



Abstract - revised

Background: Genetic polymorphisms in drug metabolizing enzymes and transporters affect antiretroviral (ARV) drug disposition. These factors along with maturational changes likely affect ARV pharmacokinetics (PK) in children; however, their potential impact on PK and clinical outcomes are not well characterized. It has been shown that the gene polymorphism cytochrome P450 2B6 (CYP2B6) G516T is associated with decreased activity of CYP2B6 in liver and increased plasma efavirenz (EFV) levels, but no data are available regarding the impact of the CYP2B6-G516T on the PK of Nevirapine (NVP) in children.

Methods: In 126 children from PACTG 366 and 377 cohorts, who received NVP and protease inhibitors (PI, nelfinavir and/or ritonavir) as a part of HAART regimens, real-time PCR was used to genotype polymorphisms of CYP2B6. PK data at week 4 was used to calculate area under the curve (AUC) by the trapezoidal rule and determine clearance (CL). Immunologic and virologic data were also collected during HAART.

Results: Of the 126 children, 49 (39%) had the CYP2B6-516-G/G (wild type), 63 (50%) had the G/T (heterozygous) and 14 (11%) had the T/T genotype (homozygous). The age, gender, race/ethnicity, concomitant PI regimens, baseline HIV-1 RNA and CD4+ T cell percents were not different among the three groups (P = .16-.89). NVP AUC in children with the T/T genotype (95.0mcg*hr/mL) was significantly higher than those with the G/G (57.8mcg*hr/mL, P=.004) and G/T genotype (58.3mcg*hr/mL, P=.003). NVP CL in children with the CYP2B6-516-T/T genotype (1.6 L/hr/m2) was significantly decreased compared to those with the G/G (2.3 L/hr/m2, P=.001) and G/T genotype (2.1 L/hr/m2, P=.003). Children with the T/T genotype had a significant increase in CD4+ T cell percents (+7.0%) compared to those with the G/G (+3.4%, P = .008) and G/T genotype (+5.5%, P = .04) at week 12. This trend continued at week 24 (P = .01). Furthermore, children with the CYP2B6-516-T/T genotype had a more rapid decline of log HIV-1 RNA (-1.92 log10 copies/mL) compared to those with the G/G (-1.31 log10 copies/mL, P=.008) and G/T genotype (-1.33 log10 copies/mL P=.04) from baseline to week 12. This trend also continued at week 24 (P = .04, P = .03, respectively).

Conclusions: The CYP2B6-G516T genotype alters NVP PK as well as immunologic and virologic responses to NVP containing HAART regimens in children. These data suggest that the CYP2B6-G516T is an important genetic variant that alters the PK of NNRTIs and may impact the response to HAART regimens containing NVP or EFV.

Background

- Nevirapine (NVP) is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) and has been used widely for treating HIV-1 infected patients as a component of highly active antiretroviral therapy (HAART).
- NVP is mainly metabolized by hepatic CYP2B6 and CYP3A4 and eliminated primarily in the urine.
- Several studies have shown the importance of the CYP2B6-G516T gene polymorphisms impacting on plasma EFV pharmacokinetics (PK) in HIV-1 infected adults as well as those in children; however, only one study has examined the impact of the CYP2B6-G516T gene polymorphisms on NVP PK in HIV-1 infected adults; however, the numbers of subjects were limited and the clinical responses to the different NVP concentrations were not evaluated.
- PK of NVP may differ greatly between children and adults due to physiological differences associated with growth and development, as well as the immaturity of enzyme systems and clearance mechanisms in children.

Objectives

1. To determine if the CYP2B6-G516T polymorphism affects NVP pharmacokinetics in HIV-1 infected children receiving HAART.
2. To investigate relationships between CYP2B6-G516T and clinical outcomes in HIV-1 infected children receiving HAART containing NVP.

Subjects and Methods

- **Subjects:** Of the subjects enrolled in PACTG 366 and 377, we selected 126 subjects who satisfied the following criteria: 1) Patients received HAART for at least 24 weeks, 2) virologic and immunologic data were available at baseline, weeks 12 and 24, 3) pharmacokinetic data for NVP were available at week 4 (intensive) and intermittently (sparse) during therapy.
- **Amplification and detection of gene polymorphisms by real-time PCR:** CYP2B6-G516T, CYP2B6-C1459T and *MDR1*-C3435T genotypes were detected using real-time PCR in LightCycler with the LightCycler FastStart DNA Master Hybridization Probe kit (Roche Diagnostics). The genotypes were determined by melting curves.
- **NVP pharmacokinetics:** Among the 126 patients: 40 had intensive sampling data available at week 4 of treatment and 86 had sparse PK data available during HAART. AUC was determined using noncompartmental analysis for intensive PK and by Bayesian post-hoc analysis (1 compartment) of sparse PK data (NONMEM). The pharmacokinetic parameter of interest was oral clearance at week 4 (CL/F) adjusted to body surface area (CL/F/m²).
- **Other clinical markers:** Plasma HIV-1 RNA was quantified using the Roche Amplicor HIV-1 Monitor assay (Roche Molecular Systems). The analysis had a detection limit of 400 copies/mL of RNA. The absolute numbers and percentages of CD4+ T cells were determined in PACTG certified laboratories by flow cytometry. Alanine transferase (ALT) and aspartate transferase (AST) were quantified as a part of study protocol at each study visit.
- **Statistical analyses:** The Kruskal-Wallis test: to determine whether the following parameters differed among the CYP2B6-G516T genotypes: i) clearance rate for NVP, ii) clinical parameters including baseline plasma HIV-1 RNA and CD4+ T cell percents, and iii) incidence of adverse effects. The Wilcoxon sum rank test: comparisons when 2 of the 3 genotypes were compared with regards to the pharmacokinetic and clinical parameters. The Spearman correlation test: to evaluate the correlation between NVP clearance and age. All P-values calculated were two-sided and a P-value of < 0.05 was considered to be statistically significant.

Table 1. Subjects from PACTG 366 and 377

PACTG	N	Experience		HAART regimens		
		PI	NNRTI	NRTIs	PI	NVP
366	N = 126	-	-	2	PI	+
	17 (14%)	-	-	2	NFV	+
	13 (10%)	-	-	2	RTV	+
	22 (17%)	+	-	1	NFV + RTV	+
377	23 (18%)	-	-	d4T	RTV	+
	51 (41%)	-	-	d4T or d4T + 3TC	NFV	+

d4T: stavudine, 3TC: lamivudine, NFV: nelfinavir, RTV: ritonavir, NVP: nevirapine

Table 2. Baseline characteristics of 126 children based on the CYP2B6-G516T genotype

Characteristics	All Patients	CYP2B6-G516T			P-value
	N = 126	G/G	G/T	T/T	
Sex no. (%)					
Male	64 (51%)	26 (21%)	32 (25%)	6 (5%)	0.80
Female	62 (49%)	23 (18%)	31 (25%)	8 (6%)	
Age (years)					
25th percentile	3.82	3.82	3.73	2.97	
Mean	6.45	6.20	6.58	6.35	0.67
75th percentile	9.08	8.58	9.63	9.73	
Race/Ethnicity no. (%)					
African-American (AA)	78 (62%)	29	38	11	0.16
Hispanic	27 (21%)	9	16	2	
White	19 (15%)	11	8	0	
Others	2 (2%)	0	1	1	
CD4+ T cell percents (%)					
25th percentile	18	17	19	14	0.44
Mean	25	26	24	24	
75th percentile	32	35	29	34	
Log plasma HIV-1 RNA (copies/mL)					
25th percentile	4.02	3.99	3.95	4.04	0.86
Mean	4.53	4.47	4.56	4.57	
75th percentile	5.04	4.95	5.17	5.10	

Figure 4. Age and NVP oral clearance

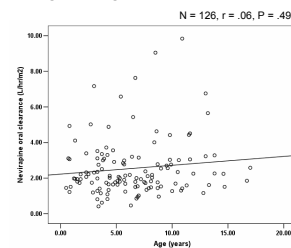
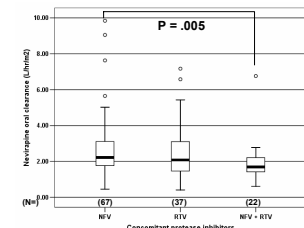


Figure 5. Concomitant PIs and NVP oral clearance



Hepatotoxicity by NVP

- Hepatotoxicity (AST or ALT: ≥5 times higher than upper normal limits) due to NVP use was observed in eight patients during the first 24 weeks of HAART (6.3%).
- The mean NVP AUC in these subjects was 61mcg*hr/mL (vs. 63 mcg*hr/mL in those without hepatotoxicity).
- CYP2B6-G516T genotype: CYP2B6-516-G/G genotype: 4 patients (50%), -G/T genotype: 2 patients (25%), and -T/T genotype: 2 patients (25%).
- *MDR1*-C3435T genotype: *MDR1*-3435-C/C genotype: 4 patients (50%), and -C/T genotype: 4 patients (50%).
- Of note, patients who experienced hepatotoxicity did not have the *MDR1*-3435-T/T genotype, or CYP2B6-1459-C/T genotype, which is consistent with findings in adults.

Conclusions

- The CYP2B6-G516T gene polymorphisms affect the oral clearance of NVP, and virologic and clinical outcomes in HIV-1 infected children receiving NVP containing HAART regimens.
- The CYP2B6-G516T genotype may be an important factor to determine the immunologic and virologic outcomes in children receiving NVP containing regimens.
- Combining with the previous EFV data, these findings support the importance of CYP2B6-G516T genotypes on NNRTI clearance and suggest that CYP2B6 gene polymorphisms may be useful in implementing strategies designed to provide optimal outcomes for children receiving NNRTI containing HAART regimens.

Acknowledgement

We thank Drs. David Haas and Richard Kim at Vanderbilt University for providing control samples for the CYP2B6-G516T and -C1459T genotypes.

Figure 1. NVP oral clearance in children with the CYP2B6-G516T genotype

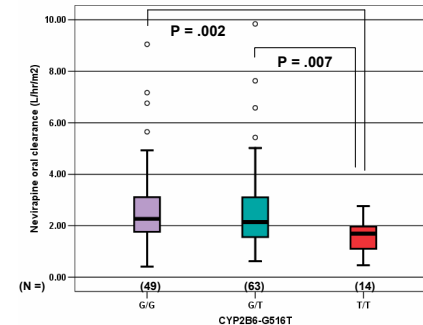


Figure 2. CD4+ T cell percent change from baseline to weeks 12 and 24 in children with the CYP2B6-G516T genotype

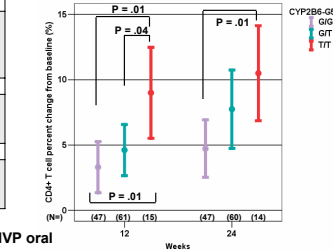


Figure 3. Log plasma HIV-1 RNA change from baseline to weeks 12 and 24 in children with the CYP2B6-G516T genotype

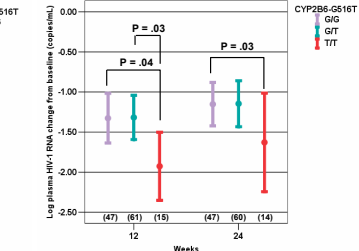


Table 3. Multivariate analysis for NVP oral clearance

Covariates	Regression Coefficient ± S.E.	P-value
Constant	3.123 ± 0.510	< .001
Age (years)	0.057 ± 0.037	.131
Sex (male)	0.339 ± 0.276	.222
Race/ethnicity (AA)	-0.377 ± 0.288	.193
Concomitant PIs (NFV + RTV)	-0.756 ± 0.366	.041
CYP2B6-516 (T/T)	-0.452 ± 0.213	.036

CYP2B6-516-T/T genotype (P = .04) and concomitant NFV + RTV (P = .04) were independently and statistically associated with NVP oral clearance.