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Original Research

Substantial Effect of Efavirenz Monotherapy on Bilirubin Levels in Healthy Volunteers

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ABSTRACT

Background: Efavirenz exhibits multiple interactions with drug-metabolizing enzymes and transporters, and for this reason efavirenz-based HIV therapy is associated with altered pharmacokinetics of coadministered drugs. Probably by the same mechanism, efavirenz-based HIV therapy affects the disposition of endogenous compounds, but this effect is difficult to directly link with efavirenz because it is used in combination with other drugs.

Objectives: To explore the effect of efavirenz monotherapy on biochemical laboratory values in a clinical trial of healthy volunteers.

Methods: Men and women (aged 18–49 years) with body mass index ≤ 32 who were assessed to be healthy based on medical history, physical examination, and standard laboratory screening received a single (600 mg) and multiple doses (600 mg/d for 17 days) of efavirenz orally. This trial was designed to determine the pharmacokinetics and drug interactions of efavirenz. As part of this study, analysis of serum chemistries that were measured at study entry (screening) and 1 week after completion of the multiple dose study (exit) is reported.

Results: Data from 60 subjects who fully completed and 13 subjects who partially completed the study are presented. Total bilirubin was substantially reduced at exit (by ~30%, with large intersubject variability) compared with screening values ($P < 0.0001$). The percent changes were in part explained by the intersubject differences in baseline total bilirubin because there was a significant correlation between baseline (screening) values and percent change at exit ($r = 0.50$; $P < 0.0001$). Hemoglobin and absolute neutropenia were also substantially decreased at exit compared with screening, but this may be due to intensive blood sampling rather than direct effect of efavirenz on these parameters. No significant correlation was found between percent change in hemoglobin versus percent change in bilirubin, indicating the effect of efavirenz on bilirubin is independent of its effects on hemoglobin.

Conclusions: Efavirenz monotherapy significantly lowers plasma total bilirubin concentration in healthy volunteers independent of its effect on hemoglobin, probably through its effects on bilirubin metabolism and transport (uptake and efflux). These findings help explain reversal by efavirenz of hyperbilirubinemia induction observed by some protease inhibitor antiretroviral drugs (eg, atazanavir). Besides its well-documented role on drug interactions, efavirenz may alter the disposition of endogenous compounds relevant in physiologic homeostasis through its interaction with drug metabolizing enzymes and/or drug transporters. ClinicalTrials.gov identifier: NCT00668395.

Introduction

Highly active antiretroviral therapy (HAART), a combination of at least 3 antiretroviral drugs, effectively suppresses viral

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replication that is linked to CD4+ T cell counts elevation in patients with HIV infection with AIDs. The introduction of HAART in 1996 was a landmark treatment change that considerably improved the prognosis of patients infected with HIV by reducing AIDS-related morbidity and mortality.[1] Because of HAART and better management of opportunistic infections, HIV, which was once fatal, is now considered a chronic manageable infection that requires lifelong drug therapy. Because HIV has gradually changed from an acute to chronic disease, HAART-associated adverse effects, nonadherence, and complex drug–drug interactions may compromise patients' quality of life.[2,3]

The nonnucleoside reverse transcriptase inhibitor efavirenz-based treatment regimens represent the cornerstone of HIV therapy and are widely used worldwide as the initial preferred therapy in treatment-naïve HIV patients.[4] Efavirenz is generally well tolerated, but common adverse effects such as central nervous system symptoms (in ~50% of patients),[5] metabolic disorders,[6] and hepatic injury[7] compromise the safe use of efavirenz. Some of these adverse effects (eg, neuropsychiatric effects) are the main reason for early discontinuation of efavirenz-based therapy in patients.[8,9] In addition, certain studies and case reports have identified other adverse effects such as gynacomastia, hematologic abnormalities, abnormal vision, osteomalacia, and hypersensitivity reactions[6,10] in patients treated with efavirenz-based therapy. These adverse effects are important because they may affect drug adherence that promotes viral resistance and may cause withdrawal of an otherwise effective drug regimen.

There is evidence that efavirenz may alter serum concentrations of endogenous compounds through its interaction with endobiotic-metabolizing enzymes and transporters. We recently reported[11] that in vivo efavirenz reduces the concentrations of bilirubin, a substrate of uridine glucuronosyltransferase (UGT) 1A1, organic anion transporting polypeptide (OATP) 1B1, and multidrug resistance-associated protein (MRP) 2. The use of efavirenz-based HIV therapy is associated with vitamin D deficiency and increased alkaline phosphatase,[12,13] probably through effects of efavirenz on vitamin D-metabolizing enzymes.[14,15] In patients with HIV-1 infection, particularly those with AIDS, hematologic adverse effects such as anemia occur at high frequency and are associated with poor prognosis.[16] It is believed that the infection itself is a risk factor, but certain combination antiretrovirals (eg, azidothymidine-containing) also lead to hematologic adverse effects.[17] In certain case reports, efavirenz-based therapy has been implicated in immune-mediated hemolytic anemia,[18] increased hemoglobin over time,[19] and neutropenia.[20] Despite the evidence that efavirenz is implicated in altered endogenous laboratory markers of safety, making a direct link between efavirenz and safety laboratory values is difficult because efavirenz is used in combination with other drugs (often used with 2 nucleoside reverse transcriptase inhibitors or a nucleoside reverse transcriptase inhibitor and nucleotide reverse transcriptase inhibitor and it remains unknown whether the changes reported are specific to efavirenz or the other components of HAART. The presence of HIV infection also confounds interpretation of these data.

The effect of efavirenz monotherapy on hematologic and biochemical safety laboratory values was assessed in a clinical trial in healthy male and female volunteers primarily designed to determine the pharmacokinetics and drug interactions of a single dose and multiple doses of efavirenz.[21,22] Serum chemistries measured at study entry (ie, screening and before efavirenz administration) and 1 week after completion of the entire study (exit and 1 week after the final dose of efavirenz) were compared.

Materials and Methods

Study subjects

Healthy nonsmoking male and female volunteers (aged 18–49 years) who fulfilled eligibility criteria (no concurrent medication use) were enrolled in a trial at the Indiana University School of Medicine Clinical Research Center. Volunteers were judged to be healthy by medical history, physical examination, and standard screening laboratory tests. Detailed inclusion and exclusion criteria have previously been described.[21,22] The Indiana University School of Medicine Institutional Review Board approved this study and all volunteers signed an institutional review board-approved informed consent and HIPAA documents before enrollment.

Study design

The protocol was primarily designed to test efavirenz (single 600-mg oral dose) and multiple dose (after 600 mg/d for 17 days orally) pharmacokinetics and interactions with the activities of selected cytochrome p450s (CYPs) (ie, CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4), as measured by simultaneous administration of a single dose of each isoform-specific probe substrate. Participants were studied in 3 phases with a total of 2 inpatient days and a total of 11 outpatient visits. Phase 1 (Day 1): A single 600-mg oral dose of efavirenz and a cocktail of isoform-selective CYP probe substrates were given to the volunteers and pharmacokinetic sampling was performed for 7 subsequent days. Phase 2 (Day 7): Participants began taking efavirenz 600 mg orally each day for 17 days and pharmacokinetic samples were collected every third day for measurement of trough concentrations of efavirenz. Phase 3 (Day 24): Participants took their final dose of efavirenz with the cocktail probes followed by 7 days of pharmacokinetic sampling. On Day 30 participants completed and exited the trial. Standard laboratory results were obtained during the screening phase (ie, baseline and before drug administration) and at exit on Day 30 (ie, approximately 1 week after the last dose of efavirenz). The overall study design is presented in **Figure 1**. Here, we present a comprehensive analysis of data that relate to the influence of efavirenz monotherapy on exit laboratory values compared with those obtained at baseline.

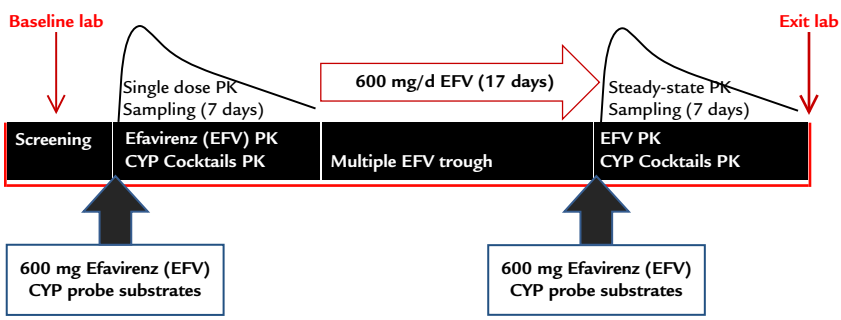

Figure 1. Study design schema. CYP = cytochrome p450; EFV = efavirenz; PK = pharmacokinetic; mg/d = mg/day.

Data analysis

Laboratory data at baseline were compared with data at exit using 2-tailed, paired Student *t* test. A *P* value < 0.05 was considered to be statistically significant. Pearson correlation coefficient was obtained. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California).

Results

The study included 73 volunteers (71.2% white, 21.9% African American, and 6.8% other races); 60 subjects who fully completed the study (30 days) and 13 subjects who partially completed the study. In **Table I**, means (SD) and percent change in laboratory values are presented for the 60 subjects who fully completed the study (38 men and 22 women).

Marked reduction in total bilirubin was observed at exit compared with screening (see **Table I** and **Figure 2A**). The mean percent change in total bilirubin was ~30%, with extensive intersubject variability (**Figure 2B**). We found no correlation between bilirubin baseline concentrations and percent change in total bilirubin concentrations at exit (after multiple doses of efavirenz) relative to screening (before efavirenz administration) in healthy volunteers (Pearson $r = 0.04$; $P = 0.76$; n = 60). The percent changes were in part explained by intersubject differences in baseline total bilirubin because there was a significant correlation between baseline (screening) values and percent change at exit (Pearson $r = -0.43$; $P = 0.0006$). At screening, 16.6% of subjects had bilirubin values > 1 mg/L, whereas only 5% (n = 3) had values > 1 mg/dL at exit. Of note, 2 subjects with known Gilbert syndrome showed a marked decreased in total bilirubin at exit (–50% and –66.7%) compared with screening. This reduction was much greater than the average percent change from the rest of subjects, which was ~29%. Because bilirubin is a breakdown product of hemoglobin, correlations between bilirubin and hemoglobin levels were performed. As shown in **Figure 3**, no significant correlation was found between percent changes in hemoglobin versus percent change in bilirubin, suggesting that the effect of efavirenz on bilirubin concentration is independent of its possible effect on hemoglobin. Similarly, percent change in bilirubin did not correlate with percent change in red blood cell counts (Pearson $r = 0.18$ and $P = 0.17$) (data not shown).

As shown in **Figure 4**, total bilirubin serum concentrations in subjects who partially completed the study phases (ie, those who received 0 or up to 8 doses of efavirenz) compared with those who fully complete the study are displayed. As the number of efavirenz doses (days of treatment with efavirenz) increased, the extent of reduction in total bilirubin concentration tended to increase.

As shown in **Table I**, hemoglobin was significantly lower at exit (approximately 1 week after the last dose of efavirenz) compared with those values at screening (before initiation of efavirenz dosing). Hemoglobin was reported as a percentage due to the differences in standard normal values for men and women. The average (SD) percent change in hemoglobin was –8.6% (5.4%), with substantial between subject variability (5.4% to –20.6%). Baseline hemoglobin did not predict percent change (Pearson $r = -0.05$;

Table I
Demographic and clinical laboratory values before and 1 week after pretreatment with efavirenz (600 mg/d for 17 days) in healthy volunteers (n = 60).

Measure	Screening	Exit	% Change*	*P* value†
Sex (M/F)	38/22			
Race (white/black/other)	44/12/4			
	←————————————Mean (SD)————————————→			
Age (y)	28.1 (9.8)	-	-	
Weight (kg)	74.4 (14.0)	-	-	
Body mass index	24.2 (3.9)	-	-	
Sodium (mmol/L)	139.7 (2.1)	139.2 (1.7)	–0.38 (1.69)	0.079
Potassium (mmol/L)	3.88 (0.23)	3.74 (0.26)	–3.13 (7.9)	0.0017
Chloride (mmol/L)	103.9 (2.6)	105.3 (2.4)	1.40 (3.22)	0.0017
Carbon dioxide (mmol/L)	28.65 (1.78)	27.27 (1.89)	–4.65 (6.55)	< 0.0001
Blood urea nitrogen (mg/dL)	10.92 (4.0)	10.78 (3.29)	7.40 (38.42)	0.77
Anion (mmol/L)	7.22 (1.95)	6.66 (1.52)	–0.91 (38.42)	0.075
Creatinine (mg/dL)	0.92 (0.20)	0.87 (0.16)	–3.41 (15.78)	0.01
Calcium (mg/dL)	9.45 (0.31)	9.11 (0.33)	–3.5 (3.95)	< 0.0001
Albumin (g/dL)	4.12 (0.29)	3.86 (0.31)	–6.44 (7.62)	< 0.0001
Protein (g/dL)	7.11 (0.47)	6.61 (0.45)	–6.93 (7.47)	< 0.0001
Total bilirubin (mg/dL)	0.89 (0.42)	0.58 (0.22)	–29.93 (27.20)	< 0.0001
Alkaline phosphatase (units/L)	59.9 (14.9)	62.8 (18.2)	5.15 (13.73)	0.0125
Aspartate aminotransferase (units/L)	22.59 (4.53)	22.92 (8.17)	3.31 (26.67)	0.69
Alanine aminotransferase (units/L)	19.15 (7.28)	20.88 (11.75)	8.56 (32.78)	0.13
Hemoglobin (g/dL)	14.38 (1.09)	13.14 (1.22)	–8.6 (5.4)	< 0.0001
Red blood cell (million/cu mm)	4.73 (0.36)	4.30 (0.40)	–8.9 (5.2)	< 0.0001
Hematocrit (%)	41.93 (3.0)	38.23 (3.38)	–8.8 (5.6)	< 0.0001
White blood cell (k/cu mm)	6.21 (1.33)	5.60 (1.51)	–9.1 (17.6)	< 0.0001
Absolute neutrophil count (k/cu mm)	3.68 (1.14)	3.11 (1.13)	–13.9 (22.8)	< 0.0001
Mean corpuscular volume (fL)	89.0 (3.4)	89.0 (3.4)	0.03 (1.8)	0.94
Mean corpuscular hemoglobin (pg)	30.5 (1.2)	30.6 (1.3)	0.3 (2.1)	0.26
Mean corpuscular hemoglobin concentration (GM/dL)	34.3 (0.7)	34.4 (0.7)	0.2 (1.8)	0.43
Red blood cell distribution width (%)	13.0 (0.8)	13.1 (0.7)	0.3 (4.0)	0.79
Meran platelet volume (fL)	8.4 (0.8)	8.4 (0.8)	0.4 (4.1)	0.63
Platelet count (k/cumm)	254.9 (61.3)	243.6 (60.5)	–3.6 (13.2)	0.02
Lymphocytes (k/cumm)	1.83 (0.4)	1.82 (0.53)	1.9 (31.8)	0.92
Monocytes (k/cumm)	0.49 (0.15)	0.46 (0.17)	–4.1 (29.2)	0.08
Eosinophils (k/cumm)	0.21 (0.51)	0.17 (0.12)	19.5 (74.2)	0.59
Basophils (k/cumm)	0.010 (0.030)	0.013 (0.034)	–33.3 (51.6)	0.42

*Percent change at exit relative to screening.
†*P* value comparing screening and exit (2-tailed, paired Student *t* test). $P < 0.05$ was considered to be statistically significant.

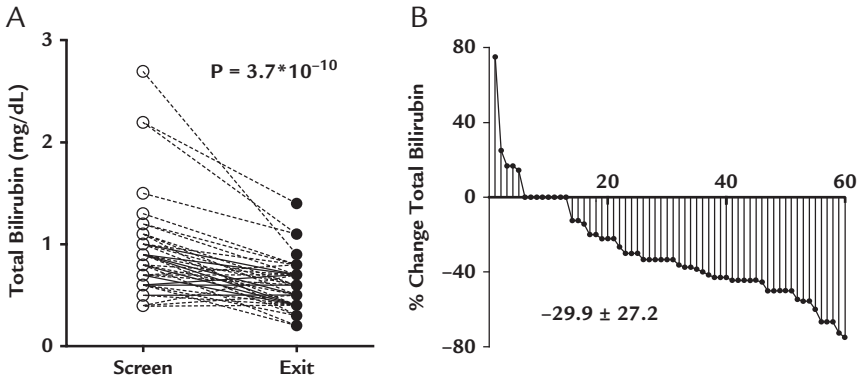

Figure 2. Effect of multiple doses (600 mg/d for 17 days) efavirenz on total bilirubin in healthy volunteers (n = 60). (A) Total bilirubin at screening (before efavirenz treatment) versus exit (1 week after the final efavirenz dose). (B) Percent change at exit relative to screening in individual subjects (average percent change [SD]).

$P = 0.69$). No subject was anemic at screening because hemoglobin count within normal range at screening was an inclusion criterion to participate in the study. Of 60 subjects who completed the entire study, 34 (56.7%) developed clinical anemia at exit, which was normocytic in each case, although subjects were asymptomatic on exit evaluation. Normal ranges for hemoglobin and hematocrit for men are 13.5 to 17.5 g/dL and 41% to 53%, respectively, whereas normal ranges for hemoglobin and hematocrit for women are 12 to 16 g/dL and 36% to 46%, respectively. Reductions in hemoglobin and hematocrit were highly significant ($P < 0.0001$) (**Table I**). As expected, red blood cell counts were significantly decreased at exit compared with screening; red blood cell indices, including mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red blood cell distribution width remained normal between screening and exit in all subjects (**Table I**).

Upon the recommendation of the data and safety monitoring board, any subject with hemoglobin levels < 12 g/dL at study exit was required to return to our clinical research center for a follow-up safety assessment. A total of 23 subjects had hemoglobin levels < 12 g/dL at study exit. None of these subjects were treated with iron. Of these, 15 subjects with evidence of anemia at exit failed to return for follow-up complete blood cell count. One subject was excluded because of incomplete data at exit. Only 7 subjects returned for follow-up and had complete blood cell analyses. **Supplemental Figure 1** shows slow recovery of hemoglobin during the follow-up period (1 month after exit) after discontinuation of efavirenz for the subjects (n = 7) who returned for repeat evaluation.

As shown in **Table I**, absolute neutrophil count (ANC) was significantly lower at exit than at screening (−13.9% [22.8%]). Whereas the majority of subjects (80%) showed decreased neutrophil count at exit (−3.2% to −54.6%), an increase was observed in 20% of subjects (2%–83%). Baseline ANC did not correlate with changes at exit (Pearson $r = -0.20$; $P = 0.12$). Reductions in total white blood cell and ANC between screening and exit were highly statistically significant ($P < 0.0001$). However these decreases in total white blood cell count and ANC were generally within the normal range in these healthy volunteers.

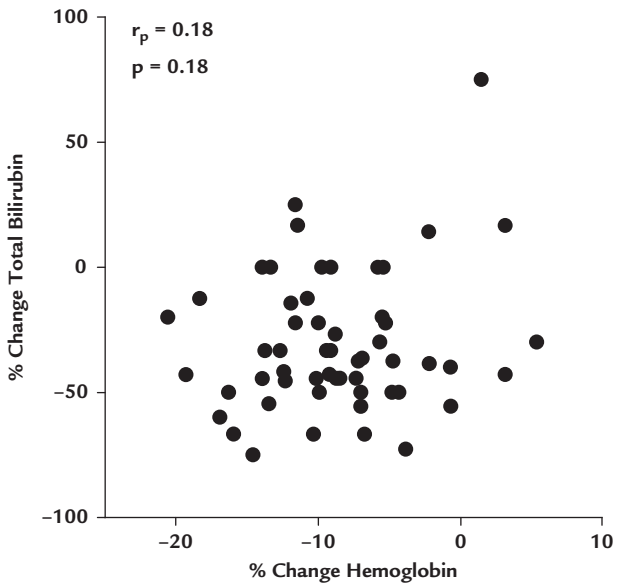

Figure 3. Pearson correlation between percent changes in hemoglobin concentrations and percent change in total bilirubin concentrations at exit (after multiple doses of efavirenz) relative to screening (before efavirenz administration) in healthy volunteers (n = 60).

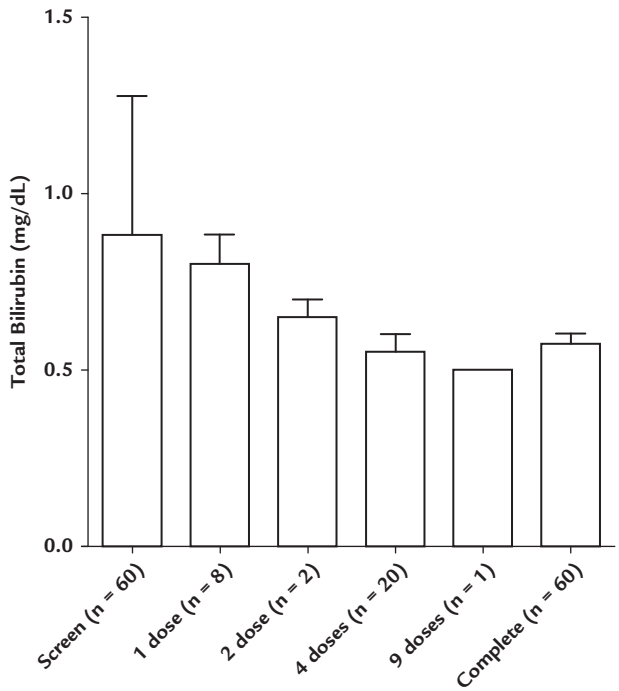

Figure 4. Total bilirubin concentrations in patients who partially completed the different phases of the study compared with those who fully completed the study and to the screening values. Subjects in the 1-dose group only completed Phase 1 (only 1 dose of efavirenz) and did not receive additional doses of efavirenz. Patients in the 2 to 9 doses groups discontinued at different stages during Phase 2 of the study; that is, after taking 1 (600 mg), 3 (600 mg/d for 3 days), and 8 (600 mg/d for 8 days) doses of efavirenz during Phase 2 at home.

Changes in other hematologic and serum electrolytes, protein, creatinine, and liver enzymes are listed in **Table I**. Marginal but statistically significant reduction in certain values was observed, whereas others showed no difference.

We explored whether efavirenz exposure predicts changes in the laboratory values tested above. Steady-state efavirenz AUC_{0-24} and the maximum efavirenz plasma concentrations did not correlate with any of the laboratory values measured. Also the percent change of efavirenz exposure (steady-state vs a single dose) did not correlate significantly with percent change of the laboratory values (exit vs screening) (**Supplemental Figure 2**).

Discussion

In our study, efavirenz monotherapy (600 mg/d) significantly lowered total plasma bilirubin levels at exit compared with screening values in healthy volunteers, independent of its effect on hemoglobin. Because exit labs were performed 1 week after the final dose of efavirenz, our data suggest a long offset effect likely due to the long half-life of efavirenz or the slow turnover of the endogenous compounds. These data are consistent with a recent study in a small number of healthy volunteers reporting that both total and conjugated bilirubin levels were reduced by efavirenz.[23]

To gain insight into the mechanisms by which efavirenz decreases bilirubin levels, it is important to briefly point out the complex hepatobiliary disposition of bilirubin. The disposition of bilirubin and its conjugated metabolites is complex, involving metabolizing enzymes and transporters.[24,25] Unconjugated bilirubin enters the hepatocytes via diffusion and/or transport where it undergoes glucuronidation by UGT1A1 to mono- and diglucuronides. Before eventually being secreted into the bile via primarily MRP2, a substantial fraction of these glucuronides are secreted into blood from the intracellular compartment and then undergoes reuptake in the cell from the blood by OATP1B1 and OATP1B3. This secretion and reuptake loop (ie, sinusoidal liver-to-blood shuttling loop or hepatocyte hopping) may prevent saturation of biliary excretion in the upstream hepatocytes, thereby ensuring efficient biliary elimination by MRP2.

It has been well documented that drugs known to activate constitutive androstane receptor (CAR) (eg, phenytoin and phenobarbital) and/or Pregnane X receptor (PXR) (eg, rifampicin and carbamazepine) upregulate the expression of proteins involved in bilirubin disposition, including UGT1A1 and MRP2,[26,27] and thus reduce serum bilirubin concentrations.[28,29] In fact, phenobarbital and rifampicin have been used in the past to treat hyperbilirubinemia.[29,30] Similarly, efavirenz is known to activate CAR[11,31] and possibly PXR,[32] and it is reasonable to suggest that efavirenz, like these PXR/CAR activators, interacts at ≥ 1 of these sites to enhance the elimination of bilirubin. Considering that bilirubin is mainly excreted in the bile after conjugation by UGT1A1,[33] one potential pathway by which efavirenz reduces bilirubin serum concentrations may be its ability to enhance UGT1A1-mediated glucuronidation of bilirubin into mono- and diglucuronides in the liver. Indeed, efavirenz-based therapy has been reported to reverse hyperbilirubinemia induction by indinavir and atazanavir, drugs that are known to inhibit UGT1A1 activity.[34] The possibility that reduced effective inhibitory concentrations (exposure) of these UGT1A1 inhibitor drugs by efavirenz may contribute to this observation cannot be ruled out given that efavirenz enhances the elimination of indinavir[35] and atazanavir.[36] However the ability of efavirenz to lower serum concentrations of bilirubin was demonstrated in the absence of these UGT1A1 inhibitors.[11,23] Of note, efavirenz has been reported to enhance the elimination of drugs that are substrates of UGT1A1, including dolutegravir.[37] The second possible mechanism by which rifampin decreases bilirubin concentrations may involve induction of MRP2, which has been shown to facilitate efflux transport of bilirubin glucuronides in to the bile.[38] Although only total bilirubin was measured in our study, there is evidence that efavirenz reduces the concentrations of bilirubin glucuronides,[23] supporting efavirenz-mediated efflux transport of the conjugate into the bile. Our data suggest that efavirenz enhances bilirubin elimination through induction of UGT1A1 and MRP2, although the contribution of other mechanisms (eg, inhibition of MRP3 or induction of uptake such as OATPs) cannot be fully excluded. Together our findings help explain reversal by efavirenz of hyperbilirubinemia induction by some protease inhibitor antiretroviral drugs (eg, indinavir and atazanavir). Efavirenz is expected to alter the pharmacokinetics of coadministered drugs through similar mechanisms (modulating UGT1A1 or drug transporters). Through activation of CAR and PXR,[11,31] two key regulators of drug and endobiotic disposition and genes, efavirenz, besides bilirubin, likely modulate the disposition of many endogenous compounds. For example, the use of efavirenz-based HIV therapy is associated with vitamin D deficiency and increased alkaline phosphatase,[12,13] and this effect is believed to be due to effect of efavirenz on 24-hydroxylase,[15] an enzyme responsible for the inactivation of 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D.[14] Complex drug interactions with efavirenz have been well documented.[6] Our data suggest that efavirenz may influence homeostasis through effects on the disposition of endogenous compounds.

According to the efavirenz package insert, different efavirenz-based therapies were reported to be associated with neutrophils $< 750/mm^3$ in 2% to 10% of subjects. In 1 case report, initiation of efavirenz-based therapy was reported to exacerbate HIV-related neutropenia, an effect that persisted for the entire time efavirenz was taken and resolved when efavirenz was discontinued.[20] In our study, efavirenz monotherapy significantly decreased ANC, consistent with the above-mentioned data. We also found substantial decrease in hemoglobin at exit compared with screening, with about 56% of subjects developing nonsymptomatic anemia. However, our data were obtained and analyzed without a control group of volunteers who took placebo instead of efavirenz. Although this anemia was observed 1 week after the intensive blood sampling during the final phase (Phase 3) inpatient visit, we cannot rule out the possibility that the low values of hemoglobin (and possibly cell counts) at exit may simply reflect blood drawn during the intensive pharmacokinetic sampling ($\sim$500 mL blood across 30 days).[39,40] Therefore our findings should be viewed as hypothesis generation that requires further validation in placebo-controlled settings, and, if found positive, the mechanisms involved need to be identified and the clinical significance in the target population (patients with HIV-1) established.

Conclusions

Multiple doses of efavirenz monotherapy reduce total bilirubin levels. These data suggest that efavirenz may influence disposition of coadministered drugs or endogenous compounds relevant in homeostasis through upregulation of genes involved in bilirubin metabolism and transport. Although anemia and decreased ANC were common during efavirenz monotherapy in our study, further study is needed to confirm whether these effects are due to blood loss resulting from intensive pharmacokinetic sampling or a direct adverse effect of efavirenz.

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Ingrid F. Metzger and Troy C. Quigg data analysis, data collection, data interpretation and manuscript writing. Abdulateef O. Aregbe data collection. Nancy Thong data collection and conducted the study. John T. Callaghan and Noam Epstein data collection and manuscript writing. Samir K. Gupta data analysis and manuscript writing. Anne T. Nguyen and Colleen K. Stevens data collection and data analysis. David A. Flockhart conducted the study and manuscript writing. Zeruesenay Desta developed study design, data analysis, data collection, data interpretation and manuscript writing.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Appendix

Supplementary data

Supplementary data associated with this article can be at http://dx.doi.org/10.1016/j.curtheres.2014.05.002.

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