Simultaneous Population Pharmacokinetic Modeling of Ketamine and Three Major Metabolites in Patients with Treatment-Resistant Bipolar Depression

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Running head: popPK model of ketamine and three major metabolites

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What is known

(R,S)-Ketamine is a phencyclidine derivative that was initially developed as an anesthetic agent and which is currently being studied in the treatment of pain and depression. After administration, the drug is extensively N-demethylated to (R,S)-norketamine. The pharmacokinetics of ketamine and norketamine have been extensively studied in volunteers and patients after the administration of anesthetic and sub-anesthetic doses. However, ketamine and norketamine are extensively transformed into a series of diastereomeric hydroxyketamines and hydroxynorketamines and (R,S)-dehydrornorketamine metabolites. The plasma kinetics of these metabolites have not been elicudated.

What this study adds

The current study expands the characterization of the disposition kinetics of (R,S)-ketamine and (R,S)-norketamine and presents a population pharmacokinetic analysis of (R)-ketamine, (S)-ketamine, (R)-norketamine, (S)-norketamine, (R)-dehydrornorketamine, (S)-dehydrornorketamine and (2S,6S;2R,6R)-hydroxynorketamine and the serum concentration-time profiles of multiple ketamine metabolites observed in the plasma of patients after a single 40-min infusion of a sub-anesthetic dose of the drug. The data demonstrate that while norketamine is an initial metabolite, it is not the major circulating metabolite and suggest that the determination of the downstream metabolites of ketamine may play a role in the pharmacological effects of the drug.
ABSTRACT

AIM

To construct a population pharmacokinetic (popPK) model for ketamine, Ket, norketamine, norKet, and dehydronorketamine, DHNK, hydroxynorketamine, (2S,6S;2R,6R)-HNK, and hydroxyketamine, HK, in patients with treatment-resistant bipolar depression.

METHODS

Plasma samples were collected at 40, 80, 110, 230 min, Day 1, 2 and 3 in 9 patients following a 40 min infusion of (R,S)-Ket (0.5 mg/kg) and analyzed for Ket, norKet, and DHNK enantiomers and (2S,6S;2R,6R)-HNK, (2S,6S;2R,6R)-HK and (2S,6R;2R,6S)-HK. A compartmental popPK model was constructed that included all quantified analytes, and unknown parameters were estimated with an iterative 2-stage algorithm in ADAPT5.

RESULTS

Ket, norKet and DHNK and (2S,6S;2R,6R)-HNK were present during the first 230 min post-infusion and significant concentrations (> 5 ng/ml) were observed on Day 1, plasma concentrations of (2S,6S;2R,6R)-HK and (2S,6R;2R,6S)-HK were below quantification. The average (S)/(R) plasma concentrations for Ket and DHNK were <1.0 while no significant enantioselectivity was observed for norKet. There were large inter-patient variations in terminal half-lives and relative metabolite concentrations; at 230 min, (R,S)-DHNK was the major metabolite in 4/9 patients, (R,S)-norKet (3/9), (2S,6S;2R,6R)-HNK (2/9). The final PK model included 3-compartments for (R,S)-Ket, 2-compartments for (R,S)-norKet and single compartments for DHNK and HNK. All PK profiles were well
described, and parameters for (R)-, (S)-Ket and (R)-, (S)-norKet were in agreement with prior estimates.

CONCLUSIONS
This represents the first PK analysis of (2S,6S;2R,6R)-HNK and (R,S)-DHNK. The results demonstrate that while norKet is the initial metabolite, it is not the main metabolite suggesting that future Ket studies should include the analysis of the major metabolites.

INTRODUCTION
(R,S)-Ketamine ((R,S)-Ket; Figure 1), is a chiral phencyclidine derivative that was initially developed as an anesthetic agent and which is currently being studied in the treatment of pain and depression [1,2]. Sub-anesthetic doses of (R,S)-Ket have been successfully used in the treatment of patients with complex regional pain syndrome (CRPS), c.f. [3,4], postoperative pain in opioid tolerant patients [5], in emergency room treatments [6] and in patients suffering from treatment-resistant bipolar depression [7-9]. In the USA, Ket is used as a racemic (50:50) mixture of (R)-Ket and (S)-Ket; however, the administration of the single isomer, (S)-Ket, is effective in the treatment of CRPS [10-12].

After administration, (R,S)-Ket is extensively N-demethylated to norketamine ((R,S)-norKet; Figure 1), which has anesthetic [13] and antinociceptive properties [14,15] and is considered as the “active” metabolite. (R,S)-Ket and (R,S)-norKet are further transformed into two diasteromeric hydroxyKet and six diastereomeric hydroxynorKet
metabolites (HK and HNK, respectively; Figure 1) and dehydronorketamine (DHNK; Figure 1), [16,17]. An early study of the anesthetic activity of a key HNK metabolite, (2S,6S;2R,6R)-HNK, indicated that this compound did not produce anesthesia in the rat [13], and there has been no subsequent characterization of the pharmacological activity of this metabolite, any of the other hydroxylated metabolites of Ket and norKet. However, recent work in our laboratories has demonstrated that (R,S)-DHNK is a potent inhibitor of the α7-nicotinic acetylcholine receptor and the data suggest that this metabolite may play a role in Ket clinical effects (unpublished data).

The pharmacokinetics of (R,S)-Ket, (R)-Ket and (S)-Ket have been extensively studied in volunteers and patients after the administration of anesthetic and sub-anesthetic doses of (R,S)-Ket and sub-anesthetic doses of (S)-Ket, c.f [11, 18-22]. Since (R,S)-norKet was identified as the “active” metabolite, these studies have often included the determination of the pharmacokinetic profiles of (R,S)-norKet or the separate enantiomers, but not the HK, HNK or DHNK metabolites. However, significant plasma concentrations of (2S,6S;2R,6R)-HNK were detected in the rat following administration of (R,S)-Ket or (R,S)-norKet [13] and (R)- and (S)-DHNK were identified in plasma samples obtained from patients [23] and rats [24] receiving (R,S)-Ket and in Shetland ponies after administration of (R,S)-Ket or (S)-Ket [25,26]. All of the major metabolites of (R,S)-Ket, with the exception of (2S,6R;2R,6S)-HK, have been identified in urine samples obtained from volunteers who received a single 50 mg oral dose of (R,S)-Ket [27] and in the plasma and urine of CRPS patients receiving a continuous 5-day infusion of (R,S)-Ket [28].
Although significant plasma and urine concentrations of (2S,6S;2R,6R)-HK, HNK and DHNK metabolites have been observed after Ket administration, their plasma concentration time profiles have not been determined. Thus, the aim of the current study was to apply a chiral-archiral LC-MS/MS assay validated for the determination of the plasma concentrations of (2S,6S;2R,6R)-HK, (2S,6S;2R,6R)-HNK and (R,S)-DHNK [28] to the analysis of plasma samples obtained from patients receiving (R,S)-Ket for the treatment of treatment-resistant bipolar depression and to use these profiles to perform a population PK (popPK) analysis of (R)-Ket, (S)-Ket, (R)-norKet (S)-norKet, (R)-DHNK, (S)-DHNK, (2S,6S;2R,6R)-HNK and (2S,6S;2R,6R)-HK.

METHODS

Patient samples

The plasma samples analyzed in this study were obtained during a previously reported study of the effect of (R,S)-Ket in the treatment of treatment-resistant bipolar depression [8]. In brief: After obtaining informed consent, the patients were entered into a randomized double-blind crossover study in which they received a 40 min infusion of 50 ml of either 0.9% saline solution or a 0.5 mg/kg dose of (R,S)-Ket hydrochloride. There was a 2-week period between infusions. Plasma samples were collected prior to the initiation of the infusion, at 40 min (end of the infusion), 80 min, 110 min, 230 min and on Days 1, 2 and 3 post-infusion and frozen at -80 °C until analysis. During the course of the study the patients were required to take either lithium or valproate within a specified range and no other psychotropic medications were allowed.
Bioanalytical methods

The plasma concentrations of (R)-Ket, (S)-Ket, (R)-norKet, (S)-norKet, (R)-DHNK, (S)-DHNK, (2S,6S;2R,6R)-HNK, (2S,6S;2R,6R)-HK and (2S,6R;2R,6S)-HK were determined using a previously described achiral-chiral liquid chromatography – mass spectrometry method [28] that was revalidated for the lower plasma concentrations observed in this study. The chromatographic experiments were carried out on a Shimadzu Prominence HPLC system (Shimadzu, Columbia, MD, USA) and total analyte concentrations were determined using an Eclipse XDB-C\textsubscript{18} guard column and a Varian Pursuit XR\textsubscript{S} 5 \textsubscript{C}18 analytical column (Varian, Inc., Palo Alto, CA, USA) and the relative enantiomeric concentrations of (R)- and (S)-Ket, (R)- and (S)-norKet, (R)- and (S)-DHNK were determined using Chrial-AGP guard and analytical columns (Advanced Separation Technologies, Whippany, NJ, USA). The MS/MS analysis was performed using a triple quadrupole mass spectrometer model API 4000 system from Applied Biosystems/MDS Sciex equipped with Turbo Ion Spray® (TIS) (Applied Biosystems, Foster City, CA, USA). The data were acquired and analyzed using Analyst version 1.4.2 (Applied Biosystems). Positive electrospray ionization data were acquired using multiple reaction monitoring (MRM) and quantification was accomplished using area ratios calculated using D\textsubscript{4}-(R,S)-Ket as the internal standard, where the concentration of the internal standard was set at 50 ng/ml.

Calibration curves and quality control standards were prepared using the racemic mixtures of the analytes. The 8-point calibration curves used to measure (R,S)-Ket and (R,S)-DHNK ranged from 3.9 to 500 ng/ml and the calibration curves used to measure (R,S)-norKet, (2R,6R;2S,6S)-HNK, (2S,6S;2R,6R)-HK and (2S,6R;2R,6S)-HK were
from 1.9 to 250 ng/ml. The calibration curves were linear for all of the analytes with $R^2$ values ranging from 0.98 to 0.99. Quality Control (QC) standards (lower, middle, upper) for (R,S)-Ket and (R,S)-DHNK were 7.8, 31.25 and 250 ng/ml, respectively, and for (R,S)-norKet, (2R,6R;2S,6S)-HNK, (2S,6S;2R,6R)-HK and (2S,6R;2R,6S)-HK the QCs (lower, middle, upper) were 20, 40 and 80 ng/ml, respectively. The coefficient of variation was < 6% for the high QC standards and <14% for the low QC standards. The LOQ for (R,S)-Ket and (R,S)-DHNK was 3.9 ng/ml and the LOQ for (R,S)-norKet, (2S,6S;2R,6R)-HNK, (2S,6S;2R,6R)-HKet and (2S,6R;2R,6S)-HK was LOQ 1.9 ng/ml.

**Pharmacokinetic model**

(R,S)-Ket was administered as a constant rate infusion ($K_0$). The model structure for each enantiomer is identical, and a schematic of the final pharmacokinetic model is shown in Figure 2. The distribution of (S)-Ket was described by a central volume ($V_{1s}$) and two peripheral compartments ($A_{2s}$, $A_{3s}$) and defined by the following system of equations:

$$\frac{dC_{1s}}{dt} = K_0/(2 \cdot V_{1s}) - \left(k_{12s} \cdot C_{1s} + k_{13s} \cdot C_{1s} + k_{14s} \cdot C_{1s}\right) \cdot \frac{C_{1s}}{V_{1s}}$$

$$\frac{dA_{2s}}{dt} = k_{12s} \cdot C_{1s} \cdot V_{1s} - k_{21s} \cdot A_{2s}$$

$$\frac{dA_{3s}}{dt} = k_{13s} \cdot C_{1s} \cdot V_{1s} - k_{31s} \cdot A_{3s}$$

where $C_{1s}$ is the plasma concentration of (S)-Ket, and drug distribution is regulated by first-order rate constants $k_{12s}$, $k_{21s}$, $k_{13s}$ and $k_{31s}$. Metabolism of (S)-Ket to (S)-norKet was described by the first-order elimination rate constant $k_{14s}$. Individual infusions were set equal to 50% of the total infusion to account for the racemic mixture. A two-
compartment model (central, C₄s and peripheral, A₅s) was used to describe the rate of change of (S)-norKet plasma concentrations (C₄s):

\[
\frac{dC_{4s}}{dt} = k_{14s} \cdot C_{1s} - (k_{45s} + k_{46} + k_{47}s) \cdot C_{4s} + k_{54s} \cdot A_{5s} / V₁s
\]

\[
\frac{dA_{5s}}{dt} = k_{45s} \cdot C_{4s} \cdot V₁s - k_{54s} \cdot A_{5s}
\]

with first-order distribution rate constants k₄₅s and k₅₄s. The central volume of norKet was assumed to be equal to the (S)-Ket central compartment volume. NorKet undergoes further hydroxylation and dehydrogenation to yield corresponding HNK and DHNK. The rate of change of plasma (S)-DHNK was described by a one-compartment model (C₆s):

\[
\frac{dC_{6s}}{dt} = k_{46s} \cdot C_{4s} - k_{60s} \cdot C_{6s}
\]

where conversion of (S)-norKet to (S)-DHNK and the elimination of (S)-DHNK are governed by first-order rate constants k₄₆s and k₆₀s. A one-compartment model was also used to characterize (2S,6S)/-(2R,6R)-HNK plasma concentrations (C₇); however, separate enantiomers were not measured for diastereomeric HNK, and both (R)- and (S)-norKet contribute to the formation of total (2S,6S)/-(2R,6R)-HNK concentrations:

\[
\frac{dC_{7}}{dt} = k_{47} \cdot (C_{4s} + C_{4r}) - k_{70} \cdot C_{7}
\]

with first-order rate constants k₄₇ and k₇₀ defining the formation and elimination of (2S,6S)/-(2R,6R)-HNK. Only the equations for (S)-enantiomers are shown, as the system is identical for the (R)-enantiomers.

Data analysis

All measurements of (R)- and (S)-enantiomers for parent drug and metabolites were...
modeled simultaneously using an iterative 2-stage (ITS) algorithm as implemented in ADAPT5 (Biomedical Simulations Resource, USC, Los Angeles, CA). Parameters were assumed to be log-normally distributed within the population and up to 1000 iterations were used to obtain parameter distributions. Model selection during the development process was based on the objective function, goodness of fit plots, and distribution of residuals. Residual variability was modeled using the additive plus proportional variance model:

\[ Var_i = \sigma_1 + \sigma_2 \cdot Y_{pred} \]

where \( Var_i \) is the variance of the \( i \)th data point, \( \sigma_1 \) and \( \sigma_2 \) are the estimated variance model parameters, and \( Y_{pred} \) is the \( i \)th model predicted value. Final estimated parameters were reported as population mean estimates and inter-individual variability (IIV; CV%).

RESULTS

Subject characteristics

The subject population in this study included 6 females and 3 males whose age ranged from 23 to 62 yr (mean 46 ± 15 yr), weight from 51 to 118 kg (mean 90 ± 19 kg), and body mass index from 19 to 39 (mean 31 ± 6). All subjects were diagnosed with treatment-resistant bipolar depression, and additional study details have been previously published [8].

Plasma concentrations of the stereoisomers of Ket and its metabolites

The achiral-chiral analytical method used in this study detected measurable quantities of (R)-Ket, (S)-Ket, (R)-norKet, (S)-norKet, (R)-DHNK, (S)-DHNK, and (2S,6S;2R,6R)-
HNK in the samples assessed in this study. The plasma concentrations of (2S,6S;2R,6R)-HK were ≤ 3.0 ng/ml in the 40 – 230 min samples and (2S,6R;2R,6S)-HK was not detected in any of the samples. Representative chromatograms from the achiral analyses of plasma samples obtained from a patient at 230 min and Day 1 are presented in Figures 3 and 4, respectively. The chromatogram obtained from the 230 min plasma sample, Figure 3, also contained peaks corresponding to 5 additional hydroxynorketamine metabolites, identified as b-f in Figure 1, but these compounds were not quantified due to a lack of analytical standards. The plasma sample obtained from this patient at 24 h after the administration of (R,S)-Ket also contained (R)- and (S)-Ket, (R)- and (S)-norKet, (R)- and (S)-DHNK, (2S,6S;2R,6R)-HNK and (2S,5R;2R,5S)-HNK, Compound f, Figure 3.

There was a wide inter-patient variation in the plasma concentrations of the target analytes, which is reflected in the average plasma concentrations presented in Figure 5. Significant concentrations (> 8 ng/ml) of all of the metabolites were observed in the Day 1 plasma and (R,S)-DHNK and (2S,6S;2R,6R)-HNK were still quantifiable in the Day 2 and Day 3 plasma samples. There were also large variations in the relative total plasma concentrations, i.e. the combined concentrations of the enantiomers, of the N-demethylated metabolites. For example, in the samples obtained 230 min – post dose, (R,S)-DHNK was the major metabolite in 4/9 patients, followed by (R,S)-norKET (3/9), and (2S,6S;2R,6R)-HNK (2/9). In this study, the concentrations of (R,S)-DHNK and (2S,6S;2R,6R)-HNK trended to increase over time relative to (R,S)-NK.

When the plasma samples were analyzed on the chiral lc system, the average enantiomeric ratio, expressed as (S)-enantiomer/(R)-enantiomer, for the 40-230 min samples were 0.84 ± 0.04 for Ket, 0.67 ± 0.09 for DHNK and 1.00 for norKet. This
Enantioselectivity is consistent with the previously reported average (S)-/(R)-Ket plasma concentrations after the administration of (R,S)-Ket [19,20,22] and the (S)-/(R)-DHNK plasma ratios of 0.71 determined in both Shetland ponies 120 min after a 2-hr infusion of (R,S)-Ket [25] and in a CRPS patient on Day 3 of a 5-day continuous infusion of (R,S)-Ket [28].

**Pharmacokinetic analysis**

Both (R)- and (S)-Ket showed a rapid initial decline in plasma concentrations after termination of the 0.67 hr infusion (Figure 6). On the other hand, the average times to peak plasma concentrations of the metabolites were about 1.33 hours for both (R)- and (S)-norKet and 3.83 hours for (R)-DHNK, (S)-DHNK and (2S,6S;2R,6R)-HNK (Figure 6). All plasma metabolite concentrations gradually decreased, but with variable terminal elimination half-lives.

The pharmacokinetics of Ket has been characterized previously with one-, two- or three-compartment models [12,14,19,22,29]. The one-compartment model failed to capture the elimination phase of (R)- and (S)-Ket concentration profiles (data not shown). Although improvements in model fitting were obtained with a two-compartment model, a slight bias was evident in diagnostic plots (e.g., residuals vs. predicted concentrations; data not shown). The ultimate three-compartment model (Figure 2) provided the best description of (R)- and (S)-Ket profiles and objective criteria, such as diagnostic plots and a lower Akaike’s information criterion (AIC). The number of compartments for norKet and the remaining metabolites were similarly optimized. The disposition of norKet has been previously described using one- or two-compartment models [11,14].
Inclusion of a peripheral norKet compartment resulted in a decreased AIC value of 160 points and significant improvements in diagnostic plots (data not shown). Some pharmacokinetic analyses have included transit compartments to confer a time-delay in the formation of norKet [11,14]; however, this model structure introduced bias in the terminal phase of norKet (data not shown). The final pharmacokinetic model, consisting of three (R)- and (S)-Ket, two (R)- and (S)-norKet, one (R)- and (S)-DHNK and one (2S,6S;2R,6R)-HNK compartment is depicted in Figure 2.

Additional first-order elimination rate constants of (R)- and (S)-Ket and corresponding norKet compartment, representing alternate elimination pathways, were also tested; however, values were estimated to be relatively small and were set to zero to avoid overparameterization. Final model parameter estimates were obtained by simultaneously fitting the model to all available concentration-time profiles. Mean population parameter estimates are listed in Table 1. (R)- and (S)-Ket distributed very rapidly from the central compartment to the peripheral compartment with distribution rate constants \( k_{13r} \) of 56.5 hr\(^{-1} \) and \( k_{13s} \) of 64.3 hr\(^{-1} \). The central volume is 14 L for (R)-Ket and 9.15 L for (S)-Ket. Interestingly, the first-order elimination rate constants for (R)- and (S)-DHNK are 1.31 and 1.1 hr\(^{-1} \), or 1.5 to 2-fold greater than that of (2S,6S;2R,6R)-HNK (0.629 hr\(^{-1} \)), which might indicate that (R)-DHNK is a major metabolite in urine. This is consistent with the observation that (R)-DHNK was the predominate urinary metabolite in samples obtained from CRPS patients treated with a 5-day infusion of (R,S)-Ket [28].

The time-course of (R)- and (S)-Ket and corresponding metabolite concentrations in a representative subject are shown in Figure 6, and individual model predicted profiles...
are in good agreement with experimental data. Model diagnostic plots are shown as supplemental figures, and observed versus individual fitted drug concentration were reasonable for parent drug and metabolites with no systematic trend or bias (Figure S1). Individual standardized weighted residuals as a function of time for (R)- and (S)-ketamine and corresponding metabolites are reasonably distributed about zero, with only a slight bias for norKet and HNK, likely due to the limited number of subjects and blood samples (Figure S2).

**DISCUSSION**

This study reports the determination of the plasma concentration-time profiles of the stereoisomers of Ket, norKet, DHNK and (2S,6S;2R,6R)-HNK after administration of a sub-anesthetic dose of (R,S)-Ket (0.5 mg/kg as a single 40-min infusion). The results demonstrate that (R)- and (S)-DHNK and (2S,6S;2R,6R)-HNK are major circulating metabolites, that significant concentrations of these metabolites are present in the 24-hr plasma samples and can be detected in the 48-hr plasma of some patients, Figure 5. In addition to (2S,6S;2R,6R)-HNK, the plasma samples also contained measurable amounts of 4 of the other known positional and stereoisomeric HNKs [16,28] with (2S,5R;2R,5S)-HNK as the most predominate. These findings confirm the extensive hydroxylation of norKet which was observed in the initial *in vitro* studies, in the urine of volunteer subjects after the administration of an oral dose of (R,S)-Ket [27], and in the plasma of CRPS patients after a 3-day continuous infusion (R,S)-Ket [28]. Interestingly, no significant concentrations of the hydroxylated metabolites of Ket, i.e. (2S,6S;2R,6R)-HK and (2S,6R;2R,6S)-HK, were detected in this study which is consistent with the data from
the analysis of CRPS patients [28, unpublished data]. In addition, there were wide variations in the relative plasma concentrations of norKet, DHNK and (2S,6R;2R,6S)-HNK Ket. For example, in the samples obtained 230 min post dose, (R,S)-DHNK was the major metabolite in 4/9 patients, followed by (R,S)-norKET (3/9), and (2S,6S;2R,6R)-HNK (2/9). These results are consistent with previous data obtained from CRPS patients in which (2S,6S;2R,6R)-HNK and (2S,6R;2R,6S)-HNK were the predominate metabolites while the plasma concentrations of (R)- and (S)-DHNK were ~5-fold greater than the corresponding norKet enantiomers.

In this study, the plasma concentrations of (R)- and (S)- DHNK and (2S,6S;2R,6R)-HNK increased from the end of the infusion to the 230 min sampling point, while the plasma concentrations of (R)- and (S)-norKet decreased. These results suggest that (R,S)-norKet is the source of (R,S)-DHNK and (2S,6S)-HNK and are consistent with the observation by Leung and Baillie [13] that the iv administration of (R,S)-norKet to rats resulted in increasing plasma and brain concentrations of (2S,6S;2R,6R)-HNK and decreasing (R,S)-norKet during the 10 min post-dose sampling period. Bolze and Boulieu [23] also demonstrated that in an intensive care patient the plasma concentration of (R,S)-DHNK increased during a 2-hr infusion of (R,S)-Ket and remained relatively constant during an 8-hr sampling period. (R)-DHNK, (S)-DHNK and multiple HNK metabolites were also identified in the plasma and urine of Shetland ponies after the iv administration of (R,S)-Ket or (S)-Ket [25,26]. However the concentrations of these metabolites were not quantitatively determined nor was the identity of the HNK metabolite(s) established due to the lack of analytical standards.
Although the presence of down-stream Ket metabolites after administration of (R,S)-Ket and (S)-Ket has been established for over 10 years, all of the previous PK studies have been limited to the analysis of the plasma profiles of Ket and norKet. The present study reports the first simultaneous popPK model that includes (R)-DHNK, (S)-DHNK and (2S,6S;2R,6R)-HNK. Interestingly, the mean first-order formation rate constant of (R)-DHNK is almost 2-fold greater than that of (S)-DHNK, but with similar elimination rate constants (Table 1). This set of parameters results in greater systemic exposures to the R-enantiomer of DHNK (Figures 5 and 6c).

The clinical relevance of expanding the pharmacokinetic assessment of down-stream ketamine metabolites is suggested by the temporal disconnect in PK-PD studies seeking to establish a relationship between plasma concentrations of Ket or norKet, as the racemic mixtures or separate enantiomers, and therapeutic response. This was observed in PK-PD studies in CRPS patients receiving (R,S)-Ket [22] or (S)-Ket [10,12] and subjective pain relief and in the antidepressant response determined in the initial study of the treatment-resistant bipolar depression patients re-analyzed in this study [8]. Although turnover of endogenous mediators likely plays a major role, one potential explanation for the apparent hysteresis in the PK-PD relationships is that one or more the hydroxylated metabolites or DHNK contribute to the efficacy of the drug. The pharmacological activities of the hydroxylated metabolites of Ket and norKet and DHNK are under investigation and data from these studies will be reported elsewhere.

In conclusion, the results of this study show that after the administration of a single sub-anesthetic dose of (R,S)-Ket to patients with treatment-resistant bipolar depression, significant plasma concentrations of DHNK and (2S,6S;2R,6R)-HNK were
detected up to 48 hr after administration. The data indicate that while norKet is the initial metabolite, it is not necessarily the major metabolite. The results also suggest that future PK, PK-PD and clinical studies of Ket should be expanded to include the analysis of the major Ket metabolites as well as patient covariates that influence inter-individual variability in exposures.

COMPETING INTERESTS

There are no competing interests to declare.

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Figure Legends

Figure 1 The metabolism of ketamine

Figure 2 Schematic of the final pharmacokinetic model of (R) and (S)-ketamine and corresponding metabolites. Abbreviations and system equations are defined under Pharmacokinetic model in Methods.

Figure 3 The chromatographic trace from the achiral analysis of a plasma sample obtained 230 min after administration of a 0.5 mg/kg dose of (R,S)-Ket where: a = (2S,6S;2R,6R)-HNK; b = (2S,6R;2R,6S)-HNK; c = (2S,5S;2R,5R)-HNK; e = (2S,4R;2R,4S)-HNK; f = (2S,5R;2R,5S)-HNK.

Figure 4 The chromatographic trace from the achiral analysis of a plasma sample obtained one day (Day 1) after administration of a 0.5 mg/kg dose of (R,S)-Ket where: a = (2S,6S;2R,6R)-HNK; f = (2S,5R;2R,5S)-HNK.

Figure 5 The average plasma concentrations, presented as ng/ml, of the stereoisomers of (R,S)-Ket and its major metabolites in 9 patients treated with a 0.5 mg/kg dose of (R,S)-Ket.

Figure 6 Pharmacokinetic profiles for ketamine and corresponding metabolites in a representative subject; a) ketamine, b) norketamine, c) dehydronorketamine, and d) (2S,6S)-(2R,6R)-HNK. Red circles are measured (S)-enantiomers concentrations, blue circles are measured (R)-enantiomers concentrations, and continuous lines are model fitted profiles.
Table 1. Population pharmacokinetic parameter estimates for \((R)\) and \((S)\)-ketamine and corresponding metabolites.

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Definition</th>
<th>((S))-enantiomer</th>
<th>Mean</th>
<th>CV%(^b)</th>
<th>((R))-enantiomer</th>
<th>Mean</th>
<th>CV%(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{12}) (hr(^{-1}))</td>
<td>Distribution rate constant of Ket</td>
<td>12.1</td>
<td>46.2</td>
<td>7.30</td>
<td>51.6</td>
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<td>(k_{21}) (hr(^{-1}))</td>
<td>Distribution rate constant of Ket</td>
<td>0.040</td>
<td>80.2</td>
<td>0.16</td>
<td>12.3</td>
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<tr>
<td>(k_{31}) (hr(^{-1}))</td>
<td>Distribution rate constant of Ket</td>
<td>64.3</td>
<td>4.74</td>
<td>56.5</td>
<td>4.23</td>
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<tr>
<td>(k_{14}) (hr(^{-1}))</td>
<td>Formation rate constant of NorKet</td>
<td>3.19</td>
<td>59.7</td>
<td>6.18</td>
<td>59.7</td>
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<tr>
<td>(k_{13}) (hr(^{-1}))</td>
<td>Formation rate constant of NorKet</td>
<td>2.37</td>
<td>33.0</td>
<td>2.65</td>
<td>34.2</td>
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<tr>
<td>(k_{46}) (hr(^{-1}))</td>
<td>Formation rate constant of DHNK</td>
<td>0.94</td>
<td>37.6</td>
<td>1.63</td>
<td>44.5</td>
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<tr>
<td>(k_{60}) (hr(^{-1}))</td>
<td>Elimination rate constant of DHNK</td>
<td>1.10</td>
<td>61.7</td>
<td>1.31</td>
<td>58.0</td>
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<tr>
<td>(k_{54}) (hr(^{-1}))</td>
<td>Elimination rate constant of NorKet</td>
<td>4.45</td>
<td>9.47</td>
<td>8.95</td>
<td>3.31</td>
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<td>(V_1) (L)</td>
<td>Central compartment volume of Ket</td>
<td>9.15</td>
<td>11.1</td>
<td>14.0</td>
<td>8.35</td>
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</tr>
</tbody>
</table>

Ket, ketamine; NorKet, norketamine; DHNK, dehydronorketamine; HNK, (2S,6S;2R,6R)-hydroxynorketamine

\(^{a}\) Shared parameter between enantiomers
\(^{b}\) % coefficient of variation representing inter-individual variability
Figure 1

- **(R,S)-Ket**
- **(R,S)-norKet**
- **(R,S)-DHNK**

(a) 
(b) 
(c) 
(d) 
(e) 
(f)
Figure 2

IV Infusion

KET C1r/s, V1r/s

NK C4r/s

HNK C7

DHNK C6r/s

Tissue A2r/s

Tissue A3r/s

Tissue A5r/s
Figure 4

HNK ISOMERS

(R,S)-Norket

(R,S)-Ket

(2R,6S;3R,6R)-HNK

(R,S)-DHNK