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## **Safety-efficacy balance of S-ketamine and S-norketamine in acute and chronic pain**

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## Chapter 5

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### **Effect of rifampicin on S-ketamine and S-norketamine plasma concentrations in healthy volunteers after intravenous S-ketamine administration**

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## **Introduction**

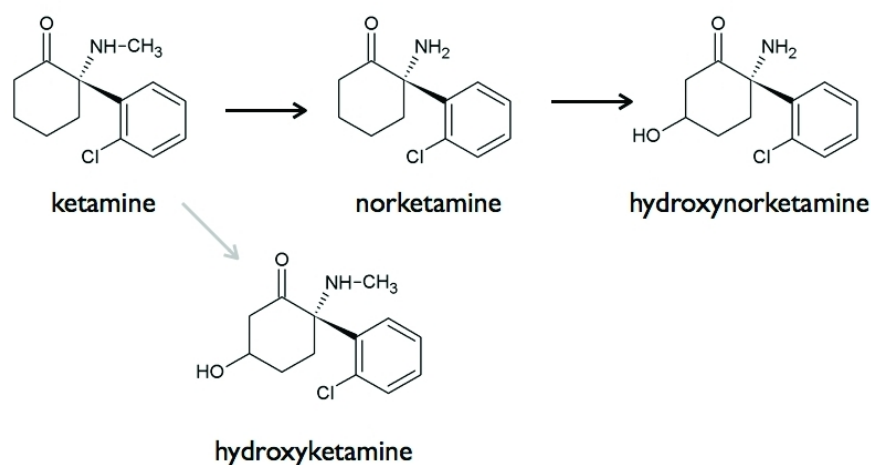
Ketamine is an arylcycloalkylamine structurally related to phencyclidine and first synthesized in 1963.<sup>1</sup> At relatively high dose, ketamine produces anesthesia while at low, subanesthetic, dose it is a potent analgesic. Ketamine analgesia is thought to result from non-competitive antagonism at the ionotropic glutamatergic *N*-methyl-D-aspartate (NMDA) receptor.<sup>2</sup> NMDA receptors are excitatory and involved in enhanced nociceptive processing at spinal and supra-spinal sites.<sup>3</sup> By blocking the NMDA receptor, ketamine effectively reduces signal propagation in the pain circuitry from the spinal cord to the cortex and consequently produces analgesia. Ketamine-induced acute pain relief is driven by its pharmacokinetics, that is, upon termination of infusion (causing a brisk decrease in plasma concentration) acute pain relief ends rapidly<sup>4,5</sup>, while in chronic pain patients the relief of spontaneous pain outlasts the treatment period by weeks to months.<sup>6-9</sup>

Ketamine undergoes extensive metabolism by hepatic cytochrome P450. In rat liver microsomes, norketamine, hydroxynorketamine, and hydroxyketamine account for 80%, 15% and 5%, respectively, of ketamine metabolism (Figure 1).<sup>10</sup> In vivo, rabbits convert 68% of ketamine to norketamine.<sup>11</sup> In humans, *N*-demethylation to norketamine is the major route of metabolism, which can undergo further metabolism via cyclohexanone ring hydroxylation to form 4-, 5-, and 6-hydroxynorketamine. Ketamine itself can be hydroxylated, forming 4-hydroxyketamine, although this is considered quantitatively insignificant.<sup>10,12</sup> Other minor metabolites have recently been reported.<sup>13</sup> Finally, norketamine and the hydroxy metabolic products are subsequently glucuronidated and eliminated via the kidney and bile.<sup>14</sup> Just 10-15% of ketamine is eliminated unchanged. Like ketamine, norketamine is a non-competitive antagonist at the NMDA receptor.<sup>15,16</sup> Non-human data indicate that norketamine passes the blood-brain barrier, has about one-fifth to one-third the potency of ketamine and is thought to contribute up to 30% of ketamine analgesia, and, albeit to a lesser extent, to the development of psychotomimetic side effects.<sup>15,17,18</sup> No pharmacological activity is attributed to the hydroxynorketamines.<sup>17</sup>

The major human hepatic cytochrome P450s catalyzing ketamine *N*-demethylation in vitro are CYP2B6 and CYP3A4, although there is ambiguity as to their comparative contributions to clinical ketamine metabolism.<sup>19,20</sup> Rifampicin is an effective and non-selective inducer of multiple hepatic P450s, including those (CYPs 2B6 and 3A4) which catalyze ketamine *N*-demethylation.<sup>21</sup>

In the current study, we performed a compartmental pharmacokinetic analysis to quantify the effect of CYP induction on the elimination of *S*(+)-ketamine (*S*-ketamine) and formation and elimination of *S*(+)-norketamine (*S*-norketamine).

We took this approach (i.e., CYP induction) as norketamine is unavailable for testing in humans. Next, using a sigmoid  $E_{MAX}$  model with S-ketamine and S-norketamine contributions, we simulated the effect of rifampicin on analgesia to obtain an indication of the importance of variations in S-norketamine concentration on analgesic effect. We tested the effect of the S(+)-enantiomer of ketamine as it is the only registered ketamine product for human use in The Netherlands. S-ketamine has greater analgesic potency than either the R(-)-variant or the racemic mixture, with possibly fewer side effects than the racemic mixture.<sup>16,22</sup>



**Figure 1** Metabolic pathways of ketamine. The black arrows indicate the major pathway; the grey arrow the minor pathway.

## Materials and methods

### Subjects

Twenty healthy male volunteers (age 19-29 years, body mass index < 30 kg/m<sup>2</sup>) were recruited to participate in the study after approval of the protocol by the local Human Ethics Committee (Commissie Medische Ethiek, Leiden University Medical Center, Leiden, The Netherlands). Written informed consent was obtained prior to inclusion in the study according to the Declaration of Helsinki. All subjects were instructed not to eat or drink for at least 8 h before the study. Alcohol, coffee and chocolate were not allowed for 24 hours and grapefruit or grapefruit juice was not allowed 6 days prior to the study day. The study was registered at [www.trialregister.nl](http://www.trialregister.nl) under number NTR1328.

### **Study design: S-ketamine infusion and blood sampling**

The study design was randomized single-blind, placebo-controlled and crossover. Subjects were treated with daily oral rifampicin 600 mg (Sandoz BV, Almere, The Netherlands) (Session I) or placebo (Session II; cellulose tablets produced by the local pharmacy) in the five days preceding the experimental testing (five 600 mg doses were given). Rifampicin/placebo was taken at bedtime. The time interval between the last dose and the ketamine administration was 10-12 hours. The order of the sessions was random with at least 3 weeks between sessions. On the study day the subjects received an arterial line in the radial artery of the non-dominant hand for blood sampling and a venous line in the contra-lateral arm for drug infusion. Total study duration was 5 h, of which S-ketamine (Ketanest S, Pfizer BV, Capelle aan de IJssel, The Netherlands) was administered during the first 2 h. The subjects were randomly allocated to receive a 20 mg/70 kg/h or 40 mg/70 kg/h S-ketamine infusion. The ketamine infusions were similar for Sessions I and II. Randomization was done by the pharmacy using computer-generated randomization lists.

Arterial blood sampling was performed at times  $t = 0$  (pre-drug baseline), 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 125, 130, 135, 150, 180, 210, 240, 270 and 300 minutes after the start of the infusion. Five ml of blood was collected per sample. The analysis has been described previously.<sup>4</sup> In brief: the samples were centrifuged at a speed of 3000 rpm for 10 minutes. Two to three ml plasma was separated within 15 min of blood collection and stored at -25 °C until analysis. For the construction of S-ketamine and S-norketamine calibration lines, solid substances were obtained from Parke-Davis (Dallas, TX, USA) and Tocris (St. Louis, MO, USA), respectively. After extraction from the specific sample, S(+)-ketamine and S(+)-norketamine concentrations were determined by HPLC on a Gemini C18 column (Phenomenex, Utrecht, The Netherlands) at 40 °C. Monitoring of the eluent was performed at 195 nm with a photodiode-array-detector (PDA 100, Dionex, Amsterdam, The Netherlands). The lower limit of quantitation was 10 ng/ml, the lower limit of detection was 3 ng/ml for both drugs. All samples with concentrations > 3 ng/ml were included in the analysis. None of the samples had ketamine concentrations below 3 ng/ml while the initial 2 norketamine samples of all subjects were below the detection limit.

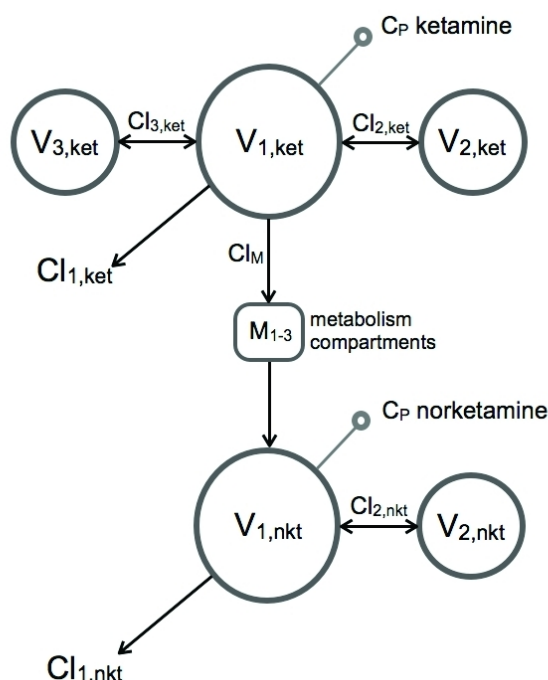
### **Pharmacokinetic analysis**

A t-test was performed to compare the maximum plasma concentration of S-ketamine and S-norketamine ( $C_{MAX}$ ), their time of occurrence ( $T_{MAX}$ ), and the area-under-the-plasma concentration-curve (AUC) divided by the duration of the experiment ( $AUC_{0 \rightarrow 300}$ ) of the placebo versus ketamine treatment. The analysis was performed in SPSS for Windows version 16.0 (SPSS Inc. Chicago, IL, USA). P-values < 0.05 were considered significant. Values reported are mean  $\pm$  SEM.

The compartmental model used for pharmacokinetic analysis was identical to a previously published model.<sup>4</sup> The analysis was performed in two steps. Initially, only the ketamine data were analyzed. Subsequently, the combined ketamine norketamine data were analyzed. The model consisted of three ketamine compartments connected to two norketamine compartments (Figure 2). Since we had found that norketamine's formation and elimination (and inter-compartmental distribution) rates were not simultaneously estimable<sup>4</sup>, the norketamine formation rate was set equal to the ketamine elimination rate. Volumes were scaled via  $WT/70$  and clearances via  $(WT/70)^{0.75}$ , where  $WT$  is body weight in kg.<sup>23</sup> In order to test for a significant rifampicin effect on clearances, we introduced factor  $F$  as follows:

$$\text{Parameter}_{\text{RIFAMPICIN}} = \text{Parameter}_{\text{PLACEBO}} \cdot (1 + F)$$

For example, an  $F$  value of 2 indicates that the parameter increased by 200% after rifampicin treatment. Significance of factors was tested using forward selection. Since experiments were performed on two separate days we estimated both inter-individual ( $\omega^2$ ) and inter-occasion ( $v^2$ ) variances. Exponential error models were used for inter- and intraindividual variability.



**Figure 2** Schematic representation of the pharmacokinetic model used to analyze the combined S-ketamine and S-norketamine data.  $V_{1, \text{ket}}, V_{2, \text{ket}}$  and  $V_{3, \text{ket}}$  are the three ketamine (ket) compartments,  $V_{1, \text{nkt}}$  and  $V_{2, \text{nkt}}$  the two norketamine (nket) compartments.  $M_{1-3}$  represents three sequential metabolism compartments.  $Cl_{1, \text{ket}}$  is the clearance from compartment  $V_{1, \text{ket}}$ ,  $Cl_{2, \text{ket}}$  the clearance from compartment  $V_{2, \text{ket}}$ ,  $Cl_{3, \text{ket}}$  the clearance from compartment  $V_{3, \text{ket}}$ ,  $Cl_{1, \text{nkt}}$  the clearance from compartment  $V_{1, \text{nkt}}$  and  $Cl_{2, \text{nkt}}$  is the clearance from compartment  $V_{2, \text{nkt}}$ .  $Cl_M$  is the S-ketamine clearance responsible for the S-norketamine formation.

The compartmental analysis was performed with the statistical package NONMEM VII with first-order conditional estimation with interaction method (ICON Development Solutions, Ellicott City, MD, USA).<sup>24</sup> Data presented are median and 95% confidence intervals. A bootstrap analysis was used to check the final model and to obtain 95% confidence intervals for the model parameters (from 1000 successful runs).

### **Side effects**

Drug high and sedation were scored using an 11-point numerical rating scale ranging from 0 (= no effect) to 10 (= maximum possible effect) at regular intervals prior, during and following ketamine infusion.

### **Simulation study**

Simulation studies enable the systematic exploration of inferences made in our study with respect to anticipated norketamine effect size. Here we performed simulations to estimate the effect of the large change in norketamine concentration and relatively modest change in ketamine concentration that we observed after rifampicin treatment on pain relief. Two sets of simulations were performed, one set on pain relief induced by short-term ketamine infusion and another set on pain relief induced by chronic ketamine administration. To that end we made a priori assumptions with respect to the norketamine contributions to ketamine effect: simulations with 0, 10 and 25% norketamine contribution were made. The difference in effect observed in the simulated pain relief with and without rifampicin treatment will give an indication of the norketamine contribution to effect.

### *Acute antinociception paradigm*

Using the pharmacokinetic model parameters the effect of rifampicin treatment on acute antinociception was simulated for different norketamine contributions to effect. To that end, analgesic effect during and following a 2 hour S-ketamine infusion of 40 mg/h was simulated using the current pharmacokinetic data set linked to pharmacodynamic data previously obtained and modeled in a similar subject population in our laboratory.<sup>4</sup> Analgesia was simulated using a sigmoid  $E_{MAX}$  model assuming an additive effect of S-ketamine and S-norketamine as follows:

$$VAS(t) = \frac{BLN}{1 + \left( \frac{C_{ket}(t)}{C_{50,ket}} + \frac{C_{nkt}(t)}{C_{50,nkt}} \right)^\gamma}$$

where VAS is visual analogue score (ranging from 0 cm = no pain to 10 cm = severe pain), BLN is baseline (or predrug) VAS,  $\gamma$  a shape parameter,  $C_{ket}$  and  $C_{nkt}$  the plasma concentrations of S-ketamine and S-norketamine, respectively;  $C_{50,ket}$  the plasma concentration S-ketamine causing 50% effect,  $C_{50,nkt}$  the concentration S-norketamine causing 50% effect. The following model parameters were used (reference 4: BLN = 6.7 cm,  $\gamma$  = 2.5, and  $C_{50,ket}$  = 375 ng/ml. The  $C_{50,nkt}$  was varied in such a way that it contributed 0, 10 and 25% to total analgesic effect. We assumed no delay between blood concentration and acute antinociceptive effect (i.e., pain relief in response to an experimental heat pain stimulus) for both S-ketamine and S-norketamine.<sup>4,5</sup>

### *Chronic analgesia paradigm*

Using pharmacokinetic model parameters (from references 6 and 7 on a study on the effect of ketamine on spontaneous chronic pain relief in complex regional pain syndrome type 1 patients), the effect of rifampicin treatment on chronic analgesia was simulated for different norketamine contributions to effect (0, 10 and 25%). To that end analgesic effect during and following a 4-day S-ketamine infusion of 5 mg/h on day 1, 10 mg/h on day 2, 15 mg/h on day 3 and 20 mg/h on day 4 was simulated.<sup>6,7</sup> Before the simulations were performed the pharmacokinetic data obtained in the healthy volunteer population and chronic pain patients were compared. The differences were sufficiently small (data not shown) to allow the application of a rifampicin effect as observed in our current study to the kinetic data obtained in chronic pain patients. The pharmacodynamic model was as given above with model parameters: BLN = 7 cm,  $\gamma$  = 1.9, and  $C_{50,ket}$  = 10 ng/ml. The  $C_{50,nkt}$  was varied in such a way that it contributed 0, 10 and 25% to total analgesic effect. We previously observed that in our group of chronic pain patients, the effect of ketamine on spontaneous pain relief lasted beyond the duration of treatment.<sup>6,7</sup> In a subgroup of these patients (i.e., responders to therapy) we estimated an effect half-life ( $t_{1/2k}$ ) of about 11 days and used this value in the chronic pain simulations.<sup>7</sup>

## **Results**

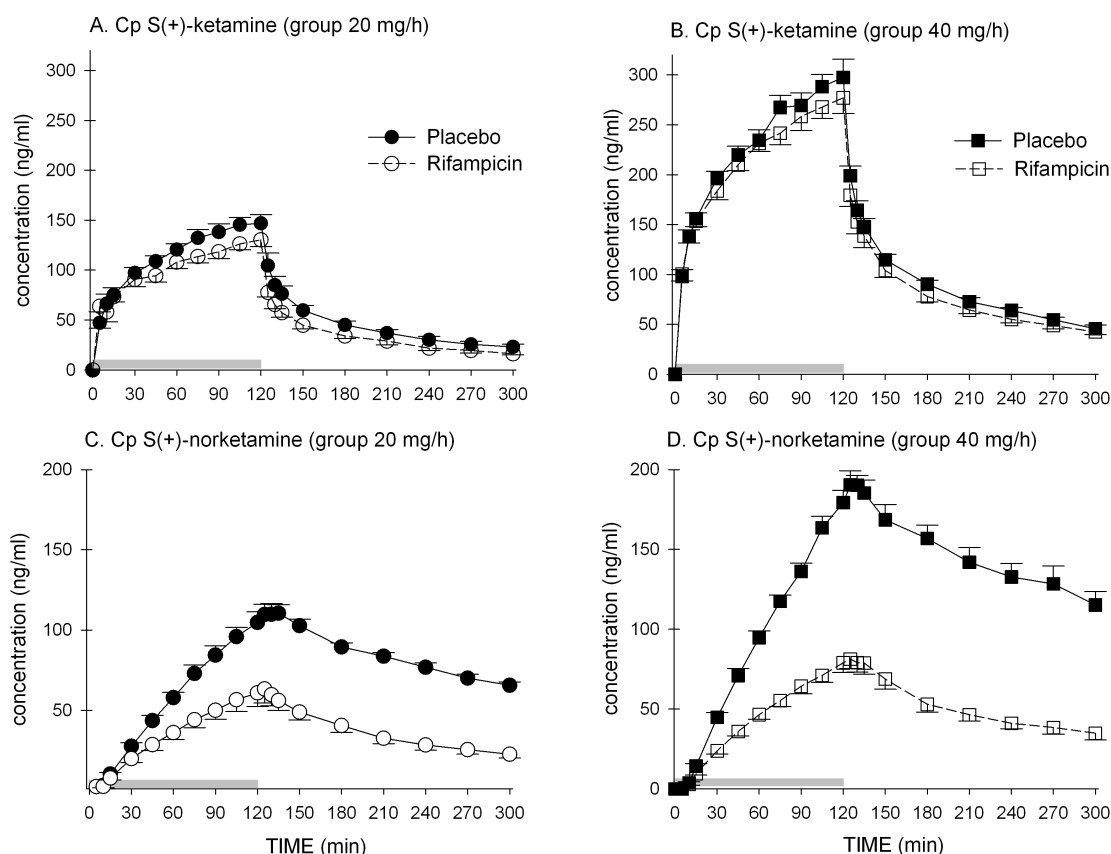
### **Subjects**

All subjects completed the protocol without unexpected side effects. One subject (in the 20 mg/h ketamine group) failed to return, for unknown reasons, for his second experimental session. The subjects' ages ranged from 20 to 29 years (mean age 22.0 years), their body mass index (BMI) from 19 to 28 (mean BMI 22.2). There were no differences in age or BMI between treatment groups.



### Descriptive pharmacokinetic analysis

In Figure 3 the mean S-ketamine and S-norketamine concentrations in plasma after rifampicin and placebo are plotted. Rifampicin decreased S-ketamine concentrations albeit the effects were modest in magnitude. The effect of rifampicin was most prominent in the lower S-ketamine infusion group: during 20 mg/h infusion rifampicin caused a 17% decrease in  $C_{MAX}$  ( $P < 0.001$ ) and a 14% decrease in  $AUC_{0 \rightarrow 300}$  ( $P < 0.001$ ). At double the ketamine infusion dose rifampicin decreased  $C_{MAX}$  by 6% (ns) and  $AUC_{0 \rightarrow 300}$  by 7% ( $P = 0.02$ ; Table 1). S-ketamine  $T_{MAX}$  values remained unaffected by rifampicin (Table 1). In contrast, the effects of rifampicin on S-ketamine's metabolite S-norketamine concentrations in plasma were large with a 44% and 58% decrease in  $C_{MAX}$  for the infusion of 20 mg/h ( $P = 0.001$ ) and 40 mg/h ( $P < 0.001$ ), and a 53% and 62% decrease in  $AUC_{0 \rightarrow 300}$  for the 20 mg/h and 40 mg/h infusions ( $P < 0.001$ ), respectively (Table 1, Figure 3). S-norketamine  $T_{MAX}$  values remained unaffected by rifampicin (Table 1). The metabolite-to-parent drug  $AUC_{0 \rightarrow 300}$  ratios ( $AUC_M/AUC_P$  in Table 1) were about 50% lowered by rifampicin compared to placebo irrespective of the S-ketamine infusion dose.



**Figure 3** Effect of rifampicin treatment on plasma concentrations of S-ketamine (A and B) and S-norketamine (C and D) during and 3 h following a 2-h S(+)-ketamine infusion of 20 mg/h (A and C) and 40 mg/h (B and D). Values are mean  $\pm$  SEM. The grey bars depict the 2-h infusion period.

**Table 1** Effect of rifampicin on S-ketamine and S-norketamine concentrations:  $C_{MAX}$ ,  $T_{MAX}$  and Area-Under-the-Curve (AUC) values.

	Placebo	Rifampicin	P value
[S-ketamine] <sub>20</sub> *			
$C_{MAX}$ (ng/ml)	150.4 ± 3.9	132.6 ± 7.1	< 0.001
$T_{MAX}$ (min)	114 ± 3	113 ± 4	0.72
$AUC_{0 \rightarrow 300}$ (ng/ml)	71.5 ± 5.4	60.8 ± 5.1	< 0.001
[S-norketamine] <sub>20</sub> *			
$C_{MAX}$ (ng/ml)	113.8 ± 5.9	64.5 ± 8.7	0.001
$T_{MAX}$ (min)	129 ± 2	125 ± 1	0.06
$AUC_{0 \rightarrow 300}$ (ng/ml)	73.3 ± 8.4	35.5 ± 11.7	< 0.001
$AUC_M/AUC_P$	1.03 ± 0.19	0.58 ± 0.19	< 0.001
[S-ketamine] <sub>40</sub> *			
$C_{MAX}$ (ng/ml)	304.7 ± 17.5	285.6 ± 14	0.27
$T_{MAX}$ (min)	110 ± 5	113 ± 3	0.51
$AUC_{0 \rightarrow 300}$ (ng/ml)	142.0 ± 15.8	132.0 ± 17.4	0.02
[S-norketamine] <sub>40</sub> *			
$C_{MAX}$ (ng/ml)	198.3 ± 8.1	83.6 ± 6.2	< 0.001
$T_{MAX}$ (min)	132 ± 2	128 ± 2	0.17
$AUC_{0 \rightarrow 300}$ (ng/ml)	124.8 ± 18.5	47.9 ± 11.6	< 0.001
$AUC_M/AUC_P$	0.90 ± 0.12	0.36 ± 0.10	< 0.001

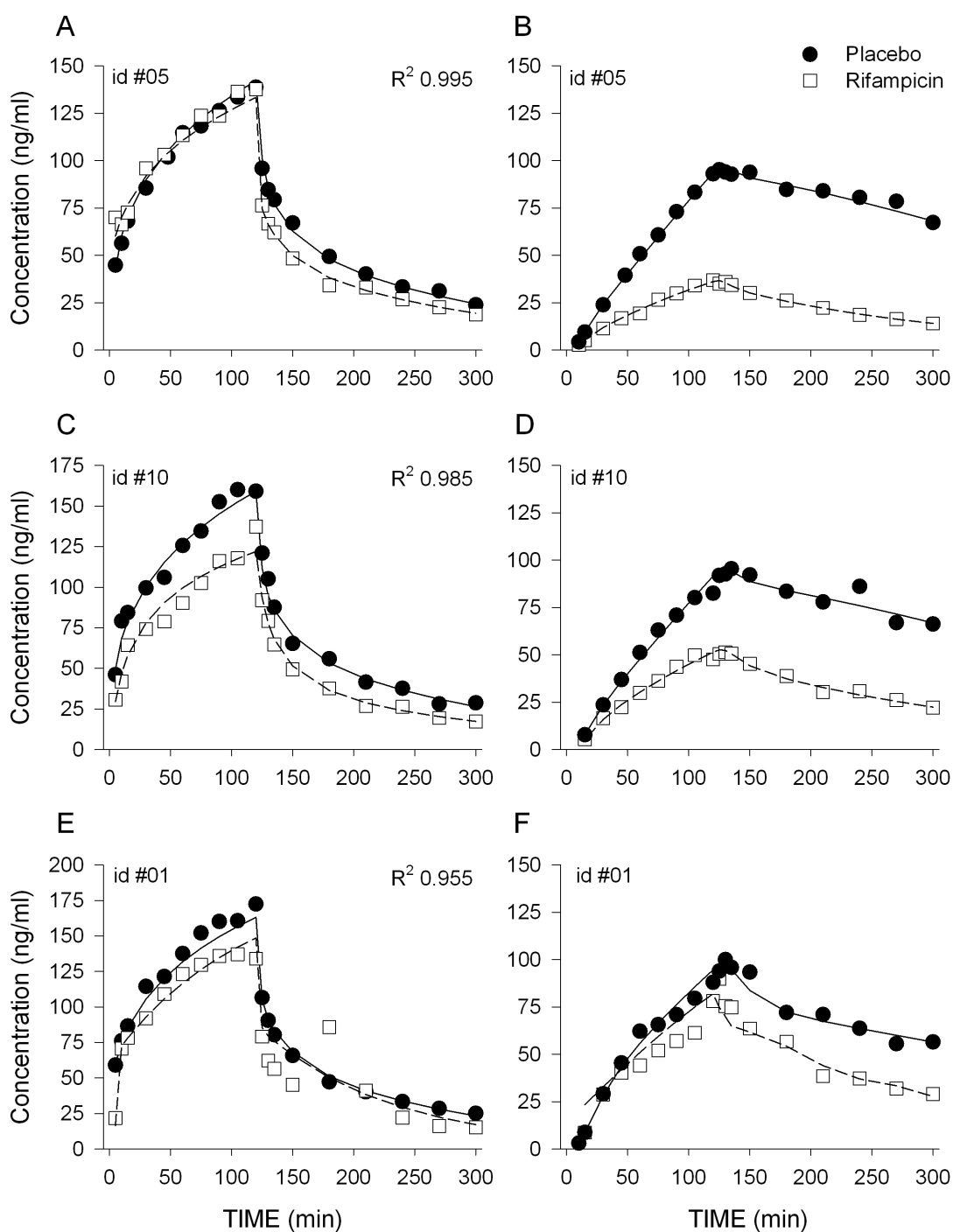
Values are mean ± SEM.

\*<sub>20</sub> and <sub>40</sub> denote data obtained during and after the 2-h infusion of 20 mg/h and 40 mg/h S-ketamine infusion (both per 70 kg), respectively.

$AUC_M$  = metabolite  $AUC_{0 \rightarrow 300}$  (S-norketamine);  $AUC_{0 \rightarrow 300}$  = the area-under-the-curve determined for the 300 min study period divided by 300 min;  $AUC_P$  = parent  $AUC_{0 \rightarrow 300}$  (S-ketamine);  $C_{MAX}$  = peak concentration;  $T_{MAX}$  = time of occurrence of  $C_{MAX}$ .

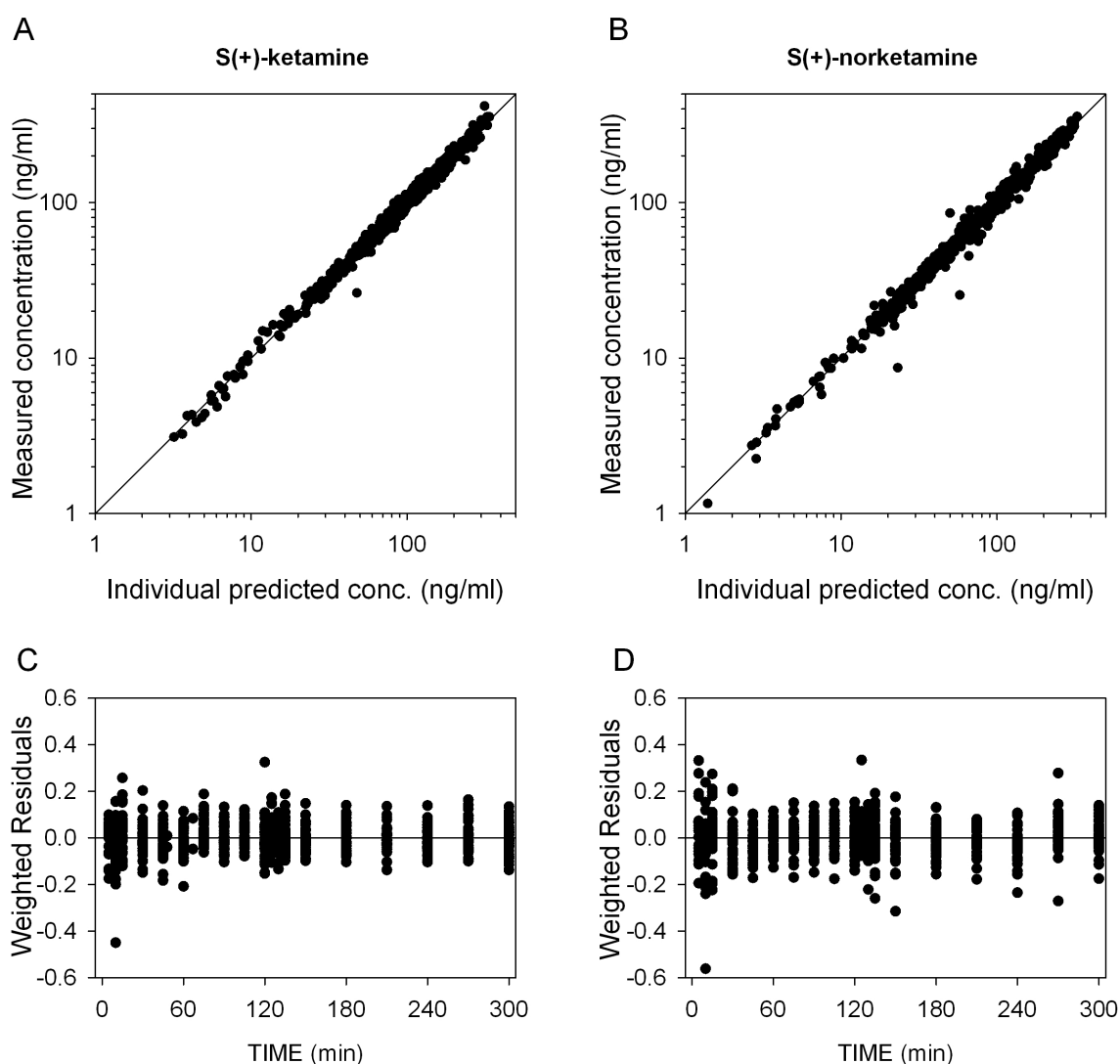
### Compartmental pharmacokinetic analysis

The pharmacokinetic model is given in Figure 2. In agreement with an earlier observation<sup>4</sup>, extending the three compartmental model (in which just the ketamine data were modeled) with a part describing the norketamine data did not cause any change in S-ketamine pharmacokinetic parameter values (data not shown). This indicates that adding the metabolism compartments ( $M_{1-3}$  in Figure 2) and the two norketamine compartments did not affect the analysis of ketamine. As judged by the eye, the model adequately described the data. Best, median and worst data fits as determined by the coefficient of determination ( $R^2$ ) for the combined S-ketamine/S-norketamine data fits are given in Figure 4.



**Figure 4** Pharmacokinetic data fits of the combined S-ketamine/S-norketamine model. Best **A** S-ketamine and **B** S-norketamine fits of subject #05; median **C** S-ketamine and **D** S-norketamine data fits of subject #10; worst **E** S-ketamine and **F** S-norketamine data fits of subject #01. Closed circles and continuous lines are the placebo data; open squares and dashed lines are the rifampicin data. Goodness of fit was determined by the coefficient of determination ( $R^2$ ).

Goodness of fit plots are given in Figure 5 (individual predicted versus measured concentration). Model parameter estimates together with their 95% confidence intervals are given in Table 2. The pharmacokinetic model parameters were in agreement with previous findings.<sup>4,25-27</sup> Rifampicin affected S-ketamine elimination (and consequently S-norketamine formation) modestly: clearance from the central compartment ( $V_{1,ket}$ ) increased by 13% ( $F Cl_{1,ket} = 0.13$  in Table 2). In contrast, rifampicin had a much greater effect on S-norketamine elimination: S-norketamine clearance from its central compartment ( $V_{1,nkt}$ ) increased by about 200% ( $F Cl_{1,nkt} = 2.02$  in Table 2).



**Figure 5** Goodness of fits plots: individual predicted PK data versus measured data for **A** S-ketamine and **B** S-norketamine. Weighted residuals,  $(Y_{\text{measured}} - Y_{\text{predicted}})/Y_{\text{predicted}}$ , versus time for **C** S-ketamine concentrations and **D** S-norketamine concentrations.

**Table 2** Pharmacokinetic model parameters of the combined ketamine-norketamine model.

S(+)-ketamine	Estimate	SEM	$\omega^2$	SEM	$\nu^2$	SEM
$V_{1, \text{ket}}$ (L)	17.0	1.91	-	-	0.148	0.048
95% CI	13.6 - 21.0				0.06 - 0.25	
$V_{2, \text{ket}}$ (L)	28.3	2.97	-	-	-	-
95% CI	23.3 - 34.1					
$V_{3, \text{ket}}$ (L)	147	10.2	0.065	0.019	0.029	0.020
95% CI	129 - 172		0.03 - 0.10		0.00001 - 0.08	
$Cl_{1, \text{ket}}$ (L/h)	93.5	2.70	0.007	0.004	0.006	0.002
95% CI	87.6 - 98.8		0.001 - 0.02		0.002 - 0.01	
F $Cl_{1, \text{ket}}^{\#}$	0.13	0.03				
95% CI	0.07 - 0.20					
$Cl_{2, \text{ket}}$ (L/h)	127	12.3	-	-	-	-
95% CI	107 - 148		-		-	
$Cl_{3, \text{ket}}$ (L/h)	91.9	5.10	0.02	0.006	-	-
95% CI	82 - 101		0.01 - 0.03		-	
$\sigma^2$	0.008	0.002				
S(+)-norketamine	Estimate	SEM	$\omega^2$	SEM	$\nu^2$	SEM
MTT (h)	0.25	0.02	0.05*	0.03*	0.02	0.01
95% CI	0.21 - 0.28		0.01 - 0.14		0.004 - 0.05	
$V_{2, \text{nkt}}$ (L)	193	10.6	-	-	0.14	0.03
95% CI	173 - 217		-		0.09 - 0.19	
$Cl_{1, \text{nkt}}$ (L/h)	64.9	2.81	0.03	0.01	0.03	0.01
95% CI	58.5 - 70.8		0.01 - 0.06		0.01 - 0.05	
F $Cl_{1, \text{nkt}}^{\#}$	2.02	0.19				
95% CI	1.67 - 2.41					
$Cl_{2, \text{nkt}}$ (L/h)	334	30.5	0.05*	0.03*	0.14	0.04
95% CI	285 - 411		0.01 - 0.14		0.07 - 0.22	
$\sigma^2$	0.007	0.001				

Units of the volumes are L at 70 kg; units of the clearances are L/h at 70 kg. Factor F denotes the significant effect of rifampicin treatment on model parameters  $Cl_{1, \text{ket}}$  and  $Cl_{1, \text{nkt}}$  ( $P < 0.01$ ).

\*One parameter was sufficient for the inter-individual error of  $Cl_{2, \text{nkt}}$  and MTT.

<sup>#</sup>  $P < 0.01$ .

$Cl_{1, \text{ket}}$  = clearance from compartment 1, ket;  $Cl_{2, \text{ket}}$  = clearance from compartment 2, ket;  $Cl_{3, \text{ket}}$  = clearance from compartment 3, ket;  $Cl_{1, \text{nkt}}$  = clearance from compartment 1, nket;  $Cl_{2, \text{nkt}}$  = clearance from compartment 2, nket; **F** = factor F; **ket** = S-ketamine; **MTT** = mean transit time; **nket** = S-norketamine;  $V_{1, \text{ket}}$  = volume of compartment 1, ket;  $V_{2, \text{ket}}$  = volume of compartment 2, ket;  $V_{3, \text{ket}}$  = volume of compartment 3, ket;  $V_{2, \text{nkt}}$  = volume of compartment 2, nket;  $\nu^2$  = interoccasion variability (in the log-domain);  $\omega^2$  = intersubject variability (in the log-domain);  $\sigma^2$  = within-subject variability (in the log domain).

### Side effects

Side effects were present during ketamine infusion and resolved promptly upon termination of infusion. The highest scores measured during ketamine infusion all occurred at the end of the infusion period and were for drug high:  $6.5 \pm 2.3$  (placebo, low-dose ketamine) versus  $5.5 \pm 3.0$  (rifampicin, low-dose ketamine;  $P > 0.05$ ),  $8.1 \pm 2.0$  (placebo, high-dose ketamine) versus  $7.8 \pm 2.6$  (rifampicin, high-dose ketamine;  $P > 0.05$ ); and for sedation  $3.8 \pm 3.6$  (placebo, low-dose ketamine) versus  $3.8 \pm 2.6$  (rifampicin, low-dose ketamine;  $P > 0.05$ ), and  $5.1 \pm 3.6$  (placebo, high-dose ketamine) versus  $4.5 \pm 3.8$  (rifampicin, high-dose ketamine;  $P > 0.05$ ).

### Simulation study

#### *Acute antinociception paradigm*

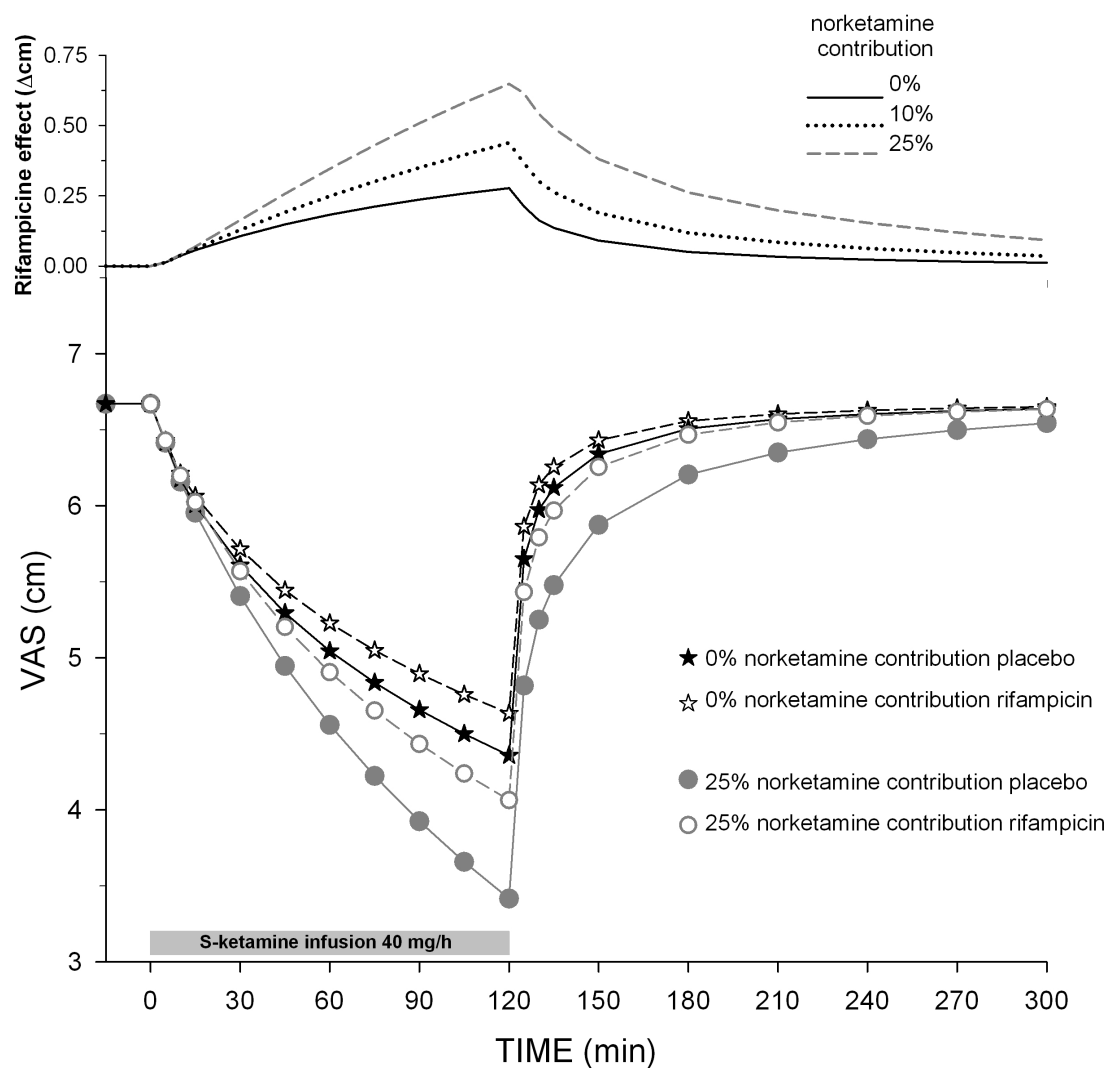
The simulation study was based on the current pharmacokinetic model parameters (Table 2) linked to a pharmacodynamic model in which both S-ketamine and S-norketamine contribute to effect. The results of the simulations are shown in Figure 6. At increasing norketamine contributions to analgesic effect, peak VAS is reduced by a maximum of 0.7 cm at 25% contribution. At 0, 10 and 25% norketamine contribution, rifampicin treatment causes an increase in peak VAS of 0.3 cm (= 5% of peak VAS), 0.4 cm (10%) and 0.7 cm (21%), respectively (Figure 6).

#### *Chronic analgesia paradigm*

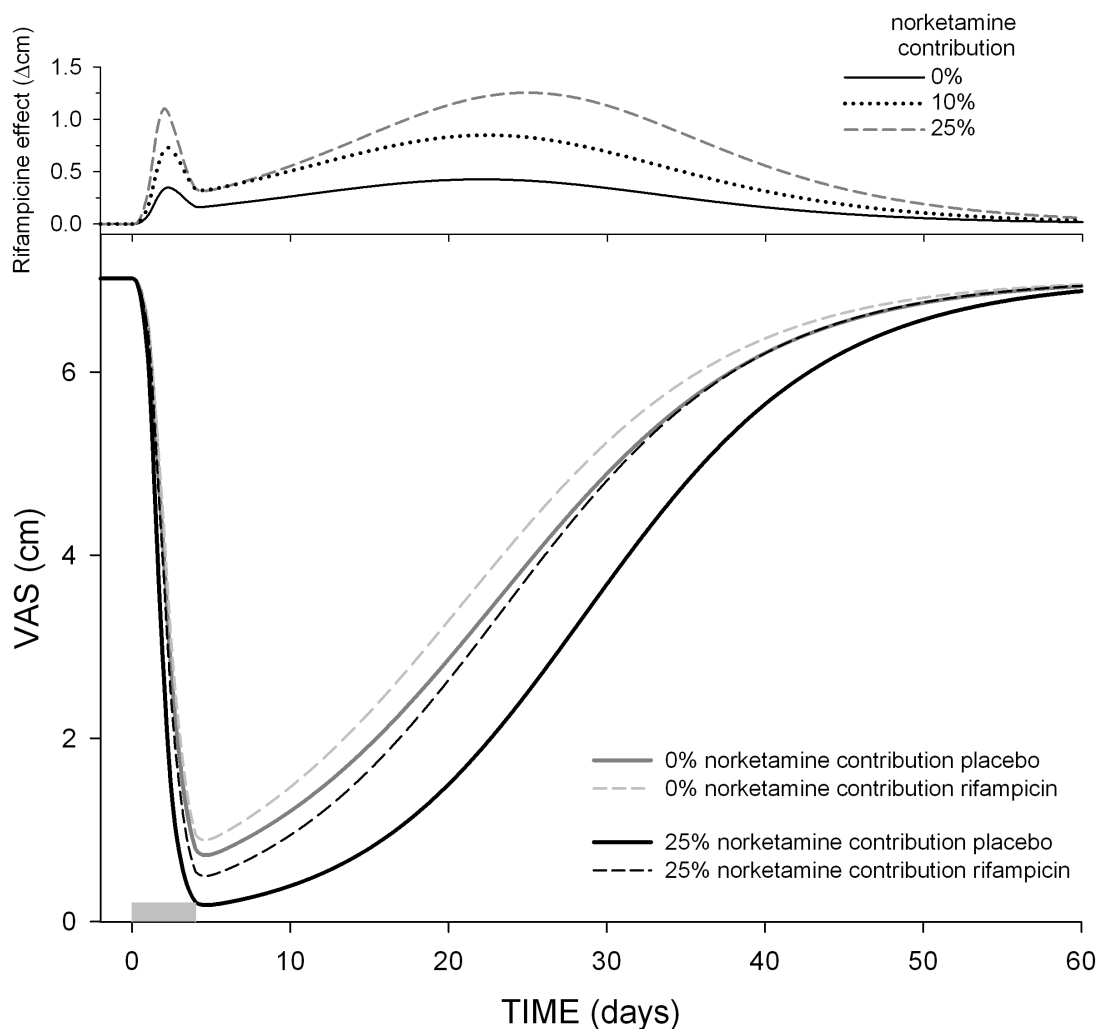
The study was based on the application of the rifampicin effect to pharmacokinetic and pharmacodynamic data obtained in chronic pain patients. Hence, the effect of S-ketamine infusion mimics the effect observed in chronic pain patients (Figure 7).<sup>7</sup> The effect of rifampicin was relatively small with maximum increases in VAS of 0.4 cm (0% norketamine contribution), 0.8 cm (10% contribution) and 1.3 cm (25% contribution, Figure 7). A biphasic effect of rifampicin on analgesia was observed with an initial peak during infusion (peak on day 2 of the infusion) and a second peak between days 20 and 30, irrespective of the magnitude of the norketamine contribution.

### Discussion

Rifampicin caused a 10% reduction in S-ketamine plasma concentrations coupled to a 50% reduction in plasma concentrations of ketamine's metabolite S-norketamine. We relate these changes to the induction of S-ketamine and S-norketamine elimination. The compartmental pharmacokinetic analysis indicated a 13% increase in S-ketamine elimination, and a 200% increase in S-norketamine elimination.



**Figure 6** Simulation study for an acute antinociception paradigm. Simulation studies enable the systematic exploration of inferences made in our study with respect to anticipated norketamine effect size. We performed simulations to estimate the effect of the large change in norketamine concentration and relatively modest change in ketamine concentration that we observed after rifampicin treatment on acute pain relief induced by a 2-h 40 mg/h ketamine infusion (grey bar). We made a priori assumptions with respect to the norketamine contributions to ketamine effect: simulations with 0, 10 and 25% norketamine contribution were made. The difference in effect observed in the simulated pain relief with and without rifampicin treatment will give an indication of the norketamine contribution to effect. The top diagram shows the difference in VAS between placebo and rifampicin data for the three norketamine contribution to effect: 0, 10 and 25%. VAS = visual analogue score.



**Figure 7** Simulation study for a chronic analgesia paradigm. We performed simulations to estimate the effect of the large change in norketamine concentration and relatively modest change in ketamine concentration that we observed after rifampicin treatment on chronic pain relief, induced by a 4-day intravenous ketamine infusion (grey bar: day 1 = 5 mg/h, day 2 = 10 mg/h, day 3 = 15 mg/h and day 4 = 20 mg/h). Simulations with 0, 10, and 25% norketamine contribution were made. The difference in effect observed in the simulated pain relief with and without rifampicin treatment will give an estimate of the norketamine contribution to effect. The top diagram shows the difference in VAS between placebo and rifampicin data for the three norketamine contribution to effect: 0, 10, and 25%. VAS is visual analogue score.

The significant reduction in norketamine plasma concentrations in rifampicin-treated subjects was an unexpected finding. Both norketamine  $C_{MAX}$  and ratio  $AUC_{norketamine}/AUC_{ketamine}$  were diminished. Several explanations, not necessarily mutually exclusive, are possible. The first is a diminished rate of norketamine formation. This seems unlikely. CYP2B6 and CYP3A4 are the major human hepatic cytochrome P450s catalyzing ketamine *N*-demethylation *in vitro*, although their relative contributions *in vivo* remain unknown.<sup>19,20</sup> At therapeutic



substrate concentrations, both the rate and intrinsic clearance of *N*-demethylation to norketamine by CYP2B6 were found to be more than 10-fold greater than by CYP3A4 in two different studies.<sup>19,20</sup> Based on this observation, using individual S(+)- and R(-)-ketamine enantiomers, Yanagihara et al.<sup>19</sup> concluded that CYP2B6 mainly mediates ketamine *N*-demethylation. In contrast, Hijazi et al.<sup>20</sup> concluded that CYP3A4 is the predominant CYP responsible for ketamine *N*-demethylation, which is based in part on the greater hepatic expression of CYP3A4 than CYP2B6. At clinical ketamine concentrations (< 5  $\mu\text{M}$ ) there is no evidence for other important CYPs involved. At supraclinical ketamine concentrations (50  $\mu\text{M}$ ) other CYP involvement has been described<sup>28</sup>, although also then CYP2B6 and 3A4 are the enzymes with the highest activity. The clinical involvement of CYP3A4 was supported by the effect of the CYP3A4 inhibitor clarithromycin, which caused a 3.6-fold increase in S-ketamine  $C_{\text{MAX}}$  while norketamine  $C_{\text{MAX}}$  was reduced by 54%.<sup>29</sup> Regardless of the relative contributions of CYPs 2B6 and 3A4 to ketamine *N*-demethylation, since rifampicin (600 mg daily for 5 days) induces the hepatic expression and catalytic activity of CYP2B6 and CYP3A4 several-fold, as well as the clinical metabolism of numerous CYP2B6 and CYP3A4 substrates<sup>21</sup>, induction of ketamine metabolism by rifampicin is expected. Patients treated with chronic barbiturates (another inducer of hepatic CYP enzymes) have lower plasma ketamine concentrations.<sup>30</sup> Together these findings do not suggest that rifampicin inhibits ketamine *N*-demethylation *in vivo*.

Another possible explanation for reduced norketamine concentrations is increased ketamine metabolism via an alternate pathway, and metabolic switching. Human liver microsomes form 4- and 5-hydroxyketamine.<sup>10</sup> Although ketamine hydroxylation is considered minor compared with *N*-demethylation, the enzymes responsible for ketamine hydroxylation and the effect of rifampicin induction are unknown.

A third potential explanation for rifampicin reduction of plasma norketamine concentrations is increased secondary metabolism of norketamine. The major secondary metabolite is 6-hydroxynorketamine, with lesser formation of 4- and 5-hydroxynorketamine. At clinical ketamine concentrations the enzymes responsible for norketamine hydroxylation and the effect of rifampicin induction are unknown. A recent study performed at supraclinical concentrations (50  $\mu\text{M}$  norketamine) indicates involvement of CYP2B6 and 2A6.<sup>28</sup> Our compartmental analysis indicates that this possible mechanism is most likely. While norketamine formation may have increased by 13%, its elimination was increased by more than 200% (Table 2). Assuming that also at clinical concentrations norketamine metabolism is predominantly regulated via CYP enzymes 2B6 and 2A6, it is highly probable that rifampicin caused induction of these enzymes and consequently increased the elimination of S-norketamine and caused the 50% lower S-norketamine plasma concentrations that we observed (Figures 3 and 4).

Finally, another potential explanation is an effect of hepatic and/or renal drug transporters on ketamine and/or norketamine disposition. In addition to induction of several CYP isoforms, rifampicin is an effective inducer of several drug transporters, including the efflux transporters P-glycoprotein, breast cancer resistance protein, and multi-drug resistance proteins 1 and 2, and an inhibitor of the hepatic uptake transporter organic anion-transporting polypeptide.<sup>21,31</sup> Inhibition of hepatic ketamine uptake or induction of efflux might reduce intracellular concentrations and hence the extent of *N*-demethylation, despite an accelerated rate. No information is available on ketamine or norketamine and hepatic drug transport.

Previously we modeled the contribution of norketamine to ketamine effect in a study on the effect of a ketamine infusion on acute antinociception using an experimental heat pain model.<sup>4</sup> In that study we were unable to estimate a contribution of norketamine to ketamine antinociception, suggesting that, in humans, norketamine is not analgesic. However, since norketamine concentrations were not manipulated there was considerable uncertainty in the estimation of norketamine contribution to effect. In order to get an indication of the pharmacodynamic effect of the variations in S-norketamine concentration, we performed a simulation study with acute analgesia as end-point. Animal data show that S-norketamine is a non-competitive NMDA receptor antagonist in the spinal cord and cortex.<sup>15</sup> Its affinity to the NMDA receptor is weaker than that of S-ketamine, but there seems consensus that, at least in animals, norketamine does contribute significantly to the antinociceptive properties of ketamine (up to 30%), irrespective whether the S(+)-isomer or the racemic mixture is tested.<sup>14,15,17,18</sup>

Extrapolating the animal data to our simulations we assumed that S-norketamine contributed to overall analgesic effect in a range of 0 to 25%. Our simulation indicates that rifampicin caused a reduction in acute pain responses with a maximum in effect in VAS increase of less than 1 cm (Figure 6). Part of this effect is attributed to changes in S-ketamine concentration (7%) while the remainder is related to the changes in the concentration of S-norketamine (up to 16%). In actual practice, such an effect is small and may not be clinically detectable or important. This then suggests that the dose of ketamine for treatment of acute pain in patients on rifampicin needs little or no adjustment.

We infused S-ketamine for 2 hours and observed a ratio  $AUC_M/AUC_P$  of about 1 after placebo treatment (Table 1). Similar ratios are observed after long-term intravenous S-ketamine infusion in patients with chronic pain.<sup>6</sup> Our simulation study revealed only a relatively small effect of variations in norketamine concentration on analgesia in chronic pain patients (maximum increase in VAS = 1.3 cm, Figure 7). However, a prolonged exposure to norketamine may cause a greater passage of the drug into the central nervous system than occurred

during our short-term infusion and this may then increase its contribution to ketamine's pharmacodynamics.<sup>18</sup> We therefore may have underestimated the norketamine effect in our estimation of analgesia from S-ketamine in chronic pain patients. Also the route of administration may play a role in norketamine's contribution to effect. For example, after oral and sublingual ketamine administration the concentration S-norketamine exceeds that of S-ketamine due to the first pass-effect in the liver and enterocyte.<sup>27</sup> These higher concentrations may increase norketamine's bioavailability to the brain compartment with a corresponding increase in contribution to ketamine's analgesic effect. Further studies are needed to assess the effect of the induction of cytochrome P450 enzymes on norketamine's contribution to ketamine-induced analgesia.

The two simulations studies that we performed used different values for effect-half life (no delay for acute pain and a  $t_{1/2k}$  of 11 days for chronic pain relief). The absence of a delay for acute pain is derived from various studies on the acute effects of ketamine (measuring ketamine-induced electroencephalographic changes, arousal and recall during anesthesia and acute pain relief)<sup>4,5,32,33</sup>, and suggests a rapid passage of ketamine across the blood-brain-barrier. The equilibration half-life ( $t_{1/2k}$ ) of 11 days used in the chronic study is not a blood-effect-site equilibration constant, but rather a disease modulatory factor. Various studies indicate that ketamine produces long-term analgesic effects beyond the treatment period with a half-life of many days.<sup>5-9</sup> We recently estimated a value for  $t_{1/2k}$  of about 11 days in patients with chronic pain from complex regional pain syndrome type 1.<sup>7</sup> Interestingly, side effects in chronic pain patients treated with ketamine still show a rapid onset and offset suggesting that the drug rapidly crosses the blood-brain barrier and that the persistent analgesia observed is unrelated to the brain ketamine concentration.<sup>5,6</sup> We recently argued that ketamine initiates a cascade of events (of which NMDA receptor antagonism is a first step) resulting in persistent analgesia.<sup>34</sup>

In conclusion, using rifampicin as inducer of specific cytochrome P450 enzymes, we observed that rifampicin induces the elimination of S-ketamine's metabolite, S-norketamine, probably via induction of the CYP3A4, CYP2B6, and/or CYP2A6 enzymes.

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