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The Pharmacokinetics of Ketamine Following Intramuscular Injection to F344 Rats

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Abstract

Ketamine is a glutamate N-methyl-D-aspartate receptor antagonist that is a rapid acting dissociative anesthetic. It has been proposed to be an adjuvant treatment along with other drugs (atropine, midazolam, pralidoxime) used in the current standard of care (SOC) for organophosphate and nerve agent exposures. Ketamine is a pharmaceutical agent that is readily available to most clinicians in emergency departments and possesses a broad therapeutic index with well characterized effects in humans. The objective of this study was to determine the pharmacokinetic profile of ketamine and its active metabolite, norketamine, in F344 rats following single or repeated intramuscular administrations of subanesthetic levels (7.5 mg/kg or 30 mg/kg) of ketamine with or without the standard of care. Following administration, plasma and brain tissues were collected and analyzed using a liquid chromatography-mass spectrometry method to quantitate ketamine and norketamine. Following sample analysis, the pharmacokinetics were determined using non-compartmental analysis. The addition of the current SOC had a minimal impact on the pharmacokinetics of ketamine following intramuscular administration and repeated dosing at 7.5 mg/kg every 90 min allows for sustained plasma concentrations above 100 ng/mL. The pharmacokinetics of ketamine with and without the SOC in rats supports further investigation of the efficacy of ketamine co-administration with the SOC following nerve agent exposure in animal models.

Keywords

Ketamine; norketamine; nerve agent; pharmacokinetics; liquid chromatography-mass spectrometry

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Introduction

Ketamine is a glutamate N-methyl-D-aspartate (NMDA) receptor antagonist that is a rapid acting dissociative anesthetic being investigated for the treatment of seizures including status epilepticus.^[1-6] Given its potential as a treatment for acute seizures, ketamine has been proposed as an adjuvant therapy to the current standard of care (SOC) following organophosphate (OP) or nerve agent exposure to reduce seizures and the resulting neural damage.^[1, 2, 7-10] The current SOC for OP and nerve agent exposure is intramuscular (IM) administration of pralidoxime (2-PAM), atropine, and midazolam. Though the pharmacokinetics (PK) of ketamine have been studied in detail in a variety of mammalian species, there are no data regarding ketamine in rats in the presence of the multiple co-administered drugs used in combination with the current SOC for exposure to nerve agents.

Drug-drug interactions in which markedly increased ketamine levels occur may result in unwanted central nervous system (CNS) toxicity, whereas those interactions that may reduce ketamine concentrations in target tissue might diminish or abolish effects. Ketamine and its active metabolite norketamine undergo metabolism via hepatic Cytochrome P450s primarily via CYP2B6 and CYP3A4, which may be impacted with the co-administration of other pharmaceuticals such as rifampicin, itraconazole and ticlopidine, though none of the SOC agents are predicted to have effects on ketamine metabolism.^[11] In addition to metabolic clearance, ketamine is a substrate of the rat and human uptake organic cation ion transporters (OCT1, OCT2, OCT3) that mediate biliary and renal elimination of organic bases.^[12-14] It is known that 2-PAM is a substrate in rats and mice of OCT1 and OCT2,^[15] atropine is a potent inhibitor of OCT1^[16-18] and OCT3,^[19] while midazolam is an OCT1 inhibitor.^[20] Thus, there is a possibility of modulation of the systemic concentrations and distribution of ketamine to peripheral tissues including the brain with co-administered SOC drugs.

The analysis of ketamine and norketamine in biological matrices has been accomplished by a number of groups using multiple approaches for sample extraction and instrumental analysis. Historically, gas chromatography–mass spectrometry (GC-MS) has been a highly useful approach and offers the ability for separation of stereoisomers.^[21-25] Liquid chromatography–mass spectrometry (LC-MS) has also been a favored approach due to its sensitivity and reduced need for complex sample extraction approaches.^[26-32] As both ketamine and norketamine are pharmacologically active, sensitive and selective methodologies are necessary to accurately quantitate both compounds.

The objective of this study was to determine the PK profile of ketamine and its active metabolite, norketamine, in rats following single or repeated intramuscular administrations of subanesthetic levels of ketamine with or without the SOC. This study was designed to enable a follow-on study evaluating the efficacy of ketamine with the SOC following nerve agent administration.

Experimental Methods

Animals

Animal studies were approved by the Lovelace Respiratory Research Institute (LRRI) Institutional Animal Care and Use Committee; conducted in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International; and carried out in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council 1996). Male F344 rats (~8-10 weeks of age during the study) were purchased from Charles River Laboratories and were randomized into four study groups. Animals were housed with 12-hour light/dark cycles at 19-26°C with humidity of 30-70%. Up to two rats per cage were housed in polycarbonate shoebox cages on racks. Municipal water and food (2016C Harlan Global Certified Rodent Chow, Harlan Teklad, Madison, WI) were available *ad libitum*.

Reagents

Ketamine dosing solution was purchased from Putney, Inc. (Portland, MA) and either used as directed or aseptically diluted using sterile saline to a final concentration of 25 mg/mL. The concentration of the ketamine dosing formulations was verified using LC-MS as described below. Atropine was purchased from Sigma Aldrich (St. Louis, MO) and prepared at a concentration of 15 mg/mL in sterile saline. The pH of the vehicle was acidified with dilute hydrochloric acid to increase solubility before being neutralized to pH 7.2 with dilute sodium hydroxide. The 2-PAM used was purchased from Sigma Aldrich (St. Louis, MO) and prepared at 50 mg/mL in sterile saline and sterile filtered. Midazolam (5 mg/mL) was purchased from Hospira (Lake Forest, IL) and used without modification.

The analytical reference standards for ketamine, norketamine, ketamine-d₄, and norketamine-d₄ in methanol were obtained from Cerilliant (Round Rock, TX). Multiple lots of K₃EDTA rat plasma and brains were obtained from Bioreclamation (Westbury, NY). Purified water was collected from an in-house Labconco (Kansas City, MO) Water Pro Plus system. Glacial acetic acid was obtained from JT Baker (Center Valley, PA). Ammonium hydroxide was obtained from Sigma Aldrich (St. Louis, MO). LC-MS-grade water and acetonitrile were obtained from EMD (Billerica, MA). Formic acid was obtained from Thermo Scientific (Waltham, MA).

Animal Administrations

Four animal groups were used in this study with all administrations of ketamine done via intramuscular injection (IM) using an insulin syringe with a 29-gauge needle as shown in Table 1. Group 1 was administered 7.5 mg/kg of ketamine without the SOC regimen. Groups 2 and 3 were administered the SOC regimen followed by a single injection of ketamine at either 7.5 mg/kg (Group 2) or 30 mg/kg (Group 3) 30 minutes post SOC administration. Group 4 was administered five (5) successive doses of ketamine at 7.5 mg/kg at 0, 90, 180, 270, and 360 min ($\pm$ 3 min). The time of first ketamine administration was defined as time zero for all groups. The SOC regimen utilized for groups 2 and 3 was of atropine (3 mg/kg, IM) and 2-PAM (25 mg/kg, IM) followed by midazolam (0.66 mg/kg, IM) 5 $\pm$ 1 min later.

Within each group, four animals were assigned to each time point. Additionally, each group had an additional two animals that were not assigned a scheduled collection time point for potential use as spares during the conduct of the study. At scheduled necropsy (± 5 min) or in cases of morbidity, animals were euthanized by intraperitoneal injection of an overdose of a barbiturate-based sedative (Euthasol[®], Virbax, Ft. Worth, TX). Blood was collected via cardiac puncture and K₃EDTA plasma was prepared following centrifugation at 2000 g for 10 minutes at 2-8 °C. Following euthanasia, a gross necropsy was performed and carcasses were perfused with phosphate buffered saline containing heparin to remove any residual blood and then collected for bioanalytical analysis. Brain and plasma samples were stored frozen (−70 to −90°C) until analysis. Prior to bioanalytical analysis, the brain samples were homogenized with 1 mL of 10 mM ammonium acetate pH 4.4 per gram of brain tissue providing a final concentration of 0.49 g tissue per milliliter using the estimated density of brain of 1.04 g/mL.^[33] Brain to plasma ratios were calculated following conversion of brain concentrations from ng/g to ng/mL with no estimation for potential residual blood remaining following perfusion.

Sample Preparation

A protein precipitation method was developed to isolate ketamine and norketamine from rat plasma and brain homogenate using a ratio of 3:1 acetonitrile:matrix. Plasma or brain homogenate (50 μ L) was combined with 50 μ L of mixed internal standard (50 ng/mL ketamine-d₄ and norketamine-d₄ in acetonitrile), 50 μ L of ketamine/norketamine spiking solution in acetonitrile, and 50 μ L of acetonitrile and then vortexed. Blanks and study samples were prepared in a similar fashion with the substitution of unspiked acetonitrile in place of the spiking solution. These samples were centrifuged at 13,000 rpm for 5 minutes. The supernatant was transferred to a new tube and dried under a gentle stream of nitrogen using a nitrogen evaporator. The dried residues were reconstituted with 50 μ L of 90/10 water/acetonitrile, vortexed, and transferred to autosampler vials with glass inserts for analysis.

Solutions and Calibration/QC Samples

Ketamine and norketamine stock solutions were supplied by the manufacturer at 1.0 mg/mL in methanol. Calibration standards and quality controls (QCs) were prepared by spiking rat plasma or brain homogenate with a working solution of ketamine and norketamine in acetonitrile at different concentration levels. Plasma used in the generation of calibration curves and QC samples was pooled from multiple lots after selectivity criteria were met.

Plasma calibration standards were prepared at the following concentrations: 2.5, 5, 10, 50, 75, 100, 250, and 500 ng/mL. QC samples were prepared at the following concentrations: 7.5, 200, and 400 ng/mL. Dilutional QC samples were prepared at 4000 ng/mL. Brain homogenate calibration standards were prepared at the following concentrations: 5.1, 10.2, 20.4, 102, 153, 204, 510, and 1020 ng/g. Brain homogenate QC samples were prepared at the following concentrations: 15.3, 408, and 816 ng/g.

Ketamine-d₄ and norketamine-d₄ internal standard solutions were supplied at 100 μ g/mL by Cerilliant (Round Rock, TX) in methanol. A mixed ketamine-d₄/norketamine-d₄ internal

standard (IS) spiking solution was prepared by diluting the IS stock solutions to 50 ng/mL with acetonitrile. Ketamine and norketamine mixed working solutions were prepared in acetonitrile.

Liquid Chromatography – Tandem Mass Spectrometry

The LC-MS/MS system used for separation and detection of ketamine and norketamine was comprised of an Agilent (Santa Clara, CA) 1200 HPLC system with a G1322A degasser, G1311A quaternary pump, G1329A autosampler, and G1316A column heater coupled to a Sciex API 4000 mass spectrometer (Sciex Instruments, Foster City, CA). The instruments were controlled using Analyst software version 1.5.2 (Sciex, Foster City, CA). Figures were generated using SigmaPlot (Version 11, Systat Software). All statistical values were calculated using Microsoft Excel or SigmaPlot.

Chromatographic separation was conducted on an Agilent 1200 HPLC fitted with a Restek (Bellefonte, PA) Allure PFPP 5 μ m 100 $\times$ 2.1 mm column with Phenomenex (Torrance, CA) Phenyl guard column, 4.0 $\times$ 2.0 mm. The column temperature was set at 40°C and the flow rate was 0.75 mL/min with an overall run time of 8 minutes. A reverse phase gradient was utilized following sample injection with the initial conditions of 90% A held for 1 minute, the percentage of organic increased over 4 minutes to 12% A which was held for 1 minute when the column was re-equilibrated to the initial conditions for 3 minutes. The injection volume was 10 μ L and the autosampler temperature was set at 4 °C. Mobile phase A was composed of 0.1% formic acid in LC-MS water, and Mobile Phase B was composed of 0.1% formic acid in acetonitrile. Ketamine (239.1 $\rightarrow$ 126.1 m/z) and ketamine-d4 (242.3 $\rightarrow$ 128.8 m/z) eluted at approximately 4.8 minutes and norketamine (224.0 $\rightarrow$ 125.1 m/z) and norketamine-d4 (228.2 $\rightarrow$ 128.8 m/z) eluted at approximately 4.3 minutes. A Sciex API 4000 mass spectrometer collected multiple reaction monitoring (MRM) spectra generated by positive-mode electrospray ionization. The source conditions entailed the following: ionization voltage – 4500V, nebulizer temperature – 400 °C, GS1 – 75 units, GS2 – 50 units, and declustering potential – 53, 32, 40, 30 for ketamine, norketamine, ketamine-d4, and norketamine-d4, respectively.

Analytical Method Validation

The LC-MS/MS method was validated to be fit-for-purpose in providing quantitative determination of ketamine and norketamine in rat plasma and brain tissue using the acceptance criteria outlined by FDA guidance for bioanalysis.^[34] The following parameters were assessed: linearity range, accuracy, precision, dilutional linearity, selectivity (six lots), matrix effects, process efficiency, recovery, and the stability of working solutions and spiked plasma. The assay was then bridged for brain homogenate with an abbreviated accuracy and precision analysis conducted at three QC levels (n=6/level).

The linear range of the assay was determined using matrix-matched calibration standards. Peak area ratios were calculated by dividing the analyte peak area versus internal standard peak area. Linear regression ($y = mx + b$) with $1/x$ weighting was utilized to calculate concentrations of standards and QCs. Matrix blanks and zero (IS in matrix) blanks were also included in each analytical run. Accuracy and precision were determined by spiking samples

with ketamine and norketamine at the three QC levels: the lower limit of quantitation (LLOQ), the upper LOQ (ULOQ), and the dilutional QC level ($n = 6/\text{level}$). Matrix effect ratios, recovery, and process efficiency were determined using peak areas of a neat standards, extracted QCs and blank matrix extracted and spiked at an identical concentration of QCs after extraction adapted from Matuszewski and colleagues.^[41]

Pharmacokinetic Analysis

Pharmacokinetic parameters of ketamine and norketamine in plasma were determined by non-compartmental analysis using WinNonlin (Phoenix Version 6.2, Pharsight Corporation, Mountain View, CA) and employing the linear trapezoidal rule with linear interpolation and uniform weighting. Individual animal PK was not assessed and all values were calculated using the mean concentration for each time point. Ketamine or norketamine concentrations below the LOQ, but still detectable, were assigned a value of half of the LOQ. For Group 4, the area under the curve (AUC) was determined using the 5th dosing (365 – 840 min) of ketamine for comparison purposes to groups 1-3. The terminal half-life was determined using the time points that provided the best-fit with a minimum of 3 points.

Results

Bioanalytical Method Validation

A LC-MS/MS method was developed and validated to determine concentrations of ketamine and norketamine in rat plasma and brain homogenate. No interferences were observed for any of the target analytes or their corresponding internal standards. Representative chromatograms for ketamine and norketamine at the 2.5 ng/mL and a plasma blank are shown in Figure 1. The plasma-based calibration curve was validated from 2.5 to 500 ng/mL. The brain homogenate based calibration curve was validated from 5.1 to 1020 ng/g. A 1/X weighting of the calibration curve was utilized to improve the percent accuracy at the low end of the calibration curves for both compounds. Table 2 shows the results of the QC checks for plasma and brain homogenate.

Matrix effects, recovery, and process efficiency were assessed during the method validation. The matrix effect ratio of ketamine is 0.280 and 0.973 for norketamine. Ketamine recovery was 76% and norketamine recovery was 86%. The process efficiency of ketamine is 21.2% and 83.2% for norketamine. Similar findings were observed for the stable isotope internal standards.

The stability of the ketamine and norketamine was assessed in plasma and they were within 15% of the original concentration at the anticipated sample handling and storage conditions at room temperature (4 hr), following freeze thaw cycles (3 cycles), and long term storage at -70 to -90°C (81 days). The stability of working solutions stored at -15 to -30°C was also assessed and the solutions were within $\pm 15\%$ of freshly prepared solutions after 4 days.

Clinical Observations

All animals from groups 1 and 4 survived to their scheduled euthanasia without any abnormal signs. As expected, these animals displayed minor signs of sedation following

ketamine administration, but appeared normal 15 min post administration. Animals in groups 2 and 3 displayed a different reaction following administration of the SOC regimen potentially due to excessive administration of atropine in the absence of a co-administered nerve agent. Three animals from Group 2 (7.5 mg/kg) and eight animals from Group 3 (30 mg/kg) became moribund and were euthanized prior to their scheduled euthanasia and were not included in the PK analysis. For Group 2, two spares were utilized at the 30 minute time point and at 240 minutes for Group 3. The use of the spare animals allowed the collection of the PK time points at the scheduled collection times. These animals became lethargic and chilled to the touch following atropine and 2-PAM administration. The severity of these observations increased following midazolam administration and the animals started to become cyanotic and had difficulty breathing. When ketamine was administered the animals went into respiratory distress and rapidly declined. To address these symptoms, animals were placed on warming pads, administered 100% oxygen, and were manually manipulated in an attempt to increase movement. This procedure was conducted on approximately 50% of Group 2 and all of the Group 3 animals. It is unclear if the additional intervention increased survival and, if it did, by how much. The results from the moribund animals that were euthanized outside of the predetermined time points were excluded from the PK analysis.

Pharmacokinetic Analysis

Following the analytical method validation, plasma and brain samples were analyzed using LC-MS/MS to determine concentrations following administration. Pharmacokinetic parameters determined using WinNonlin are shown in Table 3. The plasma concentration versus time curves for ketamine and norketamine for the groups 1–3 are shown in Figure 2. Brain ketamine and norketamine concentrations for groups 1–3 are shown in Table 4. The plasma and brain concentration versus time curves for ketamine and norketamine for Group 4 are shown in Figures 3 and 4, respectively.

Discussion

The LC-MS/MS approach developed offers a robust and fast sample extraction and analysis of ketamine and norketamine in plasma and brain homogenate. The limit of quantitation of 2.5 ng/mL and 5.10 ng/g for ketamine and norketamine in plasma and brains is comparable to other methods using more time-consuming approaches such as solid phase extraction and liquid-liquid extraction, respectively.^[28, 29, 35] This method allows for the rapid analysis of biological matrices with minimal sample preparation, providing quantitative results that can be utilized for future studies.

The effects of the SOC protocol utilized in this study were surprising but not unexpected. Previous study indicates that a high dose (3 mg/kg) of atropine is the major contributor to the effects observed in groups 2 and 3, possibly due to the lack of competing excess acetylcholine that would be present following nerve agent exposure. Normal doses of atropine in humans exposed to nerve agents are a total dose of 2–6 mg, while larger doses have been used in OP pesticide exposures to have sufficient pharmacological activity.^[36, 37] The dose of atropine used in this study has been used by our laboratory with previous

success in nerve agent exposure to rodents and was selected as a potential upper boundary of anticipated atropine administration in people undergoing treatment for nerve agent or OP exposure who are unresponsive to lower doses. To verify that our high dose of atropine was not detrimental when animals were exposed to a nerve agent, a small pilot study in sarin challenged rats (LD50) treated with SOC followed by ketamine was undertaken. In contrast to the non-sarin challenged rats, all animals in this pilot study survived to at least 6 hours post treatment (data not shown). These data support the hypothesis that the effects observed in non-sarin challenged rats is related to the lack of excess acetylcholine. These data and further studies will be reported elsewhere.

The PK results suggest a rapid absorption of ketamine into the systemic circulation at therapeutic concentrations following an IM injection and rapid metabolism of ketamine to norketamine with a gradual reduction in systemic concentrations. The calculated terminal elimination half-life was $\sim 40\text{--}75\text{ min}^{-1}$ depending on the dose group. The observed half-lives were comparable to those observed following intravenous (IV) (38 min^{-1}) and intraperitoneal (IP) (75 min^{-1}) administration at 10 mg/kg and 125 mg/kg, respectively. [38, 39]

Group 1 had a higher ($\sim 2.5\times$) $C_{\max}$ as compared to Group 2, though the AUCs were comparable, suggesting there was little difference in total systemic exposure of ketamine with or without the SOC across the observed time period. It should be noted that Group 2 plasma ketamine concentrations at 5 (range 157-1096 ng/mL) and 15 min (range 442-1216 ng/mL) were considerably more variable as compared to Group 1 (5 min: range 1580-2040 ng/mL; 15 min: range 1440-1700 ng/mL) and this variability likely contributed to the differences in the observed $C_{\max}$. Additionally, the similarity in dose normalized AUCs for groups 1–3 (see Table 3) suggests that there is little drug-drug interaction affecting the systemic PK parameters. The observed PK parameters parallel previous studies investigating the pharmacokinetics of ketamine via IM administration (85 mg/kg) in Sprague Dawley rats with a half-life of approximately 30 min, and a $T_{\max}$ of approximately 20 mins, though the AUC_{last} at $\sim 291,000\text{ ng min/mL}$ was approximately equal to the calculated AUC at 30 mg/kg used in our study with nearly a quarter of the dose.^[40] Group 4 data suggests that there is modest dose accumulation in both plasma following repeated administration every 90 minutes, and this accumulation quickly decreases with only small differences in the calculated PK parameters between single and repeat dose studies (Table 3). This suggests sustained systemic concentrations above 100 ng/mL with dosing every 90 mins.

The brain concentrations of ketamine and norketamine following a single dose of ketamine with or without the SOC are shown in Table 4. Group 2 had a lower average brain concentration (2740 ng/g) as compared to Group 1 though there was variability in the data. This effect was absent at the later time points, suggesting that inhibition of OCT1-3 is not likely a major concern for ketamine and SOC co-administrations. Accordingly, the impact of SOC co-administration on ketamine and norketamine brain concentrations appears to be modest, if present at all. It should be noted that these interpretations are from a limited number of animals ($n = 3/\text{time point}$) and further analysis is required before the potential for transporter inhibition in humans can be fully elucidated. Ketamine is metabolized by CYP2B6, which is a highly polymorphic enzyme with allelic variants that have reduced

clearance of ketamine and may impact its use in a mass casualty situation.^[11] The ketamine brain to plasma ratios were assessed and they varied from ~1 to 3 with all groups being ~2.8-3.3 at 15 minutes after the final administration, indicating that ketamine was able to rapidly partition into the brain following IM administration. In general, norketamine had lower concentrations with the majority of brain to blood ratios being below 1. Other studies investigating ketamine and norketamine in rat brains following IP administration found similar results with ketamine brain to serum ratios of ~3 to 5, while norketamine had lower ratios below 1 for all points other than 10 and 20 minute following administration.^[38]

In repeat dose studies (Group 4), brain concentrations of ketamine and norketamine show a similar temporal and quantitative peak and trough relationship, as was observed in the plasma with concentrations, approaching steady state after the 5th dosing. The observed terminal half-lives of both ketamine ($\sim 67 \text{ min}^{-1}$) and norketamine ($\sim 147 \text{ min}^{-1}$) in brain were similar to the results observed in the blood. These data also indicate that ketamine and norketamine are rapidly detectable in the brain (within 10 min), suggesting that the anti-seizure effects may be rapidly initiated following IM administration.

Ketamine is a U.S. FDA approved pharmaceutical that is readily available to most clinicians in emergency departments. It initially was suggested for use as an adjuvant therapy for nerve agent exposures over 15 years ago.^[10] Further, Ketamine possesses a broad therapeutic index and has been used clinically for over 40 years with well characterized effects in humans. These properties make ketamine an ideal candidate for potential adjuvant therapy to the current SOC for the treatment of nerve agent intoxication.

Conclusion

A LC-MS/MS method was developed and used to analyze and quantitate ketamine and norketamine from rat plasma and brain samples. The PK of ketamine following IM administration of ketamine with or without the utilized SOC for nerve agents was determined. Critically, these data suggest that the addition of the utilized SOC had a minimal impact on the pharmacokinetics of ketamine and its metabolite following IM administration. It should be noted that these findings may not apply to other dosing regimens utilized for nerve agent exposures and additional research may be warranted if different combinations are utilized. Additionally, following IM administration, ketamine and its metabolite were rapidly found in brain tissues with steady-state levels easily achieved. Favorable pharmacokinetics, controlled blood levels, a large body of clinical experience, and the general clinical availability of ketamine make it an ideal adjuvant treatment for nerve agent intoxication.

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Figure 1: Upper image, representative ketamine (left) and norketamine (right) chromatogram for Low (2.5 ng/mL) standard. Lower image, representative ketamine (left) and norketamine (right) chromatogram for a blank plasma sample.

Figure 2: Ketamine and norketamine concentration vs. time curves in rat plasma following a single administration of ketamine. Group 1 received ketamine (7.5 mg/kg) only. Group 2 received Standard of Care (SOC) consisting of atropine, 2-PAM, midazolam, and ketamine (7.5 mg/kg). Group 3 received SOC and ketamine (30 mg/kg). Data is expressed as AVG $\pm$ SD.

Figure 3:
Ketamine and norketamine concentration vs. time curves in rat plasma following repeated dosing at 7.5 mg/kg. Data is expressed as AVG $\pm$ SD

Figure 4:
Ketamine and norketamine concentration vs time curves in rat brains following repeated dosing at 7.5 mg/kg. Data is expressed as AVG $\pm$ SD

Table 1.

Pharmacokinetic study design

Group ID	Treatment Group	Ketamine Dose (Dose Timing)	Targeted Blood Collection Time (min)	Animals per time point
1	Ketamine	7.5 mg/kg (0 min)	5, 15, 30, 60, 120, 240, 360	4
2	Ketamine+ SOC ^a	7.5 mg/kg (0 min)	5, 15, 30, 60, 120, 240, 360	4 ^b
3	Ketamine+ SOC ^a	30 mg/kg (0 min)	5, 15, 30, 60, 120, 240, 360	4 ^c
4	Ketamine	7.5 mg/mg (0, 90, 180, 270, 360 min)	10, 90, 100, 180, 190, 270, 280, 360, 365, 375, 390, 420, 540, 600, 720, 840	4

^a– Standard of Care: Atropine (3 mg/kg, IM), 2-PAM (25 mg/kg, IM), Midazolam (0.66 mg/kg, IM)

^b– n = 3 animals at 240 min

^c– n = 3 animals at 15 min; n = 2 animals at 120 and 240 min

Table 2.

Liquid chromatography-mass spectrometry method validation

Sample Name	Nominal Conc. (ng/ml)	Ket. Avg. Calc. Conc. (ng/ml)	Ket. % RSD	Ket. % Diff. from Nominal	Norket. Avg. Calc. Conc. (ng/ml)	Norket. % RSD	Norket. % Diff. from Nominal
Day 1							
QC 1	7.5	7.32	4.32	2.47	6.84	4.48	8.87
QC 2	200	204	2.56	2.17	205	5.22	2.25
QC 3	400	401	2.13	0.333	389	2.67	2.79
Day 2							
QC 1	7.5	7.27	8.86	3.11	7.29	3.53	2.87
QC 2	200	191	3.13	4.33	205	1.59	2.67
QC 3	400	399	2.66	0.333	391	2.77	2.25
Day 3							
QC 1	7.5	8.36	4.95	11.5	8.14	7.28	8.47
QC 2	200	195	4.14	2.42	205	4.68	2.33
QC 3	400	412	0.78	3.08	394	3.30	1.50
Additional Validation Parameters							
LLOQ	2.5	2.73	6.11	9.07	2.38	4.89	4.47
ULOQ	500	514	2.11	2.70	503	5.18	0.533
Dil QC ^a	4000	438	3.51	9.42	426	3.15	6.58
Rat Brain Tissue							
QC 1	15.3	15.7	4.56	2.78	15.6	3.08	1.89
QC 2	408	417	3.01	2.13	424	1.74	4.04
QC 3	816	798	2.08	2.25	819	2.24	0.375

^a—Sample diluted 1:10 to assess dilutional linearity.

Table 3.

Selected pharmacokinetic parameters

	Dose	C _{max}	T _{max}	t _{1/2}	AUC _{last}	AUC _{∞_D}
Group	mg/kg	ng/mL	min	min ⁻¹	min*ng/mL	Min*ng*kg/mL/mg
Pharmacokinetic Parameters for Ketamine in Rat Plasma						
1	7.5	2100	5	68.4	71600	9640
2	7.5	884	15	74.6	58300	7840
3	30	4250	15	39.7	271000	9060
4	7.5	1380	5	89.7	63300	8550
Pharmacokinetic Parameters for Norketamine in Rat Plasma						
1	7.5	954	15	89.2	90300	12600
2	7.5	850	30	65.8	96200	13300
3	30	2160	60	63.9	311000	10700
4	7.5	709	15	110	76200	10600

C_{max} – maximal concentrationT_{max} – time of maximal concentrationt_{1/2} – Terminal elimination half-lifeAUC_{last} – Area under the concentration vs time curveAUC_{∞_D} – Dose Normalized Area under the concentration vs time curve

Table 4.

Ketamine and norketamine brain concentrations

Group	Time Point (min)	Ketamine		Norketamine	
		Average (ng/g)	SD (ng/g)	Average (ng/g)	SD (ng/g)
1	15	4470	1130	847	149
	120	160	53.5	224	36.5
	360	14.8	9.52	30.7	12.1
2	15	2740	501	579	118
	120	107	13.9	194	30.9
	360	6.1	4.28	32.7	5.77
3	15	13500	2950	1040	126
	120	2850	2750	782	49.2
	360	22.4	4.70	69.3	17.4

n = 3 animals per time point