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Factors influencing plasma nevirapine levels: a study in HIV-infected children on generic antiretroviral treatment in India

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Background: Nevirapine is an important component of paediatric combination HIV therapy. Adequate drug exposure is necessary in order to achieve long-lasting viral suppression.

Objectives: To study the influence of age, drug dose and formulation type, nutritional status and *CYP2B6* 516G>T polymorphism on blood concentrations of nevirapine in children treated with generic antiretroviral drugs.

Methods: A multicentre study was conducted at four sites in India. HIV-infected children receiving generic nevirapine-based fixed-dose combinations were recruited. Trough and 2 h nevirapine plasma concentrations were determined by HPLC. Characterization of the *CYP2B6* gene polymorphism was performed using direct sequencing. Clinical and nutritional status was recorded. Groups were compared using the Mann–Whitney *U*-test and multivariable logistic regression analysis was performed to identify factors contributing to low drug levels.

Results: Ninety-four children of median age 78 months were studied; 60% were undernourished or stunted. Stunted children had a significantly lower 2 h nevirapine concentration compared with non-stunted children ($P<0.05$); there were no significant differences in trough concentrations between different nutritional groups. Nevirapine levels were significantly higher in children with TT compared with GG and GT *CYP2B6* genotypes ($P<0.01$). Children ≤3 years had a 3.2 (95% confidence interval 1.07–9.45) times higher risk of having sub-therapeutic nevirapine concentrations.

Conclusions: Nevirapine blood concentrations are affected by many factors, most notably age ≤3 years; a combination of young age, stunting and *CYP2B6* GG or GT genotype could potentially result in sub-therapeutic nevirapine concentrations. Dosing recommendations for children should be reviewed in the light of these findings.

Keywords: nutritional status, CYP2B6 516G>T polymorphism, pharmacokinetics

Introduction

There are an estimated 2.5 million HIV-infected children worldwide, most of them living in countries with high rates of poverty and malnutrition. While access to antiretroviral treatment (ART) for adults has increased dramatically since the WHO's 3 by 5 initiative, that of HIV-infected children has lagged behind. This is due partly to lack of diagnostic facilities and partly to lack of affordable and appropriate antiretroviral formulations.

India rolled out its free ART programme in April 2004 scaling up rapidly: >350000 patients had been initiated on treatment, including ~21000 children, as of August 2010.[1] In 2006, the WHO published guidelines for the treatment of children in resource-poor settings and these were updated in 2008.[2] Specifically, the dose of nevirapine was revised to a higher range of 300–400 mg/m^2/day, based on experience and emerging data that children required a higher mg/kg dose of nevirapine.[3] In India, ART was initially provided using adult formulations; paediatric

formulations were made available by the National AIDS Control Organisation (NACO) at government ART centres from November 2006. These specially formulated paediatric generic drugs are available as fixed-dose combinations (FDCs), consisting of stavudine with lamivudine and nevirapine formulated in two different ratios (6:30:50 and 10:40:70 mg). A weight-based dosing card has been developed for ease of use in the ART centres, which aims to provide the correct dose of all three drugs to children in a simplified way using weight bands (Table 1).[4]

It has been suggested that adult FDCs, while suitable for older children, are not appropriate for young children.[5] Not many studies have specifically examined the bioavailability of paediatric FDCs[5,6] and none has been performed in India. The aim of this study was to examine the influence of age, sex, drug dose, type of formulation, nutritional status and *CYP2B6* 516G>T polymorphism on the steady-state plasma concentrations of nevirapine in HIV-infected children receiving treatment with generic adult or paediatric antiretroviral formulations in India.

Methods

Patients

HIV-infected children (6 months–12 years) receiving ART for at least 15 days at the Government Rajaji Hospital (Madurai), BJ Wadia Hospital (Mumbai), Government Hospital of Thoracic Medicine (Chennai) and Kilpauk Medical College and Hospital (Chennai) were recruited to the study. None was on concurrent rifampicin-containing anti-tuberculosis treatment. The study was conducted at the outpatient clinics of the hospitals, after obtaining approval from the institutional ethics committees of the individual study sites, and written informed consent from parent/guardian. Blood was drawn when the child came in to the clinic (trough concentration) and 2 h after directly observed administration of antiretroviral drugs. CD4 cell counts and viral load values were noted from the patients' records wherever available.

Treatment

All children were receiving a generically formulated combination of lamivudine and nevirapine with stavudine. Dosing was based on body weight bands as per NACO guidelines (Table 1). Further, while the majority of children received either one of two paediatric formulations, 39 children received an adult formulation. The parent or guardian was questioned regarding adherence and whether the child had been administered the previous night's dose.

Assessment of nutritional status

Height (to the nearest cm) and weight (to the nearest 0.1 kg) were measured. The Z scores for weight and height were computed based on the child's age and gender using the EPI-NUT component of the EPI-INFO 2002 software package (version 3.4.3) from the CDC [based on the National Centre for Health Statistics (NCHS) reference median values]. The Global Database on Child Growth and Malnutrition recommends a cut-off Z score lower than −2 to classify low weight-for-age (WAZ) (underweight), low height-for-age (HAZ) (stunting) and low weight-for-height (WHZ) (wasting) as moderate and a Z score lower than −3 SD to define severe under-nutrition.[7] A Z score lower than −2 indicates that a child's HAZ, WAZ or WHZ is 2 SD below the age- and gender-specific median for the normal population.

Drug estimations

Plasma concentration of nevirapine was determined by a validated HPLC (Shimadzu Corporation, Kyoto, Japan) method with UV detection.[8] 3-Isobutyl 1-methyl xanthine was used as the internal standard. Calibration curves using known concentrations of nevirapine (0.05–10 μg/mL) were constructed on each assay day. Inter-day variations of the calibration curve standards ranged from 98% to 104% with an accuracy of 101%. Unknown concentrations were derived from linear regression analysis of the peak height ratios (analyte/internal standard) versus concentration curve.

Genotyping of CYP2B6 G516T

Genetic characterization of the *CYP2B6* gene was performed using genomic DNA extracted from whole blood. A 204 bp fragment in exon 4 of the *CYP2B6* gene containing the target site (position 516) was amplified by a single round of PCR, using the following oligonucleotide primers:[9] *CYP2B6* forward primer (5′-CTTGACCTGCTGCTTCTTCC-3′); and *CYP2B6* reverse primer (5′-TCCCTCTCCGTCTCCCTG-3′). The amplicon was directly sequenced using a 3100 Avant Genetic Analyzer (Applied Biosystems, USA).

Table 1. NACO revised paediatric dosing schedule (2007)

Weight (kg)	Type of tablet	No. of tablets daily		Nevirapine daily dose (mg)	Nevirapine daily dose mg/m^2	Nevirapine daily dose mg/kg
		AM	PM			
3–4.9	stavudine 6+lamivudine 30+nevirapine 50	1	1	100	189–426	5–20
5–5.9		1.5	1	125		
6–9.9		1.5	1.5	150		
10–11.9		2	2	200		
12–13.9	stavudine 10+lamivudine 40+nevirapine 70	1.5	1.5	210	83–445	3.3–18.7
14–16.9		2	1.5	245		
17–19.9		2	2	280		
20–24.9	stavudine 30+lamivudine 150+nevirapine 200	1	0.5	300	222–750	4.8–22.3
≥25.0		1	1	400		

Statistical evaluation

Analysis of data was performed using SPSS version 14. Comparison of nevirapine concentrations between different groups of children was performed using the Mann–Whitney *U*-test. Multiple group comparisons were performed using the Kruskal–Wallis test. A χ^2 test was used to test the difference between the proportions of children with sub-therapeutic nevirapine trough concentrations (<3.0 μg/mL) receiving < and ≥300 mg/m^2/day, and with different grades of malnutrition. Multiple logistic regression analysis by a backward elimination method was carried out to identify the contribution of various factors resulting in sub-therapeutic nevirapine trough concentrations. A *P* value of ≤0.05 was considered statistically significant.

Results

Ninety-four HIV-infected children participated in the study (patient characteristics given in Table 2). Trough and 2 h nevirapine concentrations were available for 88 and 87 children, respectively (a few children had only one sample collected). The majority of the children were malnourished with 37% and 20% moderately and severely underweight, and 21% and 40% moderately and severely stunted, respectively (sub-groups combined for analysis). The daily prescribed nevirapine dose in mg/m^2/day was significantly lower in the formulation with 70 mg of nevirapine compared with the other formulations with 50 and 200 mg of nevirapine. However, the type of formulation used did not have any impact on peak or trough concentrations.

Table 3 shows plasma nevirapine in children grouped based on age, sex, dose, formulation, HAZ and WAZ. Stunted children had a significantly lower 2 h nevirapine concentration compared with non-stunted children ($P<0.05$), but trough concentrations were not different. Children ≤3 years of age had both lower trough and 2 h nevirapine concentrations compared with those >3 years; the differences were statistically significant ($P<0.05$). Though a higher proportion of children ≤3 years had sub-therapeutic levels compared with those >3 years, this was not statistically significant (59% versus 31%, respectively). On multivariable logistic regression, among the variables studied such as age, nevirapine dose in mg/m^2/day, HAZ and WAZ, age was the only factor found to be significantly associated with sub-therapeutic nevirapine trough concentrations of <3 μg/mL (odds ratio 3.2; 95% confidence interval 1.07–9.45; $P<0.05$).

CYP2B6 516G>T genotyping was performed in 28 children in whom adequate blood samples were available; 11, 12 and 5 had GG, GT and TT genotype, respectively. The demographic details and nevirapine concentrations of these 28 children are shown in Table 4. No significant association was observed between the genotypes and age or nevirapine dose, though children in the GG and GT groups were apparently younger and more malnourished. Trough and 2 h nevirapine concentrations were significantly higher in the children with TT genotype compared

Table 2. Demographic characteristics of study participants ($n=94$)

Variables	Median (range)
Age (months)	78.1 (9.6–184.5)
Weight (kg)	16.0 (5.7–36.4)
WAZ score	−2.1 (−4.9–2.2)
Height (cm)	109.0 (64.0–156.8)
HAZ score	−2.6 (−6.6–3.8)
WHZ score	−0.4 (−4.6–10.0)
Body mass index (kg/m^2)	14.5 (8.8–20.2)
Body surface area (m^2)	0.7 (0.3–1.3)
Duration of ART (months)	17 (1–54)
CD4, %	12 (3–30)
Daily prescribed NVP dose (mg/m^2/day), d4T:3TC:NVP	
6:30:50	294.7 (178.6–476.2)
10:40:70	280.0 (83.3–567.5)[a]
30:150:200	353.1 (222.2–750.0)
Daily prescribed NVP dose (mg/m^2/day)	
≤3.0 years	312.5 (178.6–567.5)
>3.0 years	318.2 (83.3–750.0)
Daily prescribed NVP dose (mg/kg/day), d4T:3TC:NVP	
6:30:50	13.6 (5.0–26.3)
10:40:70	13.5 (3.3–25.9)
30:150:200	14.2 (8.7–18.8)

d4T, stavudine; NVP, nevirapine; 3TC, lamivudine.
[a]$P<0.05$.

Table 3. Plasma nevirapine concentrations (median and range) in different groups of children

Groups	*n*	Trough (μg/mL)	*n*	2 h (μg/mL)
Sex				
female	41	3.6 (0.6–12.6)	39	5.0 (1.5–16.3)
male	47	3.7 (0.1–10.1)	48	6.0 (1.6–18.9)
Dose				
<300 mg/m^2/day	40	3.3 (1.3–10.9)	40	5.3 (2.1–18.9)
≥300 mg/m^2/day	48	4.1 (0.1–12.6)	47	6.2 (1.5–16.3)
Drug formulations, d4T:3TC:NVP				
6:30:50	24	3.5 (0.1–10.9)	22	5.2 (1.5–13.5)
10:40:70	25	3.3 (1.2–10.1)	27	5.3 (1.6–18.9)
30:150:200	39	4.5 (0.6–12.6)	38	6.1 (1.6–16.3)
HAZ score				
stunted (<−2 HAZ)	55	3.6 (0.6–12.6)	55	5.3 (1.6–14.3)[a]
normal	33	3.9 (0.1–10.0)	32	6.1 (1.5–18.9)
WAZ score				
underweight (<−2 WAZ)	51	3.7 (0.6–12.6)	53	5.6 (1.6–18.9)
normal	37	3.2 (0.1–10.9)	34	5.6 (1.5–16.3)
Age				
≤3 years	17	2.5 (0.1–10.9)[a]	14	4.2 (1.5–13.5)[a]
>3 years	71	4.0 (0.6–12.6)	73	5.7 (1.6–18.9)

[a]$P<0.05$.

Table 4. Demographic details and plasma nevirapine concentrations among children with different genotypes of *CYP2B6* 516G>T polymorphism

	Median (range)		
Variables	GG ($n=11$)	GT ($n=12$)	TT ($n=5$)
Age (months)	77.7 (16.4–140.8)	80.1 (48.5–134.1)	99.9 (63.3–127.3)
Weight (kg)	17.4 (7.5–23.5)	17.5 (14.0–27.0)	16.0 (11.0–28.0)
WAZ score	−2.4 (−4.0 to −1.5)	−2.0 (−3.0–0.4)	−1.8 (−3.8 to −1.2)
Height (cm)	104.0 (72.0–136.0)	109.5 (95.0–131.0)	113.0 (97.0–134.0)
HAZ score	−2.7 (−4.1 to −1.3)	−2.0 (−4.6–2.0)	−1.7 (−3.2–1.2)
WHZ score	−1.2 (−2.7–10.0)	−0.5 (−4.6–10.0)	−1.3 (−3.0–10.0)
Body mass index (kg/m^2)	13.9 (12.0–16.1)	14.5 (9.1–17.1)	13.7 (11.7–15.6)
Body surface area (m^2)	0.7 (0.4–0.9)	0.7 (0.6–1.0)	0.7 (0.6–1.0)
Duration of ART (months)	14 (1–54)	14 (1–54)	13.5 (1–33)
CD4, %	12.5 (5–23)	18 (5–27)	7 (3–11)
Daily prescribed NVP dose (mg/m^2/day), d4T:3TC:NVP			
6:30:50	279 (195–320)	270[a]	293[a]
10:40:70	314 (302–327)	280 (83–400)	342 (239–446)
30:150:200	238 (222–430)	299 (233–388)	375 (361–388)
Daily prescribed NVP dose (mg/kg/day), d4T:3TC:NVP			
6:30:50	15.6 (13.3–16.0)	11.4[a]	13.0[a]
10:40:70	14.3 (14.1–14.5)	12.5 (3.3–18.7)	18.0 (13.6–22.3)
30:150:200	10.8 (8.7–17.0)	12.4 (9.1–16.6)	14.6 (14.3–15.0)
Trough concentration (μg/mL)	3.1 (1.7–7.7)	4.0 (2.3–10.0)	9.5 (6.7–10.2)[b]
2 h concentration (μg/mL)	5.7 (2.5–13.2)	5.6 (2.5–13.0)	12.6 (8.1–16.3)[b]

[a]Value represents a single observation.
[b]$P<0.01$ (Kruskal–Wallis test).

with GG and GT genotypes ($P<0.01$). None of the children in this study had any nevirapine-associated toxicity.

Overall, 32 children (35%) had sub-therapeutic nevirapine trough concentrations; of those, 10 children were ≤3 years. *CYP2B6* 516G>T genotype status was known for only seven children, of whom four and three belonged to GG and GT genotypes, respectively. Post-ART viral load values were available for 10 of these 32 children; four had detectable viral load after 6 months of ART. Post-ART viral load was also available for nine of the remaining children with adequate blood concentrations; two of these nine children had a detectable viral load; this difference (4/10 versus 2/9) was not significant.

Discussion

In this cohort of children being treated with generic paediatric FDCs in India, a substantial proportion (35%) had sub-therapeutic nevirapine trough levels (<3 μg/mL), and this was more pronounced in young children. Of the factors investigated, age was the major predictor of low nevirapine levels, confirming previous reports.[5,6] However, most previous studies were in children receiving fractions of adult FDC tablets and there has been limited information on children receiving paediatric formulations so far. While low blood drug concentrations could be due to poor bioavailability, poor compliance, low dose or an increase in metabolism, the first three factors are unlikely in this case. Metabolism of drugs varies with age but the relationship of age and drug dosage is not linear. In general, newborns and infants <1 year metabolize drugs more slowly, while for children >1 year of age, significantly higher weight-corrected doses compared with adults are needed for drugs eliminated by the cytochrome P450 (CYP) isozymes CYP1A2, CYP2C9 and CYP3A4 (including nevirapine). In contrast, weight-corrected doses for drugs eliminated by renal excretion or metabolism by CYP2C19, CYP2D6, *N*-acetyltransferase and UDP glucuronosyltransferase in children are similar to those in adults.[10,11] Our finding of younger children being at higher risk of having sub-therapeutic plasma nevirapine trough concentrations (in spite of using nevirapine doses in the recommended dosing range of 300–400 mg/m^2) is similar to that reported by Poerksen *et al.*[12] They reported that while using adult Triomune 30 tablets, the majority of children achieved a therapeutic nevirapine concentration; however, treatment was suboptimal in younger children receiving dosages of less than half-tablets twice daily. Ellis *et al.*[5]

reported that adult FDCs were not well suited to children, particularly at younger ages, while L'homme *et al*.[6] observed that certain paediatric formulations (Triomune baby and junior) were appropriate for children weighing ≥6 kg. Mulenga *et al*.[13] indicated that blood concentrations may be lower in infants weighing 3–6 kg taking paediatric FDCs. While we confirmed the vulnerability of children ≤3 years to being dosed inadequately, in our study, the formulation type did not influence drug levels, though we did not have enough children weighing <6 kg to test this sub-group properly.[13]

The weight-based dosing card developed by NACO[4] is based on the dosing schedule recommended by the WHO. However, about half of the patients in this study were receiving less than the minimum recommended dose of 300 mg/m^2/day. The mean nevirapine doses received by the children in the present study were 290, 269 and 357 mg/m^2/day for the 50, 70 and 200 mg nevirapine formulations, respectively. While the 50 mg (paediatric) and 200 mg (adult) combinations resulted in doses closer to the recommended range, the 70 mg formulation resulted in the lowest doses received by the children. However, we did not observe any difference in plasma concentrations of nevirapine among children receiving the different formulations, which limits us from drawing any firm conclusions about the unsuitability of that combination. Several generic manufacturers now produce paediatric three-drug FDCs: two combinations are available in India (stavudine, lamivudine and nevirapine in the ratios 1:5:8 and 1:4:7). From the studies of Poerksen *et al*.[12] and Pollock *et al*.[14] and the present study, it appears that both the baby and adult formulations would result in adequate dosing in children >3 years of age.

In our study, although stunted children had lower 2 h nevirapine concentrations, the clinical significance of this is unclear, since we were not able to determine virological outcomes in all cases. Ellis *et al*.[5] found that stunted children in Malawi and Zambia had lower nevirapine concentrations, while wasted children tended to have higher concentrations. A similar trend emerged from our logistic regression analysis with stunting and wasting influencing nevirapine levels in opposite directions; however, neither was significant after adjusting for other variables. It is known that pathophysiological changes in the gut, liver and kidneys associated with malnutrition can alter pharmacokinetic processes, but the exact mechanisms involved are unclear.[15] Stunting is a chronic condition and is associated with gut mucosal changes that could cause malabsorption of drugs and altered levels of drug-metabolizing enzymes in the liver leading to enhanced clearance and a decrease in serum concentrations of protein-bound drugs. Wasting, being an acute condition, could possibly lead to fatty liver, causing a reduced rate of hepatic metabolism and an increase in plasma concentrations of drugs. The impact of malnutrition on antiretroviral drug levels and its role in drug pharmacokinetics and response to treatment deserves further study, since a large proportion of children initiating treatment in resource-poor settings are malnourished, and this is likely to be an important risk factor.[16] Pollock *et al*.[14] studied the pharmacokinetics of nevirapine in HIV-infected children and found that while nevirapine exposure was strongly related to dose administered and age, malnutrition did not have any effect.

The finding of higher nevirapine concentrations in children with the TT genotype of *CYP2B6* 516G>T compared with those with GG or GT is consistent with the observations of Saitoh *et al*.[17] While pharmaco-genotypes could be determined in only a small subset of children in this study, it is likely to be an important determinant of nevirapine concentrations. We and others have shown that adults with the TT mutant genotype have significantly higher concentrations of nevirapine than individuals with the GG or GT genotype.[17–19]

Ultimately, what is important are patient outcomes and response to treatment. Chokephaibulkit *et al*.[20,21] have demonstrated the effectiveness of adult FDCs in treating HIV-infected children, with appropriate nevirapine exposure and satisfactory virological and immunological responses. In the present study, while the majority of children initiating ART showed good short-term clinical and immunological response, our finding of detectable viraemia in 4 of 10 children among those with sub-therapeutic nevirapine levels, after 6 months of ART, is worrying and indicative of either suboptimal efficacy or suboptimal adherence. All the children recruited in this study were receiving treatment at government ART centres with no specific measures to monitor adherence. However, self-adherence as reported by care-givers was >90%. The possibility of sub-therapeutic nevirapine concentrations leading to development of viral resistance in the long term cannot be ruled out.

Our study had some limitations. Viral load values were available in only a small number of children; therefore correlation with treatment response could not be studied. Burger *et al*.[22] recently demonstrated that a limited sampling model using three timepoints (1, 2 and 6 h) could be used to predict nevirapine, stavudine and lamivudine AUC accurately and precisely in HIV-infected children. However, due to practical considerations, blood sampling was limited to only two timepoints in our study and the 2 h timepoint was chosen based on adult pharmacokinetic data.[23] Further, our cohort was treated using one of three different formulations, adding to the heterogeneity of the population studied. Finally, pharmacogenetic testing was possible only in 28 children due to limited availability of blood specimens—this limited the power of our analysis as this factor is likely to be an important determinant of non-nucleoside reverse transcriptase inhibitor drug levels.

To our knowledge, this is the first study to evaluate the blood concentrations of nevirapine in an Indian paediatric population receiving treatment with generic paediatric antiretroviral drugs at government ART centres. We found that the main factor affecting nevirapine blood concentrations was age, with pharmacogenetics and possibly nutritional status also contributing. A combination of factors (younger age, stunting, *CYP2B6* GG or GT genotype) could potentially result in sub-therapeutic nevirapine concentrations in some children. The study findings have important clinical implications and raise the issue of whether higher dose recommendations are required for malnourished children and those ≤3 years old. With the recent revision in guidelines to initiate treatment in infancy as soon as diagnosis of HIV has been made, dosing of nevirapine in the very young child is a priority issue.[2] A prospective study on a large number of children receiving generic drugs is required to correlate drug levels with treatment outcomes.

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Transparency declarations

None to declare.

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