Efavirenz-mediated induction of omeprazole metabolism is CYP2C19 genotype dependent

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Efavirenz increases CYP2C19- and CYP3A-mediated omeprazole metabolism. We hypothesized that CYP2C19 and CYP2B6 genetic polymorphisms influence the extent of induction of omeprazole metabolism by efavirenz. Healthy subjects (n = 57) were administered a single 20 mg oral dose of omeprazole on two occasions: with a single 600 mg efavirenz dose; and after a 17-day treatment with efavirenz (600 mg per day). DNA was genotyped for CYP2C19*2, *3 and *17 alleles and CYP2B6*6, *4 and *9 alleles using Taqman assays. Omeprazole, its enantiomers and metabolites were measured by liquid chromatography/tandem mass spectrometry. Our results showed that efavirenz increased omeprazole clearances in all CYP2C19 genotypes in non-stereoselective manner, but the magnitude of induction was genotype dependent. Metabolic ratios of 5-hydroxylation of omeprazole were reduced in extensive and intermediate metabolizers of CYP2C19 (P < 0.05). No significant associations were observed between CYP2B6 genotypes and induction by efavirenz on omeprazole metabolism. Our data indicate how interplays between drug interactions and CYP2C19 genetic variations may influence systemic exposure of CYP2C19 substrates.

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INTRODUCTION

Efavirenz-based regimens are often the preferred first-line therapy in naive HIV-infected patients. Complex clinically relevant drug–drug interactions associated with efavirenz are of major concern. Efavirenz is primarily metabolized by CYP2B6 into 8-hydroxyefavirenz and to a lesser extent via pathways involving CYP2A6, CYP3A4/5 and UGT2B7.1–5 Upon repeated administration, efavirenz enhances its own metabolism primarily through induction of CYP2B6 and the elimination of many co-administered drugs by upregulating drug metabolizing enzymes co-regulated with CYP2B6, including CYP3As and CYP2C19.6–8 In some cases, short-term and long-term exposures to the drug can produce mixed inhibitory/inductive properties like the one observed with CYP2C19.9 After a single dose of efavirenz, extensive metabolizers (EMs) for CYP2C19 had a reduced metabolic ratio of 5-hydroxyomeprazole compared with those previously reported following administration of omeprazole alone suggesting small competitive inhibition of CYP2C19 activity.8 Consistent with these findings, efavirenz was reported to be a moderate inhibitor of CYP2C19 in vitro, with an IC50 value of 16 μM.9

The proton pump inhibitor omeprazole is widely used in the treatment of various gastric acid-related disorders. Omeprazole is chiral and mainly metabolized by CYP2C19 to 5-hydroxyomeprazole (94 and 27% for the R- and S-omeprazole, respectively) and to omeprazole sulfone by CYP3A4 (2 and 27% for the R- and S-omeprazole, respectively).10,11 Thus, omeprazole metabolism has been suggested as a dual probe of CYP2C19 and CYP3A activities. CYP2C19 activity is highly variable among individuals, mainly due to genetic and non-genetic factors including drug interactions. The CYP2C19 gene is highly polymorphic; currently, 28 alleles have been identified (http://www.cypalleles.ki.se/cyp2c19.htm), exhibiting interethnic differences in frequencies. The most frequent alleles contributing to the majority of poor metabolizer (PM) phenotype are CYP2C19*2 and CYP2C19*3.12 The variant allele CYP2C19*17, which is more common in Caucasians than in other ethnic population, is associated with increased CYP2C19-dependent metabolism of drugs such as omeprazole, voriconazole, escitalopram and clopidogrel; some authors assigned carriers of the CYP2C19*17 allele as ultra-rapid metabolizer (UM).13–17

Recently, we reported that repeated administration of efavirenz to healthy volunteers enhances CYP2C19- and CYP3A-catalyzed omeprazole metabolism, but the extent of this induction varies widely among subjects.6 Literature evidence suggests that genetic variants in CYPs influence the extent of inhibition drug interactions18 as well as drug–drug interactions caused through induction of CYPs, including those involving CYP2C19,19–22 although the later has been generally less studied. We reasoned that variants in the CYP2C19 gene may contribute to the variable induction by efavirenz of CYP2C19 and CYP3A8. Assuming positive relationship between plasma efavirenz exposure and enzymatic induction, it is also conceivable that CYP2B6 genetic variations known to affect efavirenz plasma exposure23–27 influence the extent of induction of these enzymes.

The main objective of this study was to determine the impact of single dose vs multiple doses of efavirenz on the pharmacokinetics of omeprazole and its metabolites in relation to CYP2C19 and CYP2B6 genotypes. Since omeprazole is administered as a racemic mixture to probe CYP2C19 (and CYP3A) activity and its metabolism is stereoselective,10,11 we also investigated whether the extent of induction of omeprazole metabolism by efavirenz occurs in a stereoselective manner among the CYP2C19 genotype groups.
MATERIALS AND METHODS

Study design

Sixty healthy subjects were enrolled in this sequential open-label design where subjects received oral racemic omeprazole (20 mg) on two occasions: with a single 600 mg efavirenz dose; and after a 17-day treatment with efavirenz (600 mg per day). Blood samples were obtained from which plasma and genomic DNA were prepared for pharmacokinetic and genotyping analyses, respectively. The study protocol was approved by the Indiana University Institutional Review Board. All subjects gave written informed consent. Subjects were judged healthy based on medical history, physical examinations and routine laboratory tests during the screening. Details of the protocol are provided in Supplementary Material.8

CYP2C19 and CYP2B6 genotyping

Genomic DNA was extracted from whole blood with the Qiagen DNA MiniKit (Qiagen, Valencia, CA, USA). Genotyping for CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893), CYP2C19*17 (rs12248560), CYP2B6*9 (516G>T, rs3745274) and CYP2B6*4 (785A>G, rs2279343) was performed by TaqMan Assay-Reagents Allelic Discrimination Kits (Applied Biosystems, Foster City, CA, USA) according to supplier’s instructions. The two CYP2B6 SNPs (785A>G and 516G>T) together form the CYP2B6*6 allele.

Analytical methods

Plasma concentrations of R- and S-omeprazole, R- and S-5-hydroxyomeprazole, and omeprazole sulfone were measured using a chiral high-performance liquid chromatography tandem mass-spectrometric method as described previously.8

Pharmacokinetic and data analyses

Pharmacokinetic parameters were calculated using a non-compartmental analysis method (Kinetica 5.0 Software; Thermo Fisher Scientific, Waltham, MA, USA). Omeprazole metabolic index (omeprazole hydroxylation index and omeprazole sulfoxidation index) was defined as log of plasma concentration ratio of omeprazole to its respective metabolite, determined in a plasma sample collected 3 h after drug administration. Metabolic ratios of omeprazole to its sulfone and metabolic ratios to its 5-hydroxyomeprazole were determined using area under the curve (AUC) ratio.

Pharmacokinetic data are reported as mean ± s.d. Data were analyzed by the paired t-test, Wilcoxon-matched paired test, and by one-way analysis of variance (ANOVA) with Dunn’s comparison and Kruskal–Wallis test as appropriate using GraphPad Prism (Graph Pad Software, La Jolla, CA, USA). P-values <0.05 were regarded as statistically significant. The sample size was calculated with a power of 95% and a two-sided level of significance at 0.05 to detect a difference of 30% in omeprazole CL (single vs multiple doses of efavirenz) for the same subjects (paired analysis) within each CYP2C19 genotype.

RESULTS

Genotyping and demographic characteristics

Of the total 60 subjects who completed the entire study, data from 57 subjects were included in the final analysis. Samples from three subjects were excluded from the analysis due to missing samples in one phase.

The genotype frequencies of CYP2C19 and CYP2B6 were all in Hardy–Weinberg equilibrium (Supplementary Table S1). The CYP2C19 genotypes were grouped based on genotype-predicted phenotypes to UM (CYP2C19*17/*17; n = 4), EM (CYP2C19*1/*1, n = 18; and CYP2C19*1/*17, n = 15), intermediate metabolizer (IM: CYP2C19*1/*2 or *2/*17, n = 18, CYP2C19*1/*3, n = 1) or PM (CYP2C19*2/*2, n = 1). Similarly, CYP2B6 genotypes were categorized as slow metabolizer (CYP2B6*6/*6, n = 5), IM (CYP2B6*1/*6 or *4/*9, n = 14; and CYP2B6*4/*6, n = 1) and normal metabolizer (CYP2B6*1/*1, n = 34; and CYP2B6*1/*4, n = 3).

There was no statistically significant differences (P > 0.05) in any of the demographic characteristics of the volunteers either among the CYP2C19 genotype groups (Table 1) or among the CYP2B6 genotype groups (Supplementary Table S2).

CYP2C19 and CYP3A phenotype frequency distributions of omeprazole administered with a single dose and multiple doses of efavirenz

The metabolic indexes for the hydroxylation and sulfoxidation of omeprazole after a single dose and multiple doses of efavirenz exhibit a large inter-individual variability (Figure 1). The log metabolic index for the hydroxylation of racemic- and R-omeprazole exhibited bimodal distribution (Figures 1a and b); no such bimodal distribution of the log metabolic index for the hydroxylation of S-omeprazole was observed (Figure 1c), probably due to preferential elimination of S-omeprazole through CYP2C19-mediated O-demethylation as opposed to R-omeprazole, which is mainly cleared through CYP2C19-mediated S-hydroxylation.10,11 The log metabolic index for the sulfoxidation of omeprazole reflecting the distribution of CYP3A activity is illustrated in Figure 1d.

Table 1. Demographic characteristics according to CYP2C19 genotypes

| CYP2C19 genotypes | P-value |
|-------------------|---------|
| UM (CYP2C19*17/*17) |         |
| Number of subjects | 4       | 33      | 19 | 1 |
| Gender (male/female) | 2/2     | 22/11   | 11/8 | 1/0 |
| Age (years) | 34.0 ± 12.3 | 27.1 ± 9.6 | 30.2 ± 9.8 | 18 | 0.15 |
| Weight (kg) | 74.5 ± 7.7 | 74.4 ± 15.5 | 74.7 ± 11.4 | 69.6 | 0.96 |
| BMI | 24.5 ± 4.4 | 23.9 ± 4.1 | 24.8 ± 3.9 | 22 | 0.61 |

| Ethnicity |         |
|-----------|---------|
| Caucasians | 3       | 27      | 13 | 1 |
| Black-Americans | 1       | 5       | 5 | 0 |
| Asians | 0       | 1       | 1 | 0 |

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer.
Figure 1. CYP2C19 phenotype frequency distribution of the log metabolic indexes (MI) of hydroxylation (a–c) and sulfoxidation (d) of omeprazole after a single 600 mg oral dose of efavirenz (EFV) or during induction by multiple doses (600 mg per day for 17 days) of EFV. Racemic (a), R-omeprazole (b) and S-omeprazole (c) hydroxylation MI are presented. The histograms were plotted using the log of omeprazole concentration divided by the metabolite concentration (5-hydroxyomeprazole or omeprazole sulfone) taken 3 h after the administration of omeprazole. The probit plots were obtained by curve fitting of the log of omeprazole hydroxylation and sulfoxidation MI from 57 subjects.
Effect of efavirenz on associations of CYP2C19 genotypes with the metabolism of racemic, R- and S-omeprazole

CYP2C19 genotypes are key determinants of omeprazole metabolism when omeprazole is given alone. We assessed whether this CYP2C19 genotype–phenotype relationship is affected in the presence of efavirenz. When omeprazole was administered with a single dose of efavirenz, the elimination parameters of racemic-, R- and S-omeprazole were dependent on CYP2C19 genotypes (Table 2a; Table 3a; Figure 2). The clearance of racemic omeprazole was on average 4.4-fold and 2.2-fold lower in IM compared with UM and EM of CYP2C19, respectively (P < 0.001). Similarly, the clearances of R-omeprazole were significantly higher in UM and EM (by 5.7- and 2.2-fold, respectively) than IM of CYP2C19 (P < 0.001). The clearances of the S-enantiomer in IM group were lower by 3.5 and 2.1 times than that of UM and EM, respectively (P = 0.003).

Multiple doses of efavirenz significantly decreased the AUC of omeprazole and its enantiomers (Table 3b) in all genotypes in comparison with UM and EM (by 5.7- and 2.2-fold, respectively) than IM of CYP2C19 (P < 0.001). The clearances of the S-enantiomer in IM group were lower by 3.5 and 2.1 times than that of UM and EM, respectively (P = 0.003).

CYP2C19 genotype–phenotype associations were also confirmed using metabolic ratios. Pharmacokinetic parameters of racemic-, R-5- and S-5-hydroxyomeprazole and omeprazole sulfone are presented in Tables 2 and 3. Metabolic ratios of omeprazole 5-hydroxylation (AUC$_{R−→}^{−→}$ omeprazole/S-hydroxyomeprazole) were significantly affected by CYP2C19 genotypes in both treatment groups (single and multiple doses of efavirenz) (Table 2b). STereoerective analysis demonstrated that the R- and S-5-hydroxy metabolic ratios of omeprazole were similarly influenced by the CYP2C19 metabolic status (Table 3). For example, the mean metabolic ratio of R-5-hydroxyoomeprazole after a single dose of efavirenz was 4.6 and 2.3 times greater in IM compared with UM and EM groups, respectively (P < 0.01). After multiple dosing of efavirenz, the mean metabolic ratio of R-5-hydroxyoomeprazole was 2.2 and 1.7 times higher in IM than in UM and EM subjects, respectively (P < 0.002). Induction by efavirenz tended to reduce the magnitude of difference between IM and other CYP2C19 genotype groups. As shown in Table 2, CYP2C19 genotypes were significantly associated with the AUC$_{S−→}^{−→}$ of omeprazole sulfone in both treatment groups (single and multiple doses of efavirenz).

The above data were further confirmed using metabolic index (an approach often used in the literature). Analysis performed with the mean log metabolic indexes confirmed the influence of CYP2C19 genotypes on phenotype of omeprazole after a single dose and during induction by efavirenz (Table 4).

Influence of CYP2C19 genotypes on the extent of induction of racemic-, R- and S-omeprazole metabolism by efavirenz

Efavirenz appears to induce omeprazole clearance in relation to CYP2C19 genotype. Compared with the single dose of efavirenz arm, treatment with multiple doses of efavirenz increased the elimination of racemic-, R- and S-omeprazole in EM and IM groups (P < 0.05) (Figures 2a–c, respectively). The percent change in oral clearance of omeprazole following multiple doses compared to single dose of efavirenz are presented (percent changes in clearance

| Table 2. Pharmacokinetic parameters of racemate omeprazole, 5-hydroxyoomeprazole and omeprazole sulfone after a single oral 20 mg dose of omeprazole when co-administered with (a) a single 600 mg oral dose of efavirenz and (b) following multiple doses of efavirenz |
| PK parameters after a single dose of Efavirenz (EFV) | CYP2C19 genotypes |
| | UM | EM | IM | PM |
| (a) Omeprazole racemate | | | | |
| AUC$_{0−→}^{−→}$ (nmol h l$^{-1}$) | 625 ± 345$^a$ | 1284 ± 820$^b$ | 2590 ± 1963$^{ab}$ | 10367 |
| CL/F (l h$^{-1}$) | 131 ± 100$^a$ | 64 ± 42$^b$ | 30 ± 16$^{ab}$ | 5.6 |
| S-hydroxyoomeprazole | | | | |
| AUC$_{0−→}^{−→}$ (nmol h l$^{-1}$) | 649 ± 227 | 847 ± 326 | 826 ± 210 | 412 |
| Metabolic ratio | 0.91 ± 0.28$^a$ | 1.44 ± 0.60$^b$ | 3.32 ± 2.71$^{ab}$ | 25 |
| Omeprazole sulfone | | | | |
| AUC$_{0−→}^{−→}$ (nmol h l$^{-1}$) | 548 ± 321$^a$ | 888 ± 783$^b$ | 2195 ± 1128$^{ab}$ | 8005 |
| Metabolic ratio | 1.22 ± 0.70 | 1.68 ± 0.68 | 1.25 ± 0.58 | 1.30 |
| (b) Omeprazole racemate | | | | |
| AUC$_{0−→}^{−→}$ (nmol h l$^{-1}$) | 337 ± 165$^a$ | 759 ± 482$^b$ | 1241 ± 615$^{ab}$ | 6118 |
| CL/F (l h$^{-1}$) | 210 ± 105$^a$ | 110 ± 82$^b$ | 61 ± 35$^{ab}$ | 9.5 |
| % change of CL | 84 ± 132$^a$ | 93 ± 100$^{b}$ | 116 ± 79$^{b}$ | 69% |
| S-hydroxyoomeprazole | | | | |
| AUC$_{0−→}^{−→}$ (nmol h l$^{-1}$) | 342 ± 97 | 658 ± 343 | 617 ± 221 | 535 |
| Metabolic ratio | 0.96 ± 0.26$^a$ | 1.18 ± 0.56$^{b}$ | 2.06 ± 0.88$^{b}$ | 11.4 |
| Omeprazole sulfone | | | | |
| AUC$_{0−→}^{−→}$ (nmol h l$^{-1}$) | 534 ± 304$^a$ | 967 ± 776$^b$ | 1692 ± 891$^{ab}$ | 10238 |
| Metabolic ratio | 0.65 ± 0.15 | 0.87 ± 0.39 | 0.85 ± 0.55 | 0.60 |

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CL/F, apparent oral clearance; EFV, efavirenz; EM, extensive metabolizer; IM, intermediate metabolizer; PK, pharmacokinetic; PM, poor metabolizer; UM, ultra-rapid metabolizer.

Percent changes in oral clearance of omeprazole following multiple doses compared to single dose of efavirenz are presented (percent changes in clearance are significantly affected by efavirenz in EM and IM of CYP2C19). The metabolic ratio was determined using the ratio of AUC$_{S−→}$ omeprazole/AUC$_{R−→}$ omeprazole metabolite P-value; comparison between genotype groups using ANOVA with Dunn’s multiple comparison analysis, P < 0.05. $^a$UM vs IM, $^b$EM vs IM.
clearance (from single to multiple doses of efavirenz) of racemic omeprazole was statistically significantly in EM (93%) and IM (116%) groups (P < 0.05); the same tendency was noted with the R- and S-enantiomers of omeprazole (Table 3b). Although an enhanced clearance of omeprazole was observed in UM subjects following multiple dosing of efavirenz (percent change of 84%; Table 2b), this difference did not reach a statistical significance level. Higher clearance was also observed in the sole PM of CYP2C19 with percent changes ranging from 62 to 79% (Figure 2; Table 2). This observation suggests that efavirenz enhances clearance in the PM subject through induction of other non-CYP2C19 elimination pathways of omeprazole. The change in clearance of R-omeprazole (single vs multiple doses of efavirenz) within each genotype group was not different from the change observed for S-omeprazole, indicating that the overall induction of omeprazole metabolism by efavirenz was not enantioselective.

The extent of induction in each genotype group was evaluated by comparing the metabolic ratios (Table 3) after multiple doses of efavirenz with those after a single dose. Multiple doses of efavirenz significantly reduced the metabolic ratios of 5-hydroxylation in EM (−23 ± 48%, P = 0.0002) and in IM (−31 ± 23%, P = 0.0004) of CYP2C19. This ratio was also reduced in UM, but it did not reach a statistically significant level. In the PM subject, the metabolic ratio of 5-hydroxylation was decreased by −52% following multiple doses of efavirenz compared with a single dose. Again, these data indicate that efavirenz induces an alternative pathway(s) of omeprazole elimination or the rate of sequential metabolism. Of note, the genotype groups associated with lower CYP2C19 activity tended to be associated with higher induction of CYP2C19 activity.

In Table 4, the mean log metabolic ratios for the 5-hydroxylation and the sulfoxidation metabolic indexes within EM and IM of CYP2C19. The metabolic ratio was determined using the ratio of AUCₐ₀-ₐ₋ of omeprazole/AUCₐ₀-ₐ₋ of omeprazole metabolite P-value; comparison between genotype groups using ANOVA with Dunn's multiple comparison analysis, P < 0.05. *UM vs IM. aEM vs IM.

### Table 3. Pharmacokinetic parameters of R- and S-omeprazole and R- and S-5-hydroxyomeprazole are presented after a single oral 20-mg dose of omeprazole when co-administered with (a) a single 600 mg oral dose of efavirenz and (b) following multiple doses of efavirenz.

| PK parameters after a single dose of EFV | CYP2C19 genotypes |
|--------------------------------------|------------------|
|                                      | UM   | EM   | IM   | PM   |
| (a) R-Omeprazole                     |      |      |      |      |
| AUCₐ₀-ₐ₋ (nmol h⁻¹)                  | 189 ± 103ᵃ | 465 ± 292ᵇ | 1059 ± 956ᵃᵇ | 5895 |
| CL/F (l h⁻¹)                         | 219 ± 177ᵃ | 85 ± 54ᵇ | 39 ± 21ᵃᵇ | 4.9 |
| S-Omeprazole                         |      |      |      |      |
| AUCₐ₀-ₐ₋ (nmol h⁻¹)                  | 451 ± 239ᵃ | 831 ± 548ᵇ | 1536 ± 1050ᵃᵇ | 4562 |
| CL/F (l h⁻¹)                         | 87 ± 61ᵃ | 52 ± 37ᵇ | 25 ± 13ᵃᵇ | 6.4 |
| R-5-hydroxyomeprazole                |      |      |      |      |
| AUCₐ₀-ₐ₋ (nmol h⁻¹)                  | 588 ± 198 | 759 ± 322 | 743 ± 191 | 352 |
| Metabolic ratio                      | 0.33 ± 0.13ᵃ | 0.66 ± 0.44ᵇ | 1.55 ± 1.50ᵃᵇ | 16.7 |
| S-5-hydroxyomeprazole                |      |      |      |      |
| AUCₐ₀-ₐ₋ (nmol h⁻¹)                  | 66 ± 29 | 79 ± 35 | 108 ± 50 | 82 |
| Metabolic ratio                      | 6.6 ± 2.9ᵃ | 10.1 ± 4.1 | 16.7 ± 12.1ᵃ | 55 |
| (b) R-Omeprazole                     |      |      |      |      |
| AUCₐ₀-ₐ₋ (nmol h⁻¹)                  | 108 ± 62ᵃ | 298 ± 179ᵇ | 516 ± 303ᵃᵇ | 3293 |
| CL/F (l h⁻¹)                         | 356 ± 214ᵃ | 142 ± 100ᵇ | 77 ± 45ᵃᵇ | 8.8 |
| % change of CL                       | 78 ± 52% | 87 ± 112ᵇ | 113 ± 93ᵇ | 79% |
| S-Omeprazole                         |      |      |      |      |
| AUCₐ₀-ₐ₋ (nmol h⁻¹)                  | 237 ± 107ᵃ | 480 ± 375ᵇ | 705 ± 329ᵃᵇ | 2825 |
| CL/F (l h⁻¹)                         | 144 ± 65ᵃ | 102 ± 106ᵇ | 54 ± 33ᵃᵇ | 10.3 |
| % change of CL                       | 84 ± 121% | 111 ± 118ᵇ | 127 ± 86ᵇ | 61% |
| R-5-hydroxyomeprazole                |      |      |      |      |
| AUCₐ₀-ₐ₋ (nmol h⁻¹)                  | 308 ± 88 | 552 ± 217 | 563 ± 206 | 428 |
| Metabolic ratio                      | 0.42 ± 0.2ᵃ | 0.55 ± 0.2ᵇ | 0.93 ± 0.5ᵃᵇ | 7.7 |
| S-5-hydroxyomeprazole                |      |      |      |      |
| AUCₐ₀-ₐ₋ (nmol h⁻¹)                  | 39 ± 13 | 57 ± 31 | 61 ± 30 | 106 |
| Metabolic ratio                      | 6.2 ± 2.2 | 8.7 ± 4.9 | 14.0 ± 8.8 | 27 |

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CL/F, apparent oral clearance; EFV, efavirenz; EM, extensive metabolizer; IM, intermediate metabolizer; PK, pharmacokinetic; PM, poor metabolizer; UM, ultra-rapid metabolizer.

Percent changes in oral clearance of omeprazole following multiple doses compared to single dose of efavirenz are presented (percent changes in clearance are significantly affected by efavirenz in EM and IM of CYP2C19). The metabolic ratio was determined using the ratio of AUCₐ₀-ₐ₋ of omeprazole/AUCₐ₀-ₐ₋ of omeprazole metabolite P-value; comparison between genotype groups using ANOVA with Dunn's multiple comparison analysis, P < 0.05. *UM vs IM.ᵃEM vs IM.
The effects of efavirenz on omeprazole metabolism could be time-dependent manner.\textsuperscript{6,28–30} We tested whether the inductive hypothesis that clearance, metabolic ratio and metabolic index) to test the inductive effect of efavirenz in all genotype groups in non-stereoselective manner and this finding concur with a similar trend observed by Baldwin et al.\textsuperscript{15} The lack of association between CYP2C19 genotypes and 5-hydroxyomprazole exposure may be in part explained by the sequential metabolism of 5-hydroxyomprazole and the mixed inhibitor/inducer properties of efavirenz on CYP2C19 activity.\textsuperscript{8} Our data show that the AUCAUC\textsubscript{EM,\textit{CYP2C19}*17} of omeprazole sulfone in EM was 1.7-fold higher than in UM of CYP2C19, consistent with a previous study showing no statistical difference in AUCAUC\textsubscript{EM} for this metabolite between the CYP2C19*17*17 and CYP2C19*11*11 groups.\textsuperscript{15} A trend toward lower AUCAUC\textsubscript{EM,\textit{CYP2C19}*17} of 5-hydroxyomprazole in UM of CYP2C19 compared with EM or IM groups after a single dose and multiple doses of efavirenz (present study) and this finding concur with a similar trend observed by Baldwin et al.\textsuperscript{15} The lack of association between CYP2C19 genotypes and 5-hydroxyomprazole exposure may be in part explained by the sequential metabolism of 5-hydroxyomprazole and the mixed inhibitor/inducer properties of efavirenz on CYP2C19 activity.\textsuperscript{8}

We found that the genotype–phenotype association is essentially maintained in the presence of efavirenz (single and multiple doses) as supported by the: (1) significant associations of CYP2C19 genetic variants and omeprazole elimination; (2) frequency distribution histogram and probit plots of the hydroxylations indexes of omeprazole showing bimodality with an antimode of ~0.6–0.8, consistent with previous reports;\textsuperscript{31,32} (3) significantly higher metabolic ratios of omeprazole hydroxylation in IM compared with EM and UM of CYP2C19, consistent with previous data showing a gene-dose effect of CYP2C19 genotypes on metabolic ratios of omeprazole 5-hydroxylation;\textsuperscript{15} and (4) analysis of the log of omeprazole hydroxylation index. Omeprazole is mainly cleared by CYP2C19-mediated 5-hydroxylation. However, we found no influence of CYP2C19 genotype on the AUCAUC\textsubscript{EM} of 5-hydroxyomprazole, consistent with a previous study showing no statistical difference in AUCAUC\textsubscript{EM} for this metabolite between the CYP2C19*17*17 and CYP2C19*11*11 groups.\textsuperscript{15} A trend toward lower AUCAUC\textsubscript{EM} of 5-hydroxyomprazole in UM of CYP2C19 compared with EM or IM groups after a single dose and multiple doses of efavirenz (present study) and this finding concur with a similar trend observed by Baldwin et al.\textsuperscript{15} The lack of association between CYP2C19 genotypes and 5-hydroxyomprazole exposure may be in part explained by the sequential metabolism of 5-hydroxyomprazole and the mixed inhibitor/inducer properties of efavirenz on CYP2C19 activity.\textsuperscript{8}

Table 4. Mean log of metabolic indexes of 5-hydroxylation (racemic-, R- and S-omeprazole) and sulfoxidation of omeprazole according to the CYP2C19 genotypes

| CYP2C19 genotypes | Log of metabolic index |
|-------------------|------------------------|
|                   | Single dose of EFV\textsuperscript{a} | Multiples doses of EFV\textsuperscript{b} | P-value\textsuperscript{\textit{c}} |
| 5-Hydroxylation   |                        |                      |                           |
| UM                | – 0.10 ± 0.29          | – 0.11 ± 0.09        | 0.9                      |
| EM                | 0.20 ± 0.23            | 0.002 ± 0.26         | 0.0003                   |
| IM                | 0.45 ± 0.31            | 0.18 ± 0.18          | 0.002                    |
| PM                | 1.52                   | 1.05                 |                          |
| Sulfoxidation     |                        |                      |                           |
| UM                | – 0.03 ± 0.49          | – 0.31 ± 0.23        | 0.4                      |
| EM                | 0.39 ± 0.31            | – 0.18 ± 0.27        | 0.0001                   |
| IM                | 0.28 ± 0.39            | – 0.15 ± 0.40        | 0.003                    |
| PM                | 0.59                   | 0.01                 |                          |
| R-5-Hydroxylation OMP |                    |                      |                           |
| UM                | – 0.55 ± 0.31          | – 0.54 ± 0.18        | 1.0                      |
| EM                | – 0.22 ± 0.27          | – 0.38 ± 0.29        | 0.004                    |
| IM                | 0.08 ± 0.35            | – 0.11 ± 0.21        | 0.01                     |
| PM                | 1.35                   | 0.89                 |                          |
| S-5-Hydroxylation OMP |                    |                      |                           |
| UM                | 0.79 ± 0.27            | 0.72 ± 0.07          | 0.6                      |
| EM                | 1.03 ± 0.22            | 0.84 ± 0.28          | 0.002                    |
| IM                | 1.19 ± 0.30            | 1.05 ± 0.28          | 0.002                    |
| PM                | 1.76                   | 1.32                 |                          |

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; EFV, efavirenz; EM, extensive metabolizer; IM, intermediate metabolizer; OMP, omeprazole; PK, pharmacokinetic; PM, poor metabolizer; UM, ultra-rapid metabolizer.

The log metabolic index for hydroxylation was determined as the log\textsubscript{10} (omeprazole\textsubscript{3h}/5-hydroxyomprazole\textsubscript{3h}) and the log metabolic index for sulfoxidation of omeprazole as the log\textsubscript{10} (omeprazole\textsubscript{3h}/omeprazole sulfone\textsubscript{3h}). Log of metabolic indexes for (racemic-, R- and S-) 5-hydroxylation and sulfoxidation of omeprazole are significantly lower in IM compared with EM and UM of CYP2C19 (P < 0.05; ANOVA with Dunn’s multiple comparison) after a single dose and multiples doses of efavirenz. \textsuperscript{9P-value; comparison between single dose vs multiple doses of efavirenz using paired t-test.

DISCUSSION

In the present study, we have used multiple markers (oral clearance, metabolic ratio and metabolic index) to test the hypothesis that CYP2C19 and CYP2B6 genetic polymorphisms may explain inter-individual variability in induction of omeprazole elimination by efavirenz. We demonstrated: (1) CYP2C19 genotype–phenotype relationship when omeprazole is co-administered with a single dose and multiple doses of efavirenz; (2) that omeprazole metabolism via CYP2C19 and CYP3A is induced by efavirenz in all genotype groups in non-stereoselective manner and (3) the degree of this induction appears to be CYP2C19 genotype dependent. This is the first study reporting the impact of the CYP2C19*17 allele on induction. These comprehensive analyses indicate how interplays between drug interactions and genetic polymorphisms may influence exposure of drugs metabolized by CYP2C19 and CYP3A.

The major role of CYP2C19 genetic variants in omeprazole metabolism when the drug is administered alone has been well documented. We found that this genotype–phenotype association is essentially maintained in the presence of efavirenz (single and multiple doses) as supported by the: (1) significant associations of CYP2C19 genetic variants and omeprazole elimination; (2) frequency distribution histogram and probit plots of the hydroxylation indexes of omeprazole showing bimodality with an antimode of ~0.6–0.8, consistent with previous reports;\textsuperscript{31,32} (3) significantly higher metabolic ratios of omeprazole hydroxylation in IM compared with EM and UM of CYP2C19, consistent with previous data showing a gene-dose effect of CYP2C19 genotypes on metabolic ratios of omeprazole 5-hydroxylation;\textsuperscript{15} and (4) analysis of the log of omeprazole hydroxylation index. Omeprazole is mainly cleared by CYP2C19-mediated 5-hydroxylation. However, we found no influence of CYP2C19 genotype on the AUCAUC\textsubscript{EM} of 5-hydroxyomprazole, consistent with a previous study showing no statistical difference in AUCAUC\textsubscript{EM} for this metabolite between the CYP2C19*17*17 and CYP2C19*11*11 groups.\textsuperscript{15} A trend toward lower AUCAUC\textsubscript{EM} of 5-hydroxyomprazole in UM of CYP2C19 compared with EM or IM groups after a single dose and multiple doses of efavirenz (present study) and this finding concur with a similar trend observed by Baldwin et al.\textsuperscript{15} The lack of association between CYP2C19 genotypes and 5-hydroxyomprazole exposure may be in part explained by the sequential metabolism of 5-hydroxyomprazole and the mixed inhibitor/inducer properties of efavirenz on CYP2C19 activity.\textsuperscript{8}
Mexican healthy subjects (mean value of 0.2 for EM and 0.8 for PM). In another study, a mean log omeprazole sulfone metabolic index in Caucasians of 0.48 (0.32–0.70) was reported in CYP2C19 PM. Based on genotype analysis, the mean log metabolic index for sulfoxidation was 0.39 for EM and 0.59 for PM in the present study. While the log of omeprazole sulfoxidation index can be used as a rough estimate of CYP3A activity, the large inter-subject variability observed and the influence of CYP2C19 variants on this index makes it less reliable and less accurate as a quantitative measure of CYP3A activity.

Influence of CYP2C19 and CYP2B6 genetic variations on induction of omeprazole metabolism by efavirenz

Comparison of omeprazole pharmacokinetics after a single vs multiple doses of efavirenz suggests that efavirenz induced
omeprazole metabolism in a CYP2C19 genotype-dependant but not in a stereoselective manner. Specifically, we provide convincing evidence that efavirenz significantly induces the metabolism of omeprazole in EM and IM of CYP2C19. While our findings are broadly consistent with data from other studies, some exceptions exist with respect to the degree of induction among CYP2C19 genotypes.

First, in contrast to our data showing a relatively higher induction of omeprazole metabolism in IM than in EM (or UM) of CYP2C19 by efavirenz, other investigators have found relatively smaller induction in IM compared with EM subjects. Rengelshausen et al. investigated the long-term effects of St-John’s Wort on the pharmacokinetics of voriconazole. They reported that the oral clearance of voriconazole was significantly enhanced in subjects with CYP2C19*1/*1 (P = 0.01) and CYP2C19*1/*2 (P = 0.03), but the effect on those that carry the variant was smaller. Similar findings have been reported with other substrates (for example, S-mephénytoïn) and inducers (for example, rifampicin). The reasons for the discrepancy regarding the extent of induction in CYP2C19 IM remain unknown but the relatively higher number of IM individuals enrolled in our study (n = 19) allowed us to conduct robust comparison between EM and IM groups.

Second, we noted a non-significant induction of omeprazole metabolism in UM of CYP2C19 by efavirenz and the magnitude of this induction tended to be lower compared with EM and IM. The lack of statistically significant change may be simply due to the small number of UM (n = 4) subjects studied. The CYP2C19*17 allele is caused by mutation (–808C>T) in the 5’-flanking region of the gene and the possibility that the impact of induction by efavirenz could be blunted in UM subjects due to the already high basal activity of CYP2C19 or altered binding could not be ruled out.

Third, reduction in AUCo−∞ of omeprazole and increase in metabolic ratio of 5-hydroxylation (changed from 0.04 to 0.08) observed in one PM subject (current study) are consistent with previous studies showing an increased omeprazole clearance in CYP2C19 PM after treatment with St-John’s Wort or artemisinin. Considering that CYP2C19*2/2 is associated with a complete absence of CYP2C19 activity, the induction observed in our PM subject and the magnitude thereof may be explained by induction of other non-CYP2C19-mediated metabolic pathways of omeprazole (for example, CYP3A-mediated sulfoxidation). Consistent with this suggestion, artemisinin enhanced the elimination of omeprazole in one subject with a poor CYP2C19 metabolizer phenotype. However, when S-mephénytoïn is used as a substrate, no or marginal induction of CYP2C19 by rifampicin and by St-John’s Wort has been reported in CYP2C19 PM. The degree of induction appears to vary with the substrate used, the fraction metabolized by CYP2C19, the intrinsic capacity of the inducer, the sensitivity of alternative pathway to induction and the indices used to evaluate enzyme activity which may be sensitive to sequential metabolism.

We have shown that efavirenz effectively induced omeprazole sulfoxidation in EM and IM subjects concurring with a previous report showing marked induction of this pathway of omeprazole by St-John’s Wort in EM and PM. However, notable differences were seen regarding the extent of induction of omeprazole elimination between the current study and that previously reported. They reported a substantial increase in the percent change of omeprazole sulfone AUCo−∞ after long-term exposure to St-John’s Wort in EM (159 ± 101%) and PM of CYP2C19 (136.6 ± 84.6%), in contrast to a much smaller change by efavirenz in the same genotype groups was observed in our study. This may be explained by a greater inductive effect of St-John’s Wort on CYP3A genes in the intestine and the liver compared with efavirenz, which predominantly induces CYP3A in the liver. This is the first study testing the influence of CYP2B6 polymorphisms on CYP2C19 induction by efavirenz. The CYP2B6*6 allele is associated with significantly higher efavirenz exposure. We found no significant difference in any of the markers of CYP2C19 activity between carriers and non-carriers of the CYP2B6*6 allele. Our finding suggest that the inductive effects of efavirenz on omeprazole 5-hydroxylation may be observed at plasma concentrations lower than those observed after the regular dose (600 mg per day) of efavirenz. We also found no significant association between the CYP2B6 variants and the extent of CYP3A induction by efavirenz. A recent study reported higher induction of CYP3A activity (using an endogenous marker) by efavirenz in CYP2B6 slow metabolizers. The difference in the markers used for CYP3A activity makes comparison with our study difficult. The presence of sequential metabolism in omeprazole elimination might have precluded statistical significance. In the present study, the impact of variants in the CYP3A4 and CYP3A5 genes on the degree of induction of omeprazole metabolism by efavirenz was not tested because the genetic component of CYP3A4 is small and the contribution of CYP3A5 to omeprazole sulfoxidation is marginal.

In summary, this study indicates that omeprazole elimination remains CYP2C19-genotype dependent after a single and multiple doses of efavirenz. We provide evidence that EM and IM of CYP2C19 were more prone to the inductive effects by efavirenz; this is the first report showing a significant induction by efavirenz on omeprazole elimination in IM subjects. The ability of efavirenz to induce CYP2C19 and CYP3A using omeprazole as the marker drug appears to be independent of CYP2B6 genetic polymorphisms. In conclusion, genetic factors should be taken into consideration when induction drug interactions are evaluated.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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