

Effects of *CYP2B6* Single Nucleotide Polymorphisms (SNPs) and Substance Abuse on Efavirenz (EFV) Pharmacokinetics

C Venuto¹, Q Ma¹, D Brazeau¹, B Zingman², R Reichman³, M Fischl⁴, B Gripshover⁵, R DiFrancesco¹, A Forrest¹, G D Morse¹

¹Univ at Buffalo, Buffalo, NY; ²Montefiore Med Ctr, Einstein-Montefiore Ctr for AIDS Res, Bronx, NY; ³Univ of Rochester, Rochester, NY; ⁴Univ of Miami, Miami, FL; and ⁵Case Western Reserve Univ, Cleveland, OH

Abstract

Background: Single nucleotide polymorphisms (SNPs) in *CYP2B6* influence efavirenz pharmacokinetics. Our objective was to further evaluate the relationship of SNPs to EFV concentrations in HIV+ patients with substance-related disorders (SRDs).

Methods: 37 HIV+ individuals with (n=18) or without (n=19) SRDs on EFV-containing regimens were enrolled. The primary analysis involved *CYP2B6* 516G>T which is associated with plasma EFV exposure. Secondary analyses included SNPs in *ABCB1*, *CYP3A4*, and *3A5*. The association between genotypes and substances of abuse, viral load, and CD4 cell counts was evaluated using Kruskal-Wallis and linear regression tests.

Results: Based on *CYP2B6* 516G>T genotypes, the patients were categorized as extensive (GG, n=19), intermediate (GT, n=13), and slow (TT, n=5) metabolizers. These genotypes with *2B6* were significantly associated with trough EFV concentrations (p=0.036), but not with *ABCB1*, *3A4* or *3A5*. Significantly lower EFV concentrations were noted in tobacco and alcohol users in the extensive metabolizer group with lower CD4 counts and higher viral loads. SRD had no significant relationship to antiviral responses.

Conclusions: In addition to an association between *CYP2B6* 516G>T and EFV pharmacokinetics, tobacco and alcohol use was associated with significantly lower EFV trough concentrations among patients with functional alleles. The mechanisms that underlie these observations may include combined pharmacogenomic and behavioral components.

Background

- Efavirenz (EFV) is considered a first-line drug for a non-nucleoside reverse transcriptase inhibitor-based regimen in the initial treatment of HIV infection. Despite the fixed daily dose of 600 mg EFV in adults, it is characterized by significant interindividual pharmacokinetic variability resulting in variable response across patients. Central nervous system toxicities reflect elevated EFV plasma concentrations >4 µg/mL. Conversely, patients with an insufficient exposure to EFV (<1 µg/mL) are at increased risk of virologic resistance and treatment failure.
- EFV is primarily metabolized by the polymorphic cytochrome P450 2B6 (*CYP2B6*) enzyme. The *CYP2B6**6 (516G>T and 785A>G) allele results in reduced levels of protein expression and activity, and has been associated with increased plasma EFV levels.
- Individuals with substance-related disorders (SRDs) have impaired adherence and imposed barriers to antiviral access. As a substrate and inducer of *CYP2B6* and *CYP3A4*, EFV may interact with substances of abuse such as tobacco, alcohol, and marijuana through various mechanisms.

Study Objective

- To further evaluate the relationship of SNPs to EFV concentrations in HIV-infected patients with substance-related disorders.
- To identify substances of abuse that have significant impact on EFV concentrations.

Methods

Study subjects were participants in a multicenter therapeutic drug monitoring and drug interactions protocol for HIV-infected individuals with or without SRDs. Enrollment was from May 15, 2003 to May 15, 2007 at 4 clinical sites including Bronx, NY; Rochester, NY; Miami, FL and Cleveland, OH. The substances of abuse included cigarettes, alcohol, marijuana, cocaine, and methadone. Each subject was instructed to complete three clinic visits, entry, trough, and directly observed therapy (DOT), separated by 1-2 week intervals. The substance abuse status was determined by clinicians at entry visit. Subjects were counseled to take scheduled doses of efavirenz (600 mg once daily) at the same time for 4 days prior to each visit. Plasma samples were collected for drug assay and pharmacokinetic evaluation following adherence assessment and counseling. Laboratory data e.g. CD4+ cell counts and HIV RNA were recorded during the clinic visits.

Genotyping: DNA extraction was performed using the QIAGEN DNA Mini Blood Kit (QIAGEN, Valencia, CA). *CYP2B6* polymorphism (516G>T in exon 4, *CYP2B6**6) was identified by real-time PCR assay using Stratagene Mx4000TM (Stratagene, La Jolla, CA) with specific TaqMan® probes (Applied Biosystem, Foster City, CA) to recognize the wild and mutant alleles.

Drug assay: Plasma EFV concentrations were measured using a HPLC method previously developed, validated, and certified by the New York State Department of Health at Core Analytical Laboratory, University at Buffalo.

Statistical analysis: Continuous variables were compared by the Kruskal-Wallis test, and categorical variables were compared by the chi-square and Fisher's exact tests. Multiple linear regression models were used to determine factors associated with EFV concentrations, immunological and virologic responses while adjusting for covariates.

Results

- Baseline characteristics are summarized in **Table 1**. No significant difference was noted between SRD and non-SRD groups in age, CD4 counts or HIV-1 viral load.
- For 516G>T genotype, the patients were categorized as extensive (GG, n=19), intermediate (GT, n=13), and slow (TT, n=5) metabolizers. The genotype was significantly associated with trough EFV concentrations adjusted with age, race, sex and BMI (p=0.036, **Figure 1**).
- ABCB1* or *CYP3A5* genotypes had no significant impact on EFV trough concentrations (**Figure 2**).
- Significantly lower EFV concentrations were noted in tobacco and alcohol users in the extensive metabolizer group (GG) with lower CD4 counts and higher viral loads (**Table 2, Figures 3 and 4**).
- SRD had no direct relationship to antiviral responses.

Results

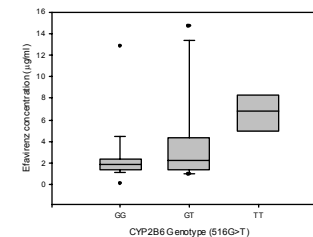


Figure 1. *CYP2B6* genotypes and efavirenz trough concentrations

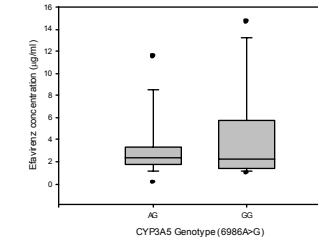


Figure 2. *CYP3A5* genotypes and efavirenz trough concentrations

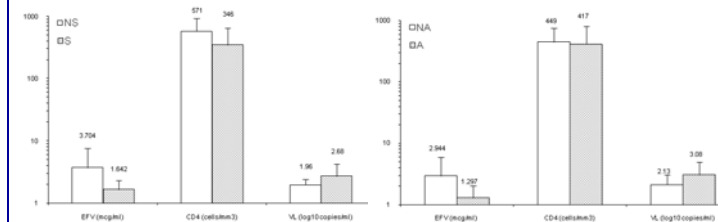


Figure 3. Effect of smoking on efavirenz concentrations, CD4 counts and viral load among HIV+ patients with *CYP2B6* 516GG

(NS=non-smoking, S=smoking)

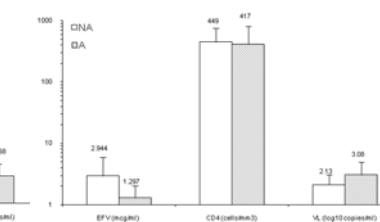


Figure 4. Effect of alcohol use on efavirenz concentrations, CD4 counts and viral load among HIV+ patients with *CYP2B6* 516GG

(A=alcohol, NA=non-alcohol)

Table 1. Clinical and demographic characteristics of HIV+ patients receiving efavirenz with and without substance related disorders (SRD)

Characteristics (Mean ± SD)	SRD	Non-SRD	P
No. of subjects	18	19	--
Males (%)	6 (33)	9 (47)	--
Age, years	46 ± 6	47 ± 7	0.801
CD4 counts, cells/mm ³	419 ± 297	428 ± 297	0.928
HIV-1 viral load, log ₁₀ copies/ml	2.4 ± 1.3	2.0 ± 0.7	0.323
Presence of HCV antibodies, %	32	17	--

Table 2. Influence of substance related disorders (SRD) on efavirenz trough concentrations – median, µg/ml (IQR)

	SRD	Non-SRD	P
Tobacco	1.761 (1.307-2.134)	2.295 (1.880-4.010)	0.043
Alcohol	1.413 (0.659-1.876)	2.247 (1.761-2.482)	0.021
Marijuana	1.732 (1.358-2.731)	2.244 (1.534-2.409)	0.430
Cocaine	1.923 (1.413-2.134)	2.045 (1.384-2.444)	0.655
Opioids	2.409 (0.678-2.487)	1.847 (1.413-2.251)	0.368

Conclusions

- These data confirm the previously reported association between *CYP2B6* 516G>T and efavirenz pharmacokinetics.
- Tobacco and alcohol use is inversely related to efavirenz trough concentrations among patients with functional allele (GG) and was associated with lower CD4 counts and higher viral loads.
- The mechanisms that underlies these observations may include both pharmacogenomic and substance related disorder components.
- Clinicians should consider these pharmacologic findings when developing ART regimens for HIV+ patients with substance related disorders.

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