Stereoselective pharmacokinetics of ketamine and norketamine after constant rate infusion of a subanesthetic dose of racemic ketamine or S-ketamine in Shetland ponies

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Objective—To evaluate pharmacokinetics of ketamine and norketamine enantiomers after constant rate infusion (CRI) of a subanesthetic dose of racemic ketamine or S-ketamine in ponies.

Animals—Five 6-year-old Shetland pony geldings that weighed between 101 and 152 kg.

Procedures—In a crossover study, each pony received a CRI of racemic ketamine (loading dose, 0.6 mg/kg; CRI, 0.02 mg/kg/min) and S-ketamine (loading dose, 0.3 mg/kg; CRI, 0.01 mg/kg/min), with a 1-month interval between treatments. Arterial blood samples were collected before and at 5, 15, 30, 45, and 60 minutes during drug administration and at 5, 10, 30, and 60 minutes after discontinuing the CRI. Plasma ketamine and norketamine enantiomers were quantified by use of capillary electrophoresis. Individual R-ketamine and S-ketamine concentration-versus-time curves were analyzed by use of a monocompartmental model. Plasma disposition curves for R-norketamine and S-norketamine were described by estimating the area under the concentration-versus-time curve (AUC), maximum concentration (Cmax), and time until Cmax.

Results—Plasma concentrations of S-ketamine decreased and biodegradation products increased more rapidly after S-ketamine CRI, compared with results after racemic ketamine CRI. The R-norketamine was eliminated faster than was the S-norketamine. Significant differences between treatments were found for the AUC of S-ketamine and within the racemic ketamine CRI for the AUC and Cmax of norketamine isomers.

Conclusions and Clinical Relevance—CRI of S-ketamine may be preferable over CRI of racemic ketamine in standing equids because the S-enantiomer was eliminated faster when infused alone instead of as part of a racemic mixture. (Am J Vet Res 2009;70:831–839)
the asymmetric environment of receptors and enzymes. In these conditions, ketamine enantiomers differ in their pharmacodynamic activities and pharmacokinetic properties. Administration of S-ketamine alone may be beneficial, compared with benefits for the administration of racemic ketamine. Studies performed in humans and ponies have revealed that the more pharmacologically active S-ketamine isomer is eliminated faster when administered as a pure enantiomer, which results in a faster recovery of psychomotor function. Furthermore, R-norketamine and S-norketamine (active metabolites of R-ketamine and S-ketamine, respectively) are also enantiosselectively metabolized. Conventional analytic techniques do not differentiate between enantiomers; therefore, total drug concentrations are monitored when racemic ketamine is administered. However, because ketamine metabolism in equine species is stereoselective, enantiosselective assays should be used to investigate the metabolism of this drug.

The pharmacokinetics of subanesthetic doses of racemic ketamine infusions have been evaluated in standing equids because of their potential analgesic and anti-inflammatory effects. However, there are no reports regarding their stereoselective disposition in the equine species. Furthermore, to our knowledge, the pharmacokinetics of S-ketamine infusions have not been reported for horses. In addition, plasma concentrations of venous blood samples were reported in pharmacokinetic studies that involved low-dose infusions of racemic ketamine. Because the pharmacodynamic action of most drugs is usually dependent on the arterial concentration in view of the fact that arterial blood delivers the drug to the specific target tissue (ie, nervous system), there is a clear rationale for the use of arterial plasma concentrations instead of venous plasma concentrations for pharmacokinetics analysis. Therefore, the objective of the study reported here was to determine the pharmacokinetics of ketamine and norketamine enantiomers for arterial plasma concentrations of Shetland ponies that received a subanesthetic dose of racemic ketamine or S-ketamine as a CRI. We also assessed the plasma ratios of the S-enantiomer to the R-enantiomer for norketamine and dehydronorketamine. The experiments were part of a study conducted to evaluate the effects of infusion of a low dose of ketamine on the nociceptive withdrawal reflex in standing ponies.

Materials and Methods

Animals—Five 6-year-old Shetland pony geldings with a mean body weight of 128 kg (range, 101 to 152 kg) were used in the study. The ponies were conventionally housed as a group at the horse clinic of the Vetsuisse Faculty in Bern and were not included in any other experiment for at least 8 months preceding this study. Approximately 4 years before the study reported here, the right carotid artery of each pony was surgically elevated to allow easy access for percutaneous puncture. Ponies were judged to be healthy on the basis of results of physical examination, a CBC, and serum biochemical analysis. Access to water and food was allowed until 1 hour prior to each experiment. The study was approved by the Committee for Animal Experimentation, County of Berne, Switzerland.

Instrumentation—One hour before each experiment, the hair over the left jugular vein and left carotid artery was clipped. A patch containing a 1:1 mixture of lidocaine base:prilocaine base was applied to the skin in each clipped area; patches were retained in place by use of a protective bandage. One hour later, the patches were removed and skin of the clipped areas was aseptically prepared. A 13-gauge catheter was then inserted into the right jugular vein, and a 20-gauge catheter was inserted into the left carotid artery. Ponies were restrained in stocks for the duration of the entire experiment.

Procedures—The experiments were conducted as a blinded randomized prospective crossover study. The ponies were randomly assigned to initially receive racemic ketamine or S-ketamine; the other treatment was administered after a 4-week washout period.

A loading dose of racemic ketamine (0.6 mg/kg; racemic treatment) or S-ketamine (0.3 mg/kg; S-ketamine treatment) diluted to a final volume of 5 mL with physiologic saline (0.9% NaCl) solution was manually administered IV during a 1-minute period via a 3-way stopcock connected to the IV catheter. Immediately after injection of the loading dose, CRI of racemic ketamine (0.02 mg/kg/min) or S-ketamine (0.01 mg/kg/min) was delivered to the ponies for the racemic and S-ketamine treatments, respectively. The CRI was administered for a period of 59 minutes by use of a syringe infusion pump via a 2-ml, 1.5-m-long, nondistensible extension tube connected to the IV catheter via the 3-way stopcock. Lactated Ringer’s solution was administered at a rate of 2 mL/kg/h through the 3-way stopcock throughout the infusion period by means of a volumetric infusion pump. For CRI administration, racemic ketamine and S-ketamine were diluted in physiologic saline solution to achieve working concentrations of 5 mg/mL and 2.5 mg/mL, respectively, which were placed in 60-mL syringes with a luer tip. To ensure accuracy in calculating the infused ketamine volumes, each extension tube was allowed with 2 mL of drug solution before loading the CRI, and the initial volume contained in the syringe was recorded. At the end of the procedure, the CRI for each drug was calculated from the initial volume minus the volume that remained in the syringe. The total amount of each drug administered was calculated by adding the amount in the bolus of the initial loading dose to the amount for the CRI of each pony.

Five milliliters of arterial blood was collected manually (collection duration, 5 seconds) from the carotid artery catheter into heparinized tubes immediately before and at 5, 15, 30, 45, and 60 minutes during drug administration and at 5, 10, 30, and 60 minutes after the end of the CRI. Aliquot collection, the arterial catheter was flushed with 10 mL of physiologic saline solution. To avoid contamination with the arterial flushing solution, 5 mL of blood was collected and discarded before each sample collection. Immediately after collection, samples were placed on ice until centrifugation (maximum time from collection until centrifugation was 60 minutes); plasma was harvested and stored frozen at −80°C until analysis.

Analysis of plasma concentrations of ketamine—Plasma samples were analyzed by use of enantioselec-
tive capillary electrophoresis, which was conducted in accordance with a protocol, with slight modifications. The assay was adapted to detect lower concentrations of the enantiomers of ketamine and norketamine and has been reported in more detail elsewhere. Briefly, ketamine, norketamine, and dehydronorketamine were extracted from 0.5 mL of plasma by use of liquid-liquid extraction at alkaline pH. The final residue was dissolved in 30 µL of 10-fold diluted Tris-phosphate electrophoresis buffer without chiral selector and analyzed on a capillary electrophoresis analyzer by use of a 50-µm (internal diameter), uncoated, fused-silica capillary of 45 cm total length; applied voltage of ~20 kV; and cartridge temperature of 20°C. Detection wavelength was 195 nm. Sample injection was accomplished by use of vacuum of 10.34 kPa for 10 seconds. A 50mM Tris-phosphate buffer (pH 2.5) that contained 10 mg of sulfated β-cyclodextrin/mL as a mixture of 2 lots was used. For ketamine and norketamine, the calibration range was between 5 and 250 ng/mL for each enantiomer. All calibration graphs were found to be linear (r > 0.998; F > 420). The quantitation limit for all enantiomers was 2.5 ng/mL, and typical intraday and interday precision values (n = 4) expressed as relative SD were < 6.5% and < 9.2%, respectively. Dehydronorketamine could not be quantified because a standard compound was not available. Ratios of the S-enantiomer to the R-enantiomer for dehydronorketamine and norketamine were calculated from the obtained absorbance data of each enantiomer as a ratio of relative peak areas (ie, peak area divided by detection time).

**Pharmacokinetic analysis**—Both compartmental and statistical moment approaches were used for the pharmacokinetic analysis of R-ketamine and S-ketamine disposition curves, which were conducted by use of a commercially available program. The individual plasma concentration-versus-time curves were fitted by use of polyexponential equations, and the number of exponents was determined by application of the Akaike information criterion and Schwartz criterion. Variables were estimated by use of nonlinear regression. Data points were weighted on the basis of the inverse of the fitted value. After application of the Akaike information criterion and Schwartz criterion, the following equation was selected:

\[ C_t = (y\cdot e^{-\lambda t}) + (C_{ss}\cdot[1 - e^{-\lambda t}]) \]

where \( C_t \) is the plasma concentration at time \( t \), \( y \) is the plasma concentration extrapolated at time 0, \( e \) is the natural logarithm, \( \lambda \) is the slope of the elimination
phase, and C_{ss} is the plasma concentration at steady state. Most pharmacokinetic variables were calculated by use of classic equations associated with compartmental analysis. The value for AUC_{\text{last}} was calculated by use of the linear trapezoidal rule. The value for AUC_{\text{last}} was estimated by use of the following equation:

$$\text{AUC}_{\text{last}} = C_{\text{last}}/\lambda_{\text{e}}$$

where $C_{\text{last}}$ is the last measured concentration. The $t_{1/2\beta}$ was calculated as 0.693$/lambda_{\text{e}}$. Value for Cl_{p} was calculated as dose/AUC_{\text{last}}, and Vd was calculated as dose/Cl_{p}. Plasma disposition curves for R-norketamine and S-norketamine were described by estimating AUC, C_{max}, and time at which C_{max} was detected.

Statistical analysis—Commercially available software packages were used for statistical analyses, and an overall value of $P < 0.05$ was considered significant. All data were analyzed for normal distribution by use of the Shapiro-Wilk W test. For all variables, statistical comparisons were conducted between the S-enantiomers with their corresponding R-enantiomers after administration of racemic ketamine and between the S-enantiomers after administration of racemic ketamine or S-ketamine. The Iman and Davenport variant of the Friedman rank test for multiple comparisons was used to compare plasma concentrations of ketamine and norketamine enantiomers between and within treatments and to analyze the difference from unity for norketamine and dehydroxynorketamine S-enantiomer–to–R-enantiomer ratios. Differences for each time point were assessed by use of the Wilcoxon signed rank test for difference in medians. Wilcoxon signed rank tests for difference in medians were also used to analyze the pharmacokinetic variables between and within treatments. For paired data, each pony served as its own control animal. Parametric data were reported as mean ± SD, and nonparametric data were reported as median and range.

Results
Mean CRI for racemic ketamine and S-ketamine was 0.02 ± 0.0004 mg/kg/min and 0.01 ± 0.0009 mg/kg/min, respectively. Mean total amount of drug (including the loading dose administered during 1 minute and the CRI administered during the subsequent 59 minutes) was 1.75 ± 0.06 mg/kg for racemic ketamine and 0.84 ± 0.02 mg/kg for S-ketamine. The S-ketamine, R-ketamine, S-norketamine, R-norketamine, S-dehydronorketamine, R-dehydronorketamine, and S-enantiomer of an unidentified hydroxymetabolite of norketamine could be detected and separated by use of capillary electrophoresis (Figure 1). No R-enantiomers were detected after administration of S-ketamine. During CRI, the plasma concentration of dehydronorketamine was already higher than that of norketamine at 30 minutes.

Except for the S-enantiomer of one of the hydroxynorketamine metabolites, no other hydroxymetabolite was detected during the assessed time interval.

For the racemic treatment, significant ($P < 0.001$) differences from unity were detected for ratios of S-norketamine enantiomers to R-norketamine enantiomers, with median ratios that increased from 1.16 to 1.42 during CRI and with larger increases (from 2.17 to 3.48) after discontinuation of the CRI (Figure 2). The median ratio for S-dehydrornorketamine to R-dehydrornorketamine gradually increased from 0.86 (after bolus injection of the loading dose) to 1.19 at 120 minutes. No significant ($P = 0.32$) differences were detected for ratios of S-dehydrornorketamine to R-dehydrornorketamine, compared with unity.

Plasma concentrations of ketamine enantiomers were plotted against time for the racemic ketamine and S-ketamine treatments (Figure 3). Plasma concentrations of S-ketamine after racemic ketamine administration (median, 10.5 ng/mL; range, 0 to 83.2 ng/mL) were comparable ($P = 0.43$) to those of R-ketamine after racemic ketamine administration (median, 9.1 ng/mL; range, 0 to 79.2 ng/mL) but were significantly ($P = 0.01$) higher than those of S-ketamine after only S-ketamine administration (median, 5.4 ng/mL; range, 0 to 65.9 ng/mL). However, analysis with Wilcoxon tests failed to identify significant differences between and within treatments at any sampling time. During the infusion, the plasma concentrations for S-ketamine after racemic ketamine administration (median, 16.7 ng/mL; range, 0 to 83.2 ng/mL) were significantly ($P = 0.01$) higher than those of its homologue after S-ketamine administration (median, 10.8 ng/mL; range, 0 to 65.9 ng/mL). The corresponding value for R-ketamine for the racemic ketamine treatment (median, 15.1 ng/mL;
The pharmacokinetics for ketamine and norketamine enantiomers could be described with a monocompartmental approach, considering a 1-minute bolus dose administered IV followed by a CRI for 59 minutes. Results of the population analysis were summarized. The AUC for S-ketamine differed significantly \( (P = 0.043) \) between treatments (Table 1). The AUC and Cmax of the norketamine enantiomers differed significantly \( (P = 0.043 \text{ for both variables}) \) with the racemic ketamine treatment (Table 2).

**Discussion**

Similar to results of other reports for ponies\(^3\) and humans,\(^16\) S-ketamine was eliminated more rapidly after administration of a single isomer, compared with elimination after administration of the racemate. This is also in agreement with results of in vitro studies,\(^4,6\) which revealed that the more rapid biotransformation of S-ketamine when administered alone may be the result of an inhibition of R-ketamine, compared with metabolism of S-ketamine when administered within the racemate. Comparable with results of other studies,\(^3,17\) \( t_{1/2el} \) and \( Vd \) did not differ significantly for the study reported here, which suggested that ketamine distribution is not stereoselective in equids.

The highest plasma concentrations for ketamine and norketamine enantiomers for both treatments were detected at 5 minutes (ie, the first time point assessed after administration of the loading dose). The plasma concentration-versus-time curve obtained for both enantiomers for either treatment did not have a clearly defined steady-state condition. Instead, a slight but constant decrease in plasma concentrations during CRI followed by a marked decrease and rapid elimination after discontinuing the CRI were detected. In this study, the infusion rate for ketamine was derived from data obtained from trials performed in humans in which the analgesic effects of the drug were evaluated\(^18\) and from infusion rates in standing horses.\(^7,8\) A value of 50% of the racemic ketamine dose for S-ketamine was chosen to compare the pharmacokinetic variables of S-ketamine in both administrations.

In standing adult horses, investigators detected venous ketamine plasma concentrations of 0.067 and 0.137 \( \mu g/mL \) after infusion of racemic ketamine at a rate of 0.4 \( mg/kg/h \) or 0.8 \( mg/kg/h \), respectively;\(^7\) and of 0.235 \( \mu g/mL \) after an infusion of racemic ketamine at a rate of 1.5 \( mg/kg/h \).\(^8\) In the present study, median arterial plasma concentrations of R-ketamine and S-ketamine for the racemic ketamine treatment (15.1 \( ng/mL \) and 16.7 \( ng/mL \), respectively) corresponded to a total racemic concentration of 0.032 \( \mu g/mL \) during a CRI of
reported that plasma concentrations of ketamine were up to 50% less in arterial blood samples, compared with concentrations in venous blood samples, after simultaneous collection of samples in ponies anesthetized with isoflurane in oxygen that received IV administration of a bolus of ketamine (2.2 mg of racemic ketamine/kg). In another study, mean arterial plasma concentrations of 0.311 µg/mL for S-ketamine and 0.319 µg/mL for R-ketamine were detected 60 minutes after administration of an induction dose of 2.2 mg of racemic ketamine/kg followed by CRI at a rate of 1 mg/kg/h to horses anesthetized with isoflurane. These 20-fold higher concentrations detected in anesthetized horses, compared with concentrations in standing ponies, could be the result of volatile agents or sedatives inducing poor perfusion in organs of elimination.

Several pharmacokinetic models, such as noncompartment, multicompartment, and physiologically based, have been applied to analyze pharmacokinetics of ketamine infusions in horses. We used a monocompartment model because the concentration-time data sets of ketamine enantiomers best fit a monoexponential decay. However, the 5-minute delay in obtaining the first plasma sample after administration of the loading dose may have resulted in overlooking the distribution phase of ketamine enantiomers, and multicompartiment models potentially could have been applied. Nevertheless, even after application of a recirculatory model in another study, it was determined that ketamine distribution is not stereoselective in dogs.

The underlying mechanism that led to the smaller AUC for S-ketamine in the S-ketamine treatment is not completely clear. In 1 in vitro study, it was found that the N-demethylation rate for the racemate was smaller than the sum of the N-demethylation rates for the enantiomers. It was postulated that S-ketamine and R-ketamine are metabolized by the same enzymes so that competitive interaction would reduce the metabolism of both stereoisomers when administered concurrently. In another in vitro study in which equine liver and lung microsomes were used, S-ketamine was metabolized to S-norketamine more rapidly when administered alone than when administered within the racemate. Interestingly, in the present study, similar plasma concentrations and calculated pharmacokinetic variables were detected for S-norketamine in both treatments, which suggested that N-demethylation of S-ketamine to S-norketamine may have been similar. A higher rate of hydroxylation of S-ketamine could explain the lower AUCs of that compound for the
S-ketamine treatment. Alternatively, a higher rate of N-demethylation of S-ketamine to S-norketamine for the S-ketamine group could have been masked by a higher rate of hydroxylation of S-norketamine, which resulted in similar S-norketamine plasma concentrations. Because the hydroxylated forms of ketamine or norketamine could not be quantified, we were unable to test these hypotheses.

Dehydronorketamine was detected but not quantified because a standard compound was not available. However, unambiguous identification of its enantiomers could be achieved.

Ratios of the S-enantiomer to the R-enantiomer are used to compare the intrinsic clearance of the 2 enantiomers, which in turn may reflect similar or different enzyme activities responsible for their metabolism. Ratios of the S-enantiomer to the R-enantiomer that diverge from unity may indicate that enantiomers are stereoselectively metabolized or excreted (or a combination of both). Analysis of the electropherograms revealed no significant differences from unity for ratios of S-dehydro and R-dehydronorketamine to R-dehydronorketamine. This differs from results of other investigations that involved a target control infusion of racemic ketamine with a mean arterial S-ketamine concentration of approximately 1.5 μg/mL, for which there was a higher concentration of R-dehydronorketamine than S-dehydronorketamine during and after drug administration. However, the temporal behavior of the ratio for S-norketamine to R-norketamine, which revealed significantly higher ratios during and after CRI, was found to be comparable with that in the latter report.

Total doses of S-ketamine (0.84 mg/kg) and racemic ketamine (1.75 mg/kg) administered in the study reported here during a 1-hour period represented 73% of the dose for each compound (S-ketamine, 1.1 mg/kg; racemic ketamine, 2.2 mg/kg) administered to ponies anesthetized with isoflurane in oxygen or sevoflurane (1.1 mg/kg). Despite the fact that the doses for S-ketamine and racemic ketamine were similar, differences in pharmacokinetic variables were obtained when results for these 3 studies were compared. Other studies on disposition of ketamine enantiomers have revealed that the pharmacokinetic results may differ markedly, depending on the modus of administration. Pharmacokinetic models derived from data obtained after administration of a single bolus may be inappropriate for application to data obtained after infusions because drugs that remain in the circulation typically have a low Vd, increased t1/2el, and reduced Clb. Indeed, reduced t1/2el values ranging between 1.3 to 3.4 minutes and increased values for Clb and Vd ranging between 2.53 to 13.8 L/kg/h and between 0.20 to 0.44 L/kg, respectively, have been detected for ketamine enantiomers after administration of a single bolus, compared with values during ketamine CRI. Nevertheless, both detection limit and interval between collection of samples can influence the calculated t1/2el and AUC. To detect the low plasma concentrations in our study, extraction prior to capillary electrophoresis analysis was used. However, despite the fact that a more sensitive analytic method was used for this study, ketamine enantiomers could not be detected 60 minutes after cessation of the CRI, which resulted in reduced AUCs of ketamine and norketamine enantiomers.
each enantiomer for both treatments, compared with reports for 2 other studies, conducted by our laboratory group in which a single bolus of drug was administered. Although the total time for collection of samples resembled that for one of the aforementioned studies, the interval between administration of the loading dose and collection of the first sample was longer (first sample collected at 5 minutes) in the study reported here. This probably resulted in lower peak plasma concentrations than in other reports, which also led to smaller AUCs. Differences in plasma concentrations and calculated AUCs obtained in other studies also can be explained by dissimilar effects on the cardiovascular system for the coadministered drugs.

Several other factors may have an impact on pharmacokinetic variables for a specific compound within a species. These include population characteristics (age, sex, body surface area, and mean body weight), plasma protein binding, coadministered drugs, sampling site, intervals between collection of samples, and analytic method. Because the ponies in the study reported here were also used in the aforementioned studies, these differences cannot be attributed to population dissimilarity nor to alteration in plasma protein concentration because the preliminary biochemical analysis performed in all ponies before each study revealed values for total plasma proteins and albumin within the respective reference ranges. Moreover, ketamine plasma protein binding is low in horses (50%), which indicates only a minor influence on the calculated pharmacokinetic variables.

Coadministered drugs may also influence metabolism of ketamine enantiomers by interacting with the CYP 450 responsible for ketamine elimination. Although coadministration of isoflurane did not alter elimination of S-ketamine or R-ketamine after administration of S-ketamine or racemic ketamine in another study, coadministration of xylazine did not alter elimination of S-ketamine or R-ketamine after administration of S-ketamine or racemic ketamine in another study. Coadministration of xylazine induced a more rapid elimination of S-ketamine with a higher Cmax than after administration of racemic ketamine. Therefore, it was suspected that N-demethylation of S-ketamine was favored as a result of coadministration of the α₂-adrenergic receptor agonist xylazine when S-ketamine was administered alone. Interestingly, in the study reported here, although a smaller AUC was detected for S-ketamine in the S-ketamine treatment, similar AUCs were detected for S-norketamine in the S-ketamine or racemic ketamine treatments. When these compounds are administered as a low-dose CRI without concomitant drug administration, it may be possible that S-ketamine is hydroxylated more rapidly after infusion of a single enantiomer than after it is administered as part of the racemate; this suggests an inhibition of the metabolic pathway by the R-enantiomer, as proposed in an in vitro study performed by use of rat liver microsomes and another study performed by use of equine liver microsomes. Because R-ketamine alone was not administered to these ponies, this hypothesis could not be tested.

Subanesthetic infusions of S-ketamine or racemic ketamine led to smaller AUCs for the S-ketamine enantiomer, which suggested a more rapid elimination of S-ketamine when administered alone than when administered within the racemate. Calculated pharmacokinetic variables differed, compared with those reported in other studies in which investigators in our laboratory group administered a single bolus of ketamine to the same ponies anesthetized by administration of isoflurane or sedated by administration of xylazine. Anesthesiologists should be aware of these differences when applying these data in a clinical setting. For subanesthetic infusions of racemic ketamine, the metabolism of ketamine is stereoselective, as indicated by the ratios of plasma concentrations of S-norketamine to R-norketamine, which increased significantly after CRI.

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