þÿ P e r i p h e r a l ± 2 - a d r e n o c e p t o r a n t a g o n i s m a f f e c t s t of intramuscularly coadministered drugs

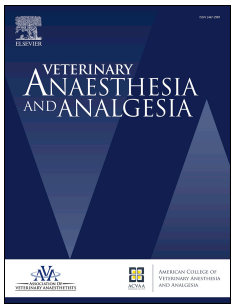
Kallio-Kujala, Ira J.

2018-07

Kallio-Kujala , I J , Raekallio , M R , Honkavaara , J , Bennett , R C , Turunen , H , Scheinin , þÿ M , H a u t a j ä r v i , H & V a i n i o , O 2 0 1 8 , ' P e r i p h e r a l ± 2 - a d r e n o c e p t o r a absorption of intramuscularly coadministered drugs ' , Veterinary Anaesthesia and Analgesia , vol. 45 , no. 4 , pp. 405-413 . https://doi.org/10.1016/j.vaa.2018.01.008

http://hdl.handle.net/10138/298627
https://doi.org/10.1016/j.vaa.2018.01.008

Accepted Manuscript



Peripheral alpha2-adrenoceptor antagonism affects the absorption of intramuscularly co-administered drugs

Ira J. Kallio-Kujala, Marja R. Raekallio, Juhana Honkavaara, Rachel C. Bennett, Heta Turunen, Mika Scheinin, Heidi Hautajärvi, Outi Vainio

PII: S1467-2987(18)30019-9

DOI: 10.1016/j.vaa.2018.01.008

Reference: VAA 237

To appear in: *Veterinary Anaesthesia and Analgesia*

Received Date: 27 June 2017

Revised Date: 18 December 2017

Accepted Date: 20 January 2018

Please cite this article as: Kallio-Kujala IJ, Raekallio MR, Honkavaara J, Bennett RC, Turunen H, Scheinin M, Hautajärvi H, Vainio O, Peripheral alpha2-adrenoceptor antagonism affects the absorption of intramuscularly co-administered drugs, *Veterinary Anaesthesia and Analgesia* (2018), doi: 10.1016/j.vaa.2018.01.008.

RESEARCH PAPER

Peripheral alpha2-adrenoceptor antagonism affects the absorption of intramuscularly co-administered drugs

Ira J Kallio-Kujala[a], Marja R Raekallio[a], Juhana Honkavaara[a], Rachel C Bennett[a], Heta Turunen[a], Mika Scheinin[b], Heidi Hautajärvi[c] & Outi Vainio[a]

[a]Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland

[b]Institute of Biomedicine, University of Turku, and TYKSLAB, Turku University Hospital, Turku, Finland

[c]Admescope Ltd., Oulu, Finland

Correspondence: Ira Kallio-Kujala, Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, P.O. Box 57, FI-00014 University of Helsinki, Helsinki, Finland. Email: ira.kallio-kujala@helsinki.fi

Authors' contributions

IKK: designed the study, collected the data, processed the data, principal writer of the manuscript; MR: designed the study, collected the data, processed the data, contributed in writing the manuscript; JH: designed the study, collected the data, processed the data, contributed in writing the manuscript; RB: designed the study, collected the data, contributed in writing the manuscript; HT: designed the study, collected the data, contributed in writing the manuscript; MS: analytical methods, contributed in writing the manuscript; HH: analytical methods, contributed in writing the manuscript; OV: designed the study, contributed in writing the manuscript.

Conflict of interest statement

HT was partly employed by Vetcare Ltd Finland at the time of the data collection. Other authors declare no conflict of interest.

Abstract

Objective We determined the possible effects of a peripherally acting alpha2-adrenoceptor antagonist, MK-467, on the absorption of intramuscularly (IM) co-administered medetomidine, butorphanol and midazolam.

Study design Randomized, experimental, blinded cross-over study.

Animals Six healthy beagle dogs.

Methods Two IM treatments were administered: 1) medetomidine hydrochloride (20 µg kg^{-1}) + butorphanol (100 µg kg^{-1}) + midazolam (200 µg kg^{-1}) (MBM), and; 2) MBM + MK-467 hydrochloride (500 µg kg^{-1}) (MBM-MK), mixed in a syringe. Heart rate was recorded at regular intervals. Sedation was assessed with visual analog scales (0 – 100 mm). Drug concentrations in plasma were analyzed with liquid chromatography - tandem mass spectrometry, with chiral separation of dex- and levomedetomidine. Maximum drug concentrations in plasma (C_{max}) and time to C_{max} (T_{max}) were determined. Paired t-tests, with Bonferroni correction when appropriate, were used for comparisons between the treatments.

Results Data from five dogs were analyzed. Heart rate was significantly higher from 20 until 90 minutes after MBM-MK. The T_{max} for midazolam and levomedetomidine (mean ± standard deviation) were approximately halved with co-administration of MK-467, from 23 ± 9 to 11 ± 6 minutes (p = 0.049) for midazolam and from 32 ± 15 to 18 ± 6 minutes for levomedetomidine (p = 0.036), respectively.

Conclusions and clinical relevance MK-467 accelerated the absorption of IM co-administered drugs. This is clinically relevant as it may hasten the onset of peak sedative effects.

Keywords alpha2-agonist, dog, medetomidine, intramuscular, MK-467, peripheral alpha2-antagonist

Introduction

Medetomidine is a selective, potent and efficacious α_2-adrenoceptor agonist (Doze et al. 1989; Maze & Fujinaga 2000) that produces sedation, muscle relaxation and analgesia in dogs (Vainio et al. 1989; Salonen et al. 1992; Kuusela et al. 2001). Medetomidine is a racemic mixture of two enantiomers, dex- and levomedetomidine, of which dexmedetomidine is the pharmacologically active component (Kuusela et al. 2000). All α_2-agonists have undesired effects on cardiovascular performance: peripheral vasoconstriction leads to arterial hypertension, and baroreflex-mediated bradycardia may result in a marked decrease in cardiac index (Bloor et al. 1992; Pypendop & Verstegen 1998).

MK-467 (previously also known as L-659'066) is an α_2-adrenoceptor antagonist that acts mainly on peripheral α_2-adrenoceptors because of its minimal ability to cross the blood-brain barrier, as directly demonstrated in rats and marmosets (Clineschmidt et al. 1988). In dogs sedated with intravenous (IV) dexmedetomidine, heart rate was higher and systemic vascular resistance lower when MK-467 was co-administered (Pagel et al. 1998, Honkavaara et al. 2011). The desired central nervous system effects of α_2-agonists, such as sedation are not affected (Honkavaara et al. 2008; Restitutti et al. 2011) whereas negative peripheral effects, such as cardiovascular effects, have been alleviated with both IV and IM administration of MK-467 (Honkavaara et al. 2011; Rolfe et al. 2012; Salla et al. 2014a).

In a recent study in dogs (Restitutti et al. 2017), it was detected that IM co-administration of MK-467 accelerated the absorption of medetomidine, resulting in faster onset and shorter duration of medetomidine-evoked sedation. The initial hemodynamic effects of medetomidine were unaffected by MK-467, but the later phases of medetomidine-related bradycardia and vasoconstriction were significantly attenuated and shortened (Restitutti et al. 2017). Elsewhere, Honkavaara et al (2017) reported that MK-467 appeared to shorten the onset and duration of sedation when it was co-administered IM with dexmedetomidine to cats. Furthermore, the addition of MK-467 significantly

shortened the T_{max} and increased C_{max} of dexmedetomidine after IM co-administration (Pypendop et al. 2017).

Furthermore, Bennett et al. (2016) reported that after IV administration of medetomidine in dogs, the levomedetomidine concentration in plasma was significantly lower than that of dexmedetomidine. Therefore, analyzing plasma medetomidine concentrations in dogs may not reflect the actual concentrations of the stereoisomers. We hypothesized that the maximum concentrations of plasma dex- and levomedetomidine, butorphanol and midazolam (C_{max}) would occur earlier (shorter T_{max}) when these drugs were co-administered IM with MK-467. Our primary objective was to evaluate whether MK-467 would enhance the IM absorption of medetomidine, butorphanol and midazolam when all four drugs were administered simultaneously from the same syringe. Co-administration of MK-467 was expected to result in an earlier C_{max} of the sedative agents which might hasten the onset of sedation. Our secondary objective was to verify the alleviation of medetomidine-induced bradycardia by MK-467, an observation reported earlier in dogs treated with IM medetomidine and butorphanol but without measurement of drug concentrations in that study (Salla et al. 2014a).

Materials and methods

Six purpose-bred, three year old beagles (four castrated males and two spayed females, mean weight 14.3 ± 1.5 kg) were used for this study. The dogs were considered healthy on the basis of clinical examination, complete blood counts and routine serum chemistry results. The National Animal Experimental board (ESAVI/7187/04.10.03/2012) provided ethical approval. Dogs were fed with commercial food and housed in groups. All experiments were performed between 8:00-12:00 AM. Food was withheld for 12 hours before the experiments, but water was freely available.

Study design

In this prospective, randomized cross-over study two IM treatments were administered to each dog, with a 14-day wash-out period. The investigated treatments were: 1) medetomidine hydrochloride (20 µg kg^{-1}) + butorphanol (100 µg kg^{-1}) + midazolam (200 µg kg^{-1}) (MBM); and 2) MBM + MK-467 hydrochloride (500 µg kg^{-1}) (MBM-MK). The dosage of MK-467 was based on previous results of our group (Restitutti et al. 2017). Randomization was obtained by drawing lots.

For the MBM-MK treatment, 1 mL of medetomidine hydrochloride (Dorbene 1 mg mL^{-1}; Vetcare Oy, Finland) and 1 mL of physiological saline solution (Natriumklorid 0.9%, B. Braun) were injected into an ampoule containing 25 mg of MK-467 hydrochloride powder. For the MBM treatment, 1 mL of the medetomidine hydrochloride solution was mixed with 1 mL of saline solution.

The medetomidine solutions with (MBM-MK) or without MK-467 (MBM), were drawn up into syringes and mixed with commercial formulations of butorphanol (Torpudor 10 mg mL^{-1}; Richter Pharma AG, Austria) and midazolam (Midazolam Hameln 5 mg mL^{-1}; Hameln Pharmaceuticals Gmbh, Germany). The final injection volume of both treatment mixtures was 0.09 mL kg^{-1}**,** resulting in an injection volume of 1.3 mL for an animal weighing 14.3 kg, which was the mean body weight of the dogs in our study. For injection, each dog was restrained in lateral recumbency and the drug mixture was injected into the lumbar epaxial muscles. Opposite sides were used for the two treatments. Aspiration was performed prior to drug injection to confirm extravascular administration.

Instrumentation and measurements

Prior to treatment administration and following aseptic preparation of the skin, 5 mg of lidocaine was administered subcutaneously over the jugular vein (Lidocain 20 mg mL^{-1}; Orion Pharma, Finland). A 13 cm long, 16 gauge single-lumen venous catheter (MILA International Inc., KY, USA) was inserted into a jugular vein for blood collection and fixed to the adjacent skin with

topical tissue adhesive. A 3-way stopcock was attached to the catheter for blood collection. The dogs used in this study were trained to allow restraint and placement of a jugular catheter.

Venous blood (6 mL into EDTA tubes, equaling a total of 66 mL) was sampled at 3, 6, 10, 15, 20, 25, 30, 40, 50, 60 and 90 minutes after drug administration. Blood samples were kept in iced water for a maximum of 30 minutes until the plasma was separated by refrigerated centrifugation. The plasma samples were stored at -20 ºC until they were analyzed for drug concentrations.

For the assessment of sedation, a visual analogue scale (VAS; analog scale of 0-100 mm) was used where (0) represented no sedation and (100) represented an animal in lateral recumbency, unresponsive to a loud hand clap. The level of sedation was assessed by a single investigator (JH) who was unaware of assigned treatment and unaware of the dogs' heart rates. Assessments were made before drug administration and 3, 6, 10, 15, 20, 25, 30, 40, 50, 60 and 90 minutes thereafter. The area under the sedation score-time curve ($AUC_{sed0-15}$) was calculated using the trapezoidal method for the first 15 minutes after injection. The first 15 minutes were chosen for comparison because in our study the T_{max} for butorphanol, midazolam, and dex- and levomedetomidine were detected with MBM-MK at approximately this time point and it was therefore expected that the greatest differences in sedation between the treatments would be detected within this time period. Maximum drug concentrations in plasma (C_{max}) and times to C_{max} (T_{max}) were determined from the concentration-time data. Areas under the concentration-time curve until 90 minutes (AUC_{0-90}) were calculated with the trapezoidal method.

Heart rates were recorded by auscultation prior to, at five minutes after treatment administration and at 10 minute intervals thereafter until 90 minutes. This was performed by another investigator (HT) who was also unaware of assigned treatment. Appropriate observer-blinding was achieved by not having either masked investigators (JH and HT) present during treatment preparation. Rectal temperature was measured with a thermometer before and 30, 60 and 90 minutes after drug injection. The animals were placed on an insulating mattress and covered with blankets while

123 sedated, and if the body temperature decreased below 36 ^{O}C, they were actively warmed with a
124 Bair-Hugger device (3M, MN, USA).

125 **Analytical Methods**

126 The concentrations of dex- and levomedetomidine (reference standard: racemic medetomidine,
127 TRC, ON, Canada) in dog plasma were determined with HPLC-MS/MS after solid phase extraction
128 with Sep-Pak tC18 96 well extraction plates (Waters Co., MA, USA) with 4,5-diphenylimidazole
129 (Sigma-Aldrich) as an internal standard. After chiral separation with a Chiralpak AGP column (4 x
130 150 mm, 5 µm, Chiral Technologies Europe, France), and 10 mM ammonium acetate (pH 4.5) and
131 acetonitrile containing 0.1% formic acid as solvents, quantitative detection was performed in multi-
132 reaction monitoring mode (MRM) with a triple quadrupole mass spectrometer (4000QTrap; MDS
133 Sciex, ON, Canada). For dex- and levomedetomidine and for the internal standard, the respective
134 precursor ions (m/z) were 201.2 and 221.1. The fragment ions (m/z) monitored and used for
135 quantitation were 95.1 for dex- and levomedetomidine and 194.0 for the internal standard. The
136 chromatograms were processed using Applied Biosystems / MDS Sciex software (Analyst version
137 1.6.1). The linear concentration range was from 0.10 ng mL^{-1} to 10.0 ng mL^{-1}. The inter-assay
138 accuracy of the quality control samples (at three different concentration levels, 0.225, 1.0 and 8.0 ng
139 mL^{-1}) ranged from 91.4% to 96.9% for dexmedetomidine and from 95.2% to 96.4% for
140 levomedetomidine.
141 After precipitation of 100 µL plasma samples on a 96-well Waters Oasis (Waters Co.) precipitation
142 plate with 200 µL of acetonitrile containing propranolol as an internal standard, concentrations of
143 butorphanol, midazolam and MK-467 in plasma were measured with HPLC coupled to tandem
144 mass spectrometry (Waters Acquity UPLC + Waters TQ-S triple quadrupole MS). The plasma
145 supernatants were transferred to 96-well plates pending analysis. Reference standards were prepared
146 in blank dog plasma by spiking the analytes at final concentrations of 0.02 – 20 000 ng mL^{-1}.
147 Quality control (QC) samples were prepared at concentrations of 0.2, 2, 20, 200 and 2000 ng mL^{-1}.

148 The temperature of the column oven was 40 °C, and the injection volume was 4 µL. The aqueous
149 eluent (A) was 0.5% formic acid in water, and the organic eluent (B) was acetonitrile. Gradient
150 elution with 2-2-90-90% (B) in 0-1-2.5-3 min was applied, followed by 1-minute equilibration. The
151 eluent flow rate was 0.5 mL^{-1}. Positive ionization mode was used with a capillary voltage of 1000V.
152 Argon was used as the collision gas, with a flow rate of 0.18 mL $minute^{-1}$. The desolvation
153 temperature was 650 °C, and the source temperature was 150 °C. Nitrogen was used as drying gas at
154 a flow rate of 900 L $hour^{-1}$ and as nebulizer gas at full flow rate. The monitored SRM transition
155 reactions were m/z 328 > 124 for butorphanol, m/z 236 > 223 for midazolam, m/z 419 > 200 for
156 MK-467 and m/z 260 > 116 for the internal standard, propranolol. The linear calibration ranges (ng
157 mL^{-1}) were fitted as follows: butorphanol 0.5-500, midazolam 0.5-1000, and MK-467 0.5-2000.
158 The QC samples in range were within 85-115% of the nominal concentrations.

159 **Statistical methods**

160 The sample size was based on a power calculation derived from earlier results for T_{max} of
161 dexmedetomidine (Restitutti et al. 2017), butorphanol (Pfeffer et al. 1980) and midazolam
162 (Schwartz et al. 2013) after IM administration in dogs. With a power of 80% and an alpha-level of
163 0.05, to detect a 50% decrease in T_{max} with pairwise one-tailed one way analysis of variance
164 (ANOVA), altogether 3 dogs would be needed for midazolam, 5 for dexmedetomidine and 6 for
165 butorphanol.

166 Shapiro-Wilk testing for normality was performed for all parametric data. The results are shown as
167 mean ± standard deviation (SD) for normally distributed data. The time of peak sedation and
168 sedation scores are expressed as median (range). Heart rate was analyzed by repeated-measures
169 ANOVA for both time and treatment effects, followed by paired samples 2-tailed t-test with
170 Bonferroni-correction. Paired samples 1-tailed t-tests were performed on C_{max} and T_{max}, and 2-tailed
171 t-testing was performed on $AUC_{0\text{-}90}$ and $AUC_{sed0\text{-}15}$. Sedation scores were compared between
172 treatments and against baseline using Mann-Whitney U-test with Bonferroni-correction.

Results

Six dogs were enrolled in the study but one was subsequently excluded, because low plasma concentrations of MK-467 (9.59 - 36.5 ng mL^{-1}) were found in this dog's samples also after MBM treatment. The source of the MK-467 contamination could not be traced. Therefore, results from only five animals are presented and were used in the analysis.

The observed concentrations of dexmedetomidine, levomedetomidine, butorphanol, midazolam and MK-467 in plasma are shown in Figures 1-2. The pharmacokinetic results, C_{max}, T_{max} and $AUC_{0\text{-}90}$ for dexmedetomidine, levomedetomidine, butorphanol, midazolam and MK-467, are summarized in Table 1.

Heart rate was significantly higher after MBM-MK than MBM between 20 and 90 minutes (Figure 3). The results for sedation scores are presented as median (range) and $AUC_{sed0\text{-}15}$ in Table 2. Peak sedation (median) was reached at 15 minutes for MBM-MK and at 20 minutes for MBM (p = 0.109). No differences were detected between treatments, but overall depth of sedation for the first 15 minutes ($AUC_{sed0\text{-}15}$) was significantly higher with MBM-MK than MBM. Rectal temperatures remained above 36 °C after both treatments. No clinically observed adverse effects were detected.

Discussion

This study demonstrated that MK-467 accelerated the absorption of co-administered midazolam and levomedetomidine when administered IM in the same syringe. As expected, it also alleviated the bradycardia attributed to dexmedetomidine.

In the present study, the first signs of sedation were observed within a few minutes after IM injection of the sedative agents, as reported earlier (Vainio et al. 1989). The times to peak sedation were in line with the plasma drug concentrations: for MBM, the median time to peak sedation was 20 minutes and the T_{max} for dexmedetomidine was 27 minutes. For MBM-MK, T_{max} of

dexmedetomidine was 17 minutes and the median time to peak sedation was 15 minutes. Also, the T_{max} of butorphanol, midazolam and levomedetomidine after MBM-MK were detected on average in the samples obtained at 15 minutes. Therefore the slightly but significantly deeper overall sedation with MK-467 during the first 15 minutes ($AUC_{sed0-15}$) probably reflected the higher plasma drug concentrations at that time. Although the racemic medetomidine contains 50% of both enantiomers, in plasma the dexmedetomidine concentration was substantially higher than that of levomedetomidine, as also earlier reported in dogs (Bennett et al 2016). As Kuusela et al. (2000) confirmed that levomedetomidine is relatively inactive in producing effects typical to alpha$_2$-adrenoceptor agonists the ratio of the enantiomers in plasma favoring dexmedetomidine is likely to attribute to the level of sedation. In addition, butorphanol and midazolam used in this study most probably added to the observed central effects.

MK-467 seemed to enhance the absorption of the other drugs: this is indicated by the statistically significantly shorter T_{max} of midazolam and levomedetomidine in the presence of MK-467 and the C_{max} and shapes of the concentration-time curves of all four analytes. A significant decrease in the T_{max} of dexmedetomidine and increase in C_{max} resulting from MK-467 co-administration was shown in a previous study from our group (Restitutti et al. 2017), in which the impact of MK-467 on plasma dexmedetomidine concentration seemed to be of similar magnitude to the present study.

Medetomidine is expected to cause local vasoconstriction at the site of injection, and MK-467 is capable of enhancing drug absorption from the injection site because of its capacity to block medetomidine's local actions on the circulation (Restitutti et al. 2017). A similar, albeit statistically indifferent, trend was detected between the T_{max} of dexmedetomidine ($p = 0.10$) and butorphanol ($p = 0.07$). For example, six minutes after administration of MBM-MK, the concentration of dexmedetomidine in plasma seemed to be at similar levels to those achieved at approximately 20 minutes after MBM. The lack of significance between treatments in dexmedetomidine and butorphanol concentrations in plasma, and the derived pharmacokinetic variables, was probably due

to the low number of dogs. In addition, as one of the datasets had to be excluded due to evident administration of MK-467 in the MBM-treatment, the amount of available data was further reduced While the lack of adequate statistical power carries the risk of inappropriately failing to reject the null hypothesis of any given investigation, as was probably the case with the apparent statistical indifference in parameters describing the disposition of dexmedetomidine and butorphanol, the authors remain of the opinion that the impact of MK-467 could still be appreciated. Unfortunately, we were unable to increase the number of animals, as the dogs had already been adopted out prior to the drug concentration analyses. In addition, the lack of additional cardiovascular data is a limitation: we only reported heart rate. Therefore, we were unable to show improvement of any global cardiovascular function by MK-467, although the alleviation of α_2-agonist-induced bradycardia by MK-467 has been associated with increased cardiac output in many previous studies (Enouri et al. 2008; Honkavaara et al. 2011; Rolfe et al. 2012; Salla et al. 2014; Restitutti et al. 2017).

There was wide variation in plasma drug concentrations between individual animals after both treatments. One of the dogs had very low plasma concentrations of all the drugs after MBM-treatment compared to the other dogs. In clinical veterinary practice, both the rate and consistency of drug absorption after extravascular administration are of practical importance. As stated before, the bioavailability of drugs is affected by the activity and blood flow of the muscle (Benet et al. 2011). The postural muscles usually have more abundant blood flow that hastens the drug absorption compared to non-postural muscles (Baxter & Evans 1973; Benet et al. 2011). In addition, the amount of perimuscular fat or intermuscular fascial planes can reduce the rate of drug absorption (Sund & Schou 1964). The epaxial muscle group contains numerous fascial planes and this may have been one of the factors causing the wide variability in our results (Dyce et al. 2002). In one study comparing the onset and quality of sedation after IM dexmedetomidine and hydromorphone in dogs, higher sedation scores were observed and faster onset of sedation was

recorded after injection in the semimembranosus and cervical sites compared to lumbar and gluteal sites (Carter et al. 2013). However, the inter-subject variability was lower after lumbar epaxial administration (Carter et al. 2013). The *Longissimus dorsi* was chosen as the injection site in our study as it does not have extensive fascial planes or surrounding adipose tissue. In addition, it was a safe place to inject because our laboratory beagles were accustomed to lie in lateral position.

Changes in heart rate reflect both medetomidine-evoked vasoconstriction in the systemic circulation and central sympatholysis. In our study, heart rate was monitored, as it is a very sensitive indicator of the cardiovascular effects of medetomidine; even very small IV doses decrease it (Pypendop et al. 1998; Pascoe 2015). MK-467 attenuated dexmedetomidine-induced bradycardia after IM injection, as also reported earlier (Rolfe et al. 2012; Salla et al. 2014a; Restitutti et al. 2017). With MBM-MK, an initial decrease in heart rate was detected: heart rate was lowest at 3-6 minutes, although no significant difference was detected between groups, after which it started to increase. As MK-467 appeared in the systemic circulation more slowly than dexmedetomidine (T_{max} for MK-467 seemed to be later than T_{max} for dexmedetomidine with MBM-MK), MK-467 probably started to alleviate the cardiovascular effects of dexmedetomidine with a delay which could explain the initial decrease in the heart rate also seen with MBM-MK. A similar phenomenon has been reported in previous studies when MK-467 has been administered IM in the same syringe with medetomidine (Salla et al. 2014b; Restitutti et al. 2017). In contrast, when medetomidine and MK-467 were administered IM, but at different injection sites, no initial decrease in heart rate was obvious (Rolfe et al. 2012), suggesting that the absorption rates of medetomidine and MK-467 from the injection sites were more similar when MK-467 did not prevent the local vasoconstriction induced by medetomidine. In another study, administration of MK-467 alone IV resulted in increased heart rate, cardiac index and tissue oxygen delivery in adult beagle dogs, but the decrease in systemic vascular resistance did not lead to hypotension, probably because of increased heart rate (Honkavaara et al. 2010).

Our primary interest in this study was to assess whether MK-467 accelerated the absorption of medetomidine, butorphanol and midazolam when administered IM in the same syringe. As we were particularly interested in the absorption phase, the follow-up period was short and no elimination phase of these drugs was observed. Thus we do not report or comment on half-lives or clearance, although it has been demonstrated that MK-467, to some extent, increases the clearance of dexmedetomidine, probably because of preserved liver blood flow (Honkavaara 2012; Bennett et al. 2016). For the same reasons, we reported $AUC_{0\text{-}90}$ for drug concentrations in plasma which were calculated based on the observed data.

Conclusions

Alpha$_2$-adrenoceptor agonists and antagonists may affect their own absorption and that of other sedatives, such as midazolam and butorphanol, when co-administered IM in the same syringe. This is clinically important as it affects the onset and depth of sedation.

References

Baxter JS, Evans JM (1973) Intramuscular injection in the cat. J Small Anim Pract 14, 297-302.

Benet LZ, Kroetz DL, Sheiner LB (2011) Pharmacokinetics: the dynamics of drug absorption, distribution and elimination. In Goodman and Gilman's The Pharmacological Basis of Therapeutics (12th edn), McGraw-Hill Education LLC, New York.

Bennett RC, Salla KM, Raekallio MR et al. (2016) Effects of MK-467 on the antinociceptive and sedative actions and pharmacokinetics of medetomidine in dogs. J Vet Pharmacol Ther 39, 336-343.

Bloor BC, Frankland M, Alper G et al. (1992) Hemodynamic and sedative effects of dexmedetomidine in dogs. J Vet Pharmacol Ther 263, 690–697.

Carter JE, Lewis C, Beths T (2013) Onset and quality of sedation after intramuscular administration of dexmedetomidine and hydromorphone in various muscle groups in dogs. J Am Vet Med Assoc 243, 1569-1572.

Clineschmidt BV, Pettibone DJ, Lotti VJ et al. (1988) A peripherally acting alpha-2 adrenoceptor antagonist: L-659,066. J Pharmacol Exp Ther 245, 32–40.

Doze VA, Chen BX, Maze M (1989) Dexmedetomidine produces a hypnotic-anesthetic action in rats via activation of central alpha-2 adrenoceptors. Anesthesiology 71, 75-79.

Dyce KM, Sack WO, Wensing CJG (2002) Textbook of veterinary anatomy (3rd edn) Saunders, Philadelphia.

Enouri SS, Kerr CL, McDonell WN, O'Sullivan ML, Neto FJT (2008) Effects of a peripheral alpha2 adrenergic-receptor antagonist on the hemodynamic changes induced by medetomidine administration in conscious dogs. Am J Vet Res 69(6), 728-736.

Honkavaara JM, Raekallio MR, Kuusela EK et al. (2008) The effects of L-659,066, a peripheral alpha2-adrenoceptor antagonist, on dexmedetomidine-induced sedation and bradycardia in dogs. Vet Anaesth Analg 35, 409-413.

Honkavaara JM, Restitutti F, Raekallio MR et al. (2011) The effects of increasing doses of MK-467, a peripheral alpha(2)-adrenergic receptor antagonist, on the cardiopulmonary effects of intravenous dexmedetomidine in conscious dogs. J Vet Pharmacol Ther 34, 332-337.

Honkavaara J, Restitutti F, Raekallio M et al. (2012) Influence of MK-467, a Peripherally Acting alpha2-Adrenoceptor Antagonist on the Disposition of Intravenous Dexmedetomidine in Dogs. Drug Metab Dispos 40, 445-449

Honkavaara J, Pypendop B, Ilkiw J (2017) The impact of MK-467 on sedation, heart rate and arterial blood pressure after intramuscular coadministration with dexmedetomidine in conscious cats, Vet Anaesth Analg http://dx.doi.org/10.1016/j.vaa.2016.08.011

Kuusela E, Raekallio M, Anttila M et al. (2000) Clinical effects and pharmacokinetics of medetomidine and its enantiomers in dogs, J Vet Pharmacol Ther, 23(1), 15-20. DOI 10.1046/j.1365-2885.2000.00245.x.

Kuusela E, Vainio O, Kaistinen A et al. (2001) Sedative, analgesic, and cardiovascular effects of levomedetomidine alone and in combination with dexmedetomidine in dogs. Am J Vet Res 62, 616-621.

Maze M, Fujinaga M (2000) Alpha2 adrenoceptors in pain modulation: which subtype should be targeted to produce analgesia? Anesthesiology 92, 934-365 936.

Pagel PS, Proctor LT, Devcic A et al. (1998) A novel alpha 2-adrenoceptor antagonist attenuates the early, but preserves the late cardiovascular effects of intravenous dexmedetomidine in conscious dogs. J Cardiothorac Vasc Anesth 12, 429-434.

Pascoe PJ (2015) The cardiopulmonary effects of dexmedetomidine infusions in dogs during isoflurane anesthesia. Vet Anaesth Analg 42, 360-368

Pypendop BH, Verstegen JP (1998) Hemodynamic effects of medetomidine in the dog: a dose titration study. Vet Surg 27, 612–622.Pypendop BH, Honkavaara J, Ilkiw JE (2017) Pharmacokinetics of dexmedetomidine, MK-467 and

their combination following intramuscular administration in male cats Vet Anaesth Analg http://dx.doi.org/10.1016/j.vaa.2017.02.008

Restitutti F, Honkavaara JM, Raekallio MR et al. (2011) Effects of different doses of L-659'066 on the bispectral index and clinical sedation in dogs treated with dexmedetomidine. Vet Anaesth Analg 38, 415-422.

Restitutti F, Kaartinen MJ, Raekallio MR et al. (2017) Plasma concentration and cardiovascular effects of intramuscular medetomidine combined with three doses of the peripheral alpha2-antagonist MK-467 in dogs. Vet Anaesth Analg, 44, 417-426 .

Rolfe NG, Kerr CL, McDonell WN (2012) Cardiopulmonary and sedative effects of the peripheral a2-adrenoceptor antagonist MK 467 administered intravenously or intramuscularly concurrently with medetomidine in dogs. Am J Vet Res 73, 587-594.

Salla K, Bennett RC, Restitutti F et al. (2014a) A comparison in dogs of medetomidine, with or without MK-467, and the combination acepromazine-butorphanol as premedication prior to anaesthesia induced by propofol and maintained with isoflurane. Vet Anaesth Analg 41, 163-173.

348 Salla K, Restitutti F, Vainionpaa M et al. (2014b) The cardiopulmonary effects of a peripheral
349 alpha-2-adrenoceptor antagonist, MK-467, in dogs sedated with a combination of medetomidine
350 and butorphanol. Vet Anaesth Analg 41, 567-57.

351 Salonen M, Reid K, Maze M (1992) Synergistic interaction between alpha 2-adrenergic agonists
352 and benzodiazepines in rats. Anesthesiology 76, 1004-1011.

353 Self IA, Hughes JML, Kenny DA, Clutton RE (2009) Effect of muscle injection site on
354 preanaesthetic sedation in dogs. Vet Rec 164, 323-326.

355 Sund RB, Schou J (1964) The determination of absorption rates from rat muscles: An experimental
356 approach to kinetic descriptions. Acta pharmacol. et toxicol 21, 313-325

357 Vainio O, Vähä-Vahe T, Palmu L (1989) Sedative and analgesic effects of medetomidine in dogs. J
358 Vet Pharmacol Ther 12, 225-231.

Figure 1 Dexmedetomidine (a), levomedetomidine (b), butorphanol (c) and midazolam (d) concentrations in plasma after administration of 1) medetomidine hydrochloride (20 μg kg^{-1}) + butorphanol (100 μg kg^{-1}) + midazolam (200 μg kg^{-1}) intramuscular (IM) (MBM), and; 2) medetomidine (20 μg kg^{-1}) + MK-467 hydrochloride (500 μg kg^{-1}) + butorphanol (100 μg kg^{-1}) + midazolam (200 μg kg^{-1}) IM (MBM-MK). Data of five dogs are shown. Both treatments were administered at 0 minutes. Data are shown as mean ± SD.

Figure 2 MK-467 concentration in plasma after administration of medetomidine (20 μg kg^{-1}) + MK-467 hydrochloride (500 μg kg^{-1}) + butorphanol (100 μg kg^{-1}) + midazolam (200 μg kg^{-1}) intramuscular (IM) (MBM-MK). Data of five dogs are shown. Treatment was administered at 0 minutes. Data are shown as mean ± SD.

Figure 3 Heart rate after administration of 1) medetomidine hydrochloride (20 μg kg^{-1}) + butorphanol (100 μg kg^{-1}) + midazolam (200 μg kg^{-1}) intramuscular IM (MBM), and; 2) medetomidine (20 μg kg^{-1}) + MK-467 hydrochloride (500 μg kg^{-1}) + butorphanol (100 μg kg^{-1}) + midazolam (200 μg kg^{-1}) IM (MBM-MK). Data of five dogs are shown. Both treatments were administered at 0 minutes. * Significant difference between treatments. Data shown as mean ± SD.

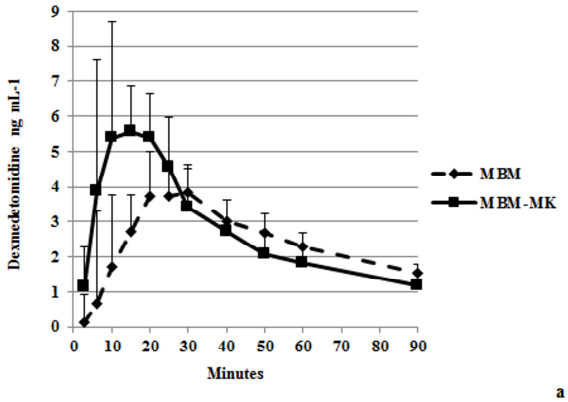
Table 1. Observed peak drug concentrations in plasma (Cmax), the time of maximum drug concentration in plasma (Tmax) and area under the concentration-time curve (AUC). Shown as mean ± SD and minimum and maximum in brackets * Significant difference between treatments.

Drug	Treatment	C_{max} (ng mL^{-1})	T_{max} (minutes)	AUC_{0-90} (min * ng mL^{-1})
Dexmedetomidine	MED	4.3 ± 2.0 (0.9 – 6.0)	27 ± 15 (10 – 50)	216 ± 92 (54 – 279)
	MED-MK	6.6 ± 2.6 (3.7 – 10.8)	17 ± 4.5 (10 – 20)	247 ± 65 (144 – 307)
	p-value	0.09	0.10	0.63
Levomedetomidine	MED	2.7 ± 1.3 (0.5 – 3.7)	32 ± 15 (10 – 50)	140 ± 63 (30 – 181)
	MED-MK	4.6 ± 1.6 (2.6 – 6.7)	18 ± 6 (10 – 25)	178 ± 47 (107 – 227)
	p-value	0.08	0.036 *	0.38
Butorphanol	MED	10.7 ± 6.1 (1.7 – 16.9)	27 ± 4.5 (10 – 30)	589 ± 305 (116 - 886)
	MED-MK	19.9 ± 9.6 (10.5 – 34.0)	15 ± 5 (10 – 20)	818 ± 246 (535 – 1143)
	p-value	0.07	0.07	0.33
Midazolam	MED	82.2 ± 43.9 (12.9 – 134.0)	23 ± 9 (10 – 40)	3743 ± 1886 (749 – 5837)
	MED-MK	157.8 ± 95.8 (82.9 – 300.2)	11 ± 6 (6 – 20)	5644 ± 2213 (3920 – 8900)
	p-value	0.11	0.049 *	0.33
MK-467	MED-MK	907 ± 173 (672 – 1051)	23 ± 6 (15 – 30)	62755 ± 11268 (49563 – 77548)

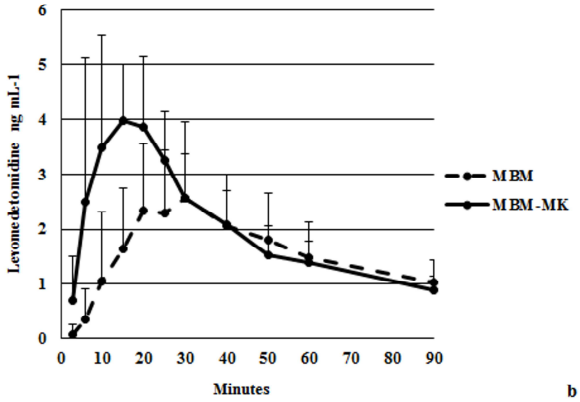
Table 2. Visual analogue sedation score (0-100) for treatments MBM and MBM-MK. Data of VAS scores are reported as median (range). $AUC_{sed0-15}$ (reported as mean ± SD) were calculated for the first 15 minutes.

*** Significant difference between treatments. † Significant difference compared to baseline.**

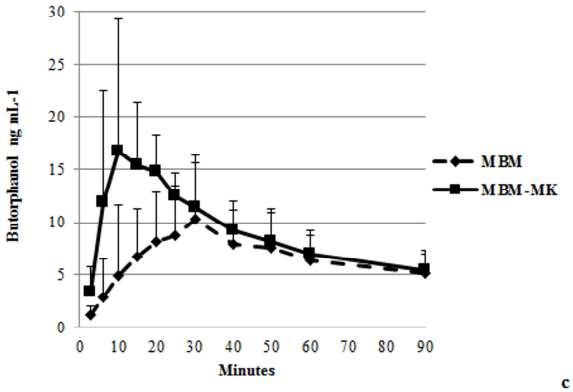
Time point (minutes)	MBM	MBM-MK
0	0 (0-0)	0 (0 – 0)
3	18 (0 – 20)	23 (3 – 85)
6	14 (0 – 51)	84 (6 – 100)
10	69 (22 – 97)	96 (72 – 100) †
15	95 (17 – 100)	100 (96 – 100) †
20	100 (58 – 100) †	100 (100 – 100) †
25	100 (76 – 100) †	100 (100 – 100) †
30	100 (79 – 100) †	100 (83 – 100) †
40	96 (78 – 100) †	94 (87 – 100) †
50	86 (77 – 100) †	78 (66 – 100)
60	85 (70 – 100)	70 (50 – 78)
90	68 (62 – 81)	26 (15 – 74)
$AUC_{sed0-15}$	598 ± 256 *	996 ± 261 *



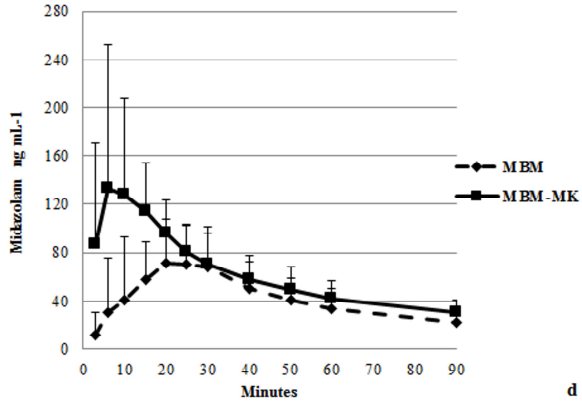
a



b



c



d