Dependence of Efavirenz- and Rifampicin-Isoniazid–Based Antituberculosis Treatment Drug-Drug Interaction on *CYP2B6* and *NAT2* Genetic Polymorphisms: ANRS 12154 Study in Cambodia

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We investigated the population pharmacokinetics and pharmacogenetics of efavirenz in 307 patients coinfected with human immunodeficiency virus and tuberculosis and included in the Cambodian Early vs Late Initiation of Antiretrovirals trial (CAMELIA) in Cambodia. Efavirenz (600 mg/d) and stavudine plus lamivudine were administered in addition to standard antituberculosis treatment, including rifampicin and isoniazid. Blood samples were obtained a mean of 14 hours after efavirenz intake at weeks 2 and 6 after initiation of efavirenz and weeks 22 (efavirenz plus antituberculosis drugs) and 50 (efavirenz alone) after initiation of antituberculosis treatment. Ten patients participated in an extensive pharmacokinetic study after week 50. *CYP2B6 G516T* and *C485-18T* polymorphisms were the most significant covariates, with weight showing a significant minor effect. Change in efavirenz apparent clearance in patients taking both efavirenz and antituberculosis treatment was highly dependent on *NAT2* polymorphism, as a possible surrogate of isoniazid exposure. Patients carrying the *CYP2B6 516 TT* genotype and slow-acetylation *NAT2* phenotype had the lowest efavirenz apparent clearance. These data suggest that the inducing effect of rifampicin is counterbalanced by a concentration-dependant inhibitory effect of isoniazid on efavirenz clearance.

Efavirenz is a nonnucleoside reverse-transcriptase inhibitor of human immunodeficiency virus (HIV) type 1 and one of the preferred components of the first-line antiretroviral treatment (ART) regimen of HIV infection worldwide. Current guidelines recommend efavirenz at a dosage of 600 mg/d combined with 2 nucleoside (or nucleotide) analogues as one of the preferred options for first-line therapy in developed as well as resource-limited countries [1]. Furthermore, it was demonstrated that efavirenz can be coadministered safely with standard antituberculosis therapy that includes rifampicin, a potent drug enzyme inducer, and isoniazid for 6 months and ethambutol plus pyrazinamide for the first 2 months. Earlier studies recommended increasing the efavirenz dosage to 800 mg/d in patients receiving efavirenz and rifampicin concomitantly [2, 3]. Later studies demonstrated the efficacy of efavirenz at a dosage of 600 mg/d along with antituberculosis drugs [4]; recently, it has been suggested that the efavirenz dosage be increased to 800 mg/d in patients weighing >50 kg [5].

Efavirenz is metabolized mainly through CYP2B6 [6], which has been demonstrated to be inducible

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and highly polymorphic [7]. The *CYP2B6 G516T* polymorphism has been associated with increased efavirenz plasma concentrations and prolonged plasma elimination half-life with consequences for tolerance and virologic efficacy [8, 9]. The prevalence of *CYP2B6 G516T* genetic polymorphism is highly variable among populations. A high frequency of the loss-of-function allele was reported in Cambodian (35%) [10] and African (42%) [11] populations.

The relationship between the *CYP2B6* genetic polymorphism and efavirenz interaction with rifampicin is poorly understood. There are currently conflicting reports in the literature; some authors report increased metabolism of efavirenz in the presence of rifampicin [12, 13], and others report the opposite [14–16]. Findings of some studies suggested that isoniazid, does not play a role in the interaction of rifampicin and EFZ itself but could explain controversial results when not accounted for [16–18]. In contrast, pyrazinamide was demonstrated not to affect CYP activities [19] and no drug interaction has been reported to date with ethambutol.

To our knowledge, the influence of antituberculosis drugs on efavirenz pharmacokinetics, taking into account pharmacogenetic characteristics of the population, has been poorly documented [16, 20]. Consequently, our study focused on drug-metabolizing enzymes and transporters involved in efavirenz disposition, CYP2B6, CYP3A4, CYP3A5, CYP2A6, ABCB1, and ABCC10 (MRP7), as described elsewhere [6, 7, 21–25]. In addition, other genetic polymorphisms, such as the *Peroxisome proliferator-activated receptor alpha* PPAR-α [26], human pregnane X receptor (hPXR; NR1I2) [27], OATP1B1 (SLCO1B1) uptake transporter [28, 29], and N-acetyl transferase type 2 (NAT2) were genotyped [30, 31], because they could be involved in efavirenz, rifampicin, or isoniazid disposition.

The aim of this study was to evaluate the variability of efavirenz concentrations in HIV-tuberculosis–coinfected patients included in the CAMELIA (Agence Nationale de la Recherche sur le Sida et les hépatites virales, ANRS 1295–CIPRA KH001) randomized clinical trial in Cambodia [32]. A population pharmacokinetic approach was used to estimate efavirenz pharmacokinetic parameters and determine whether antituberculosis treatment and patient characteristics, including pharmacogenetics of drug-metabolizing enzymes, transporters, and nuclear receptors, were significant covariates affecting efavirenz pharmacokinetic parameters.

MATERIALS AND METHODS

Patient Population and Study Design

ll patients included in the CAMELIA trial [32] gave written informed consent to participate in this ANRS 12154-PECAN pharmacogenetic-pharmacokinetic study, which was approved by the National Ethics Committee for Health Research of the Cambodian Ministry of Health. The CAMELIA trial was designed to determine whether earlier initiation of ART (2 weeks after the onset of tuberculosis treatment), as compared with later initiation (8 weeks afterward), could reduce mortality among patients with advanced immunodeficiency [32]. ART consisted of efavirenz (600 mg daily in the evening) combined with stavudine (30 mg) and lamivudine (150 mg) administered twice daily. Tuberculosis treatment consisted of a World Health Organization–recommended daily regimen of isoniazid (4–5 mg/kg/d), rifampicin (10 mg/kg/d), ethambutol (15–20 mg/kg/d), and pyrazinamide (20–30 mg/kg/d) as a fixed-dose combination during the first 2 months, followed by daily administration of isoniazid (4–5 mg/kg/d) and rifampicin (10 mg/kg/d) as a fixed-dose combination during the ensuing 4 months. For this study, blood samples were obtained 2 and 6 weeks after starting ART and 22 and 50 weeks after starting antituberculosis treatment. An additional blood sample was obtained for DNA extraction. Ten patients participated in an extensive pharmacokinetic study conducted after 50 weeks of follow-up while patients were still receiving efavirenz-based ART. Samples were obtained before efavirenz intake and 1, 2, 4, 12, and 18 hours after evening efavirenz intake.

Quantification of Plasma Efavirenz Concentrations

Plasma samples for efavirenz quantification and whole blood samples were kept frozen at −80°C until analysis. Efavirenz concentrations were assayed in plasma by validated reverse phase high-performance liquid chromatography with liquid-liquid extraction and UV detection [33]. The limit of quantification was 0.05 mg/L. The interrun variability of the low-, medium-, and high-quality controls inserted in each analytical run was <15%.

Genotyping Analysis

DNA was extracted from patient buffy coats by using Gentra Puregene Blood Kits according to the manufacturer's protocol (Qiagen). The subjects were genotyped using the Taqman allelic discrimination assay, as described elsewhere [10]. The single-nucleotide polymorphisms (SNPs) of CYP, transporters, and nuclear receptor genotyped are listed in Table 1. We aimed to find the smallest set of SNPs within NAT2 that enabled the most efficient classification of individuals into "rapid" and "slow" acetylators (rs1801280, rs1801279, rs1799930, and rs1799931). NAT2 alleles were classified based on the current knowledge of the functional impact of the variant alleles. Consequently, *NAT2*4* was considered as functional allele, and *NAT2*5, NAT2*6,* and *NAT2*7* were considered loss-of-function or slow alleles. Individuals with 2 slow activity alleles were phenotyped as slow acetylators, and those with 1 or 2 functional alleles as rapid acetylators [30, 31]. For each polymorphism, Hardy-Weinberg equilibrium was tested for using the χ^2 test.

Statistical Analyses

The number of patients required for this study was calculated by an expected decrease in efavirenz clearance between *CYP2B6*

Table 1 SNPs Genotyped on Genes Coding for Cytochromes, Transporters and Nuclear Receptors Involved in Efavirenz, Rifampicin and Isoniazid Metabolism and Disposition and Expected Effect of Loss of Function Allele (LOF) Compared to Reference Allele (Ref).

Variant	rs Number	Genetic Variant Change (Nomenclature)	Ref. Allele	LOF Allele	Expected LOF Allele Effect on Drugs Plasma Concentrations	References
CYP2B6 516	rs3745274	G > T	G	T	Increased efavirenz concentration	[6, 7]
CYP2B6 1459	rs3211371	C > T	C	T		[6, 7]
CYP2B6 485-18	rs4803419	C > T	C	T		[6, 21]
CYP3A4	rs4646437	G > A (*1A > *1B)	G	A		[22]
CYP3A5 6986	rs776746	A > G	A	G		[23]
CYP2A6	rs8192726	G > T	G	T		[24]
ABCB1 3435	rs1045642	C > T	C	T	Decreased transporter mediated efflux and possible increased efavirenz concentration	[23]
ABCC10 (MRP7)	rs2125739	T > C	T	C	Decreased transporter mediated efflux and possible increased efavirenz concentration	[25]
PPARα	rs4253728	G > A	A	G	Decreased enzyme induction and possible increased efavirenz concentration	[26]
PXR (NRI2) 7635	rs6785049	A > G	A	G	Decreased enzyme induction and possible increased efavirenz concentration	[27]
SLCO1B1 521	rs4149056	T > C	T	C	Decreased transporter mediated influx into hepatocyte for rifampicin as substrate and decreased rifampicin induction with possible increased efavirenz concentration	[28, 29]
NAT2 341	rs1801280	T > C (*5)	T	C	Increased isoniazid concentration	[30, 31]
NAT2 590	rs1799930	G > A (*6)	G	A		[30, 31]
NAT2 857	rs1799931	G > A (*7)	G	A		[30, 31]
NAT2 191	rs1801279	G > A (*14)	G	A		[30, 31]

516GG and *TT* of 50%, an intersubject variability of 60%, a power of 90%, a type I error rate of 5%, and a *t* test analysis. These assumptions led to a sample size of 12 patients per genotype group. An estimate of *CYP2B6 516T* allelic frequency of 0.2% was deduced using a HapMap Panel of 90 unrelated control samples of Asian origin. Based on Hardy-Weinberg proportions, we expected approximately 4% of patient to be carriers of *CYP2B6 516TT* in the study population and therefore included 300 of the 661 patients who participated in the CAMELIA main trial [32]. Plasma concentrations of efavirenz were analyzed using the nonlinear mixed-effects software NONMEM, version 7.1.2 (ICON Development Solutions) with the stochastic approximation expectation maximization algorithm. The structural model (ie, the choice of number of disposition compartments as well as the absorption and elimination processes) was decided using the data from the 10 patients included in the extensive pharmacokinetic substudy. Subsequently, the between- and within-subject random effect and the residual error models were developed on the data set, including all patients at all occasions.

For the absorption process, we explored first-order and zero-order models with lag time and transit compartments, with and without an absorption constant. The subject- and occasion-specific random effects were assumed to follow an exponential model. Derivation and justification of structural and variance models were guided by improvement in data prediction assessed with likelihood ratio tests using a threshold of 5%.

All genetic covariates were analyzed using a genotypic model, as follows:

$$\theta_{IV} = \theta_{PV} \times (1 + \theta_{HET} + \theta_{HOMr}),$$

where θ_{IV} is the individual value; θ_{PV}, the population value of the pharmacokinetic parameter; and θ_{HET} and θ_{HOMr}, the effect coefficients associated to the heterozygous and rare homozygous genotypes, respectively. The *ABCB1 3435* polymorphism was recoded in 2 groups: defective *ABCB1 3435T* allele carriers and noncarriers.

In addition to the genetic polymorphisms, the effects of weight, determined using a power model (as recommended in [34]) with estimation of the scaling exponent, and age were considered as covariates during model building. These covariates were centered to their median values and when missing were imputed to the corresponding occasion median. The effect of sex and antituberculosis coadministration were also investigated. The *NAT2* phenotype (rapid or slow) and the *SLCO1B1 T521C* polymorphism were considered only in the presence of a significant effect of antituberculosis treatment.

Covariates were included in the model in a stepwise approach, using likelihood ratio test at a 5% significance

threshold. Starting from the second step, all covariates were tested for interaction as well as for marginal association with the covariate previously selected. The most conservative model was taken forward.

RESULTS

This study included 307 HIV-tuberculosis–coinfected adult patients from the CAMELIA (ANRS 1295-CIPRA KH001) trial. The demographic and pharmacogenetic characteristics of these patients are presented in Table 2 and Table 3, respectively. A total of 1111 plasma concentrations of efavirenz were modeled, of which 0.71% were below the limit of quantification (0.05 mg/L) and therefore discarded from the analysis. The average sampling time was 14 hours (range, 8.4–18.0 hours) after efavirenz evening intake and was referred to as middose concentration. Less than 5% of the patients had middose concentrations <1 mg/L, about 65% had concentrations of 1–4 mg/L, and 30% had concentrations >4 mg/L. Most patients (n = 181) were observed on 4 occasions (3 times during antituberculosis treatment and once without treatment); 11 were observed once; 27 and 80 were observed on 2 and 3 occasions, respectively (all while taking antituberculosis drugs); and 8 were observed on 5 occasions. Figure 1 illustrates steady concentrations at the different sampling times, indicating that there was low intrasubject variability but large intersubject variability in efavirenz concentrations. On average, coadministration of antituberculosis drugs had no influence on efavirenz disposition ($P \leq .48$; Wilcoxon test).

A 1-compartment model with zero-order delayed absorption and linear elimination was used to describe the extensive pharmacokinetic profiles in 10 patients. Because the study design included mostly 14-hour concentrations, inter- and intrasubject variances were estimated only for the apparent clearance (CL/F). For the residual error variability, a parsimonious proportional model was selected.

In the first round of inclusion, *CYP2B6 G516T* genetic polymorphism was the most significant covariate ($P < .001$). When the remaining covariates were explored in interaction with the *CYP2B6 G516T* polymorphism, *CYP2B6 C485-18T* genetic polymorphism was the most significant ($P < .001$), but we chose to model its effect as independent of *CYP2B6 G516T* because that model required fewer parameters and was just as predictive. Of note, no subject was identified who carried the *CYP2B6 516TT* genotype and ≤1 allelic variant *CYP2B6 485-18T* or carried the *CYP2B6 516GT* genotype and 2 allelic variants of *CYP2B6 485-18T*. In the third step of analysis, the effect of weight (similar across *CYP2B6 G516T* genotypes) was added to the model ($P < .001$). Finally, the antituberculosis comedication was found to significantly increase efavirenz CL/F in carriers of *CYP2B6 516GG* genotype, but CL/F was decreased in carriers of *CYP2B6 516GT* and *TT* genotypes ($P < .02$). In patients taking antituberculosis drugs, a decrease in efavirenz CL/F was observed in slow NAT2 metabolizers, whereas an increase was observed in rapid NAT2 metabolizers ($P < .001$). The decrease in efavirenz CL/F in slow NAT2 metabolizers is higher in patients carrying the *CYP2B6 516T* allele than in those

Table 2. Baseline Demographic and Laboratory Characteristics of Study Participants at Inclusion, at Weeks 2 and 6 After Start of Efavirenz-Based Antiretroviral Treatment, and at Weeks 22 and 50 After Start of Antituberculosis Drugs

Characteristic	Inclusion	CAMELIA Randomization Arm	Week After Start of Efavirenz[a]		Week After Start of Antituberculosis Drugs	
			2	6	22	50
No. of patients[b]	307	Early	139	142	279	235
		Late	131	133		
Weight, mean (range), kg	44 (29–72)	Early	45 (29–73)	45 (32–74)	51 (28–75)	54 (36–80)
		Late	46 (32–74)	46 (28–71)	. . .	. . .
AST, mean (range), IU/L	46 (15–199)	Early	45 (16–420)	44 (18–293)	44 (15–204)	30 (14–243)
		Late	43 (20–275)	45 (13–240)	. . .	. . .
ALT, mean (range), IU/L	31 (3–255)	Early	34 (3–422)	33 (3–153)	32 (4–178)	27 (5–267)
		Late	33 (8–183)	33 (5–324)	. . .	. . .
CD4 T-cell count, mean (range), cells/μL	27 (1–196)	. . .	. . .	. . .	. . .	201 (25–710)
Patients with plasma HIV RNA > 240 copies/mL, No. (%)	. . .	. . .	. . .	. . .	. . .	. . .
Yes	307 (100)	. . .	. . .	. . .	. . .	1 (1)
No	0	. . .	. . .	. . .	. . .	233 (99)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HIV, human immunodeficiency virus.

[a] Week 2 was week 4 or 10 after the start of antituberculosis drugs according to the CAMELIA randomized arm (early or late initiation of antoretrovirals), and week 6 was week 8 or 14 after the start of antituberculosis drugs.

[b] The number of patients with an efavirenz concentration and ≤1 genotype available.

Table 3 Observed Frequencies of *CYP2B6* (rs3745274, rs3211371, rs4803419), *CYP3A4* (rs4646437), *CYP3A5* (rs776746), *CYP2A6* (rs8192726), *ABCB1* (rs1045642), *ABBC10* (*MRP7* rs2125739), PPARa (rs4253728), *PXR 7635* (rs6785049) and *SLCO1B1* (rs4149056) With the Corresponding Hardy-Weinberg P Values and the Observed NAT2 Genotypes.

Gene (Ref/LOF)	Hom-Ref, N (%)	Het-LOF, N (%)	Hom-LOF, N (%)	LOF allele (%)	H-W P Value
CYP2B6 516 (G/T)	133 (46.3)	123 (42.9)	31 (10.8)	T (32)	0.78
CYP2B6 1459 (C/T)	270 (94.7)	15 (5.3)		T (3)	1
CYP2B6 485-18 (C/T)	131 (48.0)	120 (44.0)	22 (8.1)	T (30)	0.56
CYP3A4 (G/A)	147 (50.7)	122 (42.1)	21 (7.2)	A (28)	0.56
CYP3A5 6986 (A/G)	30 (10.3)	143 (49.3)	117 (40.3)	G (65)	0.19
CYP2A6 (G/T)	228 (80)	50 (17.5)	7 (2.5)	T (11)	0.06
ABCB1 3435 (C/T)	115 (39.8)	131 (45.3)	43 (14.9)	T (38)	0.61
ABCC10 (MRP7) (T/C)	235 (85.1)	37 (13.4)	4 (1.4)	C (8)	0.09
PPARα (A/G)	274 (99.3)	2 (0.7)	0 (0)	A (0.4)	1
PXR (NRI2) 7635 (A/G)	36 (12.4)	123 (42.4)	131 (45.2)	G (76)	0.43
SLCO1B1 521 (T/C)	215 (74.1)	73 (25.2)	2 (0.7)	C (13)	0.19
*NAT2 (*4/*5*6*7)*[a]	*4/*4, 57 (20.4)	*4/*5, 22(7.9) *4/*6, 45(16.1) *4/*7, 43(15.4) *5/*6, 21(7.5) *5/*7, 14(5.0) *6/*7, 23(8.2)	*5/*5, 3 (1.1) *6/*6, 43 (15.4) *7/*7, 8 (2.9)	*5 (11) *6 (31) *7 (17)	

Abbreviations: Het-LOF, heterozygous for the loss-of-function (LOF) allele; Hom-LOF, and homozygous for the LOF allele; Hom-Ref, homozygous for the functional allele; NAT2, N-acetyl transferase type 2; Ref/LOF, Reference (i.e. functional)/loss of function allele.

[a] Acetylator phenotypes are predicted from *NAT2* genotypes as follows: patients carrying at least 1 allele *4 allele are phenotyped as rapid, and all others are slow.

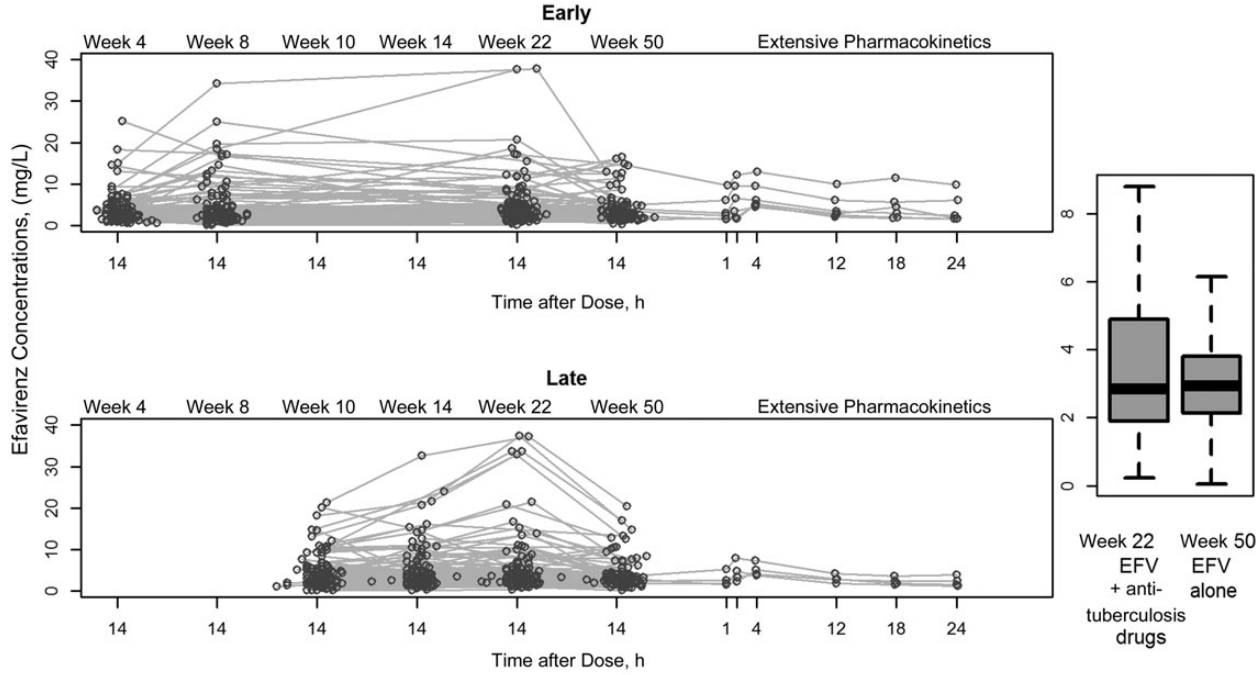

Figure 1. Observed efavirenz (EFV) 14-hour concentrations at week 4 or 8 after antituberculosis drug initiation in the treatment arm Early (for early initiation of antiretroviral treatment), at week 10 or 14 after antituberculosis drug initiation in the treatment arm Late (for late initiation of antiretroviral treatment), and at week 22 and 50 after antituberculosis drug initiation as well as the concentrations collected during a 24-hour dosing interval in 10 patients (extensive pharmacokinetic study). Concentrations from the same subject are connected by a gray line. Box plots represent the minima, the 25th, 50th, and 75th percentiles, and the maxima at weeks 22 (efavirenz plus antituberculosis treatment) and 50 (efavirenz alone).

genotyped *CYP2B6 516GG*. This association captured in the final covariate model could already be observed on the individual CL/F estimates from the base model, as illustrated in Figure 2.

The pharmacokinetic parameter estimates from the base and the final covariate model are shown in Table 4. In the base model, CL/F was 7.5 L/h. The addition of the *CYP2B6 G516T* polymorphism decreased the intersubject variability by 20%. Patients carrying the *CYP2B6 516GG* genotype had the highest efavirenz clearance (12.5 L/h) when not receiving tuberculosis treatment. In homozygous patients carrying the loss-of-function alleles (*CYP2B6 516 TT*), clearance was 80% lower (2.5 L/h). The dominant effect of the *CYP2B6 C485-18T* polymorphism explained an additional 4% intersubject variability and decreased efavirenz clearance by 30%, whatever the *CYP2B6 G516T* polymorphism. The final addition of the *NAT2* phenotype in interaction with the *CYP2B6 G516T* polymorphism explained a supplementary 4% intersubject variability. During tuberculosis treatment, in patients who carried the *CYP2B6 516GG* genotype, the apparent clearance of efavirenz was increased by 24% in rapid NAT2 metabolizers and decreased by 10% in slow NAT2 metabolizers. In *CYP2B6 516TT* carriers, efavirenz apparent clearance was increased by 8% in patients who were rapid NAT2 metabolizers and decreased by 16% in slow NAT2 metabolizers. Consequently, when tuberculosis treatment was discontinued, efavirenz concentrations increased or decreased according to the patient's rapid or slow NAT2 phenotype status.

Overall, in patients receiving efavirenz alone (week 50), efavirenz concentrations were <1 mg/L in 3%, 1–4 mg/L in 75%, and concentrations >4 mg/L in 23%, compared with 4%, 65%, and 31%, respectively, in patients receiving efavirenz plus antituberculosis drugs (week 22), which hints at higher efavirenz concentrations in presence of antituberculosis drugs. However, as illustrated in Figure 3, when stratifying across genotypes we observed that 27% of the patients with *CYP2B6 516GG* who were rapid NAT2 metabolizers had efavirenz concentrations <1 mg/L while receiving antituberculosis drugs, compared with 6% receiving efavirenz alone. In comparison, none of the patients carrying the *CYP2B6 516GG* genotype who were slow NAT2 metabolizers had a concentration <1 mg/L while receiving antituberculosis drugs, compared with 15% receiving efavirenz alone. This increase in efavirenz concentrations observed in patients who were slow NAT2 metabolizers receiving concomitant

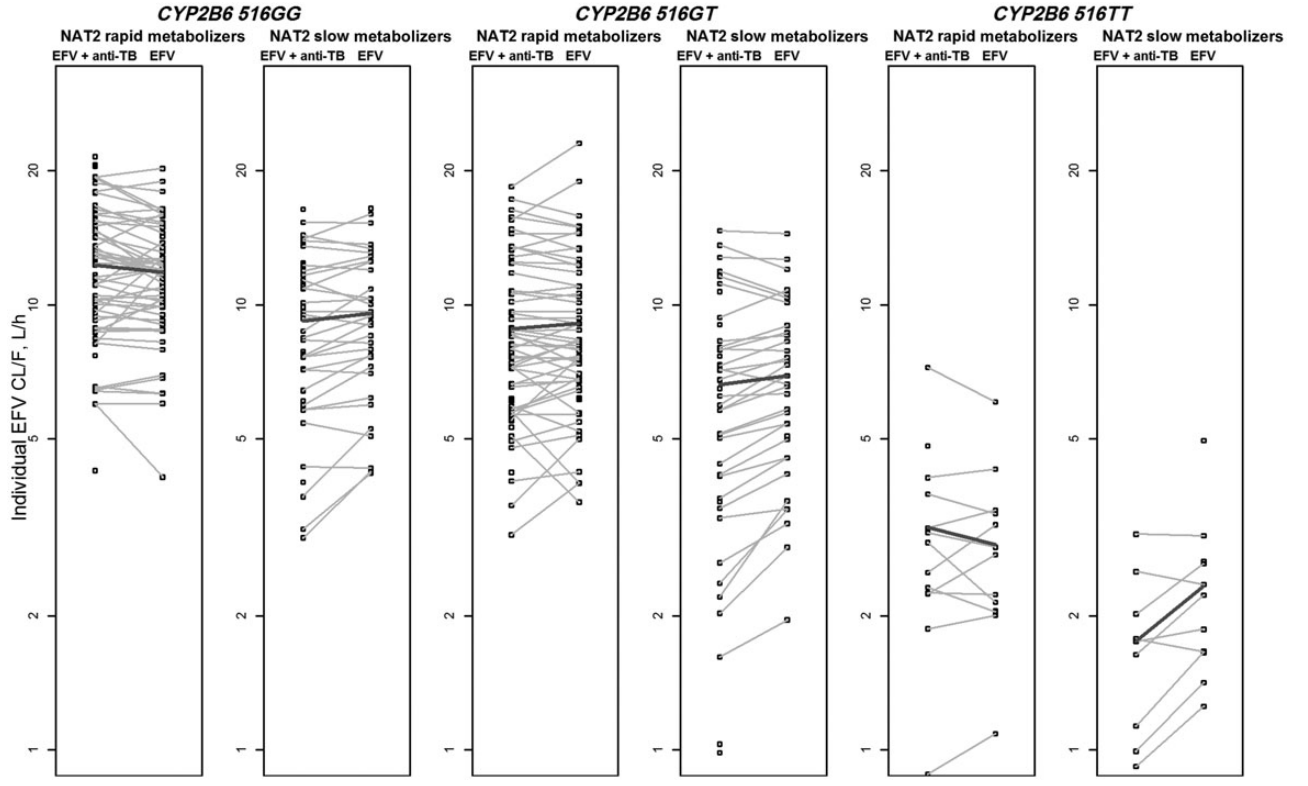

Figure 2. Efavirenz (EFV) apparent clearance (CL/F) individual estimates from the base model at weeks 22 (efavirenz plus antituberculosis [anti-TB] drugs) and 50 (efavirenz alone), grouped by genotype for the *CYP2B6 G516T* polymorphism (GG, extensive metabolizers; GT, intermediate metabolizers; and TT, poor metabolizers) and the N-acetyl transferase type 2 (*NAT2*) phenotype. The median (*dark lines*) and efavirenz CL/F individual estimates (*gray lines*) in the different phenotypes are connected.

Table 4. Pharmacokinetic Parameter Estimates of the Base and Final Models for the Population

Parameters	Estimate (RSE, %)	
	Base Model (N = 307)	Final Model (N = 263)
Duration of zero-order absorption, h	1.5	1.5
Lag time, h	0.8	0.8
Apparent volume of distribution, L	267 (13)	247 (13)
CL/F, L/h	7.5 (4)	. . .
CYP2B6 516GG		
Efavirenz alone	. . .	12.5 (6)
Efavirenz plus antituberculosis drugs	. . .	11.2 (6) (slow); 15.5 (6) (rapid)
CYP2B6 516GT		
Efavirenz alone	. . .	8.8 (6)
Efavirenz plus antituberculosis drugs	. . .	6.6 (6) (slow); 9.9 (5) (rapid)
CYP2B6 516TT		
Efavirenz alone	. . .	2.5 (10)
Efavirenz plus antituberculosis drugs	. . .	2.1 (11) (slow); 2.7 (10) (rapid)
CYP2B6 485-18T allele in % of CL/F		−30 (15)
Weight in % per 1 kg from 43 kg		+1 (27)
Between-subject variability in CL/F, %	62 (9)	34 (12)
Between-occasion variability in CL/F, %	18 (20)	19 (15)
Residual variability proportional error, %	29 (4)	28 (4)

Abbreviations: CL/F, apparent clearance; slow, slow N-acetyl transferase type 2 (NAT2) metabolizers; rapid, rapid NAT2 metabolizers; RSE, relative standard error.

antituberculosis treatment was higher in patients carrying the *CYP2B6 516T* and *CYP2B6 485-18T* alleles. For instance, 79% of *CYP2B6 516GT* and *CYP2B6 485-18 CT/TT* carriers had efavirenz concentrations >4 mg/L while receiving antituberculosis treatment, compared with 13% receiving efavirenz alone.

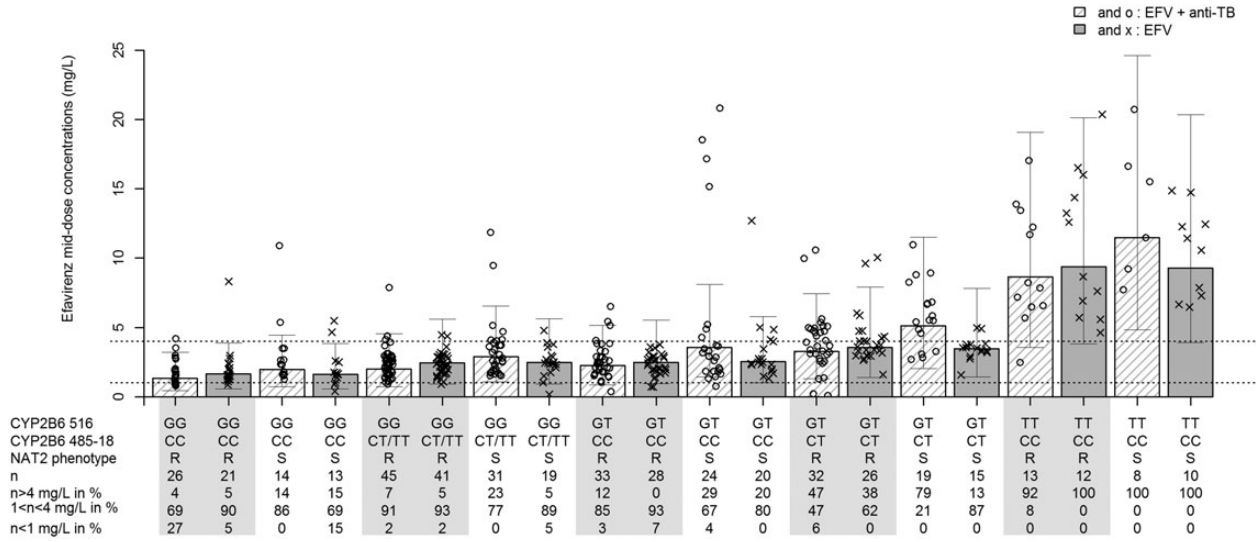

Figure 3. Observed efavirenz (EFV) 14-hour concentrations (for efavirenz plus antituberculosis treatment at week 22 and for efavirenz alone at week 50) and mean predicted middose concentrations (*bars*) with 90% population prediction interval for each *CYP2B6 516* (GG, common allele homozygote; GT, heterozygote; TT, loss-of-function allele homozygote) and *CYP2B6 485-18* (CC, common allele homozygote; CT/TT, heterozygote/loss-of-function allele homozygote) genotype and N-acetyl transferase type 2 (NAT2) phenotype (R, rapid; S, slow) combination during therapy with efavirenz plus antituberculosis drugs or efavirenz alone; No. indicates number of individuals carrying allelic or phenotypic combinations; >4 mg/L (etc), proportion of subjects with observed concentration >4 mg/L, given as a percentage. Dotted lines represent the therapeutic margin (1–4 mg/L). The gray columns regroup predictions and observations of rapid NAT2 metabolizers in the different *CYP2B6 G516T* and *CYP2B6 C485-18T* genotypes.

DISCUSSION

Efavirenz is the preferred ART backbone in HIV-tuberculosis–coinfected patients worldwide [5], although the exact mechanism of the interaction between efavirenz and standard antituberculosis drugs is not fully understood. Our study demonstrates not only that the inducing effect of the rifampicin-isoniazid combination is highly dependent on the *CYP2B6 G516T* genotype but also that the *NAT2* genotype influences the direction of the interaction, which is likely to be related to isoniazid exposure. Our pharmacokinetic-pharmacogenetic model corroborates the recent findings showing that efavirenz concentrations were higher in patients carrying the *CYP2B6 516TT* genotype and receiving a standard 4-drug antituberculosis regimen containing rifampicin and isoniazid versus efavirenz alone, although concentrations were lower in patients with the *CYP2B6 516GG* genotype [17, 18]. Only 1 of the evaluated patients had detectable HIV RNA at week 50, so no attempt was made to link efavirenz concentrations and virologic efficacy.

Efavirenz pharmacokinetic parameters estimated by a population modeling approach were similar to those reported elsewhere [35–39]. In this study, the first samples of efavirenz were collected 2 weeks after the onset of treatment in combination with antituberculosis drugs, initiated ≤4 weeks earlier. Therefore, efavirenz was modeled at a steady state of induction processes [37], and its variability was decomposed in between-occasion (or within-patient), between-subject, and residual elements in our analysis.

Because of the sparse design, only 1 compartment was used to model efavirenz distribution in the body; such a simplification has already been used by Viljoen et al [35] and Sanchez et al [39]. The estimate of 247 L for the apparent volume of distribution in the final model, lies within the range of estimates reported for caucasian or African subjects [35, 39, 40] and indicates tissue distribution despite high protein binding [41]. In our base model, CL/F was 7.5 L/h, in agreement with previous reports with values ranging from 4 to 15 L/h [35, 38–40]. As in the study of Arab-Alameddine et al [36], weight was associated with efavirenz CL/F, but here mostly by decreasing the interoccasion variability. Sex and/or age effects were not found in our study, but these demographic markers have rarely been shown to be associated with efavirenz CL/F [35].

Efavirenz is metabolized mainly through CYP2B6 and secondarily through CYP2A6 and CYP3A5/4 [6]. Genetic associations between efavirenz CL/F and polymorphisms of the *CYP2A6* and *CYP3A4* gene reported in the study of Arab-Alameddine et al [36] could not be replicated either in our study or in the study by Holzinger et al [42]. All SNPs of other enzymes and transporters listed in Table 1 were not found to affect our model. Similarly, as in the study by Swart et al [43], no relationship was found between PXR genotype and efavirenz CL/F intersubject variability. We cannot exclude phase II enzymes, such as UGT2B7, which has been described to be involved in the direct N-glucuronidation of efavirenz [44] and O-glucuronidation of 8-hydroxy efavirenz [45] but the N-glucuronidation pathway to the overall clearance of efavirenz was demonstrated to be minimal [44].

The *CYP2B6 G516T* polymorphism proved critical to explaining efavirenz CL/F intersubject variability, as highlighted elsewhere [46]. The 32% frequency of T allele loss of function found in our population agrees with findings of a study conducted in Cambodia [10] and is close to the frequency found in subjects from border countries such as Vietnam and Thailand and in African Americans and some Africans as discussed previously [10]. The important effect of *CYP2B6* allele led us to consider all other covariates in interaction in further analyses. Interestingly, none of our patients carried the *CYP2B6 T983C* variant, which was found to decrease efavirenz CL/F in subjects of African descent [42].

The new genetic polymorphism on the *CYP2B6* gene, *C485-18T,* was first discovered in a recent genome-wide association study [42]. *CYP2B6 C485-18T* is in linkage disequilibrium with rs7251950, which was found to be involved in nevirapine pharmacogenetics [21]. Here, *CYP2B6 C485-18T* was modeled as being associated with efavirenz CL/F independently of *CYP2B6 G516T*; this statistical finding was supported by the presence of these polymorphisms on mutually exclusive haplotypes.

Modeling the impact of antituberculosis treatment in each of the *CYP2B6 G516T* genotypes independently highlighted the opposite effects of isoniazid and rifampicin, with both drugs combined during the 6 months of antituberculosis therapy. First, we observed that efavirenz CL/F increased in patients taking antituberculosis drugs if they carried *CYP2B6 516GG*, and decreased if they carried *CYP2B6 516GT* and *CYP2B6 516TT*. Such data corroborate the finding of Ngaimisi et al [18], who compared 2 cohorts of patients receiving either efavirenz or efavirenz plus antituberculosis drugs including rifampicin and isoniazid. Such discrepancies could occur because rifampicin is an inducer of CYP2B6 whereas isoniazid is an inhibitor of several cytochromes, P-450 (CYP), CYP1A2, CYP2A6, CYP2C19, and CYP3A4, as reported elsewhere [47, 48].

We observed that the increase in efavirenz CL/F due to rifampicin was indeed compensated for by the inhibitory effect of isoniazid in slow NAT2 metabolizers, this effect being even more sensitive in *CYP2B6 516TT* carriers in whom alternative metabolic pathways are predominant. There are uncertainties regarding cytochromes or other enzymes involved in efavirenz metabolism, besides CYP2B6, and several minor pathways, including CYP3A, may contribute to the low efavirenz CL/F in *CYP2B6 516TT* carriers [36, 42].

Isoniazid has been found to decrease the clearance of several drugs, such as triazolam, primarily metabolized by CYP3A4/5 [47]. In vitro studies demonstrated that isoniazid inhibits CYP1A2, CYP2A6, CYP3A4/5, and CYP2C19 in a concentration-dependent manner, and it was concluded that slow

acetylators of isoniazid may be at greater risk for adverse drug interactions [47, 49]. Such dual-mechanism induction of rifampicin and inhibition by isoniazid is somewhat surprising. Whether the immediate inhibitory effect of isoniazid and the time-dependent induction could be a reason remains to be determined.

Findings of a recent *in vitro* study by Xu et al [50] suggest that the *CYP2B6 516TT* genetic polymorphism influences metabolic activity by altering substrate binding and catalytic activity and also confers susceptibility to inhibition, as demonstrated with voriconazole. Whatever the exact mechanism, our study underlines the interest of drug-drug interaction studies conducted in patients. Overall, in this Asian population, the effect of the rifampicin-isoniazid combination on efavirenz biotransformation seemed to be small. However, our study identified a small population at risk of low efavirenz concentrations while receiving rifampicin-isoniazid–based antituberculosis therapy. Plasma HIV RNA levels should be monitored, because patients who carry the *CYP2B6 516GG* and the *CYP2B6 485-18CC* genotypes and who are rapid NAT2 metabolizers may have low concentrations of efavirenz.

This study has a number of limitations. First, our population model was built from sparse sampling, and only 10 patients had extensive pharmacokinetic data available after week 50. However, the estimated CL/F values were within the reported range, supporting our methods. Second, concentrations of antituberculosis drugs were not measured. Kinzig-Scippers et al [31] have demonstrated the relationship between isoniazid CL/F and the number of *NAT2*4* alleles. Patients with no *NAT2*4* alleles have a 3-fold decrease in CL/F compared with those with 2 alleles, which supports our pharmacogenetic approach. Finally, none of the efavirenz metabolites were measured, particularly 8-hydroxy efavirenz, and these measurement would have been useful in determining whether the metabolic ratio (with or without antituberculosis drugs) would discriminate between induction and inhibition processes.

In conclusion, the findings of this pharmacokinetic-pharmacogenetic study, conducted in 307 HIV-tuberculosis–coinfected patients, add new evidence on the effect of rifampicin- and isoniazid-based antituberculosis treatment on efavirenz disposition and emphasize the importance of *CYP2B6* and *NAT2* genetic polymorphism. When tuberculosis treatment was discontinued, efavirenz concentrations rose in patients who were rapid NAT2 metabolizers and unexpectedly decreased in those who were slow NAT2 metabolizers. This phenomenon was more pronounced in patients carrying the loss-of-function genotype *CYP2B6 516TT*, suggesting that the mild rifampicin-inducing effect on CYP2B6 is counterbalanced by a concentration-dependent inhibitory effect of isoniazid on non-CYP2B6 metabolic pathways. Whether efavirenz dosing could be optimized in some patients based on their pharmacogenetic characteristics warrants further research.

Notes

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