Estimation of the effect of *SLCO1B1* polymorphisms on lopinavir plasma concentration in HIV-Infected Adults

Alessandro Schipani[1], **Deirdre Egan**[1,2], **Laura Dickinson**[1,2], **Gerry Davies**[1], **Marta Boffito**[3], **Mike Youle**[4], **Saye H. Khoo**[1], **David J. Back**[1], and **Andrew Owen**[1,*]

[1]Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK.

[2]NIHR Biomedical Research Centre, Royal Liverpool & Broadgreen University Hospital Trust, Liverpool, UK.

[3]St Stephen's Centre, Chelsea and Westminster Hospital NHS Foundation Trust, London, UK.

[4]Department of HIV Medicine, Royal Free NHS Trust, London, UK.

Abstract

Background—The organic anion transporting polypeptides (OATP)/SLCO family represents an important class of hepatic drug uptake transporters that mediate the sodium independent transport of a diverse range of amphipathic organic compounds, including the protease inhibitors. The *SLCO1B1* 521T>C (rs4149056) single nucleotide polymorphism (SNP) has been consistently associated with reduced transport activity *in vivo*, and we previously showed an association of this polymorphism with lopinavir plasma concentrations. The aim of this study was to develop a population pharmacokinetic (PK) model to quantify the impact of 521T>C.

Methods—A population PK analysis was performed with 594 plasma samples from 375 patients receiving lopinavir/ritonavir. Non-linear mixed effects modelling was applied to explore the effects of *SLCO1B1* 521T>C and patient demographics. Simulations of the lopinavir concentration profile were performed with different dosing regimens considering the different alleles.

Results—A one-compartment model with first-order absorption best described the data. Population clearance was 5.67 L/h with inter-patient variability of 37%. Body weight was the only demographic factor influencing clearance, which increased 0.5 L/h for every 10 kg increase. Homozygosity for the C allele was associated with a 37% lower clearance, and 14% for heterozygosity, which were statistically significant.

Conclusion—These data show an association between *SLCO1B1* 521T>C and lopinavir clearance. The association is likely to be mediated through reduced uptake by hepatocytes leading to higher plasma concentrations of lopinavir. Further studies are now required to confirm the association and to assess the influence of other polymorphisms in the SLCO family on lopinavir PK.

Introduction

Organic anion transporting polypeptides (OATP), coded for by the *SLCO* genes, are a family of solute carrier membrane transport proteins which influx numerous endogenous and xenobiotic compounds. Many of the OATPs (of which there are currently 13 known members) have wide substrate specificity and are expressed ubiquitously [1]. The main OATPs associated with hepatic drug uptake are OATP1B1 (OATP-C) [2, 3] and OATP1B3, which are localized on the basolateral membrane of hepatocytes. The *SLCO1B1* gene encoding OATP1B1 is polymorphic, which may affect OATP1B1 transport function and, consequently, the hepatic uptake and plasma concentrations of OATP1B1 substrates. *SLCO1B1* polymorphisms may also modify drug-drug interactions mediated by OATP1B1 [4]. Many drugs, such as statins, and more recently HIV protease inhibitors (PIs) have been identified as OATP1B1 substrates [5, 6].

HIV PIs have marked inter-individual variability in plasma concentrations and therapeutic drug monitoring (TDM) can be considered for optimizing therapy in some clinical situations. Achieving target plasma PI and non-nucleoside reverse transcriptase inhibitor concentrations is vital for HIV therapy as suboptimal concentrations can lead to the emergence of drug resistance [7-9], and high concentrations may lead to toxicity [8, 10]. Lopinavir is currently one of the most widely used PIs. Lopinavir is mainly metabolized by CYP3A enzymes and is also a substrate for the efflux transporters ABCB1, ABCC1 and ABCC2, which contribute to its low and variable oral bioavailability. To overcome these pharmacokinetic limitations and obtain adequate viral suppressive concentrations, lopinavir is co-administered with a low dose of ritonavir (lopinavir/ritonavir co-formulation is approved at a dose of 400/100 mg twice daily and additionally at 800/200 mg once daily for treatment-naïve patients in Europe and the United States [11, 12]). Ritonavir is a potent inhibitor of ABCB1, CYP3A4 and CYP3A5 mediated metabolism and thereby increases the exposure of lopinavir. Many PIs, have also been shown to activate the nuclear receptor pregnane X receptor (PXR) [13], which is a key regulator of the expression of CYP3A4 [14] and ABCB1 [15], and there is also some evidence that PXR regulates OATP1B1 expression [16, 17]. Drug transporters, like OATP1B1, play a fundamental role in drug uptake delivering the drug metabolic enzymes and facilitating clearance [5, 18]. The main purpose of this study was to develop and validate a population PK model for boosted lopinavir, and to quantify the influence of the *SLCO1B1* 521T>C (rs4149056) polymorphism, which has been shown to affect lopinavir plasma concentrations [1, 19, 20].

Methods

Patients

A total of 375 HIV-positive patients on lopinavir/ritonavir 400/100 mg (tablets) based regimens were included from the Liverpool Therapeutic Drug Monitoring Registry (Liverpool, United Kingdom). Samples were taken at random time points post dosing, plasma was obtained and stored at −80°C before analysis. All the patients had HIV viral load <50 copies/mL at the time of sampling. Exclusion criteria included pregnancy, undetectable plasma lopinavir concentrations (suggesting non-adherence to the regimen) and concomitant use of known enzyme inducers. Of the patients included in the analysis, 82% were male, median age was 40 (range 19–66), median weight was 72Kg (range 45–117). Associations of *SLCO1B1* polymorphisms with lopinavir plasma concentrations in this cohort have previously been published [1].

Lopinavir plasma concentrations and *SLCO* genotyping

Plasma obtained from blood samples was heat inactivated (58°C; 40 min) and lopinavir concentrations were determined using a validated HPLC-MS/MS method as previously described [21]. The lower limit of quantification of lopinavir was taken as the lowest point on the standard curve (95 ng/mL). Intra-assay and inter-assay coefficients of variation at the limit of quantification were between 5% and 7%, respectively. The Liverpool laboratory participates in an external quality assurance scheme (KKGT, The Hague, The Netherlands).

The frequency of the minor alleles for *SLCO1B1* 521 (C) was 11%. This SNP was in Hardy–Weinburg equilibrium as assessed by χ^2 test of observed versus predicted genotype frequencies. In total, 295 (78%) patients carried 521TT allele, 73 (20%) had 521CT and 7 (2%) patients had 521CC. Total genomic DNA was isolated using a GenElute Blood Genomic DNA kit (Sigma-Aldrich, Poole, UK), according to manufacturer's instructions. Genotyping was carried out on 2ml of the final eluent (100ml) using a real-time qPCR-based allelic discrimination assay as described previously [22]. All genotyping experiments were performed in duplicate and contained negative controls (no template) and three positive controls for each of the possible genotypes. Genotype was assigned only when both duplicates were in agreement.

Population pharmacokinetic analysis

The pharmacokinetic model was developed using NONMEM® (version VI; Icon Development Solutions, Ellicott City, MD, USA) installed under nmqual (Metrum institute) [23]. Data processing and graphical analyses were carried out using Microsoft Office Excel 2007 for Windows (Microsoft Corporation, Redmond, WA, USA). The model-building strategy was as follows. One- and two-compartment models with first- or zero-order absorption without and with lag time were fitted to the data using the first-order conditional method of estimation with interaction. Proportional, additive, and combined proportional and additive error models were evaluated to describe residual variability. In the model, residual variability was best described by a purely proportional structure. Interindividual random effects were described by an exponential model: $\theta_i = \theta \times \exp(\eta_i)$, where θ_i is the pharmacokinetic parameter of the ith individual, θ is the average population value, η_i is the interpatient random effect assumed to have a mean of zero and variance ω^2. The minimal objective function value (OFV; equal to –2log likelihood) was used as a goodness-of-fit diagnostic with a decrease of 3.84 points corresponding to a statistically significant difference between models (P=0.05, χ^2 distribution, 1 degree of freedom). Residual plots were also examined. Once the appropriate structural model was established, the following covariates were explored: body weight, age, gender, and *SLCO1B1* genotypes. Graphical methods were used to explore the relationship of covariates with individual predicted pharmacokinetic parameters. Each covariate was introduced separately into the model and retained only if inclusion in the model produced a statistically significant decrease in OFV of 3.84 (P 0.05) and was biologically plausible. A backward elimination step was then carried out once all relevant covariates were incorporated, and covariates were retained if their removal from the model produced a significant increase in OFV (>6.63 points; P 0.01, χ^2 distribution, 1 degree of freedom). Dichotomous and continuous variables, here defined as X, were introduced into the model using the following parameterizations, respectively:

$$TVCL = \theta_0 \times (1 + \theta_1 \times X) \quad 1$$

$$TVCL = \theta_0 + \theta_1 \times (X - \text{median value}) \quad 2$$

where TVCL is the typical value of lopinavir clearance (CL/F) of the population; in equation 1, θ_0 is the value of CL/F for the individuals X=0 and θ_1 is the relative difference in CL/F for the individuals X=1. In equation 2, for example, θ_0 is the typical CL/F at the median body weight and θ_1 is the change in CL/F per kg.

The effect on CL/F for variant genotype 521T>C was expressed as $CL=CL_0+\theta_1\times HET+\theta_2\times HOM$, where HET and HOM indicate genotypic status being equal to 1 for heterozygous and homozygous individuals, respectively, and 0 for the wild-type patients. CL_0 is the typical value of CL/F for individuals carrying 521TT alleles.

Model qualification

To assess the stability and performance of the model, a visual predictive check was carried out, and 1,000 data sets were simulated using the parameter estimates defined by the final model with the SIMULATION SUBPROBLEMS option of NONMEM®. Data sets were simulated for boosted lopinavir 400 mg twice daily. From the simulated data, 90% prediction intervals (P5 to P95) were constructed and observed data from the original data set were superimposed. If 90% of data points fell within the prediction interval, that was indicative of an adequate model. In addition, in order to confirm the stability and robustness of the model, a bootstrap re-sampling was used. Bootstrapping was performed with the software package Perl-speaks-NONMEM 5.1 [24]. The median values and 90% CIs for the parameter estimates were obtained from 200 bootstrap replicates of the original data set and compared with the original population parameters.

External model qualification

External validation was performed for the final model based on qualitative and quantitative evaluations of the predictive performance in an external data set of 42 observations from 6 patients that received lopinavir-based antiretroviral therapy. Two individuals had 521TC genotype and four individuals 521TT. These patients were recruited from the Department of HIV Medicine, Royal Free NHS Trust, London, and the samples were analyzed by the Liverpool laboratory. The fixed and random estimates obtained from the model were used to predict individual lopinavir concentrations, which were plotted and compared with actual concentration values. Mean prediction error and its 95% confidence interval (CI) was calculated as a measure of bias.

Simulation of the genetic effect on lopinavir at different doses

To investigate the effect of genetics on lopinavir concentration at the end of the dosing interval (C_{trough}), simulated pharmacokinetic data for lopinavir, stratified for SLCO1B1 genotype, were generated. Ninety percent prediction intervals of the simulated concentrations for each category were calculated. Additionally, 1000 individuals were simulated using the fixed and random effects of the final model that we previously published [25] at a lopinavir/ritonavir dose of 200/50 mg twice daily and also to evaluate a missed dose during a twice daily regimen (dosed 400/100 mg twice daily, evening dose missed, it will be referred to as 400/100 mg once daily); individuals were simulated considering different genetics using the estimated effect of *SLCO1B1* 521T>C on CL/F found in this study. The simulation was carried out using a sequential model with a direct concentration-dependent relationship of the form of $CL/F_{LPV} = CL_0/F_{LPV} * [1 - ((I_{MAX} * C_{RTV}) / (IC_{50} + C_{RTV}))]$, where CL/F_{LPV} is lopinavir clearance, CL_0/F_{LPV} is lopinavir clearance in the absence of ritonavir, C_{RTV} is the ritonavir plasma concentration at each time point, I_{MAX} is the maximum inhibitory effect of ritonavir on CL/F_{LPV} and IC_{50} is the C_{RTV} producing 50% of the I_{MAX}.

Results

Data from 594 plasma samples were collected from the patient population. The lopinavir plasma concentrations were in a range of 0.114 to 22.432 mg/L. An external data set of 42 samples from 6 HIV positive individuals was used to validate the model, in addition to an internal validation. A 1-compartment model described the data better than a 2-compartment model and therefore was kept as the base model. The inter-individual variability was supported only for apparent clearance. The introduction of a lag time did not significantly improve the fit. In the basic model the mean population estimates for CL/F, apparent volume of distribution (V/F) and absorption constant (k_a) were 5.7 L/h, 45.5 L and 0.2 h, respectively.

A total of four covariates (weight, age, gender and *SLCO1B1* 521T>C) were analysed using a stepwise forward-backward elimination. CL/F significantly (P<0.001) correlated with two covariates: weight and *SLCO1B1* 521T>C. In the forward inclusion age and gender were also selected. However, during the backward elimination these covariates did not meet the inclusion criteria and therefore were excluded from the final model. The mean population estimate for CL/F was 5.67 L/h; the final covariate model is detailed in table 1. Inclusion of body weight resulted in an improvement of the goodness of the fit (ΔOFV = 20.5, P < 0.001), a slight decrease in inter-individual variability of 1% and CL/F increased by 0.5 L/h with body weight increases of 10 kg.

The inclusion of this polymorphism decreased the OFV by 15 units (P < 0.001) and the IIV by 2% compared with the base model. In subjects who were heterozygous for 521TC CL/F decreased by 0.8 L/h, and in homozygotes the model showed a more significant decrease in CL/F of 2.1 L/h. The diagnostic plots for the final model showed that predicted and observed data were in agreement (figure 1). The individual conditional weighted residuals did not reflect any particular systematic trends.

Internal model qualification

A 90% prediction interval was generated from 1000 simulations for boosted lopinavir 400 mg twice daily, with the covariate values of those individuals used in the model building process. Observed data from patients used in the model-building process were superimposed onto the prediction interval. Of 323 plasma concentrations, 4.3% were above P95 and 4.6% were below P5, which suggests that overall the final model performed adequately (figure 2).

In addition, from the original data set, 200 bootstrap replicate data sets were generated and used to evaluate the stability of the final model. The median values of the parameter estimates from the bootstrapping were very similar to the mean population estimates for the final model (table 1). The 90% confidence interval (CI) for the parameter estimates obtained from the bootstrap procedure revealed adequate estimation of the pharmacokinetic parameters for both fixed and random effects and robustness of the final model.

External model qualification

External validation of the model was performed in a total of 42 observations from 6 patients. Individual lopinavir predictions versus actual concentrations in the model validation dataset are shown in figure 2. The small number of patients for the external validation can be considered a limitation. However, the performance of the individual predictions was satisfactory, with a bias (95% CI) of 2.1% (−1.1 to 5.3).

Simulation of the impact of genetics on lopinavir at different doses

In order to evaluate the clinical impact of the pharmacogenetic factors, simulated concentration-time courses of lopinavir (400mg twice daily) at steady-state were performed. The simulations were performed separately with a population of individuals homozygous for the common alleles (521TT) and with the variant alleles 521 TC and 521CC (figure 3). The predicted 95th percentile of lopinavir (median [P5 –P95] 400 mg twice daily) trough concentrations (C_{trough}) for individuals carrying alleles 521TT, 521TC and 521CC were: 5.0 (1.1-13.2), 5.11 (1.4-15.6) and 7.4 (2.0-22.1), respectively.

The effect of a missing dose on patients stable on 400/100 mg twice daily was also evaluated, mean lopinavir C_{trough} from simulated concentrations of lopinavir/ritonavir 400/100 mg once daily for individuals carrying the 521CC SNP were higher than the alleged minimum effective concentration in treatment naive patients (MEC) of 1 mg/L (1.23 mg/L, 95%CI 1.16-1.29). The number of individuals 521CC below the MEC was 40% of the total simulated population. The simulations were repeated using 521TC individuals and the results of mean lopinavir C_{trough} were significantly below the MEC (0.43 mg/L, 95%CI 0.39-0.46), the number of individuals below the MEC was 83% of the total simulated population. A second series of simulations were carried out with a reduction of dose of lopinavir/ritonavir to 200/50 mg twice daily. Individuals with 521CC and 521TC achieved a mean C_{trough} of 2.36 mg/L (95%CI 2.32-2.41) and 1.49 (95%CI 1.46-1.53), respectively (figure 4). The number of individuals below the MEC was 2% and 18%, respectively.

Discussion

A model has been developed and validated to describe lopinavir pharmacokinetics in HIV-infected individuals and used to quantify the influence of *SLCO1B1* 521T>C on CL/F. Simulations of lopinavir/ritonavir 200/50 mg twice day and 400/100 mg once daily associated with the polymorphism 521T>C generated a lopinavir mean C_{trough} above the MEC (1 mg/L) for trreatment-naive patients. The simulations were performed using a sequential model, which was developed previously [25], and allowed incorporation of the effect of both ritonavir and *SLCO1B1* 521T>C on lopinavir plasma concentrations.

OATP1B1 is predominantly expressed at the sinusoidal membrane of hepatocytes. The 521T>C polymorphism has been consistently associated with reduced transport activity *in vivo* [6, 19], leading to reduced uptake by hepatocytes and higher plasma concentrations of different classes of therapeutic drugs, such as hypocholesterolaemic statins and meglitinide analogue hypoglycaemic agents [19, 26]. Recently, we identified lopinavir as a substrate of *SLCO1B1* and showed that a variant in *SLCO1B1* (rs4149056) was associated with impaired transport activity, and therefore increased lopinavir plasma concentrations. The model that best described lopinavir pharmacokinetics was a one-compartment model with first-order absorption and elimination. Although the V/F was slightly under estimated, the k_a and CL/F were consistent with values previously published [20, 25, 27]. The dataset included sparse data from a TDM cohort and only a limited number of samples in the absorption phase. This could explain the lack of inter-individual variability for ka and may also affect the determination of inter-individual variability for V/F.

Body weight was the only demographic covariate significantly related to CL/F, which increased by 8.8% with a body weight increase of 10 kg. The inclusion of *SLCO1B1* genetics also improved the model fit. The effect of the 521CC genotype was particularly significant, decreasing CL/F by 37%, and there was a gene–dose effect with 521TC decreasing CL/F by 14% compared with the 521TT genotype.

There are limited data on the interaction between PIs and the *SLCO1B1* 521T>C polymorphism [6]. Recently, Lubomirov et al. [6, 20] incorporated population PK and 521T>C and showed a decrease in CL/F consistent with our finding for 521TC (CL/F 14% decrease), but the change in CL/F was less for 521CC (21% vs 37%). Kohlrausch et al. [19] reported an increment in lopinavir C_{trough} of 49% in individuals carrying 521CC; the average of the simulated C_{trough} in our study showed an increase of 57% compared with 521TT. In these studies the number of individuals carrying 521CC was limited, therefore to better define the impact of this SNP further studies are warranted. One of the limitations of the present study is the lack of ethnicity data and this also has implications for the Hardy Weinburg Equilibrium calculations. However, previous studies did not observe an association between trough concentrations of lopinavir and ritonavir and ethnicity [19, 20, 25]. The allele frequency of this polymorphism is comparable between Hapmap Asian populations (e.g. 14% in Han Chinese and 12% in Japanese) and the Caucasian population (15%). However, it is considerably lower in some African Hapmap populations (E.g. 2% in Yoruban and 1% Luhya) and therefore, it should be noted that this population PK analysis may not be universally applicable. The present results must also be interpreted in the context of the inherent limitations of TDM cohorts resulting from selection bias and a lack of information on factors such as ethnicity. However, given these limitations, TDM data represents a potentially valuable resource for hypothesis generation in pharmacogenetic studies. Nonetheless, as with any pharmacogenetic association, the findings should be validated in other cohorts.

In order to simulate different doses and evaluate the possible clinical impact of the *SLCO1B1* 521T>C SNP, the effect of ritonavir had to be taken in account. The data used in this study, did not include the respective ritonavir concentrations. Thus, an external model, which we have recently published, was used to simulate lopinavir and ritonavir concentrations, in order to consider the effect of ritonavir on lopinavir concentrations over time. In the simulation the effect of the 521T>C, which was determined in the present study, was included. This effect was evaluated only on lopinavir plasma concentrations, since the previous study indicated no correlation between 521T>C polymorphism and ritonavir plasma concentrations [1]. Interestingly, the results indicated that the mean C_{trough} concentrations for lopinavir/ritonavir 200/50 mg twice day, in heterozygous and homozygous individuals were above the MEC of 1 mg/L for treatment-naive patients. Additionally, for the missed dose simulation (400/100 mg once daily), the simulated C_{trough} at 24 hours had a lopinavir mean C_{trough} concentration above the MEC for C homozygous individuals.

Modelling and simulation incorporating genetics alone may help to inform future clinical studies and provide an insight into whether dose optimization of lopinavir/ritonavir is possible considering the genetic constitution of an individual. A recent clinical study [28] showed that therapeutic plasma concentrations of lopinavir can be achieved with 200/150 mg of lopinavir/ritonavir twice daily and that the majority of volunteers were above the lopinavir MEC following 200/50 mg twice daily. Dose adjustment based on *SLCO1B1* 521T>C or a panel of drug disposition SNPs may be considered as a dosing strategy with the potential to reduce both costs and toxicity However, these data are based purely on pharmacokinetic analysis and the effect of the alternate lopinavir/ritonavir dose on viral suppression requires further investigation.

Acknowledgments

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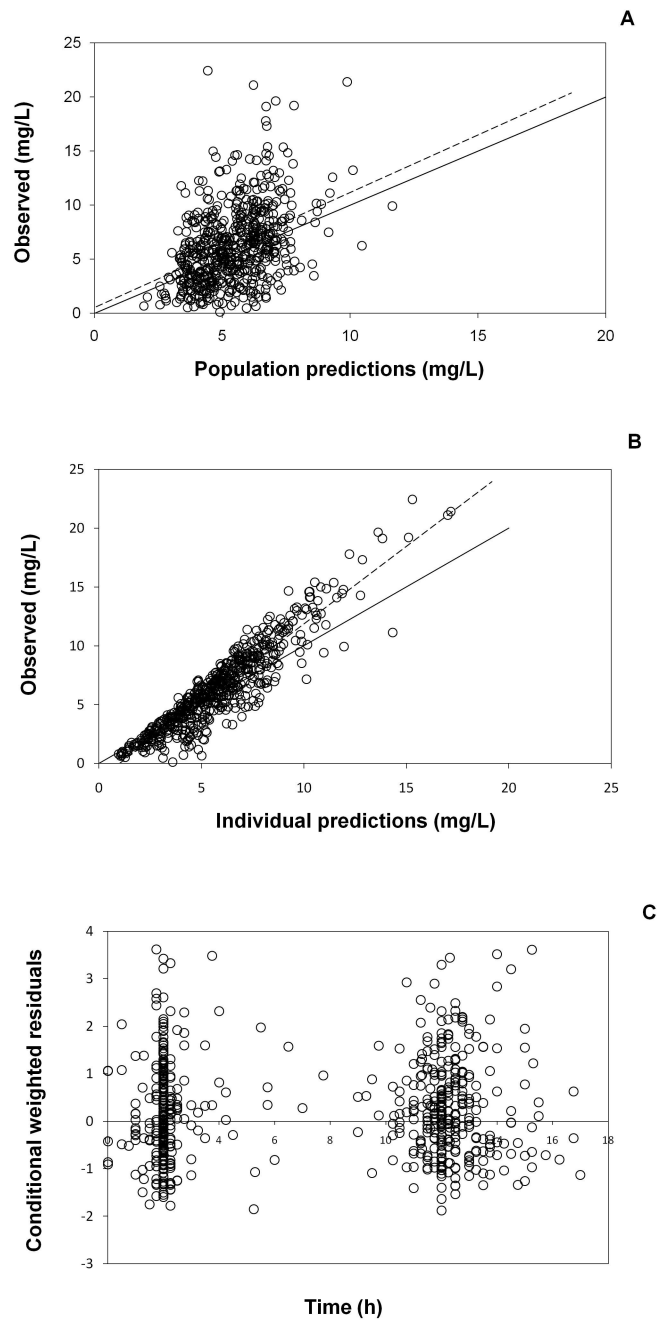
Figure 1. Goodness of fit plots for the final pharmacokinetic model
(**A**) population predictions of lopinavir *vs.* observed concentrations, (**B**) individual predictions of lopinavir *vs.* observed concentrations. The continuous line shows the line of unity and the broken line shows the line of regression. (**C**) Conditional weighted residuals *vs.* time post-dose, the continuous line shows the line at ordinate values zero.
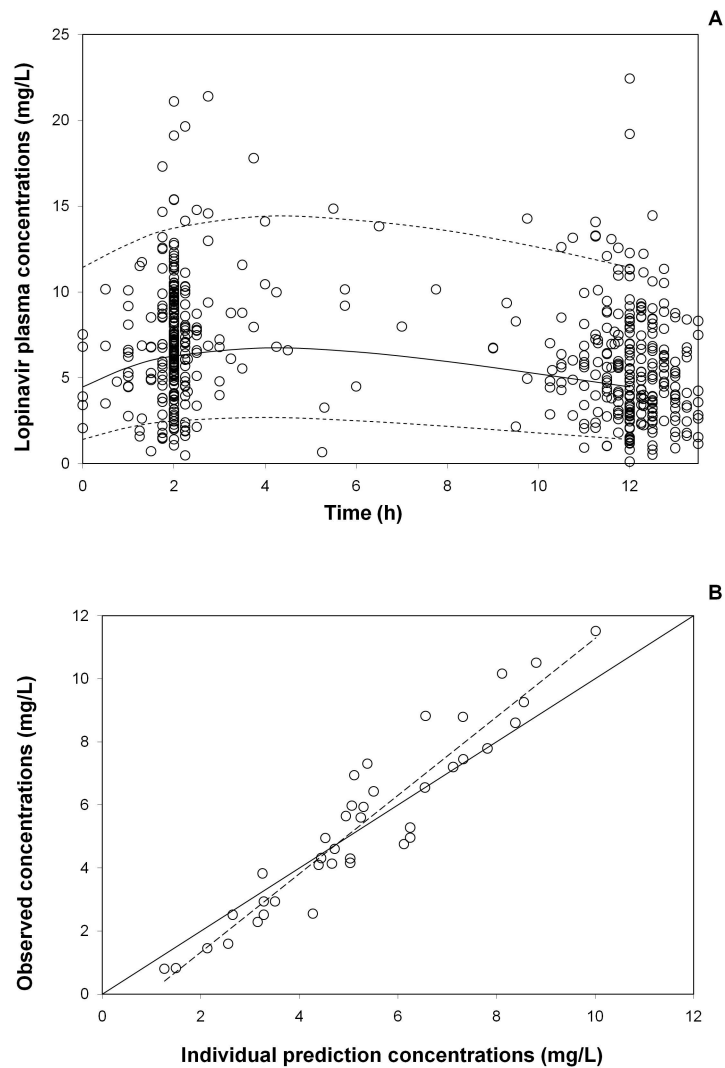
Figure 2.
(A) 90% prediction intervals determined from simulated data of boosted lopinavir 400 mg twice daily. The median population prediction is shown as a continuous line and the 90% prediction interval is shown as a broken line. **(B)** Goodness of-fit plots of the validation of the lopinavir model performed with an external data-set in a total of 42 observations from 6 patients. The broken line is the line of regression of the observed concentrations, and the continues black line is the line of identity.
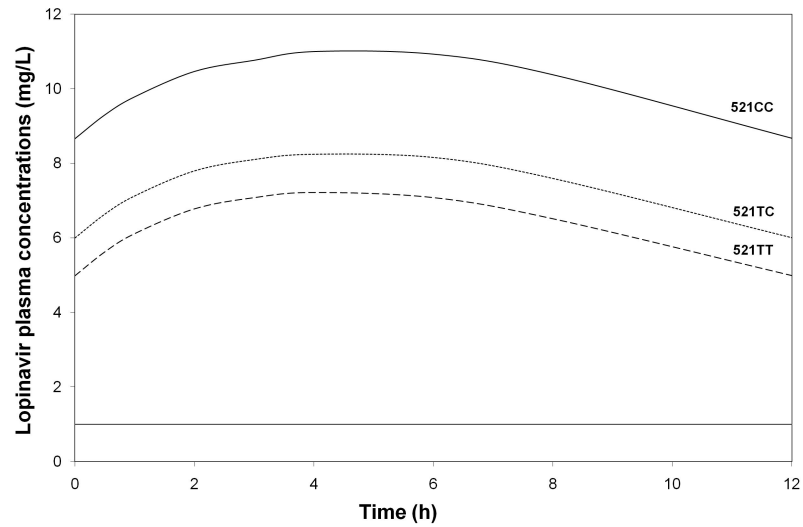
Figure 3. Steady-state lopinavir mean plasma concentration predictions for lopinavir/ritonavir 400/100 mg twice daily determined from simulated data in individuals with different genotypes
The continuous line describes mean population prediction for individuals carrying the 521CC genotype. The thin dotted line describes mean population prediction for individuals carrying the 521TC genotype. The dashed line describes mean population prediction for individuals carrying the 521TT genotype. The horizontal continuous line is at an ordinate value of 1 mg/L (proposed minimum effective concentration).
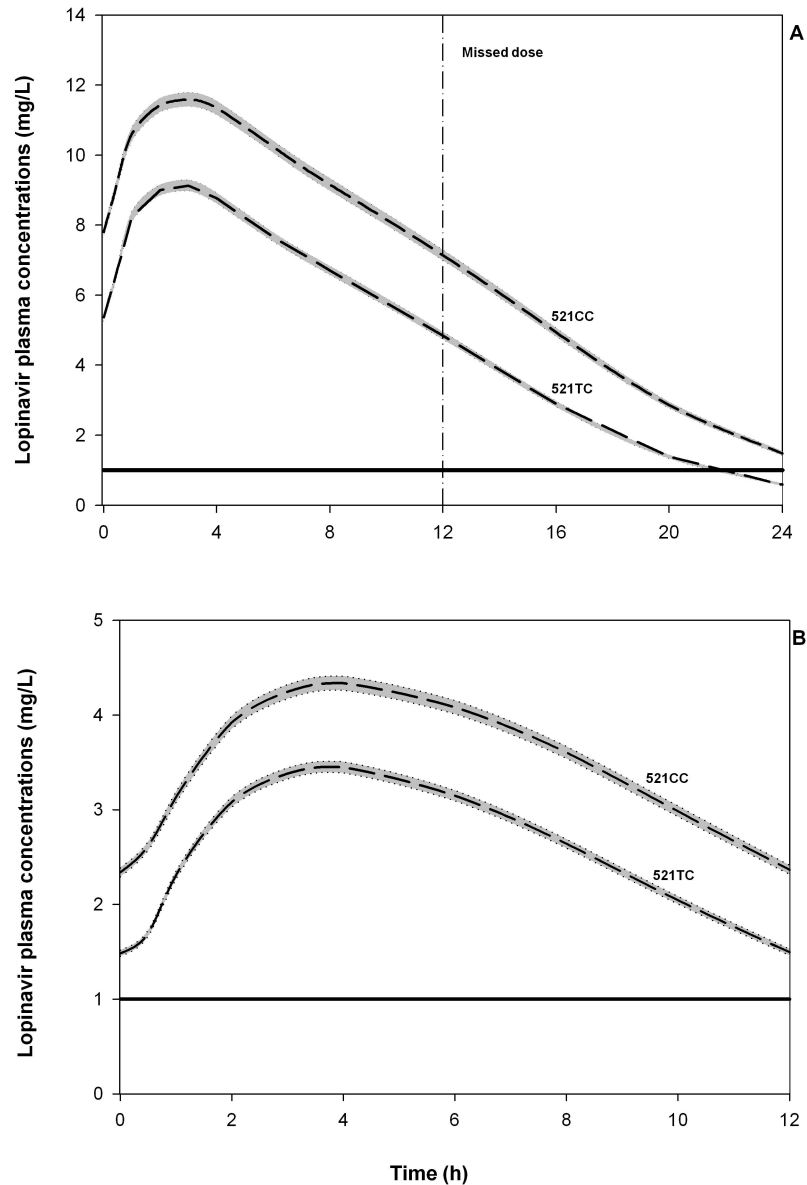
Figure 4. Lopinavir mean plasma concentration predictions determined from 1000 simulations
(**A**) Prediction for lopinavir/ritonavir 400/100 mg once day for individuals with the 521CC
and 521TC genotype. (**B**)Prediction for lopinavir/ritonavir 200/50 mg regimen twice a day
for individuals with the 521CC and 521TC genotype. Grey areas represent the 95% CI. The
horizontal continuous line is at an ordinate value of 1 mg/l (proposed minimum effective
concentration).

Table 1

LPV parameter estimates and standard errors obtained from the final population pharmacokinetic model.

Parameter	Basic Model Estimate (RSE %)	Final Model Estimate (RSE %)	Bootstrapped median (90% CI) in the final model
CL/F (L/h)	5.29 (3)	5.67 (4)	5.67 (5.22, 6.12)
V/F (L)	42.4 (17)	45.5 (14)	44.30 (16.66, 74.33)
k_a (h^{-1})	0.18 (14)	0.20 (6)	0.19 (0.07, 0.32)
IIV CL/F %	40 (38)[a]	37 (44)[a]	36 (29.5, 43.9)
Factor associated with BW on LPV CL/F	-	0.0457 (22)	0.046 (0.025, 0.066)
Factor associated with T/C on LPV CL/F	-	−0.791 (36)	−0.757 (−1.34, −0.23)
Factor associated with C/C on LPV CL/F	-	−2.09 (29)	−1.97 (−3.28, −0.89)
Proportional residual error, %	32.2 (12)	33.1 (13)	33.3 (25, 44)

[a]Relative standard errors (RSEs), defined as (SE_estimate/estimate)×100, of the CV, taken as (SE/estimate) and expressed as percentage. BW: body weight; CL/F: apparent oral clearance; IIV: interindividual variability; LPV: lopinavir; k_a: absorption rate constant; V/F: apparent volume of distribution.