Model-based approach to dose optimization of lopinavir/ritonavir when co-administered with rifampicin

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Doubling the dose of lopinavir/ritonavir overcomes the effect of rifampicin on lopinavir concentrations. However, lopinavir concentrations are highly variable and side effects occur commonly. Hence optimized dosing could limit the number of patients exposed to high lopinavir concentrations while maintaining adequate lopinavir concentrations.

WHAT THIS STUDY ADDS

- We built an integrated population pharmacokinetic model of lopinavir and ritonavir, describing the drug–drug interactions between lopinavir, ritonavir and rifampicin. Based on this model, we have predicted that lower doses of lopinavir/ritonavir can be used in patients weighing less than 50 kg. Also, diurnal variations on lopinavir and ritonavir were investigated for both bioavailability and clearance.

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AIMS

Rifampicin, a key component of antitubercular treatment, profoundly reduces lopinavir concentrations. The aim of this study was to develop an integrated population pharmacokinetic model accounting for the drug–drug interactions between lopinavir, ritonavir and rifampicin, and to evaluate optimal doses of lopinavir/ritonavir when co-administered with rifampicin.

METHODS

Steady-state pharmacokinetics of lopinavir and ritonavir were sequentially evaluated after the introduction of rifampicin and gradually escalating the dose in a cohort of 21 HIV-infected adults. Intensive pharmacokinetic sampling was performed after each dose adjustment following a morning dose administered after fasting overnight. A population pharmacokinetic analysis was conducted using NONMEM 7.

RESULTS

A simultaneous integrated model was built. Rifampicin reduced the oral bioavailability of lopinavir and ritonavir by 20% and 45% respectively, and it increased their clearance by 71% and 36% respectively. With increasing concentrations of ritonavir, clearance of lopinavir decreased in an $E_{\max}$ relationship. Bioavailability was 42% and 45% higher for evening doses compared with morning doses for lopinavir and ritonavir, respectively, while oral clearance of both drugs was 33% lower overnight. Simulations predicted that 99.5% of our patients receiving doubled doses of lopinavir/ritonavir achieve morning trough concentrations of lopinavir $> 1 \text{ mg l}^{-1}$ during rifampicin co-administration, and 95% of those weighing less than 50 kg achieve this target already with 600/150 mg doses of lopinavir/ritonavir.

CONCLUSIONS

The model describes the drug–drug interactions between lopinavir, ritonavir and rifampicin in adults. The higher trough concentrations observed in the morning were explained by both higher bioavailability with the evening meal and lower clearance overnight.

Introduction

Lopinavir/ritonavir (LPV/r), together with two nucleoside reverse transcriptase inhibitors (NRTIs), is the most widely used second-line antiretroviral regimen in low and middle income countries that follow World Health Organization (WHO) guidelines for adults [1]. A target lopinavir trough concentration greater than 1 mg l^{-1} is recommended for protease inhibitor-naïve patients [2]. Rifampicin, a key component of antitubercular therapy, reduces lopinavir trough concentrations by approximately 99% through induction of CYP3A4 and P-glycoprotein (P-gp) expression [3]. In adults this induction by rifampicin can be overcome by doubling the dose of LPV/r [4, 5]. However, side effects, such as nausea, vomiting and elevated transaminases, occur commonly when adjusted doses of LPV/r and rifampicin are co-administered [4, 6]. The antiretroviral activity of LPV/r is derived from lopinavir, but adverse effects are often related to ritonavir exposure [7]. Although altered lipid profiles and elevated liver enzymes have been associated with increased concentrations of lopinavir, the available data do not support an upper limit for the therapeutic range [2], and the presence of rifampicin, and possibly the sequence of treatment introduction with rifampicin and LPV/r, respectively, alters the risk of toxicity. Variability in lopinavir and ritonavir concentrations is high and may be accentuated when rifampicin is given concurrently [4, 5]. Hence optimized dosing may be better tolerated whilst maintaining adequate lopinavir concentrations. Therefore, alternative dosing approaches should be explored in specific sub-populations to limit the upper range of lopinavir concentrations.

Mixed-effect model-based approaches are particularly useful when applied to heterogeneous biological data because of their ability to characterize many sources of variability. These approaches allow simulation of treatment scenarios and thus the investigation of different dosing regimens and drug–drug interactions. Moreover, accurate predictions can be provided if the conditions of the simulated scenario are within the range of the experimental data. They can also be used to offer insight about scenarios outside the previously observed range, e.g. higher doses and/or other target populations. However, extrapolation beyond the range of the data should be applied with caution [8, 9].

We developed an integrated population model describing the interactions between lopinavir, ritonavir and rifampicin in HIV infected patients. Using the model, which included relevant patient and treatment covariate factors, we predicted optimal doses of LPV/r in patients with co-administered rifampicin, according to individual characteristics.

Methods

Study design and drug analysis

The study design, pharmacokinetic sampling and determination of analytes have been described previously [5]. All

patients gave written informed consent and the Cape Town University Human Research Ethics Committee approved the study. Briefly, 21 South African HIV-infected volunteers without tuberculosis were enrolled. The subjects were protease inhibitor-naïve and virologically suppressed on LPV/r plus two NRTIs. Steady-state pharmacokinetics of lopinavir and ritonavir were evaluated under four sequential treatment conditions: LPV/r (tablet formulation) in standard doses (400/100 mg 12 hourly) without rifampicin; LPV/r in standard doses and rifampicin 600 mg daily for 7 days; 1.5 times the standard dose of LPV/r (600/150 mg 12 hourly) and rifampicin 600 mg daily for 7 days; and 2 times the standard dose of LPV/r (800/200 mg 12 hourly) and rifampicin 600 mg daily for 7 days.

Intensive pharmacokinetic sampling was performed 1 week after each dose adjustment. Lopinavir and ritonavir plasma concentrations were determined using validated liquid chromatography-tandem mass spectrometry in plasma samples collected prior to an observed dose of LPV/r and at 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 12 h after drug administration. Morning doses were administered after fasting overnight, while the evening doses were given with a meal. The patients were admitted overnight for pharmacokinetic sampling and both morning and evening doses were directly observed. The lower limits of quantification (LLOQ) were 0.05 mg l^{-1} for lopinavir and 0.025 mg l^{-1} for ritonavir. Accuracy ranged from 97.6% to 104.9% for lopinavir and from 97.5% to 105.3% for ritonavir. The intra-day and inter-day precisions of both assays ranged from 0.1% to 4.7% and from 1.6% to 4.2%, respectively.

Population pharmacokinetic analysis

Population pharmacokinetic analysis was performed using the nonlinear mixed-effects modelling software, NONMEM (Version VII) [10]. The first order conditional estimation method (FOCE) with eta-epsilon INTERACTION was used for the estimation of pharmacokinetic parameters. Perl speaks NONMEM (PsN) 3.3.2 and Xpose (version 4.3.2) were used for model diagnostics [11, 12]. Different model structures and features were evaluated: one- and two-compartment disposition; zero, first order absorption, with and without lag, or through a series of transit compartments, as proposed by Savic *et al.* [13]. The inter-individual variability (IIV) and inter-occasional variability (IOV) [14] of the pharmacokinetic parameters of lopinavir and ritonavir were modelled as lognormal. Since variability in such a model can be approximately interpreted as a deviation proportional to the typical value, it will be reported as % coefficient of variation (CV). For the description of the residual unexplained variability (RUV), the presence of both an additive and a proportional component was tested.

Model development was guided by the objective function value (OFV) provided by NONMEM, which is assumed to be χ^2 distributed, precision in parameter estimates and

scientific plausibility. Goodness-of-fit plots and visual predictive checks were used during model building. In order to account for body size differences, allometric scaling was applied to apparent clearance (CL/F) and volume of distribution (V/F), investigating the use of total body weight, normal fat weight or fat free mass, as described by Holford *et al.* [15, 16].

First, two separate population pharmacokinetic models for lopinavir and ritonavir were developed. Once the basic model including allometric scaling was developed, the inclusion of the other candidate covariates (age and gender) was tested following a step wise approach similar to Wählby *et al.* [17] ($P \leq 0.05$ for forward inclusion and $P \leq 0.01$ for backward elimination). Reductions in IIV, IOV and RUV, improvement of goodness-of-fit plots and visual predictive check were also considered to decide on covariate inclusion.

These two separate models were then integrated in a combined model in which the dynamic pharmacokinetic interaction between lopinavir and ritonavir was explored. A sigmoid relationship was used to describe the effect of ritonavir concentrations on the CL/F of lopinavir (CL_{LPV}) as:

$$CL_{LPV} = CL_0 \times \left(1 - \frac{E_{\max} \times C_{RTV}}{EC_{50} + C_{RTV}} \right) \quad (1)$$

where CL₀ is the CL/F of lopinavir when no ritonavir is present, E_{max} is the maximum inhibition effect of ritonavir on lopinavir, EC₅₀ is the ritonavir concentration required to reach half of E_{max}, and C_{RTV} is the concentration of ritonavir. The presence of a delay in the onset of inhibition of lopinavir CL/F by ritonavir was tested by using an indirect model [18].

The effects of rifampicin administration on the bioavailability and CL/F of both lopinavir and ritonavir were evaluated. The oral bioavailability of the standard dose of LPV/r without rifampicin co-administration was assumed as the reference (100%) to which the other conditions were compared. Also nonlinearity in bioavailability with dose for lopinavir and ritonavir was investigated.

Since the morning pre-dose concentrations (C₀) were on average higher than the evening trough concentrations (C₁₂), differences in bioavailability and CL/F after the evening and morning doses were tested in our model to capture the diurnal variation.

To evaluate the final combined model, a non-parametric bootstrap re-sampling method was used in order to obtain 95% confidence intervals (CI) of parameter estimates. However, due to the complexity of the model, leading to long computation times, only 10 samples were included.

The final model was used to simulate concentrations in 2000 *silico* subjects (with covariates values matching the study population) to evaluate dose regimens achieving lopinavir C₀ > 1 mg l⁻¹ in patients during rifampicin-based antitubercular co-treatment. To explore the possibility that

lower doses of LPV/r may be sufficient to achieve target concentrations in smaller patients, additional simulations using 2000 subjects were performed to predict the proportion of subjects with body weight 35–49 kg achieving lopinavir C₀ > 1 mg l⁻¹ on a 600/150 mg dose of LPV/r. Simulations were also conducted to evaluate the adequacy of doubled doses (800/200 mg twice daily) of LPV/r in patients with weights 111–130 kg. The body weight used in simulations for each range was distributed evenly.

Results

Patients and data description

The demographic characteristics of patients and trough concentrations observed (C₀ and C₁₂) are summarized in Table 1 and Figure 1 respectively. Twenty-one patients, of whom 18 were females, were enrolled in the study, but three patients experiencing adverse events were withdrawn before they completed the study, so only partial data are available for them. Of these three patients, two developed grade 3/4 asymptomatic transaminitis (one on standard dose of LPV/r with rifampicin and the other on LPV/r 600/150 mg with rifampicin), while another patient withdrew consent after developing grade 2 nausea (on LPV/r 600/150 mg with rifampicin). Other adverse events were mild and resolved spontaneously. All participants had 100% adherence according to regular pill counts and questioning throughout the study period. Fat free mass was calculated using the approach proposed in Janmahasatian *et al.* [19]. A total of 800 concentrations were collected for both lopinavir and ritonavir. Data below the LLOQ of the assay were few in the dataset (1% of total data for lopinavir and 2% for ritonavir) and were set to LLOQ/2 values (0.025 mg l⁻¹ for lopinavir and 0.0125 mg l⁻¹ for ritonavir) in model building.

Model description

The structure of the final combined pharmacokinetic model is illustrated in Figure 2, while parameter estimates

Table 1

Demographic characteristics of patients (n = 21)

Characteristic	Median (range)
Gender (F/M)	18/3
Age (years)	36 (26–58)
Body weight (kg)	64.5 (43.0–110.0)
Height (cm)	160.5 (148.0–186.5)
Body mass index (BMI, kg m ⁻²)	26.7 (17.4–41.4)
Fat free mass (kg)	39.5 (30.6–65.9)
Haemoglobin (g dl ⁻¹)*	11.5 (8.4–15.0)
Total bilirubin (μmol l ⁻¹)†	7.0 (2.0–24.0)

*normal range 11.5–15.0 g dl⁻¹. †normal range 0–21 μmol l⁻¹.

Figure 1

Observed morning and evening trough concentrations of lopinavir (A) and ritonavir (B). predose concentration C_0 (▨); trough concentration C_{12} (□)

Figure 2

Structure of the final integrated lopinavir-ritonavir pharmacokinetic model. (LPV: lopinavir; RTV: ritonavir; MTT: mean transit time; CL/F apparent oral clearance, V/F apparent volume of distribution, k_a absorption rate constant, k_{tr} transit absorption rate constant, E_{max} the maximum inhibitory effect on lopinavir oral clearance by ritonavir, EC_{50} the ritonavir concentration needed to reach half of E_{max} , C concentration)

are shown in Table 2. A one compartment model with first order absorption and elimination best described the pharmacokinetics of lopinavir. For ritonavir, a two compartment model was appropriate and a series of transit compartments was used to describe the complex kinetics in the absorption phase. IIV was supported in CL/F and V/F of both drugs, while IOV was characterized in absorption parameters (absorption rate and absorption mean transit time), bioavailability, and to a lesser extent in CL/F .

In the model, the CL/F of lopinavir was influenced by ritonavir concentration, according to an E_{max} function. This

dynamic interaction between ritonavir and lopinavir significantly improved the model fit ($\Delta OFV = -120$). An indirect model describing delay of the interaction was not supported by our data. The maximum inhibitory effect of ritonavir on lopinavir CL/F was estimated to be 95%, and EC_{50} was estimated to be 0.04 mg l^{-1} . The model estimated a value of 37.9 l h^{-1} for lopinavir CL/F when no ritonavir effect is present and ritonavir clearance was estimated to 19.2 l h^{-1} . The dynamic effect between ritonavir concentrations and lopinavir CL/F in a typical patient given different dose regimens is shown in Figure 3.

Table 2

Population pharmacokinetic parameter estimates based on the final model simultaneously fitting both lopinavir and ritonavir

Parameters	Lopinavir Estimates	95% CI*	Ritonavir Estimates	95% CI*
CL/F (l h ⁻¹)†	37.9	28.5, 52.1	19.2	18.4, 22.2
RIF on CL/F (+)‡	71.0%	65.7%, 75.4%	36.0%	35.2%, 40.0%
V _c /F (l)	54.7	50.5, 64.7	22.6	21.9, 24.6
k _a (h ⁻¹)	0.991	0.63, 1.43	3.28	2.90, 3.38
Relative bioavailability when given with RIF	0.80	0.76, 0.85	0.55	0.54, 0.59
Residual variability (proportional %)	12.7	11.6, 13.6	18.8	17.1, 20.3
Evening effect on bioavailability (+)‡	42.0%	38.0%, 48.2%	45.0%	41.4%, 53.6%
Evening effect on CL/F (-)‡	32.7%	29.6%, 38.4%	32.7%	29.6%, 38.4%
Bioavailability/10 mg ritonavir(+) ^c			8.1%	5.7%, 11.2%
Intercompartmental clearance (Q/F) (l h ⁻¹)			31.0	25.7, 34.7
V _p /F (l)			56.6	50.8, 66.0
NN			2.03	1.83, 2.37
MTT (h)			1.44	1.39, 1.53
IIV CL/F (%CV)	20.2	12.7, 25.1	21.5	11.5, 31.7
IIV V/F (%CV)	27.2	10.3, 41.4	10.2	9.85, 10.5
IOV F (%CV)	21.9	17.1, 24.0	30.3	24.1, 40.7
IOV k _a (%CV)	94.2	46.5, 150.1		
IOV CL/F (%CV)	11.8	4.55, 16.1	20.4	15.5, 25.1
IOV RUV (%CV)	17.1	9.63, 25.2		
IIV F (%CV)			30.3	17.4, 49.6
IOV MTT (%CV)			27.9	19.6–38.2
Lopinavir-ritonavir interaction				
E _{max}	95.3%	94.5%, 96.3%		
EC ₅₀ (mg l ⁻¹)	0.0351	0.0194, 0.0438		

*CI confidence intervals based on mean and SD from 10 bootstrap samples. †CL/F of lopinavir without ritonavir; ‡(+) the effect is increased (-) the effect is decreased; ^c increase in bioavailability per every 10 mg ritonavir dose. CL central clearance, V_c central volume of distribution, V_p peripheral volume of distribution, F bioavailability, k_a absorption rate, RIF rifampicin, NN number of transit compartment, MTT mean transit time, IIV interindividual variability, IOV interoccasion variability, RUV residual unexplained variability, E_{max} the maximum inhibition effect of ritonavir on lopinavir, EC₅₀ is the ritonavir concentration required to reach half of E_{max}.

Rifampicin was found to increase the CL/F of lopinavir and ritonavir by 71.0% and 36.0%, respectively. Rifampicin treatment also significantly reduced the bioavailability of both lopinavir and ritonavir, by 20.2% and 45.0%, respectively. The bioavailability of ritonavir increased with increasing doses. When co-administered with rifampicin, the relative bioavailability of ritonavir increased by 8.1% for each 10 mg increment of dose. Total body weight was found to be appropriate for allometric scaling of central and peripheral volumes of distribution, while fat free mass was more appropriate for the scaling of clearances. The inclusion of allometric scaling had a significant impact on the goodness of fit, improving the OFV by about 30 points and about 10% of IIV in clearance was explained for both drugs.

The median C₀ of both drugs was higher than C₁₂ on each occasion (Figure 1). Diurnal variations were detected on both bioavailability and CL/F of both drugs in our model. Bioavailability was higher for the evening dose than for the morning dose, being increased by 42% for lopinavir and 45% for ritonavir. The CL/F of both drugs was 32.7% lower overnight than during the dosing interval following the morning dose.

Model evaluation and simulation

The robustness and precision of the final model parameter estimates were confirmed by the 95% CIs from the bootstrap results (Table 2). Visual predictive check plots (1000 simulations) for the different sampling occasions are shown in Figure 4. The 5th, 50th and 95th percentiles of the observed data are in agreement with the 95% CI of each percentile for the simulated data. This supports the adequacy of the model and its suitability to investigate alternative dosing strategies using simulation. The model predicted that 99.5% of the subjects given 800/200 mg LPV/r during rifampicin co-administration would achieve the lopinavir target concentrations (≥ 1 mg l⁻¹) for C₀, but only 77.9% of subjects for C₁₂. Ten percent of the study population weighed <50 kg, and the maximum weight in the study population was 110 kg. Simulations showed that the target C₀ would be achieved with a 600/150 mg dose of LPV/r 12 hourly, in 95% of patients weighing <50 kg during rifampicin co-administration. An 800/200 mg dose of LPV/r was sufficient for patients weighing > 110 kg (95% achieve C₀ ≥ 1 mg l⁻¹ during rifampicin co-administration).

Figure 3

The influence of ritonavir concentrations (indicated in black) on the oral clearance of lopinavir (grey) in a typical patient. (LPV/r: lopinavir/ritonavir; rif: rifampicin). 400/100 mg LPV/r no rifampicin (.....); 400/100 mg LPV/r with rifampicin (---); 600/150 mg LPV/r with rifampicin (—); 800/200 mg LPV/r with rifampicin (---)

Figure 4

Visual predictive check (VPC) of the final combined model for lopinavir (A) and ritonavir (B) stratified by occasion (PK1–PK4) from 1000 simulations. The solid line is the median of the observed data and the dotted lines are the 5th and 95th percentiles of the observed data. The grey shaded areas are the 95% CIs for the median, 5th percentile and the 95th percentiles of the simulated data. Observed concentrations are displayed as circles. PK1: 400/100 mg LPV/r no rifampicin; PK2: 400/100 mg LPV/r with rifampicin; PK3: 600/150 mg LPV/r with rifampicin; PK4: 800/200 mg LPV/r with rifampicin

Discussion

In this study, we report an integrated population pharmacokinetic model, which includes covariate effects and describes the complicated induction and inhibition interactions between lopinavir, ritonavir and rifampicin. We also explain the diurnal variation of lopinavir and ritonavir concentrations that were observed. Finally, we have extended our previously reported findings that double dose LPV/r counteracts the inducing effect of rifampicin to predict alternative dose regimens of LPV/r for low and high weight patients.

The use of mixed-effects modelling allowed us to account for the concomitant effects of body size, food on bioavailability, diurnal variation of bioavailability and clearance, and complex drug–drug interactions. The same would have been very difficult to achieve by means of a non-modelling analysis. While this model-based approach provided a powerful analysis tool, it should be kept in mind that the model was developed and thus remains related to a specific population, study design, drug doses and formulations. Therefore, caution should be exercised when trying to apply the model outside the range of conditions in which it was developed.

As shown by la Porte *et al.* [4], either doubling the dose of the capsule form of LPV/r or boosting LPV/r with additional ritonavir (such that the ratio of lopinavir : ritonavir = 1) can sufficiently counteract the effect of rifampicin induction. Doubling the dose of LPV/r is more practical to implement, and may be associated with less hepatotoxicity than ritonavir boosting. However increased doses of LPV/r appear to be poorly tolerated in healthy volunteers given rifampicin [4, 6]. As reported by Decloedt *et al.* [5], we found that doubling the dose of the tablet form of LPV/r achieved adequate trough concentrations of lopinavir during rifampicin co-administration and was better tolerated in HIV infected individuals established on a LPV/r antiretroviral regimen.

Previous studies have reported single compartmental elimination kinetics for ritonavir [8, 20–22]. However, possibly because of the rich sampling schedule used in our study, a two compartment model was more appropriate to describe the kinetics in our data. The OFV drop of about 100 points and the improvement in the fit of the individual plots supported the inclusion of a peripheral compartment. A transit compartment absorption model described the delay in absorption better than a model using a lag time.

Full induction of drug metabolizing enzymes by rifampicin has been thought to be reached about 1 week after starting daily doses of rifampicin [23]. Hence in our study blood samples were taken 1 week after each dose adjustment. A recent report amongst tuberculosis patients started on a rifampicin-containing regimen suggests that autoinduction of rifampicin metabolism may be incomplete after 7 days (the half-life of the induction process was

estimated to be 6–8 days) [24, 25]. However, as autoinduction approached maximal levels (~85% of total) after 2 weeks, near full induction should have been achieved when patients in our study underwent pharmacokinetic evaluation on 1.5 and 2 times the standard dose of LPV/r with rifampicin. Besides the effect of rifampicin, the relative bioavailability of ritonavir was found to be influenced by its own dose. When standard doses of ritonavir (100 mg) were given with rifampicin, the relative bioavailability of ritonavir was 55.0%. This increased to 77.3% and 99.6% when 1.5 times and doubled doses, respectively, were given. Therefore the dose escalation results in a more than proportional increase in ritonavir plasma concentrations, which may also lead to a stronger inhibitory effect. This effect of ritonavir dose on its bioavailability might be explained by saturation of first pass metabolism. Moreover, as ritonavir is an inhibitor and a substrate of P-gp [21], it could inhibit its own efflux from enterocytes and hepatocytes.

The model estimated a value of 37.9 l h^{-1} for lopinavir CL/F when no ritonavir effect was present, but it should be kept in mind that this value was only an extrapolation, since lopinavir was never given without ritonavir. Figure 3 depicts the dynamic effect between ritonavir concentrations and lopinavir CL/F in a typical patient given different dose regimens, and can be used to provide real-life values of lopinavir CL/F when the two drugs are administered concomitantly, as is always the case during treatment. The sigmoidal inhibition model which was finally selected estimates both a very high value of E_{max} (95%) and a low EC_{50} (0.04 mg l^{-1}). This points towards the high inhibitory potency of ritonavir at low concentrations. Ritonavir is thought to be an irreversible inhibitor [26], but Ernest *et al.* reported that reversible mechanisms are also involved [27]. Like previous models, our model did not support irreversible inhibition [8, 9]. The drug–drug interactions between lopinavir and ritonavir are mediated by both enzymes and transporters. Lopinavir is known to have induction effects on ritonavir [28], but this phenomenon was not detectable in our data.

Body size was the only covariate found to have a significant effect in our model, and it was implemented through allometric scaling. For both lopinavir and ritonavir, fat free mass was used to scale CL/F, while total body weight was better suited for V/F. Since fat tissue normally contributes little to metabolism (fat weight fraction estimated 0.001), fat free mass is expected to be a better predictor for CL/F when there are wide variations in body composition [15]. This was the case for our cohort of subjects, in which the BMI ranged from 17.4 to 41.4 kg m^{-2} . On the other hand, fat contributes to V/F for lipophilic drugs like lopinavir and ritonavir. Jullien *et al.* [29] found CL/F of lopinavir to be related to age, gender and body weight, while V/F was related to body weight in children younger than 18-years-old. In our study, gender did not explain variability after inclusion of fat free mass. However, as few

patients were male (3/21), we cannot exclude an independent effect of gender.

In our study, median C_0 concentrations were higher than C_{12} . Similarly, Heeswijk *et al.* [30] reported that some C_0 concentrations were higher than C_{12} trough concentrations of lopinavir in HIV infected patients, but the effect did not reach statistical significance. Robbin *et al.* [31] also found diurnal variation in lopinavir pharmacokinetics, with higher concentrations in the morning than in the evening. They attributed this phenomenon to reduced hepatic blood flow during sleep or to changes in plasma lipid concentrations during the overnight fast, which may alter the rate of drug absorption or clearance. This pattern of diurnal variation has been identified by other investigators for ritonavir and other protease inhibitors [20, 32–34]. Since in our study patients received a meal before the evening dose was taken, while the morning dose was taken after a 10 h fast, food could be a possible explanation for this kind of variation. We found that lopinavir bioavailability was increased by 42.0% after the evening dose, which is in agreement with a previous study which described a 26.9% increase in the AUC of lopinavir when the tablet formulation was given with moderate fat content food [35]. The difference between morning and evening trough concentrations in our subjects was more pronounced than that reported by Awni *et al.* [35], which indicates the higher C_0 concentrations are unlikely to be entirely explained by a food effect. Besides the difference we detected in lopinavir and ritonavir bioavailability, lopinavir and ritonavir oral clearance was found to be slower overnight compared with that following the morning dose. Moreover, one may speculate that other effects play a role, for example in our study we cannot exclude an absorption interaction between rifampicin (which was given with the morning dose) and LPV/r.

Guidelines for therapeutic drug monitoring recommend a lopinavir trough concentration $\geq 1 \text{ mg l}^{-1}$ for patients who are naïve to protease inhibitors [36]. For practical reasons, morning trough concentrations are usually used for therapeutic drug monitoring studies linking antiviral effect to antiretroviral concentrations [4, 5, 32, 33]. In our study, all patients given 800/200 mg LPV/r with rifampicin co-administration achieved this target. Therefore, 800/200 mg LPV/r is sufficient for most patients during rifampicin-based antitubercular treatment, including patients weighing $>110 \text{ kg}$. For patients with body weight below 50 kg, 600/150 mg LPV/r is sufficient to maintain lopinavir C_0 concentrations above the target in 95% of subjects during rifampicin treatment. This reduced dose of LPV/r may improve safety. In contrast, it has previously been reported that doubling the dose of LPV/r oral solution in young children failed to achieve target concentrations [37]. Further research is necessary to elucidate the reasons underlying this difference between children and adults.

For protease inhibitor-experienced patients the minimum recommended trough concentration is based

on the phenotypic or genotypic viral resistance patterns. Our model could be used to estimate the number of patients achieving various target minimum concentrations. For example, an estimated 84% of the patients in our population given 800/200 mg doses of LPV/r 12 hourly during rifampicin administration would achieve a target trough concentration of 3.6 mg l^{-1} , for patients with four protease inhibitor associated mutations [2]. Therapeutic drug monitoring should be advised in this context. Safety concerns limit further increases in the dose of LPV/r with concomitant rifampicin, and alternative treatment strategies will be needed for protease inhibitor-experienced patients not meeting target concentrations with doubled doses of LPV/r.

In conclusion, a population pharmacokinetic model was developed to describe simultaneously the pharmacokinetics of lopinavir and ritonavir in adults, capturing the drug-drug interactions between the two drugs and rifampicin. The model proposed has good prediction properties and can be used to simulate alternative dosage regimens when lopinavir/ritonavir is co-administered with rifampicin. Doubling the dose of LPV/r is required for most protease inhibitor-naïve patients during rifampicin-based antitubercular treatment, but for patients weighing less than 50 kg, 600/150 mg LPV/r maintains lopinavir C_0 concentrations above the recommended minimum concentration. The higher trough concentrations detected in the morning are explained by both higher bioavailability due to a food which was given with the evening dose and lower clearance overnight.

Competing Interests

There are no competing interests to declare.

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