

ORIGINAL RESEARCH

Cytochrome P450 2B6 (CYP2B6) G516T influences nevirapine plasma concentrations in HIV-infected patients in Uganda

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Objectives

Polymorphisms in the cytochrome P450 (CYP) 2B6 gene have been shown to influence nevirapine plasma concentrations in HIV-infected European Caucasians. Although nevirapine is used extensively in Africa, the influence of CYP2B6 genotype on nevirapine exposure has not been assessed in this population. We aimed to determine the influence of CYP2B6 genotype at position 516 on nevirapine trough concentrations in HIV-infected patients in Kampala, Uganda. Additional polymorphisms in the CYP and multidrug resistance protein-1 (MDR-1) genes were also assessed for their impact on nevirapine concentrations.

Methods

The following genotypes were determined in all subjects using polymerase chain reaction–restriction fragment length polymorphism: CYP2B6 G516T, MDR-1 C3435T and G2677T, CYP3A4*1B and CYP3A5*3. Nevirapine plasma concentrations were determined using high-performance liquid chromatography in 23 HIV-infected patients who were generally healthy and had been taking nevirapine 200 mg twice daily for at least 14 days. Analysis of variance with post hoc testing was used to compare nevirapine concentrations among CYP2B6 genotype groups.

Results

The median nevirapine trough concentration in individuals homozygous for the variant allele (TT) was 7607 ng/mL vs 4181 and 5559 ng/mL for GG and GT individuals, respectively (GG vs TT median ratio = 1.82; $P = 0.011$). The mean ratio for TT vs GG individuals (95% confidence interval) was 1.51 (1.18, 1.84). No associations were observed between the other polymorphisms studied and nevirapine concentrations.

Conclusions

CYP2B6 G516T significantly influenced nevirapine trough concentrations in HIV-infected patients in Uganda. Additional studies in larger patient populations are necessary to further define the potential clinical impact of these preliminary findings.

Keywords: Africa, CYP2B6, HIV, nevirapine, pharmacogenomics

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Introduction

Nevirapine is a nonnucleoside reverse transcriptase inhibitor (NNRTI) that is widely used in combination with nucleoside reverse transcriptase inhibitors (NRTIs) for the treatment of HIV infection. Nevirapine has also proved to be effective in reducing mother-to-child transmission (MTCT) of HIV-1 by 50% [1]. For these reasons, and because nevirapine is generally affordable and available in a fixed-dose combination, it is used extensively in African nations.

The relationship between nevirapine plasma concentrations and virological response and/or toxicity has not been

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clearly established. Some investigations have found a relationship between nevirapine exposure and virological response, while others have not [2,3]. Recently, Chaix and colleagues observed that higher nevirapine plasma concentrations were associated with the development of NNRTI resistant mutations (K103N and Y181C) in women who received single-dose nevirapine for prevention of MTCT [4]. Also noteworthy is the fact that approximately 65% of women who take nevirapine for prevention of MTCT of HIV-1 will have detectable NNRTI-associated resistance mutations at some point between 6 and 36 weeks postpartum [5]. The development of NNRTI resistance with single-dose nevirapine is presumed to occur secondary to prolonged detectable concentrations of the drug in plasma; this is consistent with the long half-life of nevirapine ($\sim$60 h) in pregnant women who receive single doses of the drug [6–8].

Among the factors that are capable of influencing nevirapine exposure is the gene that encodes the cytochrome P450 (CYP) 2B6 enzyme. The CYP2B6 isoform, which plays a significant role in nevirapine metabolism, is characterized by marked interindividual variability in expression and activity as a result of the presence of genetic polymorphisms [9–14]. A single nucleotide polymorphism (SNP) in exon 4 (G516T) is associated with a significant reduction in CYP2B6 catalytic activity [13,15,16]. This SNP (G516T) was recently noted to influence the pharmacokinetics of efavirenz, another NNRTI and a CYP2B6 substrate, in Japanese, European American, and African American individuals [17,18]. Similarly, CYP2B6 516TT was recently associated with greater nevirapine plasma concentrations in a Swiss cohort [19]. Despite the widespread use of nevirapine in the developing world, the influence of CYP2B6 genotype on nevirapine exposure, and whether it contributes to prolonged detectable nevirapine concentrations and NNRTI resistance, have not been investigated in Africans. The purpose of this pilot investigation was to determine whether interindividual differences in CYP2B6 516 genotype could influence nevirapine trough concentrations in a Ugandan cohort. The results of this 'proof of concept' study will influence the design of future pharmacokinetic and pharmacogenetic investigations of nevirapine in HIV-infected Africans.

Methods

Study participants

The study was conducted as a single-period investigation in which trough nevirapine concentrations were determined and compared among CYP2B6 genotypes at position 516. Nevirapine trough concentration (C_{12}) values were collected exactly 12 h after a witnessed dose the previous evening. To be considered for study inclusion, candidates had to be HIV positive, $\geq$ 18 years old, and in good general health as determined from medical history, physical examination, and serum chemistry values. Subjects had to be receiving full-dose nevirapine (200 mg twice daily) for at least 14 days prior to pharmacokinetic sampling in order to achieve steady-state conditions. Subjects refrained from taking medications known to modulate CYP2B6 and CYP3A4 and/or nevirapine metabolism. The study was approved by the Joint Clinical Research Center Institutional Review Board, the Uganda National Council for Science and Technology, and the National Institute of Allergy and Infectious Diseases Institutional Review Board. Informed consent was obtained from all participants.

A total of 15 subjects (five subjects in each of the three genotype groups, CYP2B6 516GG, 516GT and 516TT) were needed to detect a 70% difference in nevirapine trough concentrations among the three CYP2B6 groups (516GG, GT and TT). Alpha (α) was selected *a priori* to equal 0.05 with $\beta = 0.2$ and $\sigma = 0.3$ [20]. Analysis of variance, with post hoc testing using the Tukey test, was used to test for differences in nevirapine C_{12} among the genotypes. A *P*-value < 0.05 was accepted as significant. SYSTAT version 11 (Systat Software Inc. Richmond, CA, USA) was used for all inferential statistical testing and Microsoft Excel was used for descriptive statistics, including the calculation of confidence intervals (CIs).

Genetic and pharmacokinetic analyses

Venous blood samples were obtained from all subjects, and DNA was isolated from peripheral leucocytes with the Qiamp system (Qiagen Inc, Valencia, CA). CYP2B6 genotype was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) as previously described [13]. Subjects were genotypically classified into groups (516GG, 516GT and 516TT) based on PCR-RFLP analysis. A secondary aim of this study was to determine whether nevirapine C_{12} was influenced by the multidrug resistance protein-1 (MDR-1) genotype at positions 3435 and 2677, the CYP3A4*1B genotype, and/or the CYP3A5*3 null allele. DNA was isolated from peripheral leucocytes and PCR-RFLP was used to determine MDR-1 and CYP3A4/5 genotypes as described above [21–23].

Nevirapine concentrations in human plasma were determined using a high-performance liquid chromatography (HPLC) liquid–liquid extraction method. Percentage errors, as a measure of accuracy, were $< 10\%$, and the inter- and intra-assay coefficients of variation were 4.35–8.55%

and 3.54–6.52%, respectively ($R^2 = 0.998$) and the limit of detection was 25 ng/mL.

Results

Twenty-three of 31 subjects screened met the inclusion criteria and were included in final analyses. The median age of the participants was 35 years (range 27–64 years) and the study population included 16 female and seven male patients. The numbers of subjects with CYP2B6 GG, GT and TT genotypes at position 516 were 13 (57%), six (26%), and four (17%), respectively. The median (mean) nevirapine C_{12} in individuals homozygous for the variant allele (TT) was 7607 ng/mL (6960 ng/mL) vs 4181 ng/mL (4617 ng/mL) for GG and 5559 ng/mL (5464 ng/mL) for GT individuals ($P = 0.011$ for GG vs TT) (Fig. 1). The mean ratio for TT vs GG individuals (95% CI) was 1.51 (1.18, 1.84). Of the seven male patients, two were homozygous for mutants at CYP2B6 516 (29%), two were heterozygous (29%) and three expressed the wild-type allele (43%). Results were similar for the female group, in which two patients were homozygous for the CYP2B6 516 mutant (13%), four were heterozygous (25%), and 10 carried the wild type (63%). These data do not suggest an obvious association between CYP2B6 genotype and gender, although the study was not powered to detect such differences. Also, there was no visible association between nevirapine trough concentration and gender (5380 vs 5027 ng/mL for male and female patients, respectively). Among the other genetic polymorphisms that were evaluated (MDR-1, CYP3A4, and CYP3A5), none was found to significantly influence nevirapine C_{12}, although the study was not

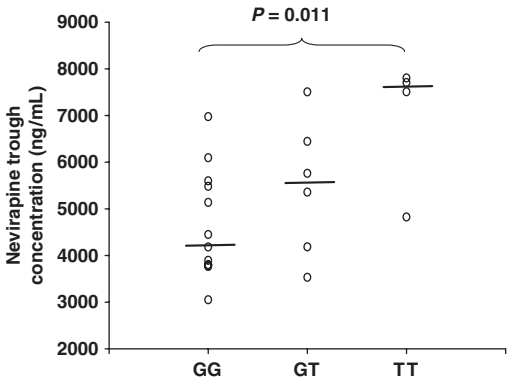

Fig. 1 Nevirapine trough concentrations in subjects with Cytochrome P450 2B6 (CYP2B6) 516GG, GT and TT genotypes. GG vs TT, $P = 0.011$; GT vs TT, $P = 0.19$; GT vs GG, $P = 0.39$ (analysis of variance with post hoc Tukey test). Open circles represent individual nevirapine trough concentrations; thick horizontal lines represent the median value.

Table 1 Influence of cytochrome P450 3A4 (CYP3A4), CYP3A5 and multidrug resistance protein-1 (MDR-1) on nevirapine trough concentrations

	Genotype														
	CYP3A4[†]			CYP3A5[‡]			MDR-1 3435				MDR-1 2677				
	*1A/*1A (AA)	*1A/*1B (AG)	*1B/*1B (GG)	*1/*1 (AA)	*1/*3 (AG)	*3/*3 (GG)	CC[§]	CT	TT	GG[§]			GT	TT	
n	0	15	8	13	8	2	19	4	0	22			1	0	
Nevirapine trough concentration (ng/mL)															
Median	–	4825	5559	5137	4688	5945	5358	4125	–	4981			6975	–	
Range	–	3532–7803	3051–7506	3051–7507	3532–7803	4184–7706	3051–7803	3764–5481	–	3051–7803			–	–	
P (ANOVA)	AG vs GG, $P = 0.84$			AA vs AG, $P = 0.96$ AG vs GG, $P = 0.76$ GG vs AA, $P = 0.83$			CC vs CT, $P = 0.21$			Not statistically evaluable					

[†]*1A is the wild-type allele.
[‡]*1 is the wild-type allele; *3/*3 (homozygous for the mutant allele) does not express the CYP3A5 enzyme.
[§]C and G are MDR-1 wild-type alleles at positions 3435 and 2677, respectively.
ANOVA, analysis of variance.

specifically powered to assess differences in nevirapine concentrations resulting from these additional polymorphisms (Table 1).

Discussion

In this study, CYP2B6 G516T significantly influenced nevirapine trough concentrations in HIV-infected African patients. Similarly, in a recent Swiss cohort study in 59 HIV-infected subjects, CYP2B6 516TT was associated with a 1.7-fold increase in nevirapine plasma concentrations compared with individuals with the GG allele ($P = 0.006$) [19]. This observation is in agreement with our data, which showed a mean (95% confidence interval) 1.5-fold increase (1.18, 1.84) in nevirapine C_{12} in TT vs GG individuals.

In addition to nevirapine concentrations, CYP2B6 516TT has also been associated with greater efavirenz exposure and central nervous system toxicity [17,19,24,25]. In a study conducted by Haas and colleagues, median efavirenz AUC_{0-24} (the area under the concentration vs time curve from zero to 24 hours post dose) values for subjects with CYP2B6 516GG, GT and TT genotypes were 44, 60 and 130 μg/h/mL, respectively ($P < 0.0001$) [18]. Results from our investigation are similar to the data reported by Haas *et al.* with regard to the relationship between CYP2B6 genotype and drug concentrations, with TT > GT > GG, although the comparative differences in drug concentrations between TT and GG individuals appear to be greater in magnitude for efavirenz (~3-fold for TT vs GG) than for nevirapine (1.5–1.7-fold for TT vs GG) [19]. This observation is consistent with *in vitro* data showing that CYP2B6 constitutes the primary metabolic pathway for efavirenz oxidation, while nevirapine is significantly metabolized by CYP3A4 in addition to CYP2B6 [26,27].

The potential limitations of this study include its relatively small sample size and the fact that trough nevirapine concentrations were collected *in lieu* of characterizing total drug exposure (AUC) and oral clearance (Cl/F). Despite the relatively small sample size ($n = 23$) of the study, it possessed sufficient power to detect a significant difference in nevirapine C_{12} between 516TT and 516GG subjects. A key characteristic of the data that added to the power of the study was the minimal variability in nevirapine concentrations that we observed in all three genotype groups. Percentage coefficients of variation (%CV) within the genotypes were 21% (TT), 27% (GT), and 25% (GG). The low variability we observed in nevirapine concentrations may have been attributable to the fact that subjects stayed overnight at the clinic and were given an observed nevirapine dose prior to trough collection the next morning. Thus, inaccuracies in sample collection did not contribute to variability in nevirapine trough concentrations. Lastly, the fact that one genotype group (TT; $n = 4$) did not achieve their predetermined sample size goal ($n = 5$ subjects per group) did not prevent us from detecting a significant difference between TT and GG individuals; this was probably attributable to the excess number of subjects in the other two genotype groups as well as the minimal variability in nevirapine concentrations noted above. Moreover, this allowed us to detect a significant difference of 51% between two of the genotype groups (vs 70% indicated in the power analysis).

To quantify the effect of CYP2B6 G516T on nevirapine disposition, it will be necessary to assess the relationship between nevirapine pharmacokinetic parameter values for AUC and Cl/F and CYP2B6 genotype at position 516. However, it is interesting to note that, in this study, nevirapine trough concentrations (such as are obtained in a therapeutic drug monitoring setting) were sensitive enough to reflect differences related to CYP2B6 516 genotype. To this end, this study provides 'proof of concept' that CYP G516T influences nevirapine C_{12} in HIV-infected African patients. Larger, definitive studies are necessary to characterize the clinical impact of CYP2B6 genetic polymorphisms on virological response and toxicity associated with nevirapine use. Such studies should also take into account the influence of additional CYP2B6 genotypes, such as C1459T and A785T, on nevirapine disposition and drug response [28].

The influence of CYP2B6 516TT on nevirapine exposure is of particular importance in the developing world in view of the widespread use of this agent. Single-dose nevirapine has proved to be effective in reducing MTCT of HIV-1 in African mothers [29,30]. However, single-dose nevirapine was recently linked to the development of NNRTI resistance mutations (K103N and Y181C) in pregnant women postpartum [31]. It should be considered whether mothers with the CYP2B6 516TT genotype who receive single-dose nevirapine are at higher risk of persisting detectable nevirapine plasma concentrations and acquisition of NNRTI resistance compared with their CYP2B6 wild-type genotype (GG) counterparts.

In addition to pregnant women receiving single-dose nevirapine, other populations at risk for persisting detectable nevirapine concentrations and subsequent NNRTI resistance include patients stopping the drug secondary to drug toxicity or for a drug holiday, and patients receiving nevirapine-based highly active antiretroviral therapy (HAART) for treatment of primary HIV infection. Although preliminary in nature, data from the current study suggest that CYP2B6 516TT individuals may be more likely to require a continued course of dual NRTI therapy (or the temporary addition of an HIV protease inhibitor) to

prevent virtual nevirapine monotherapy and NNRTI resistance, when nevirapine therapy is discontinued.

The data obtained in the present study suggest that knowledge of the CYP2B6 genotype may be useful in identifying HIV-infected patients at risk for higher nevirapine concentrations. Further characterization of the relationship among CYP2B6 genotype, nevirapine exposure (AUC) and the development of NNRTI resistance mutations is necessary to confirm the significance of the CYP2B6 genotype in this context. Because of the limited HIV treatment options in developing nations, it is prudent to assess the individual patient characteristics (e.g. CYP2B6 genotype) that may eventually help to reduce the acquisition of HIV resistance and preserve the efficacy of these medications.

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