Simultaneous pharmacokinetic modeling of unbound and total darunavir with ritonavir in adolescents: a substudy of the SMILE trial

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Abstract

Darunavir (DRV) is an HIV protease inhibitor commonly used as part of antiretroviral treatment regimens globally for children and adolescents. It requires a pharmacological booster, such as ritonavir (RTV) or cobicistat. To better understand the pharmacokinetics (PK) of DRV in this younger population and the importance of RTV boosting effect, a population PK substudy was conducted within the SMILE trial. A joint population PK model that simultaneously used total DRV, unbound DRV and total RTV concentrations was developed. Competitive and non-competitive models were examined to define RTV influence on DRV pharmacokinetics. Linear and non-linear equations were tested to assess DRV protein binding. A total of 443 plasma samples from 152 adolescents were included in this analysis. Darunavir PK was best described by a one compartment model with first-order absorption and elimination. Influence of RTV on DRV pharmacokinetics was best characterized by ritonavir AUC on DRV clearance using a power function. Association of non-linear and linear equations were used to describe DRV protein binding to alpha-1 glycoprotein and albumin, respectively. In our population, simulations indicate that 86.8 % of total and unbound DRV trough concentrations were above 0.55 mg/L (10 times protein binding-adjusted EC_{50} for WT HIV-1) and 0.0243 mg/L (10 times EC_{90} for WT HIV-1) targets, respectively. Predictions were also in agreement with observed outcomes from adults receiving 800/100 mg DRV/r once a day. Administration of 800/100 mg of DRV/r once daily provides satisfactory concentrations and exposures for adolescents aged 12 years and older.

Introduction

Current international antiretroviral treatment guidelines continue to recommend 3-drug antiretroviral therapy (ART) as the preferred first-line treatment for children and adolescents living with HIV.(1, 2) These triple ART drug combinations are primarily composed of two nucleos(t)ide reverse transcriptase inhibitor (NRTI) backbone plus either a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor (PI) or an integrase strand transfer inhibitor INSTI anchor drug. The relative tolerability and potential complications associated with long-term NRTI treatments has led to investigation of NRTI-sparing drug combinations.(3, 4) Darunavir is a PI administered with a pharmacological booster, cobicistat or ritonavir (RTV), and is included in potential NRTI-free regimens, such as dolutegravir plus ritonavir-boosted DRV (DRV/r).(5, 6) Pharmacokinetic studies of DRV/r in children and adolescents already exist and a population PK model has been built using adult and paediatric data.(7) In adults, boosting effect of ritonavir was described by different types of inhibition models(8, 9) showing that ritonavir effect on darunavir clearance is not proportional to ritonavir concentrations or exposures. Giving 100 mg daily of ritonavir instead of 200 mg daily (100 mg twice daily) may result in a substantial difference from the expected boosting effect. In addition, boosting behavior of ritonavir was not studied yet in adolescents and limited data are available concerning the influence of the RTV boosting on DRV pharmacokinetic in adolescents when administered once instead of twice daily. Darunavir is highly bound to plasma protein, primarily to alpha-1 glycoprotein (AAG), with saturation of binding at high therapeutic concentrations,(10) which may lead to changes in the unbound fraction. Unbound DRV concentrations, the pharmacological active form of the drug, has been investigated but mostly in specific adult populations (e.g. pregnant women or patients with hepatic cirrhosis).(10–13)

SMILE (PENTA 17-ANRS 152) was an international multicenter clinical trial evaluating the safety and efficacy of dolutegravir combined with DRV/r once a day in adolescents aged 12

years and older. This pharmacokinetic substudy of the SMILE trial aimed to (1) characterize the pharmacokinetics of DRV and RTV, (2) define the influence of RTV on DRV pharmacokinetics, (3) establish the relationship between unbound and total DRV concentrations and determine plasma protein-binding behavior, and (4) evaluate DRV/r fixed-dose of 800/100 mg once daily in adolescents.

Results

Darunavir/ritonavir quantification and population characteristics

Twelve samples had DRV, RTV (and dolutegravir) concentrations below the lower limit of quantification and were excluded from the analysis due to suspected non-adherence to trial medication. The final dataset included a total of 443 plasma samples from 152 participants, with a mean of 3 samples per patient. Median (range) age was 15 (12 – 18) years old and weight was 50 (39 – 97) kg. Table 1 summarizes baseline characteristics of the population.

Regarding the distribution of time sampling, 15.8 % of blood samples were collected in the first 10 hours post-dose, 78.6 % were collected within 10 hours and 20 hours post-dose; and 5.6 % of blood samples were collected after 20 hours post-dose. Figure 1 displays time points distribution of blood collections.

Total DRV, RTV and unbound DRV concentrations were measured for each plasma sample and used for model building. Concentrations below the lower limit of quantification (LLOQ) represented 2.0 %, 4.7 % and 3.6 % of the dataset for total DRV, RTV and unbound DRV, respectively. Median [IQR] total DRV concentration measured was 3.27 [2.19 – 4.71] mg/L,

and median [IQR] unbound DRV concentration measured was 0.173 [0.112 – 0.261] mg/L. The median [IQR] DRV free fraction (whatever the delay between administration and sampling), calculated for each blood sample as $\frac{Unbound\ concentration}{total\ concentration} \times 100$, was 5.4 [4.2 – 6.9] %.

Pharmacokinetics of darunavir and ritonavir

Two separate models were built for DRV and RTV using total DRV and RTV concentrations, respectively. A one-compartment model with first-order absorption and elimination best described the data for both drugs. The PK parameters of the models were absorption constant (*ka*), apparent volume of distribution (*V/F*) and apparent clearance (*CL/F*). PK parameters were well estimated (i.e., relative standard error, RSE < 30%). Inter-individual variability (IIV) on V/F_{DRV}, CL/F_{DRV} and CL/F_{RTV} were kept in the models. Other PK parameters IIVs were fixed to zero. Residual variability was defined with a proportional error model for both models.

For the DRV model, great influence of alpha-1 glycoprotein concentrations on CL/F_{DRV} was observed. Inclusion of alpha-1 glycoprotein concentrations in the model led to an objective function value (OFV) decrease of 95.4 units and an IIV decrease on CL/F_{DRV} of 4 %.

An allometric model, standardised on an adult weight of 70 kg and with an effect of weight fixed to 1 on volume of distribution and fixed to 0.75 on apparent clearance, was implemented for ritonavir model. Estimating allometric parameters rather than fix them did not improve the model. No other covariates were retained.

Influence of ritonavir on darunavir: total darunavir/ritonavir joint model

Different competitive and non-competitive interaction models of ritonavir on darunavir clearance were tested using the previous DRV and RTV models. Ritonavir exposure (AUC) with a power function on ritonavir clearance best described influence of RTV on DRV pharmacokinetics.

Equation of darunavir oral clearance from the interaction model was:

$$CL/F_{DRV,i}(L/h) = 9.7 \times \left(\frac{[AAG]_i}{0.66}\right)^{-0.73} \times \left(\frac{AUC_{0-24,RTV,i}}{5.8}\right)^{-0.38} \quad (9)$$

While equations of ritonavir oral clearance and volume of distribution were:

$$CL/F_{RTV,i}\,(L/h) = 21.8 \times \left(\frac{WEIGHT_i}{70}\right)^{0.75} \quad (10)$$

$$V/F_{RTV,i}\,(L) = 107.6 \times \left(\frac{WEIGHT_i}{70}\right) \quad (11)$$

Ritonavir AUC represents the AUC between 0 and 24h post-dose at steady-state and was obtained by dividing the dose with the apparent clearance of ritonavir. Median ritonavir $AUC_{0\text{-}24h}$ in our population was 5.8 mg*h/L.

The model showed acceptable performance with good diagnostic plots and prediction-corrected Visual Predictive Check (pcVPC). PK parameter estimates, diagnostic plots and pcVPC of this *"intermediate"* model, using only total concentrations, are presented in supplemental material.

DRV protein binding behavior: total/unbound darunavir and ritonavir final joint model

Same modeling process as for total DRV concentrations was used for unbound DRV concentrations. The structural model was defined, and the inclusion of other potential covariates

was explored. The interaction of darunavir and ritonavir was then added using the interaction model previously established. A one-compartment model best described unbound DRV concentrations. The effect of alpha-1 glycoprotein was reassessed to refine the relationship between unbound and total DRV concentrations. Unbound DRV concentrations was linked to total DRV concentrations using several protein-binding behavior models. The relationship between unbound and total DRV concentrations was best described using a non-linear model regarding darunavir binding to alpha-1 glycoprotein (AAG), and a linear model regarding darunavir binding to albumin (HSA). Figure 2 shows a schematic representation of the final joint model that simultaneously used unbound DRV, total DRV and RTV concentrations. Parameters estimated of total/unbound relationship were dissociation constant (k_d) for alpha-1 glycoprotein and a binding constant θ_{HSA} for albumin. Equation for total/unbound relationship was:

$$C_{DRV} = \left(\frac{N_{AAG} \times [AAG] \times C_{DRV,u}}{K_{d,AAG} + C_{DRV,u}}\right) + \left(\theta_{HSA} \times [HSA] \times C_{DRV,u}\right) + C_{DRV,u} \qquad (12)$$

The number of drug-binding sites on alpha-1 glycoprotein (N_{AAG}) was fixed to 1 as the estimate was near this value and was reported in the literature.(14) Moreover, fixing this parameter to 1 did not significantly increase the OFV. Parameter estimates of the final model, using total/unbound DRV concentrations and RTV concentrations, are detailed in Table 2. Diagnostic plots and predictions-corrected Visual Predictive Check of this final model are shown in Figures 3 and 4, respectively.

Implication of alpha-1 glycoprotein and albumin in DRV protein binding vary according to DRV concentrations. Figure 5 illustrates the darunavir free fraction, as well as the fractions

bound to alpha-1 glycoprotein and albumin, with respect to darunavir and plasma protein concentrations. At high DRV concentrations, DRV binding to albumin is more important, and unbound fraction increases more or less, depending on protein concentrations. On average, at a median total DRV concentration of 3.27 mg/L in our population, darunavir is 73.7 % bound to alpha-1 glycoprotein and 20.8 % to albumin, for a total plasma protein binding of 94.5%.

Simulations and predictions

Simulation from the final model indicated that administration of 800/100 mg of DRV/r once daily lead to total trough darunavir concentration (C_{DRV}) above the protein-adjusted WT EC_{50} (0.055 mg/L) for 98 % of participants. The recommended target for adults with no documented PI-resistant HIV-1 strains (0.55 mg/L)(15, 16) was reached by 86.8 % of the adolescents; while the recommended target with proven or suspected PI-resistance HIV-1 strains (2 mg/L)(16, 17) was attained by only 47.4 %.

Similar results of target attainment were found with unbound DRV concentrations when using WT EC_{90} as target. Trough $C_{DRV,u}$ were above the WT EC_{90} (0.00243 mg/L) for 98% of the patients, and above 10 times the WT EC_{90} for 86.8 % of them.

Individual-predicted trough DRV concentrations and exposures were comparable with reported observations in treatment-experienced adults receiving the same dose (Table 3).

Discussion

Several darunavir pharmacokinetic models have been published for adults using cobicistat(16) or ritonavir for boosting.(7–9) To our knowledge, Brochot et al.(7) work is the only darunavir model published including children and/or adolescents. No more data was available in adolescents and the effect of ritonavir boosting was not evaluated in this study. Furthermore, no model has studied darunavir free fraction, which is the pharmacological active part of the drug, except for pregnant women.(10) Our model highlighted the binding behavior regarding plasma protein and its potential influence on darunavir pharmacokinetics.

Our study reported a detailed pharmacokinetic study of DRV/r in adolescents of 12 years and older. We brought a novel approach on DRV interactions with plasma protein in clinical settings, which improves our understanding of darunavir elimination and overall pharmacokinetics according to plasma protein levels of patients, and we evaluated the rationale of using adult doses in adolescents.

Through this study, population PK models were built from total plasma DRV concentrations, from unbound DRV concentrations, and from total RTV concentrations. DRV and RTV interaction was assessed using total DRV and RTV concentrations, and DRV plasma protein binding behavior was defined using unbound and total DRV concentrations. Oral clearance of total DRV and RTV were 9.7 L/h and 21.8 L/h, respectively, which is consistent with reported values (e.g. 10.9 L/h(9), 10.7 L/h(8) for darunavir clearance and 20.5 L/h(9), 16.4 L/h(8) for ritonavir clearance) in the literature. Volumes of distribution of darunavir and ritonavir were however significatively higher than previously reported. Brochot et al. observed an important elevation of peripheral volume of darunavir (from 83 L to 254 L) by adding children and adolescents in its modeling dataset. We were not able to define a second compartment for darunavir or ritonavir, but it might explain why our estimates of volume of distribution are higher compared to above cited models in adults.

Darunavir is almost exclusively metabolized by cytochrome P450 (CYP) 3A4, and 2D6 to a lesser degree. By inhibiting CYP3A4, ritonavir decreases darunavir elimination and provides higher darunavir plasma trough concentrations and overall exposures. PK boosting effect of RTV on DRV concentrations was evaluated by testing different competitive and non-competitive inhibition models on darunavir clearance. Ritonavir AUC with a power function model was found best to characterize its effect on darunavir clearance compared to time-point concentrations. While precise inhibition mechanisms of CYP3A4 by ritonavir have not been clearly established; competitive, mixed-non-competitive and mechanism-based inhibition have been reported.(18, 19) Ritonavir inhibits CYP2D6 enzyme and P-gp efflux transporter that can also contribute to the boosting effect.(20, 21) The complexity of all possible ritonavir and darunavir interactions could probably be the reason that makes a direct competitive inhibition model with time-point ritonavir concentrations unsuitable, or would demand a more in-depth mechanistic model. Ritonavir AUC, which reflects overall dose exposure, fits more reasonably with a population PK model and matches with mechanism-based inhibition of CYP3A4.(9, 18, 21, 22)

Darunavir is mainly bound to alpha-1 glycoprotein in human plasma. Common values for alpha-1 glycoprotein concentrations are between 0.5 and 1.2 g/L. This relatively low alpha-1 glycoprotein concentrations and the one single drug-binding site available on each alpha-1 glycoprotein molecule(14) explain the saturation pattern observed in darunavir plasma protein binding. Nevertheless, darunavir binds to both alpha-1 glycoprotein and albumin.(23) Saturation of alpha-1 glycoprotein binding is partially compensated by albumin binding, which limits the exponential increase of unbound fraction. Our model was able to define the implication of both alpha-1 glycoprotein and albumin in DRV protein binding behavior. Indeed, unbound fraction and proportion of DRV bound to alpha-1 glycoprotein or albumin are highly

variable and depend greatly on variation of DRV, alpha-1 glycoprotein and albumin concentrations (Figure 5).

Several studies indicate that alpha-1 glycoprotein concentrations interfere with DRV PK parameters.(7, 8, 16) Our total Darunavir PK model showed that alpha-1 glycoprotein concentrations had influence on oral DRV clearance. By adding unbound DRV concentrations to the model, alpha-1 glycoprotein was found to explain the relationship between total and unbound concentrations, and its effect on darunavir clearance was no longer visible. This finding informs us about the involvement of alpha-1 glycoprotein on darunavir pharmacokinetics. DRV presents properties of low extraction type of drug. In the therapeutic DRV concentration range, plasma free fraction is mainly driven by alpha-1 glycoprotein concentrations. Predictions showed that alpha-1 glycoprotein does not clearly affects unbound DRV trough concentrations. However, total DRV trough concentrations are positively correlated with alpha-1 glycoprotein (AAG) concentrations, although AAG concentrations do not prevent target attainment (supplemental material). Thus, alpha-1 glycoprotein concentrations variations influence indirectly total DRV clearance but do not influence unbound DRV clearance.

No direct association between darunavir exposure/concentration and viral load decrease had been demonstrated, challenging the necessity of therapeutic drug monitoring for this drug.(24, 25) However, clinical practices suggest 0.55 mg/L, or 2 mg/L for patients that are PI-experienced with HIV strains expressing PI-resistant gene, as good trough concentration targets, although the 0.55 mg/L cut-off can be considered conservative for patients that are PI-naïve.(15–17, 26) No investigation was made to define potential link between unbound DRV exposure/concentration and viral load change. We, therefore, decided to use ten times the WT EC_{90} for unbound trough concentrations, which resulted to the same findings as ten times the *protein-adjusted* WT EC_{50} for total trough concentrations.

For a daily DRV/r dose of 800/100 mg, simulated PK outcomes indicate good trough concentrations and exposures. Predictions for our population are also relatively similar to PK outcomes recorded for adults receiving the same dose in ODIN trial (Table 3).(25) Our findings, considering the targets used, are also consistent with the primary outcome of the SMILE trial, where 95% of participants were maintained with a suppressed viral load (VL < 50 copies/mL) by week 48.(27)

All these results encourage the use of once daily adult dose in adolescents of 12 years and older but caution is necessary for patients presenting proven or suspected PI-resistant strains. The 2 mg/L target for trough C_{DRV} was scarcely attainable for more than half of our population with this current fixed dose. A twice-daily DRV/r dose of 600/100 mg would be more adequate for patients with probable or confirmed HIV PI-resistance. This suggestion is primarily based on the equivalence of trough concentrations and exposures observed between adolescents and adults. The similarity in the PK outcomes led us to suggest that a 600/100 mg twice daily is very likely to be adequate for adolescents with PIs resistance as it is for adults with PIs resistance. No relationship between exposure or concentration and toxicity were identified in adults(28) but investigation in children and adolescents, in regard to this topic, may be necessary at such dose.

Our study has several limitations. Blood samples were collected unequally over dosing intervals. More than half of blood samples were collected between 12h and 15h post-dose, which may have restrained identification of more elaborate absorption model or of a second compartment. Still, the current models presented in this study were well defined and showed good prediction performance via validation tools. Moreover, one-compartment models for darunavir and ritonavir have already been described in other publications.(9, 16) Assessment of the impact of ritonavir on unbound DRV clearance were performed using total RTV concentrations/exposures, although it would ideally be done using unbound RTV

concentrations/exposures. Considering the RTV plasma concentrations range, unbound and total RTV concentrations relationship are very likely to be linear,(10) therefore the use of unbound instead of total RTV concentrations would probably not have modified our findings. An important amount of albumin is present in human plasma, suggesting that DRV binding to albumin could not be saturated at therapeutic concentrations, it was thus described by a linear model and albumin affinity constant or maximal protein binding capacity could not be determined. In addition, albumin carries more than one potential drug-binding site, each of them with different affinity for darunavir. Predictions of protein binding behavior with our model should only be within the DRV, alpha-1 glycoprotein and albumin concentration ranges observed in our study.

In summary, we were able to characterize darunavir and ritonavir pharmacokinetics in adolescents receiving 800/100 mg DRV/r once daily. Protein binding of darunavir was also described by the relative implication of both alpha-1 glycoprotein and albumin. Influence of RTV on DRV clearance was defined and highlights the importance of ritonavir to attain targets. Administration of 800/100 mg of ritonavir-boosted darunavir once daily for adolescents aged 12 years and older provides satisfactory concentrations and exposures, similar to those observed in adults.

Materials and methods

Study design and population

SMILE (Strategy for Maintenance of HIV suppression with once daiLy integrate inhibitor + darunavir/ritonavir in childrEn) is a phase 2/3, multicenter and open-label trial. SMILE trial has

previously been described.(29) Children and adolescents with HIV-1 aged between 12 and 18 years were included in the trial. Before inclusion, patients were virologically controlled (HIV-1 RNA viral load <50 copies/mL for at least 12 months) with no evidence of DRV or INSTI resistance associated mutations. Prior to inclusion, informed consent was obtained from patient's legal representatives after an oral and written communication. Ethical approval was obtained from local and/or National Ethics Committees and relevant Competent Authorities.(27) All information on study design are detailed on clinicaltrial.gov (NCT02383108) and at penta-id.org.(30)

This PK substudy focused on the NRTI-sparing regimen arm where participants weighing ≥ 40 kg received 50 mg of dolutegravir in combination with 800/100 mg DRV/r once daily. Darunavir and ritonavir formulations were film-coated tablets (Prezista® 800 mg + Norvir® 100 mg). Darunavir was provided by Janssen.

Sample collection and analytical method

Blood samples were collected at different time points following a sparse sampling scheme. For each participant, one or two blood samples were collected at designated time points (depending on if they took their medications in the morning or evening) during follow-up visits at weeks 4, 12 and 24. Blood samples were centrifuged, and plasma were stored at –25°C until analysis. Drug concentrations were measured at the laboratory of clinical pharmacology of Cochin Hospital in Paris, France.

Total and unbound DRV concentrations were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS) assays. After thawing and incubation at 37°C for 20 min, unbound DRV was obtained using ultrafiltration with Centrifree® tube for 10 min to collect

protein-free plasma. The assays used for total and unbound concentration measurements were developed in the laboratory and were validated according to the Food and Drug Administration (FDA) guidance(31).

For ritonavir and total darunavir quantification, calibration curves were linear and ranged from 0.01 mg/L to 2.5 mg/L, and from 0.06 mg/L to 15 mg/L, respectively. For unbound darunavir quantification, calibration curve was quadratic and ranged from 0.01 mg/L to 4 mg/L.

The quantification methods were described in detail in Zheng et al. publications.(32, 33)

Pharmacokinetic analysis and data handling

Population pharmacokinetic models were developed using nonlinear mixed-effect modeling software MONOLIX (version 2023R1), along with stochastic approximation expectation-maximization (SAEM) algorithm. Simulations were performed using SIMULX (version 2023R1) and all graphical outputs were managed using R software (version 4.0.5).

Some samples with DRV and RTV concentrations below the lower limit of quantification (LLOQ) were removed due to suspected non-compliance. Concentrations below the LLOQ were left censored and handled using MONOLIX algorithm.(34) Missing time-point covariates for participants were replaced by the most recent observation or by the median observation in the population when a covariate is completely missing for the patient.

The modeling objective was to develop a single PK model that combines unbound DRV, total DRV and RTV concentrations. To do so, the model-building process included several steps: Firstly, DRV and RTV pharmacokinetics were characterized with two separate PK models using total DRV and RTV concentrations. Secondly, a joint model was constructed to define

RTV influence on total DRV concentrations. Thirdly, unbound DRV concentrations were added to the previous model to study darunavir protein-binding.

1. Darunavir and ritonavir pharmacokinetic model

Two separate population pharmacokinetic models were developed to describe total DRV and total RTV concentrations.

Stepwise procedure was used to find models that best suited the data. One or two-compartment models with first-order absorption and elimination were tested with analytical solutions. Inter-individual variability (IIV) was defined by exponential model and only significant IIV of PK parameter were retained. Proportional, additive, and combined models were considered for the residual variability.

IIV on a parameter was kept in the model when their deletion led to an increase of at least 3.84 units (equals to chi-squared, 1 degree of freedom, $P \leq 0.05$) of the objective function value (OFV).

The covariates included age, sex, weight, body mass index, plasma albumin, alpha-1 glycoprotein, bilirubin, creatinine concentrations (determined with Abbott Jaffe or enzymatic methods) and estimated glomerular filtration rate (eGFR). The eGFR was calculated using Schwarz formula.(35)

Continuous covariates were integrated as:

$$\theta_i = \theta_{pop} \times \left(\frac{Cov_i}{median(Cov)}\right)^{\beta} \quad (1)$$

Where θ_{pop} is the typical value of clearance or volume of distribution for a patient with the median covariate value, Cov_i is the covariate value for the individual *i*, and β is the influential factor for the continuous covariate estimated by the modeling software.

Categorical covariates were tested as:

$$\theta_i = \theta_{pop} \times \beta^{Cov_i} \quad (2)$$

Where the covariate value is set to 0 or 1.

Covariate selection is based on a stepwise forward inclusion and backward deletion. Acceptance of a biologically plausible covariate requires a minimal OFV decrease of 3.84 units (chi-squared, 1 degree of freedom, $P \leq 0.05$) in the inclusion phase associated with an IIV decrease of the considered parameter, and a minimal OFV increase of 6.63 units (chi-squared, 1 degree of freedom, $P \leq 0.01$) in the deletion phase.

2. Ritonavir influence on darunavir

Interaction between DRV and RTV was evaluated with a joint model estimating simultaneously PK parameters for both total DRV and RTV. Ritonavir AUC and time-point concentrations were used to evaluate influence of ritonavir on total DRV clearance. Several non-competitive and competitive inhibitions models were tested.

Non-competitive inhibition models link ritonavir AUC with total darunavir clearance (CL_{DRV}) while competitive models link ritonavir time-point concentrations with CL_{DRV} using power or maximum effect functions described as follows:

$$CL_{DRV,i} = CL_{DRV,pop} \times \left(\frac{RTV_i}{median(RTV)}\right)^{-\beta_{RTV}} \quad (3)$$

$$CL_{DRV,i} = CL_{DRV,pop} \times \left(1 - \frac{I_{max} \times RTV_i}{IC_{50} + RTV_i}\right) \quad (4)$$

Where $CL_{DRV,pop}$ is the typical value of DRV clearance, RTV_i is ritonavir AUC (AUC_{RTV}) or time-point concentration (C_{RTV}) for the individual i, β_{RTV} is the power factor representing the influence of RTV on CL_{DRV}, I_{max} is the maximum inhibitory effect of ritonavir and IC_{50} is the RTV value producing half of I_{max}.

3. Darunavir protein binding behavior

Protein binding behavior of darunavir was determined by adding unbound DRV concentrations ($C_{DRV,u}$) to the previous model where the interaction model of RTV on DRV clearance is already set. Total DRV were linked to the unbound DRV concentrations using linear or non-linear relationships between $C_{DRV,u}$ and total DRV concentrations (C_{DRV}). Linear and non-linear protein binding models were defined by the equations below:

$$C_{DRV} = \frac{1}{f_u} \times C_{DRV,u} \quad (5)$$

$$C_{DRV} = \frac{B_{max} \times C_{DRV,u}}{K_d + C_{DRV,u}} + C_{DRV,u} \quad (6)$$

Where f_u is the unbound fraction, B_{max} the maximum protein-binding capacity and K_d the constant of dissociation of darunavir from plasma protein.

Implication of plasma albumin (HSA) and alpha-1 glycoprotein (AAG) in DRV protein binding was also evaluated with the inclusion of HSA- or AAG-dependent parameters in the previous equations, expressed as:

$$C_{DRV} = \theta_{protein} \times [protein] \times C_{DRV,u} \quad (7)$$

$$B_{max} = N_{protein} \times [protein] \quad (8)$$

Where $[protein]$ is the plasma protein (albumin or alpha-1 glycoprotein) concentration, $N_{protein}$ the number of binding sites per protein and $\theta_{protein}$ an hybrid constant integrating $N_{protein}/K_d$ ratio(36). Concentrations were all converted in mmol/L in order to estimate the binding parameters.

4. Model selection and evaluation

For each population PK model developed, main selection criteria were improvement of diagnostic plots, model stability and relative decrease of OFV and IIV when applicable.

Final model evaluation was performed and visual examination was made on diagnostic plots and on generated prediction-corrected visual predictive checks.

5. Target attainments

One thousand Monte Carlo simulations from the final total DRV, unbound DRV and RTV joint model were performed for each patient following steady-state 800/100 mg of DRV/r once-daily and compared to different target trough concentrations.

For total darunavir, trough C_{DRV} targets were set at 0.055 mg/L, the protein binding-adjusted EC_{50} for wild type (WT) HIV-1; at 0.55 mg/L (ten times the protein binding-adjusted EC_{50} for WT HIV-1) recommended for patients with no documented PI-resistant HIV-1 strains; and at 2 mg/L, the recommended trough C_{DRV} for patients with proven or suspected PI-resistance HIV-1 strains.(15–17, 26)

For unbound darunavir, trough $C_{DRV,u}$ target was set at 0.0243 mg/L, which is 10 times the *in vitro* EC_{90} for the WT virus,(11, 37) and coherent with 5% (typical unbound fraction value)(10) of 0.55 mg/L.

Predictions were also compared to PK outcomes in treatment-experienced adults receiving the same doses in the ODIN trial.(25)

Supplementary data

Supplemental data are available online only.

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