EFFECTS OF PROTEIN CALORIE MALNUTRITION ON THE PHARMACOKINETICS OF KETAMINE IN RATS

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ABSTRACT:
The effect of protein calorie malnutrition (PCM) on the pharmacokinetics of ketamine (KET) enantiomers has been investigated. Six control and six PCM rats were administered 85 mg/kg racemic KET by intramuscular injection, and plasma concentrations of (S)- and (R-)KET, norketamine (NKET), and 5,6-dehydrokortaminate (DNK) were measured using enantioselective gas chromatography. Pharmacokinetic profiles were analyzed using standard noncompartmental and compartmental modeling methods. The volumes of distribution were similar between control and PCM rats for (S)- and (R-)KET. However, total clearance of both KET enantiomers was decreased, resulting in an increase in systemic exposure (p < 0.05). The KET absorption rate was also increased in PCM rats. A decrease in the clearance of both NKET enantiomers led to a significant increase in exposure in PCM rats (p < 0.005), and modeling results could not exclude the possibility that PCM induced an increase in the fraction of KET following the NKET pathway, which may further contribute to this increase in exposure. An increase in exposure to DNK enantiomers was also evident in PCM animals compared with controls (p < 0.005 (DNK1); N.S. (DNK2)), which was in concordance with the decrease in apparent clearance values. These results show that PCM significantly alters the pharmacokinetics of KET and several of its metabolites.

Protein calorie malnutrition (PCM) is found in developed as well as underdeveloped countries (Torris and Chew, 1994), and is the state reached when the body’s need for protein and energy fuels is not satisfied by the diet. PCM can result simply from inadequate food intake or can be the product of a disease state. In the latter case, diseases such as anorexia nervosa, cancer, and AIDS often lead to low food intake, inadequate nutrient absorption/utilization, or increased requirement of, or exaggerated loss of, nutrients. Approximately 50% of cancer patients will become malnourished during the course of their disease (Greene, 1988), and this can lead to impaired tolerance to chemotherapy and impaired immune function (Reilly et al., 1999). PCM also occurs in up to 65% of elderly hospitalized patients (Morley, 1998; Sullivan et al., 1999).

PCM has been shown to alter drug metabolism and disposition in children and in animal models (Parke and Ioannides, 1981; Anderson, 1988). In children, PCM reduced the clearance of antipyrine (Narang et al., 1977; Buchanan et al., 1979a), isoniazid, and acetanilide (Buchanan et al., 1979b, 1980). Animal models of PCM have shown reductions in the clearance of doxorubicin (Cusack et al., 1992) and acetaminophen (Jung, 1985), and altered oxidative drug metabolism (Campbell and Hayes, 1974; Fernandez et al., 1996; Walter-Sack and Klotz, 1996; Zhang et al., 1999), conjugation (Zhang et al., 1999), and protein binding (Varma, 1979). PCM induced alterations in drug metabolism and disposition appear to be multifactorial processes (Anderson, 1988; Zhang et al., 1999), and a complete understanding of the effect of nutritional status on drug pharmacokinetics would be highly desirable for effective clinical treatment.

Ketamine (KET; Fig. 1) is a chiral molecule that is often marketed as a racemic mixture [i.e., a 50:50 mixture of (S)-KET and (R)-KET enantiomers]. KET is a dissociative anesthetic agent, and the (S)- and (R)-enantiomers have significantly different pharmacokinetic and pharmacodynamic properties (White et al., 1982; Reich and Silvay, 1989). For example, the therapeutic (hypnotic, ataxic, and analgesic) potency of (S)-KET is between 2 and 4 times greater than that of the (R)-enantiomer (Marietta et al., 1977; White et al., 1985). Conversely, the posthypnotic stimulatory properties and agitated behavior that have limited the clinical use of KET are more common with (R)-KET (Marietta et al., 1977; White et al., 1982, 1985).

ABBREVIATIONS: PCM, protein calorie malnutrition; KET, ketamine; NKET, norketamine; DNK, dehydronorketamine; BrKET, bromoketamine; TFAA, trifluoroacetic acid anhydride; GC, gas chromatography; PH, peak height; PHR, peak height ratio; PK, pharmacokinetic; V, volume of distribution; kₐ, first-order absorption rate constant; kₑ₀, first-order elimination rate constant; CI, clearance; t₁/₂, half-life; Tₚmax, time to maximal concentration; Cₛmax, maximal concentration; AUC, area under the concentration-time curve.
KETAMINE PHARMACOKINETICS IN PROTEIN-DEFICIENT RATS

The remaining KET and NKET metabolites. (Trevor et al., 1983). NKET enantiomers have activity similar to that of
metabolic pathways appear to exhibit some degree of stereoselectivity
analog of NKET, and it is suggested that its levels reflect nonenzymatic
pathways involve the direct oxidation of KET at the C4, C5, and C6
Marietta et al., 1977; Adams et al., 1981; Trevor et al., 1983). In vitro
studies confirm that the major pathway for both enantiomers involves
N-demethylation to norketamine (NKET; Fig. 1) (Wieber et al., 1975;
FIG. 1. Proposed scheme for the metabolism of ketamine and norketamine enantiomers (derived from Adams et al., 1981; Trevor et al., 1983). In the present study, chromatographic and pharmacokinetic analyses were carried out for (R,S)-ketamine, (R,S)-norketamine, and (R,S)-dehydrodornorketamine (shown in bold). Chiral centers are shown for these analyzed species only (*).

KET undergoes extensive first-pass metabolism, and a number of studies confirm that the major pathway for both enantiomers involves N-demethylation to norketamine (NKET; Fig. 1) (Wieber et al., 1975; Marietta et al., 1977; Adams et al., 1981; Trevor et al., 1983). In vitro studies indicate that this is followed by further oxidation to 4-, 5-, and 6-hydroxy-NKET derivatives (Adams et al., 1981; Trevor et al., 1983). These studies also discuss the fact that other minor metabolic pathways involve the direct oxidation of KET at the C4, C5, and C6 positions. 5,6-Dehydrodornorketamine (5,6-DNK; Fig. 1) is an unsaturated analog of NKET, and it is suggested that its levels reflect nonenzymatic dehydration of 5-hydroxy-NKET (Adams et al., 1981). All of the KET metabolic pathways appear to exhibit some degree of stereoselectivity (Trevor et al., 1983). NKET enantiomers have activity similar to that of the parent drug, but with a shorter duration of action (Cohen and Trevor, 1974; Hong and Davission, 1982). Little is known about the activity of the remaining KET and NKET metabolites.

Recently, studies have shown that CYP2B6, 2C9, and 3A4 mediate the N-demethylation of both (S)- and (R)-KET in human liver microsomes (Yanagihara et al., 2001; Hijazi and Bouliou, 2002); however, the P450s responsible for this transformation in rat liver microsomes have not been established. The enzymes that mediate the hydroxylation pathways also have not been identified.

During the course of a study on the effect of PCM on the metabolism of the anti-cancer agent ifosfamide (Granvil et al., 1994), it was observed that KET immobilized PCM rats for a longer period of time than control rats. The purpose of this study is to investigate the effects of PCM on the pharmacokinetics of (R)- and (S)-KET, as well as the enantiomers of NKET and 5,6-DNK.

Materials and Methods

Chemical and Biological Reagents. Racemic and individual enantiomers of KET and NKET, along with 5,6-DNK and bromoketamine ([R,S]-BrKET), were generous gifts from Pfizer Global Research and Development (Groton, CT). Trifluoroacetic acid anhydride (TFAA) was purchased from Pierce Chemical (Rockford, IL), and both triethylamine and 2,2-dimethoxypropane were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol and toluene (“Baker Analyzed”) were obtained from J. T. Baker (Phillipsburg, NJ). Rat plasma for assay validation and calibration purposes was obtained from Pel Freez (Rogers, AR).

Animals and Treatment Protocol. This study was carried out in accordance with a protocol approved by the McGill University Animal Care Committee. Male Sprague-Dawley rats (specific pathogen free), 39 to 42 days old with an initial weight of 150 to 175 g, were obtained from Charles River Canada (Montreal, PQ, Canada). Rats were housed in a conventional facility with a 12-h light/dark cycle. After 1 to 3 days acclimation, rats started to receive isocaloric diets containing either 22.5% (control) or 5.5% (deficient) protein, as described by Varma (1979). The control diet (Rat Chow 5012) was purchased from Purina (St. Louis, MO) and the low protein diet (TD 77210) from Harlan (Indianapolis, IN). All animals were fed the control or deficient diets ad libitum for 17 to 20 days before KET administration and blood sampling. On the 15th day, plasma protein levels for control rats were, on average, 5.69 g of protein per 100 ml of plasma (range 5.32–6.01) and for PCM rats were 4.34 g/100 ml (range 4.01–4.94). Immediately before KET administration (at an age of 56–63 days), control rats weighed, on average, 345 g (range 327–363 g) and PCM rats, 199 g (range 186–208 g). Ketamine hydrochloride [100 mg of ketamine base/ml of aqueous solution, pH 3.5–5.5; Ketaset; Wyeth-Ayerst (Princeton, NJ)] was administered intramuscularly (thigh muscle) at a dose of 85 mg/kg. Blood sampling took place for up to and including 180 min post-KET dosing. Six of the rats (three control and three PCM) had samples taken at 10, 40, 90, 120, and 150 min postdose, whereas the other six rats (three control and three PCM) had blood samples removed at 20, 50, 90, 120, and 180 min postdose. Blood samples (approximately 0.3 ml each) were collected with a 21 to 23 gauge needle from the tail vein into heparinized tubes, and the samples were kept on ice until centrifugation (5 min at 10,000 g). Plasma samples were alkalinized using sodium hydroxide (10 M/9262 l) and mixed with 100 l of triethylamine (0.01 M in dry toluene) and 2,2-dimethoxypropane was purchased from Sigma-Aldrich (St. Louis, MO). Ethanol and toluene (“Baker Analyzed”) were obtained from J. T. Baker (Phillipsburg, NJ). Rat plasma for assay validation and calibration purposes was obtained from Pel Freez (Rogers, AR).

Sample Preparation. Trifluoroacetic acid anhydride was used for the preparation of trifluoroacetyl derivatives of KET, NKET, and 5,6-DNK before analysis by enantioselective gas chromatography (GC). Sample Preparation. Trifluoroacetic acid anhydride was used for the preparation of trifluoroacetyl derivatives of KET, NKET, and 5,6-DNK before analysis by enantioselective gas chromatography (GC). A 10-μl aliquot of an aqueous solution of (R,S)-BrKET (5 μg/ml) was added to 50 μl of rat plasma. Plasma samples were alkalinized using sodium hydroxide (10 M/9262 l, 0.5 M). KET and metabolites were then extracted twice into toluene (350 μl x 2). A few crystals of anhydrous sodium sulfate were added to the separated toluene fraction to ensure a completely water-free environment. Dry toluene (500 μl) was then transferred to a capped vial and mixed with 100 μl of triethylamine (0.01 M in dry toluene) and 100 μl of TFAA. Immediately after addition of TFAA, the sample was mixed gently and sealed vials were transferred to a heating block. Following reaction at 55°C for 15 min, the reaction mixture was cooled and then shaken with 1 ml of 5% sodium bicarbonate solution for 5 min. Subsequent to centrifugation, the toluene layer was removed and dried down under a steady nitrogen flow in the presence of 20 μl of propan-2-ol, and the residue was reconstituted with 50 μl of ethanol, awaiting analysis by GC.

Chromatography and Drug Assay. The plasma concentrations of the derivatized KET, NKET, and 5,6-DNK were determined using a validated enantioselective GC assay on a Hewlett Packard (Palo Alto, CA) 5890 series II GC with electron capture detection, equipped with an HP7673 autosampler, operating in the splitless mode. The column contained a cyclodextrin-based...
chiral stationary phase [Chiralpak GTP capillary column, 20 m, 0.25-mm i.d.;
Advanced Separation Technologies Inc. (Astec), Whippany, NJ], and the
carrier gas was helium at a flow rate of 1.7 ml/min. The injector and detector
temperatures were 250 and 300°C, and 0.5 μl of each sample in ethanol was
injected onto the gas chromatograph. The total duration of each run was 55
min, involving a series of temperature gradients. At zero time, the temperature
was 40°C, ramping up to 140°C following sample injection at a rate of 20°C
/min. After holding at 140°C for 10 min, the oven was taken from 140°C to
150°C at a rate of 0.5°C/min and held at 150°C for 14 min. After this time, a
temperature of 170°C was achieved at a rate of increase of 10°C/min which
was held for 4 min, resulting in a total run time of 55 min.

Due to high levels of KET and, particularly, NKET enantiomers in rat
plasma, standard curves were taken up to 20 μg/ml racemic mixture, whereas
up to 10 μg/ml was sufficient for DNK. Thus, blank plasma was spiked with
racemic KET and NKET at concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 7.5,
10.0, 12.0, 15.0, and 20.0 μg/ml and DNK at concentrations of 0.1, 0.2, 0.5,
1.0, 2.0, 5.0, 7.5, and 10.0 μg/ml. The concentration range for the separate
enantiomers of KET and NKET was 0.05 μg/ml to 10.0 μg/ml and 0.05 to 5.0
μg/ml for DNK enantiomers. Standards were prepared and processed along
with each set of experimental and control samples.

Drug peak heights (PHs) were used for concentration calculations. Since
the internal standard (BrKET) is a racemic mixture, the two enantiomer PHs
were added together before peak height ratio (PHR) calculations: KET, NKET,
and 5,6-DNK enantiomer PHR = specific enantiomer PH/(S)-BrKET PH +
(R)-BrKET PH). For plots of PHR against concentration, linearity was
observed for KET, NKET, and 5,6-DNK enantiomers over the concentration
range of 0.05 μg/ml to 5 μg/ml (r² ≥ 0.99).

The calibrators used for assay validation consisted of KET and NKET concen-
trations of 0.1, 0.2, 0.5, and 10.0 μg/ml for each enantiomer, and the calibrators
for each DNK enantiomer were 0.1 and 5.0 μg/ml. Intraday (n = 4 samples) and
interday (n = 4 days) validation studies were conducted using the calibrators.
The limit of detection was 0.01 μg/ml for KET and 5,6-DNK enantiomers and
0.015 μg/ml for DNK enantiomers. All enantiomers could be accurately quantified at
the lowest calibration point of 0.05 μg/ml. Inter- and intraday coefficients of variation
were <4% for (S)- and (R)-KET and <9% for both DNK enantiomers. For (S)-
and (R)-NKET, the intraday coefficients of variation were <9%, but the interday
coefficients of variation were not satisfactory (>20%). To overcome the interday
variability, a calibration curve was run with each set of pharmacokinetic samples,
which constituted an intraday analysis.

Pharmacokinetic Data Analysis: Pharmacokinetic parameters were calcu-
lated via a standard noncompartmental analysis using WinNonlin v2.1 (Phar-
sight, Mountain View, CA). The maximum plasma concentration (Cmax), time
to reach Cmax (Tmax), area under the plasma concentration-time curve (AUC;
log-linear trapezoidal rule extrapolated to infinity), total systemic clearance
(CL = Dose/AUC), and half-life (t1/2 = ln 2/λz; where λz is the terminal slope of
the PK profile) were calculated for individual rat KET concentration-time
profiles. The terminal phase of the metabolite enantiomers was not easily
identifiable for individual rats. Therefore, only Cmax, Tmax, and AUC (from 0
to 3 h) were calculated for individual metabolite enantiomer profiles.
Differences in pharmacokinetic parameters were tested using a Kruskal-Wallis
one-way analysis of variance by ranks, with post hoc comparisons of median
values using the Mann-Whitney test (α = 0.05). For comparison purposes, t1/2
values for the metabolite enantiomers were calculated using pooled PK data.

To assess the kinetic relationships between specific compounds, concentra-
tions of (R)- and (S)-KET, NKET, and DNK were fitted sequentially to
one-compartment linear mammillary plasma clearance models (Fig. 2). The
(R)- and (S)-enantiomer pathways were modeled separately. The rate of change
of KET concentrations in the central compartment (C_{KET}) was described by the
following equation:

\[
\frac{dC_{KET}}{dt} = k_a \cdot \text{Dose} \cdot e^{-k_{el} \cdot t} - k_{em} \cdot C_{KET}
\]

where k_a is a first-order absorption rate constant, k_{el} is a first-order elimination
rate constant, and V_{KET} is the volume of distribution of ketamine. The fraction
of KET absorbed into the blood stream following i.m. injection was assumed to
be complete (Clements et al., 1982), and total systemic clearance was
secondarily calculated, where Cl = k_a \cdot V_{KET}.

The pharmacokinetics of KET enantiomers were fixed according to eq. 1
and the resulting estimated parameters, and the concentrations of NKET
(C_{NKET}) were characterized subsequently using the following equation:

\[
\frac{dC_{NKET}}{dt} = k_a \cdot C_{NKET} - k_{em} \cdot C_{NKET}
\]

where k and k_{em} are first-order rate constants of formation and elimination. The
concentrations of DNK (C_{DNK}) were modeled in a similar fashion, such that

\[
\frac{dC_{DNK}}{dt} = k_a \cdot C_{DNK} - k_{em2} \cdot C_{DNK}
\]

where k_2 and k_{em2} are first-order rate constants of formation and elimination
of DNK enantiomers and C_{DNK} is fixed according to eq. 2. Authentic standards
of (S)- and (R)-DNK were not available during GC analysis; thus, the first
enantiomer to elute from the chiral column was named DNK1 and the second
DNK2 (Fig. 3B). However, initial modeling efforts gave evidence to suggest
that DNK1 was (R)-DNK and DNK2 was (S)-DNK, inasmuch as a fit could be
achieved only when DNK1 was fitted with (R)-NKET, and
DNK2 with the (S)-DNK enantiomer pathway and not vice versa. The total AUC
values of the fitted profiles for NKET and DNK enantiomers were calculated from
the estimated parameters according to

\[
\text{AUC} = \lim_{t \to -\infty} (C_{t=0})
\]

where C is C_{NKET} or C_{DNK} in the Laplace domain. Thus,

\[
\text{AUC}_{\text{NKET}} = \lim_{t \to -\infty} \left( \frac{k_a \cdot \text{Dose}}{V \cdot (s + k_d) \cdot (s + k_{el}) \cdot (s + k_{em})} \right) = \frac{k_a \cdot \text{Dose}}{V \cdot k_d \cdot k_{em}}
\]

and

\[
\text{AUC}_{\text{DNK}} = \lim_{t \to -\infty} \left( \frac{k_a \cdot k_2 \cdot \text{Dose}}{V \cdot (s + k_2) \cdot (s + k_d) \cdot (s + k_{el}) \cdot (s + k_{em})} \right) = \frac{k_a \cdot k_2 \cdot \text{Dose}}{V \cdot k_d \cdot k_{em} \cdot k_{em2}}
\]
Hence, the apparent clearance ($\text{Cl}/F$) of the metabolites was calculated as $\text{Dose}/\text{AUC}$, and the apparent volumes of distribution ($V/F$) were calculated as the ratio of the $\text{Cl}/F$ terms to their respective elimination rate constant ($k_{em}$ or $k_{em2}$). In this manner, the $F$ term for NKET is $f_r$, and $(f_r \times f_{r2})$ for DNK (Fig. 2).

Concentration data were analyzed using a naive-pooling approach, and all model parameters were estimated using WinNonlin. Nonlinear regression analysis was conducted with data weighted by $1/Y$, and goodness-of-fit was assessed by system convergence, Akaike information criterion, correlation coefficients ($r^2$), examination of residuals, and visual inspection.

**Results**

This study comprised, in total, six control and six PCM rats, all receiving an 85 mg/kg intramuscular dose of KET hydrochloride. PCM rats were under the anesthetic effects of KET for considerably longer than control rats. For five of the control rats, the duration of immobilization was between 60 and 90 min, with one of the control rats not undergoing complete anesthesia at all, being hypoactive but still mobile. In contrast, whereas one PCM rat started to move approximately 60 min after KET
dosing, the remaining five were immobilized for ≥90 min (90, 105, 115, >120, >120 min).

Under the analytical conditions, the enantioselective separation of the enantiomers of KET, NKET, DNK, and BrKET was achieved in a single run without significant interference from the plasma matrix. Representative chromatograms from blank plasma and from 120 min postadministration are presented in Fig. 3, A and B. The retention times of the enantiomers of KET were 36.9 (R) and 37.6 (S) min; of NKET, 19.9 (S) and 21.9 (R) min; of 5.6-DNK, 29.6 and 35.0 min (R- and S-enantiomers not defined); and of BrKET, 47.4 and 48.1 min (R- and S-enantiomers not defined). The enantioselective separation values (ε<sub>ка</sub>) were 1.02, 1.11, 1.19, and 1.02, respectively. In addition to peaks corresponding to enantiomers of KET, NKET, DNK, and BrKET, peaks were observed at 21.5, 26.5, and 31.0 min that increased with sampling time and were not present in blanks (Fig. 3B). However, the compounds yielding these peaks were not characterized.

The net exposure to (S)-KET, (R)-KET, (S)-NKET, (R)-NKET, and DNK1 was significantly increased due to PCM. Although exposure to DNK2 was increased, the difference between control and PCM rats was not statistically significant (Table 1). For NKET enantiomers, the exposure was increased approximately 4-fold (p < 0.005) and for DNK enantiomers, the AUC was almost doubled. PCM did not affect peak 15.55) l 8.75 (6.03

<p>| TABLE 1 | Noncompartmental analysis of ketamine and metabolite enantiomers in control and protein calorie malnourished rats |
|---------------------------------|-------------------------------------------------|---------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>(S)-Ketamine</th>
<th>(R)-Ketamine</th>
<th>(S)-Norketamine</th>
<th>(R)-Norketamine</th>
<th>Dehydronorketamine 1</th>
<th>Dehydronorketamine 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg·ml&lt;sup&gt;-1&lt;/sup&gt;) 4.81 (2.52–5.88)</td>
<td>5.00 (2.46–6.00)</td>
<td>3.26 (2.02–3.52)</td>
<td>2.08†† (1.19–2.68)</td>
<td>1.49 (1.15–1.65)</td>
<td>1.04 (0.22–1.98)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h) 0.33 (0.17–0.67)</td>
<td>0.33 (0.17–0.67)</td>
<td>0.81 (0.66–1.50)</td>
<td>0.81 (0.66–0.83)</td>
<td>0.81 (0.67–0.83)</td>
<td>0.81 (0.67–0.83)</td>
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<tr>
<td>---------------------------------</td>
<td>AUC (mg·h·1&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;1)&lt;/sup&gt; 5.85 (3.73–7.03)</td>
<td>5.38 (3.11–7.83)</td>
<td>8.19 (3.45–6.21)</td>
<td>8.88 (3.11–7.83)</td>
<td>3.11 (2.07–2.07)</td>
<td>1.47 (0.29–2.78)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h) 0.54 (0.33–0.71)</td>
<td>0.55 (0.33–0.71)</td>
<td>0.76&lt;sup&gt;a&lt;/sup&gt; (0.33–0.71)</td>
<td>0.76&lt;sup&gt;a&lt;/sup&gt; (0.33–0.71)</td>
<td>0.55&lt;sup&gt;a&lt;/sup&gt; (0.33–0.71)</td>
<td>0.51&lt;sup&gt;a&lt;/sup&gt; (0.33–0.71)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg·ml&lt;sup&gt;-1&lt;/sup&gt;) 5.08 (2.94–7.04)</td>
<td>5.04 (2.94–6.46)</td>
<td>5.81&lt;sup&gt;a&lt;/sup&gt; (2.94–4.46)</td>
<td>5.67&lt;sup&gt;a&lt;/sup&gt; (2.94–4.46)</td>
<td>5.67&lt;sup&gt;a&lt;/sup&gt; (2.94–4.46)</td>
<td>1.97&lt;sup&gt;a&lt;/sup&gt; (1.70–2.59)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h) 0.33 (0.17–0.90)</td>
<td>0.33 (0.17–0.90)</td>
<td>1.17 (0.78–2.00)</td>
<td>1.17 (0.78–2.00)</td>
<td>1.17 (0.78–2.00)</td>
<td>1.47 (1.50–2.50)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>AUC (mg·h·1&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;1)&lt;/sup&gt; 7.32&lt;sup&gt;a&lt;/sup&gt; (6.75–16.3)</td>
<td>7.53&lt;sup&gt;a&lt;/sup&gt; (6.6–13.92)</td>
<td>16.88&lt;sup&gt;a&lt;/sup&gt; (13.47–22.25)</td>
<td>16.88&lt;sup&gt;a&lt;/sup&gt; (13.47–22.25)</td>
<td>16.88&lt;sup&gt;a&lt;/sup&gt; (13.47–22.25)</td>
<td>2.87 (3.61–1.81)</td>
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<td>---------------------------------</td>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h) 0.99&lt;sup&gt;a&lt;/sup&gt; (0.73–2.48)</td>
<td>0.82&lt;sup&gt;a&lt;/sup&gt; (0.73–2.48)</td>
<td>2.29&lt;sup&gt;a&lt;/sup&gt; (0.73–2.48)</td>
<td>2.29&lt;sup&gt;a&lt;/sup&gt; (0.73–2.48)</td>
<td>2.29&lt;sup&gt;a&lt;/sup&gt; (0.73–2.48)</td>
<td>2.01&lt;sup&gt;b&lt;/sup&gt; (1.34–3.12)</td>
</tr>
</tbody>
</table>

* Calculated from 0 to infinity for KET, and 0 to 3 h for NKET and DNK.

† Calculated using pooled concentration-time data.

†† Significantly different between control and PCM rats (p < 0.005).

** Significantly different between (S)-KET and (R)-KET for the k or V<sub>NKET</sub>/F terms. However, the apparent clearance and elimination rate constant of (R)-Norketamine were approximately 1.5 to 2 times higher than for the (S)-enantiomer. The apparent clearance was slightly higher for DNK2 than for DNK1 as well.

** Discussion**

This is the first study in which the pharmacokinetics of (S)- and (R)-KET in rats, including metabolites, are described, with the aim of
elucidating the effects of PCM on the pharmacokinetics of KET. Although the number of animals in the study was small, this descriptive study suggests that PCM not only leads to a reduction in the clearance of both (S)- and (R)-KET, but also affects the pharmacokinetics of several KET metabolites by altering the clearance and quite possibly formation mechanisms.

### FIG. 4

Concentration-time profiles of ketamine (first row), norketamine (second row), and 5,6-dehydronorketamine (third row) enantiomers following ketamine administration to control and PCM rats. The filled circles represent the observed concentration values. The lines through the data depict the model-fitted profiles.

### TABLE 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>PCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k ) (min(^{-1}), g(^{-1}))</td>
<td>0.0976 (34)</td>
<td>0.195 (54)</td>
</tr>
<tr>
<td>( k_e ) (min(^{-1}))</td>
<td>0.0202 (15)</td>
<td>0.0124 (14)</td>
</tr>
<tr>
<td>Cl/F (l h(^{-1}), kg(^{-1}))</td>
<td>7.39 (7.1)</td>
<td>6.08 (15)</td>
</tr>
<tr>
<td>V/F (l kg(^{-1}))</td>
<td>6.08 (15)</td>
<td>5.90 (11)</td>
</tr>
</tbody>
</table>

The values are given as mean (CV), where CV represents the coefficient of variation.

### Footnotes:

1. Represents \( k_a \), \( k \), and \( k_2 \) for KET, NKET, and DNK, respectively.
2. Represents \( k_{el} \), \( k_{em} \), and \( k_{em2} \) for KET, NKET, and DNK, respectively.
3. \( F = 1 \) (KET), \( f_r (NKET) \), and \( f_r \times f_r (DNK) \).
Following both noncompartmental analysis and fitting of concentration data from control rats to the selected pharmacokinetic model, it appeared that there were no significant differences in the volume of distribution, clearance, half-life or AUC between (R)- and (S)-KET. A lack of enantiomerspecific difference in the exposure to KET is in accordance with previous findings in rats in which KET plasma levels were determined after i.v. administration of the separate KET enantiomers (Marietta et al., 1977; Ryder et al., 1978). A value of approximately 6 l/kg for the volume of distribution of KET enantiomers (Table 2) was the same whether determined using modeling techniques or noncompartmental analysis (data not shown). However, the volume of distribution of racemic KET reported in the literature for species other than rat is approximately 3 l/kg (Kaka and Hayton, 1980; Domino et al., 1982, and references therein). A species difference in plasma protein or tissue binding could lead to such an effect, although KET has been found to exhibit very low protein binding (Duvaldestin, 2001; Wagner and O’Hara, 1997).

Upon pharmacokinetic analysis of concentration data from rats with PCM, the absorption of KET into the systemic circulation after i.m. injection appeared to be enhanced compared with that seen in control rats (Table 2). The increase in $k_p$ for both KET enantiomers is most likely due to the smaller muscle mass of PCM animals (Table 2). Protein deficiency, and the loss of lean body mass that accompanies it, did not seem to affect the volume of distribution of KET. However, a reduction in clearance of both KET enantiomers is probably the major cause of the increased systemic exposure and half-life of (R)- and (S)-KET observed in PCM animals (Fig. 4; Table 1), potentially contributing to the increased anesthetic effects of KET in this rat group. Studies illustrating a PCM-related inhibition of P450s in rodents have been previously reported (Fernandez et al., 1996; Zhang et al., 1999). This inhibition includes the rodent counterparts of those reportedly associated with KET N-demethylation in humans (CYP3B, CYP3A, and CYP2C) (Yanagihara et al., 2001; Hijazi and Bouliue, 2002). It follows that the apparent reduction in the overall clearance of KET in PCM rats would occur as a result of such a reduction in phase 1 metabolism.

N-Demethylation is considered the major metabolic pathway for both (S)- and (R)-KET in rat and in humans (Wieber et al., 1975; Marietta et al., 1977; Adams et al., 1981; Trevor et al., 1983). N-KET enantiomers are active metabolites of KET and, despite having a shorter duration of action at equivalent doses (Cohen and Trevor, 1974), their pattern of activity follows that of their parents, such that the (S)-enantiomer has the greater anesthetic properties, whereas (R)-NKT appears to cause more pronounced posthypnotic stimulation (Hong and Davisson, 1982). Within the control rat group, the net exposure to (S)-NKT was 57% higher than that of (R)-NKT (Table 1), which is consistent with the work of Ryder et al. (1978). Although (S)- and (R)-NKT formation and apparent volume of distribution ($k$ and $V_{NKT}/F$) appeared to be similar, the elimination rate constant ($k_{em}$) and apparent clearance for (R)-NKT ($CIF$) were double the estimation for (S)-NKT (Table 2), offering an explanation for the observed higher plasma levels of (S)-NKT (Fig. 4).

PCM led to a 4-fold increase in systemic exposure to both NKT enantiomers and an equivalent increase in half-life compared with controls (Table 1). The median AUC ratios of NKT to KET in PCM rats were more than double those observed for control animals (2.31 versus 1.01 and 1.48 versus 0.58 for (S)- and (R)-enantiomers). These changes are most likely a result of a decrease in the clearance of (R)- and (S)-NKT due to PCM ($CIF$ decreased approximately 4-fold) (Table 2). However, it is possible that an increase in the fraction of (R)- and (S)-KET following the N-demethylation pathway also occurs. Assuming that PCM does not alter NKT volume of distribution, the decrease in $V_{NKT}/F$ might indicate an increase in the fraction ($fr$) of (S)- and (R)-KET following the N-demethylation pathway in PCM animals. One possible explanation for this phenomenon could be an inhibition of the alternative metabolic pathways of KET (i.e., 4-, 5-, or 6-hydroxylation; Fig. 1), leaving more KET available to N-demethylation. The concept of “compensatory” increases in one metabolic pathway due to inhibition of a competing pathway has previously been observed for glucuronidation versus sulfation (Morris and Pang, 1987, and references therein). The increase in NKT exposure and half-life suggests that plasma concentrations of NKT might remain above some minimum effective concentration for an extended period of time, thus contributing to the increased anesthetic effects of KET in PCM rats.

The other metabolite that was measured in this study, DNK, is proposed to arise from nonenzymatic dehydration of 5-hydroxy-NKT (Adams et al., 1981) and quite probably 6-hydroxy-NKT (6-OH-NKT), as an artifact of GC analytical procedures (Fig. 1). From in vitro studies, Trevor et al. (1983) indicate that only the (R)-enantiomer of 5-OH-NKT and the (S)-enantiomer of 6-OH-NKT are formed in rat liver. Thus, it would follow that the nonenzymatic formation of (R)-DNK should reflect and give some insight into the 5-hydroxylation of (R)-NKT and that of (S)-DNK should correspond to the 6-hydroxylation of (S)-NKT. Although, after GC analysis, chromatograms displayed two measurable DNK peaks (DNK1 and DNK2; Fig. 3B), their enantiomeric identity could not be ascertained due to the lack of separate authentic standards of (S)- and (R)-DNK. However, in an attempt to include DNK in the PK model, it was determined that DNK1 fitted only with the (R)-enantiomer pathway, thus possibly arising from (R)-5-OH-NKT, and DNK2 fitted only with the (S)-pathway, thus possibly arising from (S)-6-OH-NKT. The systemic exposure to DNK1 was approximately 40% higher than that to DNK2 within control rats and 30% higher within PCM rats (Table 1).

PCM led to an approximate 2-fold increase in systemic exposure of rats to both DNK enantiomers (Table 1; Fig. 4), and there was a lack of any obvious terminal phase by the end of the 3-h experiment. In concurrence with these findings, modeling results suggested that the clearance ($CIF$) of both DNK enantiomers was halved in PCM animals compared with controls (Table 2). These results could reflect an inhibition in the clearance of (R)-5-OH- and (S)-6-OH-NKT due to PCM. Interestingly, median DNK/NKT AUC ratios in PCM rats were approximately half the values for control rats (0.33 versus 0.67 and 0.17 versus 0.30 for DNK1 and DNK2), which might suggest that there was a decrease in the fraction of NKT passing through the DNK pathway. This is supported indirectly by the increase in $V_{DNK}/F$ terms calculated for PCM rats (Table 2). Since the F term for $V_{DNK}$ is $fr \cdot fr_2$, we hypothesize that the decrease in $fr_2$ is more significant than any potential increase in $fr$ (see $V_{DNK}/F$ in Table 2), obviously further relying on the assumption that PCM does not affect $V_{DNK}$. Taken together, the decreased clearance of DNK sufficiently offsets any decrease in DNK formation, resulting in augmented DNK exposure in PCM animals. The delay before maximal plasma levels of either DNK enantiomer were achieved in PCM rats, illustrated by $T_{max}$ values (Table 1), could reflect a delay in the formation of both (R)-5-OH-NKT and (S)-6-OH-NKT from NKT. In an attempt to improve the fitting of DNK enantiomers to the model, an additional transit (intermediary) compartment between NKT and DNK was included, but this failed to improve model fitting criteria (data not shown).

In conclusion, it appears that PCM alters the pharmacokinetics of KET, leading to changes in absorption and elimination of both (R)- and (S)-KET. PCM also affects the pharmacokinetics of the KET...
metabolites, primarily via clearance mechanisms but possibly also by affecting the fraction of drug and intermediate metabolites following a particular metabolic pathway. These changes offer an explanation for the increase in immobilization time seen in PCM rats following KET administration, compared with controls. Although the specific P450s responsible for these PCM-related effects have not been identified, it can be suggested that this condition has the potential to directly impact the pharmacokinetics of many therapeutic agents that are metabolized by P450s.

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References


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