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Mechanism of efavirenz influence on methadone pharmacokinetics and pharmacodynamics II: Hepatic and intestinal CYP2B6, CYP3A and transporter activities

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Abstract

Efavirenz diminishes methadone plasma concentrations, an effect attributed to CYP3A induction, but actual mechanisms are unknown. This investigation determined the effects of two weeks of efavirenz (600 mg daily) on hepatic and intestinal CYP3A4/5 (probed with intravenous and oral alfentanil), hepatic CYP2B6 (oral efavirenz hydroxylation) and intestinal transporter (oral fexofenadine) activities, and on methadone pharmacokinetics and pharmacodynamics in healthy volunteers. It also assessed efavirenz effects on CYP expression and activity in human hepatocytes. Efavirenz significantly induced systemic and oral alfentanil clearance 2- to 5-fold, increased alfentanil hepatic and intestinal extraction ratios, and significantly induced apparent 8-hydroxyefavirenz formation clearance. Efavirenz also moderately decreased fexofenadine plasma concentrations, suggesting decreased intestinal uptake and/or increased P-glycoprotein-mediated efflux. Efavirenz induced CYP2B6 and CYP3A4 expression, activity, and methadone metabolism in human hepatocytes. Efavirenz coinduces hepatic CYP2B6 and CYP3A4/5 activities, coinduces hepatic and intestinal CYP3A4/5, and coinduces gastrointestinal CYP3A and xenobiotic efflux transporters.

Keywords

cytochrome P450 3A; CYP3A; cytochrome P450 2B6; CYP2B6; efavirenz; methadone; alfentanil; in vivo probe; phenotyping

Efavirenz is a first-generation non-nucleoside reverse transcriptase inhibitor (NNRTI), used for over a decade as an essential component of highly-active antiretroviral therapy.[1,2] Efavirenz is a first-line drug used worldwide in the treatment of HIV and postexposure prophylaxis, and the preferred NNRTI. It is recommended in the United States as a third antiretroviral added to an initial two-drug regimen,[3] and in the United Kingdom as initial

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therapy.[4] Efavirenz is one of three drugs coformulated together (with emtricitabine and tenofovir), which comprises the most popular antiretroviral regimen due to the convenience of a single pill taken once daily and resultantly high treatment adherence.

Efavirenz is a well-known victim and perpetrator of drug-drug interactions. It is extensively metabolized, with cytochrome P450 (CYP) CYP2B6-catalyzed primary (to 8-hydroxyefavirenz) and secondary hydroxylation the principal (>90%) route of inactivation and systemic clearance.[5,6] A minor route, CYP2A6-catalyzed 7-hydroxylation, may also influence disposition under certain circumstances.[5,7] While a few drugs induce (rifampin) or inhibit (voriconazole) efavirenz elimination,[8,9] drug interactions perpetrated by efavirenz are more numerous and clinically significant. For example, efavirenz decreased the area under the curve of several HIV protease inhibitors, anticonvulsants, steroids, buprenorphine, and statins,[2,10,11] increased the metabolism of oral erythromycin and midazolam,[12,13] and induced oral bupropion hydroxylation and clearance.[14] Of significant importance is the reduction of methadone plasma concentrations by efavirenz, which can precipitate opioid withdrawal.[15–17] In addition to causing drug-drug interactions, efavirenz also induces its own metabolism and clearance.[18–20] Apparent transporter-mediated efavirenz drug-drug interactions have also been reported, such as the reduction in plasma concentrations of the non-CYP3A4-metabolized pravastatin.[11]

The mechanism of efavirenz-mediated drug interactions in general is incompletely understood. In human hepatocytes, efavirenz upregulated CYP3A mRNA and/or protein expression[21–23] and activity.[21] Efavirenz also upregulated CYP2B6 mRNA and protein expression in human hepatocytes,[23] but not LS180 human colon cancer cells.[22] Efavirenz has been described as both an inhibitor and inducer of CYPs 2B6, 2C9, 2C19, and CYP3A.[10,12,132,14] In addition, efavirenz caused dose-dependent, tissuespecific induction of hepatic but not intestinal CYP3A4,[12] an apparent paradox which has remained unexplained. Efavirenz was also reported to influence the expression or activity of several transporters, although the data have been characterized as sparse and conflicting.[24] *In vitro*, efavirenz induced P-glycoprotein (P-gp, ABCB1)[22,24–26] and breast cancer resistance protein (BCRP, ABCG2) expression,[22] variably induced expression of multidrug resistance proteins 1–6 (MRP1-6, ABCC1-6),[22,24] and inhibited activity of MRPs 1–3.[27] However efavirenz did not affect P-gp activity in cultured cells or rat intestine.[28,29] Little or no information is available on the clinical effect of efavirenz on transporters.

The specific mechanism of efavirenz reductions in methadone plasma concentrations and opioid withdrawal described above is unidentified.[15–17] CYP2B6 and CYP3A4 have the greatest activity towards methadone N-demethylation *in vitro*, while CYP3A5 is comparatively inactive,[30–34] and these two CYPs are considered to mediate methadone clearance with disputed degrees of relative importance. Antiretroviral drug effects on methadone clearance have traditionally been attributed to alterations in CYP3A4 activity, although this concept has been challenged, and greater importance of CYP2B6 suggested.[35–37] Methadone is also a substrate for P-gp, which influences methadone intestinal absorption in animals,[38] although the role of P-gp in human methadone disposition and clinical effects is poorly understood. Therefore, efavirenz effects on methadone disposition and clinical effects may involve modulation of CYP2B6, CYP3A, and/or drug transporters.

A combined laboratory and clinical investigation was performed to determine (i) mechanism(s) of efavirenz alterations in methadone disposition and clinical effect, including altered CYP2B6-, CYP3A4/5- and/or P-gp-mediated methadone bioavailability, first-pass metabolism, and systemic clearance; (ii) effects on CYP2B6 and CYP3A expression and activity and methadone metabolism in human hepatocytes; (iii) efavirenz influence on

methadone pharmacodynamics; (iv) efavirenz effects on hepatic CYP2B6 and CYP3A4/5, first-pass CYP3A4/5, and intestinal transporter activities, using validated *in vivo* probes; and (v) the ability of a noninvasive *in vivo* CYP3A4/5 probe to detect efavirenz drug interactions and predict methadone disposition.

This article reports a comprehensive crossover investigation in healthy volunteers to assess efavirenz effects on CYP2B6, CYP3A4/5 and transporter activities. Hepatic and first pass CYP3A4/5 activities were evaluated using intravenous and oral alfentanil (a nonselective CYP3A4/5 substrate, henceforth collectively referred to as CYP3A) as the *in vivo* probe.[39,40] Fexofenadine, a substrate for P-gp and other transporters, was the *in vivo* transporter probe.[41,42] Pupil diameter change (miosis) was used as a surrogate for alfentanil plasma concentrations to noninvasively estimate alfentanil clearance and hence CYP3A activity. Efavirenz effects on CYP induction and activity in human hepatocytes were also studied. An accompanying article describes efavirenz effects on methadone pharmacokinetics and pharmacodynamics.[43]

Results

Efavirenz significantly induced hepatic and first-pass CYP3A activity. Efavirenz effects on alfentanil plasma concentrations are shown in Figures 1A–B, and pharmacokinetic parameters provided in Table 1. The AUC ratio (efavirenz/control) for IV alfentanil was reduced to 0.5 by efavirenz, and systemic clearance and the hepatic extraction ratio were both doubled, indicating 2-fold induction of hepatic CYP3A activity. Efavirenz reduced the AUC ratio (efavirenz/control) for oral alfentanil to 0.2, increased apparent oral clearance 5-fold, increased the intestinal extraction ratio by 50%, and decreased oral bioavailability in half, cumulatively indicating 5-fold induction of first-pass CYP3A activity.

Pupil data were available before plasma alfentanil concentrations, and used as an early surrogate for alfentanil clearance to assess efavirenz effects on CYP3A activity (Figure 1C–D and Table 1). Efavirenz decreased the magnitude and duration of alfentanil miosis, and significantly decreased the AUEC ratio for intravenous but not oral alfentanil by approximately half. Efavirenz had no influence on alfentanil pharmacodynamics, measured as the concentration-miosis relationship (Figure 1E). The EC_{50} for miosis was approximately 40 ng/ml, maximum miosis was not reached at the highest alfentanil concentrations observed (100 ng/ml), and little miosis occurred at low alfentanil concentrations (<10 ng/ml).

Metabolism of efavirenz was used to probe the activity of CYP2B6. Efavirenz and 8-hydroxyefavirenz plasma concentrations are shown in Figure 2, and pharmacokinetic parameters provided in Table 2. After two weeks of efavirenz, compared with the first dose, there were significant increases in efavirenz apparent oral clearance and 8-hydroxyefavirenz apparent formation clearance, and a significant decrease in the mid-dose interval efavirenz/8-hydroxyefavirenz plasma concentration ratio.

Disposition of oral fexofenadine was used to evaluate the activity of the intestinal efflux pump P-gp, and other intestinal transporters. Efavirenz had no effect on fexofenadine peak plasma concentrations, but decreased AUC (Figure 3, Table 3).

Efavirenz effects on CYP3A and CYP2B6, and the consequences for methadone metabolism, were measured *in vitro* using primary hepatocytes from three human livers. CYP3A activity was determined by metabolism of alfentanil to noralfentanil, and CYP2B6 activity was measured by the hydroxylation of bupropion. Efavirenz caused concentration-dependent induction of both CYP3A and CYP2B6 mRNA expression and catalytic activity (Figure 4). At 10 μM efavirenz, CYP3A and CYP2B6 mRNA expression were increased 9-

and 7-fold, respectively, and CYP3A and CYP2B6 catalytic activity was increased 6- and 3-fold, respectively, compared with vehicle controls. Efavirenz also caused concentration-dependent induction of methadone N-demethylation (EDDP formation), which was induced 4-fold.

Discussion

One purpose of this investigation was to quantify effects of 2 weeks of oral efavirenz on hepatic and first-pass CYP3A activity. The major finding, as expected, was that efavirenz significantly induced hepatic CYP3A. For the hepatic CYP3A probe intravenous alfentanil, AUC was decreased to half that of control, while systemic clearance and the hepatic extraction ratio were doubled. Thus efavirenz caused a 2-fold induction of hepatic CYP3A activity. Efavirenz induction of hepatic CYP3A was nearly equivalent to the 2.5-fold increase in alfentanil clearance caused by rifampin (600 mg for 5d).[39]

The unexpected finding was that efavirenz also significantly induced intestinal CYP3A activity. For the first-pass CYP3A probe oral alfentanil, AUC was decreased to 22% of control, apparent oral clearance was increased 5-fold, and the intestinal extraction ratio increased 50%. Efavirenz induction of first-pass and intestinal CYP3A was similar to that by 75 mg rifampin for 5d,[44] but less than by 600 mg for 6d, which decreased AUC to 5% of control, caused a 30-fold increase in apparent oral clearance, and a 75% increase in intestinal extraction ratio.[39] Whether these differences in extent of rifampin and efavirenz induction reflect differences in the intrinsic efficacy or the time course of induction (maximal induction by rifampin and efavirenz occur after approximately 2 weeks and 3months, respectively[19,45]), cannot be determined from the present data.

This is the first investigation to demonstrate efavirenz clinical coinduction of both hepatic and intestinal CYP3A activity. Although previous studies showed that efavirenz decreased the AUC of several oral CYP3A drugs, including HIV protease inhibitors, anticonvulsants, steroids, and statins,[2,10,11] and increased the metabolism of oral erythromycin and midazolam,[12,13] the mechanism was unknown. The present results suggest that the mechanism is induction of both hepatic and intestinal CYP3A. Efavirenz co-induction of hepatic and intestinal CYP3A is different from the previous report,[12] and general consideration that efavirenz induces hepatic but not intestinal CYP3A4.[1,2] These differences may reflect dose- (or time-) dependency of intestinal induction, since the previous investigation,[12] which measured CYP3A4 protein content in intestinal biopsies, evaluated 200 and 400 mg efavirenz (for 10d), while the present investigation studied the currently used clinical dose of 600 mg (for 2 weeks).

The second major finding of this investigation was that efavirenz induced hepatic CYP2B6 activity, based on efavirenz autoinduction. The phenomenon of efavirenz autoinduction is frequently mentioned, although it is less described quantitatively. Two approaches have been used previously to evaluate efavirenz autoinduction. One evaluated changes in apparent oral efavirenz clearance.[18,19] After multiple doses, efavirenz plasma AUC decreased 22–42%, presumably reflecting a commensurate increase in clearance.[18] After 14d of efavirenz, autoinduction caused a 32% increase in oral clearance.[19] The estimated average time to 50% of maximal autoinduction was 10d, with 81% of steady-state clearance reached after 14d, and up to 3 months required to reach full steady-state induction.[19] A second approach used the mid-dose interval efavirenz/8-hydroxyefavirenz metabolic ratio.[20] Because efavirenz is typically taken at bedtime to minimize side effects, daytime (mid-dose interval) sampling is often used for convenience in clinical studies.[7] Long-term (4 vs 1 month) efavirenz autoinduction was associated with a decreased efavirenz/8-hydroxyefavirenz metabolic ratio,[20] although autoinduction beginning with initialization of

efavirenz was not evaluated. In the present investigation, efavirenz autoinduction was clearly observed, evidenced by significant increases in both efavirenz apparent clearance and 8-hydroxyefavirenz apparent formation clearance, and by a significant decrease in the efavirenz/8-hydroxyefavirenz metabolic ratio. Efavirenz 8-hydroxylation, which accounts for more than 90% of overall efavirenz oxidation, is catalyzed principally by CYP2B6, is considered a CYP2B6-selective pathway, and suggested to be a phenotypic probe for clinical CYP2B6 activity.[2,5,20] Therefore, the several metrics of increased efavirenz 8-hydroxylation and clearance observed in this investigation support the conclusion that efavirenz autoinduced CYP2B6 activity. Indeed, other studies have also shown efavirenz upregulation of CYP2B6, assessed by heteroinduction. For example, the AUC ratio of hydroxybupropion/bupropion, a specific probe for CYP2B6, was increased 2.3-fold after 2 weeks of efavirenz.[14]

This investigation is therefore the first to demonstrate efavirenz clinical coinduction of both hepatic CYP2B6 and CYP3A activities. Although efavirenz autoinduction of CYP2B6 could theoretically be attributed to upregulation of intestinal as well as hepatic CYP2B6 activity, little support exists for this hypothesis. For example, efavirenz clinically did not induce intestinal CYP2B6 expression, based on immunoblot analysis of intestinal biopsies, albeit at 200 and 400 mg (not 600 mg) efavirenz.[12] In human LS180 cells, efavirenz upregulated CYP3A4 but not CYP2B6 mRNA expression.[22] More generally, in human intestinal precision-cut slices, the pregnane X receptor (PXR) and/or constitutive androstane receptor (CAR) ligands rifampin and phenobarbital upregulated CYP3A4 activity but had no significant effect on CYP2B6 activity.[46] Overall, CYP2B6 is considered to be negligibly if at all expressed in human intestine.

Coinduction of hepatic CYP2B6 and CYP3A clinically is consistent with the present and prior *in vitro* studies. Efavirenz caused concentration-dependent induction of both CYP2B6 and CYP3A4 mRNA and catalytic activity in primary hepatocytes (Figure 4). In the same model, efavirenz previously caused concentration-dependent induction of CYP2B6 and CYP3A4 mRNA and protein expression[21,23,47] and CYP3A4 activity.[21] Efavirenz was a more effective inducer of hepatocyte CYP2B6 compared with CYP3A4 mRNA expression.[23,47] In contrast, it was reported to be a more effective inducer of CYP3A4 than CYP2B6 protein expression.[23] Nevertheless, previous studies showed that Western blot analysis of hepatocyte CYP2B6 protein expression was not as sensitive or quantitative, and underestimated the degree of induction, compared with mRNA and enzyme activity.[48] In the present investigation, efavirenz increased hepatocyte CYP3A activity more than CYP2B6, although there was considerable variability between livers. Efavirenz is a ligand for both PXR and CAR, and coinduction of hepatocyte CYP2B6 and CYP3A4 activities has been variably attributed to preferential activation of either PXR or CAR. While PXR-mediated reporter gene activation by efavirenz was similar for CYP2B6 and CYP3A4,[23,47] induction of both isoforms was variably attributed to preferential activation of PXR[23] or CAR.[47] Regardless of the mechanism, efavirenz is an inducer of both hepatic CYP2B6 and CYP3A4 *in vitro* and *in vivo*. Efavirenz coinduction of hepatic CYP2B6 and CYP3A4 is similar to that seen with the PXR and CAR activators phenobarbital and carbamazepine.[19] Efavirenz induction of hepatocyte methadone N-demethylation in this investigation is consistent with induction of either CYP3A and CYP2B6. Both expressed isoforms have the greatest catalytic activity towards methadone N-demethylation *in vitro*,[30–34]

Another major purpose of this investigation was to assess the ability of alfentanil miosis to noninvasively detect alterations in CYP3A activity and drug interactions. Efavirenz decreased alfentanil miosis, and the AUEC ratio by approximately half. The difference was statistically significantly for intravenous alfentanil but narrowly missed significance for oral alfentanil. Several reasons explain this observation. First, there was greater variability in the

disposition of oral compared with intravenous alfentanil, resulting in a wider confidence interval for the geometric mean ratio of induced/control. Second, plasma concentrations were lower after oral compared with intravenous alfentanil. Previous results showed that the optimal (linear) range for measuring miosis is ~20–100 ng/ml alfentanil, and that changes in miosis underestimate the changes in plasma alfentanil at lower concentrations.[39,40,44] In the present protocol, alfentanil concentrations were much lower after oral administration, and exceeded 20 ng/ml only briefly. Third, because of the concentration-response relationship, the sensitivity of alfentanil miosis for detecting CYP3A induction, where plasma concentrations decrease, is less than that for detecting CYP3A inhibition, where plasma concentrations increase.[39,44] Nevertheless, alfentanil miosis did predict efavirenz effects on hepatic CYP3A activity, and with a somewhat larger sample size might have predicted efavirenz effects on first-pass CYP3A activity.

The last major finding of this investigation, which is the first to evaluate efavirenz effects on P-gp and other transporters, was that efavirenz appeared to moderately induce P-gp. Efavirenz decreased fexofenadine AUC, which was not due to enhanced elimination, suggesting increased fexofenadine intestinal efflux. When this investigation was designed, fexofenadine was used to probe intestinal P-gp-mediated interactions based on published recommendations.[49] Originally considered selective for P-gp, fexofenadine is now known to be a substrate for multiple transporters. Intestinal fexofenadine absorption is influenced by both P-gp-mediated efflux and organic anion transporting polypeptide (OATP) 1A2-mediated uptake.[50] Hepatic excretion of unchanged drug is the primary route of fexofenadine elimination. Hepatic uptake of fexofenadine involves OATPs 1B1, 1B3 and 2B1.[51] Fexofenadine is a substrate for hepatic sinusoidal MRP3 (but not MRP4)-mediated efflux, and BSEP- (but not BCRP-, and possibly not MRP2-) mediated hepatic canicular efflux.[51] Polymorphisms of P-gp, MRP2, OATP2B1 influence fexofenadine pharmacokinetics. Modeling and simulation studies suggested that hepatic uptake, rather than basolateral efflux or biliary excretion, is the principal determinant of fexofenadine clearance and systemic concentrations.[52] Clinical evidence also supports the liver as a site of fexofenadine drug interactions.[53] Together, therefore, drug interactions which change fexofenadine disposition may reflect altered intestinal permeability (OATP1A2-mediated uptake and/or P-gp-mediated efflux), or OATP 1B1-, 1B3- and/or 2B1-mediated hepatic uptake, and thus fexofenadine may more aptly be considered a more generalized probe for transporters function. Thus efavirenz effects may reflect decreased intestinal OATP1A2-mediated uptake and/or increased P-gp-mediated efflux, and/or increased OATP 1B1-, 1B3- and/or 2B1-mediated hepatic uptake. Since fexofenadine elimination rates were not affected, the present results suggest decreased intestinal uptake and/or increased P-gp-mediated efflux.

Some *in vitro* data corroborate the above considerations. In LS180 intestinal cells, efavirenz induced P-gp expression and activity,[22,25] and 2 to 3-fold induction of BCRP, MRP3, and MRP5 mRNA.[22] Efavirenz was not a substrate for P-gp, MRP1, MRP2, or BRCP,[22] but inhibited BCRP and MRPs 1–3[26,27] but not P-gp,[28] in cells overexpressing these transporters. Concomitant efavirenz upregulation of P-gp, CYP3A and CYP2B6 is consistent with its activation of PXR, and very high induction potential index (calculated based on potency for PXR activation and clinical concentration).[54]

There are potential limitations of this investigation. Efavirenz effects were evaluated in healthy volunteers rather than in HIV-infected patients. This was deliberate, because standard antiretroviral therapy involves several drugs, thereby precluding a careful mechanistic evaluation and attribution of results to any one specific drug. Pharmacogenetic influences on efavirenz induction of CYPs and transporters were not evaluated in this small

investigation. Lastly, a longer duration of efavirenz administration may have somewhat greater effects on induction.[19]

In summary, efavirenz significantly induced hepatic, intestinal, and first-pass CYP3A4/5 activity, and significantly induced hepatic CYP2B6 activity. Efavirenz also altered the activity of intestinal transporters.

Methods

Clinical Protocol

Additional Methods information is provided in an accompanying article.[43] The protocol was a two-session sequential crossover approved by the University of Washington Institutional Review Board. Twelve normal HIV-negative volunteers (22±4 yr, 71±12 kg) were studied before and after 2 weeks of oral efavirenz (600 mg nightly, continued for the duration of the study). Hepatic and first-pass CYP3A activities were evaluated using intravenous and oral alfentanil, and P-gp activity assessed using oral fexofenadine, as described previously.[36,37,39,40,42,43] Plasma alfentanil and fexofenadine concentrations were simultaneously quantified using solid-phase extraction and electrospray liquid chromatography-mass spectrometry. Dark-adapted pupil diameter was measured just prior to blood sampling.

Plasma and urine efavirenz and 8-hydroxyefavirenz concentrations were determined by liquid-liquid extraction and liquid chromatography-mass spectrometry. To plasma (100μl) was added aprobarbital (62.5 ng) (Restek, Bellefonte, PA) and 30μl of 50 mM sodium carbonate, then twice extracted with 1 ml ethyl acetate:pentane (1:1). After centrifugation, organic layers were combined, evaporated under nitrogen (60°C), resuspended in 500μl acetonitrile, centrifuged, transferred to a new tube, evaporated to dryness, and resuspended in 250μl acetonitrile. Calibration standards contained 0, 5, 10, 25, 50, 100, 500, 1000, 2500 and 5000 ng/ml efavirenz (NIH AIDS Research and Reference Reagent Program) and 0, 0.5, 1, 2.5, 5, 10, 50, 100, 250 and 500 ng/ml 8-hydroxyefavirenz (Toronto Research Chemicals, Ontario, Canada) in blank plasma. Samples (40μl injection) were analyzed using an Agilent (Palo Alto, CA) 1100 series HPLC with vacuum degasser, binary solvent pump and 96-well plate auto sampler, and SunFire C18 column (4.6 × 150mm, 5μm) (Waters Corp., Milford, MA), interfaced to an Agilent 1100 series mass spectrometer operated in negative electrospray ionization mode. Mobile phase was (A) 0.04% formic acid in water and (B) 98% acetonitrile at 1 ml/min. Gradient conditions were initially 50% B, increased to 75% at 3 min, 100% at 4 min, held for 3.5 min, then decreased to 50% for 5 min re-equilibration. Parameters were: nitrogen drying gas (11 L/min, 350°C), nebulizer pressure 40 psi, capillary 3000 V, and fragmentor 120 V. Elution times for aprobarbital (*m/z* 209), 8-hydroxyefavirenz (*m/z* 330), and efavirenz (*m*/z 314) were 2.5, 4.8, and 5.4 min, respectively. Interday coefficients of variation were 9 and 10% for 10 and 1,000 ng/ml efavirenz, and 6% and 9% for 2.5 and 100 ng/ml 8-hydroxyefavirenz. For analysis of urine 8-hydroxyefavirenz, samples (10 μl) were hydrolyzed overnight at 37°C with 62.5 units β-glucuronidase, the internal standard aprobarbital (10 ng) added, diluted 1:50,000 with acetonitrile, and analyzed (20 μl) similar to plasma. Calibration standards contained 0, 10, 25, 50, 100, 200, 300, or 400 ng/ml 8-hydroxyefavirenz. Interday coefficients of variation were 8% and 6% for 25 and 300 ng/ml 8-hydroxyefavirenz.

Pharmacokinetic data were analyzed using noncompartmental methods.[43] Formation clearance of 8-hydroxyefavirenz was the product of the molar fraction of dose recovered in urine and efavirenz apparent oral clearance. Paired t-tests assessed differences between groups for pharmacokinetic and effect parameters. Non-normal data were log transformed for analysis but reported as nontransformed results (arithmetic mean ± SD). Statistical

significance was assigned at $p < 0.05$. Plasma AUC and urine data were also assessed as ratios (efavirenz/control) and the geometric mean and 90% confidence interval.

Hepatocyte Experiments

Alfentanil and methadone were from the National Institutes of Drug Abuse through Research Triangle Institute (Research Triangle Park, NC). Efavirenz was from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Noralfentanil-d5, d3-EDDP, and d6-hydroxybupropion were from Cerilliant (Round Rocks, TX). Fresh plated human hepatocytes and Hepatocyte Supplement were from Cellzdirect/Invitrogen (Durham, NC). Williams E media was from Lonza (Walkersville, MD). Bis-tris acrylamide gels (4–12%) and nitrocellulose membranes were from Invitrogen (Carlsbad, CA). Mouse antihuman monoclonal CYP3A4, rabbit antihuman polyclonal CYP2B6 and mouse antihuman monoclonal β-actin antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Goat anti-rabbit and goat anti-mouse secondary antibodies, and SDS-cell lysis and blocking buffers were from Li-COR (Lincoln, NE). Other reagents were from Sigma (St. Louis, MO).

Upon hepatocyte arrival, media was changed to supplemented Williams E and cells allowed to equilibrate for 24 hr at 37°C/5% CO_2/95% humidity. Cells were incubated in supplemented Williams E media containing drugs (efavirenz, phenobarbital, rifampin) or vehicle control for 72 hr, with media/drug changed daily. Efavirenz and phenobarbital were dissolved in DMSO (final concentration 0.1%). Prior to testing CYP activity cells were incubated with drug-free media for 1 hr. Media was then changed to that containing 500 μM bupropion and cells incubated for 40 min at 37°C with shaking, to determine CYP2B6 activity. Media was removed, analyzed for hydroxybupropion, then replaced with drug-free media for a 1 hr wash-out period. Media was replaced with that containing 500 μM racemic methadone and cells incubated for 40 min. Media was removed, analyzed for EDDP, and replaced with drug-free media for a 1 hr wash-out. To determine CYP3A4/5 activity, media was replaced with that containing 200 μM alfentanil, cells incubated for 40 min, and media analyzed for noralfentanil. Each determination was performed in triplicate using hepatocytes from 3 livers. After each experiment, hepatocytes were frozen for later quantification of mRNA.

Metabolite analysis was performed on an API 3200 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA)-Shimadzu LC-20AC HPLC (Shimadzu, Columbia, MD) (EDDP and noralfentanil) or an API 4000 QTRAP mass spectrometer-Agilent 1100 HPLC (hydroxybupropion). Both mass spectrometers were equipped with a Turbo Ion Spray ionization source and a Waters T3 HPLC column (50 × 2.1mm, 3.5μm). Injection volume was 20 μl and the oven temperature was 25°C. The HPLC mobile phase (0.3 ml/min) was (A) 0.1% formic acid and (B) 0.1% formic acid in acetonitrile. The gradient program for EDDP was 35% B for 0 min, linear to 60% B over 1.0 min, held at 60% until 2 min, linear to 100% until 3 min, 100% B until 4 min, then re-equilibrated to initial conditions for 1 min; for noralfentanil it was 35% B for 0 min, linear to 40% B until 0.5 min, held at 40% until 2.5 min, linear to 100% until 3 min, held at 100% B until 4 min, then re-equilibrated to initial conditions over 1.5 min; for hydroxybupropion it was 10% B for 0 min, linear to 30% B over 1.0 min, held at 30% until 1.5 min, linear to 100% until 2 min, held at 100% B until 2.5 min, then re-equilibrated to initial conditions over 3.5 min. Retention times were 2.7, 2.9 and 1.5 min, respectively, for EDDP, noralfentanil and hydroxybupropion. Mass spectrometers were operated in positive-ion mode, ion spray voltage 5500, curtain gas at 20, ion source gas 1 at 30, ion source gas 2 at 30 and collision gas at 5, and unit mass resolution. Multiple reaction monitoring transitions for analytes and standards were *m/z* 278.2→234.2 and 281.2→234.2 for EDDP and d3-EDDP; *m/z* 277.0→128.0 and 282.0→128.0 for noralfentanil and d5-noralfentanil; *m/z* 256.1→238.1

and 262.2→167.2 for 8-hydroxybupropion and d6-8-hydroxybupropion. Metabolites were quantified using area ratios and standard curves prepared using calibration standards in blank media.

Hepatocyte mRNA expression was determined by real-time reverse transcription polymerase chain reaction using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA), according to the manufacturers instructions.

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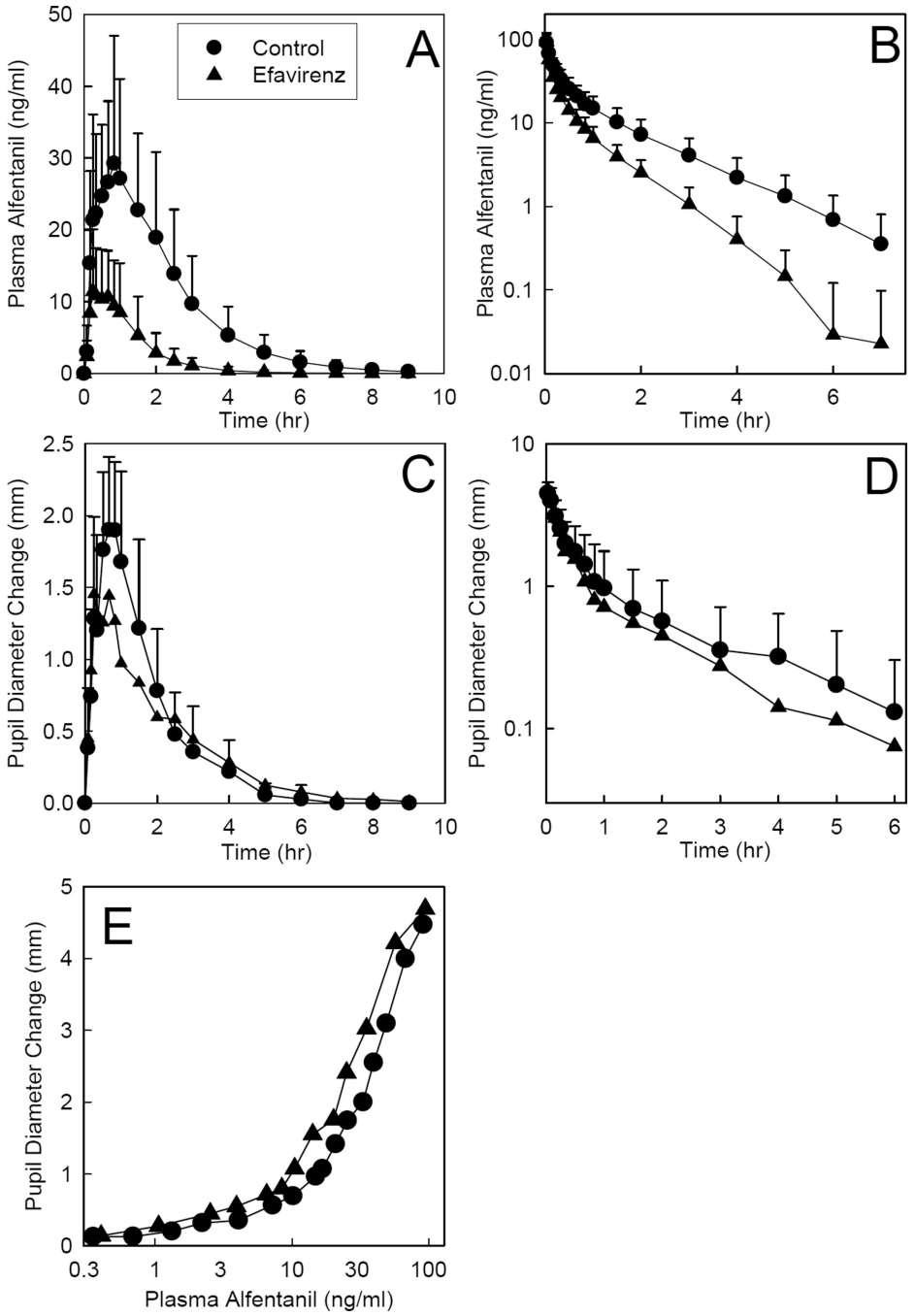

Figure 1.

Efavirenz effects on first-pass and hepatic CYP3A activity, assessed using alfentanil as a CYP3A probe. Subjects received 43 μg/kg oral or 15 μg/kg intravenous alfentanil. Panels A and B show alfentanil plasma concentrations after oral and intravenous administration, respectively. Panels C and D show dark-adapted pupil diameter change from baseline (miosis), used as a surrogate for alfentanil plasma concentrations, oral and intravenous administration, respectively. Panel E shows the relationship between miosis and plasma concentration after intravenous alfentanil administration. Each data point is the mean ± SD (n = 12). Some SD are omitted for clarity.

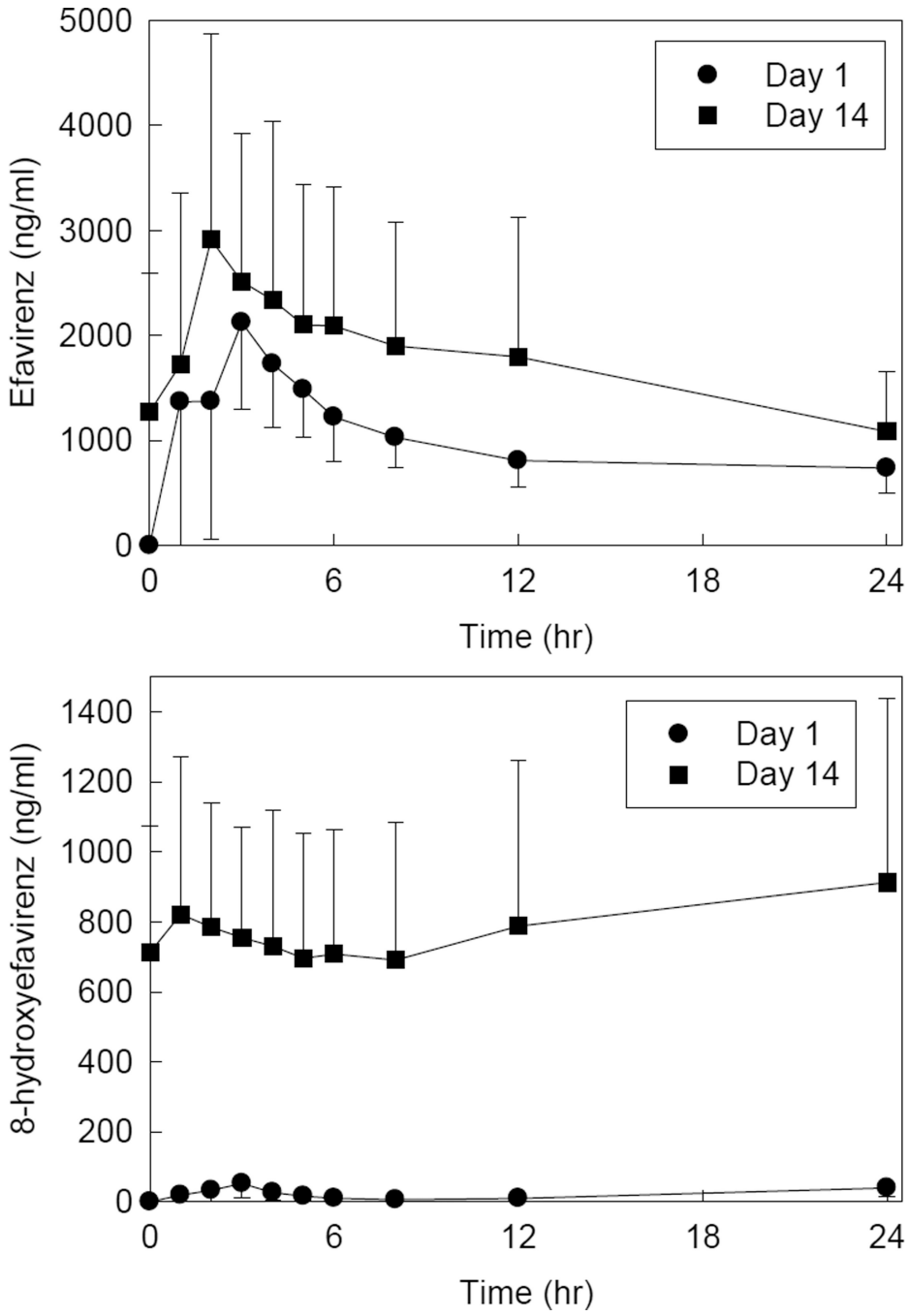

Figure 2.
Efavirenz effects on CYP2B6 activity, assessed using efavirenz disposition as a probe. Shown are efavirenz and 8-hydroxyefavirenz plasma concentrations after the first efavirenz dose and after 2 weeks of efavirenz. Each data point is the mean ± SD (n = 12).

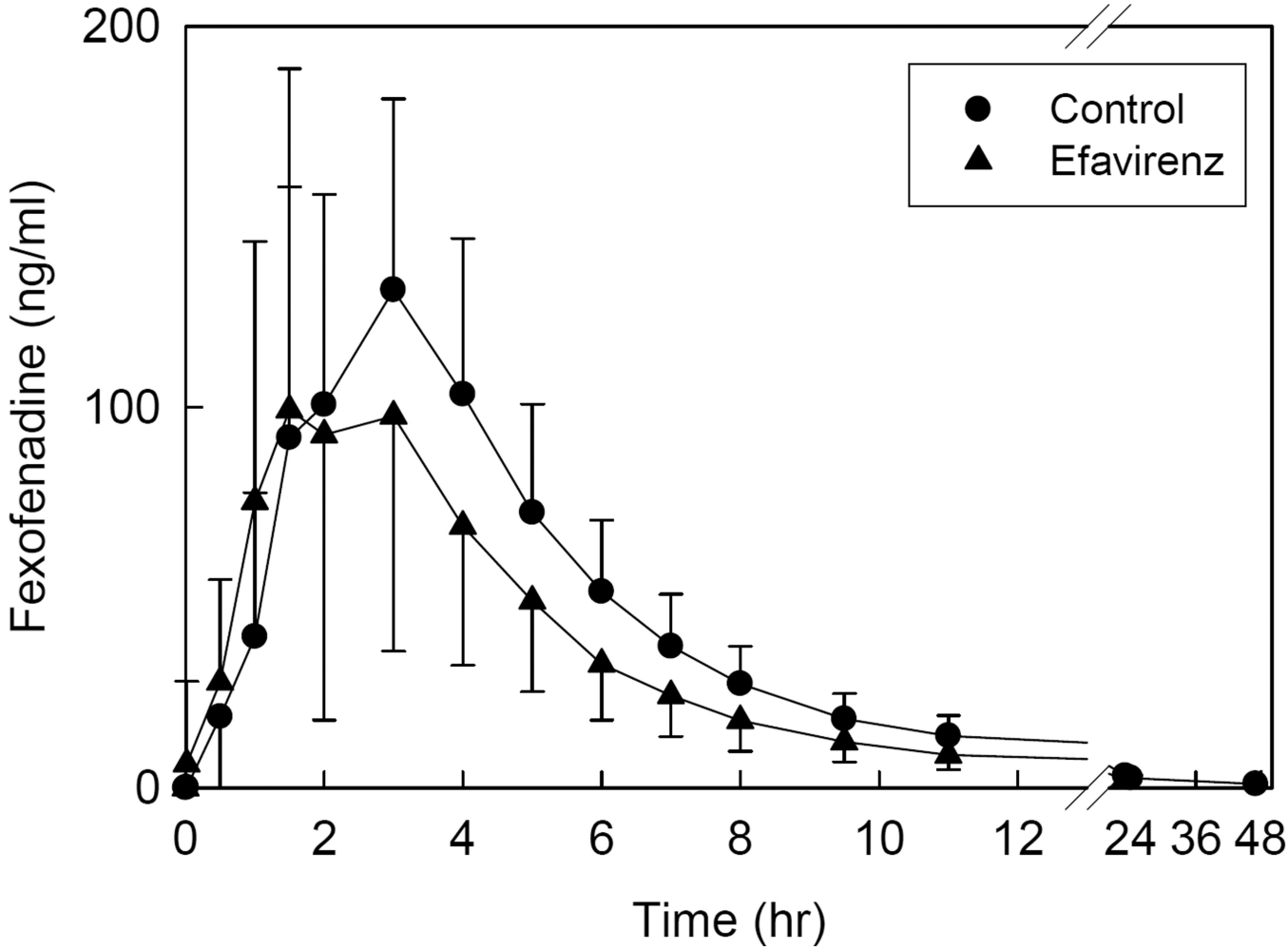

Figure 3.
Efavirenz effects on gastrointestinal transporter activity, assessed using fexofenadine as a transporter probe. Each subject received 60 mg oral fexofenadine on all occasions. Each data point is the mean ± SD (n = 12).

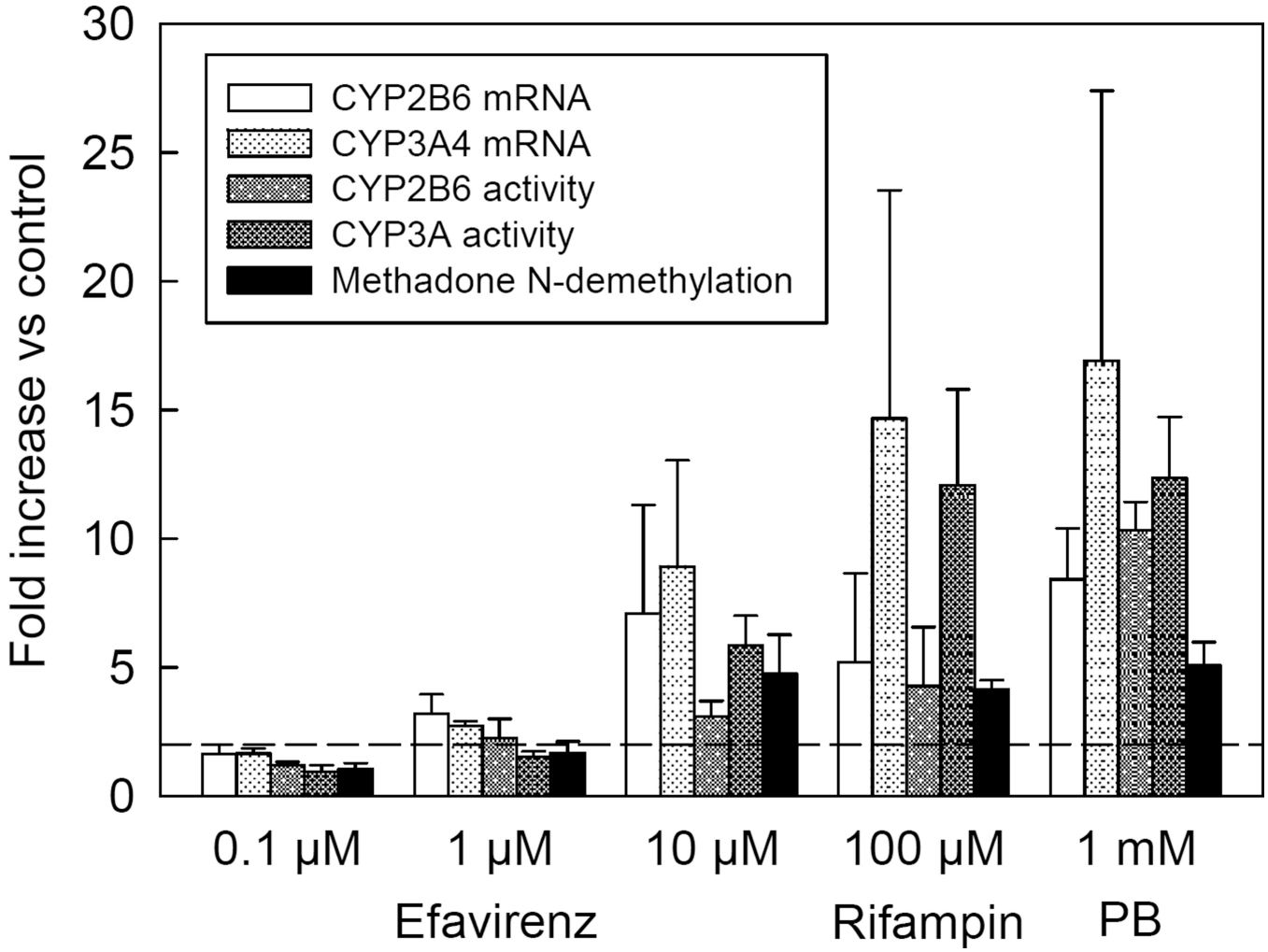

Figure 4.

Efavirenz effects on primary human hepatocyte CYP2B6 and CYP3A4 mRNA expression and catalytic activity, and methadone metabolism. Hepatocytes were incubated with efavirenz for 72 hr and then washed. CYP2B6 activity was determined by measuring bupropion hydroxylation, CYP3A activity was determined by measuring alfentanil dealkylation to noralfentanil, and methadone metabolism was determined by measuring EDDP formation. CYP2B6 and CYP3A4 mRNA was measured by RT-PCR. Rifampin (100 μM) and phenobarbital (PB, 1 mM) were used as positive controls. Hepatocytes were from a 51 year old male (nonsmoker, no ethanol use, taking escitalopram, 52 year old female (nonsmoker, no ethanol use, medication history unavailable), and 75 year old male (smoker, ethanol use, taking 25 duloxetine and cyclobenzaprine). Each data point is the mean ± SD of three livers, where mRNA and catalytic activity were determined in triplicate for each liver. The dotted line represents a 2-fold increase in expression or activity.

Table 1

Intravenous and oral alfentanil pharmacokinetics and effects

	Control	Efavirenz
IV alfentanil		
C_{max} (ng/ml)	92 ± 23	101 ± 32
$AUC_{0-\infty}$ (ng •hr •ml^{-1})	55 ± 22	30 ± 9 *
$AUC_{0-\infty}$ ratio (efavirenz/control)		0.54 (0.47, 0.62)
CL_{IV} (ml•kg^{-1}•min^{-1})	5.0 ± 1.5	9.3 ± 3.0 *
Elimination $t_{1/2}$ (hr)	1.1 ± 0.2	0.85 ± 0.47 *
E_H	0.32 ± 0.09	0.59 ± 0.17 *
Maximum miosis (mm)	4.6 ± 0.8	4.6 ± 0.7
Miosis $AUEC_{0-\infty}$ (mm•hr)	4.3 ± 2.8	3.2 ± 2.5 *
Miosis $AUEC_{0-\infty}$ ratio (efavirenz /control)		0.58 (0.38, 0.89)
Oral alfentanil		
C_{max} (ng/ml)	35 ± 17	15 ± 8 *
$AUC_{0-\infty}$ (ng •hr •ml^{-1})	75 ± 40	18 ± 13 *
$AUC_{0-\infty}$ ratio (efavirenz/control)		0.22 (0.16, 0.30)
CL/F (ml•kg^{-1}•min^{-1})	12.4 ± 6.4	58.2 ± 32.6 *
Elimination $t_{1/2}$ (hr)	1.1 ± 0.3	0.65 ± 0.18 *
F_{oral}	0.46 ± 0.15	0.20 ± 0.09 *
E_G	0.37 ± 0.15	0.55 ± 0.18 *
Maximum miosis (mm)	2.3 ± 0.5	1.6 ± 0.9
Miosis $AUEC_{0-\infty}$ (mm•hr)	4.2 ± 1.6	4.3± 4.6
Miosis $AUEC_{0-\infty}$ ratio (efavirenz/control)		0.63 (0.40, 1.01)

Subjects received 15 μg/kg IV alfentanil and 43 μg/kg oral alfentanil. Results are the arithmetic mean ± SD (n=12), except the AUC and AUEC ratio (efavirenz/control), which is the geometric mean (90% CI). AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; CL_{IV}, systemic clearance of IV alfentanil; CL/F, apparent oral clearance of alfentanil; E_H, hepatic extraction ratio; E_G, intestinal extraction ratio; F_{oral}, bioavailability. AUEC, area under the effect (miosis)-time curve

* Significantly different from control ($p<0.05$)

Table 2

Efavirenz pharmacokinetic parameters

	First dose	Two weeks
CL/F (L/hr)	12.3 ± 6.1	20.1 ± 9.5 *
12 hr efavirenz/8-hydroxyefavirenz concentration ratio	152 ± 116	2.9 ± 2.5 *
8-hydroxyefavirenz apparent formation clearance (L/hr)	1.0 ± 0.6	10.7 ± 5.7 *

CL/F, apparent oral clearance of fexofenadine.

* Significantly different from control ($p<0.05$)

Table 3

Fexofenadine pharmacokinetics

	Control	Efavirenz
C_{max} (ng/ml)	141 ± 56	118± 81
$AUC_{0-\infty}$ (ng •hr •ml^{-1})	716 ± 242	554 ± 302
$AUC_{0-\infty}$ ratio (efavirenz/control)		0.73 (0.59,0.91)
CL/F (ml•kg^{-1}•min^{-1})	23.2 ± 12.6	33.2 ± 18.5 *
Elimination $t_{1/2}$ (hr)	5.4 ± 0.8	5.3 ± 0.8

Results are the arithmetic mean ± SD (n=12), except the $AUC_{0-\infty}$ ratio (efavirenz/control), which is the geometric mean (90% CI). AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; CL/F, apparent oral clearance

* Significantly different from control ($p<0.05$)