RESEARCH PAPER

Effects of vatinoxan in dogs premedicated with medetomidine and butorphanol followed by sevoflurane anaesthesia: a randomized clinical study

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Abstract

Objective To investigate effects of vatinoxan in dogs, when administered as intravenous (IV) premedication with medetomidine and butorphanol before anaesthesia for surgical castration.

Study design A randomized, controlled, blinded, clinical trial.

Animals A total of 28 client-owned dogs.

Methods Dogs were premedicated with medetomidine (0.125 mg m^{-2}) and butorphanol (0.2 mg kg^{-1}) (group MB; $n = 14$), or medetomidine (0.25 mg m^{-2}), butorphanol (0.2 mg kg^{-1}) and vatinoxan (5 mg m^{-2}) (group MB-VATI; $n = 14$). Anaesthesia was induced 15 minutes later with propofol and maintained with sevoflurane in oxygen (targeting 1.3%). Before surgical incision, lidocaine (2 mg kg^{-1}) was injected intratesticularly. At the end of the procedure, meloxicam (0.2 mg kg^{-1}) was administered IV. The level of sedation, the qualities of induction, intubation and recovery, and Glasgow Composite Pain Scale short form (GCPS-SF) were assessed. Heart rate (HR), respiratory rate (f_R), mean arterial pressure (MAP), end-tidal concentration of sevoflurane ($F_{E'}$Sevo) and carbon dioxide ($P_{E'}CO_2$) were recorded. Blood samples were collected at 10 and 30 minutes after premedication for plasma medetomidine and butorphanol concentrations.

Results At the beginning of surgery, HR was 61 ± 16 and 93 ± 23 beats $minute^{-1}$ ($p = 0.001$), and MAP was 78 ± 7 and 56 ± 7 mmHg ($p = 0.001$) in MB and MB-VATI groups, respectively. No differences were detected in f_R, $P_{E'}CO_2$, $F_{E'}$Sevo, the level of sedation, the qualities of induction, intubation and recovery, or in GCPS-SF. Plasma medetomidine concentrations were higher in group MB-VATI than in MB at 10 minutes ($p = 0.002$) and 30 minutes ($p = 0.0001$). Plasma butorphanol concentrations were not different between groups.

Conclusions and clinical relevance In group MB, HR was significantly lower than in group MB-VATI. Hypotension detected in group MB-VATI during sevoflurane anaesthesia was clinically the most significant difference between groups.

Keywords anaesthesia, butorphanol, dog, medetomidine, sevoflurane, vatinoxan.

Introduction

α_2-Adrenoceptor agonists, such as medetomidine, are widely used for sedation and premedication in dogs (Murrell & Hellebrekers 2005). However, all drugs in this class have relevant cardiovascular adverse effects including vasoconstriction and marked bradycardia followed by decreases in cardiac output and oxygen delivery (Bloor et al. 1992; Pypendop & Verstegen 1998). Although the desired effects of sedation and antinociception of α_2-adrenoceptor agonists originate in the central nervous system (CNS), the initial impact on the cardiovascular system is mainly mediated peripherally via activation of α_2-adrenoceptors within vascular smooth muscle leading to vasoconstriction (Clough & Hatton 1981; Horn et al. 1982). In addition, the centrally mediated general sympatholysis after the administration of α_2-adrenoceptor agonists could contribute to the bradycardia (Honkavaara et al. 2008).

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Vatinoxan is a selective α_2-adrenoceptor antagonist, which penetrates poorly into the mammalian CNS (Clineschmidt et al. 1988; Honkavaara et al. 2020) limiting the pharmacological effects to tissues outside the blood–brain barrier. Numerous studies have shown that vatinoxan prevents the vasoconstriction and the following cardiovascular depression without marked effects on sedation attributed to dexmedetomidine or medetomidine in dogs (Pagel et al. 1998; Enouri et al. 2008; Honkavaara et al. 2008, 2011; Restitutti et al. 2011). Furthermore, vatinoxan substantially reduces the plasma concentrations of α_2-adrenoceptor agonists when administered simultaneously intravenously (IV). This effect is likely mediated via improvement of global haemodynamic function resulting in higher clearance and increased volume of distribution (Honkavaara et al. 2012; Bennett et al. 2016). Interestingly, equal plasma dexmedetomidine concentrations were detected with medetomidine (20 μg kg^{-1}) alone and with a twofold dosage (40 μg kg^{-1}) of medetomidine administered IV concomitantly with vatinoxan (Huuskonen et al. 2020).

The benefit of various dosages of vatinoxan on cardiovascular function was demonstrated in Beagle dogs premedicated with medetomidine before general anaesthesia with isoflurane (Salla et al. 2014a, 2017). Isoflurane reduces mean arterial pressure (MAP) in dogs, mainly attributed to decrease in systemic vascular resistance (Brahim & Thut 1984; Mutoh et al. 1997). The vasoconstriction and isoflurane-sparing effects of medetomidine may counteract the vasodilation effects of isoflurane (Bloor et al. 1992).

At present, only one clinical study has been published regarding the effects of vatinoxan in dogs sedated with intramuscular (IM) medetomidine and butorphanol (Kallio-Kujala et al. 2018). Further investigation of the effects of vatinoxan in dogs premedicated with α_2-adrenoceptor agonists before general anaesthesia is important. Our aim was to document effects of vatinoxan in healthy, client-owned dogs, when administered with a routine IV premedication of medetomidine and butorphanol prior to general anaesthesia. We hypothesized 1) that vatinoxan would decrease the incidence of bradycardia after premedication with IV medetomidine and butorphanol; and 2) that concomitant administration of vatinoxan with medetomidine and butorphanol will not change the intensity of sedation, the maintenance of a surgical plane of anaesthesia or the quality of recovery.

Materials and methods

This study was performed at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Helsinki and was approved by Animal Experiment Board (ESAVI/6851/2017). Informed owner consent was obtained. Based on power analysis (two-sided equality) according to the previous results from experimental dogs (Salla et al. 2014a), 10 dogs per group were required to detect a 20 ± 15 beats $minute^{-1}$ difference in heart rate (HR) and a 20 ± 15 mmHg difference in MAP with a power of 80% and an alpha-level set at 0.05 (http://powerandsamplesize.com). The study design was a randomized, controlled, blinded, clinical trial. Randomization was performed in blocks for body weights (5–10, 10.1–30 and 30.1–50 kg) by blindly picking a paper note from an envelope. Randomization in blocks aimed to ensure relatively homogenous populations between groups.

Male dogs assigned to class I or II according to the criteria described by the American Society of Anesthesiologists (Dugdale 2010) scheduled for prescrotal castration were included. Dogs with body weight <5 or >50 kg, body condition score ≥4/5, breed-related risks for general anaesthesia (e.g. brachycephalic syndrome), age <6 months or >10 years, and intolerance to IV catheter placement without sedation were excluded.

After physical examination, a catheter was placed in a cephalic or saphenous vein to obtain blood samples for haematology and basic serum chemistry tests. After IV catheterisation, dogs rested in their cages for at least 30 minutes. The dogs were assigned to group MB, medetomidine [0.125 mg m^{-2}; Medetomidine Hydrochloride, 0.5 mg mL^{-1} (Recipharm, Sweden) or Cepetor, 1 mg mL^{-1} (CP-Pharma, Germany)] and butorphanol (0.2 mg kg^{-1}; Butordol, 10 mg mL^{-1}; Intervet International BV, The Netherlands) IV or group MB-VATI, medetomidine (0.25 mg m^{-2}) and butorphanol (0.2 mg kg^{-1}) and vatinoxan (5 mg m^{-2}; MK-467 HCl, 10 mg mL^{-1}; Vetcare Ltd, Finland) IV. The body surface area (BSA) was calculated using:

$$\text{BSA} = 10.1 \times (\text{body weight in kg})^{0.67} \times 10^{-2}\ (\text{m}^2)$$

(White & Kearney 2014; Pypendop & Jones 2015). The dose of medetomidine (0.125 mg m^{-2}) in group MB corresponds to a dose of 7 μg kg^{-1} for a 5 kg dog and 4.5 μg kg^{-1} for a 50 kg dog.

Drugs were prepared by another investigator not involved in data collection. All premedication drugs were drawn up separately and mixed in a single syringe before administration. In group MB, saline was added to reach equal administration volumes. The syringes were covered with opaque tape for blinding.

Before drug administration (baseline; T0), HR, from the electrocardiogram, and noninvasive oscillometric blood pressure (NIBP) were recorded in the recumbent dog (Life Scope TR; Nihon Kohden, Japan). The blood pressure cuff, width approximately 40% of the limb circumference, was placed over the metatarsus.

Premedication was administered IV as bolus over 10 seconds and flushed with 10 mL saline. After premedication, IV fluid therapy (5 mL kg^{-1} $hour^{-1}$) with balanced crystalloid solution

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(Ringer-Acetat Baxter Viaflo; Baxter Ltd, Finland) was provided until the end of the surgical procedure. Blood samples (3 mL for ethylenediamine tetra-acetic acid tube) for analysis of plasma drug concentrations were collected at 10 and 30 minutes after premedication from the jugular vein by using 5 mL syringe and 25 gauge needle. Actual times of blood sampling were recorded.

Sedation was assessed at 10 minutes (T10) using a composite simple descriptive sedation score ranging from 0 (no sedation) to 15 points (deep sedation) (Raszplewicz et al. 2013). After the sufficient level of sedation was achieved, a 22 gauge, 25 mm catheter (Terumo Europe NV, Belgium) for monitoring invasive blood pressures (IBP) was placed in the dorsal pedal artery. Only two attempts were allowed. For each dog, a new pressure transducer kit (Gabarith PMSET; Becton Dickinson, UT, USA) was used, flushed with saline, zeroed to atmospheric pressure and positioned level with the manubrium.

General anaesthesia was induced with propofol (PropoVet, 10 mg mL^{-1}; Abbott Laboratories Ltd, UK) administered with a loading dose (0.5 mg kg^{-1}) followed by constant rate infusion (CRI; 1 mg kg^{-1} $minute^{-1}$). Start of induction was 15 minutes after premedication, with the actual time recorded. Achieved loss of consciousness was evaluated by assessing palpebral reflexes and the rotation of eyes every 5−10 seconds. The trachea was intubated when palpebral reflexes disappeared and the mandible could be pulled down without resistance. Propofol CRI was stopped after successful endotracheal intubation and the total dose of propofol was recorded. The quality of induction and intubation were scored ranging from 1 (ideal induction, or smooth intubation) to 4 (induction not reached) or 5 (intubation failed) (Casoni et al. 2015). General anaesthesia was maintained with sevoflurane in 60% oxygen in air−oxygen mixture set on flowmeters with rebreathing circle system (Dräger Perseus A500; OneMed Ltd, Finland). End-tidal sevoflurane concentration ($F_{E'}$Sevo) was targeted to 1.3% but was adjusted if needed to maintain surgical plane of anaesthesia (absent palpebral reflex, rotated eye globe, relaxed jaw tone and no movement in response to surgery). $F_{E'}$Sevo was recorded.

During anaesthesia, HR, f_R, SpO_2, NIBP and IBP (when applicable) and oesophageal temperature (T_{OES}) were monitored (Life Scope TR; Nihon Kohden), and inspired fraction of oxygen, end-tidal partial pressure of carbon dioxide ($P_{E'}CO_2$) and $F_{E'}$Sevo with anaesthesia station (Dräger Perseus A500, OneMed Ltd; gas analyser calibrated during yearly maintenance) and recorded at 5-minute intervals until the end of surgery. The duration of surgical procedure was recorded. Dogs were breathing spontaneously, but if apnoea >30 seconds occurred or $P_{E'}CO_2$ increased >55 mmHg (7.3 kPa), mechanical intermittent positive pressure ventilation (IPPV) was instituted. The need for IPPV was recorded as yes or no. Surgical site infiltration and intratesticular injection of lidocaine (total dosage 2 mg kg^{-1}, divided into three volumes; Lidocain, 20 mg mL^{-1}; Orion Pharma, Finland) was performed. Dogs were covered with hot-air devices, electrical heating mattress and blankets to maintain T_{OES} >36 °C.

Rescue analgesia was implemented if two of three variables (HR, f_R, MAP) were increased during the surgery >20% from the values recorded at a stable plane of anaesthesia before incision. In that case, another dose of lidocaine was injected into the surgical site and spermatic cords. If lidocaine (4 mg kg^{-1}) did not produce sufficient analgesia, fentanyl (3 μg kg^{-1}; Fentanyl-Hameln, 50 μg mL^{-1}; Hameln Pharma Plus Gmbh, Germany) was administered IV. Intraoperative rescue analgesia was recorded as yes or no.

In case of concomitantly recorded invasive (when applicable) or noninvasive MAP <60 mmHg and SAP <90 mmHg during anaesthesia, a step-based approach was initiated according to the study anaesthesia guidelines (Bednarski et al. 2011) with 10 mL kg^{-1} crystalloid bolus administered within 5 minutes, followed by a 2 mL kg^{-1} colloid bolus (Gelofusin, 40 mg mL^{-1}; B Braun Melsungen AG, Germany) and dopamine (Dopmin 40 mg mL^{-1}; Orion Corporation, Finland) CRI at 2.5−10 μg kg^{-1} $minute^{-1}$. The correction of hypotension using crystalloid, colloid bolus or dopamine CRI were recorded as yes or no.

At the end of general anaesthesia, the dog recovered in a dedicated room. Supplemental oxygen was administered via loose face mask and SpO_2, HR and f_R were monitored by the same anaesthetist (KS) until the dog was able to lift the head and was responsive to verbal stimuli. The time of extubation and head lift were recorded. The quality of the early recovery phase was scored approximately 30 minutes after extubation with a simple numerical score ranging from 1 (smooth transition to alertness) to 5 points (extreme excitement, re-sedation necessary) (Jiménez et al. 2012; Appendix A). Atipamezole (0.5 mg m^{-2}; Antisedan, 5 mg mL^{-1}; Orion Pharma Ltd) was administered IM if the dog was deeply sedated and bradycardia (HR <60 $minute^{-1}$) persisted after tracheal extubation for more than 10 minutes. In case of severe excitation during early recovery, dexmedetomidine (0.5 μg kg^{-1}; Dexdomitor, 0.1 mg mL^{-1}; Orion Pharma Ltd) was administered IV.

Meloxicam (0.2 mg kg^{-1}; Metacam, 5 mg mL^{-1}; Vetcare Ltd) IV was administered for postoperative analgesia at the end of surgery, followed by 0.1 mg kg^{-1} orally once per day at home for 3−5 days. Pain assessment was performed by the same anaesthetist (KS) 1 hour after extubation with Glasgow Composite Pain Scale short form (GCPS-SF) (Reid et al. 2007). A dog with a GCPS-SF pain score ≥6/24 was treated with IV buprenorphine (0.015 mg kg^{-1}; Bupaq Multidose, 0.3 mg mL^{-1}; Richter Pharma, Austria). The dogs were closely observed and discharged from hospital when their locomotor ability was normal.

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On the following day, a researcher (HT, VP) contacted the owner by phone and requested responses to a questionnaire regarding lethargy, appetite, nausea or vomiting, consistency of the faeces and the signs of pain or distress of their dog. Neither the researcher nor the owners were aware of the assigned group during the telephone call.

Plasma drug concentration analysis

Plasma was separated by centrifugation (3000 ***g*** for 15 minutes) and stored at −20 °C until analysed. The concentrations of medetomidine, butorphanol and vatinoxan were determined using liquid chromatography−tandem mass spectrometry as reported earlier (Kallio-Kujala et al. 2018). The precursor ions ($m\,z^{-1}$) for medetomidine, butorphanol, vatinoxan and internal standard (phenacetin) were 201, 328, 419 and 180, respectively. Fragmented ions ($m\,z^{-1}$) used for quantification were 95, 310, 82 and 110 for medetomidine, butorphanol, vatinoxan and internal standard, respectively. The interassay accuracy of the quality control samples ranged from 92.1% to 105.7% for medetomidine, from 87.8% to 105.5% for vatinoxan and from 94.9% to 102.8% for butorphanol.

Statistical analysis

All statistical tests were performed by using SPSS Version 27 (IBM SPSS Statistics; IBM Corp., NY, USA). The normality of data distribution was evaluated with Shapiro−Wilk test. Repeatedly measured variables were analysed with mixed model of analysis of variance with *post hoc* Bonferroni correction at selected time points. Parametric variables measured only once were compared with unpaired two-tailed *t* tests. Categorical variables and non-normally distributed variables were compared between treatments with Mann−Whitney *U* test. For categorical variables recorded yes/no, Fisher exact test was used. Data are presented mean ± standard deviation or median (range) as appropriate. An alpha-level <0.05 was considered statistically significant.

Results

A total of 28 dogs scheduled for prescrotal castration were included in the study and equal numbers assigned to groups MB and MB-VATI. Dogs in groups MB and MB-VATI were aged 3.1 ± 2.5 and 3.8 ± 2.7 years ($p = 0.50$) and weighed 21.1 ± 10.5 and 22.8 ± 11.7 kg ($p = 0.429$), respectively. Most of the dogs ($n = 17$) weighed 10.1−30 kg, five dogs weighed >30 kg and six dogs <10 kg. The dog breeds were mixed breed ($n = 8$), Border Collie ($n = 4$), Labrador Retriever ($n = 3$), German Shepherd ($n = 2$) and one each Australian Kelpie, Belgian Shepherd, Collie, Dachshund, Giant Schnauzer, Keeshond, Lagotto Romagnolo, Mittelspitz, Schapendoes, Welsh Corgi and Whippet.

There were no significant differences between groups regarding the qualities of sedation, induction, intubation and recovery, or in the induction, incision, end of surgery, extubation and head lift times or in the duration of surgery (Table 1). Total doses of propofol for intubation were 2.8 ± 0.6 mg kg^{-1} in group MB and 2.8 ± 0.9 mg kg^{-1} in group MB-VATI ($p = 0.158$).

In both groups, HR decreased from baseline after the administration of IV premedication. HR was significantly higher in group MB-VATI than in group MB between T5 and T90 ($p < 0.001$; Fig. 1). After 5 minutes from the administration of premedication, HR were 43 ± 15 and 67 ± 17 beats $minute^{-1}$ in groups MB and MB-VATI, respectively ($p = 0.002$). In group MB-VATI, an abrupt decrease in HR from 80 to 30−35 beats $minute^{-1}$ occurred in one dog during ligation of a spermatic cord, 98 minutes after premedication, and atropine (0.02 mg kg^{-1}; Atropin, 1 mg mL^{-1}; Takeda Ltd, Finland) was administered IV. The HR increased and subsequent values of cardiovascular variables were excluded from further analysis.

During the procedure, both IBP MAP ($p < 0.001$) (Fig. 2) and NIBP MAP ($p = 0.001$) (Table 2) were significantly lower in group MB-VATI than in group MB. More than half of the dogs in group MB-VATI (eight out of 14) needed an intervention for hypotension in comparison with one dog in group MB ($p = 0.013$). In group MB-VATI, four out of eight hypotensive dogs required dopamine CRI in addition to the fluid boluses.

No dog required intraoperative rescue analgesia or IPPV. There were no differences in f_R ($p = 0.452$), $P_{E'}CO_2$ ($p = 0.811$) or $F_{E'}$Sevo ($p = 0.158$) between groups (Table 2). T_{OES} were >36 °C in both groups.

Table 1 The sedation scores 10 minutes after sedation (T10), qualities of induction, intubation and recovery, and induction, incision, end of surgery, length of surgery, extubation and head lift times in minutes in dogs premedicated intravenously with either medetomidine 0.125 mg m^{-2} and butorphanol 0.2 mg kg^{-1} (group MB, $n = 14$) or medetomidine 0.25 mg m^{-2}, butorphanol 0.2 mg kg^{-1} and vatinoxan 5 mg m^{-2} (group MB-VATI, $n = 14$) followed by propofol induced sevoflurane anaesthesia for surgical castration.

Variables	Group		*P*
	MB	MB-VATI	
Sedation score at T10	14 (11−14)	14 (4−14)	0.210
Induction time (minutes)	16.4 ± 0.7	16.2 ± 1.0	0.250
Quality of induction	1 (1−2)	1 (1−2)	1.000
Quality of intubation	1 (1−2)	1 (1−3)	0.610
Incision time (minutes)	52.5 ± 7.0	53.2 ± 8.7	0.813
End of surgery (minutes)	98.6 ± 16.7	100.7 ± 14.9	0.723
Length of surgery (minutes)	46.1 ± 12.8	47.3 ± 8.3	0.772
Extubation time (minutes)	115.8 ± 20.7	116.8 ± 16.3	0.783
Head lift time (minutes)	121.4 ± 22.9	121.5 ± 16.0	0.472
Quality of recovery	2 (1−3)	2 (1−5)	0.147

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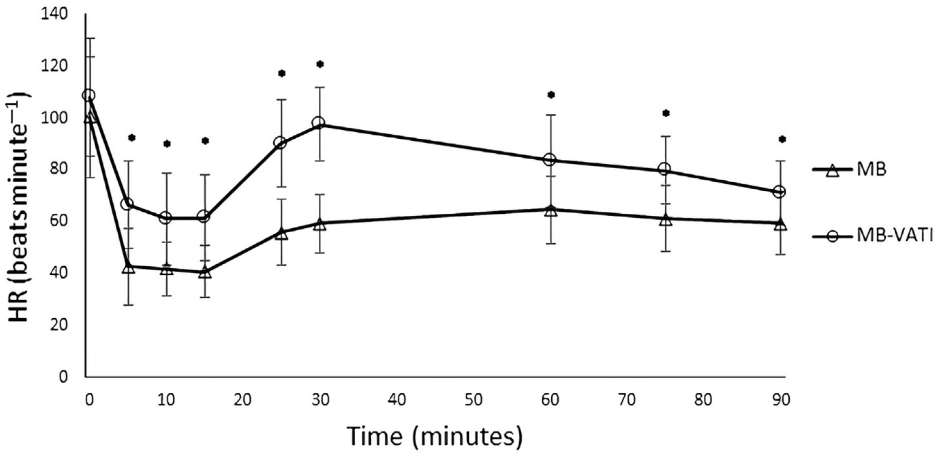

Figure 1 Mean ± standard deviation of heart rates (HR) in dogs before (T0) and after premedication (T5−T15) with intravenous medetomidine (0.125 mg m^{-2}) and butorphanol (0.2 mg kg^{-1}; group MB, $n = 14$), or medetomidine (0.25 mg m^{-2}), butorphanol (0.2 mg kg^{-1}) and vatinoxan (5 mg m^{-2}; group MB-VATI, $n = 14$), followed by propofol induction and sevoflurane anaesthesia before (T25−T30) and during surgical castration (T60−T90). *Significantly different between groups ($p < 0.05$).

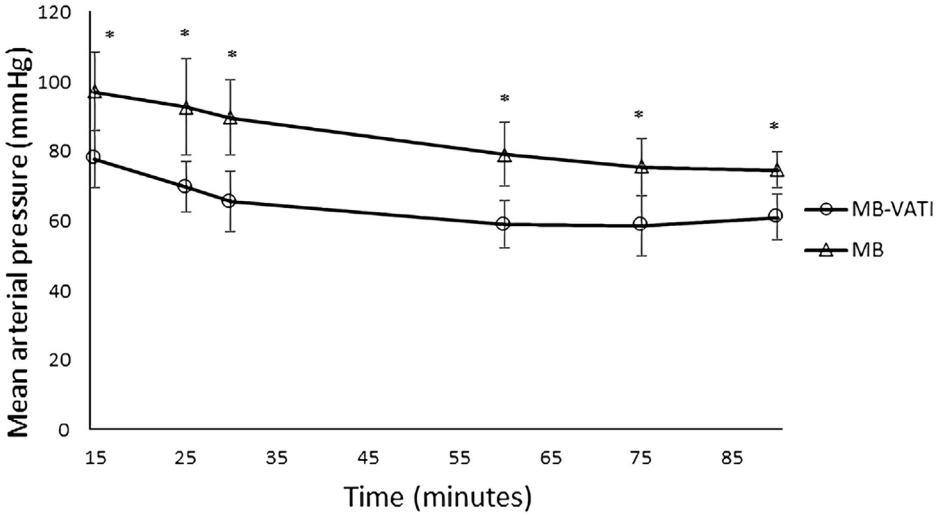

Figure 2 Mean ± standard deviation invasive mean arterial pressures in dogs after premedication (T15) with intravenous medetomidine (0.125 mg m^{-2}) and butorphanol (0.2 mg kg^{-1}; group MB, $n = 13$) or medetomidine (0.25 mg m^{-2}), butorphanol (0.2 mg kg^{-1}) and vatinoxan (5 mg m^{-2}; group MB-VATI, $n = 13$) followed by propofol induction and sevoflurane anaesthesia before (T25−T30) and during surgical castration (T60−T90). *Significantly different between groups ($p < 0.05$).

Atipamezole was administered during recovery in four dogs in group MB and five dogs in group MB-VATI ($p = 1.000$). At the time of extubation, additional dexmedetomidine was needed in two dogs in group MB-VATI ($p = 0.481$). The median GCPS-SF was 4 (1−8) and 2.5 (1−8) in groups MB and MB-VATI, respectively ($p = 0.168$). Rescue analgesia with buprenorphine was administered to four dogs in group MB and two dogs in group MB-VATI ($p = 0.648$). As several dogs in both groups needed additional drugs shortly after extubation (atipamezole or dexmedetomidine), no comparisons were made for HR, SpO_2 or f_R during the postoperative period.

Plasma medetomidine concentrations were significantly higher in group MB-VATI than in group MB at 10 ($p = 0.001$) and 30 minutes ($p < 0.001$) after drug administration, whereas no differences were detected in plasma butorphanol concentrations or blood sampling times between groups at those time points (Table 3).

In the telephone call the day after surgery, decreased appetite ($p = 0.015$) and soft faeces ($p = 0.030$) were reported more often in group MB than in MB-VATI (Table 4). Owners (one from each group) could not be reached for the interview.

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Table 2 Noninvasive mean arterial blood pressure (NIBP, MAP), respiratory rate (f_R), end-tidal partial pressure of carbon dioxide ($P_{E'}CO_2$), and end-tidal sevoflurane concentration ($F_{E'}$Sevo) in dogs at baseline (T0) and at 15 minutes (T15) after intravenous premedication with either medetomidine (0.125 mg m^{-2}) and butorphanol (0.2 mg kg^{-1}; group MB) or medetomidine (0.25 mg m^{-2}), butorphanol (0.2 mg kg^{-1}) and vatinoxan (5 mg m^{-2}; group MB-VATI), and after induction of anaesthesia with propofol and sevoflurane for surgical castration (T30, skin incision, Surgery start; skin sutures, Surgery end). Data are from 14 dogs in each group except where indicated. Data are presented as mean ± standard deviation. *n*, number of dogs; na, not assessed.

Variable	Group	Time point				
		T0	T15	T30	Surgery start	Surgery end
NIBP, MAP	MB ($n = 13$)	111 ± 21	96 ± 20*	80 ± 17*	70 ± 14*	69 ± 10
(mmHg)	MB-VATI ($n = 13$)	110 ± 22	80 ± 12	62 ± 14	58 ± 12	62 ± 13
f_R (breaths $minute^{-1}$)	MB	na	15 ± 3	9 ± 5	10 ± 6	15 ± 11
	MB-VATI	na	14 ± 6	7 ± 3	7 ± 2	14 ± 15
$P_{E'}CO_2$	MB	na	na	48.6 ± 6.5	47.9 ± 5.1	46.1 ± 4.8
(mmHg)	MB-VATI	na	na	48.5 ± 6.8	46.1 ± 7.0	46.3 ± 7.8
$P_{E'}CO_2$	MB	na	na	6.5 ± 0.9	6.4 ± 0.7	6.4 ± 0.6
(kPa)	MB-VATI	na	na	6.5 ± 0.9	6.1 ± 0.9	6.2 ± 1.0
$F_{E'}$Sevo (%)	MB	na	na	1.5 ± 0.4	1.5 ± 0.2	1.5 ± 0.2
	MB-VATI	na	na	1.3 ± 0.2	1.4 ± 0.2	1.5 ± 0.2

*Significant difference between groups ($p < 0.05$).

Discussion

This randomized, clinical trial describes the perianaesthetic effects of vatinoxan with a combination of medetomidine and butorphanol in client-owned dogs scheduled for prescrotal castration under sevoflurane anaesthesia. The most significant findings in this study were the differences in HR and blood pressures between groups.

In the present study, HR was significantly higher in MB-VATI than MB group throughout the procedure. This is in line with results obtained from previous study in laboratory dogs sedated IV with the combination of medetomidine, butorphanol and vatinoxan (Salla et al. 2014b). Moreover, HR was significantly higher during isoflurane anaesthesia in laboratory Beagle dogs when vatinoxan was combined only with medetomidine (Salla et al. 2014a, 2017). Adverse cardiovascular effects, such as tachyarrhythmias and hypertension, have been reported after administration of an anticholinergic agent to treat or prevent bradycardia after medetomidine in dogs (Enouri et al. 2008; Salla et al. 2017). Therefore, these drugs are not suitable for routine correction of bradycardia induced by medetomidine. However, despite the initial decrease in HR and cardiac output after a bolus of α_2-agonist, these variables return close to baseline values during subsequent isoflurane anaesthesia in dogs (Grasso et al. 2015; Salla et al. 2014a, 2017). These findings present the anaesthetist with the dilemma of whether to treat the initial bradycardia after medetomidine in dogs during inhalation anaesthesia. In practice, the decision is driven by the concurrent measurements of blood pressure and the severity of bradyarrhythmia.

Table 3 Blood sampling times and plasma concentrations for medetomidine, butorphanol and vatinoxan in dogs at 10 (T10) and 30 (T30) minutes after intravenous premedication with either medetomidine 0.125 mg m^{-2} and butorphanol 0.2 mg kg^{-1} (group MB, $n = 12$) or medetomidine 0.25 mg m^{-2}, butorphanol 0.2 mg kg^{-1} and vatinoxan 5 mg m^{-2} (group MB-VATI, $n = 12$). Anesthesia was induced with propofol 15 minutes after premedication and maintained with sevoflurane. Data are presented as mean ± standard deviation.

Variable	Time point	Group	
		MB	MB-VATI
Blood sampling time (minutes)	T10	10.6 ± 0.7	10.7 ± 1.5
	T30	30.6 ± 0.8	30.6 ± 1.0
Medetomidine (ng mL^{-1})	T10	3.1 ± 0.5*	5.1 ± 1.8*
	T30	1.4 ± 0.3*	2.2 ± 0.5*
Butorphanol (ng mL^{-1})	T10	78.8 ± 20.6	64.0 ± 20.8
	T30	36.5 ± 8.5	30.0 ± 7.5
Vatinoxan (ng mL^{-1})	T10		741 ± 235
	T30		482 ± 144

*Significant difference between groups ($p < 0.05$).

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Table 4 Responses from dog owners on the day following sevoflurane anaesthesia and castration obtained during a telephone call. Investigator and owners were unaware which premedication was assigned to the dog; medetomidine (0.125 mg m^{-2}) and butorphanol (0.2 mg kg^{-1}; group MB, $n = 13$) or medetomidine (0.25 mg m^{-2}), butorphanol (0.2 mg kg^{-1}) and vatinoxan (5 mg m^{-2}; group MB-VATI, $n = 13$). Data are presented as the number of dogs with the observed behaviour.

At-home behaviour	Group	
	MB	MB-VATI
Lethargy	11	8
Decrease in appetite	6*	0
Nausea	1	0
Soft faeces	7*	1
Pain or distress	9	11

*Significantly different between groups ($p < 0.05$).

The most significant finding in the present study was the moderate to marked hypotension in group MB-VATI. Hector et al. (2021) demonstrated that the presence of vatinoxan attenuates dexmedetomidine-induced decrease in sevoflurane minimum alveolar concentration (MAC) in dogs. In the present study, $F_{E'}$Sevo was approximately 35% lower (at 1.5%) in both groups than the reported MAC (2.3%) of sevoflurane in dogs (Kazama & Ikeda 1988). Moreover, the required $F_{E'}$Sevo was higher than the targeted $F_{E'}$Sevo of 1.3%, indicating that the sevoflurane administration could not be decreased further. In addition, despite the fact that increased medetomidine dose in group MB-VATI was associated with higher plasma concentration during sevoflurane anaesthesia, hypotension and higher HR were more common in group MB-VATI than in group MB. Therefore, we consider that these differences in cardiovascular effects between groups are the result of including vatinoxan. Similarly, several studies with experimental dogs, horses and cats have recorded lower blood pressures during inhalation anaesthesia with the presence of vatinoxan in comparison with α_2-adrenoceptor agonists alone (Salla et al. 2014a, 2017; Pakkanen et al. 2015; Martin-Flores et al. 2018; Jaeger et al. 2019; Tapio et al. 2019).

In previous studies, the increased HR and decreased blood pressures following the administration of various doses of vatinoxan with an α_2-agonist in dogs resulted in improved cardiac outputs and oxygen delivery in comparison with α_2-agonist treatment alone (Honkavaara et al. 2012; Salla et al. 2014a,b; Bennett et al. 2017). However, cardiac output or oxygen delivery were not measured in the present clinical study, and interventions were required to treat clinically unacceptable MAP in more than half of the dogs in MB-VATI group.

The dose ratio of medetomidine to vatinoxan (1:20) was chosen based on the previous studies in laboratory Beagle dogs premedicated with various dose ratios followed by isoflurane anaesthesia (Salla et al. 2014a, 2017). However, it should be noted that in these experimental studies the dose of medetomidine was higher and butorphanol was not administered (Salla et al. 2014a, 2017) in comparison with the present study. Therefore, further investigations are needed to explore whether different doses or dose ratios of medetomidine and vatinoxan, another administration route such as IM, or the use of other anaesthetic agents for maintenance could provide clinically satisfactory blood pressures during general anaesthesia in various dog populations.

In the present study, the dose of medetomidine in group MB-VATI was twice that in group MB intended to achieve similar plasma concentrations of medetomidine in both treatments, based on earlier results in laboratory Beagle dogs (Honkavaara et al. 2012; Huuskonen et al. 2020). However, this goal was not reached and plasma concentrations of medetomidine were higher in group MB-VATI, although less than twofold higher than in group MB. In addition, plasma concentrations of butorphanol were not different between groups. The probable cause of the variability in drug plasma concentrations for administered doses, alongside the minor inaccuracy in blood sampling times among individual dogs, could be physiological and metabolic differences across dog breeds that are responsible for breed-related diversity in the dose—exposure relationship of drugs (Martinez et al. 2013). In horses premedicated with detomidine and butorphanol with or without vatinoxan, plasma concentrations of butorphanol during isoflurane anaesthesia were not statistically different between treatments at selected time points (Pakkanen et al. 2015). However, the area under the plasma concentration time curve for butorphanol was less with vatinoxan treatment. Moreover, the present study was not designed to compare pharmacokinetic variables, and owing to the clinical nature of the study only two blood samples were collected for plasma drug concentrations analysis, preventing a thorough interpretation of the pharmacokinetic behaviour of the drug combinations. Nevertheless, the dose of propofol, the level of sedation, the qualities of induction, intubation or recovery or GCPS-SF did not differ between groups. It is possible that the sedation scoring system used in the study may not have discriminated between slight differences in the deep levels of sedation. Also noted is that this study was not powered to detect a difference in GCPS-SF and procedural times varied among individual dogs as expected in clinical practice, which could have influenced the results regarding recovery quality and GCPS-SF. However, some dogs required postoperative rescue analgesia, similarly reported by Huuskonen et al. (2013). In addition, administration of either atipamezole or dexmedetomidine in the early recovery phase was indicated for several dogs, consequently, routine monitoring during the recovery phase is recommended. Further studies are warranted to investigate the influence of vatinoxan on the plasma drug concentrations at the postoperative phase and on the need for postoperative analgesic

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agents. Gastrointestinal side effects such as decreased appetite or soft faeces were reported less frequently by the owners of dogs in group MB-VATI. By contrast, in a previous study the administration of α_2-adrenoceptor antagonist was accompanied with soft faeces in sedated dogs (Kallio-Kujala et al. 2018). Therefore, further studies regarding the incidence of gastrointestinal side effects after general anaesthesia in small animal medicine are also warranted.

Conclusions

HR decreased less in dogs premedicated with medetomidine—butorphanol—vatinoxan than in dogs premedicated without vatinoxan. Increased incidence of hypotension was the most clinically relevant effect of vatinoxan during sevoflurane anaesthesia. Therefore, monitoring blood pressure with a plan for treatment of hypotension is recommended when vatinoxan is combined with medetomidine and butorphanol and anaesthesia is maintained with sevoflurane in dogs.

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Authors' contributions

KMS: study design, data collection, analysis and interpretation of data, writing the original draft of the manuscript. HAT and IJK: study design, data collection, writing the original draft of the manuscript. VP, JL and PB: study design, data collection. DCC, MRR and OV: study design. All authors contributed to review and editing of the manuscript.

Conflict of interest statement

At the time of the study design and data collection, HT was a PhD student in University of Helsinki, Finland, but is currently employed by Vetcare Ltd, Finland. Vetcare Ltd provided the drugs used in this study but did not contribute to the study design, data collection, analysis of data or interpretation of the results. The authors declare no conflict of interest.

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Appendix A

Scoring of early recovery 30 minutes after sevoflurane anaesthesia. Modified from Jimenez et al. (2012).

Score	Description
1	After extubation smooth transition to alertness. No paddling, vocalization, trembling or vomiting. Coordinated movements within 10 minutes.
2	Fairly smooth or slow transition to alertness. No paddling, vocalization, trembling or vomiting. No body movements attempted in 10 minutes, may need atipamezole.
3	Moderately smooth with mild excitement. Mild paddling, vocalization, trembling or vomiting. Some incoordination in body movements.
4	Excitement. Uncoordinated whole body movements. Paddling, vocalization or vomiting observed.
5	Extreme excitement, emergence delirium. Uncoordinated whole body movements that cannot not be easily restrain. Dogs needs re-sedation.

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