

# Population pharmacokinetics of nevirapine in combination with rifampicin-based short course chemotherapy in HIV- and tuberculosis-infected South African patients

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## Abstract

**Objective** The aim was to develop a model to describe the population pharmacokinetics of nevirapine in South African human immunodeficiency virus (HIV)-infected patients who were taking nevirapine-based antiretroviral therapy concomitantly or in the absence of rifampicin-based tuberculosis therapy.

**Methods** Patients were divided into two groups: (1) patients receiving nevirapine-containing antiretroviral regimen (200 mg twice daily) and continuation phase rifampicin-containing tuberculosis therapy ( $n=27$ ) in whom blood samples were obtained before and not less than 14 days after they completed tuberculosis therapy; (2) patients without tuberculosis who were receiving a nevirapine-containing antiretroviral regimen for at least 3 weeks ( $n=26$ ). The population pharmacokinetics of nevirapine was described using nonlinear mixed effects modelling with NONMEM software. Based on the developed model, plasma concentration profiles after 300, 400 and 500 mg of nevirapine twice daily were simulated.

**Results** Concomitant administration of rifampicin increased nevirapine oral clearance (CL/F) by 37.4% and reduced the absorption rate constant ( $k_a$ ) by almost sixfold. Rifampicin reduced the nevirapine average minimum concentration by 39%. Simulated doses of 300 mg twice daily elevated nevirapine concentrations above subtherapeutic levels in most patients, with minimum exposure above the recommended maximum concentration. The area under the concentration–time curve of 12-hydroxynevirapine was not different in the presence of rifampicin. 2-, 3- and 8-Hydroxynevirapine were not detectable (LLOQ=0.025 mg/L).

**Conclusion** The developed model adequately describes nevirapine population pharmacokinetics in a South African population when taken with/and in the absence of rifampicin treatment. The simulations suggest that an increased dose of 300 mg twice daily would achieve adequate nevirapine concentrations in most patients during rifampicin-containing treatment for tuberculosis.

**Keywords** HIV · Pharmacokinetics · Nevirapine · Rifampicin · Tuberculosis

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## Introduction

Human immunodeficiency virus (HIV) infection has led to a global increase in the incidence of tuberculosis (TB). Almost one third of African patients with TB develop the disease as a result of HIV infection, and TB is the main cause of death in African patients with HIV/acquired immune deficiency syndrome (AIDS). The majority of patients with HIV-associated TB are in sub-Saharan Africa, with about 2 million South Africans coinfecting with TB/HIV

[1, 2]. In South Africa, a regimen consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor, either efavirenz or nevirapine, is first line antiretroviral therapy, as is recommended for resource-limited settings by the World Health Organization (WHO). Nevirapine is a component of inexpensive generic fixed-dose antiretroviral combinations given twice daily; these combinations are used extensively in the developing world. The treatment of TB with a rifampicin-based regimen is standard practice in South Africa. The regimen consists of a 2-month intensive phase with rifampicin, isoniazid, pyrazinamide and ethambutol, followed by a 4-month continuation phase with rifampicin and isoniazid. Ethambutol is included in the continuation phase in those patients who have had a previous episode of TB. Nevirapine is both metabolized by and induces cytochrome P450 (CYP) isoenzymes 3A4 and 2B6 [3]. The plasma concentrations of nevirapine initially decrease after the start of therapy due to auto-induction, reaching a steady state during the second week of treatment [3, 4]. Rifampicin is a potent inducer of CYP3A4 and increases the expression of CYP2B6 [5, 6]. It therefore enhances drug metabolism, leading to a risk of obtaining sub-therapeutic concentrations of nevirapine and the selection of resistant viral strains due to persistent viral replication [7]. Viral resistance to nevirapine results from a single mutation on the reverse transcriptase gene [8]. The mutation is observed after 4 weeks of monotherapy and may occur in just 2 weeks if viral replication is not well controlled in the presence of nevirapine [4]. Several reports on the effect of rifampicin on nevirapine pharmacokinetics have been published [4, 9–14]. However, to our knowledge this is the first modelling report on the effect of rifampicin on nevirapine pharmacokinetics in South African patients.

The *in vitro* metabolism of nevirapine by human hepatic microsomal preparations results in four metabolites—2-, 3-, 8-, and 12-hydroxynevirapine—with 2- and 12-hydroxynevirapine being the major metabolites produced. The pharmacokinetics of nevirapine in eight healthy male volunteers receiving oral nevirapine 200 mg daily for 2 weeks initially, then 200 mg twice daily for another 2 weeks, followed by a single oral dose of 50 mg containing 100  $\mu$ Ci of [ $^{14}$ C]nevirapine has been reported. The major metabolites in plasma were 2-, 3- and 12-hydroxynevirapine glucuronide followed by 12-hydroxynevirapine and then 3-hydroxynevirapine [15]. The nevirapine-induced skin rash in rats is hypothesized to be caused by a reactive metabolite of 12-hydroxynevirapine [16].

The aim of this study was to develop a model to describe the pharmacokinetics of nevirapine and its metabolites in South African HIV-infected patients on nevirapine-based antiretroviral therapy during the continuation phase of a rifampicin-based short-course chemotherapy.

## Methods

The data were obtained from a cross-sectional study which included intensive and sparse sampling in a total of 53 patients.

### Study design

Twenty-seven HIV-infected patients with a minimum age of 18 years were enrolled during the continuation phase of standard TB treatment. They were established on an antiretroviral regimen consisting of 200 mg nevirapine [11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido (3,2-b:2'', 3''-e) [1, 4] diazepin-6-one; Viramune (Boehringer Ingelheim, Ingelheim am Rhein, Germany) or Aspen (Durban, South Africa) generic] given orally twice daily and two NRTIs, stavudine or zidovudine, together with lamivudine. Patients with Karnofsky scores (quantifies patients' general well-being, with 100 being perfect health and 0 indicating death) below 70, known severe renal, hepatic or gastrointestinal disease, including malabsorption or severe diarrhoea, or those receiving other medication that might have (on the basis of a known interaction, or strong theoretical basis) affected nevirapine, rifampicin or isoniazid plasma concentrations were excluded. The patients were asked if they were taking over the counter drugs, natural products, traditional medicine or any other drug apart from the drugs involved in this study. They all responded with a negative answer. The patients were receiving standard, recommended weight-based doses of rifampicin and isoniazid. They were enrolled and admitted for pharmacokinetic blood sampling. A minimum of 14 days after TB treatment was completed, the patients were re-admitted for pharmacokinetic blood sampling.

Twenty-six HIV-positive patients who did not have TB were enrolled as a control group using the same inclusion and exclusion criteria. The patients were on an antiretroviral regimen consisting of two NRTIs and nevirapine (200 mg orally twice daily) for at least 3 weeks.

Seventeen TB/HIV-coinfected patients and eight controls underwent intensive pharmacokinetic sampling. Blood samples were taken just before drug administration and at approximately the following time points 0.5, 1, 1.5, 2, 4, 6, 10, 12, 12.25, 12.75, 14, 22 and 24 h after drug administration. Sparse sampling was used for the remainder of the patients in both groups: two blood samples, a minimum of 1 h apart, were obtained on each sampling occasion. Venous blood was collected in 4-mL LiH Pst Gel plastic vacuum tubes, stored on ice until sample collection at that time point was complete for all patients, then immediately centrifuged for 10 min at 3000 rpm. The blood for the intensive samples taken after 12 h and for the sparse samples was not immediately centrifuged or stored on ice

before centrifugation. Plasma was transferred to labelled 1.2-mL microcentrifuge tubes, which were stored at -80°C until analysis.

Standardized meals were given during admissions for blood sampling. Adherence during the week prior to admission was assessed by means of a questionnaire. Patient covariates, such as gender, age, weight, alanine aminotransferase, albumin, total bilirubin, haemoglobin, WHO HIV disease stage and concomitant medications, were recorded (Table 1). For patients coinfecting with TB/HIV, covariates were recorded on both pharmacokinetic sampling occasions.

The study was conducted according to the guidelines of the Helsinki declaration of 2000, and the protocol and informed consent documents were approved by the Research Ethics Committee of the University of Cape Town, South Africa before patients were enrolled in the study.

**Table 1** Characteristics of South African patient population

Characteristics	Number of patients	Median (range)
Total number of patients (male/female)	53	(12/40)
TB/HIV-coinfecting patients		
Intensive	17	
Sparse	10	
HIV-infected patients without tuberculosis		
Intensive	8	
Sparse	18	
Continuous covariates		
Age (years)	51	34.6 (21.1–58.4)
Weight (kg)	52	67.6 (42.5–105)
Albumin (g/L)	25	41.0 (24.0–60.0)
Alanine aminotransferase (U/L)	25	25.0 (10.0–127)
Total bilirubin ( $\mu\text{mol/mL}$ )	25	5.00 (2.00–17.0)
Haemoglobin (g/dl)	25	12.4 (6.00–17.1)
Categorical covariates (TB/HIV patients during TB treatment/ TB/HIV patients after TB treatment/HIV patients)		
WHO disease stage		
2	0/0/2	
3	13/8/15	
4	14/14/9	
Concomitant medications		
Stavudine	16/13/7	
Zidovudine	0/2/1	
Lamivudine	16/14/8	
Cotrimoxazole	13/10/4	
Vitamin B complex	5/3/2	
Multivitamins	3/0/1	
Pyridoxine	5/1/0	
Warfarin	2/0/0	
Ethambutol	5/0/1	

TB, Tuberculosis; HIV, human immunodeficiency virus

A noncompartmental analysis of part of this study, including intensively sampled TB/HIV-coinfecting patients (up to 12 h after nevirapine administration), has been reported earlier [17].

#### Drug quantification

The method used to measure plasma concentrations of nevirapine and its metabolites 2-, 3-, 8- and 12-hydroxynevirapine was developed based on a previously published method [18].

Nevirapine plasma concentrations were measured using a liquid chromatography–mass spectrometry method. Liquid chromatography was performed on a Waters Alliance (model 2690; Milford, MA) high-performance liquid chromatography (HPLC) system and a Hypersil Gold C18 column (20×2.1 mm; Thermo Fisher Scientific, Waltham, MA). The mobile phase consisted of 50% acetonitrile in 4 mM ammonium acetate, 0.1% trifluoroacetic acid. The flow rate was 0.3 mL/min. For detection, a Waters/Micro-mass ZMD single quadrupole mass spectrometer was used in positive ionization mode. The detection of nevirapine was at 276.2 *m/z* and that of neostigmine (internal standard) at 223.2 *m/z*. A 20- $\mu\text{L}$  volume of sample or the control was precipitated with 50  $\mu\text{L}$  acetonitrile containing 1 mg/L neostigmine. After vortexing for 30 s and centrifuging for 5 min at 750 *g*, a 2- $\mu\text{L}$  volume of the supernatant was injected. The standard curve was linear in the range 0.2–20 mg/L, and the lower limit of quantification (LLOQ) was 0.2 mg/L. In each run, three quality control samples of 0.8, 3 and 9 mg/L were included.

Plasma concentrations of the nevirapine metabolites, 2-, 3-, 8- and 12-hydroxynevirapine, were determined by a tandem mass spectrometry method. The system consisted of an Applied Biosystems (Foster City, CA) API 3200 linear ion trap. Liquid chromatography was performed using an Agilent 1200 series HPLC instrument and a Gemini C18 column (particle size 3  $\mu\text{m}$ , 2.0×50 mm; Phenomenex, Torrance CA). The mobile phase contained ammonium formate (5 mM) and acetonitrile in the ratio of 15:85. The flow rate was set to 0.3 mL/min. The selected reaction monitoring transitions of  $[\text{M} + \text{H}]^+$  precursor ions to product ions were *m/z* 283.2–214.1 for 3-hydroxynevirapine, *m/z* 283.2–160.9 for 2-hydroxynevirapine, *m/z* 283.2–265.2 for 12-hydroxynevirapine, *m/z* 283.2–242.3 for 8-hydroxynevirapine and *m/z* 276.3–162.3 for physostigmine (internal standard). A 100- $\mu\text{L}$  volume of plasma was precipitated with 300  $\mu\text{L}$  acetonitrile containing 0.5  $\mu\text{g/mL}$  of the internal standard. After mixing for 10 s and centrifugation for 5 min at 750 *g*, 10  $\mu\text{L}$  of the supernatant was diluted with 1000  $\mu\text{L}$  of the mobile phase. The injected volume was 5  $\mu\text{L}$ . The standard curves were linear in the range 0.025–5 mg/L, with a regression coefficient greater than 0.999 for

all compounds. In each run, three quality control samples of 0.12, 0.9 and 4.5 µg/ml were included. The LLOQ was 0.025 mg/L.

For nevirapine and its metabolites; the inter- and intraday precision was less than 12% across the range of quantification and less than 10% for the control samples. Accuracy ranged from 89 to 115%. Samples showed no loss of stability of either nevirapine or its metabolites when stored at either –20 or –80°C for a period of 12 months. At room temperature, nevirapine in whole blood was stable for up to 1 week, and the metabolites in spiked plasma were stable for up to 24 h while 12-hydroxynevirapine in whole blood was stable up to 24 h.

### Data analysis

Population pharmacokinetics of nevirapine were described using nonlinear mixed effects modelling within NONMEM version VI (GloboMax, Hanover, MD) [19]. The typical population pharmacokinetic parameters and interindividual and residual variabilities were estimated by the first-order conditional estimation method with interaction.

One- and two-compartment models with linear elimination and first-order absorption were fitted to the data. Elimination was assumed to take place from the central plasma compartment.

An exponential error model was used to describe interindividual variability (IIV) to avoid negative individual parameter estimates:

$$P_i = P_{pop} \cdot \exp(\eta_i) \quad (1)$$

where  $P_i$  is the individual parameter,  $P_{pop}$  is the parameter for a typical individual and  $\eta_i$  is the IIV that describes the difference between the typical predicted value and the individual estimate. The variable  $\eta_i$  was normally distributed with mean 0 and variance  $\omega^2$ . The difference between predicted and observed concentrations was expressed as residual variability. The different error models that were investigated were additive, proportional and slope–intercept models. Uncertainty in the parameter estimate was expressed as relative standard error (RSE), expressed as the ratio of the estimated standard error (SE) of the parameter relative to its value.

The potential covariate relationships of the recorded covariates were tested. Covariates were then tested in the model by stepwise addition using an objective function value (OFV) change of >10.83 ( $p < 0.001$ ) as the cut-off for inclusion, followed by stepwise deletion using an OFV change of >12.12 ( $p < 0.0001$ ) to retain the effect on the selected parameters. Only two individuals had WHO disease stage 2; this category was therefore merged with WHO stage 3, the most common category. In addition,

when a concomitant medication was taken by only one individual, it was excluded from the covariate analysis.

Model selection was based on the OFV, which is minus twice the log likelihood of the data. A drop in the OFV of 6.63 between two nested models corresponding to  $p < 0.01$  was regarded as being statistically significant. Other model selection criteria were graphical analysis using XPOSE ver. 3.104 and ver. 4 [20], standard error of parameter estimates obtained from the output of the NONMEM software and scientific plausibility.

Model validation was done by bootstrap resampling [21]. Parameter estimates were re-estimated for each of the 1000 bootstrap samples. The median and 95% confidence intervals were also compared with the NONMEM estimates from the final model. Model validation was also done by simulations and a predictive check, where the final model and parameter estimates were used to simulate 1000 data sets based on the original design. Observed concentrations were compared to the simulated median and 95% nonparametric prediction intervals.

Nevirapine plasma concentrations after twice-daily doses of 300, 400 and 500 mg, respectively, were simulated in intensively sampled TB/HIV-coinfected patients during rifampicin-containing TB treatment based on the individual pharmacokinetic parameters from the final model.

The area under the plasma concentration–time curve within a dosing interval (AUC) of the nevirapine metabolites were calculated with linear trapezoids for ascending intensive data and log trapezoids for descending intensive data. The AUCs were calculated separately for concentrations following the morning dose (AUC-day) and following the night dose (AUC-night). The paired  $t$  test (S-PLUS ver. 6.2 for Windows; Insightful, Seattle, WA) was used for comparing AUC-day for TB/HIV patients during TB treatment to AUC-day for the same patients after TB treatment. The comparison for AUC-night was carried out in the same manner. A  $p$  value of <0.05 was considered to be statistically significant.

### Results

Nevirapine population pharmacokinetics in TB/HIV- and HIV-infected s ( characteristics provided in Table 1) was best described by a one-compartment model with first-order absorption and elimination. Concomitant administration of rifampicin increased nevirapine oral clearance (CL/F) by 37.4% and reduced the absorption rate constant ( $k_a$ ) by almost sixfold. In order to describe the circadian rhythmicity in the observed concentration–time data, a covariate for day or night was used. Day was defined as the time between sunrise and sunset, while night was the time between sunset and sunrise. The study was conducted over

a range of 11 months where sunrise ranged between 5.29 a.m. and 7.52 a.m., and sunset sunrise ranged between 5.48 p.m. and 7.41 p.m. The shortest day was 9 h 56 min long, while the longest day was 14 h 9 min long. Circadian rhythm in the different parameters was tested and found to be significant for oral clearance (CL/F) and absorption rate constant ( $k_a$ ). Circadian rhythm was also tested by different coding of day and night where day was defined as the time between 8 a.m. and 10 p.m. while night was the time between 10 p.m. and 8 a.m. This latter approach to coding resulted in higher OFV compared to the sunrise/sunset approach; consequently, the sunset/sunrise approach was adopted. Nevirapine  $k_a$  at night was coded as the sum of the elimination rate constant ( $k$ ) and an additional estimate to avoid flip-flop. The need for an absorption lag time was also tested and determined to be need only at night; it was therefore fixed to zero during the day. The estimates and the RSE are summarized in Table 2. The CL/F increased by 1.56% with every 1-year increase in age relative to the population median age of 34.6 years. In addition, the CL/F increased by 2.84% with every 1 g/L increase in albumin relative to the population median albumin of 41 g/L. Concomitant administration of the vitamin B complex was significant for CL/F as well. The volume of distribution (V/F) increased by 1.42% with every 1 kg increase in weight relative to the population median weight of 67.6 kg (Table 2). The

precision in IIV for CL/F at night in the HIV s group was low. However, we decided that it needed to be included in the final model due to a significant drop in OFV and, most importantly, an improvement in the visual predictive check plot, which indicated that the parameter helped to explain variability in the data.

The results of the bootstrap validation are represented in Table 2. Figure 1 shows visual predictive check plots obtained from the simulation of 1000 data sets based on the final model. The percentage of the observed concentrations above the simulated median and the percentage of the observed concentrations below the simulated median was (57:43), (51:49) and (47:53) in TB/HIV-infected patients during TB treatment, after TB treatment and in HIV-infected patients, respectively. This indicates that there was a good agreement between the medians of the simulated and observed concentrations based on the final model. The percentage of observed concentrations outside the simulated 90% prediction interval was 9, 5 and 5% in TB/HIV-infected patients during TB treatment, after TB treatment and in HIV-infected patients, respectively. Most of the discrepancy was in the higher concentration range where the simulations from the model resulted in somewhat higher concentrations compared to the observed, indicating that the model is overpredicting variability. Figure 2 shows the individual predicted nevirapine concentrations versus

**Table 2** Parameter estimates from the final pharmacokinetic nevirapine model

Parameter	NONMEM results		Bootstrap results	
	Mean	RSE	Median	95% CI
CL/F day (L/h)	2.76	0.057	2.73	2.49–3.02
CL/F night (L/h)	3.78	0.11	3.71	3.07–4.29
Increase in CL/F with rifampicin (%)	37.4	0.26	42.2	26.5–59.5
V/F (l)	137	0.072	134	117–153
$k_a$ day ( $h^{-1}$ )	3.57	0.61	3.01	2.01–32.2
$k_a$ night ( $h^{-1}$ )	$k+0.24$	0.39	$k+0.201$	$k+(0.084-0.355)$
$k_a$ day with rifampicin ( $h^{-1}$ )	0.613	0.42	0.618	0.357–1.13
$k_a$ night with rifampicin ( $h^{-1}$ )	$k+0.813$	0.55	$k+0.629$	$k+(0.215-1.70)$
Absorption lag time at night (h)	0.73	0.0016	0.636	0.344–0.731
Effect of age on CL/F ( $\theta_{CL/F, AGE}$ )	0.0156	0.67	0.0202	0.00718–0.0274
Effect of albumin on CL/F ( $\theta_{CL/F, ALB}$ )	0.0284	0.18	0.0291	0.0172–0.0397
Effect of Vitamin B complex on CL/F ( $\theta_{CL/F, BCOM}$ )	0.118	0.93	0.109	-0.0525–0.322
Effect of body weight on V/F ( $\theta_{V/F, WT}$ )	0.0142	0.3	0.0133	0.00753–0.0196
IIV in CL/F (TB/HIV group without rifampicin) and in CL/F day (HIV group)	0.307	0.27	0.291	0.203–0.362
IIV in CL/F (TB/HIV group with rifampicin)	0.314	0.24	0.289	0.217–0.351
IIV in CL/F night (HIV group)	0.14	2.3	0.172	0.031–0.491
IIV in $k_a$ day	0.71	0.85	0.625	0.179–1.14
IIV in $k_a$ night (TB/HIV group)	2.0	0.53	1.62	0.656–2.56
IIV in $k_a$ night (HIV group)	1.2	0.53	1.25	0.953–1.60
Proportional residual variability	0.128	0.095	0.126	0.112–0.146

RSE, Relative standard error; CI, confidence interval; CL/F, oral clearance; V/F, volume of distribution;  $k_a$ , absorption rate constant;  $k$ , elimination rate constant that is equal to the oral clearance divided by the volume of distribution; IIV, interindividual variability.  $(CL/F)_{pop} = (CL/F) \cdot \{1 + [\theta_{CL/F, AGE} \cdot (AGE - 34.6)]\} \cdot \{1 + [\theta_{CL/ALB} \cdot (ALB - 41.0)]\} \cdot \{1 + [\theta_{CL/F, BCOM}]\} \cdot (V/F)_{pop} = (V/F) \cdot \{1 + [\theta_{V/F, WT} \cdot (WT - 67.6)]\}$

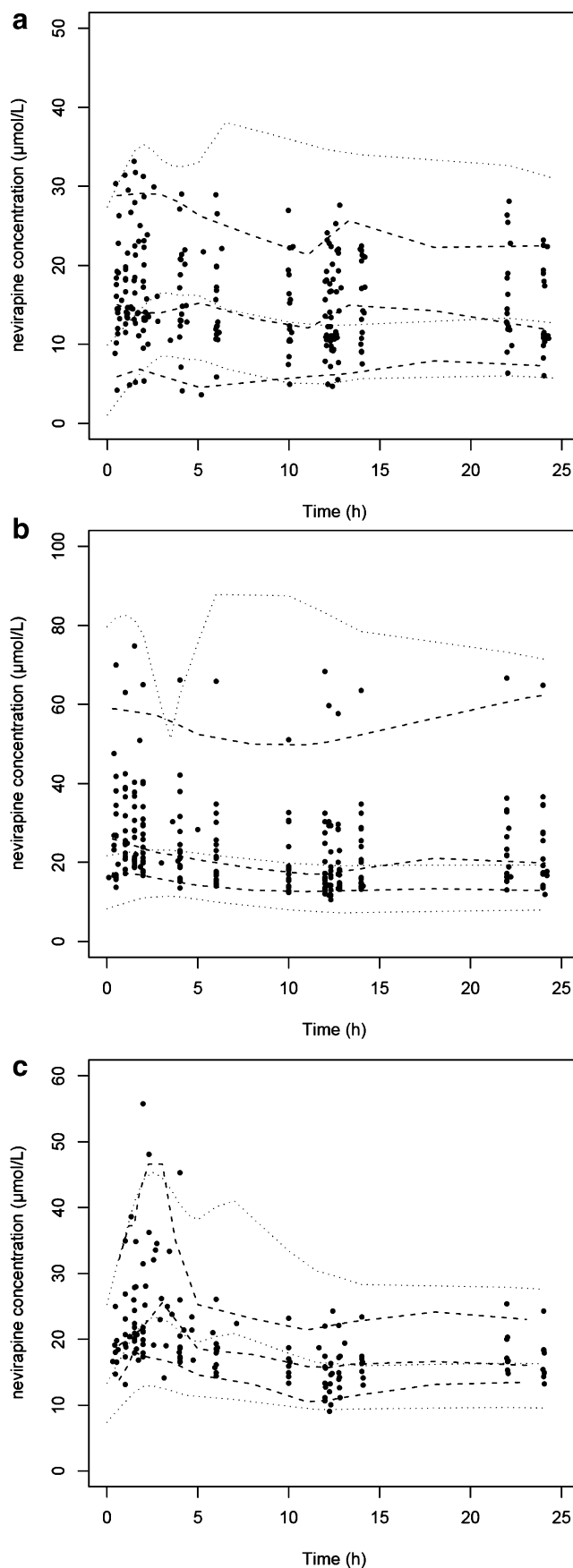


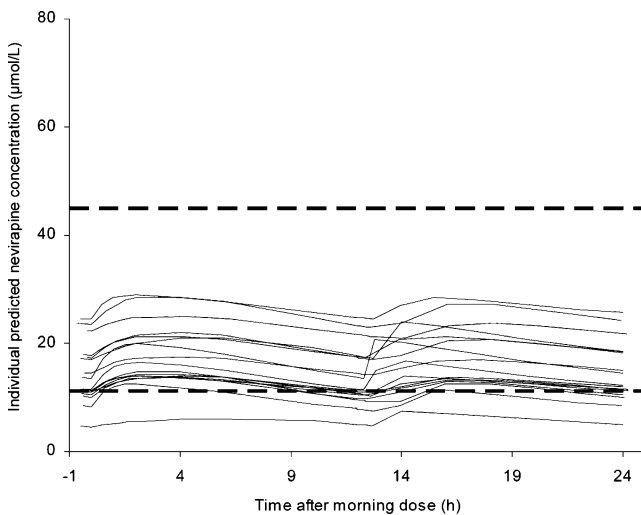
**Fig. 1** Observed nevirapine concentration compared to simulated median and prediction interval of 1000 simulations in tuberculosis/human immunovirus (TB/HIV)-coinfected patients during the continuation phase of standard tuberculosis treatment (a), after completion of TB treatment and a washout period of more than 14 days (b) and in HIV-infected patients (c). Filled circles are the observed concentrations. The highest and lowest dashed lines demarcate the 95% range of the observed concentrations, the middle dashed line is the median of the observed concentrations. The highest and lowest dotted lines demarcate the simulated 95% nonparametric prediction interval, the middle dotted line is the median of the simulated prediction interval

time after the morning dose in TB/HIV-infected patients during TB treatment. In the intensively sampled TB/HIV-infected patients during TB treatment, the observed concentrations of one patient were always below 3 mg/L, while eight patients had at least one time point below 3 mg/L. In one patient of the sparsely sampled TB/HIV-infected patients during TB treatment, the two observed concentrations were below 3 mg/L. Three patients had at one time point each—a plasma concentration below 3 mg/L. All observed concentrations of sparsely sampled patients were within the range of the observed concentrations of the intensively sampled patients. Simulations were therefore only carried out for intensively sampled patients. The simulation of different nevirapine doses, based on the individual pharmacokinetic parameters from the final model, is shown in Fig. 3a–c. The simulation of the 300 mg dose twice daily resulted in one out of 17 patients having all plasma concentrations below 3 mg/L. After simulation of the 400 mg dose twice daily, only one of the patients had simulated plasma levels below 3 mg/L, and after a simulated dose of 500 mg twice daily none of the simulated plasma levels were below 3 mg/L. Upon simulation of 400 and 500 mg nevirapine twice daily, a number of patients had plasma concentrations above 12 mg/L (Fig. 3b and c).

The minimum concentration ( $C_{\min}$ ) of nevirapine was calculated for the intensively sampled TB/HIV-infected patients: it was 39% lower with co-administration of rifampicin-containing TB therapy than without.

Of the four analysed nevirapine metabolites, namely 2-, 3-, 8- and 12-hydroxynevirapine, only 12-hydroxynevirapine was found in concentrations above the LLOQ of 0.025 mg/L. Data for 12-hydroxynevirapine were only available from 16 intensively sampled TB/HIV-infected patients during and after TB treatment. There was no consistent trend in the percentage change of individual AUC-day or AUC-night between these two sampling periods. In some patients, the metabolite concentrations were higher after TB treatment, and in some patients they were lower. The concentration–time profiles of the two individuals with highest and lowest percentage change are shown in Fig. 4. Several attempts were made to extend the





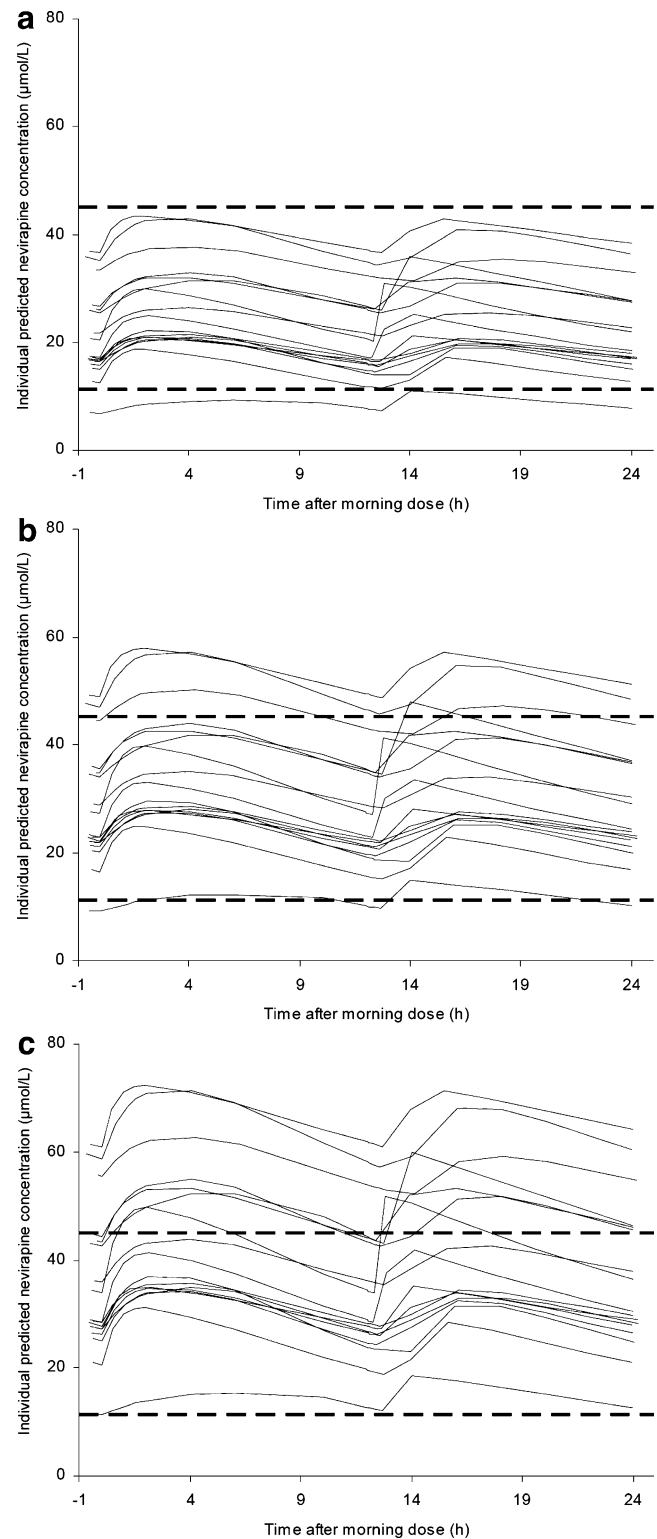
**Fig. 2** Individual predicted nevirapine concentrations versus time in TB/HIV-coinfected patients during the continuation phase of standard TB treatment. The lower dashed horizontal line is the cut-off value of 11.3  $\mu\text{mol/L}$  (3 mg/L) below which dose adjustment has been suggested, the upper dashed horizontal line is the cut-off value of 45.1  $\mu\text{mol/L}$  (12 mg/L) above which dose adjustment has been suggested

parent compound pharmacokinetic model to include the metabolite. However, the concentrations were not well predicted. Noncompartmentally derived AUC-day, median (interquartile range) for TB/HIV-infected patients during TB treatment was 2.9 (2.2–3.2) h.mg/L; for TB/HIV-infected patients after TB treatment, it was 2.8 (2.6–4.0) h.mg/L. The median AUC-night median (interquartile range) for TB/HIV-infected patients during and after TB treatment was 2.3 (1.7–3.6) and 2.7 (2.1–3.7) h.mg/L, respectively. There was no statistical significant difference between these sampling periods (during and after TB treatment) for AUC-day or AUC-night.

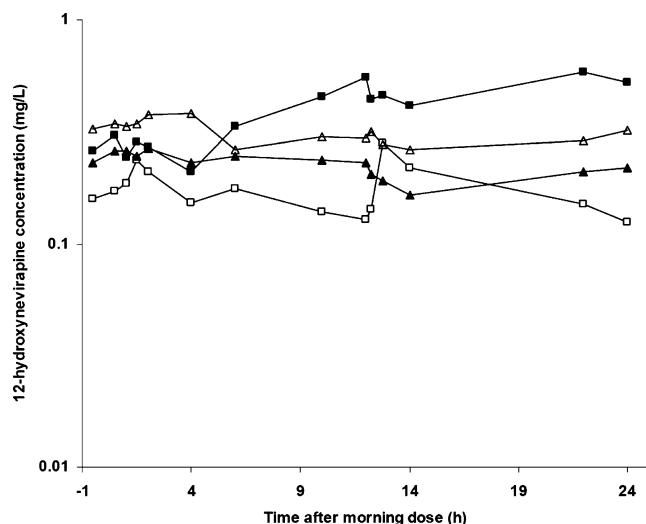
In the TB/HIV group, one of the intensively sampled and three of sparsely sampled patients failed to return after termination of the TB treatment. The data of one of the sparsely sampled patients was not available during TB treatment. One intensively sampled TB/HIV-infected patient was re-admitted for blood sampling 10 days after the TB treatment was completed.

## Discussion

Nevirapine concentrations below 3 mg/L increase the risk of virological failure by fivefold [7], and dose adjustment has been suggested if nevirapine  $C_{\text{min}}$  is lower than 3 mg/L or higher than 12 mg/L [22, 23]. Our results show that nevirapine concentrations following twice daily doses of nevirapine 200 mg can be lower than the recommended 3 mg/L when taken concomitantly with rifampicin (Fig. 2). The observed concentrations in two patients were always



**Fig. 3** Individual predicted nevirapine concentrations versus time in TB/HIV-coinfected patients, during the continuation phase of standard TB treatment simulated, based on the individual pharmacokinetic parameters from the final model, with a dose of 300 mg twice daily (a), 400 mg twice daily (b) and 500 mg twice daily (c). The lower dashed horizontal line is the cut-off value of 11.3  $\mu\text{mol/L}$  (3 mg/L) below which dose adjustment has been suggested, the upper dashed horizontal line is the cut-off value of 45.1  $\mu\text{mol/L}$  (12 mg/L) above which dose adjustment has been suggested



**Fig. 4** Observed 12-hydroxynevirapine concentration–time profiles in the two individuals with the highest (*squares*) and lowest (*triangles*) percentage change of the area under the curve (*AUC*) with rifampicin treatment (*filled symbols*) compared to after washout (*open symbols*)

below 3 mg/L, while 11 patients had at least one plasma concentration below 3 mg/L. On average, rifampicin reduced nevirapine  $C_{\min}$  by 39%. Simulations of different doses of nevirapine revealed that increasing the dose to 300 mg twice daily resulted in fewer patients being exposed to subtherapeutic levels and there was a minimum exposure to levels above the recommended threshold of 12 mg/L for dose reduction (Fig. 3a). At doses of 400 mg and 500 mg twice daily, few or no patients, respectively, will be exposed to subtherapeutic levels, but there will be a risk of high plasma levels in some of the exposed patients (Fig. 3b and c). From a pharmacokinetic point of view, increasing the dose to 300 mg twice daily seems to be a satisfactory approach that needs to be studied in patients with respect to nevirapine efficacy and toxicity.

In a previous study, the concomitant use of nevirapine and rifampicin in HIV-infected patients led to a significant reduction in nevirapine AUC (by 58%), maximum concentration  $C_{\max}$ ; by 50%—and  $C_{\min}$ — (68%) [4]. In another group of 13 HIV-infected patients, rifampicin reduced the nevirapine AUC by 46%, the  $C_{\max}$  by 42% and the  $C_{\min}$  by 53%, and in eight of the 13 patients the  $C_{\min}$  was below 3 mg/L. Increasing the nevirapine dose to 300 mg twice daily elevated  $C_{\min}$  above 3 mg/L [9]. Co-administration of nevirapine and rifampicin in five patients coinfecting with HIV and TB led to a significant reduction in nevirapine AUC<sub>0–12</sub> (by 31%) and  $C_{\max}$  (by 36%) [10], but there was no significant decrease in  $C_{\min}$ . This result is consistent with the results of another study in seven patients coinfecting with HIV and TB showing no decrease in nevirapine  $C_{\min}$  [11]. In 74 Thai patients coinfecting with HIV and TB, rifampicin reduced the mean plasma concentrations of nevirapine, but more than 86% of the patients

had their concentrations above 3.1 mg/L [12]. The authors of the study suggested that this result was due to the low body mass index of such patients. In another study on 70 Thai patients coinfecting with HIV and TB, rifampicin reduced the nevirapine  $C_{\min}$  by 17%, but more than 70% of the patients had concentrations above 3.4 mg/L [13]. These patients were followed after the discontinuation of rifampicin, and their  $C_{\min}$  increased by 16.7% [14].

The estimates from the final population pharmacokinetic model of CL/F, V/F and  $k_a$  are consistent with previous reports of nevirapine population pharmacokinetics in other populations [24–26]. In our study, rifampicin induced the metabolism of nevirapine, seen as an increase in its CL/F by 37.4%, which is in agreement with previous reports of rifampicin altering the pharmacokinetics of nevirapine [4, 9, 10, 12, 13]. This may be due to the induction of CYP3A4 and CYP2B6 leading to an enhancement of nevirapine metabolism. The absorption rate of nevirapine, after the morning dose, was almost sixfold lower when co-administered with rifampicin. Rifampicin has been shown to decrease the rate of absorption of cyclosporine [27]. Further, in the presence of P-glycoprotein (P-gp) inhibitors, the  $k_a$  of [<sup>14</sup>C]bepotastine in the small intestine has been shown to increase [28]. Nevirapine may be a substrate for P-gp [29, 30]. It is possible that rifampicin alters the absorption rate of nevirapine since it induces intestinal P-gp [31].

The three parameters found to be different during the night compared to the daytime were the CL/F,  $k_a$  and absorption lag time. The CL/F and the absorption lag-time were found to be higher during the night, whereas  $k_a$  was found to be lower during the night than during the daytime. The circadian rhythm has previously been reported to be a small trend of a higher AUC due to a higher  $C_{\max}$  that is reached earlier and of a lower oral clearance after the morning dose [32]. Nevirapine is lipophilic [33], and it is known that some lipophilic drugs show an earlier and higher  $C_{\max}$  after the morning dose than when the drug is given in the evening. Rapid absorption by passive diffusion of drugs given in the morning may be due to faster gastric emptying and a higher perfusion of the gastrointestinal tract [34]. Nevirapine is metabolized by CYP3A4, which is found in the intestine. The rapid absorption in the morning may decrease the time of contact between nevirapine and the metabolizing enzymes, thus resulting in decreased first-pass metabolism and increased bioavailability of nevirapine, which is seen as a lower CL/F in the morning compared to evening. However, this increased bioavailability was not seen in V/F. The model predicted a higher absorption rate, in the presence of rifampicin, after the evening dose in comparison to the morning dose. A possible hypothesis is that isoniazid might act as P-gp inhibitor [35]. Given the delayed absorption of isoniazid



[36], isoniazid could increase nevirapine absorption since it was given once daily with the morning dose of nevirapine and not at night.

Enterohepatic recycling of nevirapine has been shown in rats [37] and is suggested to be present in humans due to the presence of a second peak concentration following the administration of oral and intravenous single doses [32, 38, 39]. In this study, a second peak concentration was seen in some patients, but not in others who may have eaten at times different from the sampling times. Therefore, the implementation of an enterohepatic recycling model was judged not to be possible.

In the final model, albumin was found to be a predictor of variability in CL/F, where CL/F increased by 2.84% with every 1 g/L increase in albumin relative to the population median albumin of 41 g/L. Human immunodeficiency virus-infected patients suffer from altered metabolism, specific disturbances in protein turnover and weight loss [40]. Consequently, weight and albumin level seem to reflect metabolic disturbances, and a positive correlation between albumin and weight has been found in HIV-infected patients [41]. An increase in clearance with weight has been reported [25, 42], but the inclusion of weight in the model did not explain variability in CL/F, neither as the only covariate for CL/F or in combination with other covariates. Weight was therefore not included in the final model as a predictor of variability in CL/F. A lack of relationship between weight and nevirapine pharmacokinetics has also been seen in other studies [13, 24, 26, 43]. Age was also found to be a predictor of variability in CL/F, where CL/F increased by 1.56% with every 1-year increase in age relative to the population median age of 34.6 years.

The biotransformation rate of 12-hydroxynevirapine is best correlated with CYP3A4 although other enzymes are involved, including CYP2D6 and CYP2C9 at clinically relevant nevirapine concentrations [44]. There was no difference in the AUC values of 12-hydroxynevirapine with or without rifampicin treatment. This may be attributed to the different expression of enzymatic activity among patients. Since 12-hydroxynevirapine is metabolized by several enzymes, net enzymatic activity in each patient and, therefore, the effect of rifampicin on the CYP3A4 formation pathway of 12-hydroxynevirapine might explain the lack of trend.

In conclusion, the model presented here adequately described the population pharmacokinetics of nevirapine in South African HIV-infected patients in the absence and presence of TB infection and concomitant administration of rifampicin. Concomitant administration of nevirapine and rifampicin enhances the oral clearance of nevirapine and slows down its absorption, resulting in potentially sub-therapeutic levels in some patients. Increasing the dose of nevirapine to 300 mg twice daily seems to elevate low

concentrations to a value above the clinical threshold of 3 mg/mL, with minimum exposure levels above 12 mg/L.

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