Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Pharmacokinetics of S-ketamine and R-ketamine and their active metabolites after racemic ketamine or S-ketamine intravenous administration in dogs sedated with medetomidine

(Article begins on next page)

26 April 2024

Journal Pre-proof

Pharmacokinetics of S-ketamine and R-ketamine and their active metabolites after racemic ketamine or S-ketamine intravenous administration in dogs sedated with medetomidine

Noemi Romagnoli, Rima N. Bektas, Annette Kutter, Andrea Barbarossa, Paola Roncada, Sonja Hartnack, Regula Bettschart-Wolfensberger

Please cite this article as: Romagnoli N, Bektas RN, Kutter A, Barbarossa A, Roncada P, Hartnack S, Bettschart-Wolfensberger R, Pharmacokinetics of S-ketamine and R-ketamine and their active metabolites after racemic ketamine or S-ketamine intravenous administration in dogs sedated with medetomidine, *Veterinary Anaesthesia and Analgesia*, https://doi.org/10.1016/j.vaa.2019.08.048.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

RESEARCH STUDY

Pharmacokinetics of S-ketamine and R-ketamine and their active metabolites after racemic ketamine or S-ketamine intravenous administration in dogs sedated with medetomidine

Noemi Romagnoli[a], Rima N. Bektas[b], Annette Kutter[b], Andrea Barbarossa[a], Paola Roncada[a], Sonja Hartnack[c], Regula Bettschart-Wolfensberger[b]

[a] Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064, Ozzano dell'Emilia, Bologna, Italy

[b] Anaesthesiology Section, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 258 c, 8057 Zurich, Switzerland

[c] Epidemiology Section, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 270, 8057 Zurich, Switzerland

Correspondence: Andrea Barbarossa, Department of Veterinary Medical Sciences, University of Bologna, via Tolara di Sopra 50, 40064, Ozzano dell'Emilia, Bologna, Italy. Email: andrea.barbarossa@unibo.it

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

NR, RB, RNB, APK participated in data acquisition; moreover NR, AB, PR and SH performed data analysis and interpretation, and drafted the paper; RB, RNB, APK participated in data's interpretation and revised the paper. RB and NR conceived the study design, revised the paper and approved the final version.

1   **Objective** To assess the differences in the pharmacokinetic profiles of S-ketamine, R-ketamine, and

2   their metabolites S-norketamine and R-norketamine, and to measure relevant physiologic variables

3   after intravenous administration of racemic ketamine or S-ketamine alone in beagle dogs sedated

4   with medetomidine.

5   **Study design** Experimental, blinded, randomized crossover study.

6   **Animals** A total of six adult beagle dogs, three females and three males.

7   **Methods** Medetomidine (450 mcg m^{-2}) was administered intramuscularly, followed 20 minutes

8   later by either S-ketamine (2 mg kg^{-1}) or racemic (RS) ketamine (4 mg kg^{-1}) both administered

9   intravenously. Blood samples were collected before medetomidine administration, and at multiple

10   timepoints 1 to 900 minutes following the ketamine administration. Plasma samples were analysed

11   using liquid chromatography-tandem mass spectrometry. Heart rate, respiratory rate, non-invasive

12   blood pressure, haemoglobin saturation with oxygen in (SpO_2) and body temperature were

13   measured at baseline, before ketamine administration, and 1, 2, 5, 10, 15, 20 and 30 minutes after

14   ketamine administration. All cardiovascular variables, blood glucose, haemoglobin and lactate

15   concentrations were analysed using different linear mixed effects models; the significance was set

16   at $p < 0.05$.

17   **Results** S-ketamine showed a two-compartment kinetic profile; no statistically significant

18   differences were observed between its concentrations or in the calculated pharmacokinetic

19   parameters following S- or RS-ketamine. When the racemic mixture was administered, no

20   differences were detected between R- and S-ketamine concentrations, but the area under the curve

21   (AUC) for R-norketamine was significantly lower when compared to that of S-norketamine.

22   Clinically relevant physiologic variables did not show statistically significant differences following

23   the administration of the racemic mixture or of S-ketamine alone.

24

25   **Conclusions and clinical relevance** This study performed in dogs showed that racemic ketamine

26   and S-ketamine combined with medetomidine, showed enantioselective pharmacokinetics as S-and

27   R-norketamine AUCs were different, but S-ketamine levels were identical.

28

29   *Keywords* canine, medetomidine, pharmacokinetic**s,** s-ketamine, s-norketamine

30 **Introduction**

31 Ketamine is a dissociative anaesthetic, widely used in human and veterinary anaesthesia. It is a

32 racemic mixture of two optical isomers, R- and S-ketamine, which have different pharmacological

33 effects (Bergman 1999). S-ketamine is available for dogs in some European countries. In humans,

34 the relative potency of S-ketamine is twice that of the racemic form, and the loss of response to

35 verbal commands is seen at half the dose of S-ketamine as compared to racemic ketamine (Ihmsen

36 et al. 2001). In species such as the dog and pony, the clearance of S-ketamine administered alone is

37 higher than that of S-ketamine or R-ketamine administered in the racemic form (Ihmsen et al. 2001;

38 Duque et al. 2008; Larenza et al. 2009). This explains the faster recovery seen in patients

39 anaesthetized with the S-enantiomer alone (Ihmsen et al. 2001; Duque et al. 2008).

40 Clinically relevant physiologic functions are usually well maintained with ketamine when in

41 combination with moderate doses of α_2-adrenoceptor agonists. Studies in ponies and horses (Filzek

42 et al, 2003; Larenza et al. 2007), however, have shown some differences in the cardiopulmonary

43 effects related to the stereoselectivity of ketamine.

44 The combination of racemic ketamine with α_2-adrenoceptor agonists has been widely used

45 in dogs to induce sedation and anaesthesia (Ueyema et al. 2008). This drug combination produces

46 an adequate quality and duration of sedation and anaesthesia for minor medical and surgical

47 procedures. α_2-Adrenoceptor agonists alter the metabolism of other co-administered drugs, such as

48 opioids and ketamine *in vitro*, mainly via an interaction with cytochrome P (CYP) enzymes

49 (Kharasch et al. 1991; Sandbaumhüter et al. 2015). This interaction can influence the intensity and

50 duration of the effects of ketamine.

51 The aims of this study were to obtain the pharmacokinetic profiles of S-ketamine and R-

52 ketamine, and their major metabolites, S-norketamine and R-norketamine, in healthy beagles

53 sedated with intramuscular (IM) medetomidine after racemic ketamine or S-ketamine intravenous

54 (IV) administration. Clinically relevant physiologic variables were also compared.

55   We hypothesized that the pharmacokinetics of ketamine's enantiomers and its metabolites,

56   when combined with medetomidine, were not stereoselective in dogs given racemic ketamine or the

57   S-isoform alone.

58

59   **Material and Methods**

60   Animals

61   The trial was approved by the committee for Animal Experimentation of XXX 67/2011. A total of

62   six healthy adult beagle dogs, three females and three males (non-castrated), 21 ± 11 [mean ±

63   standard deviation (SD)] months of age, weighing 15.0 ± 1.1 (mean ± SD) kg, were used in the

64   study. A *post-hoc* power calculation on the pharmacokinetic (PK) data was used to verify the

65   adequacy of the number of dogs included, and the power was 99.5% for six dogs.

66   Complete blood cell count and blood chemistry were checked two days before the

67   experiment. The dogs were fasted overnight but always had free access to water.

68   Drug administration and Monitoring

69   This was a blinded, randomized crossover trial with a 3-week washout period between treatments.

70   A random order generator (GraphPad Software, CA, USA) determined treatment allocation. The

71   study was performed following good clinical practice guidelines (Flecknell, 1993).

72   Following standard aseptic preparation, two peripheral venous catheters (Surflo IV Catheter

73   22-gauge, Terumo, Belgium) were inserted into both cephalic veins of each dog, one catheter for

74   drug administration, and the other as a reserve. An additional catheter was placed in the jugular vein

75   (16-gauge, 16 cm long, Blue Flex Tip Catheter, Arrow International, Teleflex Medical GmbH,

76   Switzerland) following infiltration of lidocaine (1 mL; Lidocain 2$^{\%}$, Streuli, Switzerland). This

77   catheter was used for central venous blood sampling. The catheter for drug administration was

78   attached to a lactated ringer infusion (Ringer-Lactat Fresenius; Fresenius Kabi AG, Switzerland) at

79   a rate of 5 mL kg^{-1} hour^{-1} for 30 minutes before anaesthesia induction. The dogs were sedated with

80   medetomidine administered IM at a dose rate of 450 μg m^{-2} (approximately 17 μg kg^{-1} in a 15 kg

81   dog, Dorbene, Fort Dodge, Italy). After 20 minutes, S-ketamine 2 mg kg^{-1} (Keta-S; Dr. E. Graeub

82   AG, Switzerland) (S-KET treatment) or racemic ketamine 4 mg kg^{-1} (Ketasol-100; Dr. E. Graeub

83   AG, Switzerland) (RS-KET treatment) were rapidly administered IV over 1-2 seconds.

84   Once anaesthesia was induced (identified by loss of laryngeal reflex), the dogs were

85   intubated and allowed to breath room air (fraction of inspired oxygen (FiO_2 = 0.21). A

86   multiparameter monitor (BN 850, GE Medical Systems, Anandic Medical Systems AG,

87   Switzerland) was used to monitor anaesthesia. The following measurements were taken heart rate

88   (HR) from an electrocardiogram, respiratory rate (f_R), indirect arterial blood pressures (systolic:

89   SAP, mean: MAP, diastolic: DAP), haemoglobin (Hb) saturation with oxygen in % (SpO_2), end-

90   tidal carbon dioxide partial pressure end-tidal CO_2 ($PE'CO_2$) and body temperature (T°). The

91   variables listed above were recorded at baseline [before medetomidine administration = timepoint

92   (T-22)], at T-1 (20 minutes after medetomidine injection and prior to ketamine administration), and

93   1, 2, 5, 10, 15, 20 and 30 minutes after the ketamine administration. Before ketamine

94   administration, and 5 and 30 minutes after ketamine injection, blood glucose (Contour, Bayer AG

95   Heathcare, Switzerland), lactate (Accutrend, Roche Diagnostics, Switzerland) and haemoglobin

96   concentration (Hemocue Hb201+, Baumann Medical AG, Switzerland) were measured.

97   The peripheral venous catheters were removed 30 minutes after drug administration., the

98   dogs were given 4 mg kg^{-1} carprofen (Rimadyl ad us. Vet, Pfizer AG, Switzerland) IV 4 hours after

99   drug administration. The dogs were observed by veterinarians and offered a commercial diet once

100   they were fully awake. During and after anaesthesia, the dogs' body temperatures were maintained

101   between 37.0-38.5°C using warm water blankets (Hico-Aquatherm 660, Nufer Medical,

102   Switzerland), a heat and moisture exchanger (HMEF1000, Anandic Medical Systems AG,

103   Switzerland) and a forced air-patient warming system (Bair Hugger Model 505, Carbamed,

104   Switzerland) as needed. After taking the last blood sample, the jugular catheter was removed.

105   Sample collection

106   A blood sample was taken for determination of plasma drug concentration 2 minutes before

107   medetomidine administration (T-22) and 20 minutes after (T-2), prior to anaesthesia induction.

108   Blood samples were also collected at anaesthesia induction (T0), 1, 2, 5, 10, 15, 20, 30, 45, 60, 75,

109   90, 105, 120, 150, 180, 210, 240, 300, 360, 450, 540, 630, 720, 810 and 900 minutes after ketamine

110   administration. Samples for determination of plasma drug levels were collected from the central

111   venous catheter and put into labelled heparinized tubes (BD 3.5 mL Vacutainer, Becton Dickinson,

112   Belgium). A total of 4 -mL of blood were drawn before each sample, and were re-injected

113   immediately after, and the catheter was then flushed with 5 mL of saline (B-Braun, Melsungen,

114   Germany). The effective time of sampling was recorded for each sample. Immediately after

115   collection, the samples were centrifuged at 4 °C and 3000 ×g for 10 minutes (Sarstedt LC 1-K,

116   Germany). The plasma was stored at -80°C in suitable tubes (Nunc 1.8 mL SI Cryotube vials; Nunc

117   A/S, Denmark) until analysis.

118   Plasma drug analysis

119   The plasma samples were analysed using the liquid chromatography-tandem mass spectrometry

120   (LC-MS/MS) method, previously described by Romagnoli et al. (2017). Briefly, after the addition

121   of labelled internal standards (Ketamine☐d4 and Norketamine☐d4, purchased from Sigma-Aldrich,

122   MO, USA), 150 μL of plasma were extracted with methanol and centrifuged. The supernatant was

123   filtered through a 0.2 μm PTFE filter (Phenomenex, CA, USA) prior to analysis.

124   The LC system consisted of a Waters Aquity UPLC binary pump (Waters, MA, USA),

125   equipped with a Phenomenex Lux 3μm Cellulose☐3 (150 x 2,00 mm, 3,0 μm) column

126   (Phenomenex, CA, USA). The mobile phase was a mixture of acetonitrile and an aqueous solution

127   containing ammonium acetate 20 mM and ammonium formate 0.1%, at a flow rate of 0.45 mL

128   minutes^{-1} under programmed conditions. The LC was interfaced to a Waters Quattro Premier XE

129   triple quadrupole mass spectrometer (Waters, MA, USA), operating in positive electrospray

130   ionisation (ESI+), and two specific transitions were observed for each analyte (Ketamine: 238 →

131   125 and 179 *m/z*, Norketamine: 224 → 125 and 207 *m/z*) and for each internal standard (Ketamine☐

132   D4: 242 → 129 and 183 *m/z*, Norketamine- D4: 228 → 129 and 211 *m/z*).

133   The analytical method was validated in accordance with EMEA/CHMP/EWP/192217/2009

134   guidelines at the beginning of the experiment. Linearity was satisfactory ($R^2 > 0.99$) over a range

135   extending from 15 to 15,000 ng mL^{-1} for both S-Ketamine and R-Ketamine, and from 15 to 3000 ng

136   mL^{-1} for the norketamine enantiomers. The lower limit of quantification (LLOQ) was 15 ng mL^{-1}

137   for all target compounds; inter- and intra-day accuracy and precision were both below 10% for all

138   the analytes.

139   Pharmacokinetics and statistical analysis

140   The aim of the statistical analysis was to detect potential differences in the repeated measurements

141   of the cardiopulmonary variables, Hb, lactate and glucose concentrations between the two

142   treatments. For each of the outcome variables, different linear mixed effects models were run. In all

143   models, dog was included as a random intercept to account for potential clustering within animals.

144   In contrast, in the different models, time was included either as a fixed effect, with or without an

145   interaction term with treatment, or as a random slope. The treatments were included as a fixed

146   effect and omitted in the null model. Model selection was based on Akaike's Information Criterion

147   (AIC) and on likelihood ratio tests which provided the *p*-values.

148   The analysis was carried out using R software (R Core Team 2018) and the packages: nlme

149   (Pinheiro et al. 2018) and lmtest (Zeileis & Hothorn T 2002). Based on the assumption of missing at

150   random, the missing values were inputted using the package missForest (Stekhoven 2013). All

151   cardiopulmonary variables, Hb, lactate and glucose concentrations are reported as mean ± SD.

152   The R-ketamine and S-ketamine concentration *versus* time curves were analysed for each

153   individual by XY plot using WinNonlin 6.3 (Pharsight Corporation, CA, USA).

154   The plasma drug concentrations obtained after IV administration were fitted using the

155   following equation:

156        $C(t) = A\ e^{-at} + B\ e^{-\beta t}$.

157   All pharmacokinetic parameters are reported as mean ± SD, and were determined using

158 WinNonlin 6.3 (Pharsight Corporation, CA, USA). The individual plasma concentration *versus* time

159 curves were fitted, and the best compartment model was determined by application of the AIC

160 (Yamaoka et al. 1978).

161   The following pharmacokinetic parameters were calculated for each dog for the ketamine

162 enantiomers: area under the curve to infinity ($AUC_{0 \to \infty}$), half-life of the distribution phase ($T_{1/2dis}$),

163 half-life of the elimination phase ($T_{1/2el}$), rate constants of the elimination phase(K_{el}), mean

164 residence time (MRT), total body clearance (Cl_B), volume of distribution of the central

165 compartment (V_c),   peak concentration(C_{max}) and time of peak concentration (T_{max}). For S-

166 norketamine and R-norketamine, non-compartmental analysis was used to determine the $AUC_{0 \to \infty}$,

167 peak metabolite concentration (C_{max}) and time of peak metabolite concentration (T_{max}). The

168 Wilcoxon signed rank test was used to detect differences between the treatments concerning the

169 pharmacokinetic parameters with a significance level of $p < 0.05$.

170

171 **Results**

172 All the dogs enrolled in the study also finished the study and recovered without any complications.

173 The dogs came from the pool of experimental dogs of the Vetsuisse Faculty of the University of

174 Zürich, where they returned 24 hours after the end of the study.

175   Sedation was considered to be profound following medetomidine administration, and S- and

176 racemic ketamine were injected according to the scheduled times. Intubation was judged to be easy

177 in four dogs in the RS-KET treatment and two dogs in the S-KET treatment, and less easy but

178 possible in the remaining dogs. In the RS-KET, treatment one dog showed several episodes of

179 muscle shaking 3.5 minutes following drug administration. All dogs recovered well from the

180 anaesthesia and were standing within 37.9 ± 16.9 (mean ± SD) minutes in the S-KET treatment and

181 42.9 ± 19.6 (mean ± SD) minutes in the RS-KET treatment. No significant difference was detected

182 between treatments.

183   The cardiovascular variables are summarised in Table 1. The SpO_2 ranged between 98% and

184   100% in all dogs. Some cardiopulmonary measurements were only possible when the dogs were

185   unconscious and tolerated the endotracheal tube (*e.g.* $PE'CO_2$), or other measurement devices.

186   No significant treatment effect was detected in the following variables: HR ($p = 0.068$), f_R ($p =$

187   0.388), SAP ($p = 0.465$), DAP ($p = 0.260$), MAP ($p = 0.355$), T° ($p = 0.317$), SpO_2 $p = 0.967$

188   Hb ($p = 0.09$), lactate ($p = 0.230$) and glucose ($p = 0.185$).

189   Plasma drug concentrations

190   Due to technical problems with the drug assay, the concentrations of the R- and the S-enantiomers

191   from the racemic mixture could not be determined in one dog.

192   The plasma drug concentrations of both the ketamine and the norketamine enantiomers were

193   plotted against the time points for both racemic ketamine and S-ketamine administration (Figs 1 and

194   2). No statistically significant differences were detected between the concentrations of S-ketamine

195   alone, and R-ketamine and S-ketamine after administration of the racemic mixture, at any time.

196   Neither R-ketamine nor R-norketamine were detected after the administration of S-ketamine.

197   Pharmacokinetic parameters

198   The S-ketamine plasma concentration following the IV administration of a bolus of racemic

199   ketamine or S-ketamine alone was best described using a 2-compartment model. The

200   pharmacokinetic parameters are summarised in Table 2. No statistically significant differences were

201   observed in S-enantiomer concentrations between treatments. Nor was there a significant difference

202   between two enantiomers in the RS-KET treatment. The $AUC_{0 \to \infty}$ for S-norketamine, after both

203   racemic ketamine and S-ketamine administration, was significantly higher when compared to R-

204   norketamine measured after injection of the racemic mixture.

205

206   **Discussion**

207   In this study, following IM administration of medetomidine, the pharmacokinetic parameters of S-

208   ketamine after a single IV injection of the racemic drug or the S-enantiomer alone, did not differ

209 significantly from one another. In addition, the pharmacokinetic results for the R-isomer did not

210 differ significantly from those of S-ketamine after RS-ketamine administration either.

211   The cardiovascular and respiratory effects observed following medetomidine and ketamine

212 administration are in accordance with those previously reported for protocols including ketamine

213 and medetomidine (Ko at al. 2001; Enouri et al. 2008). α_2-Adrenoceptor agonist administration

214 commonly causes bradycardia, as observed in our study. This results from both an increase in

215 systemic vascular resistance and a decrease in sympathetic tone (Pypendop & Verstegen 1998). In

216 the present study, it was not possible to evaluate the non-invasive blood pressure before RS-

217 ketamine or S-ketamine administration, due to the temperament of the dogs. However, the

218 administration of both the racemic mixture and S-ketamine induced an increase in HR, as

219 previously reported in dogs by Enouri et al. (2008). In the present study, the cardiovascular effects

220 of the two different anaesthetic protocols were comparable, as were the recovery times. In human

221 volunteers sedated with midazolam, S-ketamine provided faster recovery as compared to the

222 racemic mixture administered at twice the dose of S-ketamine alone (Doenicke et al. 1992).

223   In the present study, significantly more S-norketamine than R-norketamine was detected in

224 plasma. These findings disagree with those reported by Sandbaumhüter et al. (2016), who did not

225 find any statistically significant differences between the two enantiomers in beagle dogs. Moreover,

226 lower overall S-norketamine and R-norketamine concentrations were observed in the dogs included

227 in the present study when compared with dogs anaesthetized with sevoflurane (Romagnoli et al.

228 2017). Mechanical ventilation and sevoflurane may influence cardiovascular function and thus drug

229 disposition and elimination (Romagnoli et al. 2017). In the present study, all the dogs were sedated

230 with medetomidine which also has an influence on the ketamine metabolism (Kharasch et al. 1992;

231 Sandbaumhüter et al. 2016). Such variation might be related to a reduction of the cardiac output

232 (CO). In a previous study, Pypendop & Verstegen (1998) reported that medetomidine administered

233 to healthy dogs significantly reduced CO and may thereby reduce hepatic perfusion. Conversely,

234 Lawrence et al. (1996) reported that dexmedetomidine, the active enantiomer of medetomidine,

235 decreased renal blood flow by 25% but did not affect liver blood flow. Furthermore, Restitutti et al.

236 (2013), observed that dexmedetomidine induced changes in the blood flow of the abdominal organs,

237 especially in the kidneys. In the study reported by Sandbaumhüter et al. (2016), no significant

238 differences were detected in the metabolic profile of ketamine of urine when dogs were

239 administered either sevoflurane or medetomidine. Therefore, it seemed likely that both

240 medetomidine and sevoflurane could decrease ketamine elimination. The hepatic metabolism of

241 both ketamine and medetomidine is catalysed by) CYP3A4 and orthologs of CYP2C9 enzymes

242 (Capponi et al, 2009; Schmitz et al, 2010), and they have also shown an elevated affinity for

243 medetomidine in dogs (Duhamel et al. 2010). α_2-Adrenoceptor agonists inhibit CYP450 by means

244 of their imidazole ring which binds to the haem iron of CYP (Sandbaumhüter et al. 2015) and they

245 therefore slow the metabolism of drugs which use the same enzymatic pathway, as demonstrated *in*

246 *vitro*.

247   In this study, neither the V_c nor Cl_B of S-ketamine and R-ketamine differed significantly

248 from one another in dogs given racemic ketamine; nor did these parameters differ when the values

249 of S-ketamine were compared to racemic ketamine. Our findings were in accordance with those

250 previously reported in dogs anaesthetized with sevoflurane and given racemic ketamine or the S-

251 isomer (Romagnoli et al. 2017). They suggest the absence of stereoselectivity in the distribution and

252 clearance of ketamine enantiomers in dogs sedated with medetomidine. Similar results have

253 previously been obtained in ponies receiving racemic ketamine or S-ketamine and anesthetised with

254 isoflurane or sedated with xylazine (Larenza et al. 2007; 2008).

255   Lower $AUC_{0\to\infty}$ and C_{max} for R-norketamine compared to S-norketamine have already been

256 reported in ponies anaesthetized with isoflurane or xylazine (Larenza et al. 2007; 2008). Some

257 authors have hypothesized the existence of differences in protein binding between the two

258 enantiomers which could have influenced their renal clearance (Larenza et al. 2007). A previous *in*

259 *vitro* study has demonstrated that the co-administration of medetomidine and racemic ketamine

260 produced a stronger inhibition of the formation of R-norketamine when compared to S-

261 norketamine (Sandbaumhüter et al. 2015). In the present study, we hypothesize that medetomidine

262 inhibited racemic ketamine demethylation to R-norketamine in preference for S-norketamine. Since

263 norketamine metabolites were not evaluated, the preference of pharmacologically inactive R-

264 norketamine over the active S-norketamine hydroxylation cannot be excluded. However, the

265 pharmacokinetics of 6-hydroxynorketamine and dehydronorketamine enantiomers have already

266 been determined in dogs sedated with medetomidine (Sandbaumhuter et al. 2016). In that study, a

267 higher C_{max} of (2R,6R)-6-hydroxynorketamine as compared to (2S,6S)-6-hydroxynorketamine was

268 observed after administration of the racemic mixture; similar results were not found for 5,6-

269 dehydronorketamine. In the study of Sandbaumhüter et al. (2016), there were no differences

270 between R- and S-norketamine pharmacokinetics. However, in the present study inhibition of the

271 formation of the R-isoform over the S-isoform of norketamine was observed following

272 medetomidine administration. In canine clinical practice, the pharmacological effect of ketamine

273 metabolites, particularly S-norketamine, is still unclear. However, an analgesic effect similar to that

274 of racemic ketamine, was reported for this metabolite in a rodent model (Holtman et al. 2008). In

275 the present study, no analgesic evaluations were performed in beagle dog.

276   Both dexmedetomidine and levomedetomidine are potent *in vitro* inhibitors of the N-

277 demethylation of S- and R-ketamine to norketamine (Kharasch et al. 1992). Hence, racemic

278 medetomidine was expected by the author of the present study to be a more potent inhibitor than

279 dexmedetomidine alone. Additional studies are needed to evaluate the effects of dexmedetomidine

280 on ketamine and norketamine disposition, and pharmacokinetics with respect to medetomidine.

281   This study has some limitations; there was a high intra- and inter-individual variability of

282 cardiorespiratory variables. In addition, some dogs did not tolerate monitoring devices being

283 attached while awake, and thus some measurements before and after sedation and anaesthesia were

284 not performed. As the variability was higher than expected, the study was underpowered with

285 regards to cardiorespiratory measurements and thus could not detect whether there were any

286   differences between treatments. Moreover, the exclusion of one dog from the statistical analysis

287   could result in a low statistical power, therefore increasing the risk of beta error.

288   Conclusions

289   This study confirmed that the distribution and clearance of ketamine enantiomers, when combined

290   with medetomidine, were not stereoselective in dogs administered racemic ketamine or the S-

291   isoform alone. However, the metabolism of ketamine was inhibited, as demonstrated by low

292   norketamine concentrations with R-norketamine being the most affected. Despite these differences

293   in metabolite disposition, no significant differences between the two treatments were observed

294   regarding the cardiopulmonary variables studied.

295

References

Bergman SA (1999) Ketamine: review of its pharmacology and its use in pediatric anesthesia. Anesth Prog 46, 10-20.

Capponi L, Schmitz A, Thormann W et al, (2009). In vitro evaluation of differences in phase 1 metabolism of ketamine and other analgesics among humans, horses, and dogs. Am J Vet Res. 70:7 77-86.

Doenicke A, Kugler J, Mayer M et al. (1992) Ketamine racemate or S-(+)-ketamine and midazolam. The effect on vigilance, efficacy and subjective findings. Anaesthesist 41, 610-618.

Duhamel MC, Troncy E, Beaudry F (2010) Metabolic stability and determination of cytochrome P450 isoenzymes' contribution to the metabolism of medetomidine in dog liver microsomes. Biomed Chromatogr 24, 868-877.

Duque JC, Oleskovicz N, Guirro EC et al. (2008) Relative potency of ketamine and S(+)-ketamine in dogs. J Vet Pharmacol Ther 31, 344-348.

Enouri SS, Kerr CL, McDonell WN et al. (2008) Cardiopulmonary effects of anesthetic induction with thiopental, propofol, or a combination of ketamine hydrochloride and diazepam in dogs sedated with a combination of medetomidine and hydromorphone. Am J Vet Res 69, 586-595.

Flecknell PA (1993) Anaesthesia of animals for biomedical research. Br J Anaest 71, 885-894.

Holtman JR Jr, Crooks PA, Johnson-Hardy JK et al. (2008) Effects of norketamine enantiomers in rodent models of persistent pain. Pharmacol Biochem Behav 90: 676-685.

Ihmsen H, Geisslinger G, Schüttler J (2001) Stereoselective pharmacokinetics of ketamine: R(−)-Ketamine inhibits the elimination of S(+)-ketamine. Clin Pharmcol Ther 70, 431-438.

Kharasch ED, Herrmann S, Labroo R (1992) Ketamine as a probe for medetomidine stereoisomer inhibition of human liver microsomal drug metabolism. Anesthesiology 77, 1208-1214.

321   Kharasch ED, Hill HF, Eddy AC (1991) Influence of dexmedetomidine and clonidine on

322   human liver microsomal alfentanil metabolism. Anesthesiology 75, 520-524.

323   Kharasch ED, Labroo R (1992) Metabolism of ketamine stereoisomers by human liver

324   microsomes. Anesthesiology 77, 1201-1207.

325   Ko Jc, Fox SM, Mandsager RE (2001) Anesthetic effects of ketamine or isoflurane induction prior

326   to isoflurane anesthesia in medetomidine-premedicated dogs. J Am Anim Hosp Assoc 37, 411-419.

327   Larenza MP, Landoni MF, Levionnois OL et al. (2007) Stereoselective pharmacokinetics of

328   ketamine and norketamine after racemic ketamine or S-ketamine administration during isoflurane

329   anaesthesia in Shetland ponies. Br J Anaesth 98, 204-212.

330   Larenza MP, Knobloch M, Landoni MF et al. (2008) Stereoselective pharmacokinetics of

331   ketamine and norketamine after racemic ketamine or S-ketamine administration in Shetland ponies

332   sedated with xylazine. Vet J 177, 432-435.

333   Larenza MP, Peterbauer C, Landoni MF et al. (2009) Stereoselective pharmacokinetics of

334   ketamine and norketamine after constant rate infusion of a subanesthetic dose of racemic ketamine

335   or S-ketamine in Shetland ponies. Am J Vet Res 70, 831-839.

336   Lawrence CJ, Prinzen FW, de Lange S (1996) The effect of dexmedetomidine on nutrient

337   organ blood flow. Anesth Analg 83, 1160-1165.

338   Pinheiro J, Bates D, DebRoy S et al. (2018). nlme: Linear and Nonlinear Mixed Effects

339   Models_. R package version 3.1-137. https://CRAN.R-project.org/package=nlme

340   Pypendop BH, Verstegen JP (1998) Hemodynamic effects of medetomidine in the dog: a dose

341   titration study. Vet Surg 27, 612-622.

342   R Core Team (2018). R: A language and environment for statistical computing. R Foundation

343   for Statistical Computing. https://www.R-project.org/.

344   Restitutti F, Laitinen MR, Raekallio MR et al. (2013) Effect of MK-467 on organ blood flow

345   parameters detected by contrast-enhanced ultrasound in dogs treated with dexmedetomidine. VAA

346   40, e48-e60.

347   Romagnoli N, Bektas RN, Kutter AP et al. (2017) Pharmacokinetics of ketamine and

348   norketamine enantiomers after racemic or S-ketamine IV bolus administration in dogs during

349   sevoflurane anaesthesia. Res Vet Sci 112, 208-213.

350   Sandbaumhüter FA, Theurillat R, Thormann W (2015) Effects of medetomidine and its active

351   enantiomer dexmedetomidine on N-demethylation of ketamine in canines determined in vitro using

352   enantioselective capillary electrophoresis. Electrophoresis 36, 2703-2712.

353   Sandbaumhüter FA, Theurillat R, Bektas RN et al. (2016) Pharmacokinetics of ketamine and

354   three metabolites in Beagle dogs under sevoflurane vs. medetomidine comedication assessed by

355   enantioselective capillary electrophoresis. J Chromatogr A 1467, 436-444.

356   Schmitz A, Thormann W, Moessner L, Theurillat R, Helmja K, Mevissen M. (2010)

357   Enantioselective CE analysis of hepatic ketamine metabolism in different species in vitro.

358   Electrophoresis. 31:1506-16.

359   Stekhoven DJ (2013) missForest: Nonparametric Missing Value Imputation using Random

360   Forest. R package version 1.4.

361   Ueyama Y, Waselau AC, Wiese AJ et al. (2008) Anesthetic and cardiopulmonary effects of

362   intramuscular morphine, medetomidine, ketamine injection in dogs. Vet Anaesth Analg 35, 480-

363   487.

364   Yamaoka K, Nakagawa T, Uno T (1978) Application of Akaike's information criterion (AIC)

365   in the evaluation of linear pharmacokinetic equations. J Pharmacokinet Biopharm 6, 165-175.

366   Zeileis A, Hothorn T (2002) Diagnostic Checking in Regression Relationships. R News 2, 7-

367   10. https://CRAN.R-project.org/doc/Rnews/

368

1   **Figure 1**. Mean plasma concentrations (error bars represent standard deviation) of R-ketamine (R-

2   ket) and S-ketamine (S-ket) for the RS-KET treatment graph A and S-ketamine (S-ket) for the S-

3   KET treatment graph B after the administration of racemic ketamine 4 mg kg^{-1} or S-ketamine 2 mg

4   kg^{-1}, respectively, to five dogs sedated with medetomidine (450 μg m^{-2}).

5

6   **Figure 2**. Mean plasma concentrations (error bars represent standard deviation) of R-norketamine

7   (R-nor) and S-norketamine (S-nor) in the RS-KET treatment graph A and S-norketamine (S-nor) in

8   the S-KET treatment graph B, after the administration of racemic ketamine 4 mg kg^{-1} or S-ketamine

9   2 mg kg^{-1}, respectively, to five dogs sedated with medetomidine (450 μg m^{-2}).

10

1 **Table 2.** Mean values ± standard deviation of the pharmacokinetic parameters of ketamine and

2 norketamine enantiomers in plasma samples obtained from five dogs sedated with medetomidine

3 and an intravenous bolus of S-ketamine (S-KET) or racemic ketamine (RS-KET) (refer to Table 1

4 for drugs doses administered). Area under the curve to infinity (AUC0-∞); half-life of the

5 distribution phase ($T_{1/2}$ dis); (half-life of the elimination phase ($T_{1/2}$ el); rate constants of the

6 elimination phase (K_{el}); mean residence time (MRT); body clearance (Cl_B); volume of distribution

7 for the central compartment (Vc); peak concentration (Cmax); time of peak concentration (Tmax).

8 Significant difference between the treatments (*) and within the groups (†), $p < 0.05$.

9

Treatment	S-KET	RS-KET	
Compound	S-ketamine	S-ketamine	R-ketamine
$AUC_{0\to\infty}$ (µg minutes mL^{-1})	37.21 ± 5.28	36.83 ± 13.00	35.58 ± 12.15
$T_{1/2dis}$ (minutes)	2.47 ± 1.09	2.44 ± 1.39	2.52 ± 1.35
$T_{1/2el}$ (minutes)	43.77 ± 20.12	46.02 ± 18.51	46.74 ± 18.08
k_{el} (1 minute^{-1})	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01
MRT (minutes)	45.00 ± 20.23	45.25 ± 16.00	46.39 ± 15.38
Cl_B (mL minute^{-1} kg^{-1})	54.62 ± 7.73	59.82 ± 20.94	61.78 ± 21.76
V_c (L kg^{-1})	1.79 ± 0.69	1.94 ± 0.52	2.03 ± 0.57
	S-norketamine	S-norketamine	R-norketamine
$AUC_{0\to\infty}$ (µg*minutes mL^{-1})	3.27 ± 0.94*	3.29 ± 0.86†	1.45 ± 0.25*†'
Cmax (µg mL^{-1})	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Tmax (minutes)	6.00 ± 2.24	7.40 ± 5.13	8.40 ± 6.14

1 **Table 1.** Mean ± standard deviation values of heart rate (HR), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial

2 pressure (MAP), respiratory rate (f_R), end-tidal carbon dioxide ($PE'CO_2$), glucose, lactate plasma concentrations and haemoglobin (Hb) in six beagle

3 dogs. The dogs were sedated with medetomidine (450 µg m^{-2}) and given a bolus of S-Ketamine [S-KET (2 mg kg^{-1})] or racemic ketamine [RS-KET
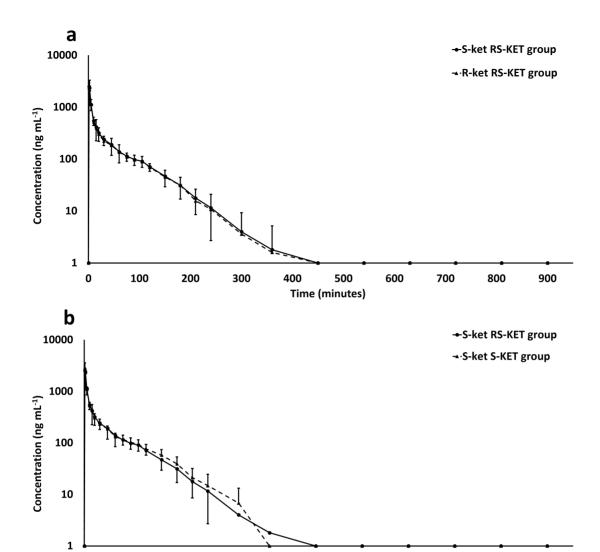
4 (4 mg kg^{-1})] 20 minutes after medetomidine administration (T0).

5

Time (minutes)	Treatment	Baseline	0	1	2	5	10	15	20	30
HR (beats minute^{-1})	S-KET	103 ± 16	48 ± 3	82 ± 16	82 ± 21	84 ± 8	75 ± 19	68 ± 21	60 ± 7	58 ± 18
	RS-KET	108 ± 19	53 ± 9	100 ± 9	108 ± 23	86 ± 9	74 ± 7	60 ± 7	54 ± 5	54 ± 5
SAP (mmHg)	S-KET			197 ± 0	197 ± 40	151 ± 25	133 ± 36	145 ± 46	137 ± 35	147 ± 5
	RS-KET				154 ± 45	162 ± 38	153 ± 26	159 ± 27	158 ± 21	141 ± 13
DAP (mmHg)	S-KET			137 ± 0	118 ± 42	98 ± 30	84 ± 23	81 ± 23	86 ± 27	10 ± 8
	RS-KET				97 ± 43	96 ± 24	96 ± 33	96 ± 24	99 ± 23	82 ± 21
MAP (mmHg)	S-KET			161 ± 0	144 ± 40	120 ± 29	102 ± 29	107 ± 33	107 ± 29	121 ± 5
	RS-KET				116 ± 39	126 ± 34	117 ± 30	120 ± 23	122 ± 20	105 ± 17
f_R (breaths minute^{-1})	S-KET	28 ± 5	19 ± 5	8 ± 7	9 ± 6	10 ± 6	17 ± 7	22 ± 7	23 ± 4	24 ± 9
	RS-KET	25 ± 7	20 ± 6	13 ± 12	8 ± 8	7 ± 4	12 ± 5	23 ± 20	23 ± 8	28 ± 6
$PE'CO_2$ (kPa)	S-KET				4.4 ± 1.3	6.3 ± 0.3	5.6 ± 1.9			
	RS-KET				5.8 ± 1.5	7.0 ± 0.4	6.6 ± 0.6	6.1 ± 0.0		
$PE'CO_2$ (mmHg)	S-KET				33.0 ± 9.9	47.6 ± 2.3	42.0 ± 14.2			
	RS-KET				43.3 ± 11.0	52.2 ± 3.0	49.3 ± 4.2	46.0 ± 0.0		
Glucose (mmol L^{-1})	S-KET		4.2 ± 0.4			4.5 ± 0.7				4.7 ±1.0
	RS-KET		4.6 ± 0.8			4.3 ± 0.7				4.6 ± 0.7
Lactate (mmol L^{-1})	S-KET		5.4 ± 3.7			2.3 ± 0.5				2.0 ± 1.1
	RS-KET		2.4 ± 1.1			1.8 ± 0.9				2.5 ± 0.3
Hb (g dL^{-1})	S-KET		14.4 ± 1.2			13.4 ± 1.5				11.9* ± 1.2
	RS-KET		13.6 ± 1.9			13.8 ± 1.0				13.7 ± 0.9

6 (*): significant difference between the treatments, $p < 0.05$.

a

b

a

b