Darunavir and Ritonavir Total and Unbound Plasmatic Concentrations in HIV-HCV-Coinfected Patients with Hepatic Cirrhosis Compared to Those in HIV-Monoinfected Patients

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Our objective was to describe the pharmacokinetic (PK) parameters of total and unbound darunavir and ritonavir concentrations in HIV-hepatitis C virus (HCV)-coinfected patients with cirrhosis, as ritonavir-boosted darunavir is mainly metabolized in the liver, and hepatic cirrhosis might modify darunavir-ritonavir concentrations. This was a prospective, case-control, and uni-center study. HIV-HCV-coinfected patients with compensated cirrhosis (cases) and HIV-monoinfected patients with normal liver function (controls) were included. Darunavir-ritonavir was given at 800/100 mg once daily. Patients were followed for 24 weeks to assess safety and efficacy. A steady-state 12-h PK study was performed. Total and unbound concentrations were determined by liquid chromatography-tandem mass spectrometry. The unbound fraction was obtained by ultrafiltration. The plasma area under the concentration-time curve (AUC) and oral clearance (CL/F) were assessed by noncompartmental models. Thirty patients (20 cases and 10 controls) were included. Among cirrhotic patients, the Child-Pugh score was C in 4 cases, B in 1 case, and A in 15 cases; the median (interquartile range) transient elastography values were 20 kPa (14 to 26 kPa), and 5 patients had prior clinical decompensations. There were no significant differences in the darunavir PK parameters between cases and controls except for longer time to maximum plasma concentrations (T_{max}) and half-lives in the cirrhotic patients. There were no significant differences in ritonavir total concentrations, but the unbound concentrations were higher in cirrhotic patients. There were significant correlations between the darunavir total and unbound concentrations in both cirrhotic patients and controls. There were no differences in PK parameters based on Child-Pugh score, liver elasticity, gender, or use of concomitant medications. In conclusion, in HIV-HCV-coinfected patients with clinically compensated cirrhosis receiving darunavir-ritonavir at 800/100 mg once daily, the darunavir total and unbound concentrations are similar to those observed in noncirrhotic patients, and dose adjustments are not necessary.

Coinfection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is highly prevalent worldwide, especially in some areas, such as southern Europe, where coinfection rates can reach as high as 35% of all HIV-infected patients (1, 2). HCV-related complications are currently one of the leading causes of morbidity and mortality in HIV-infected patients, and good and early control of both infections is crucial in order to avoid progression to liver cirrhosis (3–6).

Liver cirrhosis can impair liver function, including cytochrome P450 (CYP) enzymatic systems that are responsible for the metabolism of many antiretroviral drugs (ARV), such as protease inhibitors (PI) and nonnucleoside reverse transcriptase inhibitors (NNRTI) (7, 8). Cirrhosis can also affect protein synthesis, potentially interfering with antiretroviral (ARV) protein binding. This might increase the proportion of unbound drug, which is the active form of the ARV (8, 9). Thus, measuring unbound rather than total plasma ARV concentrations may be a better indicator of the amount of active drug in cirrhotic patients.

Some ARV do not have their pharmacokinetic (PK) parameters significantly modified in cirrhotic patients, especially integrase inhibitors, such as raltegravir (10) and newer NNRTI, such as rilpivirine or etravirine (11). However, as most of the HIV-HCV-coinfected patients with cirrhosis have gone through many prior ARV regimens and have accumulated resistance mutations, it is necessary to use drugs with higher genetic barriers, like PIs.

There is limited experience with dose adjustment of the different PIs in patients with hepatic cirrhosis. In most cases, treatment guidelines recommend using these drugs with caution or even avoiding them, especially in cases of severe hepatic impairment (12, 13).

Ritonavir-boosted darunavir (darunavir-ritonavir) is one of the most widely used PIs, and it is the PI of choice in patients with protease inhibitor-associated resistance mutations. However, there are scarce data regarding darunavir use in patients with cirrhosis, and the recommendations are fundamentally based on a study performed in HCV-monoinfected patients with mild to moderate hepatic impairment (14). Furthermore, there are very

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limited data on unbound darunavir concentrations in HIV-infected patients with or without HCV coinfection (15).

The aim of our study was to describe the PK parameters of total and unbound darunavir and ritonavir in HIV-HCV-coinfected patients with cirrhosis compared to those of a control group of HIV-monoinfected patients with normal liver function.

(Preliminary results of this work were presented at the 13th International Workshop on Clinical Pharmacology of HIV Therapy, 16 to 18 April 2012, Barcelona, Spain [16] and at the 14th European AIDS Conference, 16 to 19 October 2013, Brussels, Belgium [17].)

MATERIALS AND METHODS

Subjects and design. This was a prospective, exploratory, and case-control study performed at a tertiary care university hospital in Barcelona, Spain.

HIV-HCV-coinfected patients with hepatic cirrhosis were included as cases. Cirrhosis was defined by at least one of the following criteria: compatible biopsy result, compatible image tests, and/or esophageal varices on fibrogastroscopy, prior clinical decompensation (ascites, encephalopathy, or variceal bleeding), and/or transient elastography (as measured by FibroScan; Echosens, Paris, France) of ≥ 14.6 kPa (18). HIV-monoinfected patients with normal liver function, no history of alcohol abuse, no use of other hepatotoxic drugs, and who were negative for hepatotropic viruses were included as controls. The controls were chosen from those patients consecutively attending the outpatient clinic in the same time frame as the cases. All patients (cases and controls) were receiving darunavir-ritonavir at 800/100 mg once daily as part of their HIV treatment for ≥ 1 month before study inclusion and had suppressed HIV load. The ARV regimen had been initiated by the physician in charge of the patient prior to their inclusion in the study.

Patients with currently clinically decompensated hepatic cirrhosis, pregnancy, or who were using other medications known to significantly interact with CYP were excluded from the study.

The primary objective was to measure total and unbound darunavir concentrations in plasma in HIV-HCV-coinfected patients with hepatic cirrhosis and in controls, comparing the concentrations between the two groups. The secondary objectives were to measure ritonavir total and unbound concentrations, to correlate total and unbound concentrations for darunavir and for ritonavir, and to correlate plasma levels with Child-Pugh and Model for End-Stage Liver Disease (MELD) scores, side effects, and other covariables, such as gender, comedications, or laboratory parameters.

The study protocol was approved by the institutional review board of the hospital, and written informed consent was obtained from all patients before study inclusion.

Study procedures. Demographic data (age, sex, and race), HIV-related data (transmission risk factor, duration of infection, previous ARV regimens and reasons for discontinuation, and prior resistance testing, if performed), other potential liver-offending agents (alcohol, other drugs, or hepatitis B virus [HBV] infection), and HCV-related data (HCV antibody status, viral load, genotype, ultrasound findings, fibrogastroscopy results, transient elastography results [FibroScan], previous clinical decompensation, Child-Pugh and MELD scores, and prior treatments and response to them) were recorded for each patient at the initial visit.

The following procedures were performed at baseline: adherence assessment with the simplified medication adherence questionnaire (SMAQ), blood tests (CD4 cell count, HIV-1 RNA, hematology, coagulation, liver and kidney function tests, fasting blood lipids, and total protein, albumin, and alpha-1-glycoprotein levels), transient elastography, and a complete 12-h PK study.

The PK study was performed at steady state at ≥ 4 weeks after having started darunavir-ritonavir. All subjects were instructed to take darunavir-ritonavir at 9:00 a.m. with breakfast during the week before intensive

PK assessment. To ensure that the dose was taken 24 h before the predose analysis, patients filled a dose diary with the exact time they had taken the preceding doses. On the PK analysis day, patients came to the hospital between 8:15 and 8:45 a.m. after overnight fasting. Drugs were administered at the hospital at 9:00 a.m. with a standard breakfast consisting of orange juice, bread with butter and jam, and coffee with milk and sugar (550 kcal). Blood samples were drawn predose and 1, 2, 3, 4, 6, 8, and 12 h after the dose. Since the predose darunavir-ritonavir concentration was determined 24 h after the preceding dose, the value obtained at this time was also used for the 24-h postdosing value in PK analysis (the concentration at 24 h [C_{24}], concentration at 0 h [C_0], and trough concentrations [C_{trough}] were all equal).

The assay for the determination of total and unbound darunavir and ritonavir concentrations was based on a previously validated method for lopinavir and ritonavir (RTV) determination (19), with minor modifications, as detailed here. For total concentrations, plasma samples were supplemented with the internal standard ritonavir A-86093.0 (final concentration, 250 ng/ml), basified, and then extracted with tert-butyl methyl ether by 45 min of vortexing. The organic phase was collected, evaporated to dryness, and reconstituted with water (final extract). The unbound fraction was obtained by ultrafiltration of plasma (with no internal standard addition) with a 30-kDa membrane (Centrifree) at $2,000 \times g$ and 37°C for 45 min.

Determination of the total and unbound concentrations was performed with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using an Acquity ultraperformance liquid chromatography (UPLC)-Xevo TQ mass spectrometer (Waters, Milford, MA, USA). The stationary phase was an Acquity UPLC BEH C_{18} column (50 by 2.1 mm, 1.7- μm particle size; Waters). Extracts (for total concentration) or ultrafiltrates (for unbound concentrations) were injected into the LC-MS/MS system and resolved at 0.5 ml/min using 2 mM ammonium formate, 0.1% (vol/vol) formic acid, and a gradient with an increasing acetonitrile proportion (gradient description available on request). Detection of the eluate components was performed using multiple reaction monitoring with positive electrospray mode with the following m/z transitions: for darunavir (DRV), $548.4 \rightarrow 392.2$; for RTV, $721.3 \rightarrow 296.1$; and for ritonavir A-86093.0, $747.5 \rightarrow 140.0$. Calibration curves (made with plasma standards for total concentrations or with ultrafiltrated plasma for unbound concentrations) were processed in parallel. Between-day precision for the whole procedures (extraction plus LC-MS/MS determinations), expressed as the percent coefficient of variation, was 23% (3,118 ng/ml) for total darunavir, 30% (146 ng/ml) for unbound darunavir, 10% (3,331 ng/ml) for total ritonavir, and 28% (7.4 ng/ml) for unbound ritonavir. The lower limits of quantification were 5 ng/ml (total DRV and total RTV), 0.5 ng/ml (unbound DRV), and 0.1 ng/ml (unbound RTV).

The area under the plasma concentration-time curve from 0 to 24 h (AUC_{0-24}) and oral clearance (CL/F) were assessed using a noncompartmental analysis using the linear/log trapezoidal rule (WinNonlin 3.3; Pharsight Corp., Mountain View, CA, USA). Our laboratory takes part in an external interlaboratory quality control program for the measurement of antiretroviral drugs in plasma by the Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology, The Hague, The Netherlands (20).

Visits were performed at baseline (considered to be the day of the complete PK study) and at 12 and 24 weeks, including physical examination and laboratory tests as part of routine clinical care. The safety and tolerability of the study medications were assessed on the basis of clinical and laboratory adverse events recorded during clinical visits, using the World Health Organization toxicity grading scales.

Statistical analysis. Descriptive values are described as the number (and percentage) for qualitative variables and median (interquartile range) and geometric means (GM) with 90% confidence intervals (CI) for quantitative variables. Geometric mean ratios (GMR) with their 90% CI or Mann-Whitney U test results were used to compare darunavir and ritonavir concentrations between groups and Spearman's test was used

TABLE 1 Patient baseline characteristics

Characteristic ^a	Cirrhosis (<i>n</i> = 20)	Controls (<i>n</i> = 10)
Gender (no. [%] of males)	15 (75)	4 (40)
Age (median [IQR]) (yr) ^c	49 (43–51)	35 (31–51)
BMI (median [IQR]) (kg/m ²)	24 (20–28)	25 (23–27)
CD4 (median [IQR]) (cells/mm ³)	343 (215–677)	414 (289–894)
HIV RNA <25 copies/ml	20 (100)	10 (100)
HIV transmission route (no. [%]) ^b		
IDU	19 (95)	1 (10)
Homosexual	1 (5)	3 (30)
Heterosexual	0	6 (60)
OH >15 g/day (no. [%])	4 (20)	0
HAV antibodies (no. [%])	15 (75)	7 (70)
HBV core antibodies (no. [%])	11 (55)	2 (20)
Backbone ARV (no. [%]) ^c		
TDF-FTC	7 (35)	8 (80)
RAL	2 (10)	2 (20)
PI monotherapy	8 (40)	0
Other	3 (15)	0
HCV genotype (no. [%])		
1	16 (80)	
3	4 (20)	
Prior cirrhosis decompensation (no. [%])	5 (25)	0
Transient elastometry (median [IQR]) (kPa) ^b	20 (14–26)	5 (4–6)
MELD score (median [IQR])	9 (8–12)	
HCV RNA (median [IQR]) (log copies/ml)	6.5 (5.9–6.8)	
Child-Pugh score A/B/C (no. [%])	15 (75)/1 (5)/4 (20)	
Albumin level (median [IQR]) (g/dl) ^c	3.94 (3.59–4.14)	4.24 (3.94–4.50)
AAG level (median [IQR]) (g/dl)	60 (48–86)	78 (68–109)
Platelet count (median [IQR]) ^c	101,700 (64,475–127,650)	202,500 (136,450–258,750)
Bilirubin level (median [IQR]) ^c	0.71 (0.55–1.18)	0.32 (0.29–0.42)

^a IQR, interquartile range; BMI, body mass index; HIV, human immunodeficiency virus; IDU, intravenous drug user; OH, alcohol intake; HAV, hepatitis A virus; HBV, hepatitis B virus; ARV, antiretroviral drugs; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; RAL, raltegravir; PI, protease inhibitor; HCV, hepatitis C virus; MELD, Model for End-Stage Liver Disease; AAG, alpha-1-glycoprotein.

^b *P* < 0.001.

^c *P* < 0.05.

for studying correlations. This is an exploratory study focused on 20 cirrhotic patients and 10 controls who fulfilled all the inclusion criteria and none of the exclusion criteria. Statistical analyses were performed with the SPSS 20.0 statistical package (IBM Corp., Armonk, NY, USA).

RESULTS

Patients and baseline characteristics. Thirty patients were included, with 20 patients with cirrhosis and 10 as controls. Their baseline characteristics are described in Table 1. In the cirrhosis group, there was a higher proportion of male patients and they were slightly older, but their body mass index (BMI) values were comparable. There were significant differences in some laboratory parameters (platelet, bilirubin, and albumin levels) between cirrhotic patients and controls, as was expected. None of the included patients had active (acute or chronic) hepatitis A or B virus infection. Five of the cirrhotic patients (25% of the cases) had had prior hepatic decompensations. All patients had excellent self-reported adherence (>95%) to the antiretroviral treatment.

Pharmacokinetics. The results of the complete PK studies for darunavir are described in Table 2. Although the darunavir total

and unbound minimum concentration ($C_{\min}$) and AUC were higher in cirrhotic patients than those in controls (39% and 51% for $C_{\min}$ and 28% and 23% for AUC, respectively), there were no significant differences in any parameter between the cases and controls, except for the time to reach the maximum concentration ($T_{\max}$) and the half-lives of total and unbound darunavir, which were longer for patients with cirrhosis. Figure 1a and b shows the total and unbound darunavir concentrations, respectively, in cases and controls.

The results of the complete PK studies for ritonavir are described in Table 3 and shown in Fig. 2.

There were significant correlations between total and unbound concentrations in both cirrhotic patients and controls: darunavir AUC ($r = 0.47$ and $P = 0.039$ for cirrhotics, and $r = 0.64$ and $P = 0.048$ for controls) and $C_{\min}$ ($r = 0.80$ and $P < 0.001$ for cirrhotics, and $r = 0.88$ and $P = 0.001$ for controls). For the maximum concentration of drug ($C_{\max}$), the correlation was significant in cirrhotic patients ($r = 0.49$ and $P = 0.029$) but not in controls ($r = -0.06$ and $P = 0.881$). Regarding correlations between ritonavir total and unbound concentrations, they were significant for $C_{\min}$

TABLE 2 Darunavir pharmacokinetic parameters^a

DRV PK parameter ^b	GM (90% CI) for ^c :		GMR (90% CI) ^d
	Cirrhosis (<i>n</i> = 20)	Controls (<i>n</i> = 10)	
<i>C</i> _{min} (ng/ml)			
Total	1,352 (938–1,954)	971 (562–1,679)	1.39 (0.72–2.69)
Unbound	112 (77–164)	75 (43–131)	1.51 (0.77–2.96)
% unbound	8 (7–10)	8 (6–11)	1.08 (0.73–1.59)
<i>C</i> _{max} (ng/ml)			
Total	7,674 (6,592–8,954)	7,430 (5,984–9,247)	1.03 (0.79–1.35)
Unbound	1,242 (1,042–1,476)	1,426 (1,114–1,820)	0.87 (0.64–1.17)
% unbound	16 (13–20)	19 (15–25)	0.84 (0.60–1.18)
AUC _{0–24} (ng · h/ml)			
Total	82,414 (65,615–103,276)	64,417 (46,774–88,920)	1.28 (0.86–1.89)
Unbound	11,350 (9,290–13,868)	9,226 (6,966–12,246)	1.23 (0.87–1.74)
CL/F (liters/h)			
Total	9 (7–11)	12 (9–17)	0.72 (0.48–1.08)
Unbound	70 (57–85)	87 (65–116)	0.80 (0.56–1.14)
<i>T</i> _{max} (h)			
Total	4.0 (2.0–4.0)	2.5 (1.0–3.0)	0.011
Unbound	2.0 (2.0–3.8)	1.0 (1.0–2.3)	0.015
<i>t</i> _{1/2} (h)			
Total	15 (11–19)	8 (6–12)	1.78 (1.15–2.76)
Unbound	10 (8–13)	6 (4–8)	1.87 (1.30–2.67)

^a Results are expressed as GMs or GMRs, except for *T*_{max}, which is expressed as medians (interquartile ranges), and differences between cirrhotic patients and controls were calculated with the Mann-Whitney U test and are expressed as *P* values.

^b DRV, darunavir; PK, pharmacokinetics; *C*_{min}, minimum concentration; *C*_{max}, maximum concentration; AUC_{0–24}, area under the plasma concentration-time curve from 0 to 24 h; CL/F, clearance; *T*_{max}, time to reach *C*_{max}; *t*_{1/2}, half-life.

^c GM, geometric mean.

^d GMR, geometric mean ratio.

in both cirrhotics and controls ($r = 0.66$ and $P = 0.002$ and $r = 0.92$ and $P < 0.001$, respectively) and for AUC in controls ($r = 0.64$ and $P = 0.048$). There were significant differences between the *C*_{min} percentage of unbound darunavir and the *C*_{max} percentage of unbound darunavir in both cirrhotic patients (8% versus 16%, respectively; $P = 0.003$) and controls (8% versus 19%, respectively; $P = 0.008$), without differences between the two groups of patients (Table 2).

Regarding laboratory and PK parameters, there was a significant correlation between albumin and unbound darunavir AUC ($r = -0.52$ and $P = 0.02$) and between albumin and unbound darunavir CL/F ($r = 0.538$ and $P = 0.014$) in cirrhotic patients. In controls, there was significant correlation between alpha-1-glycoprotein levels and total and unbound darunavir AUC ($r = 0.77$ and $P = 0.016$ and $r = 0.7$ and $P = 0.036$, respectively) and between alpha-1-glycoprotein levels and total and unbound darunavir CL/F ($r = -0.77$ and $P = 0.016$, and $r = -0.7$ and $P = 0.036$, respectively).

Among cirrhotic patients, there were no differences in darunavir PK parameters based on Child-Pugh score. In a comparison of Child-Pugh C versus A scores, the total darunavir GMR (90% CI) was 0.67 (0.29 to 1.56) for *C*_{min}, 0.76 (0.53 to 1.07) for *C*_{max}, and 0.63 (0.40 to 1.00) for AUC; for unbound darunavir, the GMR (90% CI) were 1.44 (0.51 to 4.06), 1.16 (0.70 to 1.93), and 1.29 (0.78 to 2.15) for *C*_{min}, *C*_{max}, and AUC, respectively. Furthermore, there were no differences based on MELD scores, liver elasticity

(FibroScan) values, gender, or concomitant medications (including raltegravir [$n = 2$], methadone [$n = 4$], proton pump inhibitors [$n = 3$], or benzodiazepines [$n = 8$]), although the number of patients included in each group was relatively small (data not shown).

Efficacy, safety, and tolerability. During the 24 weeks of follow-up, none of the patients in the cirrhosis or control group had grade 2 to 4 side effects, and none of the cirrhotic patients had clinical decompensations. All patients had undetectable HIV RNA levels at baseline (<25 copies/ml) and maintained viral suppression during follow-up. Regarding laboratory parameters, the only significant changes were in albumin and triglyceride concentrations in cirrhotic patients (decreases of 0.23 mg/dl and 22 mg/dl, with $P = 0.036$ and 0.032, respectively), with no significant changes in CD4 cell counts, renal or hepatic function, or other lipid parameters. In 3 cirrhotic patients, treatment for HCV was started during the follow-up, 2 with peg-interferon plus ribavirin (2 and 4 months after PK analysis) and 1 with peg-interferon plus ribavirin plus telaprevir (3 months after PK analysis).

DISCUSSION

We have seen that darunavir total and unbound concentrations, when administered with ritonavir at 800/100 mg once daily in HIV-HCV-coinfected patients with compensated cirrhosis, are not significantly different from those observed in HIV-monoinfected patients with strictly normal liver function.

FIG 1 Darunavir plasma concentration-time curves. (a) Total darunavir concentrations in patients with cirrhosis (black solid line with filled squares) and controls (black dashed line with open squares). (b) Unbound darunavir concentrations in patients with cirrhosis (black solid line with filled circles) and controls (black dashed line with open circles). The results are expressed as the geometric means and 90% confidence intervals. Note the difference in scale between panels a and b.

To our knowledge, the main published data on darunavir PK parameters in patients with cirrhosis come from a study by Sekar et al. (14) in HCV-monoinfected patients (8 with Child-Pugh A scores and 8 with Child-Pugh B scores) and 16 healthy subjects, all receiving darunavir-ritonavir at 600/100 mg twice daily. In that study, no significant differences were observed between groups, and those authors concluded that no dose adjustments seemed necessary. In contrast, Tommasi et al. (21) reported higher darunavir concentrations in 5 HIV-HCV-coinfected patients, 3 of whom had a cirrhosis Child-Pugh B score, compared to 24 HIV-monoinfected controls, all receiving darunavir-ritonavir at 600/100 mg twice daily. The darunavir trough concentrations were significantly higher in cirrhotic patients than in the controls (8,519 versus 3,236 ng/ml, respectively), and the authors recom-

mended caution when using darunavir in these patients. However, these darunavir trough concentrations are much higher than those observed in our cirrhotic patients, even when analyzing only our 4 patients with a Child-Pugh C score. One potential explanation is the twice-daily dosing. It is possible that with the higher darunavir and ritonavir levels obtained with twice-daily dosing, the effect of cirrhosis may become more important than with lower once-daily administration. Furthermore, differences in trough concentrations might also be due to the small sample size and noncontrolled characteristics of the patients. For instance, in the study by Sekar et al. (14), which also used twice-daily dosing, darunavir trough concentrations in patients with a Child-Pugh B score were 3,681 ng/ml. Thus, it is very important to have more PK data from HIV-HCV-coinfected patients, especially with daruna-

TABLE 3 Ritonavir pharmacokinetic parameters^a

RTV PK parameter ^b	GM (90% CI) for ^c :		GMR (90% CI) ^d
	Cirrhosis (n = 20)	Controls (n = 10)	
$C_{\min}$ (ng/ml)			
Total	93 (67–129)	69 (42–111)	1.36 (0.76–2.44)
Unbound	0.72 (0.51–1.01)	0.29 (0.17–0.49)	2.50 (1.33–4.69)
% unbound	0.80 (0.63–1.02)	0.30 (0.22–0.43)	2.65 (1.75–4.03)
$C_{\max}$ (ng/ml)			
Total	789 (618–1,007)	865 (612–1,222)	0.91 (0.60–1.39)
Unbound	7.38 (5.94–9.16)	5.45 (4.01–7.38)	1.36 (0.93–1.97)
% unbound	0.86 (0.66–1.12)	0.50 (0.34–0.73)	1.71 (1.08–2.72)
AUC_{0-24} (ng · h/ml)			
Total	6,124 (4,732–7,925)	6,531 (4,539–9,397)	0.94 (0.60–1.47)
Unbound	63 (50–79)	36 (26–49)	1.76 (1.18–2.61)
CL/F (liters/h)			
Total	15 (12–18)	15 (11–21)	0.95 (0.65–1.41)
Unbound	1,419 (1,044–1,923)	3,055 (1,986–4,710)	0.46 (0.27–0.79)
$T_{\max}$ (h)			
Total	3.5 (1.0–6.0)	4.0 (1.8–4.0)	0.983
Unbound	3.0 (2.0–5.5)	2.0 (1.8–3.0)	0.307
$t_{1/2}$ (h)			
Total	13 (10–18)	7 (4–10)	2.06 (1.20–3.54)
Unbound	11 (9–14)	5 (4–7)	2.13 (1.42–3.19)

^a Results are expressed as GMs or GMRs, except for $T_{\max}$, which is expressed as medians (interquartile ranges), and differences between cirrhotic patients and controls were calculated with the Mann-Whitney U test and are expressed as *P* values.

^b RTV, ritonavir; PK, pharmacokinetics; $C_{\min}$, minimum concentration; $C_{\max}$, maximum concentration; AUC_{0-24} , area under the plasma concentration-time curve from 0 to 24 h; CL/F, clearance; $T_{\max}$, time to reach $C_{\max}$; $t_{1/2}$, half-life.

^c GM, geometric mean.

^d GMR, geometric mean ratio.

vir-ritonavir at 800/100 mg once daily, to be able to make dosing recommendations and not extrapolate from non-HIV-infected subjects with twice-daily administration.

In our study, the only significant differences in total and unbound darunavir concentrations between patients with cirrhosis and controls were in the $T_{\max}$ and half-lives. This delay in the $T_{\max}$ has also been described with darunavir in the study by Sekar et al. (14) and also in patients with cirrhosis receiving other protease inhibitors, such as nelfinavir (22), but not with lopinavir-ritonavir (23) or fosamprenavir (24). There are no data regarding the half-life of darunavir in cirrhosis patients, although a longer half-life in these patients than that in controls seems plausible.

The total darunavir concentrations found in our cirrhotic patients are also similar to those observed in prior studies in noncirrhotic patients with the same darunavir dose (15, 25–27). In all of these studies, including ours, the $C_{\min}$ values are well above the darunavir 50% effective concentration (EC_{50}) for wild-type virus (55 ng/ml) (27) and even the EC_{50} for viruses with a 10-fold change in resistance for darunavir (550 ng/ml). Only 3 of our cirrhotic patients had darunavir trough concentrations of <550 ng/ml, with values of 433, 438, and 476 ng/ml, and all of them had undetectable HIV RNA loads throughout the study.

There are scarce available data for darunavir unbound concentrations. In our study in HIV-HCV-coinfected patients, we did not see significant differences in unbound concentrations between patients with cirrhosis and controls. In the study by Sekar et

al. (14), the mean unbound percentages of darunavir in HIV-negative patients with mild (11.7% unbound) or moderate (16.4% unbound) hepatic impairment were higher than those in controls (7.6% unbound). In the RADAR study (15) with darunavir-ritonavir at 800/100 mg once daily, 11 patients without known hepatic impairment underwent intensive PK evaluation. The total and unbound darunavir C_{24} values in plasma were similar to those observed in our patients (1,340 and 173 ng/ml, respectively), with a median unbound percentage of 13%.

We have observed significant differences between the $C_{\min}$ and $C_{\max}$ percentages of unbound darunavir, suggesting nonlinearity in protein binding, probably due to protein saturation, with increases in the free fraction of the drug when its plasmatic concentrations increase. Regarding the correlation between proteins and unbound darunavir, it is difficult to explain with certainty why there was a correlation between the unbound darunavir AUC and albumin levels in cirrhotic patients and with alpha-1-glycoprotein levels in controls. A potential explanation is that as darunavir is bound both to albumin and alpha-1-glycoprotein, in cirrhotic patients with lower albumin concentrations, this can affect darunavir unbound concentrations. On the other hand, in controls with higher albumin values, alpha-1-glycoprotein levels might offset potential correlations with albumin concentrations.

In our study, the total ritonavir plasma concentrations were similar between patients with cirrhosis and controls. The main differences between the two groups were seen in ritonavir un-

FIG 2 Ritonavir plasma concentration-time curves. (a) Total ritonavir concentrations in patients with cirrhosis (black solid line with filled diamonds) and controls (black dashed line with open diamonds). (b) Unbound ritonavir concentrations in patients with cirrhosis (black solid line with filled triangles) and controls (black dashed line with open triangles). The results are expressed as the geometric means (90% confidence intervals). Note the difference in scale between panels a and b.

bound concentrations, which were significantly higher, in both absolute and percentage values, in cirrhotic patients. In the study by Sekar et al. (14), no significant differences were seen between patients with mild liver impairment and controls, but 50% increases in ritonavir AUC were seen if there was moderate hepatic impairment (8 patients in each group). These results are consistent with a prior study with fosamprenavir-ritonavir in cirrhotic patients (28) but not in another with lopinavir-ritonavir (23). However, none of these studies measured unbound ritonavir concentrations. A potential explanation for the higher absolute and percentage unbound ritonavir concentrations in cirrhotic patients not seen with darunavir is that ritonavir is mainly bound to albumin, which is significantly decreased in cirrhotics. Both darunavir and ritonavir are drugs that are hepatically cleared at a low level, but while ritonavir is >99% protein bound to albumin, darunavir is only 92% protein bound, and it is bound to both albumin and

alpha-1-glycoprotein. Thus, the alpha-1-glycoprotein binding by darunavir might offset changes in albumin concentrations. The implications of these differences in ritonavir unbound concentrations might be relevant, as higher boosting might be achieved, but in the clinical setting, the key factor is achieving adequate darunavir plasma concentrations.

We found statistically significant correlations between total and unbound DRV concentrations in cirrhotic patients. However, the degree of correlation varied, depending on the parameter, and it did not impact the outcomes. As a determination of unbound concentrations is technically more challenging, time-consuming, and expensive than a determination of total concentrations, it is probably not necessary to determine unbound concentrations in clinical practice.

Most of the cirrhotic patients in our study had a Child-Pugh score of A and preserved liver function. Although patients with

current clinically decompensated cirrhosis were excluded from study participation, a previous episode of liver decompensation was recorded in five cases, and 4 patients had a Child-Pugh score of C at study entry. There were no significant differences in concentrations between cirrhotic patients with Child-Pugh scores of A or C, but this might have been due to the small number of patients included with Child-Pugh C scores. Specific PK studies in larger groups of HIV-HCV-coinfected patients with moderate and severe liver impairment should be performed to recommend dosages and look for correlations, but this will be difficult given the characteristics and heterogeneity of these patients, with many confounding factors that hamper a generalization of results. Based on our findings, we would recommend starting darunavir-ritonavir at 800/100 mg once daily and monitoring closely those patients with Child-Pugh scores of B or C, if the use of darunavir-ritonavir is necessary.

Despite initial warnings of the potential increase in liver toxicity with darunavir (29), we have not seen any moderate or severe side effects with darunavir use in our cirrhotic patients, which is in line with the data from other cohorts of coinfecting patients (30, 31).

Some limitations of our study must be pointed out. Patients were already receiving and tolerating darunavir-ritonavir without side effects and with virologic suppression, and they had mainly cirrhosis Child-Pugh scores of A. Also, not all HIV-HCV-coinfecting patients with cirrhosis receiving darunavir-ritonavir controlled at our center have undergone a complete PK study. Thus, the results regarding efficacy and safety might entail a selection bias and might not be generalizable to all cirrhotic patients starting darunavir-ritonavir. We have inferred, for the convenience of the patients, that darunavir and ritonavir C_{24} values equaled C_0 and C_{trough} values in this study with once-daily administration. Some inpatient variability in darunavir plasma concentrations might exist, and thus this equation might not be exact. However, since the predose darunavir-ritonavir concentration was determined exactly 24 h after the preceding dose, this value probably is a very close approximation. Adherence was self-reported, which might have inflated the adherence rate and influenced the results, but this was minimized through dose diaries and direct observation of pill intake on the day of the PK study. Some baseline characteristics between cases and controls differed, and this might have influenced the results. However, no differences in darunavir PK parameters based on sex or race have been observed in prior studies (32); other variables that may influence PK results, such as body mass index, were well balanced, and the other parameters that showed differences between cases and controls (CD4 cell count, use of nucleos(t)ide reverse transcriptase inhibitors, and HIV transmission risk factors) are not known to influence darunavir PK parameters.

In conclusion, in HIV-HCV-coinfecting patients with clinically compensated cirrhosis, there were no significant differences in the darunavir total and unbound concentrations compared to those of HIV-infected patients with normal hepatic function. Once-daily darunavir-ritonavir at 800/100 mg was very well tolerated, and no serious adverse events were seen. According to our results, dose adjustments in these patients do not seem necessary.

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