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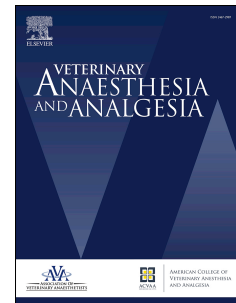
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RESEARCH STUDY

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

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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.

Objective To assess the differences in the pharmacokinetic profiles of S-ketamine, R-ketamine, and their metabolites S-norketamine and R-norketamine, and to measure relevant physiologic variables after intravenous administration of racemic ketamine or S-ketamine alone in beagle dogs sedated with medetomidine.

Study design Experimental, blinded, randomized crossover study.

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

Methods Medetomidine (450 mcg m^{-2}) was administered intramuscularly, followed 20 minutes later by either S-ketamine (2 mg kg^{-1}) or racemic (RS) ketamine (4 mg kg^{-1}) both administered intravenously. Blood samples were collected before medetomidine administration, and at multiple timepoints 1 to 900 minutes following the ketamine administration. Plasma samples were analysed using liquid chromatography-tandem mass spectrometry. Heart rate, respiratory rate, non-invasive blood pressure, haemoglobin saturation with oxygen in (SpO_2) and body temperature were measured at baseline, before ketamine administration, and 1, 2, 5, 10, 15, 20 and 30 minutes after ketamine administration. All cardiovascular variables, blood glucose, haemoglobin and lactate concentrations were analysed using different linear mixed effects models; the significance was set at $p < 0.05$.

Results S-ketamine showed a two-compartment kinetic profile; no statistically significant differences were observed between its concentrations or in the calculated pharmacokinetic parameters following S- or RS-ketamine. When the racemic mixture was administered, no differences were detected between R- and S-ketamine concentrations, but the area under the curve (AUC) for R-norketamine was significantly lower when compared to that of S-norketamine. Clinically relevant physiologic variables did not show statistically significant differences following the administration of the racemic mixture or of S-ketamine alone.

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, pharmacokinetics, s-ketamine, s-norketamine

Introduction

Ketamine is a dissociative anaesthetic, widely used in human and veterinary anaesthesia. It is a racemic mixture of two optical isomers, R- and S-ketamine, which have different pharmacological effects (Bergman 1999). S-ketamine is available for dogs in some European countries. In humans, the relative potency of S-ketamine is twice that of the racemic form, and the loss of response to verbal commands is seen at half the dose of S-ketamine as compared to racemic ketamine (Ihmsen et al. 2001). In species such as the dog and pony, the clearance of S-ketamine administered alone is higher than that of S-ketamine or R-ketamine administered in the racemic form (Ihmsen et al. 2001; Duque et al. 2008; Larenza et al. 2009). This explains the faster recovery seen in patients anaesthetized with the S-enantiomer alone (Ihmsen et al. 2001; Duque et al. 2008).

Clinically relevant physiologic functions are usually well maintained with ketamine when in combination with moderate doses of α_2 -adrenoceptor agonists. Studies in ponies and horses (Filzek et al. 2003; Larenza et al. 2007), however, have shown some differences in the cardiopulmonary effects related to the stereoselectivity of ketamine.

The combination of racemic ketamine with α_2 -adrenoceptor agonists has been widely used in dogs to induce sedation and anaesthesia (Ueyema et al. 2008). This drug combination produces an adequate quality and duration of sedation and anaesthesia for minor medical and surgical procedures. α_2 -Adrenoceptor agonists alter the metabolism of other co-administered drugs, such as opioids and ketamine *in vitro*, mainly via an interaction with cytochrome P (CYP) enzymes (Kharasch et al. 1991; Sandbaumhüter et al. 2015). This interaction can influence the intensity and duration of the effects of ketamine.

The aims of this study were to obtain the pharmacokinetic profiles of S-ketamine and R-ketamine, and their major metabolites, S-norketamine and R-norketamine, in healthy beagles sedated with intramuscular (IM) medetomidine after racemic ketamine or S-ketamine intravenous (IV) administration. Clinically relevant physiologic variables were also compared.

We hypothesized that the pharmacokinetics of ketamine's enantiomers and its metabolites, when combined with medetomidine, were not stereoselective in dogs given racemic ketamine or the S-isoform alone.

Material and Methods

Animals

The trial was approved by the committee for Animal Experimentation of XXX 67/2011. A total of six healthy adult beagle dogs, three females and three males (non-castrated), 21 ± 11 [mean \pm standard deviation (SD)] months of age, weighing 15.0 ± 1.1 (mean \pm SD) kg, were used in the study. A *post-hoc* power calculation on the pharmacokinetic (PK) data was used to verify the adequacy of the number of dogs included, and the power was 99.5% for six dogs.

Complete blood cell count and blood chemistry were checked two days before the experiment. The dogs were fasted overnight but always had free access to water.

Drug administration and Monitoring

This was a blinded, randomized crossover trial with a 3-week washout period between treatments. A random order generator (GraphPad Software, CA, USA) determined treatment allocation. The study was performed following good clinical practice guidelines (Flecknell, 1993).

Following standard aseptic preparation, two peripheral venous catheters (Surflo IV Catheter 22-gauge, Terumo, Belgium) were inserted into both cephalic veins of each dog, one catheter for drug administration, and the other as a reserve. An additional catheter was placed in the jugular vein (16-gauge, 16 cm long, Blue Flex Tip Catheter, Arrow International, Teleflex Medical GmbH, Switzerland) following infiltration of lidocaine (1 mL; Lidocain 2%, Streuli, Switzerland). This catheter was used for central venous blood sampling. The catheter for drug administration was attached to a lactated ringer infusion (Ringer-Lactat Fresenius; Fresenius Kabi AG, Switzerland) at a rate of $5 \text{ mL kg}^{-1} \text{ hour}^{-1}$ for 30 minutes before anaesthesia induction. The dogs were sedated with medetomidine administered IM at a dose rate of $450 \mu\text{g m}^{-2}$ (approximately $17 \mu\text{g kg}^{-1}$ in a 15 kg

dog, Dorbene, Fort Dodge, Italy). After 20 minutes, S-ketamine 2 mg kg⁻¹ (Keta-S; Dr. E. Graeb AG, Switzerland) (S-KET treatment) or racemic ketamine 4 mg kg⁻¹ (Ketasol-100; Dr. E. Graeb AG, Switzerland) (RS-KET treatment) were rapidly administered IV over 1-2 seconds.

Once anaesthesia was induced (identified by loss of laryngeal reflex), the dogs were intubated and allowed to breathe room air (fraction of inspired oxygen (FiO₂) = 0.21). A multiparameter monitor (BN 850, GE Medical Systems, Anandic Medical Systems AG, Switzerland) was used to monitor anaesthesia. The following measurements were taken: heart rate (HR) from an electrocardiogram, respiratory rate (f_R), indirect arterial blood pressures (systolic: SAP, mean: MAP, diastolic: DAP), haemoglobin (Hb) saturation with oxygen in % (SpO₂), end-tidal carbon dioxide partial pressure end-tidal CO₂ (PE'CO₂) and body temperature (T°). The variables listed above were recorded at baseline [before medetomidine administration = timepoint (T-22)], at T-1 (20 minutes after medetomidine injection and prior to ketamine administration), and 1, 2, 5, 10, 15, 20 and 30 minutes after the ketamine administration. Before ketamine administration, and 5 and 30 minutes after ketamine injection, blood glucose (Contour, Bayer AG Healthcare, Switzerland), lactate (Accutrend, Roche Diagnostics, Switzerland) and haemoglobin concentration (Hemocue Hb201+, Baumann Medical AG, Switzerland) were measured.

The peripheral venous catheters were removed 30 minutes after drug administration., the dogs were given 4 mg kg⁻¹ carprofen (Rimadyl ad us. Vet, Pfizer AG, Switzerland) IV 4 hours after drug administration. The dogs were observed by veterinarians and offered a commercial diet once they were fully awake. During and after anaesthesia, the dogs' body temperatures were maintained between 37.0-38.5°C using warm water blankets (Hico-Aquatherm 660, Nufer Medical, Switzerland), a heat and moisture exchanger (HMEF1000, Anandic Medical Systems AG, Switzerland) and a forced air-patient warming system (Bair Hugger Model 505, Carbamed, Switzerland) as needed. After taking the last blood sample, the jugular catheter was removed.

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- d_4 and Norketamine- d_4 , purchased from Sigma-Aldrich,
122 MO, USA), 150 μ L of plasma were extracted with methanol and centrifuged. The supernatant was
123 filtered through a 0.2 μ m 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 3 μ m Cellulose- β 3 (150 x 2,00 mm, 3,0 μ m) 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-

D4: 242 \rightarrow 129 and 183 m/z , Norketamine- D4: 228 \rightarrow 129 and 211 m/z).

The analytical method was validated in accordance with EMEA/CHMP/EWP/192217/2009 guidelines at the beginning of the experiment. Linearity was satisfactory ($R^2 > 0.99$) over a range extending from 15 to 15,000 ng mL⁻¹ for both S-Ketamine and R-Ketamine, and from 15 to 3000 ng mL⁻¹ for the norketamine enantiomers. The lower limit of quantification (LLOQ) was 15 ng mL⁻¹ for all target compounds; inter- and intra-day accuracy and precision were both below 10% for all the analytes.

Pharmacokinetics and statistical analysis

The aim of the statistical analysis was to detect potential differences in the repeated measurements of the cardiopulmonary variables, Hb, lactate and glucose concentrations between the two treatments. For each of the outcome variables, different linear mixed effects models were run. In all models, dog was included as a random intercept to account for potential clustering within animals. In contrast, in the different models, time was included either as a fixed effect, with or without an interaction term with treatment, or as a random slope. The treatments were included as a fixed effect and omitted in the null model. Model selection was based on Akaike's Information Criterion (AIC) and on likelihood ratio tests which provided the p -values.

The analysis was carried out using R software (R Core Team 2018) and the packages: nlme (Pinheiro et al. 2018) and lmerTest (Zeileis & Hothorn T 2002). Based on the assumption of missing at random, the missing values were inputted using the package missForest (Stekhoven 2013). All cardiopulmonary variables, Hb, lactate and glucose concentrations are reported as mean \pm SD.

The R-ketamine and S-ketamine concentration *versus* time curves were analysed for each individual by XY plot using WinNonlin 6.3 (Pharsight Corporation, CA, USA).

The plasma drug concentrations obtained after IV administration were fitted using the following equation:

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

All pharmacokinetic parameters are reported as mean \pm SD, and were determined using WinNonlin 6.3 (Pharsight Corporation, CA, USA). The individual plasma concentration *versus* time curves were fitted, and the best compartment model was determined by application of the AIC (Yamaoka et al. 1978).

The following pharmacokinetic parameters were calculated for each dog for the ketamine enantiomers: area under the curve to infinity ($AUC_{0\rightarrow\infty}$), half-life of the distribution phase ($T_{1/2dis}$), half-life of the elimination phase ($T_{1/2el}$), rate constants of the elimination phase (K_{el}), mean residence time (MRT), total body clearance (Cl_B), volume of distribution of the central compartment (V_c), peak concentration (C_{max}) and time of peak concentration (T_{max}). For S-norketamine and R-norketamine, non-compartmental analysis was used to determine the $AUC_{0\rightarrow\infty}$, peak metabolite concentration (C_{max}) and time of peak metabolite concentration (T_{max}). The Wilcoxon signed rank test was used to detect differences between the treatments concerning the pharmacokinetic parameters with a significance level of $p < 0.05$.

Results

All the dogs enrolled in the study also finished the study and recovered without any complications. The dogs came from the pool of experimental dogs of the Vetsuisse Faculty of the University of Zürich, where they returned 24 hours after the end of the study.

Sedation was considered to be profound following medetomidine administration, and S- and racemic ketamine were injected according to the scheduled times. Intubation was judged to be easy in four dogs in the RS-KET treatment and two dogs in the S-KET treatment, and less easy but possible in the remaining dogs. In the RS-KET, treatment one dog showed several episodes of muscle shaking 3.5 minutes following drug administration. All dogs recovered well from the anaesthesia and were standing within 37.9 ± 16.9 (mean \pm SD) minutes in the S-KET treatment and 42.9 ± 19.6 (mean \pm SD) minutes in the RS-KET treatment. No significant difference was detected between treatments.

183 The cardiovascular variables are summarised in Table 1. The SpO₂ 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'CO₂), 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° ($p = 0.317$), SpO₂ $p = 0.967$
 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→∞} 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

significantly from one another. In addition, the pharmacokinetic results for the R-isomer did not differ significantly from those of S-ketamine after RS-ketamine administration either.

The cardiovascular and respiratory effects observed following medetomidine and ketamine administration are in accordance with those previously reported for protocols including ketamine and medetomidine (Ko et al. 2001; Enouri et al. 2008). α_2 -Adrenoceptor agonist administration commonly causes bradycardia, as observed in our study. This results from both an increase in systemic vascular resistance and a decrease in sympathetic tone (Pypendop & Verstegen 1998). In the present study, it was not possible to evaluate the non-invasive blood pressure before RS-ketamine or S-ketamine administration, due to the temperament of the dogs. However, the administration of both the racemic mixture and S-ketamine induced an increase in HR, as previously reported in dogs by Enouri et al. (2008). In the present study, the cardiovascular effects of the two different anaesthetic protocols were comparable, as were the recovery times. In human volunteers sedated with midazolam, S-ketamine provided faster recovery as compared to the racemic mixture administered at twice the dose of S-ketamine alone (Doenicke et al. 1992).

In the present study, significantly more S-norketamine than R-norketamine was detected in plasma. These findings disagree with those reported by Sandbaumhüter et al. (2016), who did not find any statistically significant differences between the two enantiomers in beagle dogs. Moreover, lower overall S-norketamine and R-norketamine concentrations were observed in the dogs included in the present study when compared with dogs anaesthetized with sevoflurane (Romagnoli et al. 2017). Mechanical ventilation and sevoflurane may influence cardiovascular function and thus drug disposition and elimination (Romagnoli et al. 2017). In the present study, all the dogs were sedated with medetomidine which also has an influence on the ketamine metabolism (Kharasch et al. 1992; Sandbaumhüter et al. 2016). Such variation might be related to a reduction of the cardiac output (CO). In a previous study, Pypendop & Verstegen (1998) reported that medetomidine administered to healthy dogs significantly reduced CO and may thereby reduce hepatic perfusion. Conversely, Lawrence et al. (1996) reported that dexmedetomidine, the active enantiomer of medetomidine,

decreased renal blood flow by 25% but did not affect liver blood flow. Furthermore, Restitutti et al. (2013), observed that dexmedetomidine induced changes in the blood flow of the abdominal organs, especially in the kidneys. In the study reported by Sandbaumbüter et al. (2016), no significant differences were detected in the metabolic profile of ketamine of urine when dogs were administered either sevoflurane or medetomidine. Therefore, it seemed likely that both medetomidine and sevoflurane could decrease ketamine elimination. The hepatic metabolism of both ketamine and medetomidine is catalysed by) CYP3A4 and orthologs of CYP2C9 enzymes (Capponi et al, 2009; Schmitz et al, 2010), and they have also shown an elevated affinity for medetomidine in dogs (Duhamel et al. 2010). α_2 -Adrenoceptor agonists inhibit CYP450 by means of their imidazole ring which binds to the haem iron of CYP (Sandbaumbüter et al. 2015) and they therefore slow the metabolism of drugs which use the same enzymatic pathway, as demonstrated *in vitro*.

In this study, neither the V_c nor Cl_B of S-ketamine and R-ketamine differed significantly from one another in dogs given racemic ketamine; nor did these parameters differ when the values of S-ketamine were compared to racemic ketamine. Our findings were in accordance with those previously reported in dogs anaesthetized with sevoflurane and given racemic ketamine or the S-isomer (Romagnoli et al. 2017). They suggest the absence of stereoselectivity in the distribution and clearance of ketamine enantiomers in dogs sedated with medetomidine. Similar results have previously been obtained in ponies receiving racemic ketamine or S-ketamine and anesthetised with isoflurane or sedated with xylazine (Larenza et al. 2007; 2008).

Lower $AUC_{0 \rightarrow \infty}$ and C_{max} for R-norketamine compared to S-norketamine have already been reported in ponies anaesthetized with isoflurane or xylazine (Larenza et al. 2007; 2008). Some authors have hypothesized the existence of differences in protein binding between the two enantiomers which could have influenced their renal clearance (Larenza et al. 2007). A previous *in vitro* study has demonstrated that the co-administration of medetomidine and racemic ketamine produced a stronger inhibition of the formation of R-norketamine when compared to S-

norketamine (Sandbaumhüter et al. 2015). In the present study, we hypothesize that medetomidine inhibited racemic ketamine demethylation to R-norketamine in preference for S-norketamine. Since norketamine metabolites were not evaluated, the preference of pharmacologically inactive R-norketamine over the active S-norketamine hydroxylation cannot be excluded. However, the pharmacokinetics of 6-hydroxynorketamine and dehydronorketamine enantiomers have already been determined in dogs sedated with medetomidine (Sandbaumhüter et al. 2016). In that study, a higher C_{max} of (2R,6R)-6-hydroxynorketamine as compared to (2S,6S)-6-hydroxynorketamine was observed after administration of the racemic mixture; similar results were not found for 5,6-dehydronorketamine. In the study of Sandbaumhüter et al. (2016), there were no differences between R- and S-norketamine pharmacokinetics. However, in the present study inhibition of the formation of the R-isoform over the S-isoform of norketamine was observed following medetomidine administration. In canine clinical practice, the pharmacological effect of ketamine metabolites, particularly S-norketamine, is still unclear. However, an analgesic effect similar to that of racemic ketamine, was reported for this metabolite in a rodent model (Holtman et al. 2008). In the present study, no analgesic evaluations were performed in beagle dog.

Both dexmedetomidine and levomedetomidine are potent *in vitro* inhibitors of the N-demethylation of S- and R-ketamine to norketamine (Kharasch et al. 1992). Hence, racemic medetomidine was expected by the author of the present study to be a more potent inhibitor than dexmedetomidine alone. Additional studies are needed to evaluate the effects of dexmedetomidine on ketamine and norketamine disposition, and pharmacokinetics with respect to medetomidine.

This study has some limitations; there was a high intra- and inter-individual variability of cardiorespiratory variables. In addition, some dogs did not tolerate monitoring devices being attached while awake, and thus some measurements before and after sedation and anaesthesia were not performed. As the variability was higher than expected, the study was underpowered with 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, McDonnell 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 Anaesth* 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 Pharmacol 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 ($450 \mu\text{g m}^{-2}$).
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 ($450 \mu\text{g m}^{-2}$).
10

Table 2. Mean values \pm standard deviation of the pharmacokinetic parameters of ketamine and norketamine enantiomers in plasma samples obtained from five dogs sedated with medetomidine and an intravenous bolus of S-ketamine (S-KET) or racemic ketamine (RS-KET) (refer to Table 1 for drugs doses administered). Area under the curve to infinity ($AUC_{0-\infty}$); half-life of the distribution phase ($T_{1/2\text{dis}}$); (half-life of the elimination phase ($T_{1/2\text{el}}$); rate constants of the elimination phase (K_{el}); mean residence time (MRT); body clearance (Cl_B); volume of distribution for the central compartment (V_c); peak concentration (C_{max}); time of peak concentration (T_{max}). Significant difference between the treatments (*) and within the groups (\dagger), $p < 0.05$.

Treatment	S-KET	RS-KET	
Compound	S-ketamine	S-ketamine	R-ketamine
$AUC_{0 \rightarrow \infty}$ ($\mu\text{g minutes mL}^{-1}$)	37.21 ± 5.28	36.83 ± 13.00	35.58 ± 12.15
$T_{1/2\text{dis}}$ (minutes)	2.47 ± 1.09	2.44 ± 1.39	2.52 ± 1.35
$T_{1/2\text{el}}$ (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 ($\text{mL minute}^{-1} \text{ kg}^{-1}$)	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
$AUC_{0 \rightarrow \infty}$ ($\mu\text{g*minutes mL}^{-1}$)	$3.27 \pm 0.94^*$	$3.29 \pm 0.86^\dagger$	$1.45 \pm 0.25^{*\dagger}$
C_{max} ($\mu\text{g mL}^{-1}$)	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
T_{max} (minutes)	6.00 ± 2.24	7.40 ± 5.13	8.40 ± 6.14

Table 1. Mean \pm standard deviation values of heart rate (HR), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), respiratory rate (f_R), end-tidal carbon dioxide ($PE'CO_2$), glucose, lactate plasma concentrations and haemoglobin (Hb) in six beagle dogs. The dogs were sedated with medetomidine ($450 \mu\text{g m}^{-2}$) and given a bolus of S-Ketamine [S-KET (2 mg kg^{-1})] or racemic ketamine [RS-KET (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 \pm 16	48 \pm 3	82 \pm 16	82 \pm 21	84 \pm 8	75 \pm 19	68 \pm 21	60 \pm 7	58 \pm 18
	RS-KET	108 \pm 19	53 \pm 9	100 \pm 9	108 \pm 23	86 \pm 9	74 \pm 7	60 \pm 7	54 \pm 5	54 \pm 5
SAP (mmHg)	S-KET			197 \pm 0	197 \pm 40	151 \pm 25	133 \pm 36	145 \pm 46	137 \pm 35	147 \pm 5
	RS-KET				154 \pm 45	162 \pm 38	153 \pm 26	159 \pm 27	158 \pm 21	141 \pm 13
DAP (mmHg)	S-KET			137 \pm 0	118 \pm 42	98 \pm 30	84 \pm 23	81 \pm 23	86 \pm 27	10 \pm 8
	RS-KET				97 \pm 43	96 \pm 24	96 \pm 33	96 \pm 24	99 \pm 23	82 \pm 21
MAP (mmHg)	S-KET			161 \pm 0	144 \pm 40	120 \pm 29	102 \pm 29	107 \pm 33	107 \pm 29	121 \pm 5
	RS-KET				116 \pm 39	126 \pm 34	117 \pm 30	120 \pm 23	122 \pm 20	105 \pm 17
f_R (breaths minute ⁻¹)	S-KET	28 \pm 5	19 \pm 5	8 \pm 7	9 \pm 6	10 \pm 6	17 \pm 7	22 \pm 7	23 \pm 4	24 \pm 9
	RS-KET	25 \pm 7	20 \pm 6	13 \pm 12	8 \pm 8	7 \pm 4	12 \pm 5	23 \pm 20	23 \pm 8	28 \pm 6
$PE'CO_2$ (kPa)	S-KET				4.4 \pm 1.3	6.3 \pm 0.3	5.6 \pm 1.9			
	RS-KET				5.8 \pm 1.5	7.0 \pm 0.4	6.6 \pm 0.6	6.1 \pm 0.0		
$PE'CO_2$ (mmHg)	S-KET				33.0 \pm 9.9	47.6 \pm 2.3	42.0 \pm 14.2			
	RS-KET				43.3 \pm 11.0	52.2 \pm 3.0	49.3 \pm 4.2	46.0 \pm 0.0		
Glucose (mmol L ⁻¹)	S-KET		4.2 \pm 0.4			4.5 \pm 0.7				4.7 \pm 1.0
	RS-KET		4.6 \pm 0.8			4.3 \pm 0.7				4.6 \pm 0.7
Lactate (mmol L ⁻¹)	S-KET		5.4 \pm 3.7			2.3 \pm 0.5				2.0 \pm 1.1
	RS-KET		2.4 \pm 1.1			1.8 \pm 0.9				2.5 \pm 0.3
Hb (g dL ⁻¹)	S-KET		14.4 \pm 1.2			13.4 \pm 1.5				11.9* \pm 1.2
	RS-KET		13.6 \pm 1.9			13.8 \pm 1.0				13.7 \pm 0.9

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

