present study was a consequence of multiple drug interactions.

The gastrointestinal distress may have influenced the cardiovascular variables after atipamezole administration. By contrast, the increases in HR and CI were much larger than those recorded in a previous study (Turunen et al. 2019). However, the effect may have been exaggerated by the atipamezole dose administered, which should have been adjusted according to the timing of previously administered medetomidine. In the present study, atipamezole was administered at five times the medetomidine dose based on the labelled dose, even though 80 minutes had elapsed. A lower dose of atipamezole may have reduced gastrointestinal side effects and cardiovascular response.

Conclusions

Vatinoxan improved haemodynamic function in dogs when co-administered with medetomidine and butorphanol as premedication for ketamine anaesthesia. Mild hypotension was recorded in 25% of the dogs using the medetomidine-to-vatinoxan dose ratio of 1:25. Vatinoxan did not influence the clinical quality of anaesthesia induction or intubation, but the duration of anaesthesia was shorter. Atipamezole hastened the recovery, but the dose should be adjusted to improve the quality of recovery.

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Authors' contributions

HT: study design, conducted the study, data analysis and drafted the manuscript. MR and RB: study design, conducted the study, reviewed and edited the manuscript. JH and OV: study design, reviewed and edited the manuscript. JJ: conducted the study, reviewed and edited the manuscript. MS, SM and HH: analytical methods, reviewed and edited the manuscript.

Conflict of interest statement

HT is currently employed by Vetcare Ltd. Vetcare Ltd was not involved in the study design, data analysis and interpretation, or writing and publication of the manuscript. MS laboratory performed contract research for Vetcare Oy.

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