Model-based evaluation of the pharmacokinetic differences between adults and children for lopinavir and ritonavir in combination with rifampicin

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Doubled doses of lopinavir/ritonavir (LPV/r) compensate for the effect of rifampicin in adults, but fail to result in adequate lopinavir concentrations in young children on rifampicin-based antituberculosis therapy. The pharmacokinetic differences between adults and children administered lopinavir and ritonavir in combination with rifampicin are not clear.

WHAT THIS STUDY ADDS

- This study described the pharmacokinetics of lopinavir and ritonavir and the drug–drug interactions between lopinavir, ritonavir and rifampicin in children and adults through developing a comprehensive population model. Lower bioavailability of lopinavir and ritonavir, as well as the more potent effect of rifampicin-based antituberculosis treatment on bioavailability in children were found, which could explain the failure of double dose LPV/r to achieve adequate concentrations in children on antituberculosis treatment. Ritonavir dose had an important influence on the bioavailability of lopinavir and ritonavir. This study highlights the potential pitfalls of applying results obtained in adult studies to predict the magnitude of drug–drug interactions in children.

AIMS

Rifampicin profoundly reduces lopinavir concentrations. Doubled doses of lopinavir/ritonavir compensate for the effect of rifampicin in adults, but fail to provide adequate lopinavir concentrations in young children on rifampicin-based antituberculosis therapy. The objective of this study was to develop a population pharmacokinetic model describing the pharmacokinetic differences of lopinavir and ritonavir, with and without rifampicin, between children and adults.

METHODS

An integrated population pharmacokinetic model developed in NONMEM 7 was used to describe the pharmacokinetics of lopinavir and ritonavir in 21 HIV infected adults, 39 HIV infected children and 35 HIV infected children with tuberculosis, who were established on lopinavir/ritonavir-based antiretroviral therapy with and without rifampicin-containing antituberculosis therapy.

RESULTS

The bioavailability of lopinavir was reduced by 25% in adults whereas children on antituberculosis treatment experienced a 59% reduction, an effect that was moderated by the dose of ritonavir. Conversely, rifampicin increased oral clearance of both lopinavir and ritonavir to a lesser extent in children than in adults. Rifampicin therapy in administered doses increased CL of lopinavir by 58% in adults and 48% in children, and CL of ritonavir by 34% and 22% for adults and children, respectively. In children, the absorption half-life of lopinavir and the mean transit time of ritonavir were lengthened, compared with those in adults.

CONCLUSIONS

The model characterized important differences between adults and children in the effect of rifampicin on the pharmacokinetics of lopinavir and ritonavir. As adult studies cannot reliably predict their magnitude in children, drug–drug interactions should be evaluated in paediatric patient populations.

Introduction

The pharmacokinetic differences between adults and children can be further complicated by drug–drug interactions. Lopinavir/ritonavir (LPV/r; co-formulated in a ratio of 4:1) together with two nucleoside reverse transcriptase inhibitors (NRTIs) is recommended as the first line antiretroviral regimen for children under 2 years of age who have been exposed to non-nucleoside reverse transcriptase inhibitors [1], and is widely used as a second line regimen in adults [2]. Rifampicin, a key component of antituberculosis therapy, reduces the trough concentrations of lopinavir in LPV/r through induction of cytochrome P450 (CYP) 3A4 and P-glycoprotein (P-gp) expression. After 1 week of rifampicin, lopinavir trough concentrations were reduced by 80–95% in adults on standard doses of LPV/r [3]. In adult healthy volunteers the effect of rifampicin on lopinavir concentrations can be overcome by doubling the dose of LPV/r to 800/200 mg 12 hourly, or by adding extra ritonavir (a potent inhibitor of CYP 3A4 and P-gp) to the standard dose of LPV/r, to give a 12 hourly dose of 400 mg of lopinavir and 400 mg of ritonavir [4]. The former approach has been confirmed in HIV infected adult volunteers who uniformly achieved pre-dose target trough concentrations ($>1 \text{ mg l}^{-1}$) on doubled doses of LPV/r after 3 weeks of rifampicin daily [3]. However, doubling the dose of LPV/r failed to achieve target lopinavir trough concentrations in 59% of young children on rifampicin-based antituberculosis treatment [5]. Given expanding access to LPV/r based antiretroviral treatment for HIV-infected children and adults in countries where tuberculosis is endemic, it is essential to develop a comprehensive understanding of the pharmacokinetics of lopinavir and ritonavir, and the drug–drug interactions associated with rifampicin, in adults and children.

Population modelling is a powerful tool to summarize large amounts of data, characterize many sources and levels of variability and quantify potential interactions. We used a population non-linear mixed effects model to integrate the data from three previously published studies [4–6] in order to elucidate the pharmacokinetic differences between children and adults taking the interacting drugs lopinavir, ritonavir and rifampicin.

Methods

Study design

The three study cohorts and clinical procedures have been previously described [3, 5, 6]. Briefly, 39 children without tuberculosis received standard recommended 12 hourly doses of LPV/r oral solution ($230/57.5 \text{ mg m}^{-2}$), with a median lopinavir dose of 11.6 mg kg^{-1} , for at least 2 weeks before pharmacokinetic sampling. In addition, the pharmacokinetic data from 35 HIV-infected children with tuberculosis were included: 15 were treated with ‘super-boosted’

LPV/r (ritonavir was added to the standard 12 hourly dose of LPV/r such that the ratio of lopinavir:ritonavir was 1:1), and 20 were given doubled doses of LPV/r. Both groups also received two NRTIs together with antituberculosis regimens including daily 10 mg kg^{-1} doses of rifampicin and isoniazid. The children received these antiretroviral regimens combined with antituberculosis treatment for at least 2 weeks before pharmacokinetic evaluation. Eleven of the children underwent pharmacokinetic evaluation on a second occasion, on standard doses of LPV/r, at least 1 month after they completed antituberculosis treatment. Intensive (pre-dose and 2, 3, 4, 5, 6, 8 and 12 h after dose administration) or sparse (pre-dose and 2, 4 and 8 h after drug administration) sampling approaches were used in the children. The steady-state pharmacokinetics of lopinavir and ritonavir were also evaluated in 21 HIV infected adult volunteers virologically suppressed on LPV/r 12 hourly plus two NRTIs. Pharmacokinetic sampling (pre-dose and 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 12 h after drug administration) was performed on the last day of each of four sequential treatment occasions: LPV/r in standard doses (400/100 mg) 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) and rifampicin 600 mg daily for 7 days and two times the standard dose of LPV/r (800/200 mg) and rifampicin 600 mg daily for 7 days. LPV/r products used for children and adults were Kaletra® oral solution and Aluvia® tablet (Abbott, USA), respectively and the dose prior to pharmacokinetic evaluation was observed by a study team member.

Lopinavir and ritonavir plasma concentrations were determined using validated liquid chromatography–tandem mass spectrometry [6] in the same laboratory of University of Cape Town. 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 precision for both assays ranged from 0.1% to 4.7% and from 1.6% to 4.2%, respectively.

Population pharmacokinetic modelling

Population pharmacokinetic analysis was performed using the non-linear mixed effects modelling software NONMEM 7 [7]. Pharmacokinetic parameters were estimated using the first order conditional estimation method (FOCE) with eta-epsilon interaction. Perl speaks NONMEM (PsN) 3.4.8 and Xpose (version 4.3.2) were used for model diagnostics [8, 9].

The structural model was chosen based on the pharmacokinetic models for adults and children, respectively, that we reported previously [10, 11]: a one compartment model with first order absorption and elimination for lopinavir and for ritonavir a two compartment model with a series of transit compartments to describe delayed absorption. The interindividual variability (IIV) and interoccasion variability (IOV) of the pharmacokinetic parameters of

lopinavir and ritonavir were assumed log-normally distributed and thus the variability can be approximately interpreted as a deviation proportional to the typical value, and it is reported as %CV. The residual unexplained variability (RUV) was described by a combined additive and proportional model. The correlations between the PK parameters both at IIV and IOV level were also investigated.

Model development was guided by the objective function value (OFV), precision in parameter estimates (relative standard error, RSE%) obtained by NONMEM output or the bootstrap procedure, graphical analysis and scientific plausibility. The difference in OFV between two nested models is assumed to be χ^2 -distributed. As in the previously published separate models, allometric scaling was applied to apparent clearance (CL) and volume of distribution (V) to account for differences in body size. The following formulas were used:

$$CL_i = CL_{STD} \times \left(\frac{WT_i}{65} \right)^{0.75} \quad (1)$$

$$V_i = V_{STD} \times \left(\frac{WT_i}{65} \right)^1 \quad (2)$$

where the WT_i is each patient's body weight and 65 kg is the median body weight in our adult population. Similar formulas were used for inter-compartmental clearance (Q) and peripheral volume (V_p).

A sequential approach was used to develop the model. Firstly, two separate pharmacokinetic models for lopinavir and ritonavir were developed using the combined data from children and adults, and assuming that the pharmacokinetic parameters for a typical child and a typical adult would be different. Secondly, for each parameter separately, we tested whether the difference in the parameter estimates between children and adults was statistically significant, i.e. $\Delta OFV > 3.84$ corresponding to $P < 0.05$. If the inclusion of separate parameters for adults and children was significant, the differences were preserved in the model. Otherwise, the same parameter value was used for adults and children. Diagnostic tools such as goodness of fit plots and visual predictive checks were also used during model selection. Finally, an integrated model including the dynamic effect of ritonavir concentration on lopinavir clearance was developed using a sigmoid relationship as reported before [10, 11]:

$$CL_{LPV} = CL_0 \times \left(1 - \frac{E_{max} \times C_{RTV}^{hill}}{EC_{50}^{hill} + C_{RTV}^{hill}} \right) \quad (3)$$

where CL_{LPV} is the clearance of lopinavir, CL_0 is the clearance of lopinavir when no ritonavir is present, E_{max} is the maximum inhibition effect of ritonavir, EC_{50} is the ritonavir

concentration to reach half of E_{max} , hill is the shape factor and C_{RTV} is the concentration of ritonavir varying with time.

Relative oral bioavailability was evaluated using adults given standard doses of LPV/r without rifampicin co-administration as reference. The effect of rifampicin on the oral clearance and bioavailability of both lopinavir and ritonavir were investigated, and the differences in the effects between adults and children were evaluated for statistical significance through OFV evaluation. Moreover, the enhanced bioavailability of ritonavir and lopinavir, respectively, with higher doses of ritonavir was investigated in children and in adults. The following model was used:

$$F = BIO + SLP \times (Dose_{RTV} - Dose_{RTV-STD}) \quad (4)$$

where F is the bioavailability; BIO is the typical value of bioavailability for the standard dose of ritonavir (which changes in children and adults), $Dose_{RTV}$ and $Dose_{RTV-STD}$ denote the individual dose of ritonavir ($mg\ kg^{-1}$) and the median ritonavir dose given without rifampicin co-administration ($1.5\ mg\ kg^{-1}$ for adults and $2.9\ mg\ kg^{-1}$ for children), respectively. BIO has been fixed to 1 for adults without rifampicin co-treatment, while the values for adults with rifampicin and for children with and without rifampicin are estimated. The linear relation between F and ritonavir dose is described by the parameter SLP .

The maturation model reported by Anderson & Holford [12] was tested for both lopinavir and ritonavir to describe changes in oral clearance with age. Diurnal variations in bioavailability and clearance were also investigated in the combined model.

A non-parametric bootstrap re-sampling approach was used to evaluate the precision of the parameter estimates. However, due to extremely long computation times, only 50 samples were executed.

Results

Patients and data description

A total of 1226 concentrations of lopinavir and ritonavir from 74 children and 21 adults from profiles at steady-state were included in the study. A summary of the subjects and their characteristics is displayed in Table 1. The median dose per kilogram of body weight for LPV/r was higher in the children than in the adult volunteers (median [range] lopinavir dose in children without tuberculosis $11.6\ [9.2 - 16.0]\ mg\ kg^{-1}$ vs. $6.0\ [3.6 - 9.0]\ mg\ kg^{-1}$ in adults prior to the introduction of rifampicin).

Model description

The structure of the final combined pharmacokinetic model is illustrated in Figure 1. The pharmacokinetic parameters for children and adults were estimated

Table 1

Summary of the data

	Children	Adults
Number of patients		
HIV	39	21
HIV/TB	35	
Number of samples	426	800
Gender (male/female)	34/40	3/18
Age [median (range)]	21 months (6 months–4.5 years)	36 years (26–58 years)
Body weight [median (range)] (kg)	10.2 (5.0–17.0)	64.5 (43.0–110.0)
Lopinavir median dose no RIF (range) (mg kg ⁻¹)	12.0 (9.2–16.0)	6.0 (3.6–9.0)
Ritonavir median dose no RIF (range) (mg kg ⁻¹)	2.9 (2.3–4.0)	1.5 (0.9–2.5)

TB, tuberculosis; RIF, rifampicin.

simultaneously, and the estimates are reported in Table 2. IIV was supported on CL, V and bioavailability for both lopinavir and ritonavir, and IOV was implemented in bioavailability, CL, and the absorption parameters [absorption rate (k_a) and absorption mean transit time (MTT)].

Since no intravenous dosing data were available, estimating absolute bioavailability was not possible. Thus, the relative bioavailability of lopinavir and ritonavir was fixed to the reference value of 1 for adults without rifampicin co-treatment and the other values are reported as a proportion of this reference value. In children given the median 2.9 mg kg⁻¹ dose of ritonavir without rifampicin, the relative bioavailability of lopinavir was estimated to be 0.79 and that of ritonavir 0.25. Rifampicin decreased the bioavailability of lopinavir and ritonavir: for adults and children respectively, the bioavailability of lopinavir was reduced to 0.75 and 0.33 and the bioavailability of ritonavir was reduced to 0.48 in adults and 0.02 in children. In the final integrated model, the relative bioavailability of lopinavir in children increased by 0.02 for every 1 mg kg⁻¹ increment in the dose of ritonavir. However, in adults the dose of ritonavir was not found to affect lopinavir bioavailability. The bioavailability of ritonavir increased by 0.46 and 0.026 in adults and children, respectively, for every 1 mg kg⁻¹ increment in the dose of ritonavir.

Allometric scaling was used to account for size differences, and the typical value of the CL and V parameters was thus estimated for an individual of 65 kg, the median weight in our adult population. After adjusting for body weight with allometric scaling, the typical value for CL of lopinavir in children was 34% lower than that in adults. This value refers to lopinavir CL in absence of ritonavir and is extrapolated from the model, since lopinavir was never administered alone. It does not include the inhibitory effect of ritonavir, which is captured separately. For ritona-

vir, the typical value for CL was about 39% lower in children (13 l h⁻¹) than in adults (22 l h⁻¹). Rifampicin therapy in administered doses increased CL of lopinavir by 58% in adults and 48% in children, and the CL of ritonavir by 34% and 22% for adults and children respectively. The addition of a maturation model to account for CL changes with age was not supported by the data. The correlations between lopinavir and ritonavir in IOV of bioavailability and CL were 87% and 100%, respectively (the same random effect was used in the latter case), and the IIV correlation between the bioavailability of the two drugs was 83%.

The absorption parameters also differed between children and adults: absorption half-life was 0.6 h and 1.8 h in adults and children respectively and MTT for ritonavir was shorter in adults (1.1 h) than in children (2.2 h). The maximum concentration is reached later (3.9 h after dosing) in children than in adults (2.5 h after dosing).

The relative bioavailability of lopinavir and ritonavir predicted by our model for children on the different dosing strategies (standard doses without antituberculosis treatment, super-boosted lopinavir with antituberculosis treatment and doubled doses of LPV/r with antituberculosis treatment) with the median doses actually received is shown in Table 3. The individual AUCs of lopinavir and ritonavir predicted by our model for adults and children are shown in Figure 2. When the dosing regimen was observed in our dataset, the individual model predictions were used, while for the unobserved regimens [adults on super-boosted approach ($n = 21$) and children on standard dose with rifampicin ($n = 39$)], data were simulated based on our cohort of patients. Similarly, the trough concentrations of lopinavir predicted by our model for adults and children are shown in Figure 3.

Model evaluation

Visual predictive check plots (1000 simulations) for lopinavir and ritonavir in adults stratified by rifampicin are shown in Figure 4. The visual predictive check plots for lopinavir and ritonavir in children stratified by dose strategy are shown in Figure 5. The 5th, 50th, and 95th percentiles of the observed data are in agreement with the 95% confidence interval of each percentile for the simulated data, which supports the adequacy of the model. The bootstrap results (Table 2) confirmed the robustness of the final parameter estimates.

Discussion

A population model was developed to describe the pharmacokinetics of lopinavir and ritonavir and the drug–drug interactions between lopinavir, ritonavir and rifampicin in children and adults. The model demonstrated important differences between adults and children in the effects of rifampicin, or rifampicin-based antituberculosis treatment, respectively, and provides evidence that drug–drug

Figure 1

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 inhibition effect on lopinavir oral clearance by ritonavir; EC_{50} , the ritonavir concentration needed to reach half of E_{max} ; C , concentration; hill, shape factor

interaction studies in adults should not be used to predict the magnitude of drug–drug interactions in children unless there is a good mechanistic understanding of the processes involved.

Young children and infants are susceptible to the development of tuberculosis especially when they also have HIV infection, and HIV-infected infants frequently present with tuberculosis in high burden settings [1]. Furthermore, reduced mortality is anticipated in HIV infected children with tuberculosis if effective antiretroviral treatment is started as early as possible. Hence the development of compatible co-treatment strategies is mandatory. LPV/r plus two NRTIs is the preferred first line regimen for children under 2 years with prior antiretroviral exposure [1], and the recently reported findings of the PACTG1060 trial suggest that LPV/r has superior antiviral activity to nevirapine in infants not previously exposed to antiretrovirals for the prevention of mother-to-child transmission [13]. Adding ritonavir to standard 12 hourly doses of LPV/r ('super-boosted' lopinavir) during rifampicin-based antituberculosis treatment achieved target concentrations of lopinavir ($>1 \text{ mg l}^{-1}$) in young children. However, this approach is not feasible in many settings as ritonavir oral solution has a short shelf-life and requires refrigeration. Our model reveals the crucial role of sufficient ritonavir in supporting lopinavir concentrations. Moreover, the model suggests that reduced bioavailability of lopinavir and, more so ritonavir, largely accounts for the failure of doubled doses of LPV/r to achieve adequate concentra-

tions of lopinavir in children. The poor bioavailability of lopinavir and ritonavir was exacerbated to a greater extent in children by concurrent antituberculosis treatment than in adults given rifampicin, and was accentuated with lower doses of ritonavir. The extent to which formulation modifications of LPV/r or the antituberculosis drugs may offset these effects is unknown. There is an urgent need for further studies to evaluate the optimal doses of lopinavir and ritonavir in children with tuberculosis of all ages, together with the development of suitable formulations.

The application of allometric scaling for body weight on oral clearance and volume of distribution accounted for the different body size of the study populations, thus allowing comparison of the pharmacokinetic parameters between children and adults. The volume of distribution of lopinavir and ritonavir were similar in children and adults after the implementation of allometric scaling. However, for both lopinavir and ritonavir, oral clearance was lower in children than in adults when size differences were taken into account. This finding could be explained by incomplete maturation of clearance in the children. The number of children aged below 1 year was 12 (16%) and 35 children (52%) were between 1 and 2 years old. Hence, although the maturation model was not supported by our data, probably due to insufficient data in children under 1 year, full maturation of enzymes and transporters may not have been achieved in some young children. Moreover, lopinavir and ritonavir are metabolized mainly by CYP3A4, which is

Table 2

Parameter estimates of final integrated lopinavir-ritonavir model using combined dataset of children and adults. (The values in parentheses are 95% confidence interval obtained from 50 samples of bootstrap results)

Parameters	Lopinavir		Ritonavir		
	Adults	Children	Adults	Children	
CL (l h ⁻¹)§	23 (20, 26)	15 (13, 17)	22 (19, 25)	13 (10, 16)	
V (l)§		57 (53, 61)		39 (32, 46)	
k _a (1 h ⁻¹)	1.1 (0.9, 1.3)	0.38 (0.30, 0.46)	2.3 (1.9, 2.7)		
Bioavailability no RIF*	1 FIX	0.79 (0.75, 0.83)	1 FIX	0.25 (0.24, 0.26)	
Bioavailability with RIF*	0.75 (0.74, 0.76)	0.33 (0.31, 0.35)	0.48 (0.47, 0.49)	0.021 (0.019, 0.023)	
RIF on CL (+)†	58% (55%, 61%)	48% (46%, 50%)	34% (32%, 36%)	22% (20%, 24%)	
Slope of RTV dose effect on bioavailability‡		0.019 (0.017, 0.021)	0.46 (0.44, 0.48)	0.026 (0.024, 0.028)	
Additive error (mg l ⁻¹)		0.054 (0.051, 0.057)			
Proportional error (%)		13 (12, 14)		21 (19, 23)	
Evening effect on CL (–)	51% (48%, 54%)	27% (25%, 29%)	51% (48%, 54%)	27% (25%, 29%)	
Evening effect on F (+)	19% (18%, 21%)				
MTT (h)			1.1 (0.94, 1.3)	2.2 (2.0, 2.4)	
Q (l h ⁻¹)§				30 (26, 34)	
V _p (l)§				53 (47, 59)	
IIV CL (%CV)		22 (20, 24)		26 (24, 28)	
IIV V (%CV)		22 (19, 25)			
IOV CL (%CV)			18 (16, 20)		
IOV k _a (%CV)		68 (63, 73)			
IIV F (%CV)		26 (24, 28)		65 (62, 68)	
IOV F (%CV)		28 (25, 31)		43 (40, 46)	
IOV MTT (%CV)				39 (36, 41)	
Lopinavir-ritonavir interaction					
E _{max}			0.82 (0.81, 0.83)		
EC ₅₀ (mg l ⁻¹)			0.098 (0.093, 0.10)		
Hill			2.8 (2.7, 2.9)		
Correlation between lopinavir and ritonavir					
IOV F	87% (83%, 91%)	IOV CL	100% FIX	IIV F	82% (76%, 88%)

CL, apparent central oral clearance; V_c, apparent central volume of distribution; Q, apparent peripheral oral clearance of ritonavir; V_p, apparent peripheral volume of distribution; MTT, mean transit time; RIF, rifampicin; IIV, interindividual variability; IOV, interoccasion variability; E_{max}, maximum of ritonavir concentration effect on clearance of lopinavir; EC₅₀, the concentration of ritonavir when half E_{max} is achieved; Hill, shape factor. *The median dose of 1.5 mg kg⁻¹ and 2.9 mg kg⁻¹ of ritonavir for adults and children respectively was considered to report these values; †Rifampicin increases the apparent clearance of lopinavir and ritonavir. ‡F = 1 + SLP × (Dose_{RTV} – Dose_{RTV-STD}); clearance and volume are estimated using allometric scaling for an individual of 65 kg. §Typical values of these parameters are reported assuming a bioavailability of 1 as reference, to estimate the actual value of, e.g. CL/F for a specific group of individuals, the reported values should be divided by the correct value of relative bioavailability F, that keeps into account effects such as child/adult, rifampicin co-treatment, dose of RTV.

Table 3

The relative bioavailability of lopinavir and ritonavir adjusted to the median doses actually received by children. (The bioavailability without rifampicin co-administration in adults is fixed to 1 and used as reference)

LPV/r dose strategy	Median dose of ritonavir	Lopinavir	Relative bioavailability Ritonavir
Standard dose without antituberculosis treatment	2.9 mg kg ⁻¹	0.79	0.25
Super-boosted dose with antituberculosis treatment	14 mg kg ⁻¹	0.53	0.31
Double dose with antituberculosis treatment	6 mg kg ⁻¹	0.38	0.10

profoundly induced by post-natal environmental factors [14], and age-related changes in the composition and amount of circulating plasma proteins may influence the plasma free drug fractions, thus affecting clearance of highly bound drugs like lopinavir and ritonavir [15].

The model predicted lower bioavailability of lopinavir and markedly reduced bioavailability of ritonavir in chil-

dren compared with adults. In children given standard doses of LPV/r without rifampicin co-administration, the bioavailability of ritonavir was 25% of that in adults on a 400/100 mg dose of LPV/r (Table 3). Meanwhile, the clearance of ritonavir in children was estimated about 61% of that of adults after considering size differences. Therefore, our model predicted concentrations of ritonavir in children

Figure 2

The individual estimates of exposure for lopinavir (A) and ritonavir (B) for adults (□) and children (▨) stratified by dose strategy. The values were obtained based on the model individual predictions for the observed dosing regimen when available, or on simulation for the unobserved dosing regimens ($n = 21$ for adult on all regimens; for children, $n = 39$ for standard dose with and without rifampicin, $n = 15$ for superboosted dose and $n = 20$ for double dose; log scale is used)

much lower than those in adults, which is consistent with the observed data. Slower gastric emptying and reduced intestinal motility in children [16] may result in reduced bioavailability. Furthermore, differences in the enzymes and transporters expressed in enterocytes and hepatocytes resulting in altered affinity for the drug substrates may contribute to the different bioavailability between adults and children. Differences in the disease status between the adult and paediatric study cohorts could also play a role. The children had recently been diagnosed with tuberculosis and tuberculosis could reduce drug absorption [17], whereas the adults were volunteers established on antiretroviral treatment and generally in a good state of health. Differences in the formulation of LPV/r used in adults and children, respectively, and food effects, are likely to have contributed to altered bioavailability. Under fasting conditions, the mean AUC of lopinavir has been reported to be 22% lower for the LPV/r oral solution relative to the capsule formulation [18]. The tablet formulation of LPV/r which was used in our adults, provides a similar lopinavir

AUC to the capsule following a single 400/100 mg dose [19]. Although such an interaction has not been described, we cannot exclude the possibility that the antituberculosis drug formulations affected the absorption of LPV/r, and the differences between the children (who received their antituberculosis drugs as dispersible fixed dose combinations) and adults (who received rifampicin in tablet form) need to be considered. Moreover, the LPV/r oral solution has poor palatability, and if the children spat out part of their dose, lower model predictions of bioavailability would result. However, the morning dose of LPV/r on the day of pharmacokinetic evaluation was observed by study personnel and failure to ingest the complete dose was not reported. Moreover, poor adherence to the evening dose (12 h before pharmacokinetic evaluation) is unlikely, since the morning trough concentrations were higher than the evening trough concentrations in both adults and children. Furthermore, the bioavailability of ritonavir seems to have been disproportionately affected, suggesting that other factors play a role.

Figure 3

The individual estimates of C_{12h} trough concentration of lopinavir for adults (□) and children (▨) stratified by dose strategy. The values were obtained based on the model individual predictions for the observed dosing regimen when available, or on simulation for the unobserved dosing regimens (log scale is used)

Compared with adults, we found that lopinavir had a smaller k_a , and ritonavir a longer MTT, in children. This is consistent with the role of physiological differences such as slower gastric emptying and reduced intestinal motility in children, in addition to formulation factors and food effects.

The induction of oral clearance of lopinavir and ritonavir by rifampicin was greater in adults than in children. In contrast, the effect of rifampicin on the bioavailability of lopinavir and ritonavir was greater in children than adults. Rifampicin potently induces the expression of CYP3A4 and P-gp both in the liver and in the intestine [20, 21]. Whether CYP3A4 and P-gp in the intestine and liver, respectively, have altered susceptibility in children to induction by PXR-activators such as rifampicin is unknown. The children in our study may not have attained full maturation of hepatic CYP3A4 and P-gp and this might account for a smaller effect of rifampicin on clearance than we found in adults. Moreover, receptor expression may be different in tuberculosis patients [22], and therefore autoinduction of rifampicin may be different between children and adults. The potential role of isoniazid (which was part of the antituberculosis treatment given to the children but was not given to the adults in our study), an inhibitor of CYP3A4 and CYP2D6 [23, 24], should also be considered. It could counteract the rifampicin induction effect to some degree. However, our data do not allow the detection or separate quantification of such an effect.

At higher doses, ritonavir bioavailability increased. As ritonavir is an inhibitor and a substrate of P-gp [25], it could inhibit its own efflux from enterocytes and hepatocytes, thus increasing its bioavailability. Increased doses of ritonavir improved the bioavailability of lopinavir in

children, but this effect was not supported by our data in adults.

Potential weaknesses in our study design include bias in the distribution of certain characteristics. Different dose strategies were used in adults and children in our study and rifampicin was given as part of the standard first line antituberculosis regimen in children. Most of the adult patients were females (86%). Gender may affect the pharmacokinetics of lopinavir and ritonavir [26]. Allometric scaling of volume and clearance with body weight may not have accurately accounted for the effects of body composition differences between adults and children. Food considerably affects the bioavailability of lopinavir and ritonavir [19], and differences in study design with regard to control of food intake could have affected our findings. Lastly, although the treatment doses immediately before pharmacokinetic evaluation were carefully observed, differences in adherence to prior doses of antiretroviral treatment could contribute to the divergent findings between children and adults. The relationships described in our model should be confirmed across patient populations with a wider range of ages and weights, before they can be used to predict accurately the appropriate ratio and doses of lopinavir and ritonavir.

In conclusion, the lower bioavailability of lopinavir and ritonavir, as well as the more potent effect of rifampicin-based antituberculosis treatment on bioavailability in children were the main findings of this study, which sought to explain the failure of double dose LPV/r to achieve adequate concentrations in children on antituberculosis treatment. Ritonavir dose had an important influence on the bioavailability of lopinavir and ritonavir. Prospective studies are needed to confirm the role formulation factors

Figure 4

Visual predictive check of the final combined model for lopinavir (A) and ritonavir (B) in adults stratified by rifampicin 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% confidence intervals for the median, 5th percentile and the 95th percentiles of the simulated data. Observed concentrations are displayed as circles

as well as age and gender related changes in enzyme and transporter activity and susceptibility to induction, food and disease effects, different dose regimens and the role of concomitant drugs on the pharmacokinetics of lopinavir and ritonavir when co-administered with rifampicin in children and adults.

Competing Interests

There are no competing interests to declare.

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Figure 5

Visual predictive check of the final combined model for lopinavir (A) and ritonavir (B) in children stratified by dose strategy 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% confidence intervals for the median, 5th percentile and the 95th percentiles of the simulated data. Observed concentrations are displayed as circles

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