

Unexpected Drug-Drug Interactions in Human Immunodeficiency Virus (HIV) Therapy: Induction of UGT1A1 and Bile Efflux Transporters by Efavirenz

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Abstract

Introduction: Efavirenz is an inducer of drug metabolism enzymes. We studied the effect of efavirenz and ritonavir-boosted darunavir on serum unconjugated and conjugated bilirubin, as probes for UGT1A1 and bile transporters. **Materials and Methods:** Healthy volunteers were enrolled in a clinical trial. There were 3 periods: Period 1, 10 days of darunavir 900 mg with ritonavir 100 mg once daily; Period 2, 14 days of efavirenz 600 mg with darunavir/ritonavir once daily; and Period 3, 14 days of efavirenz 600 mg once daily. Serum bilirubin (conjugated and unconjugated) concentrations were obtained at baseline, at the end of each phase and at exit. **Results:** We recruited 7 males and 5 females. One subject developed grade 3 hepatitis on efavirenz and was excluded. Mean serum unconjugated bilirubin concentrations were 6.09 $\mu\text{mol/L}$ (95% confidence interval [CI], 4.99 to 7.19) at baseline, 5.82 (95% CI, 4.88 to 6.76) after darunavir/ritonavir, 4.00 (95% CI, 2.92 to 5.08) after darunavir/ritonavir with efavirenz, 3.55 (95% CI, 2.58 to 4.51) after efavirenz alone and 5.27 (95% CI, 3.10 to 7.44) at exit ($P < 0.01$ for the efavirenz phases). Mean serum conjugated bilirubin concentrations were 3.55 $\mu\text{mol/L}$ (95% CI, 2.73 to 4.36) at baseline, 3.73 (95% CI, 2.77 to 4.68) after darunavir/ritonavir, 2.91 (95% CI, 2.04 to 3.78) after darunavir/ritonavir with efavirenz, 2.64 (95% CI, 1.95 to 3.33) after efavirenz alone and 3.55 (95% CI, 2.19 to 4.90) at exit ($P < 0.05$ for the efavirenz phases). **Conclusion:** Efavirenz decreased unconjugated bilirubin by 42%, suggesting UGT1A1 induction. Efavirenz also decreased conjugated bilirubin by 26%, suggesting induction of bile efflux transporters. Ritonavir-boosted darunavir had no effect on bilirubin concentrations. These results indicate that efavirenz may reduce concentrations of drugs or endogenous substances metabolized by UGT1A1 or excreted by bile efflux transporters.

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Key words: Drug-drug interactions, Drug transporters, Efavirenz, HIV Therapy, UGT1A1

Introduction

Major improvements in antiretroviral therapy have transformed human immunodeficiency virus (HIV) infection into a manageable chronic disease. More and more patients are starting therapy earlier as studies continue to show benefit by treating at higher CD4 counts. Furthermore, HIV patients increasingly require treatment for comorbid conditions. Pharmacokinetic interactions between antiretrovirals (ARVs) and other drug classes or herbal compounds are thus an increasing concern.¹

Protease inhibitors and non-nucleoside reverse transcriptase inhibitors are involved in the cytochrome P450 (CYP450) or transporter systems, and may be associated with higher risk of clinically significant drug interactions.²

The non-nucleoside reverse transcriptase inhibitor efavirenz is currently one of the most commonly used ARVs, and is included as preferred first line therapy in HIV treatment guidelines, in combination with 2 nucleoside reverse transcriptase inhibitors. Efavirenz has now been widely used for more than a decade with an established track record of efficacy and tolerability.³

Efavirenz is a moderately potent inducer of various metabolic enzymes, especially CYP450 subtypes 3A4 and 2B6. This phenomenon also leads to auto-induction, so that steady state concentrations of efavirenz are lower than after a single dose. This induction effect has also led to recommendations to increase the dose of some other drugs when used concomitantly with efavirenz.

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These recommendations include HIV protease inhibitors fosamprenavir, atazanavir and lopinavir, which are metabolised by the CYP450 3A4 enzymes induced by efavirenz.⁴ While efavirenz is known to reduce indinavir concentrations, we have previously shown that a moderate dose of indinavir of 600 mg with ritonavir 100 mg is still sufficient to maintain adequate concentrations.⁵

The effect of efavirenz on other metabolic enzymes and transporters are less understood. We therefore aimed to study the effect of efavirenz and ritonavir-boosted darunavir on UDP-glucuronosyl transferase (UGT1A1) and bile efflux transporters, using unconjugated and conjugated bilirubin as probes.

Materials and Methods

Twelve healthy men and women between 21 and 65 years of age were eligible for enrollment in the study. HIV-infected patients, individuals with a clinically significant medical condition, smokers, and individuals with a known history of alcohol and/or drug abuse were excluded from the study. Exclusion criteria also included recent participation in an investigational drug study and inability to refrain from the use of prescription and non-prescription medications.

The subjects agreed to participate in the studies by giving written informed consent prior to commencement of the study. The study protocol was reviewed and approved by the Domain Specific Review Board, National Healthcare Group. Written informed consent was obtained from all volunteers. The studies were conducted in accordance with the guidelines on good clinical practice and with the ethical standards for human experimentation established by the Declaration of Helsinki.

We conducted an open-label single-sequence 3-period pharmacokinetic study with healthy HIV-seronegative adults (Fig. 1).⁶ Blood was collected for clinical chemistry assays at baseline (pre-treatment). During Period 1, the healthy volunteers received darunavir-ritonavir at 900/100 mg once daily for 10 days (completion of Period 1). Clinical chemistry assays were performed on day 10. During Period 2, efavirenz administered at 600 mg once daily was given with the darunavir-ritonavir regimen for 14 days. Clinical chemistry assays were performed on days 24 (completion of Period 2). At the start of Period 3, the darunavir-ritonavir was stopped and the healthy volunteers received efavirenz at 600 mg once daily only from days 25 to 38. Clinical chemistry assays were performed on days 38 (completion of Period 3) and one week later (exit visit).

Clinical chemistry assays included the full liver function panel. Serum bilirubin (conjugated and total) concentrations were measured using a standard spectrophotometric method. Serum samples were mixed with detergent and

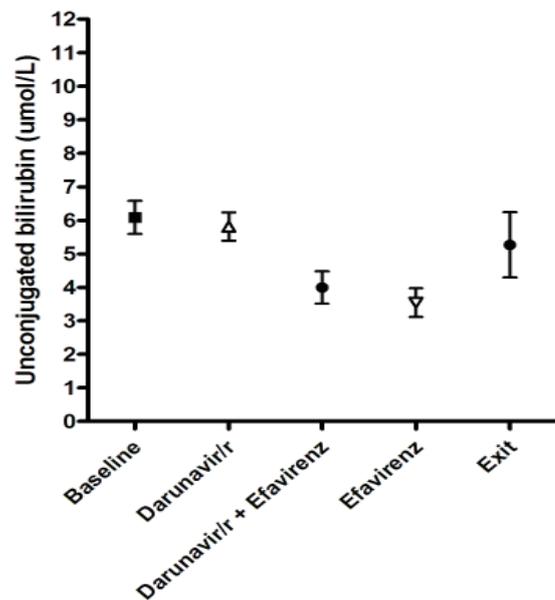


Fig. 1. Mean and standard error of unconjugated bilirubin concentrations at baseline and after the different periods.

vanadate at an approximate pH of 3.0. Oxidation of total bilirubin to biliverdin causes the absorbance of yellow, specific to bilirubin, to decrease. The total bilirubin in the sample is obtained by measuring the absorbance at 450 nm before and after vanadate oxidation. For measurement of direct bilirubin, before the addition of the vanadate, an inhibitory reagent is added to prevent the oxidation of the direct bilirubin. The same method was then used to measure bilirubin. Unconjugated bilirubin was calculated by subtracting conjugated bilirubin from total bilirubin.

Paired t-tests were performed to compare unconjugated and conjugated bilirubin in periods 1, 2 and 3 versus baseline. Ninety percent confidence intervals were calculated for the difference between the different intervention periods, and baseline. Statistical analyses and figures were prepared using Prism version 4.0 (Graphpad Software, San Diego, CA, USA).

Results

We recruited 7 males and 5 females, aged 24 to 49 years, weighing 50 to 83 kg. All subjects completed all phases of the study. One female subject developed grade 3 hepatitis with ALT more than 9 times the upper limit of normal while in Period 3 (efavirenz alone). This adverse event resolved completely after 150 days. Her data were excluded from the final analyses. One female subject developed grade 2 maculopapular rash on day 9 of darunavir/ritonavir, which resolved in 2 weeks. Three female subjects developed grade 1 papular rashes while in Period 3, which resolved in 6 days.

Table 1. Effect of 10 Days of Darunavir 900 mg and Ritonavir 100 mg Once Daily, Addition of Efavirenz 600 mg Once Daily and Efavirenz 600 mg Once Daily Alone on Unconjugated and Conjugated Bilirubin Concentrations, Values from Paired Data

Period	Unconjugated Bilirubin				Conjugated Bilirubin			
	Mean (SD) ($\mu\text{mol/L}$)	Change from baseline	90% Confidence interval of difference	<i>P</i> value vs baseline	Mean (SD) ($\mu\text{mol/L}$)	Change from baseline	90% Confidence interval of difference	<i>P</i> value vs baseline
Baseline	6.09 (1.64)	Ref			3.55 (1.21)	Ref		
DRV/RTV	5.82 (1.40)	-0.27	-1.12 to 0.58	0.574	3.73 (1.42)	0.18	-0.50 to 0.87	0.640
DRV/RTV with EFV	4.00 (1.61)	-2.09	-2.66 to -1.52	<0.001	2.91 (1.30)	-0.64	-1.08 to -0.19	0.026
EFV	3.55 (1.44)	-2.55	-3.53 to -1.56	0.001	2.64 (1.03)	-0.91	-1.62 to -0.20	0.043
Exit	5.27 (3.23)	-0.82	-2.26 to 0.62	0.328	3.55 (2.02)	0.00	-0.88 to 0.88	1.000

DRV: darunavir; RTV: ritonavir; EFV: efavirenz; SD: standard deviation of mean; Exit: exit visit 7 days after stopping efavirenz

All subjects developed grade 1 neuropsychiatric symptoms, especially giddiness, while on efavirenz.

Mean serum unconjugated bilirubin concentrations were 6.09 $\mu\text{mol/L}$ (95% CI, 4.99 to 7.19) at baseline, 5.82 (95% CI, 4.88 to 6.76) after darunavir/ritonavir, 4.00 (95% CI, 2.92 to 5.08) after darunavir/ritonavir with efavirenz, 3.55 (95% CI, 2.58 to 4.51) after efavirenz alone and 5.27 (95% CI, 3.10 to 7.44) at exit. Unconjugated bilirubin concentrations were significantly lower after both the efavirenz phases ($P < 0.01$) compared to baseline (Table 1, Fig. 1).

Mean serum conjugated bilirubin concentrations were 3.55 $\mu\text{mol/L}$ (95% CI, 2.73 to 4.36) at baseline, 3.73 (95% CI, 2.77 to 4.68) after darunavir/ritonavir, 2.91 (95% CI, 2.04 to 3.78) after darunavir/ritonavir with efavirenz, 2.64

(95% CI, 1.95 to 3.33) after efavirenz alone and 3.55 (95% CI, 2.19 to 4.90) at exit. Conjugated bilirubin concentrations were also significantly lower after both the efavirenz phases ($P < 0.05$) compared to baseline (Table 1, Fig. 2).

Discussion

In this study, we found that efavirenz significantly decreased unconjugated bilirubin by 42% and conjugated bilirubin by 26%.

Unconjugated bilirubin is converted to conjugated bilirubin by the endogenous metabolic enzyme UGT1A1. Thus the reduction of unconjugated bilirubin suggests that efavirenz induces UGT1A1. Previously, another group found that efavirenz causes a 36% reduction of the raltegravir area under curve-time concentrations.⁷ There was no recommendation to increase the dose of raltegravir when given with efavirenz. Raltegravir is also a substrate of UGT1A1, forming raltegravir glucuronide,⁸ and therefore our results, consistent with the current literature, were expected. Caution may be warranted if efavirenz is administered with other UGT1A1 substrates such as irinotecan. UGT1A1 is an important phase 2 metabolic enzyme and we have shown an increasingly role of phase 2 enzymes in the metabolism of ARVs.^{9,10}

We found that efavirenz might also increase conjugated bilirubin excretion possibly by inducing bile efflux transporters. This is a novel finding. The effect of transporters on HIV drugs and drug-drug interactions are increasingly being recognised and studied. We have previously shown unexpected interactions between 2 protease inhibitors and 2 statins, which could at least partially be attributed to transporter effects.¹¹

Bile transporters include bile salt efflux pumps (BSEP) and MRP 2 and 3. Therefore, efavirenz may reduce concentrations of substrates of these transporters. An

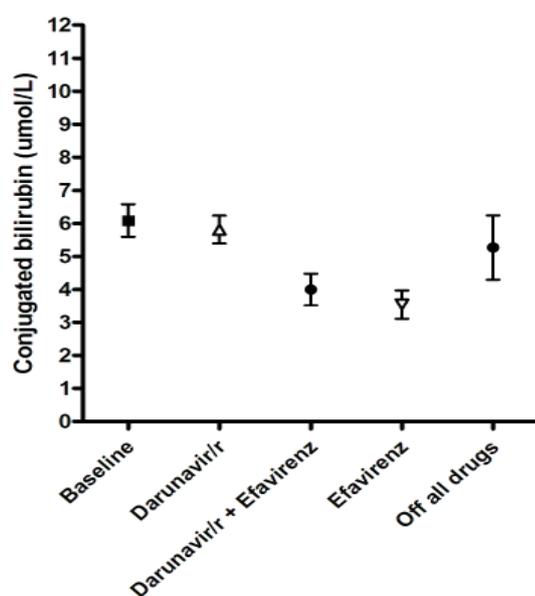


Fig. 2. Mean and standard error of unconjugated bilirubin concentrations at baseline and after the different periods.

increasing number of drugs are being discovered to be cleared by these transporters. These include some statins,¹² methotrexate¹³ and some beta-blockers.¹⁴ Therefore, efavirenz may reduce the efficacy of these other drugs and perhaps even cause treatment failure. This is an area which would need further investigation, using both in vitro and clinical studies.

Efavirenz is now recognised as an activator of the nuclear constitutive androstane receptor (CAR) and pregnane X receptor (PXR).¹⁵ This activity can lead to a increase in a broad spectrum of metabolic enzymes and transporters including UGT1A1. Changes in drug disposition with long-term efavirenz use or with efavirenz and statin use have been attributed to CAR-mediated induction of drug metabolism. As it is impossible to conduct drug-drug interaction studies with every drug substrate of these enzymes, caution would be advised whenever efavirenz or other activators of CAR or PXR such as rifampicin or phenobarbital are used concomitantly.

Ritonavir-boosted darunavir had no significant effect on bilirubin concentrations. Therefore darunavir/ritonavir had no apparent net induction or inhibition effect on UGT1A1 or these transporters. However, the effect of darunavir/ritonavir on other transporters cannot be ruled out in this study, and we have in fact found effects of darunavir/ritonavir on another transporter, MRP1.¹⁶

These results indicate that efavirenz may reduce the concentrations of drugs or endogenous substances metabolised by UGT1A1, or which are excreted by bile efflux transporters. This may lead to unexpected drug-drug interactions leading to possible treatment failure. An increased level of vigilance would be prudent in patients on efavirenz who need to take concomitant medications. This would be a common scenario in HIV patients who are commonly on multiple medications.

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