

# Effect of Milk Thistle on the Pharmacokinetics of Darunavir-Ritonavir in HIV-Infected Patients

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**The aim of this open-label, fixed-sequence study was to investigate the potential of the botanical supplement milk thistle (silymarin) to interact with the boosted protease inhibitor combination darunavir-ritonavir. Fifteen HIV-infected patients receiving antiretroviral therapy with darunavir-ritonavir (600/100 mg twice daily) for at least 4 weeks were included. Silymarin (150 mg every 8 h) was added to the antiretroviral treatment from days 1 to 14. Darunavir concentrations in plasma were determined by high-performance liquid chromatography immediately before and 1, 2, 4, 6, 8, 10, and 12 h after a morning dose of darunavir-ritonavir on day 0 and darunavir-ritonavir plus silymarin on day 14. Individual darunavir pharmacokinetic parameters were calculated by noncompartmental analysis and compared between days 0 and 14 by means of the geometric mean ratio (GMR) and its 90% confidence interval (CI). The median age was 48 years (interquartile range, 44 to 50 years), and the median body weight was 70 kg (interquartile range, 65 to 84 kg). Silymarin was well tolerated, and all participants completed the study. The GMRs for darunavir coadministered with silymarin relative to darunavir alone were 0.86 (90% CI, 0.70 to 1.05) for the area under the concentration-time curve from 0 to 12 h, 0.83 (90% CI, 0.80 to 0.98) for the maximum concentration, and 0.94 (90% CI, 0.73 to 1.19) for the concentration at the end of the dosing interval. In summary, coadministration of silymarin with darunavir-ritonavir seems to be safe in HIV-infected patients; no dose adjustment for darunavir-ritonavir seems to be necessary.**

The use of alternative medicines, including botanical supplements, is common among HIV-infected patients. More than one-third of patients have been reported to take herbal remedies, usually as a self-prescribed treatment and without medical supervision (26, 30a, 38). Although botanical supplements are often perceived to be innocuous, in fact they can modulate various cytochrome P450 enzymes (e.g., CYP3A4) and drug transporters (e.g., P-glycoprotein [P-gp]), providing a reason to suspect potential herb-drug interactions (22, 24, 25).

The most widely recognized herb to interact with drugs is St. John's wort (*Hypericum perforatum*), which is a potent inducer of CYP3A4 and P-gp activity (18, 19) and which has been related to significant decreases in exposure to different drugs, including the antiretrovirals indinavir and nevirapine (5, 35). Additionally, numerous *in vitro* studies have shown that other botanical supplements are also capable of modulating CYP and P-gp activity and, thus, interact with drugs (3, 22, 25), although results from human *in vivo* studies have been less convincing (14, 15, 22, 25).

*Silybum marianum*, an herb commonly known as milk thistle, currently ranks among the top-selling botanical supplements (27). The main active components of milk thistle are flavonolignan complexes, collectively known as silymarin. Silymarin has been reported to exert hepatoprotective properties against a variety of xenobiotics (20, 31, 32, 42) and may be administered for the treatment of liver diseases (11, 39). Noteworthy, there is recent evidence of the inhibition of hepatitis C virus (HCV) RNA polymerase by silymarin (1, 28), and results from several small clinical trials suggest that silymarin could be used as an adjunctive therapy for HCV infection (10, 11, 29, 34). Therefore, a number of HIV-infected patients, particularly those coinfecting with HCV, may be interested in taking milk thistle in addition to antiretroviral therapy (30a).

*In vitro* studies have shown that silymarin may function as a substrate and inhibitor of CYP3A4 and P-gp (2, 3, 41, 43, 44), and

this may result in interactions with many drugs, including HIV protease inhibitors. However, clinical studies with milk thistle have produced discordant results regarding its ability to modulate these proteins *in vivo* (7, 12, 14, 15, 17, 37), and evidence on the interaction between milk thistle and antiretroviral drugs comes from clinical trials that have not used currently recommended treatment regimens (8, 30, 36).

The aim of the present study was, therefore, to evaluate the potential of silymarin to interact with a boosted protease inhibitor such as darunavir-ritonavir.

## MATERIALS AND METHODS

**Study design.** This open-label, fixed-sequence study enrolled 15 HIV-infected patients who were receiving antiretroviral therapy with darunavir-ritonavir at a dosage of 600/100 mg twice daily for at least 4 weeks and whose HIV-1 RNA load in plasma was <50 copies/ml. All patients gave written informed consent before enrollment, the protocol was approved by the ethics committee of the Hospital Universitari Germans Trias i Pujol, and the study was performed according to the stipulations of the Declaration of Helsinki and registered (Clinicaltrials.gov NCT01346982).

According to the package insert, patients received one capsule containing 150 mg of silymarin every 8 h from days 1 to 14 (Legalon; lot no. D0003; Rottapharm Madaus, Barcelona, Spain). All pills came from a single lot, which was externally controlled and certified to contain 100% of the labeled content of silymarin. Antiretroviral treatment remained unchanged. Serial blood samples to determine darunavir and ritonavir

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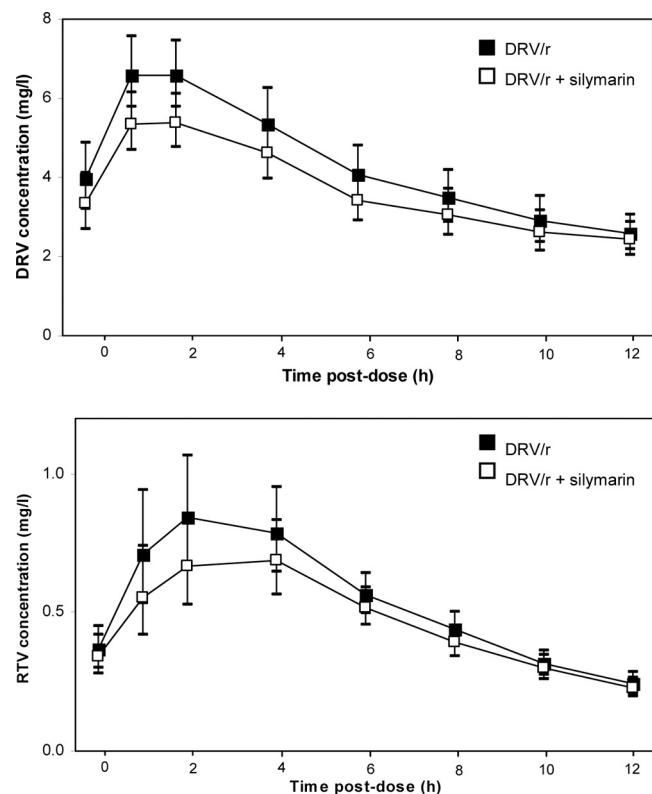


FIG 1 Geometric mean darunavir (DRV) and ritonavir (RTV) plasma concentration profiles of darunavir-ritonavir (DRV/r) with or without coadministration of multiple doses of silymarin. Error bars represent the 90% confidence interval.

concentrations in plasma were collected immediately before and 1, 2, 4, 6, 8, 10, and 12 h after a witnessed morning dose of darunavir-ritonavir on day 0 and at the same times before and after darunavir-ritonavir plus silymarin on day 14. Darunavir and ritonavir were dosed with a standard breakfast consisting of 550 kcal (43% carbohydrate, 39% fat, 18% protein).

Demographic and clinical variables (including age, body weight and height, and use of concomitant drugs, including over-the-counter medications) were recorded. Safety was evaluated by clinical interview and physical examination as well as by laboratory assessment (blood counts, chemistry, CD4<sup>+</sup> T cell count, and HIV-1 RNA load) on days 0, 14, and 28. To enhance adherence to scheduled clinical visits and the treatment protocol, patients were provided with a visit calendar. Apart from days 0 and 14, drug intake was not directly observed; adherence was assessed by means of a diary in which the patient recorded medication intake and by pill count on day 14.

**Analytical and pharmacokinetic analysis.** Blood samples for darunavir and ritonavir determinations were collected into K-EDTA-containing 10-ml tubes. Plasma was isolated by centrifugation (3,200 × *g* for 15 min) and stored at −20°C until analysis. Darunavir and ritonavir concentrations were determined by high-performance liquid chromatography with a photo diode array detector (HPLC-PDA 2996; Waters, Barcelona, Spain), according to a validated method. The analytical column was a NovaPak C<sub>18</sub> 3.9- by 150-mm with a NovaPak C<sub>18</sub> guard column (Waters). The method involved liquid-liquid extraction of drug from plasma with methyl *tert*-butyl ether. The mobile phase consisted of a gradient elution with phosphate buffer in acetonitrile (pH 6.70). The method was linear over the range of 0.05 to 10.0 mg/liter for both drugs (lower limit of quantification, 0.05 mg/liter; intra- and interday variation, <10%). Our laboratory subscribes to the external quality assurance program organized

by the Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology of Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands (9).

Darunavir and ritonavir pharmacokinetic parameters were calculated for each individual using a noncompartmental approach by means of the WinNonlin software application (version 2.0; Pharsight, Mountain View, CA). The area under the concentration-time curve during the dose interval of 0 to 12 h (AUC<sub>0-12</sub>) was calculated by means of the linear trapezoidal rule. Maximum concentrations (*C*<sub>max</sub>) and the concentrations at the end of the 12-h dosing interval (*C*<sub>12</sub>) were obtained by inspection of the concentration data.

**Statistical analysis.** Data analysis was carried out using SPSS (version 15.0) statistical software (Chicago, IL). Darunavir and ritonavir pharmacokinetic parameters were described by the geometric mean and compared between days 0 and 14 by the geometric mean ratio (GMR) and its 90% confidence interval (CI). Pharmacokinetic parameters were natural log transformed before analysis, and confidence intervals for means (and for the difference between two means) were constructed on the natural log scale on the basis of an analysis-of-variance model with treatment as a fixed effect. The results were exponentiated and reported with lower and upper limits of the 90% CIs.

A power calculation indicated that 15 patients would provide an 80% chance of detecting a 25% difference in the AUC<sub>0-12</sub> for darunavir at a level of significance of *P* equal to 0.1.

## RESULTS

A total of 15 Caucasian HIV-infected males were enrolled; 4 were coinfecting with HCV. The median age was 48 years (interquartile range, 44 to 50) years, and the median body weight was 70 kg (interquartile range, 65 to 84 kg). Antiretroviral treatments in addition to darunavir-ritonavir included raltegravir in 12 patients, tenofovir in 10, emtricitabine in 8, etravirine in 7, and maraviroc in 1. The median CD4<sup>+</sup> T cell count was 439 cells/mm<sup>3</sup> (interquartile range, 360 to 614 cells/mm<sup>3</sup>).

Silymarin was well tolerated, and all participants completed the study. The only adverse event reported was mild heartburn in one patient. All patients maintained an HIV-1 RNA load of <50 copies/ml until the end of the study.

**Darunavir and ritonavir pharmacokinetics.** Darunavir and ritonavir plasma concentration-time curves following administration of darunavir-ritonavir alone or in combination with multiple doses of silymarin are shown in Fig. 1. Darunavir and ritonavir pharmacokinetic parameters with and without silymarin coadministration are summarized in Tables 1 and 2, respectively. Coadministration of silymarin resulted in a slight, though not statistically significant, decrease in darunavir and ritonavir exposure, as both *C*<sub>max</sub> and AUC<sub>0-12</sub> decreased by about 15%, on the average. There was no substantial effect on the darunavir *C*<sub>12</sub> (Fig.

TABLE 1 Comparison of darunavir pharmacokinetic parameters with and without coadministration of multiple doses of silymarin<sup>a</sup>

Drugs or parameter	AUC <sub>0-12</sub> (mg·h/liter)	<i>C</i> <sub>max</sub> (mg/liter)	<i>C</i> <sub>12</sub> (mg/liter)
DRV/r	53.21 (46.03–61.38)	7.08 (6.28–7.98)	2.58 (2.17–3.07)
DRV/r + silymarin	45.60 (39.54–52.72)	5.86 (5.20–6.61)	2.42 (2.04–2.87)
GMR	0.86 (0.70–1.05)	0.83 (0.70–0.98)	0.94 (0.73–1.19)
<i>P</i>	0.215	0.07	0.654

<sup>a</sup> Data are expressed as geometric mean (90% confidence interval). DRV/r, darunavir-ritonavir; GMR, geometric mean ratio; AUC<sub>0-12</sub>, area under the time-concentration curve from 0 to 12 h after dosing; *C*<sub>max</sub>, maximum concentration; *C*<sub>12</sub>, concentration at the end of the 12-h dosing interval.

**TABLE 2** Comparison of ritonavir pharmacokinetic parameters with and without coadministration of multiple doses of silymarin<sup>a</sup>

Drugs or parameter	AUC <sub>0-12</sub> (mg · h/liter)	C <sub>max</sub> (mg/liter)	C <sub>12</sub> (mg/liter)
DRV/r	6.73 (5.82–7.80)	0.95 (0.80–1.12)	0.24 (0.21–0.28)
DRV/r + silymarin	6.00 (5.19–6.93)	0.86 (0.73–1.01)	0.23 (0.19–0.26)
GMR	0.89 (0.72–1.09)	0.90 (0.72–1.14)	0.94 (0.75–1.16)
<i>P</i>	0.346	0.465	0.601

<sup>a</sup> Data are expressed as geometric mean (90% confidence interval). DRV/r, darunavir-ritonavir; GMR, geometric mean ratio; AUC<sub>0-12</sub>, area under the time-concentration curve from 0 to 12 h after dosing; C<sub>max</sub>, maximum concentration; C<sub>12</sub>, concentration at the end of the 12-h dosing interval.

2), which remained above the protein-binding-corrected 50% inhibitory concentration (IC<sub>50</sub>) for viral strains with protease inhibitor resistance-associated mutations (0.55 mg/liter) (6) in all participants.

## DISCUSSION

Although *in vitro* inhibition of CYP3A4 and P-gP by silymarin (2, 3, 41, 43, 44) suggests that coadministration of this drug with HIV protease inhibitors such as darunavir could theoretically result in higher drug exposure, our findings show that the *in vivo* effect seems to be negligible. We found that there was a close correspondence of the pharmacokinetic profiles of darunavir in the presence and absence of coadministered silymarin.

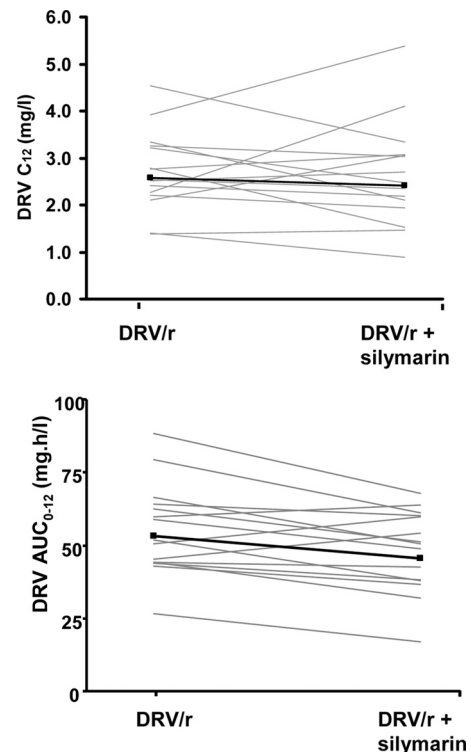
Coadministration of silymarin with darunavir-ritonavir did result in a mean darunavir C<sub>max</sub> and an AUC<sub>0-12</sub> that were non-significantly lower by 15%. The magnitude of this interaction is on the same order as that reported between efavirenz and darunavir in a study by Sekar and colleagues (40), who concluded that no darunavir dose adjustment was required. The potential impact of this interaction on the clinical outcome of HIV-infected patients seems to be quite limited in patients without darunavir resistance-associated mutations. Even when darunavir is dosed once daily, darunavir trough concentrations have been shown to be nearly 40 times above the protein binding-corrected IC<sub>50</sub> for wild-type viral strains (39a). The only scenario where this tendency toward interaction might be clinically relevant could be in treatment-experienced patients harboring viral strains with decreased susceptibility to protease inhibitors. Even small decreases in drug concentration could put such patients at higher risk of treatment failure and further selection of resistance mutations. In the present study, however, using the darunavir-ritonavir dose of 600/100 mg twice daily, darunavir concentrations at the end of the dosing interval remained above the protein binding-corrected IC<sub>50</sub> for viral strains resistant to other protease inhibitors (6).

The interaction between milk thistle and antiretroviral drugs was studied several years ago in healthy volunteers who received indinavir with or without multiple doses of silymarin (8, 30, 36). Administration of silymarin also failed to influence the indinavir AUC in these studies, although slight reductions in indinavir trough concentrations were observed. Indinavir was given at a dose of 800 mg every 8 h without ritonavir in these studies, however, reflecting dosing that is no longer recommended for routine clinical care of HIV-infected patients (33). Protease inhibitors are currently boosted by means of coadministered low doses of ritonavir, which acts as a potent CYP3A4 inhibitor (21, 33). This strategy helps maintain drug concentrations far above the concen-

tration needed to inhibit viral replication. Additionally, strong inhibition of CYP3A4 by ritonavir may also limit the potential of other agents to interact with protease inhibitors, as seems to have been the case in our study.

Our results are in line with those reported by Gurley and various coauthors (14–17), who did not observe any significant interaction between silymarin and midazolam, debrisoquine, or digoxin, used as probes for CYP3A4, CYP2D6, and P-gp, respectively, in humans. Various factors may help explain discrepancies between results from *in vitro* experiments (2, 3, 41, 43, 44) and the above-mentioned *in vivo* studies. First, there may be great variability in silymarin content among different milk thistle brands available on the market. Furthermore, discrepancies between the labeled and the actual content of active constituents have been reported for many botanical preparations (13). For our study, we purchased a single lot of silymarin from a sole vendor that used an external laboratory to certify that pills contained 100% of the labeled content of the drug. A second, more plausible explanation for discrepancies between *in vitro* and *in vivo* findings may be related to the poor and erratic oral bioavailability of silymarin, given that milk thistle flavonolignans exhibit low solubility in water (4, 23). Since we did not measure silymarin concentrations in plasma, we cannot confirm its solubility and bioavailability in our study. For the same reason, it remains to be determined whether our findings may be extrapolated to other milk thistle formulations.

In summary, our results suggest that coadministration of silymarin with darunavir-ritonavir and probably with other HIV



**FIG 2** Darunavir (DRV) concentration at the end of the 12-h dosing interval (C<sub>12</sub>) and area under the time-concentration curve from 0 to 12 h (AUC<sub>0-12</sub>) values after administration of darunavir-ritonavir with or without multiple doses of silymarin. Gray lines represent individual values, and black lines represent the geometric mean.



boosted protease inhibitors is safe and well tolerated and does not result in significant pharmacokinetic interactions. We therefore believe that no dose adjustment for darunavir-ritonavir seems to be necessary.

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We have no conflicts of interest that are directly relevant to the context of this study.

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