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Stereoselective biotransformation of ketamine in equine liver and lung microsomes

A. Schmitz^{*}, C. J. Portier[†], W. Thormann[‡], R. Theurillat[‡], and M. Mevissen^{*}

^{*}Division of Veterinary Pharmacology and Toxicology, Vetsuisse Faculty, University of Bern, Bern, Switzerland [†]Environmental Systems Biology and Risk Assessment, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA [‡]Department of Clinical Pharmacology, University of Bern, Bern, Switzerland

Abstract

Stereoselectivity has to be considered for pharmacodynamic and pharmacokinetic features of ketamine. Stereoselective biotransformation of ketamine was investigated in equine microsomes *in vitro*.

Concentration curves were constructed over time, and enzyme activity was determined for different substrate concentrations using equine liver and lung microsomes. The concentrations of R/S-ketamine and R/S-norketamine were determined by enantioselective capillary electrophoresis. A two-phase model based on Hill kinetics was used to analyze the biotransformation of R/S-ketamine into R/S-norketamine and, in a second step, into R/S-downstream metabolites.

In liver and lung microsomes, levels of R-ketamine exceeded those of S-ketamine at all time points and S-norketamine exceeded R-norketamine at time points below the maximum concentration. In liver and lung microsomes, significant differences in the enzyme velocity ($V_{\max}$) were observed between S and R-norketamine formation and between $V_{\max}$ of S-norketamine formation when S-ketamine was compared to S-ketamine of the racemate.

Our investigations in microsomal reactions *in vitro* suggest that stereoselective ketamine biotransformation in horses occurs in the liver and the lung with a slower elimination of S-ketamine in the presence of R-ketamine. Scaling of the *in vitro* parameters to liver and lung organ clearances provided an excellent fit with previously published *in vivo* data and confirmed a lung first-pass effect.

Introduction

Ketamine, a noncompetitive *N*-methyl-D-aspartate receptor (NMDA) antagonist, is one of the most frequently used injectable anesthetics in veterinary medicine. Ketamine produces a so-called dissociative anesthesia in humans, a state with suggested inhibition of thalamocortical pathways and stimulation of the limbic regions of the brain (White *et al.*, 1982). In horses, ketamine in combination with a sedative-hypnotic and/or muscle relaxant is used for short-term chemical restraint or for induction to general anesthesia. Recent studies have evaluated the effect of subanesthetic doses of ketamine on pain in horses that has already been shown for humans (Fielding *et al.*, 2006; Knobloch *et al.*, 2006). Ketamine consists of a mixture of two optical isomers: S- and R-ketamine. Differences in pharmacological effects and pharmacokinetic properties between the two enantiomers have been described *in vivo* and *in*

vitro (White *et al.*, 1985; Klepstad *et al.*, 1990; Oye *et al.*, 1992; Arendt-Nielsen *et al.*, 1996; Himmelseher *et al.*, 1996). S-ketamine has been reported to exert a fourfold greater affinity for the NMDA receptor compared to R-ketamine, resulting in a likewise increase in the hypnotic properties of the S-enantiomer (White *et al.*, 1985). In human medicine since 10 years, the pure S-enantiomer is preferentially used because of its higher potency together with fewer unwanted side effects. The pure S-enantiomer has recently been introduced to the veterinary market as a formulation for cats, where it already proved to be advantageous because of higher potency and shorter recovery times compared to the racemic mixture (Eichenberger, 2005). Investigations on the properties of the pure S-enantiomer in other species are necessary.

Pharmacokinetic studies of ketamine have been performed in humans (White *et al.*, 1985; Geisslinger *et al.*, 1993; Ihmsen *et al.*, 2001; Hijazi *et al.*, 2003), rats (White *et al.*, 1976), dogs (Schwieger *et al.*, 1991), cats (Waterman, 1983) and horses (Kaka *et al.*, 1979; Waterman *et al.*, 1987). Most of these studies used a conventional two- or three-compartment model for analysis. To our knowledge, the stereoselective pharmacokinetics of ketamine in equines derive entirely from four *in vivo* studies (Delatour *et al.*, 1991; Knobloch *et al.*, 2006; Larenza *et al.*, 2007a,b). A physiologically based pharmacokinetic (PBPK) model describing the distribution and biotransformation of ketamine and norketamine in horses receiving a ketamine infusion under isoflurane anesthesia was developed recently (Knobloch *et al.*, 2006). The authors showed that the equine lung, an organ with a strong first-pass effect, probably plays an important role in ketamine pharmacokinetics.

The present *in vitro* study addresses possible differences between biotransformation rates of ketamine in equine liver and lung microsomes and at different concentrations. Secondly, biotransformation of pure S-enantiomer and S-enantiomer in the racemic mixture was investigated. A two-phase model of ketamine biotransformation was established. *In vitro* intrinsic clearances of liver and lung were scaled to organ clearance and compared with data from a recently published *in vivo* study.

Materials and Methods

Preparation of microsomes

Equine liver and lung tissue were obtained from a local slaughterhouse. Animals were either cross-breeds or Franches-Montagnes from both sexes aged 13–30 years. Liver tissue was taken from five healthy horses that had not received any drug treatment recently. Liver microsomes were prepared in three different batches: batch 1 = horse 1, batch 2 = horse 2, batch 3 = horses 3–5. Pooled microsomes from batch 3 were analyzed to diminish the possibility of aberrant results due to polymorphisms in the individual microsomes (batches 1 and 2). Lung tissue was taken from two horses and microsomes prepared in two batches: batch 1 = horse 2 and batch 2 = horse 6. Tissue samples were taken from varying anatomical locations.

Liver and lung tissue samples were taken less than half an hour after stunning and placed on dry ice immediately for transportation to the laboratory. Samples were kept at –80 °C until use for microsome preparation as previously described (Mackinnon *et al.*, 1977). For the preparation of lung microsomes, homogenization was started by grinding the deep-frozen lung tissue followed by homogenizing in a glass potter. Microsome preparations were aliquoted and stored at –80 °C until use.

The total protein concentration was measured by the Biuret method using bovine serum albumin as a standard. Total cytochrome P450 proteins in the microsomes were determined using the method of Omura and Sato (1964). Cytochrome P450 could not be quantified in the lung microsomes due to an unusual spectrometric pattern.

Microsomal reactions: general procedure

A mixture containing substrate, either racemic ketamine (Inselspital, Bern, Switzerland) or S-ketamine (Dr Graeb, Bern, Switzerland), and NADPH regenerating system (Gentest, Woburn, MA, USA) (1.25 mM NADP⁺, 3.3 mM G-6-P, 0.4 U/mL G-6-P dehydrogenase, 3.3 mM MgCl₂) in 100-mM potassium phosphate buffer (pH 7.4) was prepared and preincubated at 37 °C. The microsomal enzymatic reaction was then started by adding liver or lung microsomal proteins to a final concentration of 0.5 mg/mL. Samples were withdrawn as described in the following two paragraphs. Reactions were terminated by adding sodium hydroxide (Merck, Darmstadt, Germany) to 0.2 M. Liver and lung microsomes from batches 1 and 2 were used for the experiments. Reactions were performed in duplicate. Control experiments with liver microsomes from batch 3 were run as single incubations to rule out polymorphisms in batches 1 and 2. Negative controls without addition of any substrate were included.

Time–concentration curves

Reactions containing liver or lung microsomes were incubated with different substrate concentrations of either the racemic mixture (100, 25 and 12.5 μM) or S-ketamine (50, 12.5 and 6.25 μM) in a final volume of 1 mL. Aliquots of 100 μL were withdrawn at different time points from the reactions after 0, 5, 9, 15, 30, 60, 90 and 120 min.

Substrate concentration-dependent microsomal activity

Kinetic studies were performed with five substrate concentrations ranging from 6 to 100 μM for the racemic ketamine and from 3 to 50 μM for pure S-ketamine in a final volume of 250 μL. Aliquots of 100 μL were withdrawn from the reaction mixture after 8 min. Linearity of the norketamine formation rate was established with respect to microsomal protein and incubation time.

Analytical procedure and assay specifications

Enantioselective analysis of ketamine and norketamine was performed by capillary electrophoresis (CE) according to Theurillat *et al.* (2005) and using the modification described by Theurillat *et al.* (2007). For analysis, samples were taken from microsomal reactions containing either racemic ketamine or S-ketamine. Samples of 100 μL were mixed with 0.5 mL 0.2-M sodium hydroxide containing 30 μL of the internal standard (+)-pseudoephedrine hydrochloride (Fluka, Buchs, Switzerland) (150 μg/mL). Residues from evaporation were dissolved in 30 or 150 μL of 10-fold diluted Tris-phosphate buffer.

Quantitation was based upon internal calibration using corrected peak areas. When analyzing samples taken from microsomal reactions with the highest concentrations of ketamine (100 μM racemic and 50 μM S-ketamine), calibration was made for every new sequence using five freshly prepared calibrators covering a range from 2 to 105 μM for each enantiomer. All calibration graphs were found to be linear within this range ($r > 0.995$, $F > 320$). For all levels, intraday relative standard deviation (RSD) values were <8%, interday RSD values beneath 9%. The detection and quantitation limits for each enantiomer were 0.25 and 0.84 μM, respectively. For samples from incubations started with lower ketamine concentrations (25, 12.5 and 6.25 μM), the calibration curve ranged from 0.4 to 21 μM for each enantiomer and all graphs were linear ($r > 0.996$, $F > 350$). For all levels, intraday RSD values were smaller than 9%, interday RSD values <14%. The detection and quantitation limits were 0.08 and 0.25 μM, respectively. Typical electropherograms for liver microsomal reactions initiated with 100-μM racemic ketamine are shown in Fig. 1.

Two-compartment model

Ketamine, when administered to ponies, is metabolized via cytochrome P450 enzymes into norketamine, which is then further biotransformed via other metabolic pathways. This leads conceptually to a binary two-compartment model to describe the pharmacokinetics of racemic ketamine in microsomal preparations as shown in Fig. 2. Such a model can be mathematically described by four ordinary differential equations:

$$\begin{aligned}\frac{dC_{kR}(t)}{dt} &= -R_{kR}[C_{kR}(t)] \\ \frac{dC_{nR}(t)}{dt} &= R_{kR}[C_{kR}(t)] - R_{nR}[C_{nR}(t)] \\ \frac{dC_{kS}(t)}{dt} &= -R_{kS}[C_{kS}(t)] \\ \frac{dC_{nS}(t)}{dt} &= R_{kS}[C_{kS}(t)] - R_{nS}[C_{nS}(t)],\end{aligned}\quad (1)$$

where $C_{kx}(t)$ refers to the concentration (μM) of x -ketamine ($x = \text{S or R}$) at time t , $C_{nx}(t)$ refers to the concentration (μM) of x -norketamine at time t , $R_{kx}[C_{kx}(t)]$ refers to the concentration-dependent rate of conversion from x -ketamine to x -norketamine and $R_{nx}[C_{nx}(t)]$ refers to the concentration-dependent rate of conversion from x -norketamine to downstream metabolites.

The four rates shown in Fig. 2 and described in equation (1) could have different functional forms. In the analyses conducted in this research, three different possible forms were assumed for the rates in the model:

$$\text{Linear } R_{wxyzq}[C_{wxyzq}(t)] = k_{wxyzq}C_{wxyzq}(t) \quad (2)$$

$$\text{Michaelis - Menten } R_{wxyzq}[C_{wxyzq}(t)] = \frac{V_{wxyzq}C_{wxyzq}(t)}{K_{wxyzq} + C_{wxyzq}(t)} \quad (3)$$

$$\text{Hill } R_{wxyzq}[C_{wxyzq}(t)] = \frac{V_{wxyzq}C_{wxyzq}^{n_{wxyzq}}(t)}{K_{wxyzq}^{n_{wxyzq}} + C_{wxyzq}^{n_{wxyzq}}(t)}, \quad (4)$$

where k_{wxyzq} is the first-order rate constant of metabolism for chemical form w (ketamine k or norketamine n), enantiomer x (R-enantiomer R or S-enantiomer S), tissue y (liver L or lung P), initial formulation z (racemic m mixture or S-only a) and dose q (high H or low L concentration of substrate) (μM of the metabolite of wx per minute); V_{wxyzq} (μM of the metabolite of wx per minute) is the maximum rate of reaction of the metabolite of wx ; and K_{wxyzq} (μM of wx) is the dissociation constant for the reaction resulting in the metabolite of wx and n_{wxyzq} is the Hill coefficient (unitless).

Parameter estimation and testing

To fit the model described above to the experimental data, we used a classical statistical likelihood for censored data as outlined by Koo *et al.* (2002). Briefly, the log of the observed concentrations were assumed to be normally distributed with a mean described by equation (1) and separate variances for each enantiomer in each tissue (eight total variances based upon measurements of S-ketamine, R-ketamine, S-norketamine and R-norketamine in liver and lung microsomes). Observed values of zero (0) concentration were assumed to be below the limit

of detection ($0.08 \mu\text{M}$) and treated as censored data points in the likelihood. Best estimates were obtained by maximizing the likelihood using the Davidon–Fletcher–Powell search algorithm (Walsh, 1975).

Hypotheses were tested using the maximum likelihood test. Several hypotheses were keys to the interpretation of the data and are best described via the rates that make up equation (1). Three hypotheses were evaluated to determine the best model within a given formulation. First, are the rates similar for liver and lung:

$$H_{01}: R_{w\text{xL}\bullet\bullet}[C_{w\text{xL}\bullet\bullet}(t)] = R_{w\text{xP}\bullet\bullet}[C_{w\text{xP}\bullet\bullet}(t)] \text{ for all } w = [k \text{ or } n] \text{ and } x = [\text{R or S}] \text{ combinations.}$$

Second, is there a difference in the metabolism of the S-ketamine when it is part of the racemic mixture versus alone:

$$H_{02}: R_{w\text{xy}\text{m}\bullet}[C_{w\text{xy}\text{m}\bullet}(t)] = R_{w\text{xy}\text{a}\bullet}[C_{w\text{xy}\text{a}\bullet}(t)] \text{ for all } w = [k \text{ or } n], x = [\text{R or S}] \text{ and } y = [\text{L or P}] \text{ combinations.}$$

Third, are the low-concentration rates different from the high-concentration rates (dose-dependent metabolism):

$$H_{03}: R_{w\text{xyz}\text{L}}[C_{w\text{xyz}\text{L}}(t)] = R_{w\text{xyz}\text{H}}[C_{w\text{xyz}\text{H}}(t)] \text{ for all } w = [k \text{ or } n], x = [\text{R or S}], y = [\text{L or P}] \text{ and } z = [\text{m or a}] \text{ combinations.}$$

In all three cases, the alternative is that at least one combination shows inequality. Hypotheses were rejected in $P < 0.05$.

A second set of hypotheses involved whether the data follow Hill kinetics, Michaelis–Menten kinetics or linear kinetics. As these are nested models (linear $\subset$ Michaelis–Menten $\subset$ Hill, where $\subset$ means a reduced form), the likelihood-ratio test was appropriate. Calculations were made using MATLAB Simulation Software (Release 7.4, MathWorks, Natick, MA, USA).

Estimation of *in vivo* total clearance

In vivo total clearance (Cl_{intr}) was calculated as the rate of biotransformation divided by the concentration and varied as a function of the concentration. To arrive at *in vivo* clearance, the biotransformation rates were scaled to the whole liver and lung organ rates. For the liver, we used a scaling factor derived from the rat of 45 mg microsomal protein/g liver (Houston, 1994). The *in vivo* scaling factor for the lung was derived after Lakritz *et al.* (2000) who described the lung microsomal protein yield to be about one-third of the liver leading to a scaling factor of 15 mg microsomal protein/g lung. Hepatic and pulmonary clearance was calculated using the venous equilibrium model (Houston, 1994). Plasma protein binding of ketamine and norketamine was taken to be 50% (Kaka *et al.*, 1979; Hijazi & Boulieu, 2004), hepatic blood flow 6.975 L/min (McConaghy *et al.*, 1996; Dyke *et al.*, 1998) and pulmonary blood flow 25 L/min (Knobloch *et al.*, 2006).

Norketamine formation curves and statistical analysis

Curves of ketamine concentration-dependent kinetics were determined using values obtained from reactions stopped after 8 min of incubation. Calculation was made according to the Hill equation (see equation 4) and estimation via the least-squares method using MATLAB Simulation Software (Release 14, MathWorks). In this analysis, V is the maximal attainable response, K stands for half-effective concentration (the concentration yielding half the maximum effect) and n describes the shape of the function (values greater than 1 describe curves with a flat low-dose region and high curvature, whereas values smaller than 1 correspond to curves that climb

rapidly). Statistical significance of comparisons made on the basis of this model was determined using the likelihood-ratio statistic (Knobloch *et al.*, 2006).

Results

Biotransformation of ketamine *in vitro*

The concentrations of the enantiomers of ketamine and norketamine were determined over a 120-min time interval in equine liver and lung microsomal incubations.

Three different biotransformation models were used to analyze the data obtained from capillary electrophoresis (CE) analysis in order to test for differences in biotransformation of ketamine to norketamine and to downstream metabolites. The best fitting model used Hill kinetics for all biotransformation rates in liver microsomes and is shown in Fig. 3. Figure 4 shows concentrations of the enantiomers of ketamine and norketamine determined during 120 min in equine liver microsomal preparations at 25- μM racemic ketamine (Fig. 4a) and at 12.5- μM S-ketamine (Fig. 4b). Figures 4c & d show ketamine and norketamine concentrations determined during 120 min in equine lung microsomal reactions at 25- μM racemic ketamine (Fig. 4c) and at 12.5- μM S-ketamine (Fig. 4d).

Intra-organ comparison between S- and R-ketamine transformation rates showed that within the liver microsomal reactions, ketamine to norketamine biotransformation rates were found to be similar for S- and R-ketamine in all batches of liver microsomes at all concentrations of ketamine investigated (Table 1). Biotransformation rates of S-ketamine compared to R-ketamine were also found to be generally the same in lung microsomes. (Table 1). In contrast to the biotransformation rate of the parent compound ($P = 0.13$), biotransformation rates of the two forms S- and R-norketamine into downstream metabolites were significantly different ($P < 0.001$) (Table 1; Figs 4a & c).

Inter-organ comparison between biotransformation rates showed that *in vitro* biotransformation rates for racemic and S-ketamine were significantly different between liver and lung microsomal reactions with a higher rate in liver compared to lung (reject H_{01} , $P < 0.001$). In both organs S-ketamine alone was metabolized faster than in the racemic mixture (reject H_{02} , $P < 0.001$). Significant differences in biotransformation rates were seen for some high and low ketamine concentrations, suggesting dose-dependent metabolism (reject H_{03} , $P < 0.001$). The same results were obtained in the batch of pooled liver microsomes.

In vivo clearance

Figure 5a shows the *in vivo* total clearances for both ketamine and norketamine when administered as the racemic mixture. At a 10- μM concentration, the predicted total clearance from the biotransformation data for ketamine is approximately 40 L/min and for norketamine is approximately 30 L/min. These clearance values were compared to those calculated using the kinetic parameters for ponies from Knobloch *et al.* (2006). As seen in Fig. 5a, the clearances are very similar for ketamine above a concentration of 20 μM and virtually identical for norketamine over the entire range of concentrations. All of the clearance curves reach peak clearance at very low concentrations ($<1 \mu\text{M}$). For the *in vivo* data from Knobloch *et al.* (2006), there is a rapid drop from peak and then a climb in ketamine clearance in the liver due to the nonlinear metabolic rates. In the lung, the clearance peaks and then drops steadily as the concentration increases.

To determine if the differences in predicted clearance have an impact on the prediction of plasma concentrations of ketamine, the PBPK model from Knobloch *et al.* (2006) was rerun substituting the biotransformation rates (suitably adjusted) for their estimated metabolic rates and compared to the original data. The resulting plots (Figs 5b & c) show that the estimated

biotransformation rates are expedient for predicting ketamine clearance from the plasma in ponies.

Norketamine formation curves

Figure 6 shows norketamine formation curves generated with five different concentrations of racemic- and S-ketamine at an incubation time of 8 min.

Intra-organ comparisons of the maximal reaction rates ($V_{\max}$) were performed. In both organs S-ketamine in the racemic mixture had a higher $V_{\max}$ than R-ketamine ($P = 0.014$ and $P = 0.011$ for liver and lung, respectively). Comparisons between racemic S-ketamine and pure S-ketamine showed a significantly higher $V_{\max}$ for the pure S-ketamine in liver ($P = 0.01$) and lung ($P = 0.004$).

When comparing $V_{\max}$ between organs, the maximal reaction rate also proved to be significantly higher in the liver for S-ketamine and R-ketamine in the racemic mixture and pure S-ketamine compared to the lung ($P = 0.003$, $P = 0.003$, $P = 0.007$, respectively). Comparing the $V_{\max}$ of racemic S-ketamine in the liver to pure S-ketamine in the lung yielded a significantly higher $V_{\max}$ in the liver ($P = 0.01$). No significant differences were seen in the K_d values. Data are given in Table 2.

Discussion

Results from the present study show that biotransformation of ketamine occurred in both equine liver and lung microsomes. The involvement of the lung in the biotransformation of ketamine was also suggested in an *in vivo* study using a PBPK model in ponies (Knobloch *et al.*, 2006). The ketamine concentrations used in our study are supposed to reflect concentrations in the specific organs *in vivo*. The concentration of 25- μM racemic ketamine used in our experiments is in accordance with calculations obtained by the PBPK model using *in vivo* data (Knobloch *et al.*, 2006). The PBPK model postulated a ketamine concentration in the lung being five times higher than the measured plasma concentration. For anesthesia, a minimal plasma level of 4.2- μM racemic ketamine is required (Fielding *et al.*, 2006), which would approximately correspond to 25- μM racemic ketamine in the lung.

Using equal amounts of the ketamine enantiomers for the reactions is in agreement with a previous study of Henthorn *et al.* (1999) in dogs, describing no enantioselectivity of ketamine in its tissue distribution. Due to identical partition coefficients of S- and R-ketamine, Knobloch *et al.* (2006) also concluded that there is no difference between S- and R-ketamine distribution in the body.

In the present report, *in vitro* biotransformation rates of ketamine in the lung reactions were found to be significantly lower than those in the liver microsomal reactions. As it was recently reported (Knobloch *et al.*, 2006) that the formation of norketamine enantiomers was predicted to be 2.8 times lower in the lung compared to the liver at steady-state, our *in vitro* results are in agreement with the estimation obtained from the *in vivo* study. In addition, the fact that CYP proteins in the lung microsomes were not measurable by our method is a further support to the study of Lakritz *et al.* (2000), reporting that in the horse the concentration of microsomal CYP proteins is threefold greater in the liver than in the lung.

Comparison of the calculated intrinsic clearances for racemic ketamine between organs showed strong agreement over most concentrations. When scaling the intrinsic clearances to organ clearances dependent on blood flow and unbound fraction of the drug, liver and lung clearance nearly reached blood flow (not shown), supporting the hypothesis of a large first-pass effect as described before. Total body clearance derived from studies using a bolus regime was

reported to be between 30 and 40 mL/min/kg (Kaka *et al.*, 1979; Waterman *et al.*, 1987; Larenza *et al.*, 2007b). In a 500-kg horse, this would mean a total clearance of 15–20 L/min, similar to the values derived here.

Of note, the half maximal rate of metabolism (K_d) for ketamine biotransformation as calculated by the model proved to be smaller than the plasma concentration that is usually required to maintain anesthesia ($>5 \mu\text{M}$). This unusual relation makes it challenging to maintain a constant plasma level during a ketamine infusion as seen in the *in vivo* study (Knobloch *et al.*, 2006), where it was necessary to start the infusion with a bolus dose. Thus, after cessation of the infusion, a rapid decline in plasma levels was seen. Most of this rapid clearance is occurring in the lung.

The difference between S- and R-ketamine transformations was more pronounced in the liver compared to the lung. This might be due to differences in the expression of each individual cytochrome P450 isoform between different tissues (Ding & Kaminsky, 2003).

Data on enantioselective pharmacokinetics of ketamine in the horse are limited compared to data in humans. Delatour *et al.* (1991) found a difference in S/R-norketamine plasma levels in horses that increased over time (3:1 after 5 min to 9:1 after 40 min) after a bolus administration of 6 mg/kg racemic ketamine. Differences between S- and R-norketamine levels were also found when administering a bolus dose of racemic ketamine to ponies under isoflurane anesthesia (Larenza *et al.*, 2007b). A similar pattern was obtained in plasma of ponies under a 2-h target-controlled infusion of racemic ketamine (Knobloch *et al.*, 2006). In all *in vivo* studies S- and R-ketamine levels did not differ over the whole observation time.

Our *in vitro* findings, which include differences in S/R-ketamine and S/R-norketamine levels, suggest that the differences in norketamine levels are due to different biotransformation rates of the norketamine enantiomers (Table 1). Like the *in vivo* PBPK model that predicted similar biotransformation rates for S- and R-ketamine but different biotransformation rates for S- and R-norketamine into downstream metabolites (Knobloch *et al.*, 2006), the biotransformation model used for the *in vitro* data predicted similar biotransformation rates for S/R-ketamine and different ones for S/R-norketamine (Table 1). Stereoselective ketamine *N*-demethylation has also been hypothesized by Delatour *et al.* (1991) in an *in vivo* study in horses and by Kharasch and Labroo (1992) in an *in vitro* study in human liver microsomes.

In vivo studies in human volunteers and surgical patients reported a higher clearance of the S-enantiomer compared to the R-enantiomer in the racemic mixture but also when the two enantiomers were administered separately (Geisslinger *et al.*, 1993; Ihmsen *et al.*, 2001; Persson *et al.*, 2002).

In the biotransformation model, S-ketamine values were significantly different when administered alone or as part of the racemic mixture. We found that the biotransformation rate of the pure S-ketamine was slightly higher than that of the S-enantiomer in the racemic mixture. This may suggest a competition of the R-enantiomer and the S-enantiomer for the same enzymatic pathway of elimination. *In vivo* studies in ponies receiving a bolus dose of racemic ketamine and pure S-ketamine only partly produced corresponding results. Depending on the co-medication, authors did or did not find higher clearances for the single S-enantiomer compared to the racemic mixture (Larenza *et al.*, 2007a,b). When administering ketamine to ponies premedicated with xylazine, differences in elimination half-life and mean residence time were significant between S-alone and S-racemic, whereas ponies receiving ketamine under isoflurane anesthesia did not show these differences. Changes in hepatic and renal enzyme activities mediated through the co-administered drug could explain the differences. Authors hypothesized a greater cytochrome-dependent *N*-demethylation for the single S-enantiomer in the ponies sedated with xylazine. More likely, as xylazine is known to decrease

heart rate and blood pressure, hemodynamic alterations caused by the co-medication could account for observed pharmacokinetic differences between ketamine in the racemic mixture and S-ketamine alone. Studies in humans have found controversial results with respect to the pharmacokinetics of S-ketamine alone vs. S-ketamine as part of the racemic mixture. Studies using an infusion regime for ketamine administration usually found higher clearances for the pure S-enantiomer, whereas bolus administration of racemic and S-ketamine did not show a difference in clearances (Geisslinger *et al.*, 1993; Ihmsen *et al.*, 2001). Other factors such as interindividual variability and different study design may also have influenced the outcome.

In conclusion, biotransformation of ketamine was shown to take place in the lung and stereoselective biotransformation of ketamine could be confirmed by our *in vitro* study. Differences in biotransformation rates and $V_{\max}$ were determined between liver and lung, between S-ketamine in the racemic mixture and S-ketamine alone and between some concentrations used in the study. The higher biotransformation rate of S-ketamine when used alone compared to the S-enantiomer in the racemic mixture indicates the possibility of a faster elimination from the body *in vivo*, and therefore a different pharmacokinetic profile than the racemic mixture. A recent study in ponies sedated with xylazine supports this assumption (Larenza *et al.*, 2007a). Clearance values derived from this *in vitro* study show a good correlation with those calculated by a PBPK model of ketamine biotransformation in ponies.

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Fig. 1. Electropherograms of samples from an incubation of equine liver microsomes with 100- μM racemic ketamine. Samples were collected at 0 min (a), 15 min (b) and 90 min (c) after beginning of the incubation. S-ket, S-ketamine; R-ket, R-ketamine; S-nor, S-norketamine; R-nor, R-norketamine. Concentrations of S-ket and R-ket in panel (a) were determined to be 47.7 and 48.5 μM , respectively. Concentrations of S-ket, R-ket, S-nor and R-nor in panel (b) were determined to be 35.6, 39.25, 7.4 and 5.1 μM , respectively, and in panel (c) 12.6, 22.3, 23.6 and 19.1 μM , respectively. The two newly appearing peaks (S/R-DHNK) in panel (c) represent S/R-dehydronorketamine, two metabolites which could not be quantified due to missing standards (Theurillat *et al.*, 2007). Panel (d) shows the temporal behavior of the current of (a).

Fig. 2.

A model for the biotransformation of ketamine in which ketamine (R and S) is metabolized to norketamine (R and S), where the rate of biotransformation from ketamine to norketamine is denoted by $R_{kR}(C_{kR}(t))$ for R-ketamine and by $R_{kS}(C_{kS}(t))$ for S-ketamine, and the rate of biotransformation from R-norketamine to other metabolites is denoted by $R_{nR}(C_{nR}(t))$ and for S-norketamine to others by $R_{nS}(C_{nS}(t))$.

Fig. 3.

Curves depict the data fits obtained from the Hill model; circles represent experimentally determined concentrations of ketamine and norketamine over time. Data are shown for liver for all three concentrations of ketamine used in the experiments. Ketamine and norketamine and the respective S- and R-forms are put in separate panels. The respective S-enantiomers pure and in the racemic mixture are depicted together in one panel. The pure S-form is always represented by a dashed line.

Fig. 4. S/R-ketamine and S/R-norketamine concentrations during 2-h incubations of equine liver microsomes with 25-μM racemic ketamine (a) and 12.5-μM S-ketamine (b). Data are shown as mean and SEM of duplicate incubations with batches 1 and 2. S/R-ketamine and S/R-norketamine concentrations during 2-h incubations of equine lung microsomes with 25-μM racemic ketamine (c) and 12.5-μM S-ketamine (d). Data are given as mean and SEM of duplicate incubations with batches 1 and 2.

Fig. 5.

Total clearance of ketamine and norketamine (a) comparing the biotransformation parameters from Table 1 (racemic mixture) with the parameters derived by Knobloch *et al.* (2006); time-concentration curves for R-ketamine (b) and S-ketamine (c) comparing the data from Knobloch *et al.* (2006) to the physiologically based pharmacokinetic model predictions (dashed line) with substitution of the biotransformation parameters in Table 1 for the estimated values from Knobloch *et al.*

Fig. 6.

Norketamine formation curves made with five different concentrations of ketamine incubated for 8 min each with liver microsomes (a) and lung microsomes (b). Symbols show the experimental data, lines the values obtained by the modeling. Data are given as mean and SEM of duplicate incubations with batches 1 and 2.

Table 1

Maximum-likelihood estimates of $V_{\max}$, K_d and n (Hill coefficient) in the two-phase biotransformation model

	Parameter	Tissue	S/R Ketamine	S-norketamine	R-norketamine
S-Ketamine	K_d (μM ketamine)	Liver	0.52	0.46	*
		Lung	1.80	2.64	*
	$V_{\max}$ (nmol metabolite/min/mg protein)	Liver	0.76	0.32	*
		Lung	0.51	0.25	*
Racemic Mixture	Hill coefficient (unitless)	Liver	1.64	1.76	*
		Lung	1.02	2.99	*
	K_d (μM ketamine)	Liver	0.51	0.59	4.78
		Lung	0.27	0.10	2.85
	$V_{\max}$ (nmol metabolite/min/mg protein)	Liver	0.97	0.53	0.47
		Lung	0.78	0.28	0.25
	Hill coefficient (unitless)	Liver	1.77	2.39	1.11
		Lung	2.00	1.71	2.50

K_d , concentration at which half-maximal activity occurs (μM); $V_{\max}$, maximal activity (nmol metabolite/min/mg protein).

* No R-ketamine biotransformation for the incubations with S-ketamine alone.

Table 2

Maximum-likelihood estimates of $V_{\max}$ (nmol/min/mg protein), K_d (μM) and n (Hill coefficient, unitless) in the dose–response analysis of norketamine formation at 8 min postincubation with five different concentrations of the appropriate substrate

	Parameter	Tissue	S-ketamine (CI)	R-ketamine (CI)
S-Ketamine	K_d (μM ketamine)	Liver	14.96 (10–22.37)	*
		Lung	6.39 (4.38–9.32)	*
	$V_{\max}$ (nmol norketamine/min/mg protein)	Liver	2.3 (1.63–3.23)	*
		Lung	1.03 (0.77–1.39)	*
	Hill coefficient (unitless)	Liver	1.46	*
		Lung	1.46	*
Racemic Mixture	K_d (μM ketamine)	Liver	7.25 (5.29–9.94)	4.56 (3.03–6.86)
		Lung	2.94 (1.59–5.45)	2.56 (1.2–5.44)
	$V_{\max}$ (nmol norketamine/min/mg protein)	Liver	1.41 (1.08–1.83)	0.85 (0.63–1.15)
		Lung	0.53 (0.35–0.81)	0.39 (0.23–0.66)
	Hill coefficient (unitless)	Liver	1.46	1.46
		Lung	1.46	1.46

K_d , concentration at which half-maximal activity occurs (μM); $V_{\max}$, maximal activity (nmol norketamine/min/mg protein); Hill coefficient is fixed; CI, 95% lower and upper bounds.

* No R-ketamine biotransformation for the incubations with S-ketamine alone.