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Ketamine and Norketamine Stability in Whole Blood at Ambient and 4°C Conditions

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Abstract

A study was implemented to describe the pharmacokinetics (PK) of ketamine (K) and its metabolite norketamine (NK) in critically ill adults. Conducting studies in these subjects is hindered by the immediate need to process and freeze samples obtained in a busy intensive care setting. The ability to store unprocessed samples at room temperature for an extended time period would overcome this barrier. Stability and blood to plasma partitioning of K and NK were investigated in whole blood up to 120 h at room temperature and 4 °C. Whole blood was spiked with K and NK (1,000 ng/mL each). Blood samples were aliquoted at different time points (0 to 120 h), extracted, and analyzed using a validated high performance liquid chromatography tandem mass spectrometry assay. The study demonstrated the stability of both K and NK in whole blood up to 120 hours. These *in vitro* studies suggest that the concentrations of K and NK measured in the PK samples are reliable. The established stability results were successfully employed to investigate K and NK pharmacology studies in critically ill adults.

Keywords

Ketamine; Norketamine; Stability; Blood; Plasma; Pharmacokinetics

INTRODUCTION

Ketamine (K) is a multifaceted drug due to its interaction with an array of receptors to produce sedation, catalepsy, somatic analgesia, bronchodilation, and sympathetic nervous system stimulation effects which have relevant utility in the critical care setting (Kurdi, Theerth and Deva 2014, Potter and Choudhury 2014). K is highly lipophilic and undergoes

extensive hepatic metabolism to its principal metabolite norketamine (NK), exhibiting one-third the anesthetic potency as the parent drug (Ebert, Mikkelsen, Thorkildsen and Borghjerg 1997).

Extracorporeal membrane oxygenation (ECMO) is used in critically ill patients with severe pulmonary and/or cardiac failure refractory to conventional therapy. Patients on ECMO typically receive high doses of fentanyl and midazolam for pain and sedation, which warrant the use of ketamine as an adjunctive agent to lessen the opioid and sedation requirements, and may translate a decrease in withdrawal and tolerance outcomes.

A study was implemented to understand the pharmacokinetics (PK) of K and NK in critically ill adults who receive ECMO support. The pharmacokinetics (PK) of drugs can change during ECMO by mechanisms of the circuit sequestration, increasing volume of distribution, and altering drug clearance and elimination (Himebauch, Kilbaugh and Zuppa 2016). The logistics of processing blood samples presented a hurdle to study conduct. The reasons for this were: 1) PK samples were drawn at nighttime and weekend hours when ancillary staff could not process and freeze plasma and 2) the intensive care unit where these samples were obtained is often busy and sample processing for research studies often falls below the priorities of fast-paced clinical care. The blood samples were drawn and stored in ICU until further processing at ambient conditions (20 to 22 °C) and the temperature range in ICU is maintained by patient comfort. A previous stability study showed K and NK were stable in blood up to 2 h at 4 °C and ambient conditions (Hijazi, Bolon and Boulieu 2001). Due to the multisite collaboration of our PK study, we investigated the stability of K and NK in blood and plasma to an extent of 120 h. In addition, we examined if blood to plasma partitioning occurred for K and NK over time employing liquid chromatography tandem-mass spectrometry assay (Brown and Melton 2011, Hasan, Hofstetter, Fassauer, Link, Siegmund and Oswald 2017, Parkin, Turfus, Smith, Halket, Braithwaite, Elliott, Osselton, Cowan and Kicman 2008, Pérez and Barceló 2008).

MATERIALS AND METHODS

Chemicals and Reagents

K, NK, K-D4, and NK-D4 ampules were purchased from Cerilliant (Round Rock, TX). Chromatographic grade methanol and acetonitrile and analytical grade formic acid were obtained from EMD Millipore (Billerica, MA). Water was purified using Barnstead Nanopure™ from Thermo Fisher Scientific (Marietta, OH, USA). Adult human blank plasma was obtained from the blood bank at The Children's Hospital of Philadelphia (Philadelphia, PA). Adult human whole blood in K2-EDTA tubes were obtained from the blood bank of Thomas Jefferson University Hospital, respectively (Philadelphia, PA).

Chromatographic Conditions

An Atlantis HILIC (hydrophilic liquid interaction chromatography) silica analytical column (3 mm×50 mm, 3 µm, Waters, Milford, MA) was used with a gradient chromatography at a flow rate of 0.7 mL/min. Column temperature was held at 40 °C and the autosampler was controlled at 10 °C. Mobile phase "A" was deionized water containing 0.2% formic acid,

and mobile phase “B” was 0.2% formic acid in acetonitrile. The gradient profile started with 90% “A” and held for 0.75 min, was then switched to 90% “B” lasting 1.8 min and held until 2.8 min, and finally switched back to “A” at 2.81 min and lasting until 3.5 min, after which the system was returned to the initial condition. Under these conditions, the analytes and internal standards eluted from the column within two minutes.

Mass Spectrometry

An AB Sciex 4000 triple-quadrupole mass spectrometer equipped with an electrospray ionization source (Framingham, MA) operating in the positive ion mode was used as a detector. Multiple reaction monitoring chromatograms were acquired with Analyst 1.6.2 (Framingham, MA). The following precursor-product ion transitions were monitored: m/z 238.7 $\rightarrow$ 125.2 for K, m/z 224.6 $\rightarrow$ 125.1 for NK, m/z 242.7 $\rightarrow$ 129.3 for K-D4, and m/z 228.2 $\rightarrow$ 129.1 for NK-D4 with a dwell time of 50 msec for each ion transition (Legrand, Roy, Monchaud, Grondin, Duval and Jacqz-Aigrain 2008). Instrument parameters were optimized to the following settings: collision-associated dissociation gas setting (high), curtain gas (35), gas 1 (80), gas 2 (50), ion spray voltage (5500 V), and source temperature (500 °C). K, NK, K-D4, and NK-D4 specific ionization parameters include 45, 54, 50, and 49 V respectively for declustering potential, 40, 36, 35, and 37 V respectively for collision energy, and 10 V for collision cell exit potential and entrance potential for all analytes and internal standards.

Preparation of Standards in Human Plasma and Blood

Standard stock solutions for K and NK (1 mg/mL in methanol) were diluted to provide 100 µg/mL (K and NK each) in methanol. Human plasma and blood standards were prepared at concentrations of 20, 50, 100, 200, 500, 1000, 2000, 4500, and 5000 ng/mL. The internal standard (IS) working solution (8 ng/mL K-D4 and 15 ng/mL NK-D4) was prepared by diluting the stock solution (1 mg/mL) with acetonitrile.

Sample Preparation

Whole Blood Stability: A single 1,000 ng/mL (K and NK) whole blood sample (20 mL) was prepared and split (10 mL each) for storage at 4 °C and ambient temperature. Six 50 µL aliquots were taken from each sample at 0, 24, 48, 72, 96, and 120 h and protein precipitated with 500 µL of IS solution. Samples were centrifuged at 15,700 rcf for 10 min. Supernatant was transferred to Eppendorf tubes and frozen at –80 °C until analysis.

Blood to Plasma Partitioning: A 750 µL aliquot of blood was taken at 0, 24, 48, 72, 96, and 120 hours and centrifuged for 5 min at 15,700 rcf to obtain plasma. The resultant plasma was split into 50 µL aliquots (n = 6) for each sample and protein precipitated with 500 µL of IS working solution. Samples were centrifuged at 15,700 rcf for 10 min and supernatant was transferred to clean Eppendorf tube and frozen at –80 °C until analysis.

After completion of the final time point, a freshly prepared plasma and blood standards (50 µL, n = 2) were extracted with 500 µL of IS solution. Standards were centrifuged at 13,000 rpm for 10 min and supernatant was transferred to a 96-well plate. Previously protein precipitated samples were thawed and aliquoted to the 96-well plate for sample analysis. The results were normalized to nominal baseline value of 1,000 ng/mL.

The method was validated in accordance with the FDA bioanalytical method validation guidelines (Food and Drug Administration Center for Drugs Evaluation and Research 2001). Assay accuracy and precision were performed using four quality control (QC) concentrations (20, 60, 800, and 4000 ng/mL) of K and NK in human plasma (n = 6). Post-processing stability (24 h in autosampler), freeze/thaw stability (3 cycles), matrix effects and selectivity (6 lots of plasma) were also assessed.

RESULTS AND DISCUSSION

K and NK Assay

A validated assay was used for analysis of K and NK in plasma. The linearity of the assay was evaluated by analyzing 8 calibration standards (n = 2) for K and NK over the nominal 20 to 5000 ng/mL concentration range ($r^2 > 0.98$). A linear regression with a $1/x^2$ weighting factor was applied to K and NK.

Intra- and inter- day precision and accuracy were determined by extracting plasma supplemented with for K and NK at four QC concentrations (n = 6). Intraday accuracy ranged between 90.8 and 108%, for both K and NK. Intraday precision ranged from 2.80–11.9% CV (coefficient of variation) for K and 2.48–6.14% CV for NK. Interday accuracy ranged from 88.9–103% for both K and NK. Interday precision ranged from 4.10–14.4% CV for K and 3.76–7.50% CV for NK. The recovery (mean $\pm$ SD %) of K and NK were 91.6 ± 9.51 and 96.5 ± 6.06 % respectively and matrix effect for 85.9 ± 6.37 and 80.4 ± 1.61 % ng/mL of K and NK respectively. Consistent with previously reported results, K showed ion suppression with protein precipitation method (Nosseir, Michels, Binder, Wiesen and Muller 2014).

Blood extraction was partially validated against the fully validated plasma assay. Intraday precision and accuracy were determined by extracting blood supplemented with for K and NK at four QC concentrations (n = 6). Accuracy and precision were between 94.6–109% and 2.09–12.1% CV, respectively, for K and NK. This assay was used to investigate the stability and partitioning of K and NK in human blood and plasma.

Stability and Partitioning of Blood and Plasma

Previous studies have shown K and NK were stable in human blood for 2 h at ambient and 4 °C conditions (Hijazi, Bolon and Boulieu 2001). Other studies have shown the stability of K and NK in plasma at –20 °C for two months and 24 h at room temperature (Sergi, Bafile, Compagnone, Curini, D'Ascenzo and Romolo 2009). The concentration of K and NK measured in clinical sample (362 samples from 15 subjects) were 640 ± 427 and 370 ± 339 ng/mL of K and NK respectively. Therefore, we selected 1,000 ng/mL of K and NK to evaluate blood stability. The stability of K and NK over time in blood and plasma is presented in Table 1. The concentration of K and NK (1,000 ng/mL each) was selected for stability studies based on the known PK profile of K and NK. For blood and plasma at room temperature and 4 °C, no significant degradation of K and NK over the 120 h was observed (Figure 1). For the partitioning effect, the ratios of blood to plasma (n=6) over time ranged from 0.97 – 1.13 for both K and NK.

CONCLUSION

This study demonstrates the stability of K and NK in whole blood up to 120 h at room temperature or 4 °C refrigeration. The results provide assurance that processing of blood PK samples can be delayed up to 120 h after collection. Lacking immediate resources to process blood and freeze plasma samples should not be a barrier to perform pharmacology studies for K and NK.

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Figure 1. Boxplot display of concentration distribution (minimum, first quartile, median, third quartile, maximum, and outlier points of concentrations greater or less than 1.5 times the interquartile range) of ketamine and norketamine in blood and plasma over time at room temperature and 4 °C

Table 1

Stability and partitioning of ketamine and norketamine in blood and plasma at room temperature and 4 °C

Drug condition	Time (h)	Blood				Plasma				Blood to Plasma Ratio
		Mean	Standard Deviation	Accuracy (%)	Precision (% CV)	Mean	Standard Deviation	Accuracy (%)	Precision (% CV)	
Ketamine 4 °C	0	1000	22	100	2	1000	24	100	2	1.07
	24	952	12	95	1	1012	27	101	3	1.01
	48	916	53	92	6	975	33	97	3	1.00
	72	957	40	96	4	1038	24	104	2	0.99
	96	1003	53	100	5	1024	46	102	5	1.05
	120	975	34	98	3	1077	38	108	4	0.97
Ketamine Room Temperature	0	1000	57	100	6	1000	42	100	4	1.06
	24	1019	70	102	7	1037	58	104	6	1.05
	48	1000	91	100	9	1021	15	102	1	1.04
	72	1006	43	101	4	1059	45	106	4	1.01
	96	1006	33	101	3	1022	40	102	4	1.05
	120	1015	61	102	6	1034	39	103	4	1.05
Norketamine 4 °C	0	1000	14	100	1	1000	36	100	4	1.08
	24	959	11	96	1	1037	35	104	3	1.00
	48	950	56	95	6	991	5	99	0	1.04
	72	977	14	98	1	1047	17	105	2	1.01
	96	1034	38	103	4	1004	24	100	2	1.12
	120	1020	29	102	3	1059	31	106	3	1.04
Norketamine Room Temperature	0	1000	39	100	4	1000	18	100	2	1.08
	24	1039	50	104	5	1028	31	103	3	1.09
	48	1007	63	101	6	1021	13	102	1	1.06
	72	1033	43	103	4	1061	46	106	4	1.05
	96	1040	27	104	3	1016	22	102	2	1.10
	120	1065	51	106	5	1012	32	101	3	1.13