Population Pharmacokinetics and Pharmacodynamics of the Therapeutic and Adverse Effects of Ketamine in Patients With Treatment-Refractory Depression

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We aimed to develop population pharmacokinetic/pharmacodynamic (PK/PD) models that can effectively describe ketamine and norketamine PK/PD relationships for Montgomery–Åsberg Depression Rating Scale (MADRS) scores, blood pressure (BP), and heart rate (HR) following i.v., s.c., and i.m. ketamine administration in patients with treatment-refractory depression. Ketamine PK/PD data were collected from 21 treatment-refractory depressed participants who received ketamine (dose titration 0.1–0.5 mg/kg as single doses) by i.v., s.c., or i.m. administration. Model development used nonlinear mixed effect modeling. Ketamine and norketamine PK were best described using two-compartment models with first-order absorption after s.c. and i.m. administration. Estimated ketamine bioavailability after i.m. and s.c. was ~64% with indistinguishable first-order absorption rate constants. Allometric scaling of body weight on all clearance and volumes of distribution improved the model fit. The delay in the concentration-response relationship for MADRS scores was best described using a turnover model (turnover time ~42 hours), whereas for the BP and HR rates this was an immediate effect model. For all PD effects, ketamine alone was superior to models with norketamine concentration linked to an effect. No covariates were identified for PD effects. The estimated half-maximal effective concentration from the MADRS score, BP, and HR were 0.44, 468, and 7,580 ng/mL, respectively. The integrated population models were able to effectively describe the PK/PD relationships for MADRS scores, BP, and HR after i.v., s.c., and i.m. ketamine administration. These findings allow for a deeper understanding of the complex relationships between route of ketamine administration and clinical response and safety.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Several trials have suggested that a subanaesthetic dose of ketamine could provide a significant antidepressant effect in patients with depression. However, there has been no population pharmacodynamic studies, and more importantly no PK/PD studies that describe and quantify the concertation-response relationships for the therapeutics and adverse effects of ketamine in treatment-refractory depression.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ The present analysis used trial data to characterize the population PK/PD relationships for the effect of ketamine on the Montgomery–Åsberg Depression Rating Scale (MADRS scores) and cardiovascular side effects of blood pressure and heart rate in patients with treatment-refractory depression.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ The results of this study indicate that the antidepressant effects of single doses of ketamine are slower in onset than its cardiovascular effects, but in contrast has a relatively long duration of action and greater potency. Thus, the antidepressant effects are more complex than the direct effects on heart rate and blood pressure.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ It is possible to model the PK/PD of ketamine in patients with treatment-resistant depression incorporating efficacy and acute adverse effects and through simulation to potentially develop alternative dose regimens and/or non parenteral formulations for efficacy optimisation.

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Major depressive disorders present a significant clinical challenge with current antidepressants achieving remission in only ~ 30% of patients.[1] Multiple trials have suggested that a subanesthetic dose of ketamine could provide a significant antidepressant effect in patients with depression.[2–4] Many studies of racemic ketamine used a fixed i.v. dose of 0.5 mg/kg given over 40 minutes, which involves some procedural complexity and requirements for medical monitoring. There have been few pharmacokinetic (PK) studies on ketamine in this therapeutic area and only a single population PK study, and in only nine patients, each receiving a single treatment of ketamine, in which several metabolites apart from ketamine enantiomers were quantified.[5] There have been no population pharmacodynamic (PD) studies, and more importantly no PK/PD studies. In addition, ketamine causes several readily quantifiable acute adverse effects, such as elevated blood pressure (BP) and heart rate (HR).[6] A recently reported dose-titration pilot study evaluated low doses of ketamine administered across multiple routes of administration (i.v., s.c. and, i.m.) in patients with treatment-refractory depression with each patient receiving multiple ketamine doses in a dose-titration protocol.[7] The present analysis uses the data reported by Loo et al.,[7] which included a small i.v. pilot study by Lai et al.[8] to characterize the population PK/PD relationships of ketamine on the Montgomery–Åsberg Depression Rating Scale (MADRS) scores and cardiovascular side effects of BP and HR.

The objectives of this analysis were to:

- Develop population PK/PD models that can describe ketamine and norketamine PK/PD relationships for MADRS scores, BP and HR after i.v., s.c., and i.m. administration of ketamine in patients with treatment-refractory depression.
- Identify covariates that are predictive for the PK/PD of ketamine.
- Use the model to examine the impact of dosage regimen on MADRS scores, BP, and HR.

METHODS

Patients and study design
PK/PD data were collected from a double-blind, controlled, crossover study involving 82 treatment sessions in 21 treatment-refractory depressed participants. For the first four participants, saline was given in the control condition.[8] This was changed to midazolam as an active comparator (n = 17, data from the first 15 participants reported in the Loo et al.[7] study). Ketamine was given at a range of doses to each participant using a dose titration approach (0.1–0.5 mg/kg) or matching volume of control drug (saline or midazolam) by one of three routes of drug administration (i.v., s.c., and i.m.) in a multiple crossover design.[7] Doses were separated by at least a week, or longer if required for MADRS scores to return to predosing criteria (MADRS score ≥ 20).[7,8] Ethics approval was obtained from the University of New South Wales Human Research Ethics Committee (Approval Number 10409).

Assessments
For all 21 subjects, the MADRS score was used to assess study entry eligibility and response to treatment, evaluated at baseline in the hour prior to each ketamine treatment (day 1) and at 4 hours, days 2, 4, and 7 after each treatment. BP and HR were measured before each treatment and at 5, 10, 30, 60, and 240 minutes after treatment. For all patients, CYP2B6 genotyping was conducted to assess *4–*8 and *13, as previously described.[9]

Blood sampling and analytical method
Venous blood samples were collected 5 minutes after the end of i.v. injection and 15 minutes after i.m. and s.c. injection, and then at 30 minutes, at 2 hours and up to 4 hours after injection for all routes. The blood was centrifuged and stored at −20 degrees prior to analysis. Ketamine and norketamine were assayed by the liquid–liquid extraction and liquid chromatography mass spectrometry (LCMS) method described previously.[7] The lower limit of quantification (LLOQ) was 0.25 ng/mL.

Software
Modeling was performed using a Dell Power Edge R910 server with 4 × 10 core Xeon 2.26 Ghz processors running Windows Server 2008 R2 Enterprise 64-bit. Model development used nonlinear mixed effect modeling using NONMEM (version 7.3; ICON Development Solutions, Ellicott City, MD)[10] with the Wings for NONMEM (version 7.3) interface (http://wfn.sourceforge.net) and IFort compiler. Processing NONMEM output and generating plots was conducted with R Software version 3.1.1 or later[11] using ggplot2, plyr, and scales packages[12–14] and associated dependencies.

General modeling approach
The PK/PD models were coded using the ADVAN13 subroutines of NONMEM and fitted to the data using the first-order conditional estimation with interaction method. Population parameter variability was modeled using a log-normal distribution. Residual variability was modeled using a combined additive and proportional error model but reduced to either additive or proportional error model if its removal did not significantly affect the overall model fit as judged by the model selection criteria. Model selection criteria were guided by mechanistic plausibility, precision of parameter estimates (%RSE < 30% and 50% for fixed and random effects, respectively), the Akaike Information Criterion (AIC) for non-nested models, the minimum objective function value, and visual inspection of standard diagnostic plots.[15]

Model development was conducted in a step-wise manner using the using the sequential "PPP&D" two-stage approach.[16] Population PK models for ketamine, and then norketamine, were first established and then used for subsequent PD modeling of the MADRS, BP, and HR data. Potential significant covariates on all PK and PD (MADRS, HR, and BP) parameters were identified using plots of covariates vs. ETAs of parameter estimates. The available covariates were body weight, sex, and CYP2B6 genotype. Route of administration was investigated as a structural covariate during PK modeling for the impact on bioavailability and absorption rate constants, whereas for PD modeling this was tested for any additional impact on PD model parameters. The influence of body weight on ketamine and norketamine PK was considered via allometric scaling, referenced to standard 70 kg, with clearances scaled to the exponent of 0.75 and volume to 1.[17] Categorical covariates were modeled with a proportional function and continuous covariates using a power function, referenced to a standard value.[15] Covariates were evaluated using a stepwise forward addition and backward elimination process,[18] with the statistical criteria of P < 0.05 during forward addition and P < 0.01 for backward elimination.

Five hundred bootstrap runs were conducted to assess the precision of final models' parameter estimates. The final PK/PD models were evaluated by constructing prediction-corrected visual predictive checks (pcVPC)[19] of 1,000 versions of the original index dataset. The observed and simulated PK/PD data were binned by time intervals based on nominal times to minimize the influence of regions of sparse data.

Pharmacokinetic modeling
Standard one-, two-, and three-compartment models, with zero-order, first-order, and transit absorption models after i.m. and s.c. ketamine, were evaluated. Between-occasion variability (BOV) on PK model parameters was investigated. As the fraction of ketamine metabolized to norketamine was not able to be estimated from the data, the metabolic

conversion ratio (f_m) was assumed to be one. The clearance and volume parameters for the norketamine PK model were, therefore, "apparent" values scaled f_m (e.g., $CL_{norketamine}/f_m$).

Pharmacodynamic modeling

MADRS scores were used as the PD metric for the antidepressant effect of ketamine, whereas BP and HR data were used as PD metrics for ketamine cardiovascular side effects. The distributions of the baseline PD parameters were assumed to have a log-normal distribution and were tested with or without between-subject variability (BSV) and BOV. The ketamine concentration-response relationship for MADRS scores, BP, and HR were modeled separately using a Sigmoid maximum effect (E_{max}) model after establishing ketamine and norketamine PK models. For all PD effects, models of ketamine or norketamine concentration were linked to the effect using direct effect, effect-compartment and turnover models.[20] All models were tested with the drug effect as either additive or proportional to the baseline value of the PD metric.

For example, the temporal delay in MADRS score changes relative to plasma concentrations was described using a turnover model with ketamine concentrations reducing the MADRS scores via an E_{max} model proportional to the baseline score. The turnover model, describing the delay in drug effect when the delay is due to a turnover of a physiological mediator, was defined by two relationships; the baseline MADRS score and the turnover time of the system. The net baseline MADRS score ($MADRS_{base}$) was represented as the ratio between the rate of production of MADRS score (i.e., the process promoting higher depression scores) a process represented by first-order rate constant K_{in}, and the rate of removal of MADRS score (i.e., the process promoting lowering of depression scores), a process represented by first-order rate constant K_{out}. The turnover time (TURNOVER) of the system was represented as the inverse of K_{out}. The system of equations describing the turnover model for ketamine effect on MADRS scores is represented in the equations below.

$$MADRS_{base} = \frac{K_{in}}{K_{out}}$$

$$TURNOVER = \frac{1}{K_{out}}$$

$$K_{in0} = E_{base} \cdot K_{out}$$

$$E_{drug} = \frac{E_{max} \times C}{(EC_{50} + C)}$$

$$K_{in} = K_{in0} \cdot \left(1 - E_{drug}\right)$$

$$\frac{d(MADRS)}{dt} = K_{in0} - K_{out} \cdot E_{drug}$$

where K_{in0} is the baseline value of the K_{in}, C is plasma ketamine concentrations, EC_{50} is the plasma ketamine concentration at half maximum effect, E_{drug} is the drug effect (E_{max} fixed to 1 for MADRS), E_{base} is the baseline *MADRS* score, and $d(MADRS)/dt$ is the rate of change of the MADRS score.

Dosing regimen simulations

The final PK/PD models were used to simulate efficacy data from a double-blind randomized placebo-controlled clinical trial in patients with treatment-resistant depression[21] in which 0.5 mg/kg was infused i.v. over 40 minutes for 3 times weekly (days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, and 26) or twice weekly (days 1, 4, 8, 11, 15, 18, 22, and 25) with efficacy metrics (1. percent change in MADRS from baseline; 2. response: percent of patients achieving a 50% reduction from baseline MADRS; 3. remission: percent of patients achieving a MADRS score of ≤ 10) all on day 15. We also compared peak plasma ketamine concentrations as well as the maximum percent increase in BP and HR from baseline. These were calculated based on 1,000 simulated subjects with the same average weights as the clinical trial participants. A similar simulation of once daily s.c. dosing (0.1, 0.3 and 0.5 mg/kg) for 7 days was performed to explore the dose–response relationship at lower more frequent doses using the least invasive route of administration in the model.

RESULTS

Study population and PK/PD data

PK/PD data used in this analysis were based on data collected from 61 treatment sessions in 21 subjects. The overall demographic and CYP2B6 genetic data are presented in **Table 1**. The number of observations per observed PK/PD variable and the average number of observations per subject is provided in **Table S1**. The evaluation times for each observed variable is provided in **Table S2**.

Overall, 14% of plasma ketamine and norketamine concentration data were missing. These were primarily due to concentrations being below the LLOQ and occurred primarily at the end of the observation period (all >50 hours postdose). Models accounting for LLOQ censored data using the YLO and M3 Methods[22] were investigated and were found to be characterized by unreliable minimization and covariate step status. No impact on parameter estimates was demonstrated as there were sufficient data points in the elimination phase of ketamine and norketamine. These below limit of quantification (BLOQ) samples were therefore excluded from the data set (M1 method).

Pharmacokinetic model

A schematic representation of the final PK/PD models of ketamine is presented in **Figure 1**. The concentration-time data for ketamine and norketamine were best described using a two-compartment model with first-order absorption for both the s.c. and i.m. routes of administration. Transit compartment models for norketamine formation were investigated and did not result in an improved fit for norketamine concentration-time data. Attempts to fit a three-compartment model to the data resulted in unstable convergence and poorly estimated parameters, which were not alleviated when fixing the small initial compartment to values from the literature.[23] When modeled separately, there were minor differences between the absorption rate constant (K_a) for the s.c. and i.m. routes,

Table 1 Participants demographic summary

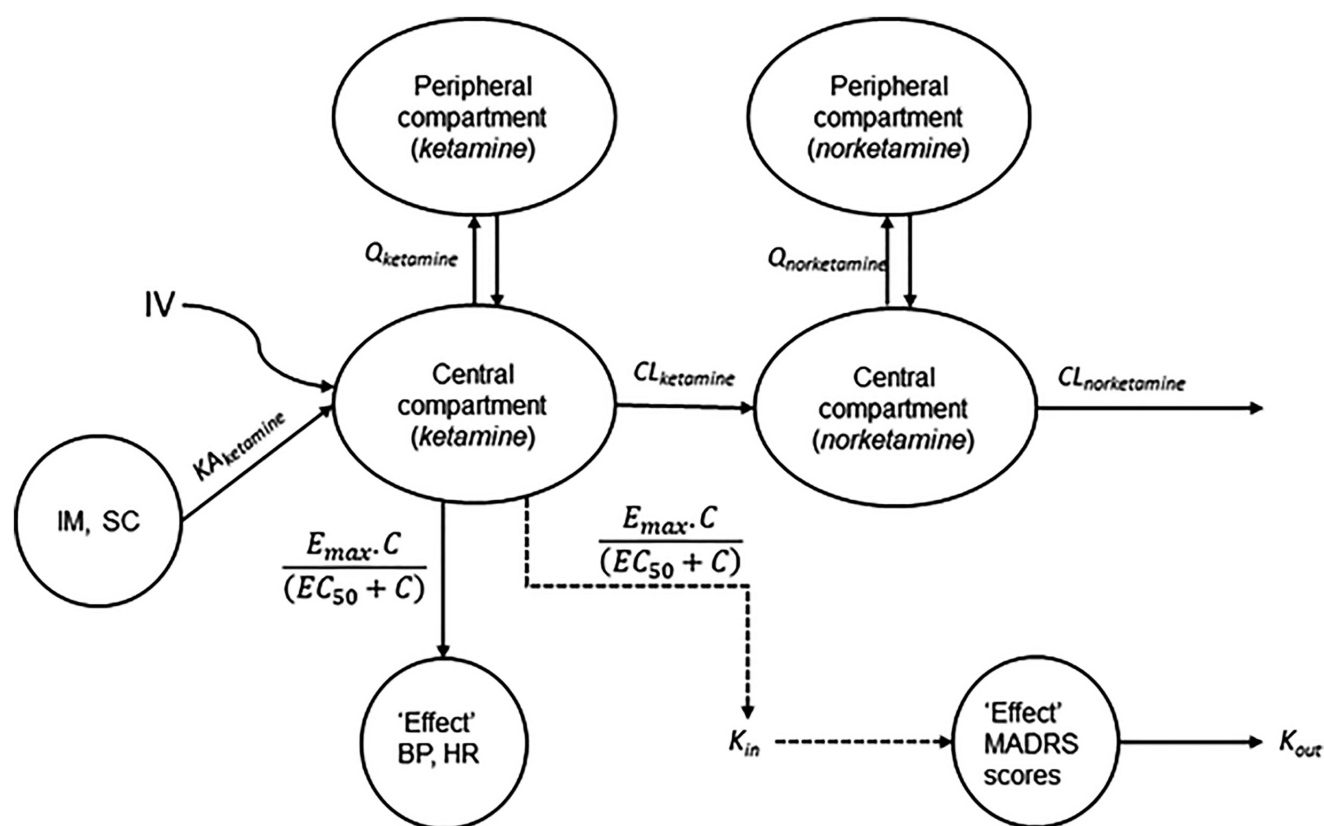| Covariate	n	Value
Sex, Female:Male	21	7:14
Age, y	21	49.5 (50, 29–69)*
Genotype (*6/*6:*1/*6,*5:*1/*1)	21	2:8:11

Mean (median, range).

Figure 1 Schematic representation of the pharmacokinetic and pharmacodynamic models of ketamine and norketamine. Q, intercompartmental clearance; KA, absorption rate constant; CL, systemic clearance; E_{max}, maximum drug effect on MADRS, blood pressure or heart rate; C, ketamine plasma concentration; EC_{50}, plasma ketamine concentration at half maximum effect; K_{in}, K_{out}, first-order rate constants for the production and removal of an effect, respectively; BP, blood pressure, HR, heart rate.

and estimation of a common K_a did not worsen the model fit (dOBJ < 2). Estimating the bioavailability of the s.c. and i.m. routes improved model fit in terms of diagnostics plots and reduced the OBJ by > 10 units. However, estimation of a common bioavailability did not worsen the model fit (dOBJ < 3). Thus, the final PK model estimated the absolute bioavailability of ketamine after i.m. and s.c. to be 64.4% with an absorption terminal half-life ($t_{1/2}$) of 6.4 minutes. The population parameter variability (PPV) was only supported on the CL, V1, and V2 of ketamine and CL/f_m and V1/f_m of norketamine. A proportional error model was used for ketamine concentrations while the norketamine model retained both a proportional and an additive residual error term. Models of BOV on PK parameters did not improve the model fit and resulted in either unsuccessful minimization or highly imprecise parameter estimates. In addition, models with BOV were characterized by low P values of random effects, which indicated a biased distribution of the Empirical Bayes Estimates (EBE-etas).

Allometric scaling on all clearance and volume of distribution parameters for both ketamine and norketamine significantly improved the model fit (dOBJ > 30) and increased parameter precision. Sex and *CYP2B6* genotype were tested as possible covariates but did not result in a significant improvement of the PK model fit.

The parameter estimates for the final PK model for ketamine and norketamine are presented in **Table 2** and show that all fixed

and random effects parameters were estimated precisely with acceptable standard errors (fixed effects % RSE < 30%, random effects % RSE < 50). Standard goodness-of-fit plots for the final ketamine and norketamine PK models (**Figures S1, S2**) demonstrate that data were adequately described by the model.

The pcVPC plots showed that the final ketamine PK model had a good predictive performance for the observed ketamine concentrations as represented by the good overlay of the median and 5th percentiles and 95th percentiles of the observed and simulated concentrations (**Figure 2a**). Similar pcVPC plots were obtained when ketamine data were stratified to the route of administration (data not shown). However, the final model tended to slightly underpredict norketamine concentrations, although the observed median, 5th, and 95th percentiles remained within the corresponding 90% confidence intervals (CIs) of the prediction intervals of the simulated data (**Figure 2b**). However, the latter may be due to the high BSV in norketamine kinetics and the amount of available data informing the variability in this analysis is limited.

Pharmacodynamic models
For all PD effects, models of ketamine alone were superior to models with norketamine concentration linked to effect. For the ketamine PD effects on MADRS scores, the turnover model was significantly better than either a direct- (unsuccessfully terminated) or effect-compartment model (delta OBJ −434). The

Table 2 Population parameter estimates for the final pharmacokinetic model of ketamine and norketamine

Code	Description	Unit	Pop value[a]	%RSE	BSV (%CV)	%RSE	ETA P value	Shrinkage, %
$CL_{ketamine}$	Central clearance of ketamine	L/h	69.6	10.5	18.5	11.1	0.356	9.7
$V1_{ketamine}$	Central volume of distribution of ketamine	L	79.3	18.7	46.8	15.1	0.846	7.1
$Q_{ketamine}$	Intercompartmental clearance of ketamine	L/h	121	14.3				
$V2_{ketamine}$	Peripheral volume of distribution of ketamine	L	87.4	14.8	42.7	23.7	0.672	22.2
$KA_{ketamine}$	Absorption rate constant	h^{-1}	6.53	19.1				
$F_{extravascular}$	Bioavailability from subcutaneous and intramuscular routes	Unitless	0.644	11.5	—	—	—	—
$CL_{norketamine}/f_m$	Central clearance of norketamine	L/h	30.3	31.2	65.1	19.1	0.476	16.3
$V1_{norketamine}/f_m$	Central volume of distribution of norketamine	L	73.6	12.4	46.6	22.4	0.811	16.2
$Q_{norketamine}/f_m$	Intercompartmental clearance of norketamine	L/h	54.2	11.3				
$V2_{norketamine}/f_m$	Peripheral volume of distribution of norketamine	L	339	34.2				
RUVPROP-ketamine	Proportional residual error	Ratio	0.294	9.6	—		—	—
RUVADD-ketamine	Additive residual error of ketamine	ng/mL	0.00001 Fix	—	—		—	—
RUVPROP-norketamine	Proportional residual error	Ratio	0.257	18.6	—		—	—
RUVADD-norketamine	Additive residual error of norketamine	ng/mL	3.88	14.7	—		—	—

BSV, between-subject variability; %CV, percent coefficient of variation; ETA P value, a reported P value to assess whether the *post hoc* individual parameters are centered on the population estimate for that parameter; Pop, population; %RSE, percent of relative standard error.
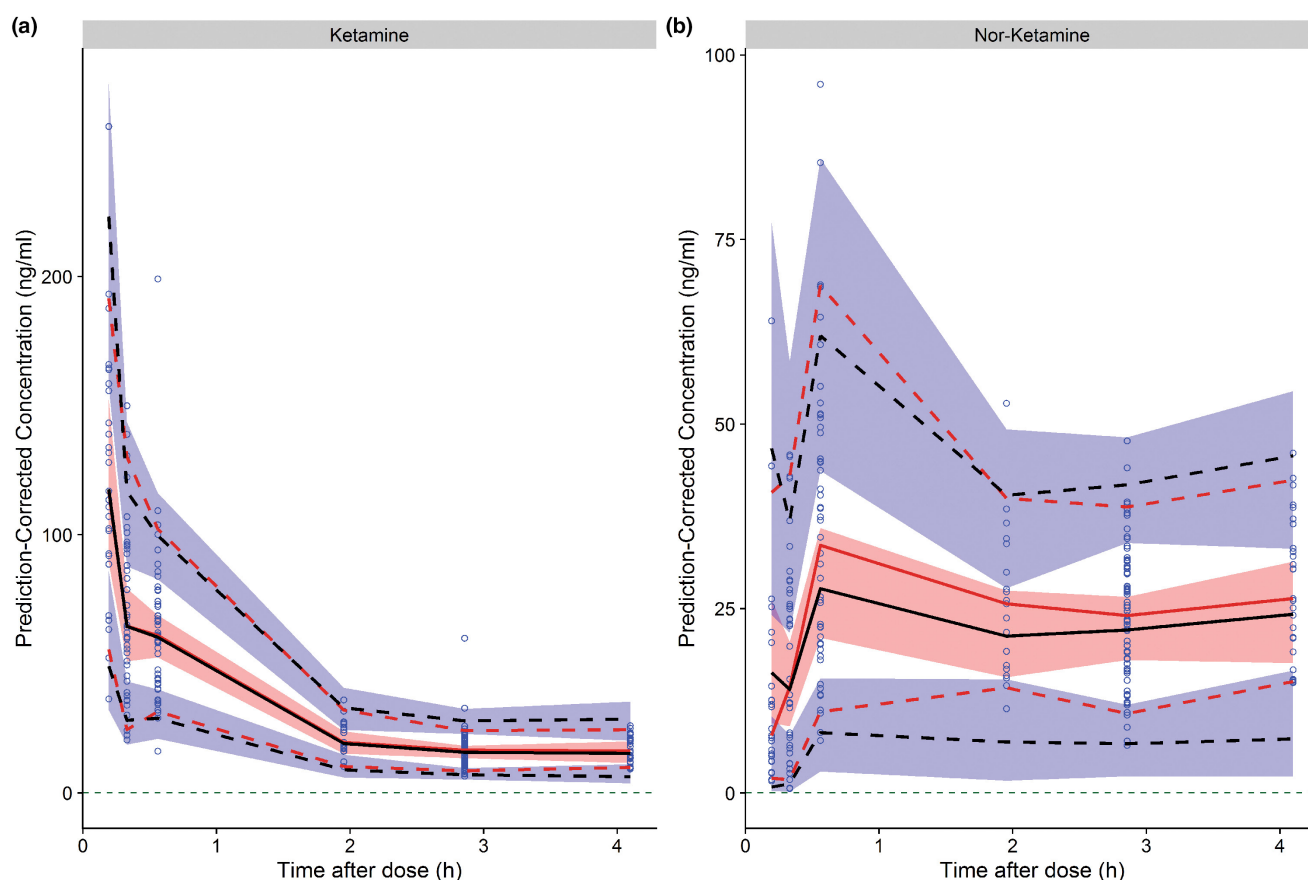[a]Population typical value.

Figure 2 Prediction-corrected visual predictive check of ketamine (**a**) and norketamine (**b**) pharmacokinetic models. Open circles represent observed concentrations. The shaded areas represent the 90% confidence interval of the 5th, 50th, and 95th percentiles of the simulated concentrations. The solid red line represents the median of the observed concentrations. The dashed red and black lines represent the 5th and 95th percentiles of the observed and simulated concentrations, respectively. The horizontal dotted lines represent the lower limit of quantification (LLOQ).

temporal delay in MADRS score changes relative to changes in plasma concentrations was best described using the turnover model with ketamine concentrations reducing the MADRS scores via an E_{max} model proportional to the baseline score (**Figure 1**). The model only supported BSV on the baseline score and turnover time parameters. Adding BOV on PD model parameters did not improve the model fit. The population estimate of the turnover time in the final model was 42.1 hours and was highly variable between subjects (95% CI: 9.17–143 hours). The population estimate for EC_{50} was 0.44 ng/mL. None of the available covariates were found to be significant in the final MADRS PD model.

The ketamine PD effects on BP and HR were best described by an immediate effect, additive E_{max} model (**Figure 1**). Both BP and HR PD models supported BSV on the baseline and EC_{50} parameters and only the BP PD model supported estimation of a Hill coefficient. Estimation E_{max} for the HR PD model resulted in implausible (> 220 bpm) parameter estimates, and, therefore, E_{max} was fixed to 220 bpm. The population estimate of EC_{50} for BP and HR were 468 ng/mL, and 7,580 ng/mL, respectively. None of the available covariates were found to be significant in either of the PD models.

The population parameter estimates and bootstrap mean and 95% CIs for the final PD models for MADRS scores, BP, and HR

rate are presented in **Table 3**. All parameter estimates from the final model fell within the bootstrap CIs. Goodness-of-fit plots for MADRS score are presented in **Figure S3** and for BP and HR in **Figures S4 and S5**, and show no major systematic bias.

The pcVPC plots showed that the MADRS, BP, and PD models had good predictive performance as represented by the acceptable overlay of the median and 5th percentiles and 95th percentiles of the observed and simulated concentrations (**Figure 3a,b**). However, the HR model tends to consistently overpredict the observed HR data (**Figure 3c**), although the percentiles of the observed data are within the 90% CIs of the corresponding prediction intervals.

Dosing regimen simulations

Singh *et al.* reported maximum plasma ketamine concentrations (C_{max}) of 219 ± 34 (mean ± SD: $n = 14$) for twice weekly and 189 ± 74 ($n = 15$) ng/mL for 3 times weekly ketamine. The model predicted C_{max} concentrations were 230 ng/mL for both regimens. The predicted median percent decrease in MADRS from baseline to day 15 was 11.5% (twice weekly) and 18.3% (thrice weekly) compared to Singh *et al.*[21] of 18% and 16%, respectively (**Figures S6, S7**). For response (percent of patients with 50% reduction from baseline MADRS), the values differed substantially

Table 3 Population parameter estimates for the final pharmacodynamic models and bootstrap mean (95% CIs) from bootstrap analyses

Code	Description	MADRS scores Pop value[a]	MADRS scores Bootstrap mean (95% CI)	BP Pop value[a]	BP Bootstrap mean (95% CI)	HR Pop value[a]	HR Bootstrap mean (95% CI)
BASE	Baseline MADRS, BP, or HR	22.3	22.3 (20.2–25.1)	97.1	96.9 (92.2–102)	72.7	72.6 (67–78)
EC_{50}	Ketamine concentration at half maximum effect (ng/mL)	0.439	0.40 (0.028–0.70)	468	542 (192–896)	7,580	6,820 (2198–8,990)
E_{max}	Maximum drug effect	1 Fix	—	51.6	61.4 (15.1–119)	220 Fix	—
HILL	Hill coefficient	1 Fix	—	2.04	2.17 (1.37–3.45)	1 Fix	—
TURN	Turnover time (h)	42.1	46.1 (22.0–82.8)	10.3	10 (7–13)	15.2	14.4 (10–19)
PPV_BASE	Population parameter variability on baseline (%CV)	27.7	26 (15–36)	—	—	156.0	135 (2–204)
PPV_EC50	Population parameter variability on EC_{50} (%CV)	—	—	60.0	56 (1–147)	—	—
PPV_TURN	Population parameter variability on turnover time (%CV)	91.7	85 (0.90–145)	—	—	—	—
RUV_ADDPD	Additive residual error	5.15	5.16 (4.30–6.02)	6.98	6.9 (5.65–8.25)	9.15	9.13 (6.25–12.7)
RUV_PROPPD	Proportional residual error model (ratio)	0.0001 Fix	—	0.0001 Fix	—	0.0001 Fix	—

Units for BP, HR, and ketamine concentration are mm Hg, beats/min, and ng/mL, respectively.
BP, blood pressure; CI, confidence interval; %CV, percent coefficient of variation; HR, heart rate; MADRS, Montgomery–Åsberg Depression Rating Scale; Pop, population.
[a]Population typical value from the original fit to the data.

(Singh et al.[21]: twice weekly 61%, thrice weekly 41%; predictions were 16.5 and 25%, respectively) and remission (MADRS <= 10; Singh et al.[21]: twice weekly 33%, thrice weekly 18%; predictions were 18 and 26%, respectively) For both simulated dosing regimens, the predicted maximum percent increases in HR and BP were ≤6.5 and 10%, respectively.

DISCUSSION

Population PK/PD models were developed to describe ketamine and norketamine concentration-response relationships for MADRS scores, BP, and HR in patients receiving ketamine across multiple routes of administration (i.v., i.m., and s.c.) over a fivefold range of doses in the context of treatment-refractory depression. To our knowledge, this paper is the first to describe the PK/PD effects of ketamine in patients with treatment-refractory depression using the population approach.

The PK of ketamine and norketamine were best described by 2-compartment linear models and the model estimated ketamine absolute bioavailability after s.c. and i.m. injection to be ~64%. This agrees with studies that reported a 2-compartment model to be the best fit for observed ketamine concentrations in both children and adults.[24–27] A previous study reported an absolute bioavailability of 93% after i.m. administration.[25] However, the latter study was performed in a small number of subjects (6 adults) and bioavailability was estimated using the traditional noncompartmental approach after i.m. and i.v. administration. In a larger population-based modeling approach study, the reported bioavailability after i.m. administration in children was 41%.[24] This and our estimated value of ~64% reflects the current dosing recommendation of i.m. ketamine for induction of anesthesia whereby the ketamine dose after i.m. administration is ~2–6 times higher than the recommended i.v. dose for adults (1–4.5 mg/kg i.v. vs. 6.5–13 mg/kg i.m. dose).[28]

A relatively high BSV was associated with the ketamine central volume of distribution of (BSV 45%) and norketamine central clearance (BSV 65%) and volume of distribution (BSV 46%); however, no covariates could be identified to explain the variability other than allometric scaling of body weight. Our study population was homogenous for age but with a small number of subjects which may not be representative of the between-subject variability across the wider population (e.g., only two subjects had CYP2B6*6/*6 genotype), making the identification of covariates difficult to detect. A larger and more heterogeneous population is needed to identify additional factors affecting the PK of ketamine and norketamine.

The active placebo data were not included in the PK/PD modeling of ketamine. The active placebo used in the study is not a placebo in the traditional sense as it does not necessarily represent the true trajectory of depression (i.e., as per "treatment as usual" in the absence of ketamine, as midazolam is not a treatment for depression). It was used to facilitate blinding, and minimize treatment expectancy effects for the evaluation of ketamine effects. Therefore, a placebo-response submodel was not included in the modeling as the main purpose of this work was to describe the concentration-response relationship for MADRS score, BP, and HR when a dose of ketamine is administered.
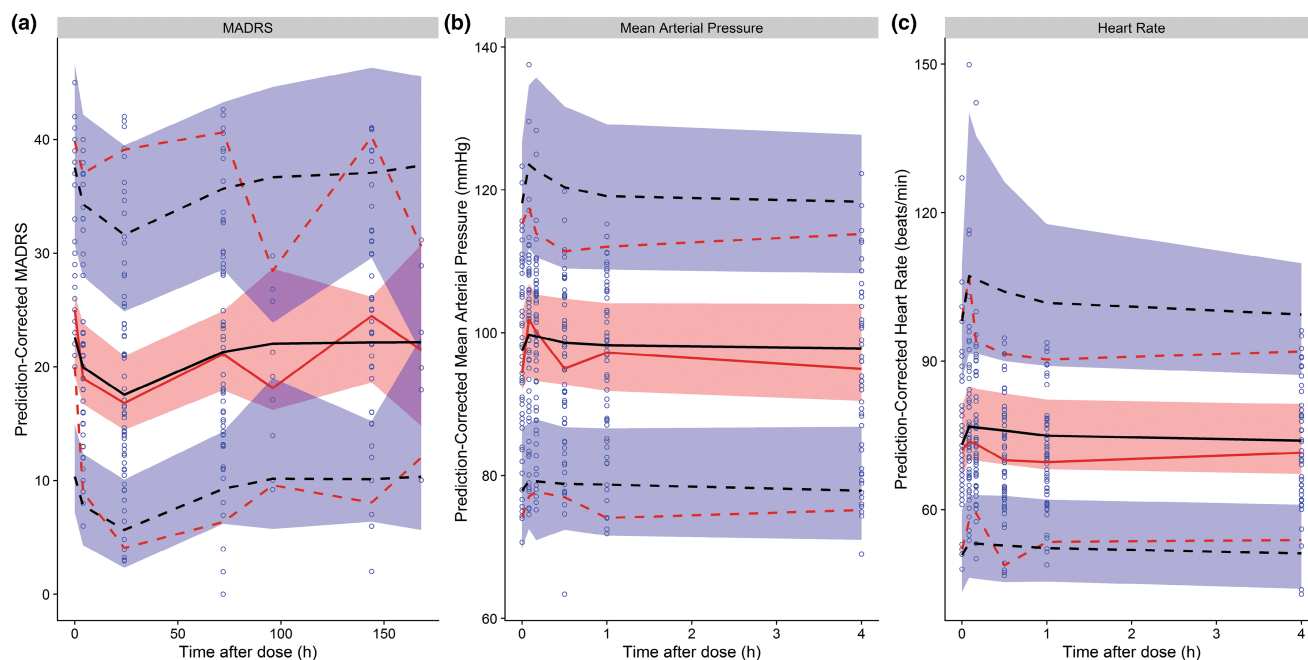
Figure 3 Prediction-corrected visual predictive check of MADRS scores (**a**), blood pressure (**b**) and heart rate (**c**) pharmacodynamic models. Open circles represent observed data. The shaded areas represent the 90% confidence interval of the 5th, 50th, and 95th percentiles of the simulated values. The solid red line represents the median of the observed values. The dashed red and black lines represent the 5th and 95th percentiles of the observed, and simulated values, respectively. MADRS, Montgomery–Åsberg Depression Rating Scale.

The PK/PD modeling showed that models of ketamine alone were superior to models with norketamine concentration linked to an effect. In a previous study of the effects of S-ketamine on cardiac output and experimental pain, Sigtermans *et al.* showed that S-norketamine made no significant contribution to the overall effects; likely due to the lower potency of S-norketamine compared to parent drug, and ketamine doses used were probably too low to cause a significant analgesic effect.[29] Similarly, Glue *et al.* demonstrated that ketamine concentrations were correlated with changes in BP and HR in patients receiving ascending ketamine doses (0.25, 0.5, and 1 mg/kg) for treatment-resistant generalized anxiety and social anxiety disorders.[30] The current analysis demonstrated that ketamine has an immediate and direct effect on HR and BP with no delay, in agreement with Sigtermans *et al.* which demonstrated an effect on cardiac output with no delay (reported EC_{20} 134 ng/mL; $EC_{50} \sim 550$ ng/mL)[29]; an effect likely attributed to ketamine's potent blocking of the ionotropic glutamate NMDA (N-methyl-D-aspartate) receptor.[31]

The HR PD model tended to consistently overpredict the observed data. Several strategies were investigated to optimize the HR PD model, including implementing semiparametric and nonparametric distributions for the BSV on PD parameters, additive BSV on PD parameters, a logit-transform function on E_{max}, mixture model for the estimated EC_{50}, and biphasic drug effect models. None of the aforementioned strategies improved the model fit. However, as the effect of ketamine on HR is not considered a major clinically significant adverse effect, and given that with the current PD model, the observed median, 5th and 95th percentiles still remained within the corresponding 90% CIs of the prediction intervals of the simulated data, we considered this model to be satisfactory for describing the available HR data.

In contrast to HR and BP, the effect of ketamine on MADRS scores was substantially delayed beyond changes in concentration, with peak effects several hours after the dose. This was best captured by a turnover model as opposed to a distributional delay with a hypothetical effect site (e.g., the central nervous system (CNS)). Although the exact ketamine (and possibly metabolites) mechanism of action in depression is not clear, it appears to increase brain-derived neurotrophic factor (BDNF) expression via several mechanisms, causing changes in pre- and postsynaptic scaffolding proteins and glutamate receptors, mainly α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA).[32–36] This is associated with decreased neurogenesis and synaptic plasticity, resulting in changes in synaptic strength and communication. We believe that the turnover model, although coarse, is biologically plausible given these complex dynamic counter-balancing processes.[33] The turnover model allows for a relatively rapid decrease in MADRS score via marked decrease of production vs. removal with a relatively slow return. This is partly due to the balance of input production vs. removal and the very low EC_{50} of ketamine in relation to plasma concentrations. Unfortunately, only norketamine concentrations were available in our dataset. This limited our ability to further explore the role of potentially active metabolites, such as 2R, 6R-hydroxynorketamine, which recent studies suggest possesses antidepressant activity.[37] We were unable to assay for this due to the unavailability of pure and labeled substance. Additional variability in the PK of such a metabolite, may have impacted upon the very large BSV in turnover time, as well as the relatively low precision of the EC_{50} estimate for MADRS scores, and possibly BP and HR.

The estimated EC_{50} from the MADRS score, BP, and HR PD models were 0.439, 468, and 7,580 ng/mL, respectively. Although

these estimates were somewhat imprecise, it is clear from the VPCs that the model predicts MADRS scores very well, and they nonetheless indicate orders of magnitude differences in the potency of ketamine for the treatment of depression vs. immediate cardiovascular adverse effects.

Our simulations of plasma concentrations were very similar to the clinical trial data of Singh *et al.*,[21] and their primary efficacy variable of percent change in MADRS scores at day 15 were also similar, response and remission data simulations were less favorable than the trial data, although numbers of participants were similar. The trial data were based on 7-day recall of depressive symptoms for MADRS at baseline, and 24-hour recall period for measurements at other timepoints. Our model reflects how MADRS was collected in the studies,[7,8] as at baseline, in the hour prior to ketamine treatment (day 1) and at 4 hours, days 2, 4, and 7 after each treatment; nevertheless, our data are based on single doses given at least 1 week apart, compared to twice and thrice weekly in the clinical trial. Whether response and remission can be more accurately predicted given the large inherent intrapatient variability in efficacy remains to be determined. Nevertheless, the model has sufficient positive features to warrant further assessment, especially in a chronic dosing setting to examine the development of tolerance.

The combination of markedly reduced potency and immediate time course of action for the adverse cardiovascular effects vs. the more potent and sustained effect on depression has important clinical implications. Via simulation, we explored daily s.c. dosing (0.1, 0.3, and 0.5 mg/kg) as this represents the least invasive route of administration in our model. As demonstrated in **Table S3**, model predicted percent of patients with clinical benefit (50% reduction from baseline MADRS) or remission (MADRS ≤ 10) by day 7 decreased from 55–50% (0.5 mg/kg) to 46–38% (0.3 mg/kg), respectively. At the same time, predicted average maximum percent increase in HR approximately halved, albeit from 4–5% at 0.5 mg/kg. At 0.1 mg/kg, benefit was reduced to ~17%, but cardiovascular effects < 1%. These findings raise the potential for very low dose sustained delivery of ketamine. Nevertheless, the development of tolerance cannot be ruled out in our work, as the trial data used to develop the model used a significant washout period between doses. As such, our simulations should be viewed with caution, but may form the foundation to informing future dose regimens.

The strengths of this study were the crossover study design and wide range of ketamine doses allowing for more robust data analysis. In addition, detailed evaluations of depression scores and measurements of BP and HR at multiple timepoints after each treatment dose allowed for a clear differentiation in the time course of effects, thereby indicating different mechanisms of actions (efficacy-adverse). Future research should explore the role of potentially active metabolites, and collect data after regular repeated doses over a longer time frame to examine the development of tolerance.

In conclusion, PK/PD models describing ketamine concentration-response relationships for MADRS scores, BP, and HR were successfully developed in patients receiving ketamine for treatment-refractory depression. The findings of this study assist in understanding the relationships between routes of ketamine administration and clinical response and safety.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

A.Y.A., A.A.S., and D.J.R.F. wrote the manuscript. C.K.L., P.G., and A.A.S. designed the research. A.Y.A., D.T.B., A.A.S., and D.J.R.F. performed the research and analyzed the data.

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