Published in final edited form as:

Clin Pharmacol Ther. 2012 March ; 91(3): 475–482. doi:10.1038/clpt.2011.249.

Induction of CYP2C19 and CYP3A Activity Following Repeated Administration of Efavirenz in Healthy Volunteers

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Abstract

Drug–drug interactions involving efavirenz are of major concern in clinical practice. We evaluated the effects of multiple doses of efavirenz on omeprazole 5-hydroxylation (CYP2C19) and sulfoxidation (CYP3A). Healthy volunteers ($n = 57$) were administered a single 20 mg oral dose of racemic omeprazole either with a single 600 mg oral dose of efavirenz or after 17 days of administration of 600 mg/day of efavirenz. The concentrations of racemic omeprazole, 5-hydroxyomeprazole (and their enantiomers), and omeprazole sulfone in plasma were measured using a chiral liquid chromatography–tandem mass spectrometry method. Relative to single-dose treatment, multiple doses of efavirenz significantly decreased ($P < 0.0001$) the area under the plasma concentration–time curve from 0 to infinity ($AUC_{0-\infty}$) of racemic-, R- and S-omeprazole (2.01- to 2.15-fold) and the corresponding $AUC_{0-\infty}$ metabolic ratio (MR) for 5-hydroxyomeprazole (1.36- to 1.44-fold) as well as the MR for omeprazole sulfone (~ 2.0) ($P < 0.0001$). The significant reduction in the AUC of 5-hydroxyomeprazole after repeated efavirenz dosing suggests induction of sequential metabolism and mixed inductive/inhibitory effects of efavirenz on CYP2C19. In conclusion, efavirenz enhances omeprazole metabolism in a nonstereoselective manner through induction of CYP3A and CYP2C19 activity.

Currently used combinations of anti-HIV drugs, known as highly active antiretroviral therapy (HAART), have considerably reduced morbidity and mortality in HIV-infected patients.¹ On the other hand, the fact that such a large number of drugs are being used to treat HIV infection and related chronic diseases has increased the risk of potential adverse drug–drug interactions. By changing the expression and activity levels of several drug-metabolizing enzymes and drug transporters, HAART medications such as efavirenz and protease inhibitors often cause adverse drug–drug interactions.

Efavirenz-based HAART is widely prescribed and among the preferred first-line therapies in drug-naïve HIV-infected patients. Efavirenz is metabolized primarily by CYP2B6 into 8-hydroxyefavirenz and to a lesser extent through pathways involving CYP2A6, CYP3A4/5, and UGT2B7.^{2–6} In addition to being a substrate of CYP2B6, efavirenz induces its own metabolism during long-term administration.^{7,8} As a result, efavirenz modulates the pharmacokinetics of many coadministered drugs, thereby potentially leading to failure of therapy or increased risk of toxicity.

The ability of efavirenz to enhance the clearance of drugs metabolized by CYP3A has been well documented.^{9–11} On the other hand, *in vitro* data suggest that efavirenz inhibits

CYP2C19, but there are limited data regarding its *in vivo* effect on CYP2C19 substrates.¹² Furthermore, *in vitro* studies demonstrated that efavirenz can activate CYP3A4 and CYP2C19 promoter activity through nuclear receptors PXR and CAR.¹³⁻¹⁵ Therefore, efavirenz may affect the pharmacokinetics of clinically important drugs metabolized by CYP2C19, including citalopram, proton-pump inhibitors, clopidogrel, voriconazole, and proguanil.¹⁶⁻²¹ The paucity of *in vivo* study data and the confusion about the inhibition and/or induction effects of efavirenz necessitate further investigations into the *in vivo* effects of this drug; a better understanding of the mechanisms underlying drug interactions is crucial, given that HIV-infected patients are particularly prone to drug-related adverse effects because they receive several concomitant medications (polypharmacy).

The aim of our study was, therefore, to determine the effects of repeated dosing of efavirenz on CYP2C19 and CYP3A4 activity levels, using omeprazole as a dual substrate probe. Because of the expected competitive inhibition of CYP2C19 by acute efavirenz administration, the pharmacokinetics of omeprazole, 5-hydroxyomeprazole, and omeprazole sulfone were compared after single and multiple doses of efavirenz to assess CYP2C19 and CYP3A activity levels. *In vitro* studies indicate that, with respect to intrinsic clearance, ~98% of R-omeprazole and ~70% of S-omeprazole is carried out by CYP2C19-catalyzed 5-hydroxylation reactions, whereas CYP3A-mediated sulfone formation seems to contribute 2 and 27%, respectively.²²⁻²⁶ As described above, efavirenz can inhibit or induce CYPs. Given the difference in relative contributions of CYP3A and CYP2C19 in omeprazole stereoselective metabolism, differential induction/inhibition of these enzymes by efavirenz could lead to enantioselective interaction. To explore this possibility, we compared the pharmacokinetic parameters of racemic, R-, and S-omeprazole, as well as the corresponding 5-hydroxylated metabolites, after single-dose treatment vs. multiple-dose treatment with efavirenz.

Results

Subject characteristics

The concentration levels of omeprazole and its metabolites were measured in plasma samples from 57 subjects (36 men and 21 women) who completed both sessions of the experiment. The subject characteristics were age ranging from 18 to 50 years with a mean of 28.8 ± 10 years, body weight ranging from 53 to 103.6 kg with a mean of 74.8 ± 13.9 kg, and body mass index ranging from 17.8 to 31.8 kg/m² with a mean of 24.3 ± 3.8 kg/m². The majority of the subjects (77.2%) were Caucasians ($n = 44$), and 19.3% ($n = 11$) and 3.5% ($n = 2$) were African-Americans and Asians, respectively. Two subjects were found to have an omeprazole hydroxylation index > 1 (omeprazole index calculated after a single dose of efavirenz), indicative of a poor CYP2C19 metabolizer phenotype.

Effects of single and multiple doses of efavirenz on the metabolism and pharmacokinetics of racemic omeprazole

The pharmacokinetics of racemic omeprazole and of its 5-hydroxyl- and sulfone metabolites were significantly altered by the repeated administration of efavirenz (Table 1). As compared with a single dose of efavirenz, multiple doses of the drug decreased the average area under the plasma concentration–time curve from 0 to infinity ($AUC_{0-\infty}$) from 1,843 nmol · h/l to 988 nmol·h/l (2.06-fold), and the maximum plasma concentration (C_{max}) of omeprazole from 704 nmol/l to 495 nmol/l (2.31-fold). The weight-adjusted apparent oral clearance of omeprazole was, on average, 1.6-fold higher after multiple doses of efavirenz as compared with that after a single dose (Table 1).

As shown in Table 1, the $AUC_{0-\infty}$ of 5-hydroxyomeprazole was significantly lower after repeated doses than after a single dose of efavirenz ($P < 0.0001$), whereas no significant difference was observed in the C_{max} of 5-hydroxyomeprazole ($P = 0.2$) between the two treatment sessions. The $AUC_{0-\infty}$ of omeprazole sulfone did not differ between the single and multiple dosing sessions ($P = 0.6$). However, the C_{max} for this metabolite was approximately twofold higher after multiple doses of efavirenz than after a single dose. The $AUC_{0-\infty}$ of 5-hydroxyomeprazole was lower than the $AUC_{0-\infty}$ of omeprazole sulfone in both sessions (single and multiple doses of efavirenz).

Figure 1 shows the individual $AUC_{0-\infty}$ metabolic ratio (MR) values calculated for hydroxylation and sulfoxidation pathways. The plasma MR of omeprazole to 5-hydroxyomeprazole (a marker of CYP2C19 activity) was significantly decreased after multiple doses of efavirenz (2.5 ± 3.6 after single dose vs. 1.7 ± 1.5 after multiple doses; $P = 0.006$). The MR of omeprazole/omeprazole sulfone (a marker of CYP3A activity) was also lower after multiple doses of efavirenz than after a single dose (1.5 ± 0.7 after single dose vs. 0.8 ± 0.4 after multiple doses; $P = 0.0001$). For the hydroxylation and sulfoxidation pathways, the induction ratios (defined as the MR after single dose of efavirenz divided by the MR after multiple doses) were 1.44 ± 0.67 and 1.97 ± 0.8 , respectively (Table 2). The induction by efavirenz through the omeprazole sulfoxidation pathway appears slightly higher than that obtained through omeprazole hydroxylation ($P = 0.005$).

Figure 2 illustrates the cumulative frequency curves of the metabolic indexes for omeprazole hydroxylation and sulfoxidation. After repeated dosing with efavirenz, the cumulative frequency curves show a shift to the left, thereby supporting the hypothesis that efavirenz induces metabolism of omeprazole. Furthermore, a greater shift is observed for the sulfoxidation metabolic index of omeprazole than for the hydroxylation index.

Effects of single and multiple doses of efavirenz on the metabolism and pharmacokinetics of omeprazole enantiomers

The plasma concentrations of R- and S-omeprazole after single and multiple doses of efavirenz are shown in Figure 3. As expected from the results of previous studies, the concentrations of R-omeprazole in plasma were lower than those of S-omeprazole. On average, after a single dose of efavirenz, R-omeprazole oral clearance was 1.7-fold greater than that of S-omeprazole ($P = 0.001$). As expected, the $AUC_{0-\infty}$ and C_{max} values of R-5-hydroxyomeprazole were much higher than those of the S-5-hydroxyomeprazole (Figure 3 and Table 1; $P < 0.0001$). No stereoselective differences in the elimination half-life were observed for R- and S-omeprazole or for R- and S-5-hydroxyomeprazole. The MR for R-5-hydroxyomeprazole was approximately tenfold lower than that of S-5-hydroxyomeprazole ($P < 0.0001$), consistent with the known stereoselective metabolism of omeprazole.

Repeated doses of efavirenz enhanced the elimination of R- and S-omeprazole (Table 1). However, no significant changes were observed in the pattern of disposition of these two enantiomers. The concentration levels of both R- and S-omeprazole in plasma were diminished to the same extent after multiple doses of efavirenz. Similarly, the ratios of $AUC_{0-\infty}$ (single dose of efavirenz) to $AUC_{0-\infty}$ (multiple doses of efavirenz) were comparable for the two enantiomers of omeprazole (2.01 and 2.15 for R-omeprazole and S-omeprazole, respectively; $P = 0.12$) (Table 1). The apparent oral clearances of both enantiomers of omeprazole were increased to a similar extent by multiple doses of efavirenz (ratios of oral clearances were 0.68 and 0.60 for R- and S-omeprazole, respectively; $P = 0.02$). In addition, the C_{max} values of R- and S-omeprazole were significantly lower (decrease of ~30%) after repeated doses of efavirenz than after a single dose (Table 1). As observed with racemic omeprazole, repeated dosing with efavirenz had no impact on the elimination half-life of either of the omeprazole enantiomers.

The $AUC_{0-\infty}$ values of R- and S-5-hydroxyomeprazole were significantly lower after repeated doses of efavirenz than after a single dose ($P < 0.0001$; Table 1). In addition, multiple doses of efavirenz caused similar changes in the $AUC_{0-\infty}$ of both enantiomers of 5-hydroxyomeprazole (1.49 ± 0.59 and 1.77 ± 1.03 for R- and S-hydroxyomeprazole, respectively). The elimination half-life of S-5-hydroxyomeprazole was slightly shorter ($P = 0.05$) after multiple doses of efavirenz than after a single dose of efavirenz, but no significant differences in C_{max} were observed for either R- or S-5-hydroxyomeprazole metabolites between the single-dose and multiple-dose sessions of efavirenz dosing ($P = 0.2$ and $P = 0.1$, respectively).

The MRs showed decreases for both isomers of 5-hydroxyomeprazole after multiple doses of efavirenz (Figure 1 and Table 2). However, the induction was nonstereoselective, as seen from the fact that the induction ratios for R- and S-omeprazole were similar (~1.4-fold) (Table 2). Further confirmation of the nonstereoselective effect of efavirenz was obtained by the absence of any difference in the $AUC_{0-\infty}$ S-omeprazole/ $AUC_{0-\infty}$ R-omeprazole ratio with single-dose treatment with efavirenz vs. multiple-dose treatment (1.8 vs. 1.7, respectively). A similar observation was made in comparing the AUC ratios of the isomers of 5-hydroxyomeprazole ($AUC_{0-\infty}$ S-5-hydroxyomeprazole/ $AUC_{0-\infty}$ R-5-hydroxyomeprazole), which were almost identical after single and multiple doses of efavirenz (0.12 vs. 0.11, $P = 0.09$). Furthermore, a similar range of shift in the cumulative frequencies of the omeprazole hydroxylation and sulfoxidation indexes was noted between R- and S-omeprazole after repeated doses of efavirenz (Figure 2). These results support the hypothesis that efavirenz has a nonstereoselective inductive effect on omeprazole metabolism.

DISCUSSION

Our study demonstrates that multiple doses of efavirenz significantly enhance the elimination of omeprazole. The significant decrease in omeprazole exposure after repeated doses of efavirenz is related to induction of CYP2C19 and CYP3A metabolic activity by efavirenz. This conclusion is based on the observed increases in omeprazole oral clearance (by 1.61-fold) and AUC induction ratio (2.1, $P < 0.001$), and on the decrease in the MRs (AUC of omeprazole/5-hydroxyomeprazole and omeprazole/omeprazole sulfone) after multiple doses of efavirenz. Furthermore, the observed decrease in the AUCs of metabolites supports the concept of induced sequential metabolism of omeprazole by efavirenz. Our study demonstrates that induction of R- and S-omeprazole metabolism by efavirenz was nonstereoselective; clearance ratios and MRs of R- and S-omeprazole are modulated to similar extents after multiple doses of efavirenz. Finally, our study suggests that efavirenz may possess a mixed inhibitory/inductive effect on CYP2C19 activity.

Previous *in vitro* and *in vivo* studies including drug–drug interaction studies showed that efavirenz could induce CYP450 activity such as CYP2B6, CYP3A4, and CYP2C activity in a concentration- and time-dependent manner. Our study demonstrates that 5-hydroxylation and sulfone formation of omeprazole are induced after repeated doses of efavirenz. The shift observed in the cumulative frequency distribution of the metabolic indexes of omeprazole hydroxylation and omeprazole sulfoxidation also supports this finding. More specifically, the results obtained in our study confirm the inductive effect of multiple doses of efavirenz on CYP3A. It has been shown that the expression levels of CYP3A4 and CYP2C19 are modulated by the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) target genes.^{13,14,27-29} On the other hand, efavirenz is known to activate PXR and CAR.^{13,15,30} Therefore, in our study, induction of CYP3A and CYP2C19 could be explained on the basis of these mechanisms. Moreover, the induction of CYP3A-mediated omeprazole sulfoxidation by efavirenz observed in our study is consistent with clinical

observations that efavirenz reduces the plasma concentrations of several CYP3A4 substrates including HMG CoA reductase inhibitors, protease inhibitor antiretrovirals, and antifungals.^{10,17,31-33}

The findings relating to induction of CYP2C19 reported in this study are also supported by recently published *in vivo* data. Efavirenz modestly decreases the exposure of drugs that are predominantly metabolized by CYP2C19, including proguanil, voriconazole, and etravirine.^{17,33-37} In one of these studies, the mean plasma concentrations of voriconazole N-oxide were diminished to a much lesser extent than those of voriconazole when the latter was coadministered with efavirenz.¹⁷ This probably reflects induction of enzymes responsible for the metabolism of voriconazole N-oxide. The authors of the study concluded that the substantial increase in N-oxide metabolite/voriconazole ratio after administration of efavirenz indicates CYP2C19 induction by efavirenz.¹⁷ These findings are consistent with our study wherein a significant decrease in plasma exposure ($AUC_{0-\infty}$) of the 5-hydroxylated metabolite of omeprazole was observed after repeated dosing with efavirenz. The decrease in metabolite exposure supports the possibility that the occurrence of sequential metabolism is also subject to induction by efavirenz. It follows that these induced sequential metabolic pathways of omeprazole underestimate the MRs and therefore also underestimate the extent of induction of CYP2C19 and CYP3A reported in this study.

However, many studies have reported conflicting results about the inducer/inhibitor mixed effects of efavirenz on CYP2C19 activity. Soyinka *et al.* showed that the coadministration of proguanil (a major substrate of CYP2C19 and a minor substrate of CYP3A4) with efavirenz resulted in an increase in plasma concentrations of the antimalarial drug.³⁸ They also reported a pronounced decrease in the MR (metabolite to proguanil), suggesting that efavirenz inhibits the metabolism of proguanil.³⁸ In addition, *in vitro* data generated using human liver microsomes showed that efavirenz was a moderate competitive inhibitor of CYP2C19 (half maximal inhibitory concentration (IC_{50}), 16 μ mol/l) and CYP3A4 (IC_{50} , 17–20 μ mol/l).¹² Consequently, the design of our study had to take into account a possible competitive inhibition of omeprazole metabolism in response to short-term administration of efavirenz. The log value of the omeprazole hydroxylation index observed in our study after a single dose of efavirenz ranged from –0.33 to 0.8, and none of our healthy subjects had a hydroxylation index <–0.5. These values are slightly higher than those observed in previous studies, in which the majority of the subjects associated with an extensive metabolizer phenotype of CYP2C19 had log values of the omeprazole hydroxylation index ranging from –1.2 to 0.7 (ref. 39) and from –0.9 to 0.6 (ref. 40). We also observed, during the study session involving omeprazole + single-dose efavirenz, that extensive metabolizers of CYP2C19 had a reduced MR of 5-hydroxyomeprazole (AUC metabolite/omeprazole; 0.7) and an increased MR of omeprazole sulfone (0.6) as compared to the values previously reported by Böttiger *et al.* (MR 5-hydroxyomeprazole: 1.38, and MR omeprazole sulfone: 0.43) after a dose of 20 mg omeprazole alone.⁴¹ These findings indicate that a small decrease in CYP2C19 activity was indeed observed in our study after a single dose of efavirenz.

The magnitude of the change observed in the MR of omeprazole sulfone after multiple doses of efavirenz appears greater than that observed for the MR of 5-hydroxyomeprazole. This could be explained in terms of the greater induction effect of efavirenz on CYP3A as compared to CYP2C19 or in terms of the simultaneous inhibition of CYP2C19 activity by efavirenz. In fact, inhibition of CYP2C19 activity by efavirenz could blunt the expected induction of the enzyme. As discussed above, our data suggest that the first dose of efavirenz exercises a small inhibitory effect on CYP2C19; this leads us to propose that our results could represent a mixed effect of induction and inhibition of CYP2C19 activity.

The proton pump inhibitor omeprazole is a chiral drug. In this study, subjects were administered the racemic mixture (50:50) of the two optical isomers R- and S-omeprazole. There is strong evidence that omeprazole metabolism is stereoselective, with differential contributions from CYP2C19 and CYP3A4 toward each enantiomer. Judging from *in vitro* data, the metabolism of R-omeprazole is catalyzed predominantly by CYP2C19 whereas S-omeprazole disposition appears to be less dependent on this enzyme.^{22,42-44} To investigate whether efavirenz modulates the enantiomers differently, a stereoselective analysis for omeprazole and 5-hydroxyomeprazole enantiomers was performed. Our data show that the clearance and AUC ratios, as well as MRs of R- and S-omeprazole, were affected to similar extents after multiple doses of efavirenz. The extents of induction of CYP3A and CYP2C19 were also similar for the two enantiomers. Therefore, efavirenz induction appears to be nonstereoselective.

A wide intersubject variability was observed in the pharmacokinetics of omeprazole in both sessions of the study (single dose and multiple doses of efavirenz). This finding could be partially explained in terms of polymorphisms within *CYP2C19* (and potentially *CYP3A5*) genes. In fact, it is well documented that the 5-hydroxylation of omeprazole co-segregates with genetic polymorphisms in the *CYP2C19* gene. The influence of *CYP3A5* polymorphisms on omeprazole disposition remains controversial, but there is evidence to show that omeprazole sulfoxidation is well correlated with CYP3A activity and with oral clearance of midazolam.⁴⁵⁻⁴⁷ In addition, the considerable variation in the degree of induction of omeprazole metabolism by efavirenz could be explained on the basis of genetic polymorphisms in *CYP2C19* and *CYP3A5*. Consequently, some subjects with low CYP2C19 activity could be less affected by the inhibitory action of efavirenz whereas individuals with functional CYP2C19 activity (extensive and intermediate metabolizers) could be more susceptible to the inductive effects of efavirenz. Currently, further analyses are ongoing to examine the role of genetic polymorphisms (*CYP2C19*, *CYP2B6*, and *CYP3A5* variants) and efavirenz exposure on omeprazole metabolism in the two sessions of the study (single dose vs. multiple doses of efavirenz).

In summary, we demonstrated that repeated doses of efavirenz substantially decrease plasma exposure (AUC) and $C_{\max}$ of omeprazole. Changes observed in the omeprazole MRs of 5-hydroxylation and sulfoxidation indicate that efavirenz induces both CYP2C19 and CYP3A. The extent of induction of omeprazole metabolism caused by efavirenz is revealed by the induction ratios of omeprazole apparent oral clearances and the ratios of omeprazole AUC (single dose/multiple doses of efavirenz). Repeated doses of efavirenz caused a moderate decrease in the concentration of 5-hydroxyomeprazole in plasma; the effect on the concentration of omeprazole sulfone in plasma was marginal. This finding may be explained on the basis of a putative inductive effect of efavirenz on sequential metabolism pathways. Our results suggest that efavirenz exhibits a mixed effect characterized by weak inhibition as well as induction of CYP2C19 activity, with the inductive effect dominating with repeated dosing. Therefore, clinically significant pharmacokinetic interactions that may lead to altered efficacy or toxicity may be anticipated when efavirenz is coadministered with drugs that are substrates for CYP3A and/or CYP2C19 (<http://medicine.iupui.edu/clinpharm/ddis/>).

METHODS

Study subjects

Subjects were ascertained to be healthy via physical examination, standard clinical laboratory tests, and medical history. They had an ideal body mass index (20–32 kg/m²); were nonsmokers; had no history of hepatic, renal, or heart diseases; had no history of or current psychiatric illnesses; had no history of gastrointestinal disorders; had experienced no serious infection within the past month; and were taking no medications. Women taking oral

contraceptives were excluded. Participants were instructed to consume no grapefruit-containing foods, fruit juices, or alcohol for at least 1 week prior to the start of the study and up to study completion. They were also instructed to refrain from ingesting foods or beverages containing xanthine (e.g., coffee, chocolate, teas, and colas) during the 48 h before the commencement of the study and during the 24 h of inpatient stay.

Although 60 subjects completed both sessions of the study, omeprazole and its metabolites were not measured in 3 of the subjects because the samples collected from them for one session of the study were physically misplaced during analysis. Therefore, the data for only 57 subjects (healthy men and women between the ages of 18 and 55 years) were included in the analysis for this paper. The study protocol was approved by the Indiana University Institutional Review Board. All subjects gave written informed consent. The trial was registered at <http://www.clinicaltrials.gov> (trial identifier NCT00668395).

Study design

The protocol used a two-session sequential design to test the influence of a single oral dose vs. multiple doses of efavirenz on omeprazole metabolism. On day 1 (first inpatient study session, namely, the single-dose efavirenz session), the subjects arrived at the Indiana Clinical Research Center at about 7 AM after an overnight fast. After predose blood collection from an indwelling catheter, the subjects were given a single 600-mg oral dose of efavirenz (Sustiva, Bristol-Myers Squibb, Princeton, NJ) with water on an empty stomach. One hour later, a single 20-mg oral dose of racemic omeprazole (Omeprazole, Kremers Urban, a division of Scharwz Pharma, Mequon, WI) was administered. Blood samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, and 24 h after efavirenz dosing. On day 7, the subjects started taking efavirenz (600 mg/day orally) in the evenings for 16 consecutive days (days 7 to 23). On the morning of day 24, they were admitted to the research center unit once more and underwent the same procedures as on day 1.

Plasma samples were separated by centrifugation for 20 min at 3,000 rpm within an hour of blood collection and stored at -80°C until analysis. After the 24-h blood sampling, the subjects were discharged.

Analytical methods

Plasma extraction was performed using a validated method developed in our laboratory. In brief, 0.5 ml of 25 mmol/l sodium chloride (pH 7.5) was added to 500 μl of plasma and the mixture was extracted with 6 ml of ethyl acetate and placed in a shaker for 5 min. After centrifugation, the organic phase was evaporated to dryness and reconstituted in 100 μl of 20 mmol/l ammonium acetate (pH 6.5):acetonitrile (95:5; vol/vol). Of this, 50 μl was injected onto the high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) system. The percentage of recovery for omeprazole (racemic, and enantiomers) was $> 90\%$, whereas that of its metabolites was $> 74\%$.

Plasma concentrations of R- and S-omeprazole, R- and S-5-hydroxyomeprazole, and omeprazole sulfone were measured using a new method of MS/MS coupled with chiral HPLC. R-lansoprazole was used as an internal standard. In brief, we used an API 2000 MS/MS triple quadrupole system (Applied Biosystems, Foster City, CA) equipped with a turbo ion spray coupled with a Shimadzu (Columbia, MD) HPLC system consisting of an LC-20AB pump and an SIL-20A HT autosampler, all controlled by Analyst 1.4.2 software (Applied Biosystems/MDS Sciex, Foster City, CA) in conjunction with Windows 2000 (Microsoft, Redmond, WA). Chiral-phase chromatography was achieved on a Chiral-AGP column, 150×4.6 mm, 5 μm , 100 Å, (Chiral Technologies West Chester, PA) equipped with a Chiral-AGP guard column, 10×4.0 mm. A gradient elution profile was used (flow

0.5 ml/min); the initial mobile phase consisted of ammonium acetate (20 mmol/l, pH = 6.5):acetonitrile (95:5; vol/vol), whereas the secondary mobile phase contained ammonium acetate (20 mmol/l, pH = 6.5):acetonitrile (10:90; vol/vol). The selected reaction-monitoring transitions of the precursor ions to selected product ions, monitored for the analytes and internal standard, were omeprazole, m/z 346.12/198; 5-hydroxyomeprazole, m/z 362.13/214.10; omeprazole sulfone, m/z 362.13/150; and R-lansoprazole m/z 370.25/252.30. The limits of quantification for omeprazole (racemic, and enantiomers), 5-hydroxyomeprazole (racemic and enantiomers), and omeprazole sulfone were 0.1 ng/ml, 0.5 ng/ml, and 1 ng/ml, respectively. For omeprazole and 5-hydroxyomeprazole, the interday and intraday coefficient of variation values were <8%, and that of omeprazole sulfone was <12%.

Chemicals

Racemic omeprazole sodium, R-omeprazole, S-omeprazole, R- and S-5-hydroxyomeprazole, omeprazole sulfone, and R-lansoprazole were purchased from Toronto Research Chemicals (North York, Ontario, Canada). The other chemicals used were of the highest quality commercially available.

Pharmacokinetic analyses

Pharmacokinetic parameters were calculated using noncompartmental analysis (Kinetica 5.0 software, Thermo, Philadelphia, PA). Plasma concentrations vs. time (AUCs) were calculated using the mixed log-linear trapezoidal method. Apparent oral clearance was calculated as dose/AUC. Two phenotypic measurements were used to determine the phenotype of CYP2C19 or CYP3A activity, using omeprazole as the probe-drug after a single dose and after multiple doses of efavirenz: (i) MR based on the $AUC_{0-\infty}$ and (ii) the metabolic indexes measured from a single plasma sample taken 3 h after the dose. MRs of omeprazole to its 5-hydroxy- and sulfone metabolites and those of R- and S-omeprazole to their R- or S-5-hydroxymetabolites were determined using $AUC_{0-\infty}$ (AUC total).

$$\text{MR hydroxylation} = \frac{AUC_{0-\infty} \text{omeprazole}}{AUC_{0-\infty} 5\text{-hydroxyomeprazole}}$$

$$\text{MR sulfoxidation} = \frac{AUC_{0-\infty} \text{omeprazole}}{AUC_{0-\infty} \text{omeprazole sulfone}}$$

The omeprazole metabolic index (MI) (comprising omeprazole hydroxylation index and omeprazole sulfoxidation index) was defined as the log of the plasma concentration ratio of omeprazole to its metabolite, determined from a plasma sample collected 3 h after drug administration. As described previously, the MI value, calculated from the concentration in a single plasma sample taken 3 h after intake of 20 mg of omeprazole, can be reliably used to phenotype both CYP2C19 (5-hydroxylation) and CYP3A (sulfoxidation) activity.^{40,45,48-50}

$$\text{MI hydroxylation} = \log_{10} \frac{\text{omeprazole}_{3\text{hours}}}{5\text{-hydroxyomeprazole}_{3\text{hours}}}$$

$$\text{MI sulfoxidation} = \log_{10} \frac{\text{omeprazole}_{3\text{hours}}}{5 - \text{omeprazole sulfone}_{3\text{hours}}}$$

The induction ratio was determined as MR (single dose)/MR (multiple doses of efavirenz).

Statistical analyses

The values of the pharmacokinetic variables are reported as mean values $\pm$ SD in the text, and geometric means with 95% confidence intervals are presented as Supplemental Tables 1 and 2. Data were analyzed using the paired *t*-test and Wilcoxon-matched pair test, and by means of one-way analysis of variance and the Kruskal–Wallis test, using GraphPad Prism 5 (GraphPad Software, La Jolla, CA). Differences were regarded as statistically significant when *P* values were <0.05 .

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project was supported by awards R01GM078501 and 3R01GM078501-04S1, and R56 grant 2R56GM067308-09A1 from the National Institute of General Medical Sciences, National Institutes of Health (NIH) and by award M01-RR00750 from the National Center for Research Resources, a component of the NIH. V.M. is the recipient of a studentship from the Canadian Institutes of Health Research.

The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the NIH.

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Figure 1. Individual metabolic ratio for the hydroxylation and sulfoxidation metabolism pathways of omeprazole (OMP). *Statistically significant changes calculated using two-tailed Student's paired *t*-test; statistically significant changes calculated using Wilcoxon signed-rank test are shown in parentheses.

Figure 2. Cumulative frequency distributions of the omeprazole metabolic indexes for (a) the hydroxylation index and (b) the sulfoxidation index of omeprazole.

Figure 3. Concentrations of omeprazole enantiomers in plasma **(a)** after a single dose of efavirenz and **(b)** after multiple doses of efavirenz.

Table 1
Pharmacokinetic parameters for plasma omeprazole, 5-hydroxyomeprazole, and omeprazole sulfone after a single oral dose or multiple doses of efavirenz

	Single dose of EFV	Multiple doses of EFV	Ratio ^a	P value ^b
Omeprazole				
$C_{\max}$ (nmol/l)	704 ± 478	495 ± 428	2.31 ± 2.69	0.0002
Elimination $t_{1/2}$ (h)	1.1 ± 0.7	1.2 ± 1.1	1.23 ± 0.70	0.4
AUC _{0→∞} (nmol · h/l)	1843 ± 1857	988 ± 905	2.06 ± 1.02	<0.0001
CL/F (l/h)	56 ± 48	99 ± 80	0.62 ± 0.36	<0.0001
CL/F/kg (l/h/kg)	0.76 ± 0.66	1.4 ± 1.2	0.62 ± 0.36	<0.0001
R-omeprazole				
$C_{\max}$ (nmol/l)	266 ± 191	197 ± 174	2.30 ± 3.02	0.002
Elimination $t_{1/2}$ (h)	1.3 ± 1.0	1.1 ± 0.8	1.47 ± 1.02	0.1
AUC _{0→∞} (nmol · h/l)	742 ± 967	414 ± 466	2.01 ± 1.09	<0.0001
CL/F (l/h)	78 ± 75	133 ± 118	0.68 ± 0.48	<0.0001
CL/F/kg (l/h/kg)	1.1 ± 1.0	1.8 ± 1.6	0.68 ± 0.48	<0.0001
S-omeprazole				
$C_{\max}$ (nmol/l)	359 ± 524	235 ± 382	2.40 ± 2.94	0.001
Elimination $t_{1/2}$ (h)	1.1 ± 0.6	1.1 ± 0.9	1.23 ± 0.65	0.4
AUC _{0→∞} (nmol · h/l)	1104 ± 939	576 ± 477	2.15 ± 1.19	<0.0001
CL/F (l/h)	44 ± 37	87 ± 90	0.60 ± 0.32	<0.0001
CL/F/kg (L/h/kg)	0.62 ± 0.52	1.2 ± 1.3	0.60 ± 0.32	<0.0001
5-Hydroxyomeprazole				
$C_{\max}$ (nmol/l)	271 ± 147	257 ± 166	1.58 ± 1.80	0.4
Elimination $t_{1/2}$ (h)	1.7 ± 0.8	1.5 ± 0.7	1.26 ± 0.69	0.2
AUC _{0→∞} (nmol · h/l)	818 ± 288	619 ± 299	1.50 ± 0.65	<0.0001
R-5-hydroxyomeprazole				
$C_{\max}$ (nmol/l)	246 ± 119	227 ± 155	1.57 ± 1.79	0.2
Elimination $t_{1/2}$ (h)	1.7 ± 0.9	1.5 ± 0.7	1.23 ± 0.59	0.1
AUC _{0→∞} (nmol · h/l)	734 ± 278	536 ± 213	1.49 ± 0.59	<0.0001
S-5-hydroxyomeprazole				
$C_{\max}$ (nmol/l)	30 ± 17	30 ± 30	1.76 ± 2.05	0.1
Elimination $t_{1/2}$ (h)	1.5 ± 0.8	1.2 ± 0.7	1.37 ± 1.14	0.05
AUC _{0→∞} (nmol · h/l)	87 ± 42	58 ± 31	1.77 ± 1.03	<0.0001
Omeprazole sulfone				
$C_{\max}$ (nmol/l)	255 ± 194	382 ± 324	0.89 ± 0.79	0.0005
Elimination $t_{1/2}$ (h)	3.3 ± 8.7	1.6 ± 0.9	2.19 ± 3.52	0.001
AUC _{0→∞} (nmol · h/l)	1435 ± 1408	1351 ± 1487	1.17 ± 0.69	0.6

Mean values are displayed $\pm$ SD.

AUC, area under the plasma concentration-time curve; CL/F, apparent oral clearance, $C_{\max}$, maximal plasma concentration; EFV, efavirenz; $t_{1/2}$, half-life.

^aRatio; single dose/multiple doses of efavirenz.

^b*P* value; statistically significant changes between pharmacokinetic parameters obtained after single dose as compared with multiple doses of efavirenz were calculated using two-tailed Student's paired *t*-test.

Table 2
Induction ratios of hydroxylation and sulfoxidation of omeprazole and of its R- and S-enantiomers

Metabolic pathway	Induction ratio ^a
5-Hydroxyomeprazole	1.44 ± 0.67
Omeprazole sulfone	1.97 ± 0.81 ^b
R-5-hydroxyomeprazole	1.41 ± 0.78
S-5-hydroxyomeprazole	1.36 ± 0.64

Mean values are displayed ±SD.

AUC, area under the plasma concentration-time curve.

^a Induction ratios were determined using the ratio of omeprazole $AUC_{0 \rightarrow \infty}$ to omeprazole metabolite $AUC_{0 \rightarrow \infty}$ after a single dose of efavirenz divided by the ratio of omeprazole $AUC_{0 \rightarrow \infty}$ to omeprazole metabolite $AUC_{0 \rightarrow \infty}$ after multiple doses of efavirenz.

^b Induction ratios were compared using one-way analysis of variance (Kruskal-Wallis with Dunn's multiple comparison). Induction ratio of sulfoxidation is significantly higher than hydroxylation induction ratios.