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Population pharmacokinetics of ritonavir-boosted atazanavir in HIV-infected patients and healthy volunteers

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***Objectives*: The aim of this study was to develop and validate a population pharmacokinetic model to: (i) describe ritonavir-boosted atazanavir concentrations (300/100 mg once daily) and identify important covariates; and (ii) evaluate the predictive performance of the model for lower, unlicensed atazanavir doses (150 and 200 mg once daily) boosted with ritonavir (100 mg once daily).**

***Methods*: Non-linear mixed effects modelling was applied to determine atazanavir pharmacokinetic parameters, inter-individual variability (IIV) and residual error. Covariates potentially related to atazanavir pharmacokinetics were explored. The final model was assessed by means of a visual predictive check for 300/100, 200/100 and 150/100 mg once daily.**

***Results*: Forty-six individuals were included (30 HIV-infected). A one-compartment model with first-order absorption and lag-time best described the data. Final estimates of apparent oral clearance (CL/F), volume of distribution (*V*/F) and absorption rate constant [relative standard error (%) and IIV (%)] were 7.7 L/h (5, 29), 103 L (13, 48) and 3.4 h^{-1} (34, 154); a lag-time of 0.96 h (1) was determined. Ritonavir area under the curve (AUC_{0-24}) was the only significant covariate. Overall, 94%–97% of observed concentrations were within the 95% prediction intervals for all three regimens.**

***Conclusions*: A population pharmacokinetic model for ritonavir-boosted atazanavir has been developed and validated. Ritonavir AUC_{0-24} was significantly associated with atazanavir CL/F. The model was used to investigate other, particularly lower, ritonavir-boosted atazanavir dosing strategies.**

Keywords: modelling, simulation, variability, pharmacokinetics

Introduction

Atazanavir (REYATAZ®, Bristol–Myers Squibb, Princeton, NJ, USA) is a protease inhibitor used as part of combination therapy in the treatment of HIV disease. It is approved in Europe and the USA at a dose of 300 mg boosted with ritonavir (NORVIR®, Abbott Laboratories, Chicago, IL, USA) 100 mg once daily (atazanavir/ritonavir 300/100 mg once daily) to be taken with food,[1,2] but is also approved in the USA unboosted at 400 mg once daily for treatment-naive patients.[1] Atazanavir benefits from once-daily dosing, low pill burden and also a favourable safety profile with less lipid abnormalities than other protease inhibitors.[3] Atazanavir is metabolized by CYP3A4/3A5[1,2] and is an inhibitor of CYP3A4, p-glycoprotein[4] and UDP-glucuronosyltransferase 1A1 (UGT1A1)[5]; therefore, the potential for drug–drug interactions is high. Important interactions with proton-pump inhibitors[6,7] and unexpectedly with tenofovir

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(VIREAD®, Gilead Sciences Inc., Foster City, CA, USA)[8,9] have been documented and patients may benefit from therapeutic drug monitoring (TDM) in this context. A therapeutic target concentration of 0.15 mg/L at trough has been defined as an optimal concentration for successful viral suppression[10] and an upper limit >0.85 mg/L has been associated with a risk of increased unconjugated bilirubin and incidences of hyperbilirubinaemia[11] due to UGT1A1 inhibition.

High inter-individual variability in atazanavir drug concentrations has been observed and can be a result of several factors including drug–drug interactions, lack of regard for food recommendations (or for whatever reason, being unable to gain access to food at the time of drug intake) and suboptimal treatment adherence. Identifying sources of variability in pharmacokinetics is important in the clinical management of HIV infection and may aid optimal dosage selection. The aim of the present analysis was to develop and validate a population pharmacokinetic model to: (i) describe ritonavir-boosted atazanavir concentrations (300/100 mg once daily) and identify important covariates that may impact pharmacokinetic variability; and (ii) simulate concentration–time profiles of lower, unlicensed atazanavir doses (150 and 200 mg once daily) boosted with ritonavir (100 mg once daily).

Methods

Patients

Data were included from three clinical studies conducted in healthy adults (one study)[12] and HIV-infected patients (two studies)[13,14] evaluating atazanavir/ritonavir pharmacokinetics dosed at 300/100 mg once daily. All individuals were recruited and assessed at one UK study centre (St Stephen's Centre, Chelsea and Westminster Foundation Trust, London, UK); all three studies were approved by the local Research Ethics Committee, and patients and volunteers provided written informed consent. Detailed accounts of study design, inclusion/exclusion criteria and pharmacokinetic findings of each study have been reported previously.[12–14] In summary, adult males and non-pregnant females (>18 years) were permitted to enrol in the studies. With the exception of HIV infection, individuals with active clinically significant conditions (such as hepatitis infections or tuberculosis) were not eligible to participate. Intake of medications known to influence protease inhibitor metabolism (such as non-nucleoside reverse transcriptase inhibitors) was not permitted, and boosted atazanavir was investigated as the sole protease inhibitor with the exception of one study in which patients also received hard-gel saquinavir (INVIRASE®, Roche Pharmaceuticals, Nutley, NJ, USA; 1600 mg once daily) in combination with atazanavir/ritonavir (300/100 mg once daily).[13] Investigations suggest that co-administration of saquinavir does not affect atazanavir pharmacokinetics.[15,16] Patients were allowed to receive tenofovir as part of their therapy backbone even though some studies have shown a decrease in atazanavir concentrations in the presence of tenofovir.[8,9]

Blood sampling and drug analysis

All individuals were stable on atazanavir/ritonavir at least 2 weeks prior to the start of each study and HIV patients received atazanavir/ritonavir in combination with two nucleoside reverse transcriptase inhibitors (NRTIs) or one NRTI plus one nucleotide reverse transcriptase inhibitor. Drug intake was directly observed and timed on the day of pharmacokinetic sampling and administered under fed conditions with a standardized meal (16–20 g fat). Venous blood samples (7 mL) were drawn and collected into heparinized tubes pre-dose (0 h) and 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose; healthy volunteers had additional samples taken at 16 and 20 h post-dose. Plasma was isolated (1000 **g**; 10 min; 4°C) within 2 h of collection and stored (−70°C) until analysed.

Plasma atazanavir and ritonavir concentrations were determined by fully validated high-performance liquid chromatography-tandem mass spectrometry methods as illustrated previously.[17,18] All concentrations were assessed at one laboratory with the exception of one study;[13] however, both laboratories participate in the same external quality assurance programme (International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV Infection, Nijmegen, The Netherlands) with acceptable performances. Details of each assay's performance have been reported previously.[17,18]

Data analysis

Non-linear mixed effects modelling was applied using NONMEM® (version VI 2.0, level 1.1, double precision; ICON Development Solutions, Ellicott City, MD, USA),[19] using first-order conditional estimation with interaction. Model fit was assessed by statistical and graphical methods. The minimal objective function value (OFV; equal to −2 log likelihood) was used as a goodness-of-fit diagnostic with a decrease of 3.84 points corresponding to a statistically significant difference between hierarchical models ($P=0.05$, χ^2 distribution, one degree of freedom). Graphical diagnostics were performed with Microsoft® Office Excel 2007 for Windows (Microsoft Corporation, Redmond, WA, USA). Standard errors of the parameter estimates were determined with the COVARIANCE option of NONMEM® and individual Bayesian parameter and concentration estimates by the POSTHOC option.

Pharmacokinetic and covariate model building

To determine the best structural model to fit the data, one- and two-compartment models with first- or zero-order absorption without and with lag-time were considered. Proportional, additional and a combined proportional–additional error model were evaluated to describe residual variability. Although the two laboratories that performed the drug analysis were part of the same quality assurance scheme, error models were explored for the two laboratories to determine whether separate assays contributed individually to the residual variability.

Once a baseline model was established, the following covariates were explored: ritonavir area under the curve over the dosing interval (AUC_{0-24}), HIV status, sex, ethnicity, weight, concomitant protease inhibitor use (saquinavir 1600 mg once daily) and tenofovir use (300 mg once daily). For continuous variables (for example, weight), plots of covariates versus individual predicted pharmacokinetic parameters were performed to determine possible relationships. Each covariate was introduced separately and only retained if inclusion in the model produced a statistically significant decrease in OFV of 3.84 ($P\leq0.05$), was biologically plausible and reduced variability (by at least 10%). A backwards elimination step was carried out once all relevant covariates were incorporated and covariates retained if their removal from the model produced a significant increase in OFV (>6.63 points; $P\leq0.01$, χ^2 distribution, one degree of freedom). Ritonavir AUC_{0-24} was determined from the concentration–time data using non-compartmental

JAC antiviral

methods (WinNonlin® 5.2, Pharsight Corporation, Mountain View, CA, USA).

Model validation

To perform a visual predictive check, 1000 datasets were simulated using the parameter estimates defined by the final model with the SIMULATION SUBPROBLEMS option of NONMEM®. Datasets were simulated for atazanavir/ritonavir 300/100, 200/100 and 150/100 mg once daily. From the simulated data, 95% prediction intervals (P2.5–P97.5) for each regimen were constructed. Observed data from the original dataset were superimposed for atazanavir/ritonavir 300/100 mg once daily. Concentration–time data from patients participating in another external clinical study receiving atazanavir/ritonavir/saquinavir 200/100/1600 and 150/100/1600 mg once daily[20] were used to evaluate the prediction of lower dose atazanavir. At least 95% of data points within the prediction interval (2.5% above and below) was indicative of an adequate model.

Bayesian estimation of trough concentrations and exposure

Using the observed data and final model parameters, single samples and combinations of two samples were used to estimate atazanavir trough concentrations, i.e. concentrations 24 h post-dose and AUC_{0-24} [determined by dose/individual predicted apparent oral clearance (CL/F)] of the HIV patients included in the analysis (300/100 mg once daily). This was achieved by the addition of the missing data variable column (MDV) to the data file to identify the concentration to be predicted by the model (i.e. concentration 24 h post-dose) and the exclusion of the COVARIANCE step. Predicted trough concentrations and AUC_{0-24} were compared with the observed values and the predictive performance was evaluated by calculating mean relative prediction error (%MPE) as a measure of bias and root mean squared relative prediction error (%RMSE) as a measure of precision.[21] Observed atazanavir AUC_{0-24} were determined by means of standard non-compartmental methods (WinNonlin® 5.2, Pharsight Corporation).

This process was also carried out with the lower dose ritonavir-boosted atazanavir regimens (200/100 and 150/100 mg once daily). Using the model and observed concentrations obtained at single timepoints (2, 4, 6, 8, 10 and 12 h post-dose) from the external atazanavir/ritonavir dataset,[20] predictions of trough concentrations (24 h post-dose) and atazanavir AUC_{0-24} were performed and compared with the observed values by the determination of %MPE and %RMSE. Observed atazanavir AUC_{0-24} at doses of 200/100 and 150/100 mg once daily were calculated by means of standard non-compartmental methods (WinNonlin® 5.2, Pharsight Corporation).

Results

Patients

Sixteen healthy volunteers (6 female) and 30 HIV-infected individuals (3 female) receiving orally administered atazanavir/ritonavir were included in the model building process, the demographics of whom are shown (Table 1). No difference in age, weight or body mass index (BMI) was observed between healthy volunteers and HIV patients ($P \geq 0.25$ for all comparisons; unpaired *t*-test). Furthermore, ritonavir AUC_{0-24} was not significantly different between healthy and HIV-infected individuals (7.36 versus 7.59 mg·h/L, $P = 0.48$; Mann–Whitney *U*-test). In total, 538 concentrations were included (one pharmacokinetic profile per patient) ranging between 0.077 and 8.763 mg/L.

Pharmacokinetic model

A one-compartment model with first-order absorption best described the data. A one-compartment model with zero-order absorption or a two-compartment model did not improve the fit. Compared with an additive error model, a proportional error model improved the fit (ΔOFV −204.2). Inclusion of an additive component further improved the model with a proportional–additive error model best describing residual variability (ΔOFV −9.4) illustrated as follows:

$$Y = \mathrm{F} * (1 + \varepsilon_1) + \varepsilon_2$$

where Y is the final prediction; F is the individual prediction; and ε_1 and ε_2 are the proportional and additive model components, respectively, with a mean of zero and variance σ^2.

Inclusion of separate error models corresponding to the two analytical laboratories did not improve the model (ΔOFV −1.2). Inclusion of inter-individual variability (IIV) on volume of distribution (*V*/*F*) significantly improved model fit (ΔOFV −84.6, compared with IIV on CL/F alone), as did IIV on the absorption rate constant (k_a; ΔOFV −122.5). Addition of an absorption lag-time further improved fit (ΔOFV −176.8), but inclusion of IIV was not significant (ΔOFV −0.8). IIV was described by an exponential model, an example of which is shown below for CL/F:

$$\mathrm{CL/F}_i = \theta_1 * \exp(\eta_i)$$

where $\mathrm{CL/F}_i$ is the atazanavir CL/F of the *i*th individual; θ_1 is the population parameter estimate; and η_i is the IIV with a mean of zero and variance ω^2.

Parameter estimates for the basic model are summarized in Table 2.

Covariate model

Once the basic structural model was defined, covariates were introduced one at a time to determine whether they influenced atazanavir pharmacokinetics. For dichotomous variables, here defined as X (such as male/female sex and absence/presence of a co-administered drug), the following equation was applied using CL/F as an example:

$$\mathrm{TVCL} = \theta_1 * \theta_2{}^{X}$$

where TVCL is the typical value of atazanavir CL/F of the population; θ_1 is the value of CL/F for the individuals $X = 0$; and θ_2 is the relative difference in CL/F for the individuals $X = 1$.

Continuous variables were introduced into the model by linear functions. Based on graphical plots of ritonavir AUC_{0-24} and pharmacokinetic parameters, exponential and power functions were explored. Following univariate analysis, ritonavir AUC_{0-24}, concomitant use of saquinavir and weight were significantly associated with atazanavir CL/F and *V*/F, and Hispanic ethnicity was significantly associated with atazanavir CL/F and k_a (Table 3). However, once multivariate analysis and

Table 1. Summary of patient demographics and baseline clinical characteristics

Parameter	*n* (%)	Median (range)
Study participants [M/F]		
healthy volunteers	16 (35) [10/6]	
HIV-infected	30 (65) [27/3]	
Ethnicity		
Caucasian	33 (72)	
Black-African	7 (15)	
Hispanic	6 (13)	
Regimen		
ATV/RTV 300/100 mg once daily	28 (61)[a]	
ATV/RTV/SQV 300/100/1600 mg once daily	18 (39)	
TDF 300 mg once daily	6 (13)	
Age (years)		
healthy volunteers		42 (25–55)
HIV-infected		43 (22–62)
all		43 (22–62)
Weight (kg)		
healthy volunteers		85 (53–115)
HIV-infected		76 (46–110)
all		76 (46–115)
BMI (kg/m^2)		
healthy volunteers		25 (20–32)
HIV-infected		24 (15–38)
all		24 (15–38)
RTV AUC_{0-24} (mg·h/L)		
healthy volunteers		7.36 (4.31–13.42)
HIV-infected		7.59 (2.41–22.05)
all		7.52 (2.41–22.05)
Baseline CD4 cell count ($cells/mm^3$)		434 (10–1181)
Baseline HIV-RNA (copies/mL)		61 (<50–72)

n, number of patients; M, male; F, female; ATV, atazanavir; RTV, ritonavir; SQV, saquinavir; TDF, tenofovir; AUC_{0-24}, area under the concentration–time curve.
[a]$n=16$ healthy volunteers.

backwards elimination were performed, only ritonavir AUC_{0-24} on atazanavir CL/F and *V*/F remained. Inclusion of ritonavir AUC_{0-24} on atazanavir *V*/F failed to reduce IIV of this parameter by >10% and therefore was removed from the final model (Table 2). The association of ritonavir AUC_{0-24} and atazanavir CL/F was described by a power relationship, illustrated below:

$$CL/F_i = \theta_1 * (RTV_i/7.52)^{\theta_2}$$

where CL/F_i is the atazanavir CL/F of the *i*th individual; θ_1 is the population parameter estimate; RTV_i is the ritonavir AUC_{0-24} of the *i*th individual; 7.52 is the median ritonavir AUC_{0-24} of all individuals expressed as mg·h/L; and θ_2 is the factor associated with ritonavir AUC_{0-24} on atazanavir CL/F.

A power relationship between atazanavir CL/F and ritonavir AUC_{0-24} was associated with a greater drop in both OFV and IIV in CL/F compared with a linear (ΔOFV, ΔIIV: −44.6, −19% versus −27.4, −13%) and an exponential function (−44.6, −19% versus −35.5, −16%). Parameter estimates for the final model are summarized (Table 2) and goodness-of-fit diagnostic plots shown (Figure 1).

Internal model validation

A 95% prediction interval was generated from 1000 simulations for atazanavir/ritonavir 300/100 mg once daily, one profile per patient ($n=46$ patients; 46000 profiles in total) with covariate values of those individuals used in the model building process (Figure 2). Observed data from patients used in the model building process were superimposed onto the prediction interval. Of 538 concentrations, 2% were above P97.5 and 4% were below P2.5 (Figure 2). This analysis suggests that the final model provided an adequate fit to the data with 94% of concentration data falling within the prediction interval.

JAC antiviral

Table 2. Atazanavir parameter estimates and relative standard errors obtained from the final population pharmacokinetic model

Parameter	Basic model		Final model	
	estimate	RSE (%)[a]	estimate	RSE (%)[a]
CL/F (L/h)	7.6	8	7.7	5
V/F (L)	103	6	103	13
k_a (h^{-1})	3.5	55	3.4	34
Lag-time (h)	0.96	3	0.96	1
IIV CL/F (%)	48	36	29	59
IIV *V*/F (%)	48	53	48	37
IIV k_a (%)	154	103	154	51
Residual error				
proportional (%)	23	18	23	27
additional (mg/L)	0.08	84	0.08	38
Factor associated with RTV AUC_{0-24} on ATV CL/F[b]	—	—	−0.8	13

RSE (%), relative standard error; CL/F, apparent oral clearance; *V*/F, apparent volume of distribution; k_a, absorption rate constant; IIV, inter-individual variability; AUC_{0-24}, area under the concentration–time curve.
[a]RSE defined as: $(SE_{estimate}/estimate)*100$.
[b]RTV AUC_{0-24} as a covariate not included in the basic model.

Table 3. Models explored to determine the influence of covariates on atazanavir pharmacokinetic parameters following univariate analysis

Covariate	Model	θ_1	θ_2	ΔOFV	*P* value
Influence of RTV AUC_{0-24} on CL/F	$CL=\theta_1*(RTV/7.52)^{\theta_2}$	7.66	−0.84	−44.6	<0.001
Influence of RTV AUC_{0-24} on *V*/F	$V=\theta_1*(RTV/7.52)^{\theta_2}$	104	−0.50	−10.1	<0.01
Influence of HIV status on CL/F	$CL=\theta_1*\theta_2^{HIV}$	8.73	0.80	−2.2	NS
Influence of HIV status on *V*/F	$V=\theta_1*\theta_2^{HIV}$	118	0.81	−1.8	NS
Influence of HIV status on k_a	$k_a=\theta_1*\theta_2^{HIV}$	2.77	1.42	−0.4	NS
Influence of SQV on CL/F	$CL=\theta_1*\theta_2^{SQV}$	8.86	0.67	−8.3	<0.01
Influence of SQV on *V*/F	$V=\theta_1*\theta_2^{SQV}$	123	0.64	−9.4	<0.01
Influence of TDF on CL/F	$CL=\theta_1*\theta_2^{TDF}$	7.75	0.83	−0.8	NS
Influence of TDF on *V*/F	$V=\theta_1*\theta_2^{TDF}$	108	0.71	−2.3	NS
Influence of TDF on k_a	$k_a=\theta_1*\theta_2^{TDF}$	3.43	1.10	−0.0	NS
Influence of sex on CL/F	$CL=\theta_1*\theta_2^{SEX}$	7.95	0.77	−2.0	NS
Influence of sex on *V*/F	$V=\theta_1*\theta_2^{SEX}$	98.7	1.26	−1.5	NS
Influence of Black-African ethnicity on CL/F	$CL=\theta_1*\theta_2^{AFR}$	7.67	0.91	−0.2	NS
Influence of Black-African ethnicity on *V*/F	$V=\theta_1*\theta_2^{AFR}$	100	1.23	−1.0	NS
Influence of Black-African ethnicity on k_a	$k_a=\theta_1*\theta_2^{AFR}$	3.28	1.44	−0.3	NS
Influence of Hispanic ethnicity on CL/F	$CL=\theta_1*\theta_2^{HSP}$	7.11	1.60	−5.0	<0.05
Influence of Hispanic ethnicity on *V*/F	$V=\theta_1*\theta_2^{HSP}$	118	0.81	−2.0	NS
Influence of Hispanic ethnicity on k_a	$k_a=\theta_1*\theta_2^{HSP}$	2.81	4.42	−4.3	<0.05
Influence of weight on CL/F	$CL=\theta_1+\theta_2*(WT-76.2)$	7.81	0.13	−8.3	<0.01
Influence of weight of *V*/F	$V=\theta_1+\theta_2*(WT-76.2)$	105	1.26	−5.8	<0.05

CL/F, apparent oral clearance; *V*/F, apparent volume of distribution; k_a, absorption rate constant; θ_1: typical value of the parameter; θ_2, estimate of the factor associated with the covariate; ΔOFV, change in objective function value; RTV, ritonavir; AUC_{0-24}, area under the concentration–time curve over 24 h; SQV, saquinavir; TDF, tenofovir; AFR, Black-African ethnicity; HSP, Hispanic ethnicity; WT, weight.

Predictions of atazanavir trough concentrations and AUC_{0-24} were made using single or a combination of two measured concentrations from each HIV-infected individual ($n=30$) and then compared with measured trough concentrations and AUC_{0-24} by means of %RMSE and %MPE (Table 4). A %RMSE <15% and %MPE not significantly different from zero were indicative of an acceptable predictive performance. An example of individual predictions of atazanavir trough and AUC_{0-24} compared with

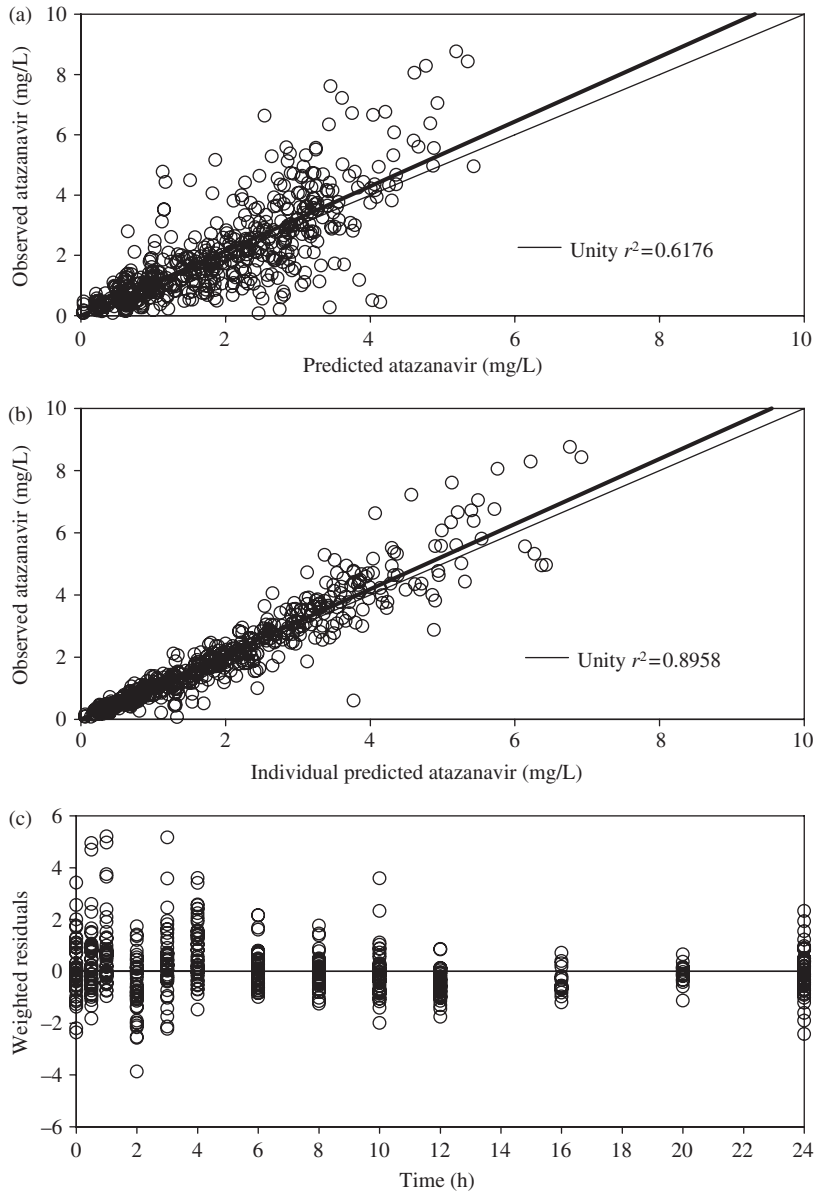

Figure 1. Goodness-of-fit plots for the final pharmacokinetic model illustrating (a) population predictions of atazanavir versus observed concentrations, (b) individual predictions of atazanavir versus observed concentrations and (c) weighted residuals versus time post-dose. The fine line describes the line of unity and the bold line describes the line of regression.

the measured values are shown when using a sample taken 4 h post-dose and following a combination of samples taken 4 and 8 h post-dose (Figure 3). The predictive performance was better for atazanavir AUC_{0-24} compared with trough concentrations with 11 of 15 chosen timepoint combinations, providing both precise and unbiased predictions (Table 4). For atazanavir trough concentrations, none of the chosen timepoints provided precise predictions (%RMSE 21.4–48.1); however, 67% (10 of 15) were unbiased.

External model validation

A 95% prediction interval was generated from 1000 simulations of the lower dose atazanavir/ritonavir regimens (200/100 and 150/100 mg once daily), one profile per patient (n=46 patients; 46 000 profiles in total) with covariate values of those individuals used in the model building process (Figure 2). Data from 18 HIV-infected patients (2 female) administered atazanavir/ritonavir/saquinavir 200/100/1600 and 150/100/1600 mg once daily as part of another external study[20] were superimposed on the 95% prediction intervals. Median (range) age, weight and BMI were 44 years (23–63), 75 kg (46–95) and 24 kg/m^2 (14–32). All patients had an undetectable viral load (< 50 copies/mL) with the exception of two individuals with viral loads of 74 and 84 copies/mL and CD4 cell count ranged between 123 and 837 cells/mm^3. Median (range) ritonavir AUC_{0-24} was 9.06 mg·h/L (3.84–15.29) and 8.86 mg·h/L (2.36–15.06) for 200/100/1600 and 150/100/1600 mg once daily, respectively. A total of 198 concentrations were available for the two lower dose regimens with 3% and 4% of concentrations lying outside

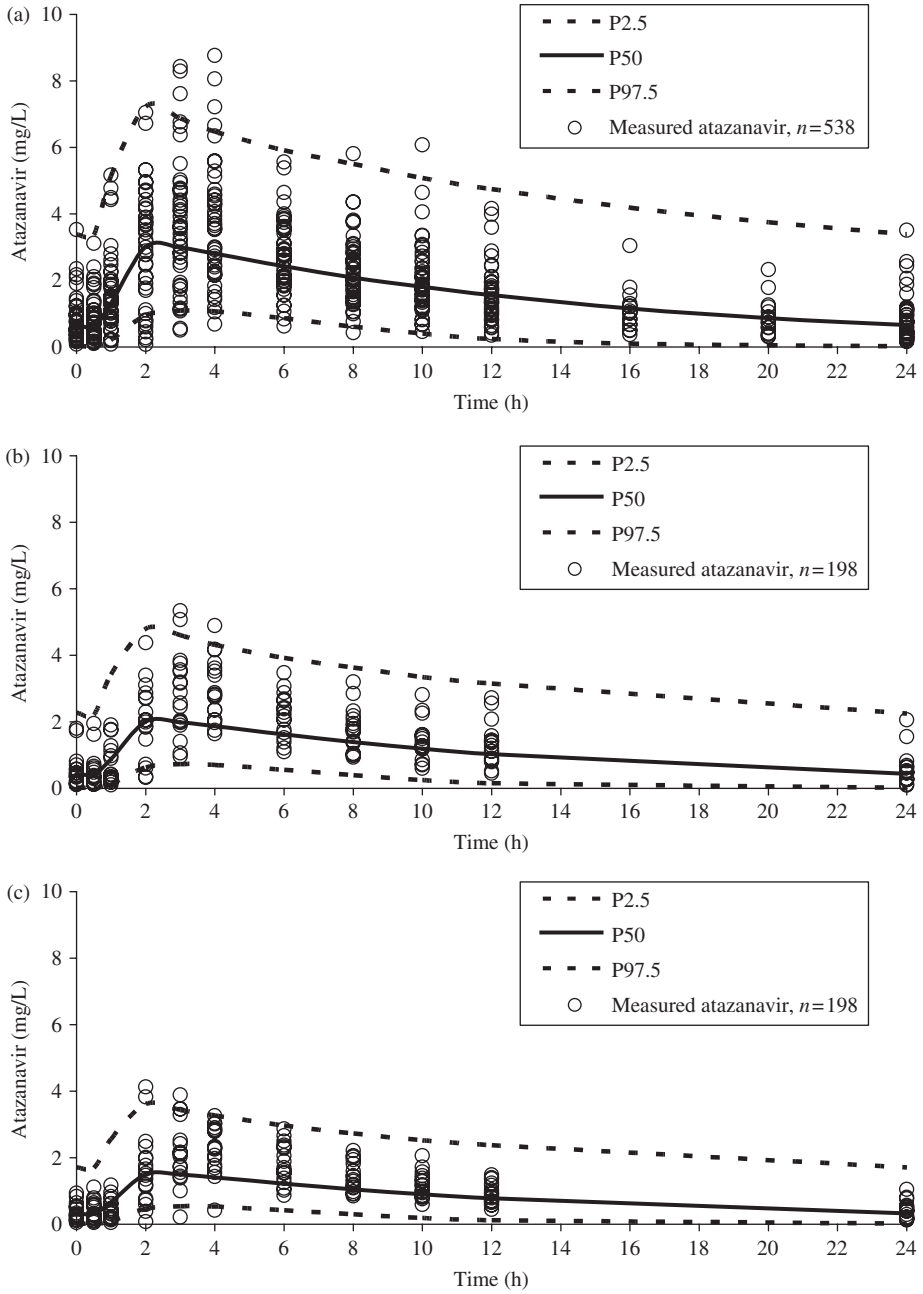

Figure 2. Ninety-five percent prediction intervals (P2.5–P97.5) determined from simulated data of atazanavir/ritonavir administered at (a) 300/100 mg once daily, (b) 200/100 mg once daily and (c) 150/100 mg once daily. Observed data are superimposed for the three evaluated regimens.

of the 95% prediction interval for atazanavir/ritonavir 200/100 and 150/100 mg once daily, respectively (Figure 2). The final model provided an adequate fit to the data with between 96% and 97% of concentration data falling within the prediction intervals for the two evaluated regimens.

Predictions of atazanavir trough concentrations and AUC_{0-24} were made using single measured concentrations from each HIV-infected individual included in the external atazanavir/ritonavir dataset (in combination with saquinavir; $n=18$)[20] and then compared with measured trough concentrations and AUC_{0-24} by means of %RMSE and %MPE as for the internal validation. A similar scenario was observed as for that of the internal validation based on single timepoint predictions (2, 4, 6, 8, 10 and 12 h post-dose). Precise and unbiased predictions of atazanavir AUC_{0-24} were obtained using the model and concentrations at 4, 6, 8, 10 and 12 h post-dose for 200/100 mg once daily and at 4, 6, 8 and 10 h post-dose for 150/100 mg once daily. As with atazanavir/ritonavir 300/100 mg once daily, none of the trough predictions were precise (%RMSE 27.4–45.4 and 21.3–49.3 for 200/100 and 150/100 mg once daily, respectively) with the exception of the 12 h post-dose for the 150/100 mg regimen, which estimated atazanavir trough with precision and accuracy (%RMSE: 14.7; %MPE, 95% CI: −1.3, −8.3 to 5.6).

Table 4. Predictive performance of the final model to predict atazanavir trough concentration (C_{trough}) and area under the concentration–time curve (AUC_{0-24}) from a single sample or combination of two samples

	Atazanavir C_{trough} prediction		Atazanavir AUC_{0-24} prediction	
Time (h)	%RMSE	%MPE (95% CI)	%RMSE	%MPE (95% CI)
2	21.4	0.1 (−7.7 to 7.9)	**11.8**	**−3.1 (−7.3 to 1.0)**
4	27.9	−3.2 (−13.3 to 6.9)	**8.3**	**2.1 (−1.0 to 4.9)**
6	48.1	−7.1 (−24.4 to 10.2)	**6.8**	**−1.1 (−3.7 to 1.2)**
8	42.1	−7.8 (−22.9 to 7.2)	**6.4**	**−2.3 (−4.1 to 0.4)**
10	31.4	−6.5 (−17.7 to 4.6)	**8.5**	**−2.0 (−5.0 to 1.0)**
12	27.3	−10.2 (−19.4 to −0.9)	8.6	−4.1 (−6.9 to −1.4)
2, 4	24.2	−0.7 (−9.5 to 8.2)	**8.5**	**1.7 (−1.4 to 4.7)**
2, 6	22.9	0.2 (−8.2 to 8.5)	**7.8**	**−2.2 (−5.0 to 0.5)**
2, 8	24.5	−3.1 (−11.9 to 3.4)	7.4	−3.8 (−6.1 to −1.5)
2, 10	26.8	−6.1 (−15.6 to 3.4)	8.3	−4.5 (−7.1 to −2.0)
2, 12	24.3	−9.7 (−17.8 to −1.6)	9.5	−6.6 (−9.1 to −4.1)
4, 6	42.5	−11.3 (−26.2 to 3.6)	**6.4**	**0.1 (−2.4 to 2.2)**
4, 8	33.1	−12.7 (−23.8 to −1.6)	**5.6**	**−0.9 (−2.9 to 1.1)**
4, 10	28.8	−12.2 (−21.7 to −2.7)	**6.4**	**−0.7 (−3.0 to 1.6)**
4, 12	26.0	−16.4 (−23.7 to −9.1)	4.8	−2.1 (−3.7 to −0.6)

Values in bold type are precise (%RMSE < 15%) and unbiased (%MPE not significantly different from zero).
C_{trough}, concentration at the end of the dosing interval, i.e. 24 h post-dose; AUC_{0-24}, area under the concentration–time curve over 24 h; %RMSE, root mean square relative prediction error (precision); %MPE, mean relative prediction error (bias); CI, confidence interval.

Discussion

A model has been developed and validated to describe ritonavir-boosted atazanavir pharmacokinetics in healthy individuals and HIV-infected patients. Of the covariates available, only ritonavir AUC_{0-24} was significantly associated with atazanavir pharmacokinetics, reducing the variability of atazanavir CL/F by 19%. Furthermore, using the model, lower dose atazanavir/ritonavir regimens (200/100 and 150/100 mg once daily) could be simulated.

Atazanavir pharmacokinetics were best described by a one-compartment model with first-order absorption, which is consistent with previous studies.[8,11,22] Population estimates for CL/F were similar, and as with previous analyses, IIV of parameters was wide, particularly k_a (154% in this analysis versus 122%–156% in other studies).[11,22] This could be partially attributed to few samples being taken in the absorption phase; however, characterization of the absorption phase was not the main focus of this analysis. Atazanavir absorption can be affected by food intake; however, all individuals included in the model-building process were part of clinical studies where food intake was standardized and carefully controlled for all participants. Furthermore, atazanavir absorption is highly dependent on gut pH, which will be variable between subjects. The median (range) individual estimate of half-life was 8.9 h (4.4–24.9) and consistent with that reported in a population analysis by Colombo *et al*. (8.8 h).[11]

There are data suggesting that atazanavir concentrations are lower in HIV patients compared with that in healthy individuals.[1,2] Atazanavir minimum concentrations are more affected (∼50% lower) than peak concentrations (∼30%) and AUC_{0-24} (∼20%), and the differences are more pronounced in the absence of ritonavir boosting.[1] Atazanavir dissolution and absorption relies heavily on an acidic environment and it has been speculated that HIV patients produce less acid due to hypochlorhydria,[23] therefore reducing atazanavir absorption. HIV status was investigated as a covariate in this analysis, but inclusion in the model did not significantly improve the fit when assessed for CL/F, *V*/F or k_a. Although it cannot be confirmed, it is plausible that HIV patients included in the analysis were not suffering from significant hypochlorhydria; therefore, no differences in pharmacokinetic parameters could be detected. Females had ∼29% lower mean individual predicted CL/F compared with males (6.40 versus 8.99 L/h), but this was not significant, which confirm data by von Hentig *et al*.[24] No association was observed between atazanavir pharmacokinetic parameters and ethnicity; however, it is possible that the analysis is underpowered to detect any disparities as the majority of the cohort were Caucasian and only seven Black-Africans and six Hispanic individuals were included. Moreover, no significant differences were observed following concomitant use of saquinavir (1600 mg once daily), which is consistent with previous studies,[15,16] or tenofovir (300 mg once daily). Tenofovir has been shown to lower atazanavir concentrations[8,9]; however, our analysis is probably underpowered to evaluate this drug–drug interaction as only 6 of the 46 individuals were receiving tenofovir, although a study has shown that tenofovir does not affect boosted atazanavir concentrations.[25] Not surprisingly, ritonavir AUC_{0-24} described some of the variability in atazanavir pharmacokinetics; however, other potentially important covariates that were not measured may also contribute.

Atazanavir/ritonavir has the lowest pill burden of all protease inhibitors, has a favourable lipid profile and is suitable for once-daily dosing. For some patients, toxicity due to hyperbilirubinaemia may be problematic and rather than switch therapies there could be potential for dose reduction, as lower ritonavir-boosted

JAC antiviral

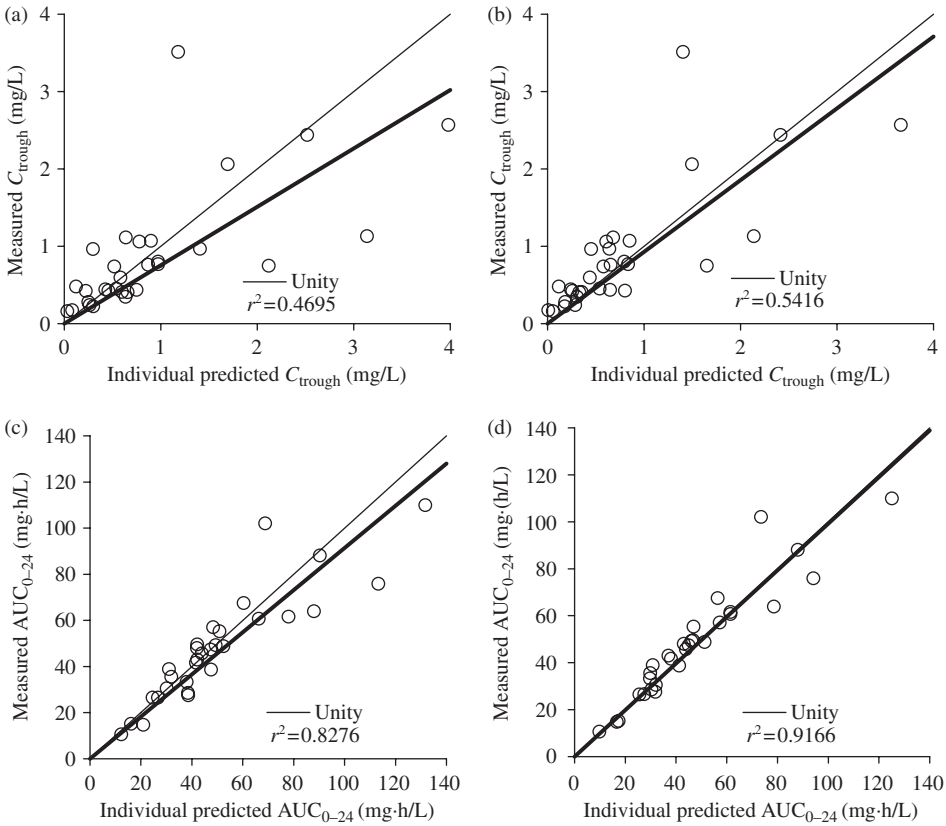

Figure 3. Individual predictions versus observed atazanavir trough (C_{trough}) using samples taken at (a) 4 h post-dose and (b) 4 and 8 h post-dose, and individual predicted versus observed atazanavir area under the curve (AUC_{0-24}) using samples taken at (c) 4 h post-dose and (d) 4 and 8 h post-dose ($n=30$). The fine line describes the line of unity and the bold line describes the line of regression.

atazanavir doses have been associated with lesser increases in total and indirect bilirubin.[20] Furthermore, a small pilot study in HIV-infected Thai patients ($n=14$) investigated the feasibility of using lower dose atazanavir/ritonavir (200/100 mg once daily) as an alternative to indinavir/ritonavir (400/100 mg twice daily), reducing pill burden and costs and improving tolerability.[26] All patients studied obtained trough concentrations above the recommended minimum effective concentration (MEC) for atazanavir (0.15 mg/L) and viral loads <50 copies/mL coupled with significant increases in CD4 cell count.[26] Here, the final model simulated atazanavir/ritonavir profiles dosed at 200/100 and 150/100 mg once daily; however, concern surrounds whether at lower atazanavir/ritonavir doses trough concentrations can remain above the MEC of 0.15 mg/L for viral suppression. Of the 46000 simulated profiles for each of the three evaluated regimens, 14%, 20% and 24% of trough concentrations were <0.15 mg/L for atazanavir/ritonavir 300/100, 200/100 and 150/100 mg once daily, respectively. Lower atazanavir/ritonavir doses may be suitable for some patients; however, efficacy data are required.

As atazanavir is administered once daily and many patients choose to take their medication in the evening, obtaining a trough concentration in the clinic for TDM can be problematic. It would therefore be advantageous to predict trough concentrations from a single sample or even estimate AUC_{0-24}, which would not only benefit TDM interpretation but would be of particular use for clinical studies incorporating genomic analyses to allow greater patient recruitment, or for studies in resource-limited settings. Solas *et al.*[22] recently described a pharmacokinetic model for atazanavir that allows Bayesian estimation of atazanavir trough, although we await details of the precision and accuracy of this approach. Taking into consideration that full pharmacokinetic profiles can have an erratic appearance, model capability to determine trough concentrations from single samples should be evaluated. We therefore used our model and single or a combination of two samples to predict trough concentrations and AUC_{0-24} of the HIV patients included in the model for which these parameters were already known. Overall, prediction of AUC_{0-24} was good for the majority of timepoints, with 73% of predictions being both precise and unbiased; however, predictions of trough concentrations tended to be unbiased but not precise, based on %RMSE and %MPE criteria. The same was true for predictions of trough and AUC_{0-24} for 200/100 and 150/100 mg, respectively. The model provides an adequate fit to the data, confirmed by the validation process; however, a number of concentration–time profiles were inconsistent, potentially making predictions of trough concentrations more difficult compared with prediction of exposure. Overall, the analysis confirms that predictions of atazanavir CL/F from sparse samples were acceptable as CL/F is a function of the area under the curve and dose of drug; however, estimation of trough concentrations from sparse sampling was not consistent, with predictions potentially being more sensitive to high variability in atazanavir absorption.

In conclusion, a population pharmacokinetic model to characterize ritonavir-boosted atazanavir (300/100 mg once daily) in HIV-infected patients and healthy volunteers has been developed and validated. Ritonavir AUC_{0-24} described some of the variability in atazanavir concentrations; however, covariates not captured in this study should be investigated. The model can be used to simulate lower dose atazanavir concentrations boosted with ritonavir; however, prediction of trough concentrations from sparse sampling may be limited. Successful prediction of AUC_{0-24} from sparse sampling would be advantageous, particularly when conducting pharmacokinetic studies in resource-limited settings, and would also be useful to investigate optimal sampling strategies for clinical studies where exposure is the parameter of interest.

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