Effect of diurnal variation, CYP2B6 genotype and age on the pharmacokinetics of nevirapine in African children

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Objectives: To characterize the effects of CYP2B6 polymorphisms, diurnal variation and demographic factors on nevirapine pharmacokinetics in African children.

Methods: Non-linear mixed-effects modelling conducted in NONMEM 7.3 described nevirapine plasma concentration–time data from 414 children aged 0.3–15 years.

Results: Nevirapine pharmacokinetics was best described using a one-compartment disposition model with elimination through a well-stirred liver model accounting for a first-pass effect and transit-compartment absorption. Intrinsic clearance was affected by diurnal variation (characterized using a cosine function with peak amplitude 29% at 12 noon) and CYP2B6 metabolizer status [extensive metabolizer (EM) 516GG/983TT, reference; intermediate metabolizer (IM) 516GT/983TT or 516GG/983TC, 17% lower; slow metabolizer (SM) 516TT/983TT or 516GT/983TC, 50% lower; ultra-slow metabolizer (USM) 516GG/983CC, 68% lower]. Age was found to affect pre-hepatic bioavailability: 31.7% lower at birth and increasing exponentially. Median (90% CI) evening Cmin values in the different metabolizer groups were 5.01 (3.01–7.47), 6.55 (3.65–13.32), 11.59 (5.44–22.71) and 12.32 (12.32–27.25) mg/L, respectively. Evening Cmin values were <3 mg/L in 43% of EM weighing <6 kg and 26% of IM weighing <6 kg, while 73% of SM and 88% of USM in all weight-bands had evening Cmin values >8 mg/L. Cmin was not markedly affected by administration time, but was altered by unequal splitting of the daily dose.

Conclusions: Diurnal variation does not greatly affect nevirapine exposure. However, when daily doses cannot be split equally, the larger dose should be given in the morning. To achieve homogeneous exposures, nevirapine doses for SM and USM should be reduced by 50%, and children weighing <6 kg with EM or IM metabolizer status should receive the same dose as children weighing 6–10 kg.

Introduction

Nevirapine was the first NNRTI available in low-income countries in a generic paediatric fixed-dose combination (FDC) tablet. This contributed to substantial cost reductions and improved the feasibility of treating HIV-infected children, and nevirapine is still widely used in resource-limited settings.¹–⁴ Nevirapine has several advantageous characteristics: it has fewer drug interactions than PIs, it does not cause adverse CNS events when compared with efavirenz, and its bioavailability is not affected by food.⁵

Despite its high potency, nevirapine has a low genetic barrier for mutations and suboptimal drug exposures increase the risks of developing drug resistance and treatment failure.⁶,⁷ Several studies have reported highly variable nevirapine concentrations, with levels <3 mg/L among children in the lower paediatric weight-bands when dosed according to WHO guidelines, increasing the risk of virological failure.¹,²,⁸–¹² Nevirapine concentrations >8 mg/L, on the other hand, were associated with an increased risk of treatment discontinuation due to adverse events among adults.⁷ However, paediatric studies quantifying nevirapine pharmacokinetic variability due to different sources and suggesting optimization of current dosing remain limited.⁸,¹³,¹⁴

Nevirapine has a complex metabolism mediated mainly by CYP3A4- and CYP2B6-coded enzymes.¹⁵ SNPs present in CYP2B6...
(516G>T and 983T>C) were identified as the main source of nevirapine variability in adults, as for efavirenz. The prevalence of 516G>T loss of function (LOF) polymorphisms differs between populations and is particularly high in black Africans, whereas 983T>C variants are not observed among Caucasians. In our previous investigation of efavirenz pharmacokinetics in African children, we showed that extensive metabolizers (EM; CYP2B6 516GG|983TT genotype) are at higher risk of developing subtherapeutic efavirenz concentrations. A similar investigation of differences in nevirapine exposures between various metabolizer groups when dosed by weight-band according to current WHO guidelines has not yet been conducted in children. CYP2B6 expression may be further modified by polymorphisms in genes coding nuclear receptors CAR (NR1I3) and PXR (NR1I2), although this has not been proved for nevirapine.

The effect of the CYP3A4 pathway on nevirapine pharmacokinetics is less studied. Although not confirmed for nevirapine, systemic exposures of CYP3A substrates have been shown to be altered by SNPs rs35599367 (CYP3A4*22) and rs7767464 (CYP3A5*1). Additionally, CYP3A activity exhibits diurnal variation, with nevirapine clearance rates increasing during the day and reducing at night. Differences between morning (AM) and evening (PM) nevirapine trough concentrations (Cmin) have been previously reported and may relate to diurnal variation in the CYP3A-mediated effects on pharmacokinetics.

The aim of this analysis was: (i) to model the steady-state population pharmacokinetics of nevirapine in the largest cohort of African children studied so far; (ii) to quantify demographic and genotypic effects on nevirapine disposition; (iii) to characterize the effect of diurnal variation on nevirapine exposures under various dosing scenarios; and (iv) to propose optimal dosing strategies for this population.

**Methods**

In this analysis, sparsely sampled data from the CHAPAS-3 trial (Children with HIV in Africa—Pharmacokinetics and Adherence of Simple Antiretroviral Regimens) was enriched with intensive data from an earlier pharmacokinetic sub-study (part of CHAPAS-1). Both studies were conducted in African children from Uganda and Zambia, as briefly described below.

**CHAPAS-1**

The trial evaluated dosing of, and adherence to, new paediatric FDC tablets: Trionune Baby (50 mg nevirapine, 6 mg stavudine and 30 mg lamivudine) and Junior (100 mg nevirapine, 12 mg stavudine and 60 mg lamivudine) in children <14 years dosed twice daily according to WHO 2006 guidelines. When the daily dose could not be split equally, the lar-

**CHAPAS-3**

Pharmacokinetics, toxicity, acceptability, adherence and virological efficacy were compared between three first-line antiretroviral regimens in children 13 years or younger. Depending on treatment allocation, patients received: Trionune Baby, Trionune Junior, Duovir-N Baby (50 mg nevirapine, 60 mg zidovudine and 30 mg lamivudine) or nevirapine (100 mg)—all paediatric formulations; or Duovir-N (200 mg nevirapine, 300 mg zidovudine and 200 mg lamivudine) or Trionune30 (200 mg nevirapine, 30 mg stavudine and 150 mg lamivudine), formulated for adults. Nevirapine-based regimens were dosed twice daily according to WHO 2010 guidelines. When the daily dose could not be split equally, the larger dose was given in the morning.

Children on nevirapine were sampled during clinic visits at week 6, week 36 and every 24 weeks thereafter until the end of the study; at each visit two samples were taken at least 2 h apart. The self-reported times of the morning and penultimate doses were recorded. Samples were stored and analysed by LC-tandem MS at the Division of Clinical Pharmacology, University of Cape Town, South Africa. The method was linear over the range of 0.0195–20 mg/L. The average intra-assay and inter-assay CV and RE were 2.9%, 2.4% and 97%, respectively.

**Genotyping**

Genotyping was performed only on patients from CHAPAS-3 by allelic discrimination real-time PCR assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR protocol involved an initial denaturation step at 95 °C for 15 min, followed by 50 cycles of amplification at 95 °C for 15 s and final annealing at 60 °C for 1 min. Genotyping Master Mx and assays for CYP2B6 516G>T (rs3745274; ID: C_7817765_60), CYP2B6 983T>C (rs28399499; ID: C_60732328_20), CYP2B6 15582C>T (rs4803419; ID: C_78177764_10), CYP3A4*22 (rs35599367, C_59013445_10), CYP3A5 (rs7767464, C_59013445_10 and rs2307424, C_25746794_20), CYP3A4*1 (rs3003596, C_16194070_10 and rs2307424, C_25746794_20), rs112 63396C>T (rs2472677, C_26079845_10), and ABC102 (rs2125739, C_16173668_10) were obtained from Life Technologies Ltd (Paisley, UK). Opticon Monitor® version 3.1 (Bio-Rad Laboratories) was used to obtain allelic discrimination plots and make allelic calls.

The distribution of the genotypes was tested for Hardy–Weinberg equilibrium using the exact test in the R ‘genetics’ package.

**Population pharmacokinetic analysis**

**Model building**

The steady-state pharmacokinetics of nevirapine was analysed using non-linear mixed-effects modelling with NONMEM 7.3 and the first-order conditional estimation method with interaction. PsN 4.4.0, Pirana and Opticon Monitor® version 3.1 (Bio-Rad Laboratories) was used to obtain allelic discrimination plots and make allelic calls.

The time of the preceding evening dose was assumed to be immediately prior to giving the morning dose and 1, 2, 4, 6, 8 and 12 h afterwards. The time of the preceding evening dose was assumed to be 12 h before the morning dose. Samples were stored and assayed using ultra HPLC with UV detection at the Department of Pharmacy of the Radboud University Medical Centre, Nijmegen, The Netherlands. The method was linear over the range of 0.1–10 mg/L. The average intra-assay and inter-assay coefficients of variation (CV) and relative error (RE) were 2.9%, 2.4% and 97%, respectively.
transit-compartment absorption. A semi-mechanistic well-stirred hepatic extraction model was tested for elimination, as in Gordin et al. This hepatic model assumed the following parameters: nevirapine fraction unbound in plasma ($f_u$) 40\%, hepatic plasma flow ($Q_H$) 50 L/h, and liver volume ($V_L$) 1 L for a typical 70 kg individual (allometrically scaled).

Between-subject variability (BSV) and between-occasion variability (BOV) were tested on all pharmacokinetic parameters assuming log-normal distribution. Residual unexplained variability (RUV) was described using a combined proportional and additive structure. We excluded from the analysis data with uncertain dosage history and nevirapine concentrations below the limit of quantification (BLQ), presumed to be due to non-compliance. (confirmed by undetectable concentrations of the companion antiretroviral drugs). Further implausible outliers were identified using visual checks and excluded based on conditional weighted residuals (CWRES) > 3).

**Covariate effects**

Allometric scaling was added to the model at an early stage (before covariate testing), as suggested by Anderson and Holford, and applied to all clearance and volume parameters. For intrinsic clearance (CLint) and pre-hepatic bioavailability ($F_{naiv}$) we tested the effect of age using a power, hockey-stick, exponential or sigmoidal function with/without Hill coefficients. The effect of diurnal variations was investigated using step or cosine functions. Besides weight and age, the other covariates tested were: study site, NRTI treatment backbone, sex, weight-for-age Z-score (WAZ), height-for-age Z-score (HAZ) and formulation. Pharmacogenetic effects were tested as individual SNPs (rs3745274, rs28399499, rs4803419, rs35599367, rs776746, rs3003596, rs2307424, rs2472677, rs21125739) and as metabolizer status determined by SNPs rs28399499, rs4803419, rs35599367, rs776746, rs3003596, rs2307424, rs2472677, rs21125739 and as metabolizer status determined by SNPs rs28399499, rs4803419, rs35599367, rs776746, rs3003596, rs2307424, rs2472677, rs21125739 and as metabolizer status determined by SNPs rs28399499, rs4803419, rs35599367, rs776746, rs3003596, rs2307424, rs2472677, rs21125739 and as metabolizer status determined by SNPs rs28399499, rs4803419, rs35599367, rs776746, rs3003596, rs2307424, rs2472677, rs21125739.

Mixture modelling with frequencies fixed to those observed in the study population was used to impute missing genotypes (predominantly in CHAPAS-1). Proportionality and correction factors were applied on RUV to test for differences between the assays and laboratories used.

**Results**

**Demographic characteristics and samples**

This analysis included 3305 samples (539 in intensive and 2766 in sparse pharmacokinetic profiles) from 414 African children (78 CHAPAS-1, 330 CHAPAS-3, 6 in both). Baseline demographic characteristics are presented in Table 1; 246 samples were excluded from the analysis (111 due to unclear dosage history, 87 outliers and 48 BLQ). Genotypes were available for 324 children (Table S1, available as Supplementary data at JAC Online); CYP2B6 metabolizer groups were 33.1% EM, 44.6% IM, 21.7% SM and 0.6% USM (Table 2); the mixture-model allocation for the remaining 96 individuals was 41.7% EM, 49.0% IM and 9.4% SM. All tested genotypes were in Hardy–Weinberg equilibrium (Table S1).

**Population pharmacokinetics**

Nevirapine pharmacokinetics were best described using one-compartment disposition, absorption through transit compartments...
and elimination using the semi-physiological model with first-pass hepatic extraction (Figure 1 and Appendix S1). The final model parameters were estimated relative to pre-hepatic bioavailability ($F_{\text{preH}}$, with typical value fixed to 1) and are presented in Table 3. All parameter estimates were found to be reasonably robust and adequate model fit was confirmed through GOF and VPC plots, which showed adequate fit of our model to the analysed data (Figures S1 and S2).

Implementing the well-stirred liver model decreased OFV by 42, without adding extra parameters. The model was parameterized with $\text{CL}_{\text{int}}$ following a circadian rhythm expressed through oscillations of the cosine function with zenith around 12 noon and amplitude of $\sim 29\%$ ($\Delta \text{OFV} = -91, \text{df} = 2, P < 0.001$) (Figure 2). The model identified distinct pre-hepatic ($F_{\text{preH}}$) and hepatic components ($F_{H}$) of bioavailability, since changes in liver activity mechanistically affected also $F_{H}$. The reference value of $F_{\text{preH}}$ was fixed to 1, and BSV and BOV were included. Estimating the diurnal effect reduced BSV in $\text{CL}_{\text{int}}$ by 34% and BOV in $F_{\text{preH}}$ by 41%. More details on the model implementation, including formulae explaining the relationship between model parameters, are presented in Appendix S1.

After applying allometric scaling to account for the effect of body size, and including diurnal effects and first-pass metabolism, the most significant covariate was the metabolizer status on $\text{CL}_{\text{int}}$, determined by CYP2B6 516G>T|983T>C genotype ($\Delta \text{OFV} = -217, \text{df} = 3, P < 0.001$), explaining 85% of remaining BSV in $\text{CL}_{\text{int}}$. Using six rather than four 516G>T|983T>C SNP-vector metabolizer groups reduced OFV by only 5 points (df = 2, $P = 0.08$) and was therefore not used.

Our data did not support a maturation effect on $\text{CL}_{\text{int}}$, but we identified age-driven differences in $F_{\text{preH}}$ which were described using an exponential model [Equation (7) in Appendix S1]. $F_{\text{preH}}$ at birth was estimated as 58.3% of the value in older children (reference fixed to 100%). 90% of $F_{\text{preH}}$ was reached by age of $\sim 3.3$ years and the half-life of the process was 1.55 years (Figure 3).

The model estimated that an average child weighing 14.5 kg and aged 4.1 years would have $F_{\text{preH}}$ 93% and their values of oral clearance ($\text{CL}_{\text{oral}}$, see Appendix S1 and Table S2) were 1.31 L/h EM (reference), 1.09 L/h IM (17% lower), 0.66 L/h SM (50% lower) and 0.42 L/h USM (68% lower). A summary of the individual exposures in children from the CHAPAS-3 trial dosed according to WHO 2010 guidelines is presented in Table 2, split by metabolizer genotype.

Higher uncertainty related to unobserved intake time (for all sparse data and pre-dose samples in intensive data) was accounted for by scaling factors (proportional model) on RUV and BOV $F_{\text{preH}}$, which were found to be respectively 1.56 and 1.54 times larger than in pharmacokinetic samples after observed intake.

No other covariates were identified as significant. The remaining stochastic variability in clearance and bioavailability was low (BSV $\text{CL}_{\text{int}}$ 21.4%, BSV $F_{\text{preH}}$ 18.7% and BOV $F_{\text{preH}}$ 17%), but absorption parameters (where no covariates improved model fit) remained highly variable [BOV absorption rate constant ($K_a$) 44.9%, BOV absorption mean transit time (MTT) 199.7%].

**Simulations**

Simulations were conducted to compare average $C_{\text{minAM}}$ and $C_{\text{minPM}}$ in weight-bands of African children divided into metabolizer groups and dosed following WHO 2010 recommendations.\textsuperscript{25}

### Table 2. Exposures of different metabolic subgroups determined by 516G>T|983T>C SNP vector

<table>
<thead>
<tr>
<th>Metabolizer status</th>
<th>$C_{\text{minAM}}$ (mg/L)</th>
<th>Median (5th–95th percentile)</th>
<th>$C_{\text{minPM}}$ (mg/L)</th>
<th>Median (5th–95th percentile)</th>
<th>Patients, n</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM, 516GG</td>
<td>T</td>
<td>983TT</td>
<td>4.58 (2.53–7.03)</td>
<td>3.8 (2.53–7.03)</td>
<td>106 (33.3)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>IM, 516GG</td>
<td>T</td>
<td>983GC</td>
<td>6.55 (4.65–12.32)</td>
<td>5.8 (4.65–12.32)</td>
<td>141 (44.2)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>SM, 516GG</td>
<td>T</td>
<td>983CC</td>
<td>11.59 (6.42–22.72)</td>
<td>10.9 (6.42–22.72)</td>
<td>70 (21.9)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>USM, 516GG</td>
<td>T</td>
<td>983CT</td>
<td>13.32 (12.32–27.25)</td>
<td>12.32 (12.32–27.25)</td>
<td>1.32 (4.1)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>EM, 516GG</td>
<td>T</td>
<td>983CC</td>
<td>5.03 (3.01–7.47)</td>
<td>4.51 (3.01–7.47)</td>
<td>59 (18.6)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>IM, 516GG</td>
<td>T</td>
<td>983TC</td>
<td>6.55 (4.65–12.32)</td>
<td>5.8 (4.65–12.32)</td>
<td>141 (44.2)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>SM, 516GG</td>
<td>T</td>
<td>983GT</td>
<td>11.59 (6.42–22.72)</td>
<td>10.9 (6.42–22.72)</td>
<td>70 (21.9)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>USM, 516GG</td>
<td>T</td>
<td>983TT</td>
<td>13.32 (12.32–27.25)</td>
<td>12.32 (12.32–27.25)</td>
<td>1.32 (4.1)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>EM, 516GG</td>
<td>T</td>
<td>983CT; IM, 516GG</td>
<td>T</td>
<td>983GT; SM, 516GG</td>
<td>T</td>
<td>983CC; USM, 516GG</td>
</tr>
<tr>
<td>EM, 516GG</td>
<td>T</td>
<td>983CC; IM, 516GG</td>
<td>T</td>
<td>983GT; SM, 516GG</td>
<td>T</td>
<td>983TC; USM, 516GG</td>
</tr>
<tr>
<td>EM, 516GG</td>
<td>T</td>
<td>983CC; IM, 516GG</td>
<td>T</td>
<td>983TC; SM, 516GG</td>
<td>T</td>
<td>983GT; USM, 516GG</td>
</tr>
<tr>
<td>EM, 516GG</td>
<td>T</td>
<td>983CC; IM, 516GG</td>
<td>T</td>
<td>983GT; SM, 516GG</td>
<td>T</td>
<td>983TC; USM, 516GG</td>
</tr>
</tbody>
</table>
Average \( C_{\text{minAM}} \) and \( C_{\text{minPM}} \) in weight-bands \( \geq 6 \) kg were \( >3 \) mg/L for most simulated individuals regardless of metabolizer status (Figure 4a). In contrast, \( >25\% \) of children in the lowest weight-band (4–6 kg) had \( C_{\text{minPM}} \) below the efficacy threshold (Figure 4b); this effect was driven mostly by EM and IM (43\% and 26\% \( <3 \) mg/L, respectively).

Table 3: Final parameter estimates (5th - 95th percentile)\(^a\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical values</th>
<th>Variability (%)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CL}_{\text{int}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM (L/h)</td>
<td>3.27 (3.00–3.69)</td>
<td>BSV ( \text{CL}_{\text{int}} ): 21.40 (20.08–32.46)</td>
</tr>
<tr>
<td>IM (L/h)</td>
<td>2.72 (2.27–2.94)</td>
<td></td>
</tr>
<tr>
<td>SM (L/h)</td>
<td>1.65 (1.47–1.89)</td>
<td></td>
</tr>
<tr>
<td>USM (L/h)</td>
<td>1.04 (0.87–1.38)</td>
<td></td>
</tr>
<tr>
<td>AMP (%)</td>
<td>29.2 (27.7–45.2)</td>
<td></td>
</tr>
<tr>
<td>SHIFT (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_c ) (L)</td>
<td>-12.30 (−13.32 to −10.38)</td>
<td></td>
</tr>
<tr>
<td>21.92 (20.24–26.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( F_{\text{preH}} ) older children(^c)</td>
<td>1 (fixed)</td>
<td>BSV ( F_{\text{preH}} ): 18.72 (6.59–20.66)</td>
</tr>
<tr>
<td>at birth (%)</td>
<td>58.30 (50.48–68.24)</td>
<td>BOV ( F_{\text{preH}} ): 17.02 (16.12–20.87)</td>
</tr>
<tr>
<td>( t_{1/2} ) (years)</td>
<td>1.54 (1.47–2.58)</td>
<td></td>
</tr>
<tr>
<td>Increased BOV ( F_{\text{preH}} ) for unobserved intake</td>
<td>1.54 (1.20–1.65)</td>
<td></td>
</tr>
<tr>
<td>MTT (h)</td>
<td>0.56 (0.49–0.70)</td>
<td>BOV MTT: 199.73 (177.23–217.70)</td>
</tr>
<tr>
<td>( K_a ) (1/h)</td>
<td>0.84 (0.67–1.12)</td>
<td>BOV ( K_a ): 44.91 (31.32–50.46)</td>
</tr>
<tr>
<td>( N_{\text{TRANS}} ) (number)</td>
<td>3 (fixed)</td>
<td></td>
</tr>
<tr>
<td>Additive error (mg/L)</td>
<td>0.32 (0.21–0.38)</td>
<td></td>
</tr>
<tr>
<td>Proportional error (%)</td>
<td>5.26 (4.26–6.18)</td>
<td></td>
</tr>
<tr>
<td>Increased error for sparse data</td>
<td>1.56 (1.49–1.81)</td>
<td></td>
</tr>
</tbody>
</table>

\( \text{CL}_{\text{int}} \), intrinsic clearance; AMP, amplitude of cosine function; SHIFT, shift in the zenith of cosine function from midnight; \( V_c \), volume of central compartment; \( F_{\text{preH}} \), pre-hepatic bioavailability; \( N_{\text{TRANS}} \), number of transit compartments (in the implementation of Savic et al.\(^39\) this would be \( NN = 2 \)); MTT, absorption mean transit time; \( K_a \), absorption rate constant; BSV, between-subject variability; BOV, between-occasion variability.

Final parameter estimates are typical population values estimated by the model. All clearance and volume parameters scaled allometrically to the median weight of 14.5 kg.

The number of transit compartments was first estimated and then fixed during the covariate analysis in order to improve model stability. The number was then re-estimated in the final model and proved not to be different from that previously fixed. The equations explaining the relation between presented parameters can be found in Appendix S1.

\(^a\) Estimated from non-parametric bootstrap (\( n = 50 \)) of the final model.

\(^b\) Expressed as approximate %CV on SD scale (\( \sqrt{\text{ETA}k100} \)).

\(^c\) Older children refers to individuals where no further age-driven increase in bioavailability can be observed (Figure 3).

Figure 1. Compartmental structure of the nevirapine pharmacokinetic model. \( \text{CL}_{\text{int}} \), hepatic clearance; \( E_{\text{int}} \), hepatic extraction; \( K_a \), absorption rate constant; \( Q_H \), hepatic plasma flow; \( V_H \), volume of the liver; \( V_c \), volume of the central compartment. The model parameters and presented relations are explained in detail in Appendix S1.
section) on average morning and evening exposures. The changes in median concentration depending on administration time and differences in systemic drug exposures are presented in Figure S3. Depending on administration time, the ratios of morning/evening exposures varied between 1.09–1.15 for \( C_{\text{min}} \) and 1.03–1.07 for AUC\(_{0–1} \), differences that are unlikely to be clinically significant.

Use of some nevirapine FDCs can lead to unequal splitting of the advised daily dose between morning and evening intakes. Simulation results showed that ratios between simulated median \( C_{\text{minAM/PM}} \) for tested dose-splitting strategies (see the Methods section) were: D1 (larger morning) 0.93, D2 (equal) 1.13 and D3 (larger evening) 1.41; and AUC\(_{0–12} \) 0.90, 1.04 and 1.22, respectively (Figure S4).

**Discussion**

We present the largest investigation to date of nevirapine pharmacogenetics, the first report of the effect of 983CC homozygosity on nevirapine pharmacokinetics and the first study in children to quantify the combined effect of CYP2B6 516G>T|983T>C. Our analysis is also the first to date to characterize the diurnal variation in nevirapine clearance through population pharmacokinetic modelling and to evaluate the effect of this phenomenon on systemic drug exposures through simulations.

The main predictor of nevirapine clearance in our cohort of African children was the combined effect of the CYP2B6 516G>T|983T>C genotype. Oral clearance estimated by our model before adjusting for the CYP2B6-SNPs was 3.8 L/h, comparable to the 3.93 L/h reported previously in children (both scaled up to 70 kg) and the 2.82–3.97 L/h found in adults. Comparing the CYP2B6 516G>T|983T>C effect with other reports is problematic, since our study is the first to use this categorization with four metabolizer subgroups for nevirapine, although it has been extensively applied to efavirenz. The 50% lower nevirapine clearance we detected for SM is greater than the 30%–37% drop previously reported for 516TT versus 516GG. Similar to efavirenz, the effect of CYP2B6 983CC (recessive homozygosity) on nevirapine pharmacokinetics is of greater magnitude than that of 516TT (68% versus 50% drop). Unsurprisingly, nevirapine clearance is affected to a lesser degree by CYP2B6 polymorphisms than efavirenz in the same population. This can be explained by a different contribution of the CYP3A4 pathway to the metabolism of these drugs.

Diurnal variation has been previously documented for several CYP3A4 substrates, consistently revealing increased clearance rates during the day as compared with during the night. Our study replicated those findings and detected significantly higher nevirapine clearance during the day, with a maximum at midday. The estimated amplitude of the diurnal variation is somewhat larger than previous reports in CYP3A4 probes. This could be due to the considerable contribution of CYP2B6 enzymes to nevirapine clearance. Although little is known about the chrono-pharmacokinetics of this pathway, our hypothesis is supported by the fact that CAR, which regulates expression of CYP2B6, exhibits a circadian rhythm linked to a 1.7-fold magnitude induction of CYP2B mRNA.

Despite the 29% amplitude of diurnal variation in nevirapine clearance, the simulated difference between morning and evening trough exposures was <15%. This lack of effect is due to nevirapine’s relatively long half-life (25–30 h at steady-state) in
Figure 4. Model-simulated exposures shown by dosing weight-bands. (a) Difference between morning and evening $C_{\text{min}}$ when dosed according to current WHO recommendations. (b) Difference in $C_{\text{min}}$ between metabolic groups when dosed according to current WHO recommendations (evening $C_{\text{min}}$ is shown). (c) Difference in $C_{\text{min}}$ between metabolic groups when dosed according to the proposed dose optimization strategy (evening $C_{\text{min}}$ is shown). Red horizontal lines correspond to the nevirapine therapeutic range, from 3 to 8 mg/L. The boxes in the percentile plots show the 25th percentiles, medians and 75th percentiles, while the whiskers correspond to the 5th and 95th percentiles of the simulated data. EM, 516GG|983TT; IM, 516GG|983TC or 516GT|983TT; SM, 516TT|983TT or 516GT|983TC; USM, 516GG|983CC. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.
To prevent suboptimal exposures, EM and IM children weighing <6 kg should receive the same dose as those in the 6–10 kg weight-band. Further homogenization of exposures can be achieved by reducing the current recommended dose for SM and USM by 50% in other weight-bands. Additionally, we characterized the effect of diurnal variation on nevirapine pharmacokinetics, and found that it is of limited clinical relevance, possibly due to nevirapine’s long half-life. However, this phenomenon should be taken into consideration when daily doses cannot be split equally and larger doses should be given in the morning.

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dosage information and BLO. The detected diurnal effect could hypothetically be further affected by food intake, which was not recorded in our study. However, food has been previously reported not to modify nevirapine bioavailability or clearance. Additionally, the analysed trials differed in the morning/evening dose-splitting strategy (see the Methods section), but the model-based approach we employed accounts for this difference.

Conclusions
This is the first study quantifying the combined effect of CYP2B6 516G>T;983T>C on nevirapine clearance in children and classifying metabolizers into four metabolic groups (EM, IM, SM and USM). To prevent subtherapeutic exposures, EM and IM children weighing <6 kg should receive the same dose as those in the 6–10 kg weight-band. Further homogenization of exposures can be achieved by reducing the current recommended dose for SM and USM by 50% in other weight-bands. Additionally, we characterized the effect of diurnal variation on nevirapine pharmacokinetics, and found that it is of limited clinical relevance, possibly due to nevirapine’s long half-life. However, this phenomenon should be taken into consideration when daily doses cannot be split equally and larger doses should be given in the morning.
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Disclaimer
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Supplementary data
Tables S1 and S2, Appendix S1 and Figures S1–S4 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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