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Stereoselective pharmacokinetics of ketamine and norketamine after racemic ketamine or S-ketamine administration during isoflurane anaesthesia in Shetland ponies

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Background. The arterial pharmacokinetics of ketamine and norketamine enantiomers after racemic ketamine or S-ketamine i.v. administration were evaluated in seven gelding ponies in a crossover study (2-month interval).

Methods. Anaesthesia was induced with isoflurane in oxygen via a face-mask and then maintained at each pony's individual MAC. Racemic ketamine (2.2 mg kg^{-1}) or S-ketamine (1.1 mg kg^{-1}) was injected in the right jugular vein. Blood samples were collected from the right carotid artery before and at 1, 2, 4, 8, 16, 32, 64, and 128 min after ketamine administration. Ketamine and norketamine enantiomer plasma concentrations were determined by capillary electrophoresis. Individual R-ketamine and S-ketamine concentration *vs* time curves were analysed by non-linear least square regression two-compartment model analysis using PCNonlin. Plasma disposition curves for R-norketamine and S-norketamine were described by estimating AUC, C_{max}, and T_{max}. Pulse rate (PR), respiratory rate (R_f), tidal volume (V_T), minute volume ventilation (V_E), end-tidal partial pressure of carbon dioxide ($P_{E'CO_2}$), and mean arterial blood pressure (MAP) were also evaluated.

Results. The pharmacokinetic parameters of S- and R-ketamine administered in the racemic mixture or S-ketamine administered separately did not differ significantly. Statistically significant higher AUC and C_{max} were found for S-norketamine compared with R-norketamine in the racemic group. Overall, R_f, V_E, $P_{E'CO_2}$, and MAP were significantly higher in the racemic group, whereas PR was higher in the S-ketamine group.

Conclusions. Norketamine enantiomers showed different pharmacokinetic profiles after single i.v. administration of racemic ketamine in ponies anaesthetised with isoflurane in oxygen (1 MAC). Cardiopulmonary variables require further investigation.

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The rational use of ketamine, alone or in combination with volatile anaesthetics, relies on the knowledge of its disposition within the body, so that dose recommendations can be established and adjusted according to the desired blood or plasma drug levels. Although the disposition of ketamine is well documented in horses,[1–4] only one study reports the plasma levels of ketamine and norketamine enantiomers obtained after racemic (R-/S-) ketamine i.v. administration for anaesthesia induction in this species.[5] This study showed higher plasma levels of S-norketamine than R-norketamine, suggesting that *N*-demethylation might be highly enantioselective in horses. The potential for discrimination between enantiomers at different stages of drug metabolism is therefore

important and highlights the need for stereospecific drug assays.[6]

In humans and mice, the S-enantiomer has been proven advantageous, reaching an identical depth of anaesthesia with only half of the necessary racemic dose.[7–9] Differences in the metabolism of the ketamine enantiomers after R-/S-ketamine administration when compared with the sole S-ketamine administration might partially explain the faster recovery of the psychomotor function observed when S-ketamine is given alone.[9–12]

As a prelude to studying ketamine infusions in horses, the main goal of this study was to determine the pharmacokinetic profile of ketamine enantiomers after a single i.v. bolus of either R-/S-ketamine or S-ketamine in healthy ponies under isoflurane anaesthesia.

Materials and methods

Study design and animals

The experiments were performed with permission of the committee for animal experimentation, Bern, Switzerland. The experimental trial was designed as a randomized prospective 2-month interval cross-over study. Seven experimental, 4 year-old, Shetland pony geldings, mean weight 122 kg (SD 16 kg), were included in this study. The ponies were administered first R-/S-ketamine (RS-k) and after 2 months of wash-out interval, S-ketamine (S-k). They were conventionally housed as a group at the equine clinic of the Vetsuisse-Faculty (Bern, Switzerland). The animals had their right carotid artery surgically elevated to allow easy access by percutaneous puncture about 2 yr before this experiment and were judged healthy on the basis of physical examination, complete blood count and biochemistry profile. On the day before the experiment, ponies were housed in a separate box to allow withholding of food for 24 h. Access to water was granted until 1 h prior to the study.

Anaesthesia and instrumentation

The whole animal experimental phase was conducted in a padded recovery stall to both induce anaesthesia and allow for a safe anaesthetic recovery. A broad rope to restrain horses was placed around the ponies' pectoralis region, then passed around their hind quarters below their quadriceps and anchored to itself over the left side of their chest. After protective bandaging of the extremities, an additional rope was placed around every metacarpus/metatarsus. By pulling and stretching the ropes gently, the ponies were smoothly placed in right lateral recumbency and restrained in that position until anaesthesia induction was achieved. Ponies were trained to be restrained with this technique from prior studies.[13] Anaesthesia was then induced with isoflurane in oxygen (O_2) via a hermetically closed face mask connected to a small-animal anaesthesia circle system (Roche Electronic Respirator 3100, F. Hoffmann-La Roche, Basel, Switzerland). The vaporizer was first set to 1% and isoflurane delivery started via the face mask (O_2 flow: 6 litres min^{-1}) and gradual increments of 0.5% were made over 5 min until the ponies reached an appropriate anaesthetic plane for endotracheal intubation (muscle relaxation, eyeball rotation, reduced palpebral reflex, absence of swallowing reflex). Subsequently, a 14 mm ID cuffed rubber–silicone tube was placed into their tracheas and connected to the breathing system. Ponies were let to breathe spontaneously throughout the whole procedure. In the event of apnoea longer than 30 s, intermittent positive pressure ventilation (IPPV) was applied by manually squeezing the reservoir bag of the breathing system twice per minute until spontaneous ventilation was restored. Anaesthesia was maintained with isoflurane in O_2 throughout the whole experiment, at each pony's predetermined individual minimum alveolar concentration (iMAC). The iMAC for each pony [mean 0.97% (SD 0.17%)] was determined in a previous study.[13]

The skin over the right jugular vein and the left carotid artery was clipped and surgically prepared for percutaneous placement of intravascular catheters. The right jugular vein was catheterized with a 13-gauge catheter (Intranule PP, Vygon, Ecouen, France), and Ringer's lactate solution 10 ml kg^{-1} h^{-1} (RLS, Laboratorium Bichsel Baar, Switzerland) was delivered intravenously throughout the procedure with the aid of two volumetric infusion pumps (Vet/IV 2.2 Heska, Ballerup, Denmark). The left carotid artery was catheterized using a 20G/1.10×45 mm arterial catheter (B-D Arterial Cannula with FloSwitch, Ohmeda, Swindon, UK). Later on, the jugular port served for test drug administration and the arterial port served for blood sampling and invasive blood pressure monitoring. To facilitate test drug administration or blood collection, three-way stopcock valves were attached to the hubs of the venous and arterial catheters between the RLS line and the invasive blood pressure line, respectively.

Anaesthesia monitoring and physiological data collection

A lead II electrocardiogram was displayed and haemoglobin oxygen saturation (Sp_{O_2}) was determined with a pulse oximeter infrared probe placed on the tongue, which also calculated the pulse rate (PR). Inspired and expired O_2 concentrations, end-expired isoflurane concentrations ($F_{E'ISO}$), capnogram and end-tidal carbon dioxide partial pressure ($P_{E'CO_2}$), tidal volume (V_T), and minute volume ventilation (V_E) were obtained from a calibrated pitot tube flow sensor and side-stream gas sampler probe (D-lite Patient Spirometry TM, Datex-Ohmeda, Helsinki, Finland) inserted between the endotracheal tube and the Y-piece of the breathing system. Respiratory rate (R_f) was calculated from the capnogram. Mean arterial pressure (MAP) was determined directly with a calibrated pressure transducer (Angiokard, Medizintechnik GmbH & Co. KG, Friedeburg,

Germany) from the catheter placed in the carotid artery. The zero level in the transducer was set at the presumed level of the heart base (point of shoulder). In addition, the difference of the pharyngeo-oesophageal temperature (δT°) between the initial value measured after endotracheal intubation and the final value measured at the end of the procedures was recorded. A portable anaesthesia monitor (Datex S-5 portable anaesthesia monitor, Datex-Ohmeda, Helsinki, Finland) continuously displayed the aforementioned data. The monitor was calibrated with a standardized calibration gas (DOT-34 NRC/375M1014, Datex-Ohmeda, Helsinki, Finland) prior to each experiment according to the manufacturers' instructions. A laptop computer connected to the monitor served to record every 5 min the displayed data from 5 min before the test drug administration (baseline data) and throughout the sampling time. In addition, the need for IPPV was also manually recorded. After complete recovery from anaesthesia, animals were returned to their boxes.

Drug administration and sample collection

Ponies were left to stabilize end-tidal isoflurane concentrations to their individual isoflurane MAC for about 20 min. A single i.v. dose of R-/S-ketamine (RS-k) 2.2 mg kg^{-1} or S-ketamine (S-k) 1.1 mg kg^{-1} were administered intravenously. Five millilitres of arterial blood were collected manually over 5 s into heparinized tubes from the carotid artery catheter immediately before and at 1, 2, 4, 8, 16, 32, 64, 128 min after test drug injection. To avoid contamination with the arterial flushing solution, 5 ml of blood was collected and discharged before every sampling event. After the samples were obtained, the arterial catheter was flushed with 10 ml of physiologic saline solution. Immediately after collection, the samples were placed on ice until centrifugation was performed (maximum: 45 min) and plasma was frozen at −80°C until the assay was performed.

Ketamine and norketamine enantiomer analysis

The plasma samples were analysed by enantioselective capillary electrophoresis (CE) according to the technique described by Theurillat and colleagues,[14] and plasma concentrations for R-ketamine, S-ketamine, R-norketamine, and S-norketamine were obtained. Briefly, this technique is based on liquid–liquid extraction of ketamine and norketamine at alkaline pH from 1 ml plasma followed by analysis of the reconstituted extract by CE in the presence of a pH 2.5 Tris–phosphate buffer containing highly sulfated β-cyclodextrin 10 mg ml^{-1} (Sigma Aldrich Chemie, Schnelldorf, Germany) as chiral selector. Analyses were performed on the P/ACE MDQ Capillary Electrophoresis Analyzer (Beckman Coulter, Fullerton, CA, USA) using a 50 μm ID fused-silica capillary of 28 cm effective length, an applied voltage of −20 kV and a cartridge temperature of 30°C. The detection wavelength was 195 nm. The limit of detection for all enantiomers was 0.01 μg ml^{-1} and intraday and interday precisions (n=5) were <8% and <14%, respectively.

Pharmacokinetic analysis

R-ketamine and S-ketamine concentration *vs* time curves were analysed for each individual by non-linear least square regression two-compartment model analysis using PCNonlin (SCI Software, Lexington, USA). Plasma concentration obtained after i.v. administration was fitted to a polyexponential equation:

$$C_{(t)} = \sum_i Y_i e^{-\lambda_i t}$$

where $C_{(t)}$ (μg ml^{-1}) is the plasma concentration at time t; Y_i (μg ml^{-1}) is the coefficient of the ith term, and λ_i (per hour) is its exponent. Initial estimates were determined using the residual method[15] and refitted by non-linear regression analysis. The number of exponents needed were determined by applying the Schwartz[16] and Akaike criteria.[17] Most pharmacokinetic parameters were calculated using classic equations associated with compartmental analysis. Distribution and elimination half-lives ($t_{1/2}$alpha and $t_{1/2}$beta, respectively) were calculated as $t_{1/2}$ alpha=0.693/λ_1 and $t_{1/2}$beta=0.693/λ_2. Area under the plasma concentration–time curve ($AUC_{(0-\infty)}$) and mean residence time (MRT, measure of drug elimination expressed as the average time a drug molecule remains in the body after i.v. injection) were calculated by the trapezoidal rule with extrapolation to infinity. The extrapolated area was estimated as $AUC_{(C_{last}-\infty)}=C_{last}/\lambda_2$, where C_{last} is the last measured concentration. Body clearance (Cl_B) was determined as Cl_B=dose/$AUC_{(0-\infty)}$ and the steady-state volume of distribution ($V_{(d(ss))}$) was calculated as $V_{(d(ss))}=Cl_B \times MRT$. Plasma disposition curves for R-norketamine and S-norketamine were described by estimating AUC, C_{max}, and T_{max}.

Statistical methods

The NCSS 2004 (Kaysville, UT, USA) and the SigmaStat 3.1 (Point Richmond, CA, USA) software packages were used to perform the statistical evaluation. All data were analysed for normal distribution with the Martinez–Iglewicz test. The significance of difference between groups for δT°, ketamine, and norketamine enantiomers plasma levels and the estimated pharmacokinetic parameters was assessed with the Wilcoxon signed rank test or, when applicable, the Student's paired t-test. Each animal served as its own match. For PR, MAP, $P_{E'CO_2}$, R_f, and V_E, a statistical comparison was made between SR-k and S-k treatment groups before and after test drug administration using an analysis of variance test and for V_T using a two-way analysis on ranks Friedman test. For all tests, overall P<0.05 was considered

the minimum level of statistical significance. Parametric data are presented as mean (SD) and non-parametric data are presented as median and range.

Results

Anaesthesia

The overall induction, maintenance, and recovery from the anaesthetic episodes were uneventful. In one pony, the administration of the S-ketamine and the RLS infusions were performed via a 16-gauge catheter placed in the right cephalic vein because of obstruction of the left jugular vein catheter. Differences between treatment groups in δT° were not statistically significant [RS-k: 0.6 (0.4)°C; S-k: 0.9 (0.6)°C].

Cardiopulmonary data

There were no statistically significant differences in the cardiopulmonary variables between groups during the baseline measurements. Trends for PR are presented in Figure 1. Ponies in the RS-k treatment group had significantly lower ($P<0.001$) overall PR compared with the S-k treatment group (Fig. 1). Trends for MAP are presented in Figure 2. Overall MAP values where higher ($P=0.006$) in the RS-k when compared with the S-k group. Trends for $P_{E'CO_2}$, V_E, and R_f are presented in Figure 3. Ponies in the S-k treatment group had a significantly lower ($P<0.001$) $P_{E'CO_2}$ compared with the RS-k treatment group (Fig. 3A). The V_E was significantly higher ($P<0.001$) in the RS-k treatment group than in the S-k treatment group (Fig. 3B). Overall R_f values were higher ($P<0.001$) in the RS-k treatment group than the S-k treatment group (Fig. 3C). Immediately after both test drug administrations, three of seven ponies in each group developed apnoea longer than 30 s. After applying IPPV for 1–2 min all of them restored to spontaneous respiration. Differences in V_T were not statistically significant between treatment groups (RS-k group, median: 1.04 litre, range: 0.24–2.61 litre; S-k group, median: 0.99 litre, range: 0.05–3.06 litre). V_T decreased in both groups after test drug administration and restored to baseline values at 10 min. Mean Sp_{O_2} for the entire anaesthetic episode was 99 (0.9)% in the RS-k treatment group and 98.8 (0.4)% in the S-k treatment group.

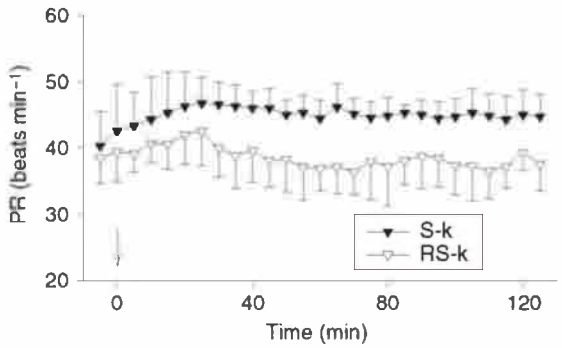

Fig 1 Mean and SD of PR plotted over time after R-/S-ketamine and S-ketamine administration to seven ponies anaesthetized with isoflurane in oxygen. The arrow marks the time of drug injection.

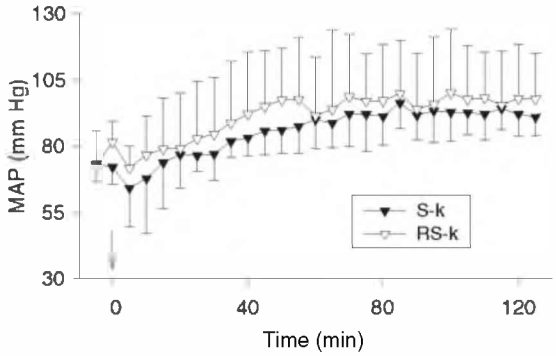

Fig 2 Mean and SD of MAP plotted over time after R-/S-ketamine and S-ketamine administration to seven ponies anaesthetized with isoflurane in oxygen. The arrow marks the time of drug injection.

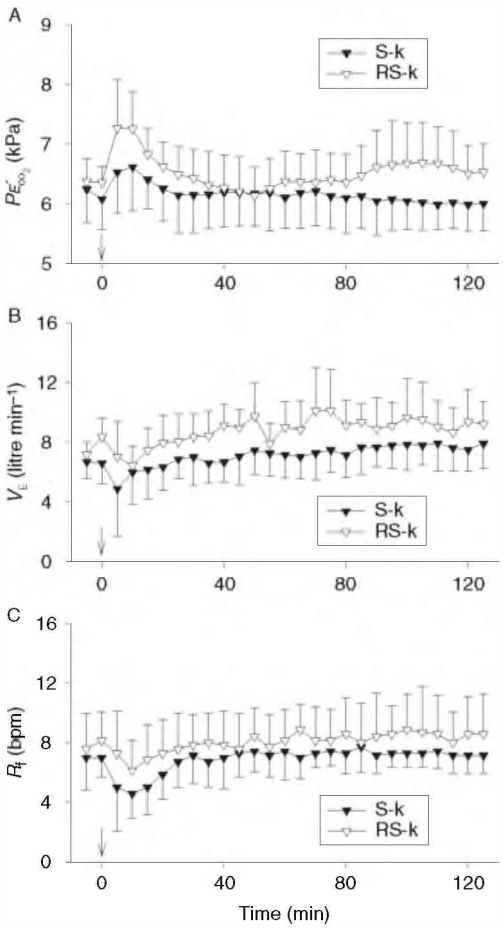

Fig 3 Mean and SD of $P_{E'CO_2}$ (A), V_E (B), and R_f (C) plotted over time after R-/S-ketamine and S-ketamine administration to seven ponies anaesthetized with isoflurane in oxygen. The arrow marks the time of drug injection.

Plasma concentrations of ketamine and norketamine enantiomers

A total of 126 plasma samples (RS-k=63, S-k=63) were analysed by CE and used for the concentration–time data evaluation. R-ketamine and R-norketamine were not detected after administration of S-ketamine. Trends for ketamine and norketamine plasma concentrations are presented in Figures 4 and 5. S-ketamine plasma concentrations were statistically significantly lower (P=0.03) in the S-k treatment group than in the RS-k treatment group at 2 min (Figs 4A and 5A). At the same time point, S-ketamine plasma concentrations were statistically significantly lower (P=0.005) when compared with the R-ketamine plasma concentrations in the RS-k treatment group (Fig. 4A). No other significant differences were determined. Plasma concentrations for S-norketamine were statistically significantly higher than plasma concentrations of R-norketamine in the RS-k group at 2 (P=0.005), 8 (P=0.007), 16 (P=0.01), 32 (P=0.005), 64 (P=0.008), and 128 (P=0.03) min. No statistical difference was noted at 1 (P=0.297) and 4 (P=0.147) min. The plasma concentrations of the S-norketamine in the S-k group were similar to its homologous enantiomer in the RS-k group (P>0.05 for all time points, Figs 4B and 5B).

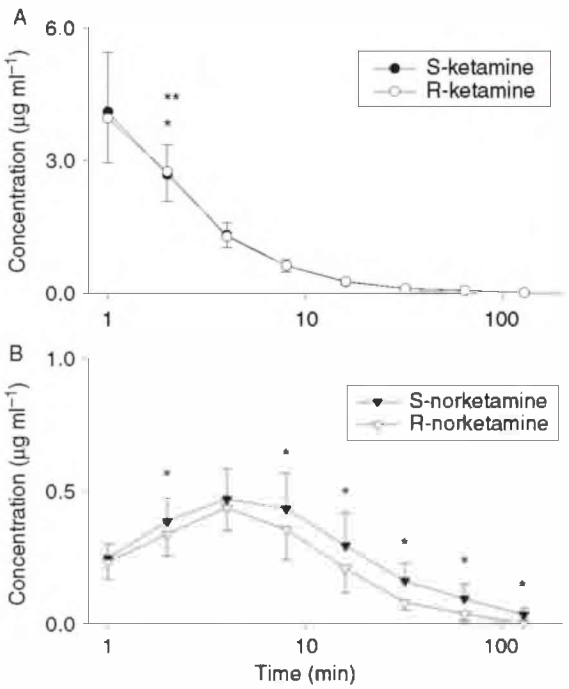

Fig 4 Mean and SD of plasma concentrations of (A) S-ketamine and R-ketamine and (B) S-norketamine and R-norketamine after R-/S-ketamine administration to seven ponies anaesthetized with isoflurane in oxygen. *P<0.05 within treatment group. **P<0.05 between the two treatment groups (comparison with data of Fig. 5A).

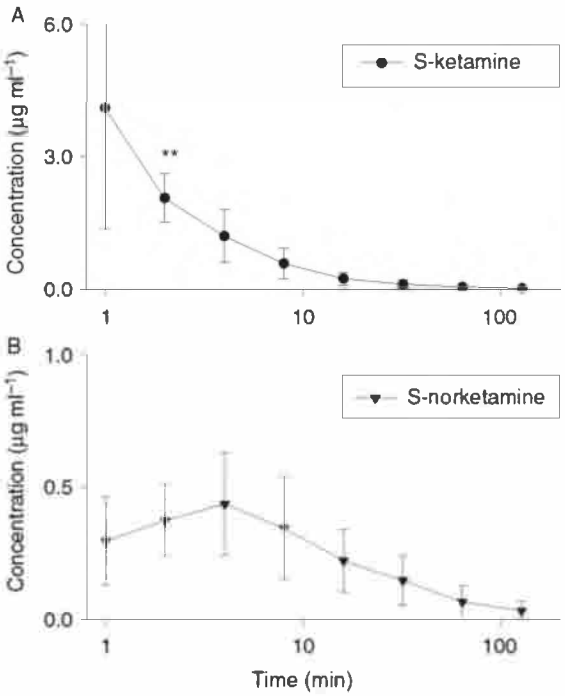

Fig 5 Mean (SD) plasma concentrations of (A) S-ketamine and (B) S-norketamine after S-ketamine administration to seven ponies anaesthetized with isoflurane in oxygen. **P<0.05 between treatment groups (comparison with data of Fig. 4A).

Pharmacokinetic analysis

The pharmacokinetics for all ketamine enantiomers could be well described with a two-compartment model. No significant differences in the pharmacokinetic variables between and within treatment groups could be detected for the ketamine enantiomers. The AUC and C_{max} of R-norketamine were found to be significantly smaller (P=0.002 and P=0.009, respectively) than those of S-norketamine in the RS-k treatment group. No other significant differences could be determined. Table 1 summarizes the results of the population analysis for all compounds.

Discussion

In the present study, the pharmacokinetic parameters of R- and S-ketamine after single i.v. injection of the racemate or the single S-enantiomer in ponies under isoflurane anaesthesia, did not significantly differ. The statistically significant differences found for the S-ketamine plasma concentrations 2 min after administration of either R-/S- or S-ketamine had no significant impact on the calculated pharmacokinetic variables. However, the statistically significant differences found in the plasma disposition of S- and R-norketamine after administration of the racemate, mirrored by their calculated pharmacokinetic parameters, suggest that in ponies anaesthetized with 1 iMAC isoflurane, the metabolism of racemic ketamine is highly

Table 1 Mean (SD) of pharmacokinetic parameters of arterial plasma S- and R-ketamine obtained from ponies (n=7) anaesthetized with isoflurane in oxygen after i.v. R-/S-ketamine (2.2 mg kg^{-1}) or S-ketamine (1.1 mg kg^{-1}) administration. *P<0.05 between enantiomers within the R-/S-ketamine treatment group. A, distributive exponential phase; B, terminal exponential phase; Alpha, hybrid rate constant of biphasic i.v. disposition curve (distribution phase); Beta, hybrid rate constant of biphasic i.v. disposition curve (elimination phase); K10, first-order elimination rate constant; K12 and K21, first-order transfer rates between central and peripheral compartments; C_{max}, maximum observed concentration value; T_{max}, time point at which C_{max} occurs

	S-ketamine	R-/S-ketamine	
		S-ketamine	R-ketamine
A (μg ml^{-1})	9.7 (14)	5.6 (2)	5.4 (1.6)
B (μg ml^{-1})	0.7 (0.6)	0.4 (0.1)	0.5 (0.3)
Alpha (min^{-1})	0.7 (0.5)	0.4 (0.08)	0.4 (0.1)
Beta (min^{-1})	0.05 (0.05)	0.03 (0.01)	0.03 (0.01)
AUC (μg min ml^{-1})	29.1 (18.1)	27.1 (6.34)	26.8 (5.3)
$t_{1/2}$alpha (min)	1.4 (0.8)	1.6 (0.3)	1.7 (0.3)
$t_{1/2}$beta (min)	26 (22.5)	23.6 (8.1)	24.2 (7.4)
K10 (min^{-1})	0.3 (0.2)	0.2 (0.05)	0.2 (0.05)
K12 (min^{-1})	0.3 (0.2)	0.1 (0.05)	0.2 (0.1)
K21 (min^{-1})	0.1 (0.1)	0.06 (0.02)	0.07 (0.04)
V_c (litre kg^{-1})	0.2 (0.1)	0.2 (0.06)	0.2 (0.1)
Cl_B (litre kg^{-1} min^{-1})	0.05 (0.02)	0.04 (0.01)	0.04 (0.01)
MRT (min)	24.6 (24.1)	18.9 (6.2)	19.1 (5.7)
$V_{(d(ss))}$ (litre kg^{-1})	0.9 (0.8)	0.8 (0.3)	0.8 (0.3)
	S-norketamine	**S-norketamine**	**R-norketamine**
AUC (μg min ml^{-1})	14.8 (0.5)	17.8 (6.4)*	10.4 (3)*
C_{max} (μg ml^{-1})	0.48 (0.2)	0.5 (0.08)*	0.4 (0.06)*
T_{max} (min)	3.6 (2.3)	7.1 (4.5)	4.3 (1.8)

stereoselective. In both treatment groups, the arterial plasma concentrations of ketamine enantiomers followed a biexponential decline, with a rapid initial distribution phase being followed by a slower elimination phase. This profile was analogous to those previously published for humans,[11] dogs,[18] and cross-bred horses.[5] Similar to humans and most other species,[1 2 4 5 10 18–21] high variability among individuals was observed in the plasma concentrations of ketamine and norketamine. Former studies on the pharmacokinetics of S-/R-ketamine in horses reported data analysed from venous samples.[1–5] In isoflurane anaesthetized ponies, large differences (up to 50%) were found between arterial and venous pharmacokinetics of ketamine isomers caused by both, distribution and elimination processes.[22] In situations in which potent short-acting drugs are used, such as anaesthetics, these differences can be of considerable importance.[23] Because the drug concentrations at the target organ are probably more closely related to arterial than to venous concentration, the controlling parameters should be based on arterial rather than venous data.[10 24] The present study is the first investigating the arterial stereoselective pharmacokinetic profile of S-/R-ketamine compared with S-ketamine in an equine species.

The iMAC is the midpoint of two end-tidal anaesthetic concentrations that just permit or just prevent gross purposeful movement after applying a supramaximal stimulation in a single anaesthetized individual.[25] Individual MAC values are normally averaged to obtain the MAC in a group of subjects. The use of each pony iMAC, instead of the MAC of the group, aimed to have a comparable degree of immobility among test animals. I.V. R-/S-ketamine at a dose of 2.2 mg kg^{-1} is well documented to induce anaesthesia in sedated horses.[2 4] Such doses, however, are seldom administered in clinical practice to horses already receiving 1 MAC of isoflurane. This dose was chosen deliberately to create a basis for further studies on constant rate infusion of ketamine in isoflurane-anaesthetized horses. As a racemate has equal proportions of each enantiomer, a dose of 1.1 mg kg^{-1} of S-ketamine was chosen to compare the profiles of the S-enantiomer in both treatment groups.

Previous investigations in rats[21] and dogs[26] anaesthetized with halogenated agents showed alterations in organ perfusion, which in turn slowed down uptake, distribution, and redistribution of ketamine. Thus, an increased apparent volume of the central compartment (V_c) could be expected, probably because of a slower elimination of the compounds by poorly perfused organs of elimination. It has long been suggested that distribution of drugs may be not only affected by blood flow but also by active transport across the endothelium.[27] It was postulated that the binding affinity of the chiral forms of ketamine to membrane receptors responsible for protein-mediated transport across the vascular endothelium might exhibit stereospecificity, showing different distribution profiles between enantiomers. However, several authors failed to demonstrate ketamine distribution to be stereoselective in humans[10 11 28] and in dogs.[18] Similarly, in the present study, the half-lives and the V_c for the ketamine enantiomers did not differ significantly between and within groups, suggesting that ketamine distribution is not enantioselective in the equine species either.

MRT and Cl_B values calculated from the arterial plasma ketamine concentrations determined after S-ketamine or R-/S-ketamine i.v. administration were similar for all enantiomers. Studies conducted in humans showed a significantly higher Cl_B for S-ketamine compared with R-ketamine after administration of either enantiomer.[11 28 29] Ihmsen and colleagues[10] showed a higher S-ketamine body clearance in men when the drug was administered as an infusion of the pure enantiomer compared with an infusion of the racemate. In contrast, Yanagihara and colleagues[30] showed no differences between the pharmacokinetics of ketamine enantiomers and this dissimilarity was attributed to variability related to human races. Inter-individual variations between comparison groups, differences in the sampling site (arterial *vs* venous), or both might also account for such discrepancies.[10]

Several factors seem to have an important impact on the metabolism of the ketamine isoforms. Ketamine is rapidly and extensively metabolized by the hepatic microsomal cytochrome P450 enzymes to norketamine, hydroxynorketamine, hydroxyketamine, and dehydronorketamine.[31–35]

In vitro studies revealed that *N*-demethylation of S-ketamine by human liver microsomes is 20% larger than that of R-ketamine and 10% greater when compared with the racemic compound.[36] Two enzymes with stereospecific preference for one enantiomer over the other, or four enzymes with different stereospecific substrate activities and different metabolic rates might account for this enantioselective *N*-demethylation.[36] In contrast another *in vitro* study with human liver microsomes suggested a non-enantioselective *N*-demethylation of R- and S-ketamine.[37]

The comparable values obtained for the calculated pharmacokinetic variables of the S-isoforms of ketamine and norketamine in both groups suggest no interference of the R-enantiomer with the overall elimination of the S-enantiomer in the racemate. However, the significant differences found for the R- and S-norketamine in the RS-k treatment group suggest that some differences exist with regard to their particular metabolic pathways. This finding corresponds with data in which similar results were obtained after a single dose of S- or R-/S-ketamine administration to human patients[11] and horses.[5] In the latter study, a S/R ratio for norketamine of 75:25 immediately after and 90:10 40 min after i.v. administration of R-/S-ketamine (6 mg kg^{-1}) was reported. The authors postulated that these ratios could be the consequence of substrate enantioselectivity of the cytochrome-dependent *N*-demethylation. In the present study, an enzyme–substrate competition seems unlikely because similar Cl_B rates were found for the S-enantiomer in both groups suggesting the presence of other enantioselective processes involved in the elimination of ketamine. Norketamine is a pharmacologically active metabolite and was found to have a potency of around 30% of that of the parent drug.[38] The hydroxylated metabolite 6-hydroxynorketamine has been reported to be a weaker anaesthetic and to produce less post-anaesthetic excitation than norketamine.[39] Consequently, norketamine elimination pathways might have a relevant influence on the overall ketamine activity. Interestingly, the hydroxylation of norketamine also exhibited enantioselectivity at different positions on the cyclohexanone ring.[35] Therefore, it could be possible that in the present study, R-norketamine hydroxylation may have prevailed, accounting for the lower plasma concentrations reflected into the lower AUC and C_{max} observed for that metabolite. Unfortunately, we were unable to quantify the plasma concentrations of the hydroxylated forms of norketamine to test this hypothesis.

Renal excretion seems to play a significant role in norketamine elimination in horses.[40] Differences in plasma protein binding leading to different renal clearance may be expected for chiral compounds.[41] Therefore, different renal clearance rates for norketamine enantiomers could also explain the higher concentrations obtained for S-norketamine in ponies' plasma when compared with those of R-norketamine. Kaka and colleagues[4] proposed involvement of extrahepatic pathways in the ketamine metabolism in horses because the ketamine Cl_B exceeded the calculated hepatic plasma flow rate. Studies performed in rats reported extrahepatic inversion of R to S-ketamine with subsequent metabolism to S-norketamine.[42] In the present study, no R-ketamine was detected after administration of the pure S-enantiomer, and after administration of the racemate, the mean plasma concentrations of R- and S-ketamine were similar, suggesting no inversion between the R- and S-enantiomers. Geisslinger and colleagues[11] proposed that a possible inversion from R- to S-ketamine could be masked by different stereoselective Cl_B rates, resulting also in similar concentration profiles for both enantiomers. However, the Cl_B of the R- and S-ketamine were similar in our study.

Although the study reported here was mainly designed to evaluate the pharmacokinetics of ketamine and norketamine enantiomers, some cardiopulmonary data were also collected. Generally, low doses of R-/S-ketamine are known to increase heart rate and MAP as a consequence of direct sympathetic stimulation.[43] The reduction in MAP observed for both compounds in the first 10 min after drug administration could have been the result of direct central nervous system depression in response to the high doses used here. S-ketamine seemed to have produced a stronger cardiac stimulation as evaluated by higher PR values observed for the S-k group compared with the RS-k group. *In vitro* studies showed an inhibition of the uptake of noradrenaline at adrenergic neuronal endings by ketamine, which occurred pre-synaptically with both enantiomers but also post-synaptically with the S-isoform.[44] On the other hand, overall mean MAP values remained significantly lower over time in the S-k treatment group compared with the racemate. The reduced MAP values observed in the S-k treatment group could have induced an autonomic reflex response that increased the heart rate and vice versa for the RS-k treatment group. Contrary to these findings, Filzek and colleagues[45] reported significantly lower heart rate and higher MAP values in horses anaesthetized with S-ketamine compared with those receiving R-/S-ketamine. In man, several studies compared R-/S-ketamine with S-ketamine and found equivalent cardiovascular stimulation.[10 46 47] Immediately after test drug administration and for about 10 min, respiratory depression occurred (apnoea, decreases in R_f, V_T, and V_E). A progressive increase in V_E was observed in both groups as test drugs were cleared from plasma. Increases in R_f might have accounted for the overall increases in the mean V_E values observed in the RS-k treatment group since V_T remained similar between groups. In turn, an overall increase in the arterial partial pressure of carbon dioxide, reflected as an increase in the overall mean $P_{E'CO_2}$ values in the RS-k treatment group, could have stimulated the respiratory system via chemoreceptor activity resulting in the observed increases in V_E and R_f. Alternatively, increases in physiologic dead space could explain the lower $P_{E'CO_2}$ values observed in the S-k treatment group.

In conclusion, the disposition of racemic ketamine in ponies anaesthetized with 1 iMAC isoflurane is stereoselective, leading to higher plasma concentrations of S-norketamine. Several factors seem to influence the metabolism of the ketamine isoforms. Further studies are required to assess the impact of different dose regimes, drug combinations, or both on the overall metabolism of the enantiomers of ketamine. Under the conditions reported here, administration of S-ketamine produced different cardiopulmonary effects than those observed with R-/S-ketamine. The underlying mechanisms responsible for these differences remain to be elucidated.

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