ORIGINAL RESEARCH ARTICLE

Comparative Population Pharmacokinetics of Darunavir in SARS-CoV-2 Patients vs. HIV Patients: The Role of Interleukin-6

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Abstract

Background Darunavir is an anti-HIV protease inhibitor repurposed for SARS-CoV-2 treatment.

Objective The aim of this study was to assess the population pharmacokinetics of darunavir in SARS-CoV-2 patients compared with HIV patients.

Methods Two separate models were created by means of a nonlinear mixed-effect approach. The influence of clinical covariates on each basic model was tested and the association of significant covariates with darunavir parameters was assessed at multivariate regression and classification and regression tree (CART) analyses. Monte Carlo simulation assessed the influence of covariates on the darunavir concentration versus time profile.

Results A one-compartment model well-described darunavir concentrations in both groups. In SARS-CoV-2 patients ($n = 30$), interleukin (IL)-6 and body surface area were covariates associated with darunavir oral clearance (CL/F) and volume of distribution (V_d), respectively; no covariates were identified in HIV patients ($n = 25$). Darunavir CL/F was significantly lower in SARS-CoV-2 patients compared with HIV patients (4.1 vs. 10.3 L/h; $p < 0.001$). CART analysis found that an IL-6 level of 18 pg/mL may split the SARS-CoV-2 population in patients with low versus high darunavir CL/F (mean ± standard deviation 3.47 ± 1.90 vs. 8.03 ± 3.24 L/h; proportion of reduction in error = 0.46). Median (interquartile range) darunavir CL/F was significantly lower in SARS-CoV-2 patients with IL-6 levels ≥ 18 pg/mL than in SARS-CoV-2 patients with IL-6 levels < 18 pg/mL or HIV patients (2.78 [2.16–4.47] vs. 7.24 [5.88–10.38] vs. 9.75 [8.45–13.79] L/h, respectively; $p < 0.0001$). Increasing IL-6 levels affected darunavir concentration versus time simulated profiles. We hypothesized that increases in IL-6 levels associated with severe SARS-CoV-2 disease may downregulate the cytochrome P450 (CYP) 3A4-mediated metabolism of darunavir.

Conclusions This is a proof-of-concept of SARS-CoV-2 disease–drug interactions, and may support the need for optimal dose selection of sensitive CYP3A4 substrates in severe SARS-CoV-2 patients.

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1 Introduction

The spread of the 2019 novel coronavirus (SARS-CoV-2) disease that emerged from China last December has reached pandemic proportions and has become a major global health concern. According to the WHO reports, as at 30 July 2020 more than 16.5 million cases and 655,000 deaths have been reported worldwide [1]. Since the beginning of the European outbreak, Italy has been deeply affected [2] and confirmed cases accounted for 1.5% of the WHO global prevalence estimate [1].

SARS-CoV-2 may cause illness of increasing severity. The earliest report of SARS-CoV-2 from the Chinese Centers for Disease Control and Prevention noted that among 72,314 cases, 81% were of a mild nature, with an overall

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Key Points

The anti-HIV protease inhibitor darunavir is one of the antiviral drugs repurposed for SARS-CoV-2 treatment. We developed and compared population pharmacokinetic models of darunavir in SARS-CoV-2 and HIV patients.

The population pharmacokinetics of darunavir may differ in SARS-CoV-2 patients compared with HIV patients. Interleukin (IL)-6 is the most significant clinical covariate affecting the oral clearance of darunavir in patients with severe disease by downregulating cytochrome P450 (CYP) 3A4 activity.

This may represent a proof-of-concept of SARS-CoV-2 disease–drug interactions, and may support the need for optimal dose selection of any sensitive CYP3A4 substrate in SARS-CoV-2 patients with severe disease.

case fatality rate of 2.3%, whereas 5% presented with respiratory failure, septic shock, and multiorgan dysfunction, resulting in a fatality rate of 50% of such cases [1]. Consequently, a three-stage classification system has recently been proposed for characterizing the increasing severity of illness with distinct clinical findings, response to therapy, and clinical outcome: stage I (mild), early infection; stage II (moderate), pulmonary involvement (IIa) without and (IIb) with hypoxia (defined as $PaO_2/FiO_2 \leq 300$ mmHg); and stage III (severe), systemic hyperinflammation [3]. Stages I and IIa have been mainly associated with viral proliferation and less severe disease, while stages IIb and III have been associated with overwhelming inflammatory response and cytokine burden, which may precipitate in multiple organ failure, with unfavorable clinical outcome and death [4, 5]. Although no approved drug treatment currently exists for SARS-CoV-2 disease, hundreds of clinical trials are currently ongoing with the intention of finding an effective treatment, looking at the available therapeutic armamentarium [6]. These trials include various drugs with antiviral activity, immunomodulatory activity, or both [6].

The anti-HIV protease inhibitor darunavir is one of the antiviral drugs repurposed for SARS-CoV-2 treatment, and is currently under investigation in six different clinical trials registered at ClinicalTrials.gov [6]. Three trials included darunavir in the darunavir/cobicistat (DRV/COBI) formulation, and the remaining three trials included darunavir/ritonavir (DRV/RTV) [6]. During the overwhelming outbreak of SARS-CoV-2 disease that occurred in March/April 2020 in Italy, some SARS-CoV-2 patients who were hospitalized at the Clinic of Infectious Diseases of the Udine University

Hospital in the Friuli Venezia Giulia Region were treated with darunavir. These patients underwent routine therapeutic drug monitoring (TDM) during treatment and we noticed that in several cases, plasma concentrations of darunavir were much higher than those usually observed among HIV patients treated with the same drug. Since darunavir is metabolized by cytochrome P450 (CYP) 3A4 in the liver and the gut [7], we hypothesized that SARS-CoV-2 disease might have affected CYP3A4 activity.

The aim of this study was to assess the population pharmacokinetics of darunavir in SARS-CoV-2 patients compared with HIV patients, for identifying any potential covariates that could affect the pharmacokinetics of darunavir in SARS-CoV-2 patients.

2 Methods

2.1 Study Design

This was a retrospective, single-center, observational study carried out at the Santa Maria della Misericordia University Hospital of Udine, Italy, between 15 March and 15 May 2020. All SARS-CoV-2 patients admitted at the Clinic of Infectious Diseases who were treated with darunavir were included in this study. The standard formulation of darunavir used was DRV/COBI (800/150 mg/day). In the case of an in-hospital shortage of DRV/COBI at the time of admission, the DRV/RTV formulation (800/100 mg/day) was used. Each SARS-CoV-2 patient received only one type of formulation (either DRV/COBI or DRV/RTV) throughout the whole treatment period. HIV patients treated with a fixed-drug combination of DRV/COBI (800/150 mg/day), emtricitabine (200 mg), and tenofovir (10 mg) [DRV/COBI/FTC/TAF, Symtuza®] who underwent TDM of darunavir in the same period as the SARS-CoV-2 subjects were included as the comparator group. The rationale for considering this study design as being reliable for population pharmacokinetic modeling is based on the findings of comparative pharmacokinetic studies between DRV/COBI (800/150 mg/day), either as single agents or in a fixed-drug combination, and DRV/RTV (800/100 mg/day). The full bioequivalence of darunavir was shown, in terms of area under the concentration–time curve (AUC) and peak concentration (C_{max}), between the two formulations (AUC geometric mean ratio range 0.96–1.02; C_{max} geometric mean ratio range 0.97–1.03) [8, 9], even if the trough concentrations (C_{min}) were approximately 30% lower for DRV/COBI.

All patients routinely underwent TDM of darunavir. SARS-CoV-2 patients underwent TDM assessment at least 48 h after commencing therapy, while HIV patients were receiving chronic and stable treatment. In both groups, each patient provided two blood samples—the first just before a

daily dose for assessing C_{min}, and the second 2 h after oral drug administration for assessing C_{max}. After blood centrifugation and plasma separation, concentrations of darunavir were measured in plasma using liquid chromatography-tandem mass spectrometry (LC–MS/MS) (Shimadzu LC-20 AB Sciex 3200 Qtrap) by means of a commercially available method (MassTox® TDM Series A Anti-HIV drugs, Catalogue No. 92924; Chromsystems GmbH, Munich, Germany). The lower limit of quantification was 6 ng/mL [10].

This study was approved by the Regional Ethics Committee of Friuli Venezia Giulia Region, Italy. In light of the retrospective nature of the study, written informed consent was waived, according to the institutional policies for studies with a retrospective design.

The following demographic and clinical data were retrieved from patient records: age, sex, weight, height, diagnosis, comorbidities, coadministered drugs, and drug-related adverse events (if any). The following laboratory parameters were collected on the day of the TDM assessment: serum creatinine, serum albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, γ-glutamyltransferase (γ-GT), and interleukin (IL)-6. IL-6 was assessed as a biomarker of acute inflammatory progression in SARS-CoV-2 patients [11] and as a biomarker of chronic inflammatory state in HIV patients [12]. The severity of SARS-CoV-2 disease was classified according to the three-stage classification system proposed by Siddiqi and Mehra [3].

2.2 Population Pharmacokinetic Modeling

Two comparative population pharmacokinetic models were created, one for SARS-CoV-2 patients and the other for HIV patients. Darunavir plasma concentrations were analyzed by means of nonlinear mixed-effects modeling using the stochastic approximation expectation maximization (SAEM) algorithm implemented within the Monolix software (version 2019R1; Lixoft, Antony, France). The timepoints of darunavir TDM assessment were exactly those related to the start of treatment in SARS-CoV-2. Conversely, six fixed drug doses were virtually added before timepoints of darunavir TDM assessment in HIV patients for simulating steady-state conditions, since those patients had been under chronic treatment for months/years.

A basic model was developed by comparing one- and two-compartment models with first-order oral absorption and elimination. All individual parameters were considered to be log-normally distributed. Exponential random effects were assumed to describe between-subject variability. Correlations between random effects were tested in the variance–covariance matrix, and implemented into the structural model accordingly. Several error models (constant, proportional, or combined) were tested for describing the residual variability. The most appropriate model was selected on the basis of the following criteria: minimum value of both the objective function value (OFV) and the Bayesian information criteria (BIC), adequacy of the goodness-of-fit plots, and minimum value of the relative standard error (RSE) of the estimated pharmacokinetic parameters.

The potential influence of covariates was tested separately on each basic model of the darunavir pharmacokinetics. The tested covariates were age, sex, weight, height, body surface area (BSA), serum albumin, serum creatinine, total bilirubin, ALT, AST, γ-GT, IL-6, and stage of SARS-CoV-2 disease according to the three-stage classification system of Siddiqi and Mehra [3]. The parameter–covariate relationship was modeled as additive or proportional shifts from the reference category for binary covariates, and the effect of continuous covariates was modeled using a power function. The covariate model was built using a stepwise procedure with forward inclusion and backward elimination. A covariate was retained in each of the two final models if the OFV decreased by at least 3.84 and both the BIC and interpatient variability of the fixed-effect parameters were decreased compared with the corresponding basic model.

2.3 Model Evaluation

Evaluation of each final model was based on the following goodness-of-fit plots: observation versus individual and population predictions, usual residual-based plots (individual-weighted residuals and population-weighted residuals), and visual predicted check (VPC) plot. The VPC plot depicts the time course of the 10th, 50th, and 90th percentiles of observed data and the corresponding 90% prediction intervals calculated from 500 Monte Carlo samples. Model performance was also assessed by means of the normalized prediction distribution error (NPDE). The 99% confidence interval of each parameter was simulated in each final model by performing 1000 nonparametric bootstraps with resampling in the Rsmlx package of R (R speaks Monolix).

2.4 Monte Carlo Simulation of the Effect of Covariates

The influence of a covariate eventually retained in the two final models on the plasma concentration versus time profile of darunavir was assessed by means of the Mlxplore package of Monolix. Overall, 1000 Monte Carlo simulations were conducted in each scenario using a 10-day standard daily dose of darunavir 800 mg (simulating either DRV/COBI 800/150 mg or DRV/RTV 800/100 mg).

2.5 Statistical Analysis

The Kolmogorov–Smirnov test was used for assessing normal or non-normal distribution of patients' data.

Consequently, descriptive statistical data were presented as mean ± standard deviation (SD) or median and 25th–75th percentiles (interquartile range [IQR]). Categorical variables were compared using the Chi square test with Yates's correction, or Fisher's exact test, as appropriate, while continuous variables were compared using the Student's *t* test or Mann–Whitney test, as appropriate. The strength of a trend between two variables was expressed by means of the Pearson correlation coefficient or Spearman's rank correlation coefficient, as appropriate. Univariate and multivariate linear regression analysis was conducted for assessing the potential association between patient covariates and darunavir pharmacokinetic parameters. A *p*-value ≤ 0.05 was required for statistical significance. Classification and regression tree (CART) analysis was carried out for assessing whether a covariate threshold could be associated with darunavir pharmacokinetic parameters. The proportion of reduction in error (PRE) was used as goodness-of-fit statistics for CART analysis, and the Kruskal–Wallis test was used to compare continuous data among groups. Bonferroni correction for multiple comparison was applied as appropriate. All statistical analysis and plotting was performed using R version 3.4.4 (The R Foundation for Statistical Computing, Vienna, Austria).

3 Results

Thirty SARS-CoV-2 patients and 25 HIV patients were included in this study. The demographic and clinical characteristics of the two groups are summarized in Table 1. The two groups were similar in terms of sex and distribution of body size metric characteristics. Median age was higher in SARS-CoV-2 patients than in HIV patients (63 vs. 47 years; *p* < 0.001). Levels of ALT, AST, bilirubin, and γ-GT were significantly higher in SARS-CoV-2 patients compared with HIV patients, and IL-6 levels were highly significantly higher in SARS-CoV-2 patients than in HIV patients (median 31.0 [IQR 10–114.75] vs. 2.0 [IQR 2.0–2.75] pg/mL; *p* < 0.001).

The clinical data of each of the SARS-CoV-2 and HIV patients are summarized in Electronic Supplementary Tables S1 and S2, respectively. Among the SARS-CoV-2 patients (Electronic Supplementary Table S1), 22 of 30 patients (73.3%) were treated with DRV/COBI, whereas 8 of 30 (26.7%) received DRV/RTV. Most of the SARS-CoV-2 patients were affected by severe disease (three at stage I, eight at stage IIa, ten at stage IIb, and nine at stage III). Seven patients had no comorbidities (7/30, 23.3%), nine had one comorbidity (9/30, 30,0%) and the remaining 14 had two or more comorbidities (14/30, 46.7%). All patients were co-treated with other drugs (the range of coadministered drugs was 1–8). Only one of the coadministered drugs

was a potential perpetrator of CYP3A4-mediated drug–drug interactions (amiodarone was started in one patient 2 days before DRV/COBI).

Among the HIV patients (Electronic Supplementary Table S2), all were treated with DRV/COBI/FTC/TAF (25/25, 100%). Ten patients had no comorbidities (10/25, 40.0%), five had one comorbidity (5/25, 20,0%), and the remaining ten patients had two or more comorbidities (10/25, 40.0%). Twenty of 25 patients (20/25, 80%) were co-treated with other drugs (the range of coadministered drugs was 1–8). None of the coadministered drugs was a potential perpetrator of CYP3A4-mediated drug–drug interaction.

3.1 Comparative Population Pharmacokinetic Analysis of Darunavir

Both the C_{min} and C_{max} of darunavir were significantly higher in SARS-CoV-2 patients than HIV patients (median C_{min} 4960.2 ng/mL [IQR 2015.0–7951.2] vs. 1010.0 ng/mL [IQR 550.0–2112.0], *p* < 0.001; median C_{max} 9476.2 ng/mL [IQR 7220.5–14835.4] vs. 6320.0 ng/mL [IQR 3888.3–7747.5], *p* < 0.001) [Fig. 1].

In both patient populations, darunavir pharmacokinetic concentrations were best described by a one-compartment model (OFV/BIC for one- vs. two- compartment models: 298.93 vs. 321.74 and 299.59 vs. 337.01, respectively, in SARS-CoV-2 patients; 181.67 vs. 214.42 and 182.55 vs. 217.96, respectively, in HIV patients). Population pharmacokinetic parameter estimates are reported in Table 2. In the SARS-CoV-2 final pharmacokinetic model, two covariates were significantly associated with darunavir pharmacokinetic parameters: IL-6 with darunavir oral clearance (CL/F), and BSA with volume of distribution (Vd). On the contrary, no covariate was found to be significantly associated with any of the darunavir pharmacokinetic parameters in the HIV final pharmacokinetic model. Either fixed-effect or random-effect estimates were accurate in both models (except for interindividual variability in Vd). All of the pharmacokinetic parameter estimates were significantly different between the two groups. Noteworthy, darunavir CL/F was approximately 2.5-fold lower (4.1 vs. 10.3 L/h; *p* < 0.001) and median darunavir exposure was more or less 2.1-fold higher (AUCs of 161,387.0 vs. 75,727.0 ng·h/mL; *p* < 0.001) in SARS-CoV-2 patients compared with HIV patients.

Linear regression of the observed versus model-predicted individual concentrations showed a very good fit, for both SARS-CoV-2 patients ($R^2 = 0.92$) and HIV patients ($R^2 = 0.98$) [Electronic Supplementary Fig. S1]. The VPC plot showed a consistent distribution between the observed and predicted concentrations in both populations (Fig. 2). The residuals were normally distributed (*p* = 0.344 and *p* = 0.159 in the Shapiro–Wilk test for SARS-CoV-2 and HIV patients, respectively), and were symmetric at approximately

Table 1 Demographics and clinical characteristics of the study groups

	SARS-CoV-2-infected patients	HIV-infected patients	p-Value
Patient demographics			
No. of patients	30	25	
Sex (male/female)	18/12 (60/40)	18/7 (72/28)	0.404
Age, years	63 (55–70.5)	47 (40–51)	< 0.001
Body weight, kg	75.0 (69.25–81.50)	75.0 (66.0–84.0)	0.904
BSA, m^2	1.86 (1.77–1.96)	1.86 (1.75–2.02)	0.689
Laboratory parameters			
Serum creatinine, mg/dL	0.86 (0.78–1.05)	0.93 (0.84–1.07)	0.424
Bilirubin, mg/dL	0.46 (0.38–0.62)	0.35 (0.23–0.48)	0.026
Alanine aminotransferase, UI/L	40.0 (21.0–71.0)	20.0 (17.0–29.0)	0.002
Aspartate aminotransferase, UI/L	36.0 (24.0–55.0)	20.0 (17.0–22.0)	0.004
γ-Glutamyltransferase, UI/L	53.5 (35.5–125.2)	22.0 (12.0–35.0)	0.035
Interleukin-6, pg/mL	31.0 (10–114.75)	2.0 (2.0–2.75)	< 0.001
Post-starting treatment time of TDM, days	3.0 (2.0–5.0)	–	–
Comedications			
No. of drugs per patient	3.5 (2.0–5.75)	4.0 (3.0–6.0)	0.151
ADRs			
No. of patients with ADRs	8 (26.7)	1 (4.0)	0.031
Type of ADRs			
Abdominal pain/diarrhea	3	1	0.617
QT prolongation	2	0	–
Asthenia	1	0	–
Tonic–clonic seizure	1	0	–
Rash	1	0	–

Data are expressed as median (interquartile range) for continuous variables and n (%) for dichotomous variables

ADRs adverse drug reactions, *BSA* body surface area, *TDM* therapeutic drug monitoring

zero ($p = 0.156$ and $p = 0.219$ in the symmetry test for SARS-CoV-2 and HIV patients, respectively).

3.2 The Prominent Role of Interleukin-6 on Darunavir Pharmacokinetics in SARS-CoV-2 Patients

IL-6 showed a trend toward a significant relationship with the three-stage classification system of SARS-CoV-2 disease severity (Spearman $\rho = 0.34$, $p = 0.07$) and was the only clinical variable highly significantly associated with darunavir CL/F at multivariate regression analysis ($p < 0.001$) [Table 3]. The multivariate model accounted for approximately 50% of the darunavir CL/F variability ($R^2 = 0.494$; $p < 0.001$). CART analysis (Electronic Supplementary Fig. S2) found that an IL-6 level of 18 pg/mL may adequately split the SARS-CoV-2 population in patients with low versus high darunavir CL/F (mean ± SD $3.47 ± 1.90$ vs. $8.03 ± 3.24$ L/h; PRE = 0.46).

The distribution of darunavir CL/F in SARS-CoV-2 patients with IL-6 levels ≥ 18 pg/mL compared with those with IL-6 levels < 18 pg/mL, and HIV patients, are depicted in Fig. 3. Median darunavir CL/F was significantly lower in SARS-CoV-2 patients with IL-6 levels ≥ 18 pg/mL than in those with IL-6 levels < 18 pg/mL, or HIV patients (2.78 [IQR 2.16–4.47] vs. 7.24 [IQR 5.88–10.38] vs. 9.75 [IQR 8.45–13.79] L/h, respectively; $p < 0.0001$). Conversely, darunavir CL/F in SARS-CoV-2 patients with IL-6 levels < 18 pg/mL was not significantly different from that of HIV patients (7.24 [IQR 5.88–10.38] vs. 9.75 [IQR 8.45–13.79] L/h; $p = 0.126$].

Figure 4 depicts the simulated median plasma concentration versus time profiles achievable with a 10-day course of darunavir in HIV and SARS-CoV-2 patients with three different increasing levels of IL-6. Median darunavir C_{min} and C_{max} estimates at steady-state were 826 (IQR 239–1824) and 5.425 (IQR 4.229–6.921) ng/mL in HIV patients; 1460 (IQR 550–2920) and 7.560 (IQR 6.147–9.242) ng/mL in SARS-CoV-2 patients with IL-6 levels of 1 pg/mL; 7020 (IQR 4090–11,380) and 13.418 (IQR 10.468–17.911) ng/mL in patients with IL-6 levels of 100 pg/mL; and 14,140 (IQR 9100–21,230) and 20.729 (IQR 15.541–27.778) ng/mL in patients with IL-6 levels of 1000 pg/mL.

Fig. 1 Box-and-whisker plots (5th and 95th percentiles) of darunavir trough and peak plasma concentrations in SARS-CoV-2 patients (blue, $n = 30$) and HIV patients (orange, $n = 25$)
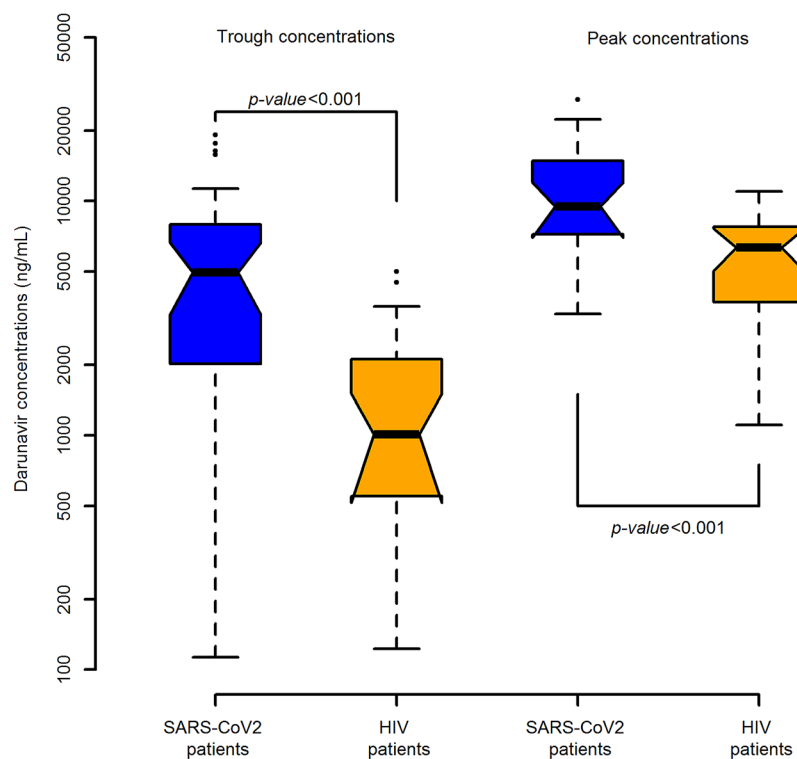

Table 2 Summary of population pharmacokinetic parameters of darunavir in SARS-CoV-2 patients compared with HIV patients

	SARS-CoV-2 patients		HIV patients		*p*-Value
	Value (RSE%)	Median (99% CI) of bootstrap	Value (RSE%)	Median (99% CI) of bootstrap	
Fixed effects					
k_a (h^{-1})	0.74 (30.6)	0.58 (0.55–0.75)	0.58 (36.6)	0.59 (0.55–0.66)	0.047
CL/F (L/h)	4.10 (10.1)	3.98 (3.93–4.11)	10.3 (10.4)	10.4 (10.2–10.6)	<0.001
$\beta_{IL6-CL/F}$	− 0.23 (24.9)	− 0.22 (− 0.23 to − 0.21)	–	–	–
V_d (L)	88.41 (7.8)	77.81 (76.13–89.17)	96.9 (11.5)	99.4 (96.7–102.0)	0.002
β_{BSA-Vd}	1.44 (35.9)	1.43 (1.31–1.71)	–	–	–
Between-subject variability					
$\omega\ k_a$ (%)	0.82 (28.3)	0.97 (0.93–1.10)	1.14 (24.6)	1.19 (1.12–1.25)	0.033
ω CL (%)	0.53 (15.2)	0.53 (0.51–0.53)	0.44 (19.5)	0.46 (0.44–0.48)	0.047
$\omega\ V_d$ (%)	0.15 (41.0)	0.13 (0.11–0.16)	0.13 (61.5)	0.18 (0.12–0.20)	<0.001
Residual variability					
b (proportional)	0.09 (22.3)	0.08 (0.07–0.09)	0.155 (64.2)	0.17 (0.16–0.19)	–

Results are expressed as estimated parameter value

β_{BSA-Vd} exponentiation coefficient of BSA on V_d, $\beta_{IL6-CL/F}$ exponentiation coefficient of IL-6 on CL/F, *CI* confidence interval, *CL/F* oral clearance, k_a first-order absorption rate constant, *%RSE* percentage relative standard error, V_d volume of distribution of the central compartment

4 Discussion

The present study investigated the population pharmacokinetics of darunavir in SARS-CoV-2 patients compared with HIV patients. The clinical covariates that may alter darunavir pharmacokinetics in SARS-CoV-2 patients were also assessed, and conceptualization of a disease-drug interaction was formulated.
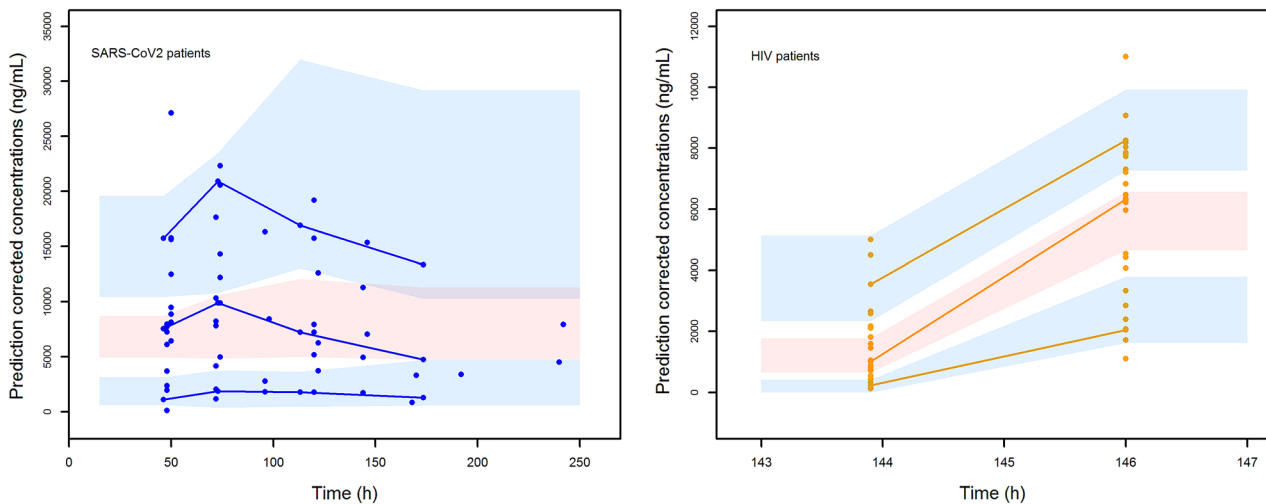
Fig. 2 Prediction-corrected visual predictive check for the population pharmacokinetic models developed in SARS-CoV-2 and HIV patients. The lines represent the median and the 10th and 90th percentiles for the observed data. The shaded areas are the prediction interval for the median (red central area) and for the 10th and 90th percentiles (light blue lower and upper areas)

Table 3 Univariate and multivariate analysis of variables potentially associated with darunavir oral clearance in SARS-CoV-2-infected patients ($n=30$)

Variables	Univariate analysis		Multivariate analysis	
	Unstandardized β-coefficient	p-Value	Unstandardized β-coefficient	p-Value
Age, years	−4.793 (−19.078 to 9.492)	0.498		
Body weight, kg	8.045 (−5.106 to 21.196)	0.221		
BSA, m^2	9.237 (−16.421 to 34.895)	0.467		
Stage of SARS-CoV-2 progression[a]	−6.330 (−15.299 to 2.640)	0.159	−0.668 (−7.898 to 6.561)	0.851
Serum creatinine, mg/dL	−0.721 (−13.672 to 12.230)	0.910		
Total bilirubin, mg/dL	0.005 (−6.673 to 6.684)	0.999		
ALT, UI/L	0.754 (−2.892 to 4.400)	0.674		
AST, UI/L	1.717 (−2.805 to 6.238)	0.442		
γ-GT, UI/L	0.549 (−2.584 to 3.681)	0.721		
Serum albumin, g/dL	−1.437 (−8.204 to 5.330)	0.663		
IL-6, pg/mL	**−2.464 (−3.783 to −1.145)**	**0.001**	**−2.513 (−3.795 to 1.232)**	**< 0.001**
Hydroxychloroquine	0.513 (−3.577 to 4.603)	0.799		

Results are expressed as slope (confidence interval). Statistical analysis by linear regression on log-transformed independent variables ($Y = \beta_0 + \beta_1 \log X + \varepsilon$). Adjusted R^2: 0.494

Variables significantly associated with darunavir oral clearance at multivariate analysis are in bold

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *BSA* body surface area, *γ-GT* γ-glutamyltransferase, *IL* interleukin

[a]Classified according to the work of Siddiqi and Mehra [3]

The population pharmacokinetic models that we developed showed darunavir CL/F was highly significantly lower in SARS-CoV-2 patients than in HIV patients, and that IL-6 was the only clinical covariate that significantly affected darunavir CL/F in SARS-CoV-2 patients.

The population pharmacokinetic model that we developed in HIV patients receiving DRV/COBI/FTC/TAF was reliable for comparison with SARS-CoV-2 patients as it provided darunavir CL/F estimates (10.3 L/h) similar to those observed in two recent population pharmacokinetic analysis carried out in HIV patients receiving DRV/RTV. In a recent multicenter, retrospective study assessing the population pharmacokinetics of darunavir among 368 HIV patients treated with DRV/RTV (either in a 800/100 mg once-daily formulation [$n=306$] or a 600/100 mg twice-daily formulation [$n=62$]) darunavir CL/F was 9.47 L/h [13], and was

Fig. 3 Scatter plot of the distribution of darunavir CL/F in SARS-CoV-2 patients with IL-6 levels < 18 pg/mL (light blue, $n = 10$), SARS-CoV-2 patients with IL-6 levels ≥ 18 pg/mL (purple, $n = 20$), and HIV patients (orange, $n = 25$). *CL/F* oral clearance, *IL* interleukin
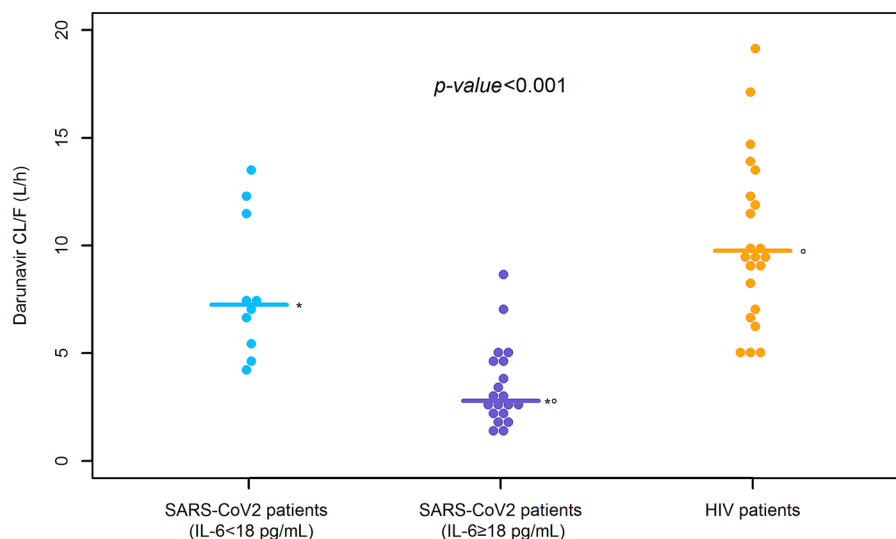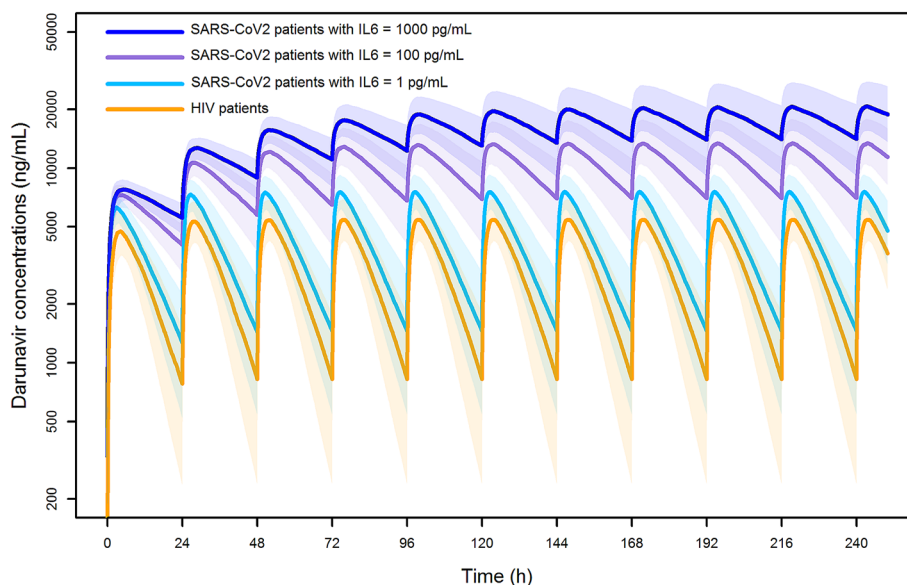
Fig. 4 Simulated median concentration–time profiles of darunavir with a daily dose of 800 mg in HIV patients (orange) and SARS-CoV-2 patients with IL-6 levels of 1 pg/mL (light blue), 100 pg/mL (purple), and 1000 pg/mL (blue). Gray shadings around simulated profiles display the 25th–75th percentiles of concentrations. *IL* interleukin

even higher (14.6 L/h) in another population pharmacokinetic study carried out using data from 716 treatment-naïve patients enrolled in a phase III comparative trial of DRT/RTV 800/100 mg once daily combined with raltegravir in one arm or FTC/TAF in the other arm [14].

To our knowledge, this is the first study assessing darunavir population pharmacokinetics in SARS-CoV-2 patients. We found that darunavir CL/F was approximately 2.5-fold lower in SARS-CoV-2 patients than in HIV patients, and that IL-6 was the only clinical covariate that significantly affected darunavir CL/F. Darunavir is metabolized by CYP3A4 in the liver and gut [7]. IL-6 is one of the most important proinflammatory cytokines regulating acute-phase protein synthesis in inflammation [15]. Previous studies

showed that IL-6 increases associated with infection or other disease states may downregulate CYP3A4-mediated activity [16–19]. Noteworthy, IL-6 burden was shown to play a pivotal role in causing systemic hyperinflammation associated with severe disease states in SARS-CoV-2 patients [20]. Recent evidence from different groups showed a twofold higher lopinavir C_{min} in SARS-CoV-2 patients [7, 21, 22]. Consequently, some observational studies showed the IL-6 receptor antagonist tocilizumab may be helpful in reducing the fatality rate among patients with severe SARS-CoV-2 [20, 23, 24]; a prospective, randomized clinical trial with tocilizumab has also been designed [25]. Most of our patients had severe SARS-CoV-2 disease (overall, 63.3% were classified as stage IIb and III) [3], and the severity of

SARS-CoV-2 disease was found to be almost significantly correlated with IL-6 levels.

CART analysis revealed that an IL-6 level ≥ 18 pg/mL allowed identifying the SARS-CoV-2 patients who had darunavir CL/F most significantly impaired. Conversely, darunavir CL/F in SARS-CoV-2 patients with IL-6 levels below this threshold was almost normal and was similar to that of HIV patients. Interestingly, a physiologically based pharmacokinetic model assessing the impact of IL-6 on sensitive CYP3A4 substrates in virtual rheumatoid arthritis patients may corroborate our hypothesis. In that study, it was estimated that the percentage increase of CYP3A4 victim drug AUC_{24} in the presence of IL-6 was 20–50% for simvastatin at IL-6 concentrations of 50 pg/mL, and 30% for cyclosporine at IL-6 concentrations of 100 pg/mL [26].

Monte Carlo simulations confirmed that the plasma concentration–time profiles of darunavir achievable with a 10-day course of darunavir may be highly influenced by IL-6 levels in SARS-CoV-2 patients. This may represent a proof-of-concept of SARS-CoV-2 disease–drug interactions, and may support the need for optimal dose selection for darunavir, as well as other sensitive CYP3A4 substrates. The findings could claim dosing adjustments of darunavir in SARS-CoV-2 patients according to their IL-6 concentrations. However, it should not be overlooked that recent studies have shown that the probably of in vivo concentrations of HIV protease inhibitors are insufficient to inhibit SARS-CoV-2 [11, 27]. Consequently, it might be hypothesized that the higher exposure observed in the presence of high IL-6 levels could be more clinically helpful in inhibiting SARS-CoV-2.

Prospective studies are urgently needed for promoting effective and well tolerated drug treatments with sensitive CYP3A4 substrates in severe SARS-CoV-2 patients with high IL-6 levels associated with stage IIb and III disease, in agreement with what has already been suggested in a recent viewpoint claiming for action [28].

We recognize that this study has some limitations. Its retrospective nature, the heterogeneity of the different types of darunavir formulations, and the small sample size of both groups must be acknowledged. Although we recognize that a unique population pharmacokinetic model could have been managed, we preferred to create two separate models because we believed that knowing the pharmacokinetic parameter estimates within each single population would have been interesting. The potential influence of CYP3A4-mediated drug–drug interactions, OATP1A2 transporter-mediated hydroxychloroquine interaction, and/or higher levels of AST/ALT on darunavir CL/F, as supposed by other authors [7], could not be completely ruled out; however, it is very unlikely that these factors might have had a major role. Only one of our patients was treated with a known perpetrator of CYP3A4, and neither hydroxychloroquine nor ALT/AST were significantly associated with darunavir CL/F at the regression analysis. Conversely, the reliability of the parameter estimates in both population pharmacokinetic models, and the robust significance of IL-6 as a clinical covariate of darunavir CL/F in SARS-CoV-2 patients, are valuable points of strength.

5 Conclusion

Our findings showed that darunavir CL/F may be significantly impaired in SARS-CoV-2 patients, and that IL-6 burden may be the clinical covariate that most significantly affects darunavir CL/F in patients with severe disease. Further prospective studies on larger SARS-CoV-2 populations are warranted to confirm our findings. A call for the appropriate application of clinical pharmacological principles in the search for well tolerated and efficacious SARS-CoV-2 treatments is urgently needed [29].

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