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PHARMACOKINETICS AND DRUG DISPOSITION

Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans

Objective: **The capacity of the non-nucleoside reverse transcriptase inhibitor efavirenz to induce either liver CYP3A4 or intestinal CYP3A4, or both, as well as intestinal P-glycoprotein, was evaluated in healthy volunteers during and after a 10-day treatment course with two different daily doses.**
Methods: **Cohorts of 12 healthy subjects were randomized (2:1) to receive either efavirenz or placebo orally for 10 days. The first cohort received 200 mg efavirenz and the second cohort received 400 mg efavirenz daily. Liver CYP3A4 activity was evaluated on 9 different occasions with use of the erythromycin breath test (ERMBT). Intestinal biopsy specimens were obtained before the first dose of efavirenz and on the day after administration of the last dose to measure intestinal CYP3A4 and P-glycoprotein contents by immunoblotting. Efavirenz plasma levels were measured by HPLC, and pharmacokinetic parameters were determined by standard noncompartmental methods.**
Results: **Efavirenz significantly increased the mean ERMBT result in a dose- and time-dependent manner, with a 55% mean induction at 400 mg and a 33% mean induction at 200 mg ($P < .01$, compared with placebo for each treatment). The efavirenz AUC on day 10 correlated with the magnitude of induction (day 11/day 1 ERMBT ratio) when the two dose groups were combined ($r = 0.509$; $P = .04$). In contrast, efavirenz treatment had no detectable effect on intestinal CYP3A4 or P-glycoprotein.**
Conclusions: **Efavirenz is an inducer of liver CYP3A4 in healthy volunteers, and interpatient differences in magnitude of induction is partly explained by variation in systemic drug exposure. However, efavirenz did not appear to induce intestinal CYP3A4 or intestinal P-glycoprotein. These results suggest that drug interactions caused by induction of CYP3A4 can be liver specific. (Clin Pharmacol Ther 2002;72:1-9.)**

Stéphane Mouly, MD, Kenneth S. Lown, MD, David Kornhauser, MD, Jeffrey L. Joseph, MS, William D. Fiske, PhD, Irma H. Benedek, PhD, and Paul B. Watkins, MD *Chapel Hill, NC, Ann Arbor, Mich, and Wilmington, Del*

Efavirenz [(*S*)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one] is an extremely potent non-nucleoside reverse transcriptase inhibitor that is widely used in the treatment of patients with human immunodeficiency virus-1 (HIV-1) infection. It is essentially completely metabolized in vivo and, in humans, 66% of the dose at 400 mg/day (5.7 mg/kg/day) has been recovered in urine as the glucuronide conjugate of the 8-hydroxymetabolite (Bristol-Myers Squibb, unpublished data, July 2000). Both CYP3A4 and CYP2B6 have been implicated in the 8-hydroxylation pathway by studies performed in vitro (Bristol-Myers Squibb, unpublished data, July 2000). CYP3A4 is usually the major cytochrome P450 present in both liver and intestine in humans.[1] CYP2B6 is present in the liver but has not been reported to be present in the intestine.[2]

During initial preclinical studies, efavirenz was noted to induce its own metabolism and to increase the activities of liver CYP3A and CYP2B isoforms in rats and rhesus monkeys (Bristol-Myers Squibb, unpublished data, July 2000). Clinical observations also make it likely that efavirenz induces CYP3A4 in humans. Efavirenz has been shown to induce the metabolism of methadone, a CYP3A4 substrate, in patients with HIV infection, leading to a 60% decrease in methadone systemic exposure and methadone withdrawal symptoms.[3,4] In addition, efavirenz treatment results in a decrease in the blood levels of indinavir, which is consistent with CYP3A4 induction.[5]

Induction of CYP3A4 by most drugs appears to involve binding to a receptor termed *hPXR* (also termed *SXR* or *PAR*), resulting in transcriptional activation of the *CYP3A4* gene.[6] The hPXR receptor also appears to mediate induction of P-glycoprotein (*MDR1* gene product), a xenobiotic transport protein present in liver and intestine involved in some drug interactions,[7] and CYP2B6.[8] The hPXR receptor has been shown to be present in enterocytes and presumably accounts for induction of intestinal CYP3A4 and P-glycoprotein when patients receive treatment with at least some inducers of liver CYP3A4.[7]

If induction of CYP3A4 by efavirenz does occur and is mediated by hPXR, we believed that efavirenz would likely induce CYP3A4 (and P-glycoprotein) in both the liver and small intestine in vivo. To test this hypothesis, efavirenz at 2 dose levels or placebo was administered to 24 healthy adults for 10 days. Liver CYP3A4 activity was measured on multiple occasions with use of the ^{14}C-erythromycin breath test (^{14}C-ERMBT).[9] Intestinal CYP3A4 and P-glycoprotein expression (in small intestinal biopsy specimens) before and after a 10-day drug treatment was also measured. As a secondary aim, we examined whether variation in liver CYP3A4, intestinal CYP3A4, or intestinal P-glycoprotein levels correlated with variations in pharmacokinetic parameters of systemic exposure to efavirenz.

METHODS

Subjects

Eligible subjects were nonsmokers between 18 and 50 years old and were within 15% of their ideal body weights. No subjects had taken any prescription or over-the-counter medications for 30 days, and they had not consumed grapefruit or grapefruit juice within 1 week before the start of the study. No subjects had any significant medical problems or laboratory test abnormalities. All subjects were screened for medical problems by history and physical examination, 12-lead electrocardiogram, hepatitis B, C, and A antibodies, HIV antibodies, urine drug screen, complete blood count, serum electrolytes, liver chemistries, and prothrombin time.

Experimental design

This was a single-center, double-blind, randomized, placebo-controlled study approved by the University of Michigan Institutional Review Board (Ann Arbor, Mich). The study required 2 separate admissions to the General Clinical Research Center. The first admission was for approximately 36 hours (2 nights) and occurred the day before the start of the study (day 0). The subjects were entered in 2 cohorts of at least 12 subjects each. Subjects were randomized within each cohort to receive either efavirenz or placebo in a 2:1 ratio, respectively. The dose levels of efavirenz studied were 200 mg (cohort 1) and 400 mg (cohort 2). The 200-mg dose was studied first and the data analyzed before we proceeded with the 400-mg daily cohort. Within each

From the General Clinical Research Center, University of North Carolina, Chapel Hill; General Clinical Research Center, University of Michigan, Ann Arbor; and Bristol-Myers Squibb, Wilmington.

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Reprint requests: Paul Brent Watkins, MD, General Clinical Research Center, University of North Carolina, School of Medicine, Campus Box 7600, Room 3005 APCF, Chapel Hill, NC 27599.

E-mail: *pbwatkins@med.unc.edu*

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cohort, 8 subjects were randomized to receive efavirenz and 4 subjects were randomized to receive matching placebo. The administration of any concomitant medication was prohibited during the entire study, starting 2 weeks before day 0 and extending to the end of the subject's participation (day 32). Alcohol- and caffeine-containing foods and beverages were prohibited beginning 48 hours before dose administration on day 1 and day 10 and throughout each in-hospital period. Subjects were to refrain from excess physical exertion throughout the study duration and from lying down during the first 5 hours after drug intake on day 1 and day 10.

After an initial screening visit, subjects were admitted to the General Clinical Research Center on day 0 of the study. They underwent endoscopy to obtain biopsy samples from the small bowel for measurements of CYP3A4 and P-glycoprotein protein concentrations at baseline (day 0) and again after 10 days of drug ingestion (day 11). Subjects then began treatment with placebo, 200 mg/day efavirenz, or 400 mg/day efavirenz for 10 days (day 1 to day 10 of the study), receiving each dose (efavirenz or placebo) with 240 mL water. An indwelling venous catheter was placed in a vein of an arm of each subject for collecting blood samples and administration of the intravenous ERMBT. After an 8-hour overnight fast, 5 mL blood was drawn (day 1 and day 10) before dosing and at 2, 3, 4, 5, 8, 12, and 24 hours after the first and last dose of the study drug for determination of efavirenz oral pharmacokinetics. The volunteers were discharged after they took their second doses on day 2 of the study. Additional blood samples were drawn on days 4 and 7. Liver CYP3A4 activity was measured with the ERMBT before the first dose of the drug on day 1 (baseline) and again on days 2, 4, 7, 11 (after the 24-hour blood sample), 12, 15, 18, and 32 of the study. The subjects were readmitted on day 9 for the 24-hour pharmacokinetic study with the final dose of efavirenz (according to the same design as day 1) and for repeated performance of small bowel biopsies on day 11. Subjects were discharged from the General Clinical Research Center the following morning (day 12). They returned to the outpatient clinic on days 15 (120 hours after dosing), 18 (192 hours after dosing), and 32 (528 hours after dosing) to receive the scheduled ERMBT.

Pharmacokinetic analysis

Plasma was separated from blood by centrifugation at 3000 rpm for 15 minutes and then transferred to labeled polypropylene cryotubes and stored at −20°C pending analysis. Efavirenz plasma concentrations were determined by HPLC with ultraviolet detection, using minor modifications to a previously validated and published method.[10] The assay had a limit of detection of 0.05 μmol/L. Quality control samples at different concentrations of efavirenz had coefficients of variation for precision and accuracy of <10% for all concentrations examined. The peak plasma concentration (C_{max}), time to reach C_{max} (t_{max}), and trough plasma level (C_{min}) were determined directly from the plasma concentration versus time data. Total area under the observed plasma concentration–time curve (AUC) was calculated by use of the linear trapezoidal method. The apparent oral clearance (CL/F) was calculated by dividing the efavirenz dose by the AUC. The terminal elimination rate constant (λn) was determined by the slope of the log-linear terminal phase of the curve (ie, last 3 concentration–time points). The drug half-life ($t_{1/2}$) was calculated as λn/0.693.

Erythromycin breath test

The ERMBT was administered as described previously.[9] In brief, a baseline breath sample was collected, 3 μCi (0.074 mmol, 0.0543 mg) of [^{14}C-*N*-methyl]-erythromycin (Metabolic Solutions Inc, Nashua, NH) was injected intravenously, and a single breath collection was made 20 minutes later. Breath test results were expressed as the percentage of administered ^{14}C that was exhaled during the first hour after the injection of erythromycin, estimated from the rate of $^{14}CO_2$ exhalation at 20 minutes as recently modified.[11]

Intestinal CYP3A4 and P-glycoprotein measurements

Endoscopy. Each subject underwent upper intestinal endoscopy after a 4-hour postbreakfast fast. While under conscious sedation induced by a combination of intravenous midazolam and meperidine (INN, pethidine), a video-endoscope was passed into the second portion of the duodenum where 5 mucosal biopsy specimens were obtained. The biopsy specimens from each set were immediately placed in an ice-cold solution of 0.05 mol/L Tris hydrochloride, 20% glycerol, 2 mmol/L ethylenediaminetetraacetic acid, and 1 mmol/L phenylethylsulfonyl fluoride, homogenized in a glass dounce, and snap frozen in liquid nitrogen. The samples were stored at −80°C pending analysis.

CYP3A4, CYP2B6, and P-glycoprotein immunoblotting. Protein concentrations were determined by immunoblotting as previously described,[12-15] with minor modifications. Proteins (15 μg of the whole biopsy homogenate) were separated with use of a 7% polyacrilamide/0.1% sodium dodecyl sulfate gel and transferred to nitrocellulose. The blots were blocked for 1

hour in Tris-buffered saline solution that contained 0.3% Tween 20 (Sigma Chemical Co, St Louis, Mo) and Carnation 5% nonfat dry milk (Nestlé, Vevey, Switzerland). After all antibody incubations were completed, the blots were developed with a chemiluminescence kit. Optical densities were converted to quantitative numbers by comparison to slot blots of serial dilutions of purified CYP3A4 protein that had been processed simultaneously with the immunoblots. To obtain accurate measures of P-glycoprotein and CYP3A4, immunoblots were repeated a total of 4 to 7 times. Final values were calculated from the arithmetic mean of all of the runs, with any outlying points (greater than 2 standard deviations from the mean for that sample) omitted. Biopsy levels of CYP3A4 and P-glycoprotein were expressed as a ratio with the villin level of the same sample (ie, CYP3A4/villin and P-glycoprotein/villin), as described previously.[12-15] For the sake of simplicity, we have termed the ratios *enterocyte concentrations*. The mean coefficients of variation for the enterocyte concentrations of CYP3A4 and P-glycoprotein were 27% (SD, 17%) and 38% (SD, 17%), respectively.

The intestinal homogenates were also blotted for CYP2B6 with the method described earlier, except that separation was performed with 9% polyacrylamide instead of 7% polyacrylamide. The filters were then incubated with WB-2B6-PEP, a rabbit immunoglobulin G monoclonal primary antibody specific for human CYP2B6 proteins (Gentest Corporation, Woburn, Mass), and goat anti-rabbit secondary antibodies conjugated with peroxidase (Zymed, South San Francisco, Calif). Optical densities were converted to quantitative numbers by comparison to slot blots of serial dilutions of purified CYP2B6 protein that had been processed simultaneously with the immunoblots.

Statistical analysis

Statistical analysis of the data was performed with SAS software (version 6.12, SAS Institute Inc, Cary, NC). A sample size of 8 subjects for each treatment group was used to have a greater than 80% power to detect a 50% difference in the ERMBT results (with $\alpha = .05$ as the level of significance), assuming a standard deviation of 25% and using a 2-sided paired *t* test. Values for efavirenz AUC, C_{max}, C_{min}, and dose were log-transformed to normalize their distribution for statistical analysis. ERMBT differences among the 3 treatments were assessed with use of a 1-way ANOVA model at each scheduled assessment time. Pairwise differences between treatments were assessed with the Fisher least significant difference. A repeated-measure ANOVA was performed to assess differences among treatments over time. To determine whether an ordered difference among treatment groups was present, the Jonckheere-Terpstra test, a nonparametric test, was performed. Intestinal biopsy measurements of CYP3A4 and P-glycoprotein were analyzed analogously to the ERMBT results without a repeated-measure analysis. The relationships between efavirenz CL/F, intestinal CYP3A4 content, intestinal P-glycoprotein content, and hepatic CYP3A4 activity (ERMBT) were examined with use of Spearman's correlation coefficient.

RESULTS

Demographic characteristics

Twenty-four healthy volunteers (1 woman and 23 men) completed the study; 1 subject was discontinued because of a protocol violation. Twenty-one (84%) subjects were white, 1 (4.0%) was black, 2 (8%) were Asian or Pacific islanders, and 1 (4%) was Hispanic. Mean age of the subjects was 29.9 ± 8.7 years (median, 27 years; age range, 19 to 49 years), with no difference between groups. Eight subjects received 200 mg/day efavirenz, 8 received 400 mg/day efavirenz, and 8 received placebo (4 in cohort 1 and 4 other in cohort 2).

Pharmacokinetics

Concentration versus time profiles of the 2 efavirenz dosing regimens on day 1 and day 10 are presented in Fig 1. Table I summarizes the main pharmacokinetics parameters for the 2 dosing regimens on day 1 and day 10. The mean C_{max}, C_{min}, and AUC, were significantly greater ($P < .05$) in the higher-dose group than in the low-dose group. However, CL/F and $t_{1/2}$ values were not significantly different between the 2 dose regimens.

Induction of liver CYP3A4 by efavirenz

Hepatic CYP3A4 activity (ie, ERMBT) was significantly increased ($P < .05$) by efavirenz compared with placebo. Induction of liver CYP3A4 was significantly higher ($P < .05$) with the 400-mg dosing regimen than with the 200-mg dosing regimen on day 7 and from day 12 to day 32 (Table II). In the higher-dose group, induction occurred as early as day 4, and dissipation of induction was observed by day 32 (400-mg dose group), 21 days after the last efavirenz dose. In the 200-mg group, the induction of liver CYP3A4 by efavirenz was evident on day 11 and had dissipated on day 18. In addition, there was a significant ordered difference ($P < .05$) between the treatment groups (higher ERMBT ratio associated with higher efavirenz dose level) for days 4 to 18 (Table II). Taken together, these results indicate a time- and dose-dependent induction of

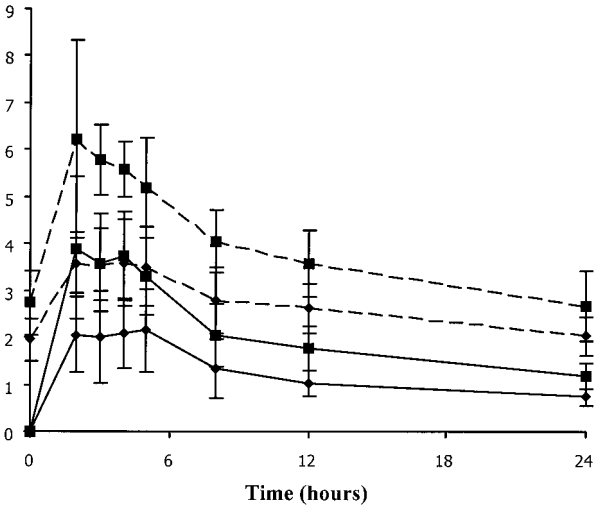

Fig 1. Concentration versus time profiles of efavirenz after single doses of 200 mg *(diamonds)* and 400 mg *(squares)* on day 1 *(solid lines)* and on day 10 *(broken lines). Error bars* represent the standard deviation (n = 8 in each group and for each point).

liver CYP3A4 activity by efavirenz, as assessed by the ERMBT.

Effects of efavirenz on intestinal variables

Enterocyte concentrations of P-glycoprotein and CYP3A4 on day 0 (before the first dose of efavirenz) and day 11 (24 hours after the last dose of efavirenz), as assessed by immunoblots of the biopsy homogenates, are presented in Fig 2, *A* and *B*, respectively. The geometric mean (ratio) of enterocyte CYP3A4 concentrations on day 11 and day 0 was 1.130 (95% confidence interval [CI], 0.865 to 1.476) for the placebo group, 1.124 (95% CI, 0.899 to 1.405) for the 200-mg efavirenz group, and 1.016 (95% CI, 0.840 to 1.228) for the 400-mg efavirenz group. Likewise, the geometric mean (ratio) of enterocyte P-glycoprotein concentrations on day 11 and day 0 was 1.138 (95% CI, 0.895 to 1.447) for the placebo group, 1.145 (95% CI, 0.888 to 1.476) for the 200-mg efavirenz group, and 1.057 (95% CI, 0.893 to 1.250) for the 400-mg efavirenz group. None of the pairwise differences between treatment groups were significant for either enterocyte concentration of CYP3A4 or enterocyte concentration of P-glycoprotein. The Jonckheere-Terpstra test did not show a significant ordered difference between the treatment groups for either enterocyte concentration of CYP3A4 or P-glycoprotein.

When all subjects were analyzed together, there was a positive correlation between enterocyte concentrations of CYP3A4 determined on day 0 and the concentration determined on day 11 ($r = 0.615$; $P < .001$; Spearman rank correlation). Likewise, there was a positive correlation between enterocyte concentrations of P-glycoprotein determined on day 0 and day 10 ($r = 0.54$; $P < .01$; Spearman rank correlation). As expected,[13,14] the ERMBT result on day 1 was not correlated to either intestinal CYP3A4 or P-glycoprotein concentrations on day 0 ($r_S = -0.17$ and P = NS for CYP3A4 and $r_S = 0.01$ and P = NS for P-glycoprotein; all patients combined). The ERMBT result on day 11 was also not correlated to either intestinal CYP3A4 or P-glycoprotein concentrations on day 11 ($r_S = -0.15$ and P = NS for CYP3A4 and $r_S = -0.017$ and P = NS for P-glycoprotein; all patients combined). Efavirenz CL/F was not correlated to enterocyte concentrations of either CYP3A4 or P-glycoprotein (data not shown).

The intestinal biopsy homogenates were also probed for immunoreactive CYP2B6 because this enzyme has been implicated in efavirenz metabolism and because CYP2B isoforms are induced in the livers of animals treated with efavirenz (Bristol-Myers Squibb, unpublished data, July, 2000). As shown in Fig 3, no CYP2B6 could be detected in human intestine either before or after drug treatment. The lower limit of detection was estimated to be 100 fmol of CYP2B6 protein per 20 μg of homogenate loaded.

Relationship between efavirenz pharmacokinetic parameters and liver CYP3A4 activity

Because CYP3A4 was believed to be the major pathway of efavirenz metabolism when this study was conducted, the relationship between efavirenz pharmacokinetic parameters and the ERMBT results was examined. There was a trend for an inverse relationship between efavirenz steady-state CL/F (day 10) and ERMBT result (day 11) within each treatment group or when all subjects were examined ($r = -0.491$ and $P = .05$ for all subjects). That is, the lower the efavirenz CL/F, the higher the liver CYP3A4 activity (high ERMBT value), contrary to expectation (see the "Discussion" section). In addition, the efavirenz AUC (day 10) was positively correlated ($r = 0.509$ and $P = .04$ for all subjects) with the ERMBT ratio (day 11/day 1; Fig 4). That is, the magnitude of liver CYP3A4 induction by efavirenz was related to the steady-state systemic exposure (ie, AUC) of efavirenz when all subjects were examined. However, this correlation was not significant for each group taken individually (data not shown).

Table I. Pharmacokinetic parameters on days 1 and 10 and the day 10/day 1 ratio after dosing regimens of 200 mg and 400 mg efavirenz (n = 8 subjects in each study group)

Pharmacokinetic parameter	*Day 1**	*Day 10**
C_{max} (μmol/L)		
Efavirenz, 200 mg	2.76 ± 0.78	4.00 ± 0.71†
Efavirenz, 400 mg	4.43 ± 1.02‡	6.98 ± 1.47†‡
C_{min} (μmol/L)		
Efavirenz, 200 mg	0.73 ± 0.18	2.03 ± 0.43†
Efavirenz, 400 mg	1.19 ± 0.28	2.67 ± 0.74†‡
AUC(0–24h) (μmol/L · h)		
Efavirenz, 200 mg	28.79 ± 6.47	64.08 ± 13.25†
Efavirenz, 400 mg	48.10 ± 11.40‡	92.59 ± 17.23†‡
t_{max} (h)§		
Efavirenz, 200 mg	3 (2–5)	3 (2–5)
Efavirenz, 400 mg	2.6 (2–5)	2.5 (2–5)
$t_{1/2}$ (h)		
Efavirenz, 200 mg	—	67.2 ± 44.0
Efavirenz, 400 mg	—	77.9 ± 29.4
CL/F (L/h)		
Efavirenz, 200 mg	—	10.25 ± 1.98
Efavirenz, 400 mg	—	14.15 ± 2.93

C_{max}, Peak plasma concentration; C_{min} trough plasma level; t_{max}, time to reach C_{max}; AUC(0–24h), area under the observed plasma concentration–time curve from 0 to 24 hours; $t_{1/2}$, half-life; CL/F, oral clearance. Statistics are presented on nontransformed data, but data were log-transformed for analysis of C_{max}, C_{min} and AUC.
*Harmonic mean and pseudo standard deviation.
†Indicates a significant difference ($P < .05$) compared with day 1.
‡Indicates a significant difference ($P < .05$) compared with the 200-mg dose.
§Summarized with use of the median with minimum and maximum in parentheses.

Table II. Geometric mean ratio and 95% CI of the ERMBT (study day to baseline [ie, day 1] ratio) for the placebo group and each efavirenz dosing regimen (n = 8 subjects in each study group)

	Placebo		*Efavirenz, 200 mg*		*Efavirenz, 400 mg*	
Study day	*Geometric mean*	*95% CI*	*Geometric mean*	*95% CI*	*Geometric mean*	*95% CI*
Day 2	1.025	0.890–1.180	1.102	0.999–1.215	1.078	0.987–1.178
Day 4	1.161	1.040–1.296	1.210	1.048–1.397	1.381*†	1.174–1.625
Day 7	1.033	0.898–1.189	1.186	1.062–1.324	1.493*†‡	1.272–1.752
Day 11	0.993	0.842–1.171	1.327*	1.127–1.563	1.546*†	1.370–1.744
Day 12	0.996	0.906–1.096	1.314*	1.186–1.456	1.550*†‡	1.339–1.795
Day 15	0.958	0.848–1.082	1.161*	0.987–1.366	1.373*†‡	1.214–1.553
Day 18	0.992	0.863–1.139	1.077	0.921–1.258	1.287*†‡	1.166–1.422
Day 32	1.024	0.916–1.144	0.909	0.769–1.075	1.069‡	0.962–1.187

95% CI, 95% Confidence interval; ERMBT, ^{14}C-erythromycin breath test.
Differences among (1-way ANOVA) and between (Fisher least significant difference) dose groups were analyzed with use of the natural log-transformation of the ratio.
Note: Statistics are presented on non-transformed data but data were log-transformed for analysis of C_{max}, C_{min} and AUC.
*Indicates a significant difference ($P < .05$) compared with the placebo group.
†According to the Jonckheere-Terpstra test, statistically significant ordered difference between the dose groups (higher ERMBT ratio associated with increasing efavirenz dose level).
‡Indicates a significant difference ($P < .05$) compared with the 200 mg efavirenz dose group.

DISCUSSION

This study was performed primarily to determine whether efavirenz is an inducer of liver CYP3A4 in humans in vivo and to characterize the time course of this induction. We also wanted to determine whether induction of liver CYP3A4 was accompanied by induction of intestinal CYP3A4 and P-glycoprotein, which we expected if liver CYP3A4 induction by efavirenz were mediated by hPXR.

Hepatic CYP3A4 activity, as measured by serial ERMBTs, was clearly induced by efavirenz administration (Table II). The magnitude of induction was

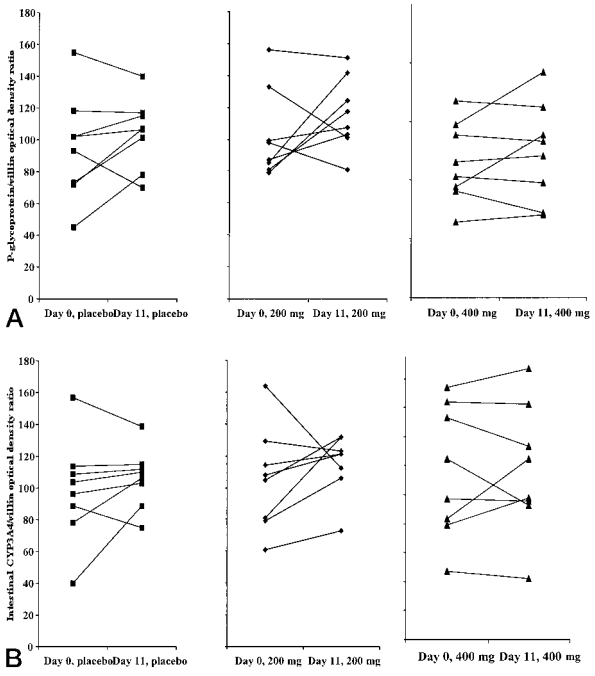

Fig 2. Intestinal content of P-glycoprotein **(A)** and CYP3A4 **(B)** on days 0 and 11 after administration of either placebo *(squares)*, 200 mg efavirenz *(diamonds)*, or 400 mg efavirenz *(triangles)* once a day for 10 days.

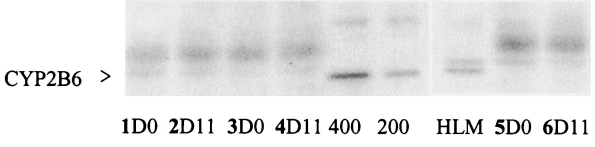

Fig 3. Representative immunoblot of CYP2B6 in intestinal homogenates. Biopsy specimens of 1 subject in each study group are shown before the first dose (D0) and after the last dose (D11) of 200 mg efavirenz (lanes 1 and 2), 400 mg efavirenz (lanes 3 and 4), and placebo (lanes 5 and 6). Two concentrations of purified CYP2B6 protein (in femtomoles) and a positive control (human liver microsome [HLM]) were analyzed at the same time.

greater in the 400-mg treatment group than in the 200-mg treatment group, and it was time dependent. The time-dependent changes in CYP3A4 activity probably largely mirrored the changes in systemic exposure to the drug, given its mean $t_{1/2}$ of 60 hours. Peak systemic exposure (steady-state plasma levels) would have been attained at around 10 days (5 $t_{1/2}$ values), the observed timing of peak induction. The dissipation in induction also followed a time course consistent with

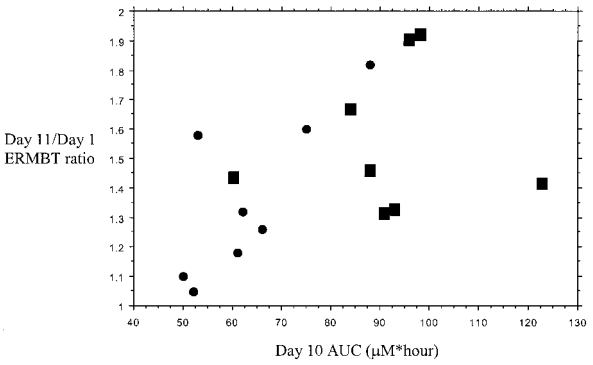

Fig 4. Relationship between efavirenz area under the observed plasma concentration–time curve (AUC) on day 10 and hepatic CYP3A4 induction on day 11 (day 11/day 1 erythromycin breath test [ERMBT] ratio). Individual nontransformed data for the 200-mg efavirenz group *(circles)* and the 400-mg efavirenz group *(squares)* are presented ($r = 0.51$ and $P = .04$ for both treatment groups combined).

the expected decrease in plasma levels. It should be noted that the magnitude of the mean change in the ERMBT result (55%) with the 400-mg dose was less than that observed with rifampin (INN, rifampicin) treatment (up to 125%),[10,16] indicating that the magnitude of induction produced by efavirenz is less.

We also found that subjects with the highest efavirenz AUC values tended to have the highest magnitudes of induction of CYP3A4 from baseline (Fig 4). This supports the reasonable conclusion that the magnitude of induction within an individual reflects in part the integrated exposure of the liver of that individual to the drug. This reinforces the importance of measurement of parameters of systemic exposure in future studies that examine potential genetic factors underlying inducer responsiveness. Because systemic exposure positively correlated with the ERMBT result, there was an inverse correlation between hepatic CYP3A4 activity and efavirenz clearance. If CYP3A4 represented a major pathway for efavirenz elimination (as was believed when these studies were undertaken), one would expect a positive correlation between liver CYP3A4 activity and drug clearance. Our opposite finding is therefore not compatible with the ability of liver CYP3A4 to have a rate-limiting effect in the elimination of efavirenz. It now seems to be likely that CYP2B6 may represent the more important pathway for elimination of this drug in humans. We also reasoned that if intestinal metabolism or transport limited efavirenz oral availability, there may exist inverse cor-

relations between enterocyte content of CYP3A4 or P-glycoprotein and systemic exposure of efavirenz. We and other researchers have used the same immunoblotting techniques to successfully detect such correlations for other CYP3A4 and P-glycoprotein substrates.[12,14,17] However, we were unable to find correlations between enterocyte levels of CYP3A4 or P-glycoprotein and parameters of systemic exposure to efavirenz.

In contrast to the substantial liver induction observed, there was no evidence for induction of intestinal CYP3A4 or intestinal P-glycoprotein in our subjects. This was unexpected because hPXR is present in both liver and intestine.[6,7] One possibility is that the increased activity of liver CYP3A4 observed resulted from posttranscriptional modification or activation of the hepatic enzyme and that the same phenomenon, which might not be detected by our protein measurements, could be occurring in the intestine. However, increases in P450 activity on this basis are unusual (with only 1 example in vivo in any species to our knowledge[18]), and efavirenz causes induction of CYP3A immunoreactive protein in animal liver (Bristol-Myers Squibb, unpublished data, July 2000). It is also possible that some induction of CYP3A4 and P-glycoprotein occurred in the intestine but was below our level of detection. However, we have previously used this same Western blot technique to demonstrate significant induction of intestinal CYP3A4 and P-glycoprotein (but not the closely related CYP3A5) in 5 adults treated with rifampin for just 2 days.[19] In addition, the 95% CI values for the day 10/day 1 ratios of enterocyte contents of CYP3A4 and P-glycoprotein were essentially identical for both treatments and placebo. In the high-dose group, the highest induction of either protein that would fall within the 95% CI is 25% above baseline (ie, greater than 25% induction was excluded with 95% confidence). It should also be noted that other investigators have concluded that the magnitude of intestinal CYP3A4 induction is greater than liver induction after oral administration of rifampin.[20] It therefore seems to be likely that efavirenz produced essentially liver-specific induction in our subjects.

The basis for discrepancy between intestine and liver responsiveness to induction is unclear, but we speculate that it may be related to a relatively short duration of exposure of the enterocyte to efavirenz during oral administration. The drug has a relatively fast absorption (t_{max} of 2 to 3 hours), it is given just once each day, and the parent drug does not appear to undergo biliary excretion.[9,21] Therefore the total duration of exposure of the enterocyte to parent efavirenz during treatment may be insufficient to cause a significant increase in gene transcription. Alternatively, the mechanism for induction of hepatic CYP3A4 in liver may involve novel mechanisms not present in the intestine.

We also anticipated that CYP2B6 may be inducible by efavirenz because CYP2B induction has been observed in animals and CYP2B6 induction can also be mediated in human liver by PXR.[8] However, we failed to detect any CYP2B6 in the small intestinal homogenates, either before or after efavirenz treatment (Fig 3). Because there is currently no validated probe for liver CYP2B6 activity, we could not determine whether efavirenz induced CYP2B6 in the livers of our subjects.

It should be noted that the relative enterocyte concentrations of CYP3A4 and P-glycoprotein of each subject remained largely unchanged during the 10-day interval between intestinal biopsies. We have previously shown that there is considerable intraindividual variation in enterocyte content of CYP3A4 in subjects maintained for 5 days on a fruit- and vegetable-free diet[12,15] and that dramatic falls in enterocyte CYP3A4 occur when subjects drink even a single glass of grapefruit juice.[12,22] This has raised the concern that enterocyte CYP3A4 levels may be so exquisitely sensitive to diet that certain studies may require inpatient hospitalization and strict dietary control. Our observations in the current study suggest that volunteers allowed to maintain their usual dietary habits as outpatients may have relatively little intrasubject variation in enterocyte levels of CYP3A4 and P-glycoprotein. Consistent with this conclusion is the observation that the magnitude of interaction between grapefruit juice and felodipine, which varies markedly between subjects and correlates with enterocyte CYP3A4 content,[12] remained relatively constant within subjects over several weeks in an outpatient setting.[23]

It is now well established that the best therapeutic strategy to treat HIV-1 infection is to combine agents with different mechanisms of action and with different dose-limiting toxicities. Such “highly active” combination therapy can increase the potential for marked drug-drug interactions to occur because most of the therapeutic agents used are substrates or modulators of liver and intestinal CYP3A4 and P-glycoprotein.[24-26] The observation that efavirenz induces CYP3A4 primarily in the liver may mean that its potential to reduce systemic exposure to high first-pass drugs is less than other inducers, such as rifampin, that induce in both the liver and the intestine.[17]

In conclusion, efavirenz is an inducer of liver CYP3A4 and the magnitude of induction appears to be a function of dose, duration of treatment, and systemic exposure to parent drug. The ability to serially follow

hepatic CYP3A4 induction at multiple time points represents one useful aspect of the ERMBT. However, efavirenz does not appear to induce intestinal CYP3A4 and P-glycoprotein, and we speculate that this may reflect a limited duration of exposure of the enterocyte to efavirenz.

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