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RESEARCH PAPER

The impact of vatinoxan on microcirculation after intramuscular co-administration with medetomidine in Beagle dogs: a blinded crossover study

Lisa Niemann[a], APN Kutter[a], FB Joerger[a], ML Wieser[a], S Hartnack[b] & B Steblaj[a]

[a]Section of Anaesthesiology, Department of Clinical Diagnostics and Services, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

[b]Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Correspondence: Lisa Niemann, Section of Anaesthesiology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 258c, 8057, Zurich, Switzerland. E-mail: lisa.niemann@uzh.ch

Abstract

Objective To measure the effects on microcirculation of medetomidine alone (MED) or combined with vatinoxan (MVX).

Study design Randomized, crossover, blinded, experimental study.

Animals A group of eight healthy purpose-bred Beagle dogs.

Methods Each dog was administered 1 mg m^{-2} MED intramuscularly (IM) or combined with 20 mg m^{-2} vatinoxan IM (MVX) with a washout period of 7 days. A sidestream dark field (SDF) camera was placed on the buccal mucosa to assess the oral mucosal microcirculation for perfused DeBacker density, proportion of perfused vessels (PPV) (both for all vessels and vessels with a diameter < 20 µm), microvascular flow index (MFI) and heterogeneity index (HI). Videos were recorded at baseline (−5) and 10, 20, 30, 40, 60, 90 and 120 minutes after treatment administration. Linear mixed-effects models were used to assess if microvascular variables were significantly associated with treatment, baseline, and sequence. Results are presented as estimated effect (95% confidence interval), and a p value < 0.05 was considered significant.

Results The interquartile range for baseline measurements was 91.49%−98.42% for PPV, 2.75−3 for MFI and 0−0.36 for HI. Significant effects of treatment and baseline were found. The estimated effect of MED against MVX was −1.98% (−3.53% to −0.42%) for PPV, −0.33 (−0.43 to −0.22) for MFI and 0.14 (0.05 to 0.22) for HI. There were no significant changes seen for perfused DeBacker density, perfused DeBacker density < 20 µm and PPV < 20 µm between treatments.

Conclusions and clinical relevance These results suggest that MVX had significantly fewer effects on buccal mucosal microcirculation than MED. The SDF camera is a useful research tool to assess the microcirculatory status of heavily sedated dogs.

Keywords anaesthesia, dog, medetomidine, microcirculation, sidestream dark field camera, vatinoxan.

Introduction

Medetomidine is an α_2-adrenoceptor agonist widely used in dogs to provide sedation and analgesia and to reduce anaesthetic requirements (Ewing et al. 1993). Medetomidine has known pronounced peripheral and central cardiovascular side effects in dogs (Pypendop & Verstegen 1998). The peripherally acting α_2-adrenoceptor antagonist vatinoxan (also known as MK-467 or L-659'066) selectively blocks the α_2-adrenoceptors outside the blood-brain barrier (Honkavaara et al. 2020) and attenuates the peripherally mediated macrovascular side effects in dogs (Enouri et al. 2008; Honkavaara et al. 2011; Rolfe et al. 2012).

Microcirculation is defined as the circulation in arterioles, venules and capillaries within tissue beds that are less than 100 µm in diameter. These so-called microvessels are ultimately responsible for the oxygen supply to the tissue and cells (Donati et al. 2013; Massey & Shapiro 2016). Therefore, regulation of the microcirculation is crucial for adequate tissue perfusion that matches the oxygen demand of the cells. Previous studies in critically ill human patients have shown that the microcirculation does not correlate with macrocirculation variables such as systemic arterial blood pressure and cardiac output (Sakr et al. 2004; Trzeciak et al. 2007). Thereby, it is not recommended to draw any conclusions from macrocirculatory measurements to the microcirculation status.

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For bedside assessment of microcirculation, a sidestream dark field (SDF) camera is commonly used both in research and clinical studies in humans. The SDF camera emits light at a wavelength of 530 nm and the light is absorbed by haemoglobin inside the erythrocytes. Using an amplifying lens, the erythrocytes become visible as black cells in real time (Goedhart et al. 2007). The SDF camera allows continuous observation of the erythrocytes in the microvessels on a display and the videos can be recorded, saved and analysed.

In humans, sublingual blood flow appears to be highly representative of the splanchnic microcirculation and is therefore commonly used to assess the microcirculatory status (Jin et al. 1998; Groner et al. 1999; De Backer et al. 2002). The microcirculation of the buccal mucosa has been investigated in a veterinary clinical study in horses and showed a significant correlation to the colonic, jejunal, oesophageal and rectal mucosal microcirculation (Hopster et al. 2018).

To date, only the effects of the peripheral vasoconstriction caused by medetomidine on microcirculation have been studied in a laser Doppler study in anaesthetized dogs (Pypendop & Verstegen 2000). That study showed reduced skeletal muscle and intestinal microcirculation but preserved renal cortical microcirculation. Whether vatinoxan attenuates not only the peripherally mediated macrovascular side effects in dogs (Enouri et al. 2008; Honkavaara et al. 2011; Rolfe et al. 2012), but also the microvascular changes, remains unknown. To the authors' knowledge, there has been no study investigating the microcirculatory effects of vatinoxan co-administration with medetomidine in dogs.

Our aim was to investigate the impact of vatinoxan on the microcirculation after intramuscular (IM) co-administration with medetomidine in healthy Beagle dogs. We hypothesized that selective blockage of the α_2-adrenoceptors outside the blood-brain barrier by vatinoxan would result in higher values of microcirculatory perfusion variables and a more homogenous blood flow compared with medetomidine sedation without vatinoxan.

Materials and methods

This project was approved by the cantonal veterinary office of Zurich, Switzerland (Licence Number 31301; ZH099/19).

Animals

A group of eight adult healthy purpose-bred Beagle dogs were enrolled in this study. Inclusion criteria prior to the study were cooperative character and good general health condition. The eight intact dogs included four females and four males, with an age range of 2.3–9.4 years and weight between 9.0 and 14.7 kg. The dogs were fasted for at least 12 hours, with free access to water, prior to anaesthesia for instrumentation. The dogs were transported in the morning of study day 0 and 7 to the study site and were returned to the animal facility the day after.

Study design

This study was a single-centre, randomized, blinded, two-sequence crossover, experimental study. The animals were randomly assigned an animal order number using a computer random number generator (Microsoft Excel; Microsoft Corporation, WA, USA), which determined the order of treatment. The investigators who made clinical observations were blinded to the treatments and a separate person (BS) prepared and administered all treatments.

Anaesthesia and instrumentation

On each treatment day, instrumentation for the concurrent macrocirculation study (unpublished data) was performed during general anaesthesia prior to administration of the study treatment. After physical examination, a catheter (Vasofix 20 gauge; BBraun, Provet, Switzerland) was placed aseptically into a cephalic vein. Anaesthesia was induced with propofol intravenously (IV) (Propofol 1% MCT Fresenius; Fresenius Kabi AG, Switzerland) titrated to effect (3.8–12.6 mg kg^{-1}) until endotracheal intubation was possible using a cuffed endotracheal tube with an internal diameter of 8 or 9 mm (Rüschelit Super Safety Clear; Teleflex Medical, Switzerland). The dogs were placed in dorsal recumbency, and the endotracheal tube was connected to an anaesthetic machine via a circle system (Aespire GE Healthcare, Finland). Anaesthesia was maintained with isoflurane to effect with an end-tidal isoflurane concentration of 1.4–2.4% in oxygen and air with an inspired oxygen fraction of 0.6. Pressure-controlled mechanical ventilation was initiated with a peak pressure of 8–12 cmH_2O and the respiratory rate adapted to achieve an end-tidal carbon dioxide partial pressure of 35–50 mmHg (4.7–6.7 kPa). Continuous monitoring of cardiopulmonary variables was established with a multiparameter monitor (Datex-Ohmeda; S5 Monitor; GE Healthcare, Finland). All dogs were administered Ringer's acetate at 5 mL kg^{-1} $hour^{-1}$ (Ringer-Acetat; Fresenius Kabi AG) IV. After clipping, applying topically lidocaine and prilocaine (EMLA Crème; Aspen Pharma Schweiz GmbH, Switzerland) and aseptic preparation, a catheter (VasoVet 22 gauge; Eickemeyer, Switzerland) was placed in a metatarsal artery. A pulmonary artery catheter (Swan-Ganz TD Catheter; Edwards Lifesciences AG, Switzerland) was placed, after local anaesthesia with lidocaine 2 mg kg^{-1} subcutaneously (Lidocaine 2%; Bichsel, Switzerland), via an introducer in the jugular vein under fluoroscopy. Thereafter all catheters were secured and flushed, anaesthetic gas administration stopped, and the dogs were allowed to recover for at least 120 minutes after extubation prior to baseline measurements of microcirculation.

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Treatment and measurements

The treatment consisted of 1 mg m^{-2} medetomidine (Dorbene; Graeub, Switzerland) (treatment MED) or 1 mg m^{-2} medetomidine with 20 mg m^{-2} vatinoxan (medetomidine 0.5 mg mL^{-1} and vatinoxan 10 mg mL^{-1} hydrochloride injection; Recipharm, Sweden) (treatment MVX). All drugs were administered by the same person (BS) IM into the gluteal muscle (after negative aspiration for blood to confirm extravascular drug administration) immediately after baseline measurements. The dose and temperature of the drugs was controlled daily before injection (BS).

Macrocirculatory variables were assessed for the concurrent study and results will be presented elsewhere (unpublished data). Body temperature was maintained between 37.4 °C and 39.1 °C with the aid of a circulating warm water mattress (Hico-Aquartherm 660; Hirtz, Germany) and forced air warming (Bair hugger; 3M, Switzerland) to effect. Blood temperature was measured using the Swan-Ganz catheter (Edwards Lifesciences AG) in the pulmonary artery and recorded.

For the evaluation of microcirculation, an SDF camera (USB3 MicroScan; MicroVision Medical, The Netherlands) was used. Microcirculation of the buccal mucosa was observed and recorded at baseline (−5) and at 10, 20, 30, 40, 60, 90 and 120 minutes after injection. The buccal mucosa above a maxillary canine tooth was gently cleaned of secretions with an isotonic saline-drenched (NaCl Bichsel Infusionslösung 0.9%; Bichsel, Switzerland) gauze sponge. The probe of the SDF camera containing a five-time magnifying objective lens was placed in slight contact with the buccal mucosa until the microvascular network was visible. Once good quality images were observed (appropriate stability, brightness and focus), videos of 7 second duration were recorded at each time point, until at least three videos with assumptive adequate quality had been obtained. The image size provided was 720 × 480 pixels with 1.45 lumen $pixel^{-1}$ spatial resolution, a field of view of 1044 × 758 µm and 30 frames per second temporal resolution.

Analysis of microcirculatory variables

Prior to further analysis, the quality of all 799 videos obtained was assessed by one video reviewer (LN) blinded to treatment and time point with the categories suggested by Massey & Shapiro (2016). The videos were screened for the following categories: illumination, focus, content, stability and pressure and excluded if the quality was subjectively decided to be unacceptable for analysis. The videos were scored for each category. For each time point, between three and 12 consecutive microcirculation videos were recorded. The first three videos with the best quality assessed by the scorer for each time point were used for the microcirculation analysis.

The following variables were obtained by automatic analysis using the automated vascular analysis (Doi et al. 2009) software 4.3C (MicroScan; MicroVision Medical): proportion of perfused vessels (PPV), PPV < 20 µm in width, perfused DeBacker density and perfused DeBacker density < 20 µm. For the automatic assessment, the software program traces all vessels and projects three horizontal and three vertical lines over the video. Consequently, the program counts all vessels crossing the lines, assesses if they are < 20 µm and if they are perfused. The PPV is calculated as the percentage of perfused vessels crossing the lines over the total number of vessels crossings the lines. The perfused DeBacker density is calculated by the total length of perfused vessels divided by the total surface (mm mm^{-2}) of the analysed area.

Further analysis of the videos was performed according to the published consensus (De Backer et al. 2007; Ince et al. 2018). The microvascular flow index (MFI) was visually analysed by the same observer blinded to time point and treatment (LN). The MFI was determined by dividing the video images into four quadrants. The predominant flow type in each quadrant was assessed (0 = absent flow, 1 = intermittent flow, 2 = sluggish flow, 3 = normal flow) using the scoring system according to the published consensus (De Backer et al. 2007). To achieve the MFI for the video, the mean of all four quadrants was calculated. The heterogeneity index (HI) is a determinant of the heterogeneity of blood flow, a characteristic of distributive abnormalities (Ince et al. 2018). The calculation of HI was performed with the formula $(MFI_{maximum} - MFI_{minimum})/MFI_{mean}$ (De Backer et al. 2007; Massey & Shapiro 2016).

Statistical analysis

A sample size calculation indicated that eight dogs were required to show a difference of 20% in cardiac output for the concurrent study (unpublished data) using a power of 80% with an α-level of 0.05.

To assess a potential difference between treatments MED and MVX, linear mixed-effects models using the software R version 3.6.3 (R Core Team 2020; R Foundation for Statistical Computing, Austria) and the package nlme (Pinheiro et al. 2013) were performed.

Treatment effects (MVX *versus* MED), sequence and the microcirculatory variables were used as fixed effects. Dog and time points were included as random effects (random intercept and slope) to account for potential clustering within a dog. Baseline was included in the analysis as a covariate for that sequence.

Model selection was based on Akaike information criterion with a lower value indicating a better model fit. Model validation was based on visually checking the residuals for normality, homogeneity and independence. If the time by

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treatment interaction was significant, time differences between treatments were evaluated; differences were deemed significant if the unadjusted p value was < 0.05. Data are presented as mean ± standard deviation (SD).

Results

Data from the eight dogs was obtained and analysed. For each time point, the first three videos of sufficient quality were chosen as described above and consequently analysed, which resulted in a total of 384 (192 per treatment) analysed videos.

The median, interquartile range and range for baseline measurements of all microcirculatory variables and for core temperature for treatments MED and MVX are presented in Table 1.

The estimated effects of treatment MED against treatment MVX and the effect of baseline are summarized in Table 2. Significant estimated effects of treatment MED against treatment MVX were found for PPV ($p = 0.01$), MFI ($p = 0.01$), HI ($p < 0.01$) and core temperature ($p < 0.01$). Significant estimated effects of baseline were found for PPV ($p = 0.03$), perfused DeBacker density ($p < 0.01$), MFI ($p = 0.01$) and core temperature ($p < 0.01$). Figs. 1–4 show PPV, MFI, HI and core temperature mean ± SD for both treatments. There was no significant effect of treatment found for perfused DeBacker density, perfused DeBacker density, < 20 µm and PPV < 20 µm.

We obtained the highest HI values and lowest MFI values 10 minutes after treatment MED administration (0.67 ± 0.47 and 1.67 ± 0.5, respectively). The lowest PPV value was obtained 60 minutes after treatment MED (88.75% ± 5.82%).

All dogs were observed to be sedated; however, no sedation scoring was performed.

Discussion

The present study examined the microcirculatory effect of medetomidine co-administered either with or without vatinoxan in dogs using an SDF camera. We found a significant negative impact of medetomidine on MFI, HI and PPV.

After medetomidine alone, the dogs had a significantly lower percentage of perfused vessels, a reduced and a more heterogeneous microvascular blood flow than that shown after medetomidine combined with the peripheral α_2-adrenoceptor antagonist vatinoxan.

MFI was assessed by quadrant, which is the most common method used to assess microcirculatory videos for flow (Pozo et al. 2012). MFI provides information about functional microcirculatory perfusion and was found to successfully differentiate between physiologic and pathologic microcirculation in humans (De Backer et al. 2002). Our results showed that when medetomidine was combined with vatinoxan, MFI was not reduced as opposed to a significant reduction of MFI after medetomidine alone.

To quantify the heterogeneity of blood flow, HI was developed. In the current study, HI was significantly increased after treatment MED compared with treatment MVX indicating a less even distribution of blood flow. We obtained the highest HI values 10 minutes after treatment MED. Similar values were measured in humans in distributive shock (0.68 ± 0.6) (Trzeciak et al. 2007). The high HI values were only seen temporarily after the high dose of medetomidine used in the

Table 1 Baseline microcirculatory measurements and core temperature of eight Beagle dogs on 2 study days before a crossover intramuscular treatment of 1 mg m^{-2} medetomidine without (MED) and with 20 mg m^{-2} vatinoxan (MVX). The proportion of perfused vessels (PPV), PPV < 20 µm, perfused DeBacker density and perfused DeBacker density < 20 µm were obtained by automatic analysis using the automated vascular analysis. The microvascular flow index (MFI) was visually analysed, and the heterogeneity index was calculated using the MFI values

Variables	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Proportion of perfused vessels (%) MED	77.50	90.99	94.28	98.81	100
Proportion of perfused vessels (%) MVX	76.92	91.99	94.76	97.94	100
Proportion of perfused vessels < 20 µm (%) MED	50.00	72.73	93.73	100	100
Proportion of perfused vessels < 20 µm (%) MVX	33.33	81.93	96.76	100	100
Perfused DeBacker density (mm mm^{-2}) MED	6.16	8.24	9.03	10.68	13.18
Perfused DeBacker density (mm mm^{-2}) MVX	5.95	8.03	9.46	11.69	16.58
Perfused DeBacker density < 20 µm (mm mm^{-2}) MED	0.21	0.21	0.53	3.08	5.53
Perfused DeBacker density < 20 µm (mm mm^{-2}) MVX	0.21	0.43	2.13	4.20	9.14
Microvascular flow index (no unit) MED	2.25	2.75	2.75	2.75	3.00
Microvascular flow index (no unit) MVX	2.00	2.69	3.00	3.00	3.00
Heterogeneity index (no unit) MED	0.00	0.00	0.36	0.36	0.44
Heterogeneity index (no unit) MVX	0.00	0.00	0.00	0.36	0.44
Temperature (°C) MED	37.7	37.9	38.2	38.3	38.5
Temperature (°C) MVX	37.6	38.1	38.2	38.3	38.3

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Table 2 Effects of treatment and baseline on microcirculatory variables and core temperature in eight Beagle dogs on 2 study days with a crossover intramuscular treatment of 1 mg m^{-2} medetomidine without (MED) and with 20 mg m^{-2} vatinoxan (MVX). The proportion of perfused vessels (PPV), PPV < 20 µm, perfused DeBacker density and perfused DeBacker density < 20 µm were obtained by automatic analysis using the automated vascular analysis. The microvascular flow index (MFI) was visually analysed, and the heterogeneity index was calculated using the MFI values. An asterisk (*) marks significant effects of treatments or baseline ($p < 0.05$). A p value < 0.05 was considered significant. The effect of baseline measures the effect of treatment and the initial values measured at time point 0

Variables	Effect of treatment MED versus MVX			Effect of baseline		
	Estimated effect	95% Confidence interval	*p*	Estimated effect	95% Confidence interval	*p*
Proportion of perfused vessels (%)	−1.98*	−3.53 to −0.42	0.01	−0.16*	−0.30 to −0.01	0.03
Proportion of perfused vessels < 20 µm (%)	−0.44	−4.43 to 3.55	0.83	−0.09	−0.22 to 0.03	0.15
Perfused DeBacker density (mm mm^{-2})	< 0.01	−0.45 to 0.45	1.00	−0.41*	−0.51 to −0.31	< 0.01
Perfused DeBacker density < 20 µm (mm mm^{-2})	0.07	−0.41 to 0.56	0.77	−0.01	−0.12 to 0.10	0.83
Microvascular flow index (no unit)	−0.33*	−0.43 to −0.22	0.01	−0.30*	−0.53 to −0.07	0.01
Heterogeneity index (no unit)	0.14*	0.05 to 0.22	< 0.01	−0.16	−0.38 to 0.06	0.15
Temperature (°C)	0.65*	0.57 to 0.73	< 0.01	−0.56*	−0.77 to −0.34	< 0.01

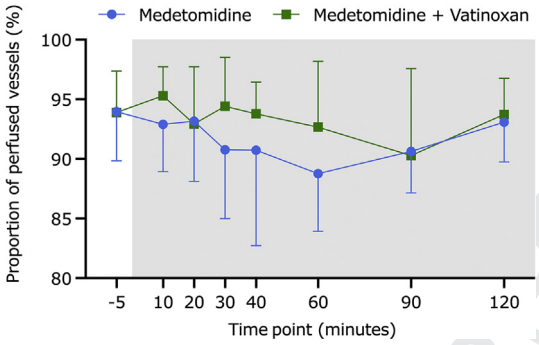

Figure 1 Proportion of perfused vessels, calculated as the percentage of perfused vessels, of eight Beagle dogs with a crossover intramuscular treatment of 1 mg m^{-2} medetomidine with (green squares) and without 20 mg m^{-2} vatinoxan (blue dots) showing a significant effect of treatment ($p = 0.01$) and baseline ($p = 0.03$). Data are presented as mean and standard deviation with values from time point 0 (= time of the injection of the treatment) shaded in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

current study and can be explained by vasoconstriction and decreased cardiac output induced by medetomidine. As this study was performed as part of a bigger study for a new drug product in registration process, the recommended dose for this product was administered as treatment.

In this study, a lower percentage of perfused vessels was measured after treatment MED compared with treatment MVX. Vasoconstrictive properties of medetomidine initiated by peripheral α_2-adrenoceptors could explain the lower PPV results in treatment MED (Pypendop & Verstegen 1998). Vatinoxan probably inhibited and resolved the medetomidine-induced vasoconstriction by binding to peripherally α_2-adrenoceptors and thereby antagonizing the effect of medetomidine.

In anaesthetized horses administered vatinoxan and dexmedetomidine or xylazine, gastrointestinal microcirculation was measured with laser Doppler flowmetry (Wittenberg-Voges et al. 2018). Vatinoxan significantly reduced

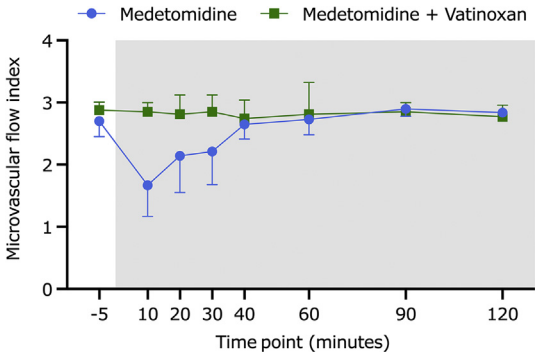

Figure 2 Microvascular flow index, assessed by the evaluation of the predominant flow type, of eight Beagle dogs with a crossover intramuscular treatment of 1 mg m^{-2} medetomidine with (green squares) and without 20 mg m^{-2} vatinoxan (blue dots) showing a significant effect of treatment ($p = 0.01$) and baseline ($p = 0.01$). Data are presented as mean and standard deviation with values from time point 0 (= time of the injection of the treatment) shaded in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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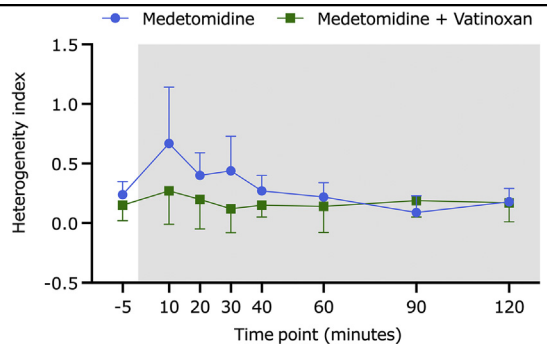

Figure 3 Heterogeneity index, calculated using the microvascular flow index, of eight Beagle dogs with a crossover intramuscular treatment of 1 mg m^{-2} medetomidine with (green squares) and without 20 mg m^{-2} vatinoxan (blue dots) showing a significant effect of treatment ($p < 0.01$). Data are presented as mean and standard deviation with values from time point 0 (= time of the injection of the treatment) shaded in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

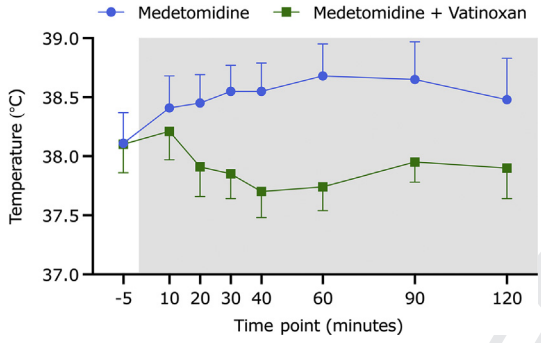

Figure 4 Core temperature obtained from the pulmonary artery catheter of eight Beagle dogs with a crossover intramuscular treatment of 1 mg m^{-2} medetomidine with (green squares) and without 20 mg m^{-2} vatinoxan (blue dots) showing a significant effect of treatment ($p < 0.01$) and baseline ($p < 0.01$). Data are presented as mean and standard deviation with values from time point 0 (= time of the injection of the treatment) shaded in grey. The dogs were actively warmed to effect. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

gastrointestinal perfusion and mean arterial pressure (MAP), although cardiac index (CI) increased after vatinoxan. The major differences between that study and the present study were the different times of drug administration and the fact that measurements were performed during inhalant anaesthesia. Additionally, a different method of microcirculation analysis was used in the equine study. Therefore, those results cannot be compared directly with values measured with an SDF probe.

In an earlier study in dogs, the effects of medetomidine on microcirculation and the subsequent administration of the peripheral and central α_2-adrenoceptor antagonist atipamezole were assessed with laser Doppler flowmetry (Pypendop & Verstegen 2000). The results showed that the intestinal and the skeletal muscular microcirculation were significantly reduced between 5 and 65 minutes after 1 mg m^{-2} medetomidine IM. The administration of 2.5 mg m^{-2} atipamezole IM 60 minutes after medetomidine did not restore microcirculation to baseline values in those dogs. Atipamezole also failed to increase macrocirculatory variables specifically heart rate and CI, while a slight decrease in MAP was induced. The decrease in blood pressure and the concomitant reduction in perfusion pressure might explain the lack of microcirculatory restoration in the study of Pypendop & Verstegen (2000). Since the dogs were anaesthetized with isoflurane, the vasodilation induced by isoflurane might have attenuated the vasoconstrictive effects of medetomidine.

In the present study, baseline microcirculatory measurements were performed in all dogs. Those values were included in the analysis as a covariate. Currently no reference values for healthy conscious dogs are available. Former published values for buccal mucosal microcirculation variables were assessed in healthy isoflurane-anaesthetized dogs (Silverstein et al. 2009). The MFI median values in that study were 2.9 (observer 1) and 3 (observer 2), which are comparable to the median values of 2.75 (treatment MED) and 3 (treatment MVX) at baseline in the present study.

No significant effects of baseline and treatment were shown for PPV < 20 µm and perfused DeBacker density < 20 µm. The small vessels with a diameter < 20 µm play an important role in gas exchange and are one of the most relevant variables for the actual microcirculation in humans (De Backer et al. 2004). However, the baseline measurements in the current study for vessels < 20 µm were variable with a range of values for PPV < 20 µm from 33% to 100% and 0.21 to 9.14 for perfused DeBacker density < 20 µm. In contrast, the median reference PPV < 20 µm in isoflurane-anaesthetized dogs was 100% (93–100) (Silverstein et al. 2009). Both the effects of vasodilation induced by isoflurane and the vasoconstrictive effect of stress on microcirculation in the conscious dogs of our study could explain these differences. Using the automatic technique, it is not possible to determine whether differences between treatment MVX and treatment MED in vessels < 20 µm were undetectable or if there were no significant differences between treatments.

The assessment of microcirculation is time consuming, not only for the acquisition of good quality videos but also for correct and complete analysis of microcirculatory variables. This makes the use of SDF cameras in clinical practice questionable. However, although the MFI is subjectively assessed, it is a useful variable for visualizing the microcirculation (Pozo et al. 2012) and corresponds well with the results of other observers (Silverstein et al. 2009). In the current study, both

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MFI and HI were significantly different between the treatments after analysis of videos in a randomized order by one evaluator blinded to treatment and time point. Although this scoring system is subjective, it was possible to identify significant changes between treatments, which were more evident during the first hour after drug administration. This agrees with the higher drug plasma concentrations reported 1 hour after drug injection (Restitutti et al. 2017).

In the current study, blood temperature of the actively warmed dogs decreased after treatment MVX. A possible explanation is that vasoconstriction after MED prevented heat loss owing to impaired peripheral perfusion. In a previous study, using thermography dogs sedated with 20 μg kg^{-1} medetomidine and 0.1 mg kg^{-1} butorphanol with or without 500 μg kg^{-1} vatinoxan maintained a higher peripheral temperature after administration of medetomidine and butorphanol (Vainionpää et al. 2013). Vatinoxan probably increased peripheral heat loss owing to increased peripheral perfusion in our study, although all dogs were actively warmed. Without active warming, the dogs in treatment MVX may have become hypothermic. However, the duration of active warming was not recorded, and this statement cannot be proven.

Hypothermia itself has shown to have a negative effect on microcirculation owing to vasoconstriction induced by hypothermia (Kamler et al. 2005). Microcirculation was better maintained in treatment MVX even if lower core temperatures were measured. If hypothermia had any negative effect on microcirculation in treatment MVX, it should have attenuated the differences between the treatments.

There are several limitations to the current study. Pressure artefacts are probably the most important limitation for the interpretation of microcirculation with the SDF technique. Such artefacts result from excessive external pressure on the microvascular bed inducing microvascular collapse and therefore creating intermittent and absent flow patterns (De Backer et al. 2007). A peculiarity of the buccal mucosa is that the soft tissue lies directly over the bone, which could also make it more prone to external vessel occlusion as a result of pressure variations caused by the examiner. These pressure variations especially affect perfusion heterogeneity.

Additionally, the video sampling for baseline values is dependent on the cooperation of the animal because it is crucial for good quality videos to have a steady video image. Therefore, obtaining good quality baseline measurements can be challenging in nervous and impatient dogs, which are not sedated or anaesthetized. However, in all dogs three videos of good quality could be analysed also at baseline, assuming adequate baseline values. Additionally, baseline was included as a covariate in the analysis to take possible differences into account.

All dogs enrolled in this study underwent general anaesthesia for instrumentation with propofol induction and maintenance with isoflurane prior to baseline measurements. This general anaesthesia could theoretically have affected the measurements of our study. In women undergoing general anaesthesia for assisted reproductive techniques, a propofol target-controlled infusion was used. Calculated propofol effect-site concentration of 6.5 (±1.8) g mL^{-1} were associated with a decrease in the total vessel density by 9.1% and the perfused capillary density by 16.7%. These alterations in microcirculation resolved 3 hours after the discontinuation of propofol administration (Koch et al. 2008). All the dogs in the current study were allowed to recover for a minimum of 2 hours before baseline measurements were made. This time period was greater than 3 hours after the single bolus propofol administration. The possible effect of isoflurane administration on microcirculation has been discussed above. Therefore, a sustained effect on the microcirculation caused by these two drugs seems improbable, also because the study was performed in a crossover blinded manner.

The administration of fluids IV during instrumentation and thermodilution measurement for the concurrent study may have affected the measurements of the microcirculation. Nevertheless, a study in healthy anaesthetized dogs showed that IV fluid therapy of 10 or 20 mL kg^{-1} $hour^{-1}$ had no significant effects on MFI and PPV compared with no fluid therapy (Silverstein et al. 2014). For this reason, the effect of IV fluids probably did not influence our results.

As a further limitation, the plasma concentrations were not measured after IM administration of the drugs. Possible variations in plasma drug concentration and their influence on microcirculation cannot be assessed within the current study. Previous IM administration of vatinoxan resulted in a faster uptake of medetomidine into the systemic circulation (Kallio-Kujala et al. 2018). However, neither local perfusion of the muscles nor level of sedation was assessed in the current study.

In the current study, the alleviation of microcirculatory changes by the addition of vatinoxan was shown. However, larger studies are necessary to determine if the smaller microcirculatory changes seen after treatment MVX will lead to fewer side effects compared with treatment MED.

The results of the current study suggest that the administration of IM medetomidine combined with vatinoxan had fewer effects on the buccal mucosal microcirculation than medetomidine alone. The SDF camera is a useful tool in research to assess the microcirculatory status of heavily sedated dogs.

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Authors' contributions

LN: study participant, data management, preparation of manuscript, critical review of manuscript. APNK and BS: study design, study participant, data management, preparation of manuscript, critical review of manuscript. FBJ and MLW: study participant, critical review of manuscript.SH: statistical analysis, critical review of manuscript.

Conflict of interest statement

Q5

The authors declare no conflict of interest.

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