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Brain penetration of ketamine: Intranasal delivery VS parenteral routes of administraion

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ABSTRACT

Ketamine is approved by the FDA to be used as an anesthetic however, recent reports have exhibited its success in the treatment of major depressive disorder (MDD). Studies have suggested that a sub-anesthetic dose produces rapid antidepressant activity providing significant symptomatic relief particularly in patients with a history of treatment resistant depression (TRD). Many reports have been published on the intranasal (IN) efficacy of ketamine in the treatment of depression, however studies that have investigated the effects of the route of administration on drug delivery to the brain appear to be absent in literature. Therefore, in this study, a single dose (15 mg/kg body weight) was administered via different routes of administration [oral (PO), intranasal (IN) and intraperitoneal (IP)] to healthy male Sprague-Dawley rats in order to determine the brain tissue pharmacokinetics of ketamine. A novel validated liquid chromatography-mass spectrometry (LC-MS) method was developed using a fused core column for the determination of ketamine in plasma and brain homogenates. While IP administration resulted in favorable concentrations in the brain and plasma; IN administration, which is supposed to favour drug delivery to the brain, exhibited moderately low drug levels post administration. PO administration produced significantly lower levels due to extensive first-pass metabolism in the liver and intestines. These results have implications for future studies exploring the use of ketamine for the treatment of MDD in order to optimize treatment regimens and suggest that parenteral administration of ketamine should be used in the treatment of depression.

1. Introduction

In the 1960s, ketamine (RS-2-2-Chlorophenyl-2-methylamincyclohexanone) was discovered to have analgesic and anesthetic effects with the potential to produce hallucinogenic and dissociative symptoms such as impaired recognition, memory and language skills as well as visual disturbances in individuals (World Health Organization, 2015); however, recent studies have exhibited intranasal ketamine's rapid and long lasting anti-depressive effects in patients with major depressive disorder (MDD) and more especially in those diagnosed with treatment resistant depression (TRD) (Kirby, 2015; Rosenbaum and Palacios, 2018; Toki et al., 2018; Zhou et al., 2014). MDD's increasing prevalence has been associated with significant public health costs and morbidity rates (Hamon and Blier, 2013; Kessler et al., 2003); the heterogeneity of this clinical disorder results in variations in symptoms experienced by individuals ranging from pervasive, low moods and loss of interest accompanied with more classical symptomology such as loss of appetite, sleep pattern alterations and diminished psychomotor and cognitive functions (Hasselmann, 2014; Kirby, 2015; Murrough, 2012). The suffocating impact on the public health system arises as a result of the disorder having a greater propensity to originate in adulthood and continue as a chronic or recurrent condition into the later stages of life; diminishing the overall quality of life of those that are affected; and often resulting in devastating outcomes such as suicide ideation (Murrough, 2012).

Approximately two-thirds of patients with Major Depressive Disorder do not receive satisfactory relief from conventional antidepressant treatments; and as many as one-third of these patients are categorized as treatment resistant; and remain untreated after numerous trials of antidepressants (Malhi et al., 2016). In an attempt to resolve this escalating resistance to conventional antidepressants, medical practitioners have resorted to the off-label use of ketamine for the treatment of MDD (Aan Het Rot et al., n.d.).

A single dose of ketamine provides symptomatic relief within hours;

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and is effective for an extensive period, however, the duration of the antidepressant effects of ketamine fluctuates across studies and is dependent on the route of administration (Aan Het Rot et al., n.d.; Malhi et al., 2016). In a trial on the antidepressant effects of ketamine, most patients experienced mood elevation approximately 120 min post-infusion of ketamine (Aan Het Rot et al., n.d.). Studies have also shown that a sub-anesthetic dose of ketamine (0.5 mg/kg) over a 40-min IV infusion produces rapid antidepressant activity in those diagnosed with TRD (Schwartz et al., 2016). Ketamine's hypothesized antidepressant effect is elicited by a neurochemical cascade involving the antagonization of the N-methyl-D-aspartate (NMDA) receptors and the subsequent activation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors; resulting in the disinhibition of glutamate signalling due to the suppression of tonic glutamate input into the GABAergic interneurons (Browne and Lucki, 2013; Hasselmann, 2014).

The route of administration of ketamine significantly affects its efficacy in the treatment of MDD (Schwartz et al., 2016). Oral bioavailability of ketamine is significantly reduced due to the extensive first-pass metabolism of ketamine in the body; therefore, other routes of administration such as sublingual and intranasal formulations are being investigated (Peltoniemi et al., 2016). Intramuscular (IM) formulations of ketamine produce maximum plasma concentrations rapidly post-administration, with comparatively high bioavailability (Peltoniemi et al., 2016; Rossiter, 2016). In a randomized controlled trial of intranasal administration of ketamine it was found that patients had significant improvements in symptoms and tolerability after 24-h of intranasal ketamine administration (Lapidus et al., 2014). Daly et al. (2018) demonstrated the rapid onset of action in TRD, with IN administration when compared to oral administration. Furthermore the are many studies that also support ketamine's IN administration for post-operative pain which supports its potential use for the treatment of MDD (Lapidus et al., 2014; Niesters et al., 2014; Rossiter, 2016). Ketamine is generally well tolerated, however, its use may be limited due to its potential to cause psychedelic (panic attacks, hallucinations, memory defects), cardiovascular, neurological and hepatoxic side effects (Clements et al., 1982; Rodriguez Rosas et al., 2003; Short et al., 2018).

Currently, several trials have been conducted to determine the analgesic, anesthetic and antidepressant effect of ketamine; however, the quantification of ketamine in the brain in order to elicit it's antidepressant activity remains elusive (Clements et al., 1982; Rodriguez Rosas et al., 2003; Toki et al., 2018). In addition to several reports that have been published on the IN efficacy of ketamine in the treatment of depression, there have been no studies investigating the effects of the route of administration on drug delivery to the brain. Furthermore, considerable limitations occur in previously reported studies as methods employed for the determination of ketamine are costly and time-consuming; with extensively long analytical run-times (Hasan et al., 2017; Toki et al., 2018). Therefore, the objectives of this study are to develop a rapid and sensitive method using a fused core column for the quantification of ketamine in rat plasma and brain tissue following to determine which route favours drug delivery to the brain.

2. Materials and methods

2.1. Chemicals and reagents

Ketamine HCL solution (100 mg/ml, Fresenius Kabi) and Ketamine D-4 HCL (0.1 mg/ml) (DLD Scientific, Durban, South Africa) were obtained. Ammonium Acetate (10 mM, pH 9.5) and LC-MS grade methanol (MeOH) were all procured from Sigma Aldrich (Steinham, Germany). Analytical Grade formic acid was purchased from Merck Millipore, South Africa. Solid-Phase extraction cartridges were purchased from Supelco-Sigma (St. Louis, MO) Ultrapure water was purified using a Milli-Q water purification system (Bedford, MA, USA) and all solvents were LC-MS grade. All Chemicals utilized in the study were of analytical grade.

2.2. Chromatographic conditions

Separation was performed on an Ascentis® Express Biphenyl column (5 cm x 2.1 i.d., 2.7 µm Particle size) with a Fused-Core® Particle design; and a gradient mobile phase changing from 50% Millipore water (0.1% v/v formic acid) (A) and 50% methanol (B). The flow rate was set at 0.4 mL/min with an injection volume of 5 µL; and a total run time of 7 min.

2.3. Mass spectrometry

An Agilent series 1100 (Agilent technologies, Waldbronn Germany) was coupled to a maXis 4G quadruple-time-of-flight Mass spectrometry instrument (Bruker Daltonics, Bremen, Germany). Results were analyzed on Bruker Data Analysis 4.0 SP 5.

Quantitative studies were conducted using MS via an ESI interface with the following settings: drying temperature, 200 °C; nitrogen nebulizer gas 0.4 bar; end plate offset, −500 V; capillary, 5500 V and drying gas, 4.0 L/min. The collision energy was optimized to 12eV for Ketamine and the IS (ketamine D-4) to achieve the required selectivity and sensitivity for the method. The mass of Ketamine and the IS were m/z 238.1 and 242.1 respectively.

2.4. Animals

All experimental animal procedures involving handling and treatment were approved by the Institutional Animal Research Ethics Committee (AREC, UKZN) (approval Reference: AREC/003/018M). 54 male Sprague-Dawley rats (weighing between 110 and 120 g) were sourced from the University of Kwa-Zulu Natal Biomedical Resource Unit and were used to conduct the study. All animals where housed under appropriate ethical standards in a well-ventilated room with humidity control systems with a 12-h light/dark cycle with the recommended enrichment.

2.5. Drug administration and sample collection

Animals were administered 15 mg/kg body weight of ketamine-HCL via either intranasal, (IN) oral (PO) or intraperitoneal routes, control animals received saline. Animals (n = 3) were euthanized at 0, 5, 15, 30, 60, 120, and 240-min post administration via IsoFor (Safeline Pharmaceuticals, South Africa) overdose. Blood samples were collected via cardiac puncture; and brain tissue surgically removed post termination. Brain tissue samples were frozen gradually with liquid nitrogen vapor; biological samples were stored at −80 °C until analysis.

2.6. Method validation

2.6.1. Calibration and quality control preparation

A primary stock solution of ketamine was prepared (10µg/10 ml) in Ultrapure water and stored at −20 °C. The lower limit of quantification (LLOQ), low quality control (LQC), medium quality control (MQC) and high-quality control (HQC) at 100 ng, 250 ng, 750 ng and 2000 ng/mL were prepared from the stock; and spiked with IS at a concentration level of 750 ng/ml. The calibration curve was prepared in triplicate (n = 3) in a range of 100–2000 ng/mL.

2.6.2. Linearity and lower limit of quantification

The linearity of this method was evaluated by analyzing standard series of samples with concentrations ranging from 100 to 2000 ng/mL of ketamine in plasma and brain homogenates. Ketamine responses were established using internal standard peak area ratio and plotted against the corresponding concentration of ketamine (expressed in ng/

mL). The calibration curves were generated in Bruker Quant Analysis (Bruker Daltonics, Bremen, Germany) using the least-square linear regression method. The benchmark for the calibration range includes, the accuracy and precision of ketamine's calibration to fall within ± 15% deviation (n = 6) as stipulated in the EMA guidelines for Bioanalytical Method Development as well as a correlation coefficient of r ≥ 0.99.

2.6.3. Accuracy, precision and recovery

Four QC levels were analyzed in order to determine the intra- and inter-day accuracy and precision parameters. The satisfactory percentage recovery standards and limit of variation on precision and accuracy should be within 15% of nominal values of the QC samples, LQC, MQC, HQC. In Accordance with the EMA guidelines the LLOQ should fall within 20% of the nominal value (n = 6).

2.6.4. Stability testing

Due to the delay in injection of extracted samples, the stability of ketamine was investigated at the LQC, MQC and HQC under different conditions i.e bench-top stability for 6 h at room temperature, autosampler stability for 24 h and freeze-thaw stability.

2.6.5. Sample preparation and recovery

During sample preparation, 100 µl of biological sample (plasma/brain homogenate) obtained from treated animals were spiked with 7.5 µl of IS. 892,5 µl of Ammonium Acetate (10 mM, pH 9.5) was added to the analyte mixture. The samples were subjected to vigorous mixing by a vortex mixer for 1 min and then centrifuged at 10,000 rpm for 15 min at 4 °C. Solid phase extraction (SPE) was performed using a C_{18}-50 mg, C_{18}-100 mg, Hydrophilic-Lipophilic-Balance and Hybrid Phospholipid Cartridges (HLB) (Sigma Aldrich, Germany). Briefly, cartridges were preconditioned with 1 ml of MeOH and ammonium acetate followed by the sample. The cartridges were washed with ultrapure water and analyte was collected with MeOH in an autosampler vial for analysis. The same procedure was followed in the construction of calibration curves of the biological matrices; in which recoveries were investigated at three-quality control (QC) levels; the low quality control (LQC), middle-quality control (MQC) and high-quality control (HQC). The QC levels were 100, 250, 750, 2000 (ng/ml or ng/g) for plasma and brain homogenate.

3. Results and discussion

This LC-MS method developed in this study was suitable for the quantification of ketamine in rat plasma and brain tissues. Chromatographic separation was achieved with a retention time of 1.0 min for the both the target analyte and the IS at m/z 238.1 and 242.1 respectively; with an optimized collision energy of 12eV for both Ketamine and the IS. Positive ion mode exhibited preferable signalling of the target analyte to that of the negative ion mode. Separation was performed on an Ascentis® Express Biphenyl column (5 cm x 2.1 i.d., 2.7 µm Particle size) with a total run-time of 7 min. A recent study utilized a CHIRAL-AGP® MS (2.0 mm i.d x 15 cm., 5 µm Particle size) column for the chiral separation of ketamine and its enantiomers, however with significantly longer run-time of 25 min (Hasan et al., 2017).

The calibration curves (n = 5) for quantification of ketamine maintained a linear range of 100–2000 ng/mL in both plasma and brain homogenates. The linear equation of ketamine in plasma was y = 0.895679x + 0.324036 with a $R^2 \geq 0.998$; the brain homogenate linear equation was y = 1.058604x + 0.462059 with a $R^2 \geq 0.999$. The lower limit of quantification was 100 ng/mL which was determined by a S/N ≥ 5 and a limit of detection (LOD) of 10 ng/mL for both brain homogenate and plasma.

The inter- and intra-day precision and accuracy in plasma and brain homogenates are presented in Tables 1 and 2. In plasma the intra- and inter-day precision ranged and from 1,05 to 3,74%; whereas in brain

Table 1
Intra- and inter-day precision and accuracy of ketamine in plasma and brain homogenate (n = 3 days).

Plasma-Ketamine				
Quality control levels	LLOQ	LQC	MQC	HQC
Concentration (ngmL^{-1})	**100**	**250**	**750**	2000
Mean	98,45	244,85	721,48	1906,34
Accuracy (%)	98,45	97,94	96,20	95,32
Inter-day precision (R.S.D., %)	2,03	2,05	1,28	2,32
Intra-day precision (R.S.D., %)	1,05	1,35	1,26	3,74
Brain-Ketamine				
Quality control levels	**LLOQ**	**LQC**	**MQC**	**HQC**
Concentration (ngmL^{-1})	**100**	**250**	**750**	2000
Mean	95,94	241,95	730,73	1957,22
Accuracy (%)	95,94	96,78	97,43	97,86
Inter-day precision (R.S.D., %)	0,83	1,41	0,75	1,82
Intra-day precision (R.S.D., %)	2,20	1,35	0,65	1,80

Table 2
Recoveries of Ketamine (n = 3) from plasma and brain homogenate.

Plasma				
Concentration (ngmL^{-1})	100	250	750	2000
	64,85	61,24	58,68	60,07
Recovery (%)	62,69	62,28	59,46	60,06
	65,71	62,90	59,98	59,45
Mean (%)	64,42	62,14	59,37	59,86
S.D. (%)	1,56	0,84	0,66	0,36
R.S.D. (%)	2,42	1,35	1,11	0,60
Brain Homogenate				
Concentration (ngmL^{-1})	**5**	**60**	**500**	**1500**
	62,87	64,75	58,68	60,07
Recovery (%)	64,43	64,68	59,46	60,06
	65,38	64,70	59,98	59,45
Mean (%)	64,23	64,71	59,37	59,86
S.D. (%)	1,27	0,03	0,66	0,36
R.S.D. (%)	1,97	1,35	1,11	0,60

Table 3
Stability of Ketamine HCL in plasma and brain homogenate (n = 3).

Ketamine Concentration ng/mL					
Bench-top stability	added		**250**	**750**	**2000**
	Plasma	Found	241,35	737,06	1912,75
		Accuracy %	96,54	98,27	95,64
		RSD %	2,99	1,51	3,53
	Brain	Found	243,44	714,62	1926,72
		Accuracy %	97,37	96,34	96,34
		RSD %	2,07	3,25	3,25
Freeze-thaw stability	Plasma	Found	241,15	722,36	1896,22
		Accuracy %	96,46	96,31	94,81
		RSD %	3,24	3,30	3,62
	Brain	Found	234,29	715,43	1910,28
		Accuracy %	93,72	95,39	95,51
		RSD %	0,49	4,24	4,05
Autosampler stability	Plasma	Found	238,26	729,92	1895,71
		Accuracy %	95,30	97,32	94,79
		RSD %	0,78	2,47	2,29
	Brain	Found	238,81	712,38	1926,53
		Accuracy %	95,53	94,98	96,33
		RSD %	0,46	0,96	2,34

Table 4
A summary of the pharmacokinetic parameters of Ketamine following a single dose of ketamine of 15 mg/kg b.w

Oral		
	Plasma	Brain
C_{max} (ng/mL or ng/g)	109,11	61,77
T_{max} (min)	5	5
$T_{1/2}$ (min)	2,5	2,35
K_{el}	0,0156	0,0060
$AUC_{0\text{-}inf}$ (ng min/mL or ng min/g)	5436,576	4060,696
IP		
	Plasma	**Brain**
C_{max} ± SD (ng/mL or ng/g)	524,58	354,06
T_{max} (min)	5	5
$T_{1/2}$ (min)	15	2,55
K_{el}	0,0126	0,0137
$AUC_{0\text{-}inf}$ (ng min/mL or ng min/g)	13059,649	10791,611
IN		
	Plasma	**Brain**
C_{max} ± SD (ng/mL or ng/g)	149,29	77,81
T_{max} (min)	15	15
$T_{1/2}$ (min)	2,85	2,6
K_{el}	0,0149	0,0083
$AUC_{0\text{-}inf}$ (ng min/mL or ng min/g)	6785,367	2892,804

homogenate it ranged from 0,65 to 2,20% with RSD values within the acceptable limits outlined by the EMA, with satisfactory percentage recovery standards and limit of variation on precision and accuracy falling within 15% of nominal values of the QC samples, LQC, MQC, HQC and the LLOQ falling within 20% of the nominal value.

The autosampler, freeze-thaw and bench-top stability of Ketamine was evaluated at the LQC, MQC and HQC levels shown in Table 3. The results exhibit that the stability and recovery found in brain homogenate and plasma were within a range of 93,72–98,27% with a RSD of 0,49–4,24% for the evaluated conditions (see Table 4).

Post IN, PO and IP administration of ketamine-HCL (15 mg/kg body weight), the pharmacokinetic parameters in plasma and brain homogenates were determined (Table 1) and Figs. 2. The pharmacokinetic parameters showed a C_{max} of 524,58; 121,07 and 109,11 ng/mL and an $AUC_{0\text{-}inf}$ of 13059,65; 6785,37 and 5436,58 in plasma within following IP, IN and PO administration respectively (Fig. 1).

The purpose of this study was to compare the plasma and brain concentrations of ketamine following various routes of administration. The pharmacokinetic parameters showed a C_{max} of 352,06; 77,81 and 61,77 ng/g and an $AUC_{0\text{-}inf}$ of 10791,61; 2892,80 and 4060,70 in brain homogenates following IP, IN. and PO administration, respectively (Fig. 2). Ketamine's concentration in brain homogenate following oral and IN administration were below the limit of quantification of 100 ng/mL.

Pharmacokinetic profiles of ketamine demonstrated that absorption is rapid in both plasma and brain homogenates with IP administration producing significantly higher plasma and brain concentrations to that of PO and IN administration. Oral bioavailability of ketamine is low due to extensive first-pass metabolism in the liver and intestine which can be compared to previous studies (Hasan et al., 2017; Clements et al., 1982), therefore this effect can be largely responsible for the low levels of ketamine observed in plasma and brain samples. Malinovsky et al. (1996) found that ketamine's bioavailability in children was approximately 50% in plasma post IN administration and; therefore, the lowered ketamine levels following IN administration could be attributed to its moderate bioavailability (Malinovsky et al., 1996). This effect may be partly caused by substantial swallowing of relatively large intranasal depositions reducing its concentration in both the brain and plasma (World Health Organization, 2015). The C_{max} reached was higher and reached quickly in both plasma and brain followed by a rapid decline until undetectable levels of ketamine were present 120 min post-administration.

4. Conclusion

We developed a novel rapid and sensitive LC-MS method for the quantification of ketamine in rat plasma and brain homogenates in order to understand the effect of route of administration on the brain distribution of ketamine for the treatment of major depressive disorder. We demonstrate that IN administration of ketamine is not be the ideal route of administration for drug delivery to the brain. However, future

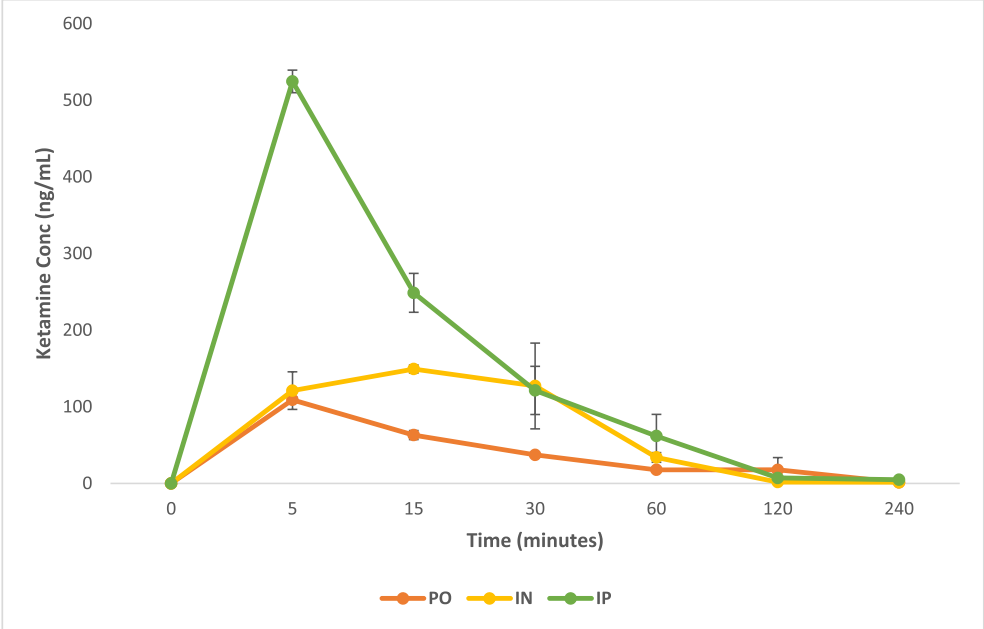

Fig. 1. Concentration-time profile of Ketamine in plasma (ng/mL) following a single dose of 15 mg/kg to male Sprague Dawley rats via PO, IP., and IN administration (n = 3, mean ± SD).

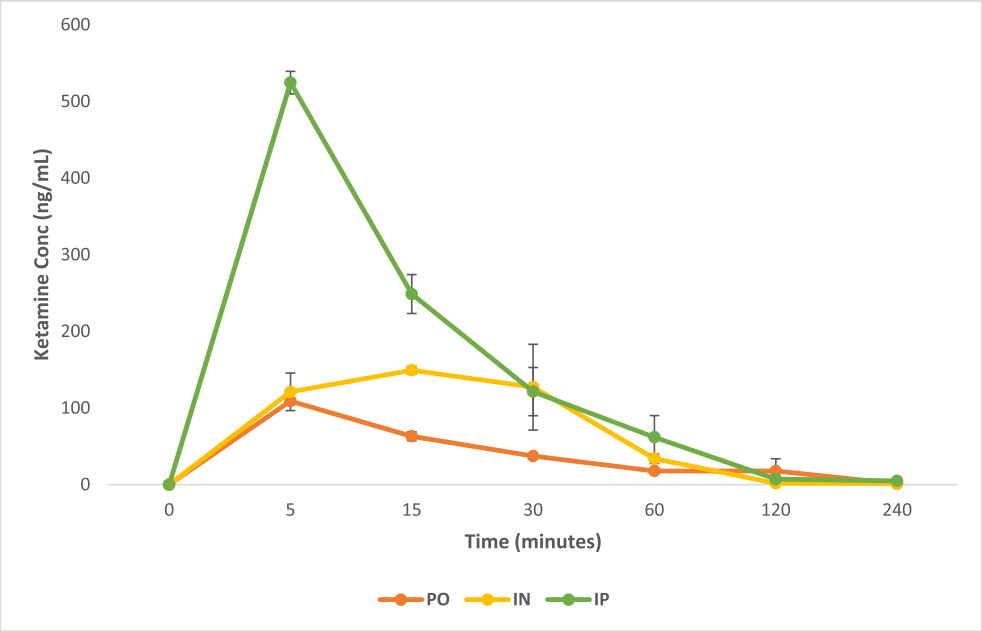

Fig. 2. Concentration-time profile of ketamine in brain tissue (ng/g) following a single dose of 15 mg/kg to male Sprague dawley rats via PO, IP, IN administration (n = 3, mean ± SD).

studies into the development of novel technologies that enhance its bioavailability may result in an easy to use IN formulation of ketamine for the treatment of MDD and TRD. Currently, parenteral administration of Ketamine exhibits more favorable brain drug delivery in order to produce satisfactory therapeutic outcomes when compared to IN and PO administration. Future investigations utilizing brain tissue imaging are required in determining the brain distributions of Ketamine and Norketamine and their benefits in the treatment of MDD.

Declaration of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.02.003.

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