**Pharmacokinetics of ketamine and three metabolites in Beagle dogs under sevoflurane
vs. medetomidine comedication assessed by enantioselective capillary electrophoresis**

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Abstract

Ketamine is often used for anesthesia in veterinary medicine. One possible comedication is the sedative α_2 -agonist medetomidine. Advantages of that combination are the compensation of side effects of the two drugs and the anesthetic-sparing effect of medetomidine. In vitro studies showed that medetomidine has an inhibitive effect on the formation of norketamine. Norketamine is the first metabolite of ketamine and is also active. It is followed by others like 6-hydroxynorketamine and 5,6-dehydronorketamine (DHNK). In an in vivo pharmacokinetic study Beagle dogs under sevoflurane anesthesia (mean end-tidal concentration 3.0 ± 0.2 %) or following medetomidine sedation ($450 \mu\text{g}/\text{m}^2$) received 4 mg/kg racemic ketamine or 2 mg/kg S-ketamine. Blood samples were collected between 0 and 900 min after drug injection. 50 μL aliquots of plasma were pretreated by liquid-liquid extraction prior to analysis of the reconstituted extracts with a robust enantioselective capillary electrophoresis assay using highly sulfated γ -cyclodextrin as chiral selector and electrokinetic sample injection of the analytes from the extract across a short buffer plug without chiral selector. Levels of S- and R-ketamine, S- and R-norketamine, (2S,6S)- and (2R,6R)-hydroxynorketamine and S- and R-DHNK were determined. Data were analyzed with compartmental pharmacokinetic models which included two compartments for the ketamine and norketamine enantiomers and a single compartment for the DHNK and 6-hydroxynorketamine stereoisomers. Medetomidine showed an effect on the formation and elimination of all metabolites. Stereoselectivities were detected for 6-hydroxynorketamine and DHNK, but not for ketamine and norketamine.

Keywords: Enantioselective capillary electrophoresis; Ketamine; Medetomidine; Pharmacokinetics; hydroxynorketamine; dehydronorketamine.

1. Introduction

The combination of the anesthetic drug ketamine and the sedative drug medetomidine is often used in veterinary medicine and sometimes also for humans. The benefits of this combination include the compensation of side effects of both drugs and the anesthetic sparing effect of medetomidine. Medetomidine decreases tachycardia, hypertension, salivation or muscular rigidity caused by ketamine whereas ketamine compensates bradycardia or hypotension induced by medetomidine [1–5]. Ketamine, a racemic drug (for chemical structure see Fig. 1A), is a N-methyl-D-aspartate- (NMDA) receptor antagonist which is well known in anesthesia but also used in analgesia and in the therapy of depressive disorders [6–9]. The S-enantiomer has a stronger affinity to the receptor [8,10–12] which is the reason that S-ketamine is registered in some countries as a drug for humans and for selected animals. Medetomidine, also a racemate, is a α_2 -receptor agonist. It is used for sedation or as an adjuvant in anesthesia. Only the S-enantiomer dexmedetomidine is pharmacologically active [1–4,13]. Both enantiomers are able to inhibit cytochrome P450 (CYP) enzymes whereas levomedetomidine is the stronger inhibitor [1,3,4]. Besides advantages, drug combinations have always the risk of pharmacokinetic or pharmacodynamic interactions. Most of the pharmacokinetic interactions can be handled by dose adaptation. For that purpose, as much as possible about the mechanisms of the interaction has to be known.

In vitro experiments with canine liver microsomes and the single canine enzyme CYP3A12 revealed an inhibitive effect of medetomidine on the N-demethylation of ketamine to norketamine [14]. Norketamine is the first and also an active metabolite of ketamine. Furthermore, hydroxylation of ketamine and norketamine at the cyclohexanone and chlorophenyl rings take place. All these metabolic steps are catalyzed by CYP enzymes [11,15–18]. Another metabolite is dehydronorketamine (DHNK). Chemical structures of selected ketamine metabolites are presented in Fig. 1A. Enzymes of the CYP family are also involved in the metabolism of medetomidine and the main metabolite is hydroxymedetomidine [1]. Thus, both drugs compete for the active site of the enzyme. Medetomidine as imidazole derivative is also able to bind at the heme iron in the active site of the enzyme which results in an inactivation of the enzyme [4].

In vitro experiments performed so far in regard to interactions of ketamine with a second drug like ketoconazole or medetomidine focused on the N-demethylation of ketamine to norketamine only [14,19–21]. The same applies to in vivo pharmacokinetics work performed in our laboratory [22,23]. Ketamine and norketamine concentrations were determined

enantioselectively by capillary electrophoresis based methods using highly sulfated γ - or sulfated β -cyclodextrin as chiral selector. The recent development of a microassay with highly sulfated γ -cyclodextrin as chiral selector permits analysis and quantification of the enantiomers of ketamine, norketamine, 6-hydroxynorketamine (6HNK) and DHNK [24]. This assay was used to analyze enantiomers of ketamine and the three metabolites in plasma samples of Beagle dogs which received a bolus injection of 4 mg/kg racemic ketamine or 2 mg/kg S-ketamine under sevoflurane anesthesia or following medetomidine sedation. Blood samples were collected up to 15 hours after ketamine administration and determined enantiomer drug and metabolite levels were used to elucidate the pharmacokinetics via compartmental analysis. Most pharmacokinetic studies are focused only on ketamine and the first metabolite norketamine [25,26]. Only a few models include also the following metabolites [12,27–29]. To our knowledge, no pharmacokinetic study which considers the effect of another drug on the pharmacokinetics of ketamine, norketamine, 6HNK and DHNK has been published thus far.

The goals of this work were (i) to demonstrate the usefulness of enantioselective capillary electrophoresis for monitoring the levels of ketamine, norketamine, 6HNK and DHNK in dog plasma after bolus drug administration, (ii) to create a pharmacokinetic model for ketamine and the three metabolites, (iii) to calculate the pharmacokinetic parameters for the dogs of the sevoflurane and of the medetomidine groups and (iv) to elucidate enantioselective effects of medetomidine on the pharmacokinetics of ketamine, norketamine, 6HNK and DHNK.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards for ketamine and norketamine (as hydrochlorides in methanol, 1 mg/mL of the free base), and DHNK (as hydrochloride 100 μ g/mL in acetonitrile), were from Cerilliant (Round Rock, TX, USA). (2S,6S)-6-hydroxynorketamine (SS-6HNK) and (2R,6R)-6-hydroxynorketamine (RR-6HNK) were from Dr. Irving Wainer (Laboratory of Clinical Investigations, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA (synthesis described by Moaddel et al. and Desta et al. [30,31]). Highly sulfated- γ -cyclodextrin (20 % w/v solution) was purchased from Beckman Coulter (Fullerton, CA, USA). D-(+)-norephedrine hydrochloride and bovine serum were from Sigma (St. Louis, Mo, USA). Disodium hydrogenphosphate, sodium hydroxide and bromothymol blue were from Merck (Darmstadt, Germany), phosphoric acid (85 %) was from Fluka (Buchs, Switzerland)

and dichloromethane (HiPerSolv Chromanorm for HPLC) was from VWR (Leuven, Belgium). Ketamine injection solutions Ketazol-100 (racemic ketamine) and Keta-S (S-ketamine) used for the dog trials were from Dr. E. Graeub AG (Bern, Switzerland).

2.2. Pharmacokinetic animal study

Dog plasma samples were collected in two trials at Vetsuisse Faculty (Zürich, Switzerland) which were performed as prospective randomised crossover studies with the permission of the Committee for Animal Experimentation, Canton Zürich, Switzerland. In both trials the washing-out period was one month. Briefly, six Beagle dogs (3 female and 3 male) with a mean age of 39 ($\pm$ 9) months under sevoflurane anesthesia (mean end-tidal concentration 3.0 $\pm$ 0.2 %) received an i.v. bolus injection of 4 mg/kg racemic ketamine (mean body mass was 15.15 $\pm$ 0.99 kg) or 2 mg/kg S-ketamine (mean body mass: 15.15 $\pm$ 1.01 kg). Another group of five Beagle dogs (2 female and 3 male) with a mean age of 21 ($\pm$ 11) months was sedated with medetomidine (450 μ g/m²) prior to bolus injection of 4 mg/kg racemic ketamine (mean body mass: 11.68 $\pm$ 2.42 kg) or 2 mg/kg S-ketamine (mean body mass: 12.14 $\pm$ 2.50 kg). Venous blood samples were collected before ketamine administration and at 25 or 26 selected time intervals up to 900 min thereafter which included the complete elimination phase. The plasma was separated and stored at -80 °C until analysis.

2.3. Sample preparation

Sample preparation followed the procedure described in Ref. [24]. Briefly, analytes were extracted with dichloromethane at alkaline pH via addition of 200 μ L water, 15 μ L of 4 μ g/mL d(+)-norephedrine hydrochloride solution (internal standard), 50 μ L of 0.5 M NaOH with 1.25 mM bromothymol blue and 1300 μ L dichloromethane to 50 μ L plasma. The vials were shaken and centrifuged. The upper aqueous phase was removed and the organic phase was transferred to a new vial and acidified with 10 μ L of 1 mM phosphoric acid prior to drying in a Speed-Vac Concentrator (Vaudaux-Eppendorf, Schönenbuch, Switzerland) at 45 °C. After reconstitution with 110 μ L water the samples were analyzed by enantioselective capillary electrophoresis.

2.4. Capillary electrophoresis instrumentation and analytical conditions

The analyses were performed on a Proteome Lab PA 800 enhanced instrument (Beckman Coulter, Fullerton, CA, USA) equipped with a photodiode array detector as described elsewhere [24]. Briefly, the 50 μ m id fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 45 cm (effective length 35 cm) was rinsed before each

experiment with bidistilled water (1 min; 20 psi) and running buffer (1 min; 20 psi). The running buffer was composed of 100 mM disodium hydrogenphosphate buffer (pH 3.0) and 0.66 % highly sulfated γ -cyclodextrin (Beckman Coulter, Fullerton, CA, USA). Before electrokinetic sample injection at 6 kV for 15 s, a plug of 50 mM disodium hydrogenphosphate buffer (pH 3.0) was injected by pressure (20 s; 1 psi). A voltage of 20 kV was applied and the current was about 64 μ A. The detection was effected at 200 nm. The temperature for all parts was set to 25 °C. Analyte quantification was based on internal calibration using corrected peak areas. Assay calibration and control was performed with fortified bovine serum samples as is described in Ref. [24]. Typical electropherograms obtained with plasma samples of two dogs are presented in Fig. 1B und 1C.

2.5. Data analysis

Data were evaluated with Microsoft Excel (Microsoft, Redmont, WA, USA) and SigmaPlot software version 12.5 (Systat Software, San Jose, CA, USA). The Student's t-test and the Mann-Whitney Rank Sum Test were used to evaluate significant differences of two data sets. An overall value of $P < 0.05$ was considered significant. Pharmacokinetic modeling with compartmental analysis and noncompartmental analysis was performed with Phoenix WinNonlin 6.4 software (Certara: Implementing Translational Science, Cary, NC, USA).

3. Results and discussion

3.1. Chiral assay and data for ketamine and metabolites

With the applied microassay based on electrokinetic sample injection and cationic separation of analytes in presence of a small amount of highly sulfated γ -cyclodextrin it was possible to determine the enantiomers of ketamine and three of its major metabolites in 50 μ L of plasma with a limit of quantification of 10 ng/mL of each enantiomer [24]. Electrokinetic sample injection associated with analyte stacking was effected across a buffer plug without chiral selector whose concentration was half of that used for analyte separation. The microassay is robust, **repeatable** (interday RSD < 9 % [24]) and was successfully applied to monitor the enantiomers of ketamine and the three metabolites in about 600 dog plasma samples of this in vivo pharmacokinetic study. Samples were collected before and after bolus injection of 4 mg/kg racemic ketamine or 2 mg/kg S-ketamine to Beagle dogs that were either anaesthetized with sevoflurane or sedated with medetomidine as is described in Section 2.2. Typical electropherograms obtained from a dog of both groups treated with racemic ketamine are presented in panels B and C, respectively, of Fig. 1.

For the group of anesthesia with sevoflurane, the sample collected prior to ketamine infusion did not reveal any peaks for the compounds of interest (Fig. 1B). However, peaks originating from sevoflurane that did not interfere with ketamine or its metabolites were detected (marked with #). Plasma samples collected 5, 20 and 90 min after infusion of 4 mg/kg racemic ketamine revealed peaks for all 8 compounds of interest that could be quantitated (Fig.1B). The same is true for the samples of the medetomidine group except that no peaks originating from medetomidine could be detected (Fig. 1C). Administration of 2 mg/kg S-ketamine instead of 4 mg/kg racemic ketamine resulted in electropherograms comprising the S-stereoisomers of ketamine, norketamine, 6HNK and DHNK only (for example see [24]) and drug levels of these S-stereoisomers were comparable to those monitored after administration of 4 mg/kg racemic ketamine. Comparison of the electropherograms between the two groups immediately revealed that medetomidine comedication resulted in the formation of much lower amounts of norketamine stereoisomers (compare e.g. 20 min electropherograms of panels B and C of Fig. 1). This aspect and those associated with stereoselectivities are further discussed in the context of the pharmacokinetic modeling described Sections 3.2 and 3.3.

As mentioned in [24], the data for RR-6HNK and SS-6HNK should be considered with some caution. First, insufficient amounts of the two standards were available to make multiple independent calibrations. Thus, deviations from the real values of up to 10 % can be expected. Second, SS-6HNK comigrates with the R stereoisomer of another hydroxynorketamine metabolite referred to as hydroxynorketamine metabolite IV in Refs. [16,24] (data not shown). Similarly, RR-6HNK comigrates with the S stereoisomer of this metabolite IV [24] (data not shown). According to in vitro work performed with canine liver microsomes, no formation of R-IV and small amounts of S-IV were observed [32]. Furthermore, metabolite IV is known to be conjugated to an appreciable extent [16]. Thus, as the microassay described does not include hydrolysis of conjugated hydroxylated norketamine metabolites, the obtained data for RR-6HNK and SS-6HNK are not much enhanced due to comigration with metabolite IV. Efforts are currently under way to find conditions to resolve all known hydroxynorketamine stereoisomers in the assay format with electrokinetic analyte injection from extracts of 50 μ L of plasma.

3.2. Pharmacokinetic modeling of ketamine and its metabolites

Pharmacokinetic models describe the concentration of a drug which undergoes the processes of liberation, absorption, distribution, metabolism and elimination in the body as function of time. Modeling can be done with the aid of a polyexponential equation for which the

coefficients have to be determined step-by-step manually or by comprehensive computer modeling using commercial software packages such as Phoenix WinNonlin [33]. Both approaches were used in our work and reveal the pharmacokinetic parameters for data comparison. For finding the best model for ketamine and the metabolites all concentration-time plots for one dog were analyzed phase by phase and compared to those obtained using Phoenix WinNonlin software. A schematic representation of pharmacokinetic modeling of ketamine and its metabolites is presented in Fig. 2.

Ketamine administered as an i.v. bolus goes immediately into blood circulation which is part of the central compartment as well as highly perfused tissues like the liver and the kidneys. The semilog plot of the determined S- and R-ketamine concentrations (logarithmic y-axis) as function of time (linear x-axis) showed two phases, the distribution and the elimination phases. This is typical for a two-compartment model and for drug application via bolus injection. The number of compartments which can be found for ketamine pharmacokinetic modeling in the literature varied between two and three and are dependent on the administration procedure used [6,26,28,29,34]. Ketamine is distributed very fast into a second compartment, the so-called tissue or peripheral compartment, which includes skin and fatty tissues. The distribution is dependent on drug characteristics like lipophilicity and the tendency for protein-binding. Ketamine is eliminated from the central compartment by metabolic reactions mediated by CYP enzymes in the liver and other organs [11,17,18] or excreted unmodified via the kidneys. The data points of the elimination phase can be analyzed by linear regression analysis thereby revealing the slope β and the y-intercept $\ln(B)$. With this linear equation and the time points of the distribution phase concentration values are calculated and subtracted from the experimentally determined concentrations. After logarithmization, these results are plotted against time and subjected to linear regression analysis thereby revealing slope α and y-intercept $\ln(A)$. Finally the pharmacokinetics of ketamine can be described with the polyexponential equation $c = A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t)$ where c and t are the concentration and time, respectively.

Norketamine is always the first metabolite found after in-vitro incubations and in blood samples after ketamine administration in different species [16,32]. Hydroxylation of ketamine to hydroxyketamines is possible as well and it can take place at different positions on the rings in the molecule [8,15,16]. Norketamine is also an antagonist at the NMDA-receptor but its affinity is only one third of that of ketamine [6]. In contrast to the behavior of ketamine, there is first a phase in which the concentration of norketamine is rising from 0 to a maximum

concentration, a fact that must be included in the kinetic model. The calculation starts as described above for ketamine, i.e. the elimination and distribution phases. Then the time points of the increasing concentration phase are inserted into the linear equation of the distribution phase. From the thereby calculated concentration the difference of the experimentally determined concentration and the concentration calculated with the linear equation of the elimination phase is subtracted. After logarithmization, results are plotted against time and analyzed with linear regression revealing the slope γ and the y-intercept $\ln(C)$. The kinetics of norketamine can thereby be described with a two-compartment model using $c=A*\exp(-\alpha*t)+B*\exp(-\beta*t)-C*\exp(-\gamma*t)$.

Elimination of norketamine proceeds via hydroxylation at different positions and unmodified excretion via the kidneys. Although the possibility of direct formation of DHNK from norketamine is discussed in the literature [12,35], DHNK is more likely formed via loss of water from hydroxynorketamine, especially 6HNK and 5HNK [16]. It is not known whether DHNK is formed enzymatically or via a chemical reaction [16,36]. Urine of a pony which received ketamine was collected and fractionated by HPLC. Two fractions comprising hydroxynorketamine metabolites (one with RR- and SS-6HNK) also contained small amounts of DHNK. The appearance of DHNK in these fractions cannot be an impurity of the fractionation procedure because DHNK eluted more than 10 min after the hydroxynorketamine metabolites [16]. Furthermore, hydroxynorketamine metabolites show up earlier compared to DHNK in time-based experimental settings in vivo and in vitro (Fig. 1; [16,32]). Based on these facts, DHNK follows hydroxynorketamine in the model presented in Fig. 2 and 6HNK was included as it was the only hydroxynorketamine analytical standard available. 6HNK is an interesting metabolite because recent studies showed its antidepressive effects [12,35,37]. 6HNK and DHNK are inhibitors of the $\alpha 7$ -nicotinic acetylcholine receptor [15]. Norketamine is not the only possible source for 6HNK. It can also be formed by N-demethylation of 6-hydroxyketamine.

Analysis of the experimental data of the 6HNK enantiomers in the semilog format revealed only one phase after the maximum concentration (elimination phase). Thus, 6HNK data can be described with a one-compartment model with first order drug absorption according to $c=(k_a*D_0*F)/(V*(k_a-k))*(\exp(-k*t) - \exp(-k_a*t))$ [33]. The elimination phase in the semilog plot is analyzed by linear regression analysis thereby revealing slope k and y-intercept $\ln((k_a*D_0*F)/(V*(k_a-k)))$. Concentration values were calculated for the remaining time points of the first part of the curve and the measured concentration values were subtracted. After

logarithmization, results are plotted against time and analyzed with linear regression to obtain the slope k_a and the y-intercept $\ln((k_a \cdot D_0 \cdot F)/(V \cdot (k_a - k)))$. Theoretically, the y-intercept for the initial and elimination phases should be the same. Typically there are not because of the lag-time and the residuals. The regression line of the elimination phase is based on the original values and on more data points. The y-intercept of the elimination phase is assumed to be more accurate and is included as an appropriate term in the equation. The same model was used for the DHNK data (Fig. 2). Good fits were obtained for both metabolites. For 6HNK and DHNK it is more difficult to make statements about the kinetics because it is not exactly known what the initial dose of these metabolites is. Thus, noncompartmental analyses were performed as well. Noncompartmental analysis is a statistical description of the experimental curves. The results were comparable with those obtained with compartmental analysis.

For all compounds there was good agreement of the data that were calculated stepwise as described above and the data produced by the software. As an example, data obtained for R-norketamine of a dog are presented in Fig. 3. Thus, the data sets of all dogs were analyzed with Phoenix WinNonlin 6.4 software using the same models (Fig. 2). Similarly, concentration – time relationships were calculated for the mean values of each compound and group and are presented in Fig. 4. With the commercial software different variations of the models were also checked and compared according to diagnostic data. The proper choice of the models presented in Fig. 2 was thereby confirmed.

3.3. Pharmacokinetic data

The α_2 - receptor agonist medetomidine has an effect on the pharmacokinetics of ketamine and the metabolites norketamine, 6HNK and DHNK. Pharmacokinetics can be described with the model shown in Fig. 2. Based on the equations of the model pharmacokinetic parameters can be calculated and compared (Tables 1-4). There is variability in the metabolism of all metabolites among the Beagle dogs which is consistent with the literature [29]. For ketamine, the values of the pharmacokinetic parameter β -half-life and the mean residence time (MRT) are different for the sevoflurane und the medetomidine group (Table 1, $P < 0.05$). Both parameters are connected to the elimination. The half-life is the time in which the concentration of a substance is reduced by 50 %. The MRT is the time a molecule of the substance stays on average in the body. The β -half-life (sevoflurane group: 29.74 ± 25.38 and 28.80 ± 25.35 min for R- and S-ketamine, respectively; medetomidine group: 68.12 ± 10.31 and 66.77 ± 12.81 min, respectively) as well as the MRT (sevoflurane: 34.04 ± 31.34 and 32.02 ± 30.15 min; medetomidine: 71.49 ± 13.12 and 69.38 ± 15.71 min) values for both

enantiomers are higher in the **medetomidine** group (Table 1). The volume of the peripheral compartment (V_{T1} , Table 1) under both conditions is higher than the volume of the central compartment (V_{central}). This is typical for lipophilic bases and stands for a high affinity of the drug to all parts of the central compartment. V_{T1} , V_{central} **and the volume of distribution at steady-state (V_{ss} , Table 1) are higher in the medetomidine group whereas the inter-compartmental transfer constants k_{10} , k_{12} , and k_{21} are higher in the sevoflurane group. For the clearance (Cl, Table 1) and the area under the curve (AUC) no significant differences in relation to the comedication could be found (Table 1; $P > 0.05$). Furthermore, no stereoselectivities were detected (Fig. 4A, $P > 0.05$).**

In contrast to ketamine, a large difference between the sevoflurane and the medetomidine group was noted for norketamine. This can be seen in the electropherograms presented in Fig. 1B and 1C, the concentration vs. time plots of Fig. 4B, and the data of Table 2. Values for the maximum concentration of norketamine (c_{max}) and the AUC are much lower in the medetomidine group. That confirms the previous in-vitro experiments in which an inhibition of the N-demethylation of ketamine to norketamine was found in presence of racemic medetomidine or dexmedetomidine [14]. Two mechanisms of inhibition are possible in this drug-drug combination. There is a concurrence on the active site of the enzyme because both drugs are metabolized by CYP enzymes. Medetomidine as an imidazole is able to block the CYP-enzymes by binding to the heme iron in the active site of the CYP enzyme [4]. Norketamine is an active metabolite. Thus, the effect on the NMDA-receptor is expected to be larger in the sevoflurane group. No stereoselectivity for norketamine was detected (Fig. 4B, $P > 0.05$).

The shorter half-life and MRT of ketamine in the medetomidine group show that the elimination of ketamine in the medetomidine group is faster. C_{max} of norketamine is indeed reached earlier but the amounts of norketamine present in the blood are much lower (Fig. 4B, Table 2). The faster formation of norketamine is expressed also in the k_{03} values, which are higher after medetomidine administration. The processes between the central and the tissue (T_2) compartments for norketamine are described with k_{34} and k_{43} . These parameters are also higher for the medetomidine group (Fig. 2, Table 2). Thus, the elimination must take another way excluding the formation of norketamine. One possibility is the increased formation of hydroxyketamine metabolites, another is the increased elimination of unmodified ketamine through the kidneys. The α_2 -receptor agonist medetomidine causes the inhibition of the release of vasopressin and the increase of the excreted urine volume [38]. Furthermore,

hyperglycemia mediated contemporaneously through the α_2 -receptor in the β -cells in the endocrine pancreas results in polyuria [39]. No significant difference in the metabolic pattern was found in urines of two dogs collected 300 min after administration of 4 mg/kg racemic ketamine under sevoflurane anesthesia or medetomidine sedation (data not shown). Thus, the second possibility could not be confirmed.

Data for the stereoisomers of 6HNK and DHNK are presented in Tables 3 and 4, respectively. For these metabolites stereoselectivity was observed ($P < 0.05$). This is also nicely seen in the data of panels C and D of Fig. 4. The concentration for RR-6HNK is higher than the concentration of the SS-6HNK in both groups (Table 3). Furthermore, the $c_{\max}$ value for RR-6HNK under sevoflurane is the same as that under medetomidine sedation (180.36 ± 52.34 and 188.01 ± 53.99 ng/ mL). The same applies for SS-6HNK (101.67 ± 4.90 and 82.68 ± 23.21 ng/ mL). The maximum concentrations, however, are reached faster under medetomidine comedication (sevoflurane group: 67.57 ± 36.23 min and 48.86 ± 19.25 min; medetomidine: 16.24 ± 1.60 and 16.65 ± 0.79 min). The AUC values are also lower in presence of medetomidine due to faster elimination. In contrast to these results with 6HNK, for DHNK under sevoflurane (Table 4) the maximum concentration of S-DHNK is significantly higher compared to that of R-DHNK. For the medetomidine group, the $c_{\max}$ value of S-DHNK is also higher than that of R-DHNK. The difference between the enantiomers is much bigger under sevoflurane, not only for $c_{\max}$ but also for the AUC. The AUC for R-DHNK in both groups is the same and smaller than the values for S-DHNK (Table 4). The velocity constants k_{05} , k_{50} , k_{06} and k_{60} show that the metabolites HNK and DHNK disperse faster under comedication with medetomidine (Tables 3 and 4). The highest concentration is reached under sevoflurane by S-DHNK (433.97 ± 152.97 ng/ mL). For both 6HNK and DHNK, pharmacological effects at the α_7 -nicotinic acetylcholine receptor were observed which makes the investigation of these compounds worthwhile [15].

For the S-ketamine (2 mg/kg) part of the two dog studies the plasma samples were also analyzed with the enantioselective capillary electrophoresis based microassay. It revealed S-ketamine and all S-metabolites but no R-ketamine and R-metabolites. For the two groups, no significant differences in the results for S-ketamine and the metabolites S-norketamine, SS-6HNK and S-DHNK were found ($P > 0.05$). Typical electropherograms are presented in Ref. [24].

4. Concluding remarks

The pharmacokinetics of S- and R-ketamine and its metabolites S- and R-norketamine, SS- and RR-6HNK and S- and R-DHNK were analyzed enantioselectively by using capillary electrophoresis in plasma of Beagle dogs under sevoflurane and under medetomidine comedication. Medetomidine has an effect on the pharmacokinetics with the elimination being always faster under medetomidine. The norketamine levels are significantly lower after administration of medetomidine. Additional experiments revealed that urinary excretion of ketamine and norketamine were unaffected by medetomidine. Stereoselectivities were detected for the 6HNK and DHNK metabolites, but not for ketamine and norketamine. Not all enzymes of the different pathways of the ketamine metabolism are identified and it is not clear which effect medetomidine has on them. It seems that some pathways work under medetomidine better than others. In previous in vitro studies the combination of S-ketamine and dexmedetomidine was found best because the pharmacologically inactive levomedetomidine is the stronger inhibitor of ketamine N-demethylation. It would be of interest to elucidate the impact of dexmedetomidine on the further metabolites in comparison to racemic medetomidine used in this study. Norketamine is also active as a NMDA-receptor antagonist. Thus, it should be investigated whether the effect on that receptor is higher in the sevoflurane group. Highly sulfated γ -cyclodextrin based enantioselective capillary electrophoresis with electrokinetic sample injection of the analytes across a short buffer plug without chiral selector provided the required ppb sensitivity to monitor the stereoisomers of ketamine and the three metabolites in extracts of 50 μ L aliquots of plasma in an efficient and **repeatable** way.

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Figures

Figure 1. (A) Chemical structures of ketamine, norketamine, 6HNK and DHNK, and electropherograms of plasma extracts of samples collected before and after 5, 20 and 90 min of injection of 4 mg/kg racemic ketamine to (B) a Beagle dog under sevoflurane anesthesia and (C) a Beagle dog under medetomidine sedation. Asterisks mark the chiral centers in the chemical structures and peaks marked with # in the electropherograms stem from sevoflurane. Key: IST, internal standard; 1, RR-6HNK; 2, SS-6HNK; 3, R-norketamine; 4, S-norketamine; 5, S-DHNK; 6, R-ketamine; 7, S-ketamine; 8, R-DHNK.

Figure 2. Schematic representation of pharmacokinetic modeling of ketamine and its metabolites based on two-compartment models for ketamine and norketamine and one-compartment models for 6HNK and DHNK enantiomers. The central compartments are labelled with ketamine, norketamine, 6HNK and DHNK. T1 and T2 are the tissue compartments for ketamine and norketamine, respectively. The k values represent inter-compartmental transfer constants.

Figure 3. Pharmacokinetic modeling of the R-norketamine data of one dog after administration of racemic ketamine under sevoflurane anesthesia using a manual step-by-step elucidation of the parameters of the polyexponential function for a two-compartment model (solid line graph) and the Phoenix WinNonlin 6.4 software for the same model (broken line graph).

Figure 4. Pharmacokinetics of (A) ketamine, (B) norketamine, (C) 6HNK and (D) DHNK for the mean plasma data of both groups after administration of racemic ketamine and evaluated with the Phoenix WinNonlin 6.4 software using the model of Fig. 2. Key: circles and solid lines, sevoflurane comedication; triangles and dashed lines, medetomidine comedication; filled symbols, R-enantiomers; open symbols, S-enantiomers.

Figure

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Table 1. Pharmacokinetic parameters of ketamine enantiomers^{a)}

Ketamine	Racemic ketamine Sevoflurane (n=6)		Racemic ketamine Medetomidine (n=5)	
	R	S	R	S
AUC (min*ng/L)	37.22 ± 7.97	35.44 ± 6.98	43.13 ± 10.52	44.85 ± 8.02
Cl (mL/min/kg)	55.69 ± 11.07	58.17 ± 10.70	47.41 ± 7.90	45.76 ± 16.61
α -half-life (min)	1.29 ± 0.58	1.35 ± 0.56	3.41 ± 1.20	3.36 ± 1.13
β -half-life (min)	29.74 ± 25.38	28.80 ± 25.35	68.12 ± 10.31	66.77 ± 12.81
A (ng/mL)	6469.47 ± 4820.36	6165.93 ± 3989.23	2845.75 ± 1147.99	3068.43 ± 1225.59
B (ng/mL)	958.39 ± 490.15	939.95 ± 510.94	311.78 ± 27.42	328.28 ± 22.19
k ₁₀ (1/min)	0.2231 ± 0.1885	0.2196 ± 0.1654	0.0738 ± 0.0302	0.0760 ± 0.0289
k ₁₂ (1/min)	0.3789 ± 0.2333	0.3467 ± 0.2268	0.1339 ± 0.0670	0.1333 ± 0.0659
k ₂₁ (1/min)	0.1242 ± 0.0694	0.1209 ± 0.0731	0.0320 ± 0.0042	0.0319 ± 0.0044
MRT (min)	34.04 ± 31.34	32.02 ± 30.15	71.49 ± 13.12	69.38 ± 15.71
V _{ss} (L/kg)	1.63 ± 1.17	1.62 ± 1.23	3.37 ± 0.66	3.13 ± 0.66
V _{central} (L/kg)	0.36 ± 0.18	0.35 ± 0.16	0.72 ± 0.34	0.67 ± 0.30
V _{T1} (L/kg)	1.24 ± 1.00	1.27 ± 1.08	2.64 ± 0.55	2.46 ± 0.61

a) Data represent mean values ± SD and were determined with the Phoenix WinNonlin 6.4 software.

Table 2. Pharmacokinetic parameters of norketamine enantiomers^{a)}

Norketamine	Racemic ketamine Sevoflurane (n=6)		Racemic ketamine Medetomidine (n=5)	
	R	S	R	S
AUC (min*ng/L)	42.91 ± 12.67	32.32 ± 16.39	3.29 ± 2.21	4.53 ± 2.04
t _{max} (min)	17.68 ± 3.45	13.33 ± 3.53	6.77 ± 2.12	6.38 ± 2.04
c _{max} (ng/mL)	316.12 ± 77.37	322.30 ± 106.50	45.35 ± 9.53	55.38 ± 6.91
α-half-life (min)	8.94 ± 1.52	7.48 ± 1.75	3.72 ± 1.27	3.42 ± 1.11
β-half-life (min)	125.71 ± 26.11	111.28 ± 34.88	78.20 ± 47.83	91.97 ± 43.58
A (ng/mL)	-29713.39 ± 55612	-13691.38 ± 242024	-7251.93 ± 19714	-16470.07 ± 30165
B (ng/mL)	212.18 ± 52.54	160.04 ± 59.10	25.71 ± 4.77	31.95 ± 4.87
k ₀₃ (1/min)	0.078 ± 0.012	0.097 ± 0.022	0.21 ± 0.07	0.22 ± 0.07
k ₃₀ (1/min)	0.018 ± 0.008	0.029 ± 0.016	0.039 ± 0.016	0.031 ± 0.009
k ₃₄ (1/min)	0.039 ± 0.007	0.049 ± 0.008	0.12 ± 0.05	0.14 ± 0.06
k ₄₃ (1/min)	0.028 ± 0.009	0.026 ± 0.010	0.058 ± 0.023	0.059 ± 0.016

a) Data represent mean values ± SD and were determined with the Phoenix WinNonlin 6.4 software.

Table 3. Pharmacokinetic parameters of 6HNK enantiomers^{a)}

6HNK	Racemic ketamine Sevoflurane (n=6)		Racemic ketamine Medetomidine (n=3) ^{b)}	
	RR	SS	RR	SS
AUC (min*ng/L)	60.08 ± 12.67	35.97 ± 7.80	20.37 ± 7.83	10.00 ± 2.27
t _{max} (min)	67.57 ± 36.23	48.86 ± 19.25	16.24 ± 1.60	16.65 ± 0.79
c _{max} (ng/mL)	180.36 ± 52.34	101.67 ± 4.9	188.01 ± 53.99	82.68 ± 23.21
k ₀₅ (1/min)	0.056 ± 0.037	0.078 ± 0.039	0.18 ± 0.04	0.19 ± 0.04
k ₅₀ (1/min)	0.0039 ± 0.0011	0.0035 ± 0.0013	0.012 ± 0.004	0.010 ± 0.004

a) Data represent mean values ± SD and were determined with the Phoenix WinNonlin 6.4 software.

b) The 6HNK data of two dogs could not be analyzed with this model.

Table 4. Pharmacokinetic parameters of DHNK enantiomers^{a)}

DHNK	Racemic ketamine Sevoflurane (n=6)		Racemic ketamine Medetomidine (n=5)	
	R	S	R	S
AUC (min*ng/L)	46.17 ± 13.51	158.55 ± 24.65	45.70 ± 13.96	60.32 ± 15.30
t _{max} (min)	80.25 ± 29.69	37.57 ± 4.79	78.86 ± 37.96	41.12 ± 4.02
c _{max} (ng/mL)	138.55 ± 68.24	433.97 ± 152.74	249.44 ± 42.89	275.50 ± 47.47
k ₀₆ (1/min)	0.033 ± 0.012	0.045 ± 0.030	0.065 ± 0.013	0.064 ± 0.008
k ₆₀ (1/min)	0.0044 ± 0.0022	0.0035 ± 0.0010	0.0087 ± 0.0057	0.0062 ± 0.0020

a) Data represent mean values ± SD and were determined with the Phoenix WinNonlin 6.4 software.