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Restitutti, Flavia

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Plasma concentration, cardiovascular and sedative effects of intramuscular medetomidine combined with three doses of the peripheral alpha-2 antagonist MK-467 in dogs

F Restitutti¹, MJ Kaartinen^{1,2}, MR Raekallio¹, O Wejberg¹, E Mikkola¹, JRE del Castillo³, M Scheinin⁴, OM Vainio¹

¹Department of Equine and Small Animal Medicine, Faculty of veterinary medicine, University of Helsinki, Finland; ²The Animal Health Trust, Kentford, UK; ³GREPAQ, Department of veterinary biomedical sciences, Université de Montréal, Quebec, Canada; ⁴Department of Pharmacology, Drug Development and Therapeutics, University of Turku, Finland

1 **Abstract**

2

3 **Objective** We investigated the plasma concentrations and cardiovascular effects of
4 intramuscularly administered (IM) medetomidine (MED), given alone and with three
5 doses of MK-467.

6 **Study design** Prospective, randomized, open, crossover trial.

7 **Animals** Eight purpose-bred healthy beagle dogs.

8 **Methods** Each dog received four treatments: MED 20 $\mu\text{g kg}^{-1}$ IM alone or mixed in
9 the same syringe with MK-467 (200, 400 or 600 $\mu\text{g kg}^{-1}$). Instrumentation was done
10 under standardized anaesthesia from which the dogs were allowed to recover before
11 baseline measurements. Composite sedation scores, cardiovascular variables, i.e. heart
12 rate (HR), cardiac output (CO), arterial and central venous blood pressures (MAP and
13 CVP), and arterial blood gases were recorded at baseline and for 60 minutes after
14 treatment. Drug concentrations in venous plasma were analysed. Generalised linear
15 mixed models for repeated measures with post-hoc Bonferroni correction were used
16 with statistical significance level set at $\alpha = 0.05$.

17 **Results** All treatments initially demonstrated the effects of MED: HR and CO
18 decreased and CVP increased. The MAP transiently increased and then significantly
19 decreased from baseline with the two highest MK-467 doses. The cardiovascular
20 effects of MED disappeared more rapidly with MK-467. Sedation scores increased
21 and decreased earlier with MK-467 than with MED alone. With MED alone, sedation
22 scores remained high until the end of the 60-minute follow-up. Maximum
23 concentrations of MED were more rapidly achieved and higher with MK-467.

24 **Conclusions and clinical relevance** The initial haemodynamic effects of MED were
25 not prevented by MK-467, but these effects were attenuated and their duration

26 shortened by MK-467, independently of dose. Absorption of MED was accelerated by
27 MK-467, when given concomitantly IM, resulting in faster sedation; addition of MK-
28 467 shortened the sedative effect of MED.

29

30 *Keywords: alpha-2 agonist, medetomidine, peripheral alpha-2 antagonist, MK-467,*
31 *dog*

32 **Introduction**

33 The alpha-2 adrenergic agonist medetomidine (MED) and its pharmacologically
34 active enantiomer, dexmedetomidine (DMED), produce sedation, muscle relaxation
35 and analgesia (Vainio et al. 1989; Salonen 1992; Grimm et al. 2000; Kuusela et al.
36 2001; van Oostrom et al. 2011), and have anaesthetic-sparing effects, but their
37 cardiovascular depressant effects (Kuusela et al. 2000; Sinclair 2003; Murrell &
38 Hellebrekers 2005; Pascoe et al. 2006) limit their use in healthy dogs. The desired
39 sedative and analgesic effects of alpha-2 agonists are mediated via centrally located
40 receptors (Doze et al. 1989; Maze & Fujinaga 2000), whereas the immediate
41 cardiovascular adverse effects are mediated via receptors situated on the peripheral
42 vasculature (Kamibayashi & Maze 2000).

43 The alpha-2 antagonist MK-467 acts mainly on peripheral receptors because of its
44 minimal capacity for crossing the blood-brain barrier, as has been demonstrated in
45 rats and marmosets (Clineschmidt et al. 1988). It also has some actions on its own, as
46 it has been shown to increase heart rate and lower systemic vascular resistance in dogs
47 (Pagel et al. 1998) and to decrease systemic blood pressures in rats (Szemerédi et al.
48 1989).

49 In an attempt to attenuate the adverse cardiovascular effects of alpha-2 agonists
50 mediated via receptors on the peripheral vasculature in dogs, the use of this peripheral
51 antagonist MK-467 is currently being investigated (Enouri et al. 2008; Honkavaara et
52 al. 2008; 2011; 2012; Rolfe et al. 2012). Pagel et al. (1998) demonstrated attenuation
53 of the early cardiovascular effects of DMED with several doses of intravenous (IV)
54 MK-467 administered prior to DMED in conscious dogs. In another study, a
55 simultaneously administered IV bolus injection of MK-467 attenuated the
56 cardiovascular effects of a DMED bolus in dogs (Honkavaara et al. 2011). In addition,

57 MK-467 altered the early disposition of DMED following concomitant IV bolus
58 administration, by doubling the apparent clearance and the apparent volume of
59 distribution of DMED (Honkavaara et al. 2012). On the other hand, MK-467
60 interfered very little with the centrally mediated sedative effects of MED or DMED in
61 dogs under laboratory conditions (Honkavaara et al. 2008; Rolfe et al. 2012), but did
62 reduce the bispectral index (Restitutti et al. 2011) in this same species.
63 To the authors' knowledge, only Rolfe et al. (2012) have reported on the effects of a
64 combination of MED and MK-467 after intramuscular (IM) administration. They
65 demonstrated attenuation of the cardiovascular effects of MED by MK-467 (MED:
66 MK-467 dose ratio of 1:20) when the drugs were injected into separate muscles.
67 Furthermore, Salla et al. (2014) demonstrated attenuation of the cardiovascular effects
68 of the combination MED and butorphanol with MK-467 after both IV and IM routes.
69 The plasma concentrations of these drugs after concomitant IM administration have
70 not been reported.
71 As the IM route of administration is commonly used for MED in clinical practice, our
72 goal was to investigate the cardiovascular effects of MED administered IM alone and
73 in the same syringe with three different doses of MK-467 in order to find the most
74 suitable dose ratio of MED: MK-467 needed to prevent the undesirable effects of
75 MED. A secondary aim was to evaluate sedation in order to demonstrate the lack of
76 MK-467 antagonism on the central effects of MED. We hypothesized that MK-467
77 would dose-dependently attenuate the cardiovascular effects of MED and also alter its
78 disposition accordingly, while administered concurrently via the IM route.

79 **Materials and methods**

80 The National Animal Experiment Board of Finland approved this study. Eight healthy
81 purpose-bred beagles, 6 neutered males and 2 spayed females, aged 1 year and

weighing 11-15 kg were used in the study. Prior to each experiment day, food was withheld for 12 hours with free access to water. The dogs were considered healthy based on clinical examination, complete haematology panel and routine serum biochemistry.

Study design and drugs

This study was a randomized, open, four-period crossover, experimental study with a washout period of at least 14 days between treatments, which consisted of:

1. Medetomidine 20 $\mu\text{g kg}^{-1}$ IM (Dorbene 1 mg mL⁻¹, Syva Laboratories S.A., Spain) (MED),
2. MED + MK-467 (MK-467 powder, PCAS, Finland) 200 $\mu\text{g kg}^{-1}$ IM (MK200),
3. MED + MK-467 400 $\mu\text{g kg}^{-1}$ IM (MK400),
4. MED + MK-467 600 $\mu\text{g kg}^{-1}$ IM (MK600).

The MK-467 powder was diluted with physiological saline solution immediately prior to administration to a concentration of 10 mg mL⁻¹. Thereafter both drug doses were mixed in one syringe for a single injection. The washout period between treatments was at least 14 days. The randomization of the dogs was performed electronically through a website (<https://www.random.org/>). Eight dogs were randomised to receive all four treatments. However the treatments were balanced in a way that two dogs received one treatment during each study period ... Five minutes after the baseline measurements, the study drugs were administered and a 60-minute assessment period was initiated.

Meloxicam 0.2 mg kg⁻¹ (Metacam 5 mg mL⁻¹; Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany) was administered IV immediately after the last blood sample, and atipamezole (100 $\mu\text{g kg}^{-1}$ IM, Antisedan, Orion Pharma, Turku, Finland)

106 was administered if an animal was still heavily sedated at the end of the follow-up
107 period.

108 Instrumentation and measurements

109 Following aseptic preparation of the skin, a 20 G catheter (Terumo Europe, Belgium)
110 was placed into a cephalic vein. Each dog inhaled 100% oxygen (4 L minute⁻¹)
111 through a face mask for 3–5 min before anaesthesia was induced with propofol
112 (Propovet 10 mg mL⁻¹, Abbott Laboratories Ltd., UK) IV to effect [median (min, max)
113 7.32 mg kg⁻¹ (4.49, 12.28); MEAN (SD) 7.8 (1.8)]. The dogs were orotracheally
114 intubated and anaesthesia was maintained with isoflurane (Isoflo, Orion Pharma Ltd.,
115 Finland) in oxygen via a circle breathing system. An arterial catheter (22G, Terumo
116 Europe, Belgium) was placed in the dorsal pedal artery and a premeasured central
117 venous catheter (a 5-Fr double-lumen catheter; AK-14502, Arrow International, PA,
118 USA) was inserted into the left jugular vein under local infiltration anaesthesia (20
119 mg of lidocain 20 mg mL⁻¹; Orion, Finland) and secured in place. During the
120 instrumentation, dogs were monitored with indirect blood pressure (Ultrasonic
121 Doppler Flow Detector Model 811-B, Parks Medical, USA), continuous lead II ECG,
122 , end-tidal CO₂, inspired and expired concentration of isoflurane and pulse oximetry
123 (S/5 Compact Anaesthesia Monitor, GE Healthcare, Finland). During instrumentation,
124 the dogs were kept under anaesthesia with expired isoflurane concentrations ranging
125 from 1.35% to 1.88% [MEAN 1.7% (0.1)] and maintaining the monitored vital
126 parameters in a physiological range. The anaesthesia for instrumentation took 45-196
127 minutes. After instrumentation, dogs were allowed to recover for at least 60 minutes
128 after the extubation prior to baseline measurements [MEDIAN (MIN, MAX) 158
129 minutes (71, 347); MEAN (SD) 172 (61)]. Body temperature was monitored after the
130 discontinuation of isoflurane, and if needed, animals were placed under blankets. The

131 experiment did not start before the body temperature was at least 38 °C. Criteria to
132 start the experiment included lack of ataxia and similar level of activity/awareness as
133 before the induction of anaesthesia.

134 During the subsequent 60-minute assessment period following the treatment
135 administration, continuous lead II ECG was monitored with patches placed on the
136 front paws and left hind paw. For continuous measurement of central venous (CVP)
137 and arterial mean, diastolic, and systolic blood pressures (MAP, DAP, SAP,
138 respectively), the transducers (Gabarith PMSET; Benton Dickinson, UT, USA) were
139 zeroed to atmospheric pressure at the level of the manubrium, and dogs were
140 positioned in lateral recumbency. Heart rate (HR), blood pressures, and respiratory
141 rate were recorded at baseline and at 5, 10, 15, 30, 45 and 60 minutes after study drug
142 administration. Cardiac output (CO) was measured at baseline, 5, 15, 30 and 60
143 minutes by the lithium dilution method (LiDCO plus Haemodynamic monitor;
144 LiDCO Ltd., UK) as previously described (Mason et al. 2001) with a standard dose of
145 0.075 mmol lithium chloride injected via the central venous catheter. Results were
146 corrected a posteriori with each dog's sodium and haemoglobin concentrations taken
147 simultaneously from the arterial catheter with heparinized syringes (PICO50,
148 Radiometer, Denmark). The samples were stored in iced water for a maximum of 20
149 minutes until analysed with temperature adjustment for blood gases (ABL 855,
150 Radiometer). Rectal temperature was monitored and maintained by insulating the
151 dogs from the table by using a foam mattress and a heating pad. The dogs received
152 isotonic fluid solution (Ringer acetate; Fresenius Kabi, Sweden) at a rate of 5 mL kg⁻¹
153 hour⁻¹ for the whole 60-minute study period.

154 A composite sedation score (Appendix 1) was obtained by a researcher (FR) who
155 remained aware of the treatments, as the sum of five parameters: resistance to

156 positioning in lateral recumbency, palpebral reflex, position of the eye, jaw and
157 tongue tone and general appearance at baseline and 5, 15, 20, 30, 45 and 60 minutes
158 after drug administration.

159 Central venous blood (3 mL, EDTA) was sampled for plasma drug
160 concentrations at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after drug
161 administration. The samples were centrifuged (3000 g, 20°C, 10 minutes), and the
162 plasma was stored at -20 °C.

163 The plasma samples were analysed for concentrations of DMED, LMED and MK-467
164 with HPLC-MS/MS after Sep-PaktC18 solid phase extraction (Waters Co., MA,
165 USA). Chiral separation of DMED and LMED was achieved with a Chiralpak AGP
166 column (4 x 100 mm, 5 µm, Chiral Technologies Europe, France). Quantitative
167 detection was performed in multi-reaction monitoring mode (MRM) with a triple
168 quadrupole mass spectrometer (API 4000, MDS Sciex, Ontario, Canada). The ion
169 mass (m/z) transitions for DMED, LMED and their internal standard,
170 diphenylimidazole (Sigma-Aldrich, Germany), were 201.2→95.1 and 221.1→194.0,
171 respectively, and those for MK-467 and its internal standard, RS-79948 (Tocris
172 Bioscience, UK), were 419.3→200.1 and 365.3→190.2, respectively. The quantitation
173 range of the DMED+LMED assay was 0.075-10.0 ng mL⁻¹, with intra-day precision
174 (CV) of 5-16 % at 0.10 ng mL⁻¹, and 2-12 % at higher concentrations of quality
175 control samples. The inter-day precision (CV) for both analytes was about 6 % at
176 0.225 ng mL⁻¹ and 6-8 % at higher concentrations (11 sample batches were analysed
177 on 11 different days). The quantitation range of the MK-467 assay was 25-10000 ng
178 mL⁻¹, with intra-day precision (CV) of 14 % at 25 ng mL⁻¹, and 3-11 % at higher
179 concentrations of quality control samples. The inter-day precision (CV) was 3-6 % at
180 concentrations of 70-10000 ng mL⁻¹ (9 sample batches were analysed on 9 different

181 days)

182 Statistics

183 Descriptive statistics (univariate and bivariate correlations) were performed for all
184 measurements, the distributions of the continuous variables were analysed by visual
185 inspection of their histograms compared with normal and log normal distribution
186 curves. For HR, RR, CO, MAP, DAP, and lactate, a natural logarithmic
187 transformation was applied to normalise the right skewed distribution, and the model
188 was fitted for the transformed response.
189 For repeated observations of continuous cardiorespiratory variables, a generalised
190 linear mixed model was used that included the fixed effects of treatment and time and
191 the interaction of treatment and time. A compound symmetry (CS), first-order
192 autoregressive (AR(1)), heterogeneous first-order autoregressive (ARH(1)), or a
193 spatial power (SP(pow)) covariance structure, taking into account the variable
194 intervals between measurement time-points and allowing for heterogeneous variances
195 was used to model the within-subject errors. Bonferroni corrections were used to
196 adjust for multiple comparisons as appropriate.

197 For sedation scores (discrete values), a generalised linear mixed model with
198 specifications for Poisson distribution for variables was used. An ARH(1) covariance
199 structure was used to model the within-subject errors.

200 The structures for generalised linear mixed models were chosen based on the
201 best model fit and smallest value of Akaike Information Criterion (AIC). The
202 Kenward-Roger approximation was used to estimate denominator degrees of freedom
203 for repeated measurements. Analyses were performed by using SAS 9.3 software for
204 generalised linear mixed models. Where differences were detected, all hypotheses
205 were tested by use of a two-sided level of significance of 0.05.

Maximum plasma concentrations ($C_{\max}$) and the time to reach maximum plasma concentration ($T_{\max}$) were derived from the concentration data. These parameters were compared between treatments with one-way ANOVA with post-hoc Tukey's test with alpha-level set at 0.05. Geometrically right-skewed data was log-transformed ($C_{\max}$) prior to statistical testing.

Results

The main haemodynamic variables (heart rate, mean arterial and central venous blood pressures, cardiac output) are presented in Figures 1-4.

There was a significant effect of treatment ($p < 0.0001$), time ($p < 0.0001$) and treatment x time interaction ($p < 0.0001$) on heart rate (HR), which significantly decreased in all treatments (Figure 1). Over time, with the highest MK-467 dose (MK600) the values returned to baseline from 20 minutes onwards. After MED and MK200, mean HR remained significantly lower than baseline during the entire 60-minute observation period ($p < 0.0001$). Two dogs receiving MED alone exhibited second-degree atrio-ventricular block throughout the follow-up period but did not require treatment.

There was a significant effect of treatment ($p = 0.0002$), time ($p < 0.0001$) and treatment x time interaction ($p = 0.0044$) on mean arterial blood pressure (MAP). It increased initially, reaching statistical significance from baseline (at 5 and 10 minutes) only with MK200 (Figure 2), and diastolic arterial blood pressure (DAP) demonstrated similar effects. Systolic arterial pressure (SAP) increased slightly after all treatments initially and later decreased over time.

There was a significant effect of treatment ($p = 0.0028$), time ($p < 0.0001$) and treatment x time interaction ($p = 0.0298$) on central venous pressure (CVP). It increased initially, reaching statistical significance from baseline values at 5 minutes

231 with all treatments (Figure 3). With MED, CVP stayed significantly above baseline
232 values during the entire 60-minute evaluation period.

233 There was a significant effect of treatment ($p < 0.0001$), time ($p < 0.0001$) and
234 treatment x time interaction ($p = 0.0084$) on cardiac output (CO). Its baseline ranged
235 widely between 1.68 and 4.81 L minute⁻¹, and it decreased significantly in all
236 treatments (Figure 4). After MED and MK200, CO was significantly decreased during
237 the entire 60-minute period, while with the two higher MK-467 doses it increased
238 towards baseline and was not significantly different from baseline at 60 minutes
239 (MK400) and 45 minutes (MK600). No significant differences emerged between the
240 three doses of MK-467.

241 The median baseline sedation scores after all treatments were from 0 (0;3) to 0
242 (0;4) [median (min;max)], with no differences between treatments. All of the dogs
243 were clinically sedated during all treatments, and the composite sedation scores
244 increased significantly from baseline (Table 1). Within the MK200 and MK600
245 treatments, the sedation scores increased significantly earlier, and later MK-467
246 shortened the duration of sedation with all given doses relative to MED alone. With
247 addition of MK-467, the maximum scores were reached within 15 minutes while with
248 MED alone it appeared to take longer. The sedation scores decreased with MK-467
249 during the 60-minute follow-up, while with MED alone the sedation scores remained
250 at the maximal level.

251 Respiratory rates were significantly lower than baseline from 10 minutes
252 onward with all doses of MK-467, but no differences were detected between these
253 treatments.

254 Arterial partial pressures of carbon dioxide (PaCO₂), oxygen (PaO₂) and
255 lactate concentration are presented in Table 2. The addition of MK-467 significantly

256 increased PaCO₂. The PaO₂ and lactate stayed relatively stable during the 60-minute
257 period. No differences were detected between or within treatments in arterial pH or
258 bicarbonate concentration, which showed high inter-individual variability (data not
259 shown).

260 Rectal temperatures decreased significantly from baseline, from 38.0 °C (0.3)
261 to 36.8 °C (0.5) [mean (SD)], but no significant differences were detected between the
262 treatments.

263 Plasma concentrations of DMED are presented in Figure 5 and those of MK-
264 467 in Figure 6, and C_{max} and T_{max} are tabulated in Table 3. MK-467 significantly
265 accelerated the rate of DMED absorption (significantly higher C_{max} and earlier T_{max}),
266 and MK-467 had slower absorption (later T_{max}) than DMED when the drugs were
267 given concomitantly. Concentrations of LMED followed a similar time course as
268 those of DMED (results not shown).

269 All treatments were relatively well tolerated. Atipamezole was administered to
270 seven dogs on MED and two on treatment MK400 out of eight , as they were deemed
271 well sedated two hours after treatment administration.,.

272 Discussion

273 In this study, intramuscularly administered MED and MK-467 showed a dynamic
274 interaction that may be more complicated than previously thought. MK-467 indeed
275 attenuated the cardiovascular effects of MED after the initial absorption phase, but
276 increasing the MK-467 dose did not significantly change the intensity of these effects.
277 However, there was a tendency for the effects of MED to return to baseline earlier
278 when the dose of MK-467 was increased. This route of administration showed that an
279 interaction of these drugs appears to exist during absorption from the site of
280 administration. Most previous studies have concentrated on intravenous

281 administration, which bypasses the absorption phase. In our study, MK-467 initially
282 facilitated the absorption of MED, reflected by a higher and more rapidly reached
283 maximum plasma concentration of DMED, and was associated with a faster initiation
284 of clinical effects, e.g. sedation scores increased more rapidly with MK200 and
285 MK600. Moreover, the initial cardiovascular effects were not prevented or attenuated
286 by antagonist administration. However, MK-467 later attenuated the effects of MED
287 and appeared to shorten their duration, reflected by significant differences at late
288 follow-up time-points between treatments with MK-467 and MED alone. However,
289 no dose-dependency for MK-467 could be verified. A pharmacokinetic drug
290 interaction may explain these findings, as our results indicate enhanced absorption of
291 DMED (and LMED) when MK-467 was added to the treatment. This is a novel
292 finding: Rolfe et al. (2012) suggested that after an IM injection, the absorption and/or
293 distribution of MK-467 were faster or similar to MED alone, as the concurrently IM
294 administered MK-467 immediately attenuated the systemic peripheral effects of
295 MED. However, they did not report plasma drug concentrations; therefore, only the
296 pharmacodynamic results can be compared.. The doses of MED and MK-467 used by
297 Rolfe et al. (2012) were similar to the middle dose used in our study (MK400).
298 However, they administered MED in a separate syringe immediately followed by
299 MK-467 injection in the lumbar muscle group, whereas we mixed MED and MK-467
300 in the same syringe. Rolfe et al. (2012) did not specify whether different injection
301 sites were used, and also the dilution of their drugs was higher. Therefore, in that
302 study, MK-467 was without local effects on the absorption of medetomidine. The
303 absorption of medetomidine from the injection site was likely to be slower in their
304 study compared to ours, which probably explains the differences in the early
305 cardiovascular events between the studies. In our study, MK-467 may have prevented

306 the local vasoconstriction caused by MED, thus initially facilitating the absorption of
307 MED at the injection site. The small differences in the absorption of MED with
308 different doses of MK-467 could at least partly be explained by the dilution of MED,
309 as the concentration of MK-467 was always the same before mixing it with MED;
310 thus, the lower the dose and volume of MK-467 given, the higher the MED
311 concentration in the injected solution.

312 It was evident that MK-467 did not immediately antagonize the cardiovascular
313 effects of MED. In fact, the increases in CVP and MAP appeared initially more
314 pronounced after MK-467 during the highest DMED plasma concentrations,
315 especially by the smallest dose. of MK-467, although these differences did not reach
316 statistical significance. The antagonising effects of MK-467 probably appeared later
317 than the systemic vascular effects of DMED due to the slower absorption (later T_{max})
318 of MK-467 compared to DMED. For example, at 5 minutes after the lowest dose of
319 MK-467 (MK200), the MK-467:DMED concentration ratio in plasma was low
320 (approximately 40:1), but later when the ratio increased also the cardiovascular effects
321 of MED were reversed. This finding is consistent with an earlier result, where an MK-
322 467:DMED plasma concentration ratio of approximately 50:1 did not significantly
323 reverse the cardiovascular effects of MED during IV infusion in dogs anaesthetized
324 with isoflurane (Karttinen et al. 2014).

325 Although no significant differences emerged between the three studied doses of MK-
326 467 at any time-point, the duration of all cardiovascular effects of MED seemed to be
327 dose-dependently shortened with increasing doses of MK-467.

328 We observed a slight but significant decrease in RR with addition of MK-467 to
329 MED, but no hypoventilation was detected, as $PaCO_2$ remained within the
330 physiological range. In addition, PaO_2 and arterial lactate concentrations remained

331 within the normal ranges, while with MED alone there was a trend for lactate to
332 increase during the follow-up. Similar findings have been reported previously
333 (Uilenreef et al. 2008; Kaartinen et al. 2010; Honkavaara et al. 2011; Restitutti et al.
334 2012). PaO₂ and lactate concentrations are often used as indirect indicators of oxygen
335 carrying capacity and tissue perfusion (Pang & Boysen 2007; Uilenreef et al. 2008;
336 Restitutti et al. 2012), when oxygen delivery and consumption are not measured or
337 calculated.

338 Rectal temperatures decreased during sedation after all treatments despite the passive
339 measures to maintain body temperature. As there were no differences between
340 treatments and none of the dogs were hypothermic, the decrease in temperature was
341 considered not to affect our results.

342 No differences between treatments were detected in the maximal sedation
343 scores, and all dogs were clinically sedated. Our results are consistent with earlier
344 ones where addition of MK-467 did not alter the clinical sedation in dogs
345 (Honkavaara et al. 2008; Restitutti et al. 2011; Rolfe et al. 2012). However, we
346 detected a shorter duration of sedation when MK-467 was administered
347 simultaneously with medetomidine. No difference was detected in the MK-467 dose
348 used for the intensity of its effects, although the duration of action appeared dose-
349 dependently reduced.

350 All cardiovascular and sedation results taken into consideration, we could not
351 prove our hypothesis of the dose-dependency of the effects of MK-467 and can offer
352 no recommendations for an IM MED:MK-467 dose ratio based on these results.

353 This study had some limitations. Because the main observer was aware of the
354 given treatments, the subjective component (sedation score) could have been
355 inadvertently affected causing error on the results. However, sedation score was only

356 a secondary parameter to be investigated and the results are similar to those from
357 previous “blinded” studies. Moreover, these subjective changes could be supported by
358 the parallel changes in objective measurements of plasma concentrations. In addition
359 to study limitations, as mentioned above, the concentrations of the administered drugs
360 were different in each treatment, as injected volumes differed in order to reduce the
361 volumes to be injected IM, which might have affected the absorption kinetics.
362 In conclusion, in this study conducted in healthy beagle dogs, the classical
363 haemodynamic effects of MED were attenuated and their duration shortened by the
364 concomitant IM administration of MK-467. The absorption of MED was enhanced
365 when it was administered IM in the same syringe with MK-467.. The initial
366 haemodynamic effects of MED were not prevented by the investigated doses of MK-
367 467, and maximal sedation was unaffected, although the duration of these
368 forementioned effects were shortened.

- 370 Clineschmidt BV, Pettibone DJ, Lotti VJ et al. (1988) A peripherally acting alpha-2
371 adrenoceptor antagonist: L-659,066. *J Pharmacol Exp Ther* 245, 32-40.
- 372 Doze VA, Chen BX, Maze M (1989) Dexmedetomidine produces a hypnotic-
373 anesthetic action in rats via activation of central alpha-2 adrenoceptors.
374 *Anesthesiology* 71, 75-79.
- 375 Enouri SS, Kerr CL, McDonell WN et al. (2008) Effects of a peripheral alpha2
376 adrenergic-receptor antagonist on the hemodynamic changes induced by
377 medetomidine administration in conscious dogs. *Am J Vet Res* 69, 728-
378 736.
- 379 Grimm KA, Tranquilli WJ, Thurmon JC et al. (2000) Duration of nonresponse to
380 noxious stimulation after intramuscular administration of butorphanol,
381 medetomidine, or a butorphanol-medetomidine combination during
382 isoflurane administration in dogs. *Am J Vet Res* 61, 42-47.
- 383 Honkavaara J, Restitutti F, Raekallio M et al. (2012) Influence of MK-467, a
384 Peripherally Acting alpha2-Adrenoceptor Antagonist on the Disposition of
385 Intravenous Dexmedetomidine in Dogs. *Drug Metab Dispos* 40, 445-449.
- 386 Honkavaara JM, Raekallio MR, Kuusela EK et al. (2008) The effects of L-659,066,
387 a peripheral alpha2-adrenoceptor antagonist, on dexmedetomidine-
388 induced sedation and bradycardia in dogs. *Vet Anaesth Analg* 35, 409-
389 413.
- 390 Honkavaara JM, Restitutti F, Raekallio MR et al. (2011) The effects of increasing
391 doses of MK-467, a peripheral alpha(2)-adrenergic receptor antagonist,
392 on the cardiopulmonary effects of intravenous dexmedetomidine in
393 conscious dogs. *J Vet Pharmacol Ther* 34, 332-337.
- 394 Kaartinen J, Del Castillo JR, Salla K et al. (2014) Haemodynamic interactions of
395 medetomidine and the peripheral alpha-2 antagonist MK-467 during step
396 infusions in isoflurane-anaesthetised dogs. *Vet J* 202, 353-360.
- 397 Kaartinen J, Pang D, Moreau M et al. (2010) Hemodynamic effects of an
398 intravenous infusion of medetomidine at six different dose regimens in
399 isoflurane-anesthetized dogs. *Vet Ther* 11, E1-E16.
- 400 Kamibayashi T, Maze M (2000) Clinical uses of alpha2 -adrenergic agonists.
401 *Anesthesiology* 93, 1345-1349.
- 402 Kuusela E, Raekallio M, Anttila M et al. (2000) Clinical effects and
403 pharmacokinetics of medetomidine and its enantiomers in dogs. *J Vet*
404 *Pharmacol Ther* 23, 15-20.
- 405 Kuusela E, Vainio O, Kaistinen A et al. (2001) Sedative, analgesic, and
406 cardiovascular effects of levomedetomidine alone and in combination
407 with dexmedetomidine in dogs. *Am J Vet Res* 62, 616-621.
- 408 Mason DJ, O'Grady M, Woods JP et al. (2001) Assessment of lithium dilution
409 cardiac output as a technique for measurement of cardiac output in dogs.
410 *Am J Vet Res* 62, 1255-1261.
- 411 Maze M, Fujinaga M (2000) Alpha2 adrenoceptors in pain modulation: which
412 subtype should be targeted to produce analgesia? *Anesthesiology* 92, 934-
413 936.
- 414 Murrell JC, Hellebrekers LJ (2005) Medetomidine and dexmedetomidine: a
415 review of cardiovascular effects and antinociceptive properties in the dog.
416 *Vet Anaesth Analg* 32, 117-127.

417 Pagel PS, Proctor LT, Devcic A et al. (1998) A novel alpha 2-adrenoceptor
 418 antagonist attenuates the early, but preserves the late cardiovascular
 419 effects of intravenous dexmedetomidine in conscious dogs. *J Cardiothorac*
 420 *Vasc Anesth* 12, 429-434.
 421 Pang DS, Boysen S (2007) Lactate in veterinary critical care: pathophysiology
 422 and management. *J Am Anim Hosp Assoc* 43, 270-279.
 423 Pascoe PJ, Raekallio M, Kuusela E et al. (2006) Changes in the minimum alveolar
 424 concentration of isoflurane and some cardiopulmonary measurements
 425 during three continuous infusion rates of dexmedetomidine in dogs. *Vet*
 426 *Anaesth Analg* 33, 97-103.
 427 Restitutti F, Honkavaara JM, Raekallio MR et al. (2011) Effects of different doses
 428 of L-659'066 on the bispectral index and clinical sedation in dogs treated
 429 with dexmedetomidine. *Vet Anaesth Analg* 38, 415-422.
 430 Restitutti F, Raekallio M, Vainionpaa M et al. (2012) Plasma glucose, insulin, free
 431 fatty acids, lactate and cortisol concentrations in dexmedetomidine-
 432 sedated dogs with or without MK-467: a peripheral alpha-2 adrenoceptor
 433 antagonist. *Vet J* 193, 481-485.
 434 Rolfe NG, Kerr CL, McDonell WN (2012) Cardiopulmonary and sedative effects of
 435 the peripheral alpha2-adrenoceptor antagonist MK 0467 administered
 436 intravenously or intramuscularly concurrently with medetomidine in
 437 dogs. *Am J Vet Res* 73, 587-594.
 438 Salla K, Restitutti F, Vainionpaa M et al. (2014) The cardiopulmonary effects of a
 439 peripheral alpha-2-adrenoceptor antagonist, MK-467, in dogs sedated
 440 with a combination of medetomidine and butorphanol. *Vet Anaesth Analg*
 441 41, 567-574.
 442 Salonen J (1992) Chemistry and pharmacokinetics of the alpha2-adrenoceptor
 443 agonist. In: Short CEP, A. (ed). *Animal pain*, Vol. Churchill Livingstone Inc.
 444 pp. 191-200.
 445 Sinclair MD (2003) A review of the physiological effects of alpha2-agonists
 446 related to the clinical use of medetomidine in small animal practice. *Can*
 447 *Vet J* 44, 885-897.
 448 Szemerédi K, Stull R, Kopin IJ et al. (1989) Effects of a peripherally acting alpha
 449 2-adrenoceptor antagonist (L-659,066) on hemodynamics and plasma
 450 levels of catechols in conscious rats. *Eur J Pharmacol* 170, 53-59.
 451 Uilenreef JJ, Murrell JC, McKusick BC et al. (2008) Dexmedetomidine continuous
 452 rate infusion during isoflurane anaesthesia in canine surgical patients. *Vet*
 453 *Anaesth Analg* 35, 1-12.
 454 Vainio O, Vaha-Vahe T, Palmu L (1989) Sedative and analgesic effects of
 455 medetomidine in dogs. *J Vet Pharmacol Ther* 12, 225-231.
 456 van Oostrom H, Doornenbal A, Schot A et al. (2011) Neurophysiological
 457 assessment of the sedative and analgesic effects of a constant rate
 458 infusion of dexmedetomidine in the dog. *Vet J* 190, 338-344.
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 460
 461

Tables.

Table 2. Selected arterial blood gas parameters in dogs exposed to medetomidine (20 µg kg⁻¹, MED) and its combination with three doses of MK-467 (MK200, MK400, MK600). Values are shown as mean (SD).

	Baseline	5 min	15 min	30 min	60 min
<u>PaCO₂ (kPa)</u>					
MED	4.6 (0.2)	4.6 (0.3)	4.5 (0.3) ^a	4.5 (0.4) ^a	4.5 (0.3) ^{abc}
MK200	4.7 (0.4)	4.8 (0.2)	4.7 (0.5)	4.8 (0.4)	5.1 (0.4) ^c
MK400	4.7 (0.4)	4.7 (0.4)	4.6 (0.3) ^b	4.9 (0.3)	5.1 (0.3) ^b
MK600	4.6 (0.3)	4.9 (0.4)	5.1 (0.4) ^{*ab}	4.9 (0.5) ^a	5.1 (0.3) ^{*a}
<u>PaO₂ (kPa)</u>					
MED	12.5 (0.8)	12.2 (1.5)	12.2 (0.8)	12.0 (1.6)	12.6 (1.0)
MK200	12.7 (0.5)	10.9 (1.4) [*]	11.6 (1.4)	12.5 (1.2)	13.0 (1.3)
MK400	13.0 (0.9)	11.7 (1.1)	12.5 (0.8)	12.6 (1.1)	13.2 (0.6)
MK600	12.8 (0.6)	11.3 (1.0)	11.5 (1.5)	12.8 (1.4)	13.1 (1.2)
<u>Lactate (mmol L⁻¹)</u>					
MED	0.6 (0.4)	0.5 (0.3)	0.7 (0.3)	0.9 (0.3)	1.1 (0.4)
MK200	0.5 (0.2)	0.4 (0.1) [*]	0.6 (0.3)	0.6 (0.3)	0.7 (0.3)
MK400	0.5 (0.2)	0.4 (0.1) [*]	0.6 (0.3)	0.6 (0.3)	0.7 (0.2)
MK600	0.5 (0.2)	0.5 (0.1)	0.5 (0.1)	0.5 (0.2)	0.6 (0.2)

*significant difference from baseline, ^{a, b, c}same letter = significant difference between treatments.

Table 3. Pharmacokinetic parameter estimates calculated for dexmedetomidine and MK-467 in eight dogs exposed to medetomidine (20 µg kg⁻¹, MED) and its combination with three doses of MK-467 (MK200, MK400, MK600).

Treatment	DMED	DMED	MK-467	MK-467
	C _{max} (µg L ⁻¹) Geo mean (CI)	T _{max} (min) Mean (SD)	C _{max} (µg L ⁻¹) Geo mean (CI)	T _{max} (min) Mean (SD)
MED	3.8 (3.0-4.9)	42.5 (16.3)		
MK200	7.9 ^{*a} (6.6-9.5)	9.4 [*] (4.2)	460 (358-590)	32.5 (14.4)
MK400	5.5 ^{*a} (4.7-6.3)	16.9 [*] (6.5)	645 (540-770)	38.1 (24.8)
MK600	5.5 (3.9-7.7)	13.1 [*] (7.5)	915 (696-1201)	32.5 (16.3)

Parameter estimates of maximum concentration (C_{max}), time to maximum concentration (T_{max}) for dexmedetomidine (DMED) and MK-467, presented as mean values and standard deviation (SD), or geometric mean (Geo mean) and ninety-five percent confidence interval for geometric mean (CI, lower and upper bound). *significant difference from MED, ^asame letter = significant difference between treatments.

463 Figure legends

464

465 Figure 1. Heart rate in eight dogs exposed to medetomidine ($20 \mu\text{g kg}^{-1}$, MED) and its
466 combination with three doses of MK-467 (MK200, MK400, MK600). Values are
467 presented as mean (SD). *significant difference from baseline, ^{a, b, c}significant
468 difference between treatments with same letter.

469

470 Figure 2. Mean arterial pressure in eight dogs exposed to medetomidine ($20 \mu\text{g kg}^{-1}$,
471 MED) and its combination with three doses of MK-467 (MK200, MK400, MK600).
472 Values are presented as mean (SD). *significant difference from baseline, ^{a, b,}
473 ^csignificant difference between treatments with same letter.

474

475 Figure 3. Central venous pressures in eight dogs exposed to medetomidine ($20 \mu\text{g kg}^{-1}$,
476 MED) and its combination with three doses of MK-467 (MK200, MK400, MK600).
477 Values are presented as mean (SD). *significant difference from baseline, ^{a, b,}
478 ^csignificant difference between treatments with same letter.

479

480 Figure 4. Cardiac output in eight dogs exposed to medetomidine ($20 \mu\text{g kg}^{-1}$, MED)
481 and its combination with three doses of MK-467 (MK200, MK400, MK600). Values
482 are presented as mean (SD). *significant difference from baseline, ^{a, b, c}significant
483 difference between treatments with same letter.

484

485 Figure 5. Composite sedation scores in eight dogs exposed to medetomidine ($20 \mu\text{g}$
486 kg^{-1} , MED) and its combination with three doses of MK-467 (MK200, MK400,
487 MK600). Values are presented as mean (SD). *significant difference from
488 baseline, ^{a, b, c}significant difference between treatments with same letter.

489

490 Figure 6. Dexmedetomidine plasma concentrations in eight dogs exposed to
491 medetomidine ($20 \mu\text{g kg}^{-1}$, MED) and its combination with three doses of MK-467
492 (MK200, MK400, MK600). Values are presented as mean (SD).

493

494 Figure 7. Plasma concentrations of MK-467 in eight dogs exposed to medetomidine
495 ($20 \mu\text{g kg}^{-1}$) combined with three doses of MK-467 (MK200, MK400, MK600).
496 Values are presented as mean (SD).

497

Appendix 1.

Composite sedation score (CSS). CSS is the sum of scores of the five parameters detailed below (maximum sedation score 16):

<p><u>A. Palpebral reflex (0-3)</u></p> <p>0 = Normal</p> <p>1= Reduced</p> <p>2= Weak</p> <p>3= Absent</p>	<p><u>D. Resistance to positioning in lateral recumbency (0-3)</u></p> <p>0 = Normal</p> <p>1= Turns back to sternal position</p> <p>2 = Some resistance, but stays in lateral recumbency</p> <p>3= No resistance or the position is already lateral</p>
<p><u>B. Position of the eye (0-2)</u></p> <p>0 = Middle</p> <p>2 = Turned down</p>	<p><u>E. General appearance (0-4)</u></p> <p>0 = Normal</p> <p>1= Slightly tired, head drooping</p> <p>2= Mild sedation, reacts clearly to surroundings</p> <p>3= Moderate sedation, reacts mildly to surroundings</p> <p>4= Deep sedation, no reaction to surroundings</p>
<p><u>C. Jaw and tongue relaxation (0-4)</u></p> <p>0 = Normal, opens jaw, but resists manipulation of the tongue</p> <p>1= Bites jaw together</p> <p>2= Opens jaw but strong resistance when tongue is pulled</p> <p>3= Slight resistance when tongue is pulled</p> <p>4= No resistance</p>	