Population Pharmacokinetic-Pharmacogenetic Study of Nevirapine in HIV-Infected Cambodian Patients

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The aims of this ANRS12154 open-label, single-center, multiple-dose pharmacokinetic study were to characterize nevirapine pharmacokinetics in a Cambodian population of HIV-infected patients and to identify environmental and genetic factors of variability, focusing on the CYP2B6, CYP3A5, and ABCB1 (MDR1) genes. A total of 170 Cambodian HIV-infected patients were included. Nevirapine trough concentrations were measured after 18 and 36 months of starting antiretroviral treatment and in samples drawn during a dosing interval in a subset of 10 patients. All data were analyzed by nonlinear mixed-effects modeling. The effect of covariates was investigated using the population pharmacokinetic model. Patients carrying homozygous loss-of-function alleles CYP3A5 6986A>G, CYP2B6 516G>T, CYP2B6 1459C>T, and ABCB1 3435C>T represent 42.4%, 9.2%, 0%, and 18% of the population, respectively. The median nevirapine trough concentrations did not differ after 18 and 36 months of treatment (5,705 ng/ml [range, ≤50 to 13,871] and 5,709 ng/ml [range, ≤50 to 15,422], respectively). Interpatient and intrapatient variabilities of nevirapine apparent clearance were 28% and 17%, respectively. CYP2B6 516G>T and creatinine clearance were found to significantly affect nevirapine apparent clearance. The estimated nevirapine apparent clearances were 2.95 liters/h, 2.62 liters/h, and 1.86 liters/h for CYP2B6 516GG, CYP2B6 516GT, and CYP2B6 516TT genotypes, respectively. The impact of creatinine clearance was small. This study demonstrates that 95% of the patients had sustained nevirapine exposure well above the 3,000-ng/ml threshold. Nevirapine clearance was shown to be affected by CYP2B6 516G>T genetic polymorphism and creatinine clearance, although this explained only part of the interpatient variability, which remains low compared to that for other antiretroviral drugs.

In resource-limited settings, nonnucleoside HIV-1 reverse transcriptase inhibitors (NNRTI) are the WHO-recommended backbone of first-line antiretroviral therapy. At the time of the study, nevirapine in combination with two nucleoside analog inhibitors of reverse transcriptase such as stavudine and zidovudine, in addition to lamivudine, was the recommended antiretroviral regimen in treatment-naïve patients, mainly because of the availability of WHO-prequalified low-cost generic fixed-dose combinations (7, 27). In Cambodia, the prevalence of HIV infection among the general population aged between 15 and 49 years peaked at 2% in 1998 and declined to 0.9% in 2006. This decrease has been attributed to many deaths among people infected during the early years of the epidemic before implementation of the continuum of care and the scaling-up of HIV prevention, care, and treatment programs. At the end of 2009, it is estimated that about 37,000 patients were on antiretroviral drug regimens and 69.5% were on a nevirapine back-
carrying the CYP3A5 6986GG (CYP3A5*3) genotype have very low or even undetectable hepatic CYP3A5 protein content. The two most relevant single nucleotide polymorphisms (SNPs) of CYP2B6 (G516T and C1459T) were demonstrated to result in a significant decrease in protein expression. ABCB1 3435C>T was associated with decreased transport function. Consequently, homozygous CYP3A5 6986GG, CYP2B6 516TT or CYP2B6 1459TT, and ABCB1 3435TT alleles are associated with loss-of-function proteins.

The aims of this ANRS12154 descriptive study were to characterize nevirapine pharmacokinetic parameters in a large Cambodian population of HIV-infected patients by using a population approach and to identify environmental and genetic factors of variability, focusing on the CYP3A5, CYP2B6, and ABCB1 (MDR1) genes. Mixed-effects models were used due to their flexibility in handling balanced and unbalanced data in a unified framework (37).

(The data in this study were presented in part at the 16th Conference on Retroviruses and Opportunistic Infections, Montreal, Quebec, Canada, 2009 [9a].)

MATERIALS AND METHODS

Patients and study design. The patients enrolled in this open-label, single-center, multiple-dose pharmacokinetic study were HIV-infected Cambodians. They have been included in the Ensemble pour une Solidarité Thérapeutique Hospitalière en Réseau (ESTHER) cohort at the Calmette Hospital (Phnom Penh, Cambodia) since 2003, where treatment and care have been provided to patients living with AIDS in Cambodia. This additional pharmacokinetic/pharmacogenetic study was approved by the National Ethics Committee of Cambodia. All patients signed an informed consent form which was explained orally in the presence of a witness for those unable to read. To be included in the study, patients consented to have an additional blood sample drawn at the 3-year evaluation for pharmacogenetics. During the first year, about 300 HIV-infected patients were included in this cohort, most of them treated with a nevirapine-lamivudine-stavudine generic fixed-dose combination. Patients were treated with 200 mg nevirapine daily for the first 2 weeks and 200 mg twice a day (b.i.d.) thereafter in addition to 30 mg stavudine b.i.d. and 150 mg lamivudine b.i.d. After 18 months of treatment, stavudine was switched to 300 mg zidovudine b.i.d. in most patients. Patients came to the clinic monthly for medical consultation and drug refills. They had to participate in at least three specific adherence consultations, prepared by nurse. All patients were routinely monitored every 6 months for standard liver and renal function tests and CD4 cell count (Cyflow; Partec, Munster, Germany) in blood. As part of the 18-month (M18) and 3-year (M36) visits for evaluation of treatment efficacy, in addition to standard laboratory tests, plasma HIV RNA (41) and nevirapine plasma trough concentration before morning drug intake were measured. Samples drawn 12 ± 2 h after evening drug intake were kept for pharmacokinetic analysis. Adherence to antiretroviral therapy was monitored using a validated visual analog scale (2). Some of the patients were tested for hepatitis C virus (HCV) and hepatitis B virus (HBV). In addition to the M18 and M36 sampling, 10 patients agreed to participate in an extensive pharmacokinetic substudy. They fasted under a steady-state regimen before antiretroviral drug administration, and blood samples were collected at predose and 1 h, 2, 4, 6, and 8 h after the nevirapine morning intake.

Genotyping. DNA was extracted from patient blood by using a QIAamp DNA minikit according to the protocol of the manufacturer (Qiagen). Genotyping for CYP3A5 6986A>G (international nomenclature for database single-nucleotide polymorphism: rs776746), CYP2B6 516G>T (rs3745274), CYP2B6 1459C>T (rs2121371), and ABCB1 3435C>T exon 26 (rs1045642) was performed using the TaqMan allelic discrimination assay (ABl Prism 7000; Applied Biosystems, Courtaboeuf, France). Primers and probes used for ABCB1 and CYP3A5 SNP detection have been described previously (10, 39). CYP2B6 genotyping was performed with the use of TaqMan validated SNP assays (C_6017765_60 and C_30634242_40) with a 7000HT sequence detection system (Applied Biosystems). Reactions were carried out as described previously (10, 39).

For each polymorphism, departure from Hardy-Weinberg proportions was tested using a χ² test with degrees of freedom equal to the number of observed genotypes minus 1.

Assay of nevirapine in plasma. Plasma nevirapine concentrations were assayed in France (M18) or Cambodia (M36) by liquid chromatography with diode array detection at 240 nm according to previously validated assays (48). The lower limit of quantification was 50 ng/ml. Standard curves were linear up to 10,000 ng/ml. The within-day and day-to-day precisions of quality control samples included in each analytical run were below 9%. Both laboratories participate in the French program of external quality controls (Asqualab).

Population pharmacokinetic analysis. Population pharmacokinetic modeling was performed using NONLIN software, version 2.4 (http://software.monolix.org). A one-compartment model at steady state, with first-order absorption (rate kₐ) and elimination parameterized in apparent volume of distribution (V/F) and clearance (CL/F), was used to describe the nevirapine concentrations. Data below the limit of quantification (50 ng/ml) were discarded from the analysis. Given the expected concentration levels, a patient with a concentration below this limit might be assumed not to have taken his pills.

In a first step, the intersubject variance matrix and the residual error model were determined using data from the 10 patients of the extended pharmacokinetic study plus the M36 nevirapine trough concentrations. The Bayesian information criterion (BIC) was used to select the residual error model (combined, proportional, or constant) and the non-null intersubject variances (6). In a second step, the concentrations collected at the M18 evaluation were added to the previous data set and intrapatient (e.g., interoccasion) variances (γ) were added to parameters with non-null intersubject variances (σ²). To model intersubject and intrapatient variabilities, we used an exponential model with Gaussian random effects.

In order to assess to what extent a model parameter is likely to be under the influence of the genetic polymorphisms, the genetic component of variability (σGC) was computed as described by Ozdemir et al. (35): \( \sigma_{GC} = 1 - (\gamma^2 + \sigma^2) \), which gets closer to 1 as the parameter is likely to be influenced by genetic polymorphisms.

The continuous covariates investigated for the CYP3A5 6986A>G, CYP2B6 516G>T, CYP2B6 1459C>T, and ABCB1 3435C>T polymorphisms were age, weight, alanine aminotransferase (ALAT), plasma creatinine, creatinine clearance, plasma HIV RNA, CD4 count, and adherence (assessed using a visual analog scale) along with sex, cotreatment ( stavudine or zidovudine), plasma HIV RNA above 400 copies/ml, HCV coinfection, HBV coinfection, and genotype.

Covariate model building was performed using an ascendant approach based on Wald tests on the effect of coefficient estimates of the population analysis. Screening of individual empirical Bayes estimates was not performed, as shrinkage is important with such a sparse design (5). For the univariate analyses, imputation of the missing covariates was performed and a 0.1 significance level was used. For final model building, the significance level was set to 0.05 and missing covariates, with the exception of genotypes, were imputed to the value obtained at the closest evaluation or to the median. A permutation approach was then performed to assess the P values associated with the covariates remaining in the final model. Permutation tests correct for the Wald test type I error inflation that has been shown to occur in such designs (5). One thousand permutations were performed to ensure the nominal level of 0.05.

In order to evaluate nevirapine clearance for each patient, it was computed as the mean over the empirical estimates at the different occasions. Simulations based on the final pharmacokinetic estimates were performed with R software, version 2.9.1 (http://cran.r-project.org), using 250 data sets to calculate the predicted 90% interval and median, which were overlaid on the observed data on a visual predictive check plot. These simulations were also used to compute normalized prediction discrepancies, using the R package (http://www.npde.biostat.fr), to be plotted versus time.

RESULTS

Characteristics of the study population. A total of 170 patients of the ESTHER cohort who were on nevirapine therapy and signed the informed consent form were included in this study. The median age of the population was 36.5 years (range, 21 to 64), and the median weight was 55 kg (range, 36 to 82). One hundred forty-five patients participated in the M18 evaluation, 161 in the M36 evaluation, and 139 in both the M18 and M36 evaluations in addition to the pharmacogenetic study. In addition, 10 patients (5 men) participated in the extensive pharmacokinetic substudy and only 3 did not participate in the M18 or M36 evaluation. The patients’ demographic and laboratory data are listed in Table 1. An undetectable viral load
Adherence was high in this population as and 91% of them still had undetectable plasma HIV RNA at in the case of increased viral load at M18 stayed on nevirapine, undetectable plasma viral load or lack of a resistance mutation patients at M18 and in 94% of patients at M36. Patients with (HIV RNA, <250 copies/ml) was achieved in 81% of the patients at M18 and in 94% of patients at M36. Patients with undetectable plasma viral load or lack of a resistance mutation in the case of increased viral load at M18 stayed on nevirapine, and 91% of them still had undetectable plasma HIV RNA at the M36 evaluation. Adherence was high in this population as 98% and 99% of the patients reported a visual analog scale of ≥8 at the M18 and M36 evaluations, respectively.

**Frequency of genetic polymorphism.** Loss-of-function alleles CYP3A5 6986A>G, CYP2B6 516G>T, CYP2B6 1499C>T, and ABCB1 3435C>T represent 65%, 35%, 1%, and 38% of the population, respectively. The test for Hardy-Weinberg proportions was nonsignificant for all four polymorphisms.

**Nevirapine exposure.** Four patients had concentrations measured at M18 and M36 and in the extensive pharmacokinetic substudy, 136 patients had concentrations measured at both M18 and M36, and 29 patients had concentrations measured at only one of these evaluations. At M18, one patient was excluded from the analysis as the only concentration was below the limit of quantification (LOQ); three other concentrations were below the LOQ, two at M18 and one at M36. Figure 1 represents the nevirapine concentrations observed at each occasion. The median nevirapine trough concentrations were 5,705 ng/ml (range, 50 to 15,422 ng/ml) and 5,709 ng/ml (range, 50 to 15,422 ng/ml) at M18 and M36, respectively. Note that 3.4% and 5.6% of the patients had nevirapine trough concentrations below 3,000 ng/ml at M18 and M36, respectively.

**Population pharmacokinetics of nevirapine.** Nevirapine concentrations were adequately described by a one-compartment model with first-order absorption and elimination. With the basic model, the apparent clearance of nevirapine was estimated to be 2.67 liters/h, with an interpatient variability of 28% and an intrapatient variability of 17%. The absorption constant and the apparent volume of distribution were 1.64/l and 213 liters (on average 3.9 liters/kg), respectively. Adding interpatient variabilities to these parameters did not improve the model. A constant residual error model with an estimated standard deviation of 519 ng/ml was selected. The estimates from the basic model as well as their relative estimation errors are given in Table 2.

The genetic component of variability, $R_{GC}$, for nevirapine clearance was 63.1%. After the first step of univariate covariate selection, **CYP2B6 516G>T** polymorphism ($P = 0.02$ and 3.10 to 10 for the GT and TT genotypes, respectively, compared with GG), creatinine clearance ($P = 0.07$), and HCV-coinfected status ($P = 0.04$) were significantly associated with the nevirapine apparent clearance (at the 0.1 level). Interestingly, in liver function tests, ALAT was not found to be a significant covariate.

Following the ascendant procedure based on the Wald test, only the effect of the **CYP2B6 516G>T** genetic polymorphism and the creatinine clearance remained in the model, so the apparent clearance of subject $i$ at occasion $k$ was predicted as follows: $CL_{ik} = CL \times e^{b_k \times [CLCR_{ik}/\text{median(CLCR)}]}$, where $b_k$ equals 0, −0.12, or −0.46 if patient $i$ has the GG, GT, or TT genotype for the **CYP2B6 516G>T** genetic polymorphism and $CLCR_{ik}$ is the patient’s creatinine clearance at occasion $k$.

$P$ values of the permutation test were 0.01 for GT versus GG, 0.001 for TT versus GG, and 0.007 for creatinine clearance. Estimates from the final model and their 95% confidence intervals derived from the standard errors are given in Table 2. The population mean clearances were estimated to be 2.95 liters/h, 2.62 liters/h, and 1.86 liters/h for patients carrying GG, GT, and TT genotypes, respectively, for the **CYP2B6 516G>T** polymorphism, which corresponds to 11% and 37% decreases in clearance from GG to the GT and TT genotypes, respectively. The lowest value of creatinine clearance was associated with a 14% decrease in CL/F, whereas the highest value of creatinine clearance was associated with a 16% increase in CL/F. The addition of the polymorphism and the creatinine clearance to the model lowered the interpatient variability by 3.1% and 0.3%, respectively.

**Figure 2** represents the effect of the **CYP2B6 516G>T** polymorphism and of creatinine clearance on individual nevirapine apparent clearances. Evaluation graphs, sorted by genotype for the **CYP2B6 516G>T** polymorphism, with the visual predictive

### TABLE 1. Characteristics of patients at 18 months and 36 months of evaluation

<table>
<thead>
<tr>
<th>Characteristic (unit)</th>
<th>M18 ($n = 145$)</th>
<th>Total no. of patients</th>
<th>M36 ($n = 161$)</th>
<th>Total no. of patients</th>
</tr>
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<tbody>
<tr>
<td>Median (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>36.0 (19.0–56.0)</td>
<td>145</td>
<td>37.0 (21.0–64.0)</td>
<td>161</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>53.5 (25.0–79.0)</td>
<td>142</td>
<td>55.0 (36.0–82.0)</td>
<td>158</td>
</tr>
<tr>
<td>ALAT (IU/ml)</td>
<td>27.5 (7.0–291)</td>
<td>134</td>
<td>29.0 (11.0–212.0)</td>
<td>161</td>
</tr>
<tr>
<td>Creatinine (µmol/liter)</td>
<td>7.0 (5.0–32.0)</td>
<td>129</td>
<td>7.0 (5.0–37.0)</td>
<td>160</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>89.6 (36.0–168.5)</td>
<td>130</td>
<td>82.0 (44.0–144.2)</td>
<td>156</td>
</tr>
<tr>
<td>CD4 (cells/ml)</td>
<td>207.0 (27.0–3,060)</td>
<td>145</td>
<td>299.0 (14.0–1,054)</td>
<td>161</td>
</tr>
<tr>
<td>Plasma HIV RNA (copies/ml)</td>
<td>20.0 (20.0–251,188.6)</td>
<td>140</td>
<td>400.0 (400.0–190,530.0)</td>
<td>156</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. (%) of patients</th>
<th></th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>65 (45) / 80 (55)</td>
<td>145</td>
<td>72 (45) / 89 (55)</td>
<td>161</td>
</tr>
<tr>
<td>Stavudine/zidovudine</td>
<td>119 (90) / 13 (10)</td>
<td>132</td>
<td>8 (5) / 153 (95)</td>
<td>161</td>
</tr>
<tr>
<td>Adherence ≥ 8</td>
<td>128 (98)</td>
<td>130</td>
<td>154 (99)</td>
<td>156</td>
</tr>
<tr>
<td>HIV RNA ≤ 400 copies/ml</td>
<td>128 (81.0)</td>
<td>140</td>
<td>147 (94.0)</td>
<td>156</td>
</tr>
<tr>
<td>HCV coinfected</td>
<td>10 (8.0)</td>
<td>125</td>
<td>11 (8.0)</td>
<td>138</td>
</tr>
<tr>
<td>HBV coinfected</td>
<td>18 (14.0)</td>
<td>125</td>
<td>20 (14.0)</td>
<td>139</td>
</tr>
<tr>
<td>HCV and HBV coinfected</td>
<td>2 (9.80)</td>
<td>125</td>
<td>2 (1.0)</td>
<td>138</td>
</tr>
</tbody>
</table>
check plot and the normalized prediction discrepancies versus the time plot are shown in Fig. 3. The predictions from the model adequately describe the observations within each genotype.

DISCUSSION

These are the first results on frequencies of genetic polymorphisms of major drug-metabolizing enzymes and transporters reported to be involved in NNRTI disposition in a large Cambodian population. Most Caucasians expressed the CYP3A5 6986GG genotype associated with a small amount of translated CYP3A5 protein, with a G allele frequency ranging from 0.87 to 0.94 in various Caucasian populations (22, 29). In contrast, in various Asian populations, G allele frequencies were lower, ranging from 0.59 in Indians to 0.65 in Cambodians, as demonstrated in this study, 0.67 in a Vietnamese population, and 0.74 to 0.78 in Japanese, Chinese, and Korean populations (23, 29). The frequency is even lower in patients of African descent (0.36) (22). Higher expression of the CYP3A5 protein will lead to an increase in clearance of CYP3A substrate drugs, such as HIV-1 protease inhibitors. Lower saquinavir, atazanavir, or

![ FIG. 1. Plasma nevirapine concentrations versus time in 170 Cambodian HIV patients at M18 and M36 (a) and in the extensive pharmacokinetic substudy (b). Values below the LOQ are represented by the asterisk at 0 on the y axis.](http://aac.asm.org/)

<table>
<thead>
<tr>
<th>TABLE 2. Parameter estimates and their 95% confidence intervals for the basic model and the final model with covariates</th>
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<tbody>
<tr>
<td><strong>Parameter</strong>&lt;sup&gt;c&lt;/sup&gt; (unit)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>$k_a$ (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>V/F (liters)</td>
</tr>
<tr>
<td>CL/F (liters/h)</td>
</tr>
<tr>
<td>$B_{CYP2B6} 516GG$</td>
</tr>
<tr>
<td>$B_{CYP2B6} 516TT$</td>
</tr>
<tr>
<td>$B_{CLCR}$</td>
</tr>
<tr>
<td>$\omega_{CL/F}$ (%)</td>
</tr>
<tr>
<td>$\gamma_{CL/F}$ (%)</td>
</tr>
<tr>
<td>$\sigma$ (ng/ml)</td>
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</table>

<sup>a</sup> CI, confidence interval.

<sup>b</sup> Permutation test of covariate effect.

<sup>c</sup> $B_{CYP2B6} 516GG$, $B_{CYP2B6} 516TT$, and $B_{CLCR}$ are the factors associated with the two CYP2B6 genotypes and the creatinine clearance on nevirapine CL/F, $\omega_{CL/F}$ is the interpatient variability for CL/F, $\gamma_{CL/F}$ is the interoccasion (or intrapatient) variability for CL/F, and $\sigma$ is the residual error.
indinavir concentrations (3, 24, 44) were demonstrated in patients who express CYP3A5, although disposition of lopinavir combined with ritonavir, which inhibits both CYP3A4 and CYP3A5, remained unaffected (15). This is of importance as lopinavir-ritonavir is the antiretroviral drug combination recommended by WHO for patients for whom a first-line NNRTI regimen fails.

The frequencies of the CYP2B6 516G/H11022T mutant allele associated with loss of catalytic activity vary greatly according to the study population, with the following average values: 0.14 to 0.18 in Korean and Japanese populations (18, 23, 25), 0.21 in a Han Chinese population (20), 0.22 to 0.25 in Caucasians (22, 25), 0.27 in a Vietnamese population (49), 0.32 in a Thai population (9, 38), 0.28 to 0.38 in African-Americans (22, 25), 0.42 in West Africans, and up to 0.62 in Papua New Guineans (32). Not surprisingly, the frequency of 0.35 in our Cambodian population is close to that reported for people living in border countries, such as Thailand and Vietnam. The T allele frequency of CYP2B6 516G>T mutant allele associated with loss of catalytic activity vary greatly according to the study population, with the following average values: 0.14 to 0.18 in Korean and Japanese populations (18, 23, 25), 0.21 in a Han Chinese population (20), 0.22 to 0.25 in Caucasians (22, 25), 0.27 in a Vietnamese population (49), 0.32 in a Thai population (9, 38), 0.28 to 0.38 in African-Americans (22, 25), 0.42 in West Africans, and up to 0.62 in Papua New Guineans (32). Not surprisingly, the frequency of 0.35 in our Cambodian population is close to that reported for people living in border countries, such as Thailand and Vietnam. The T allele frequency of CYP2B6 1459C/H11022T polymorphism is very low in our Cambodian population, as described for other East Asian populations (25, 49). The importance of the P glycoprotein, an efflux transporter, in drug disposition has been reviewed (46). The T allele frequency of ABCB1 3435C/H11022T in the Cambodian population is close to what was reported for the Vietnamese population (49) but is lower than that for other Asian populations (4) or European Americans (46). All these data indicate marked differences in SNP frequencies between Cambodian and other Asian populations, such as Han Chinese, or Caucasian and African populations. They are in agreement with genomewide association studies, which show the genetic substructure between different East Asian groups and a low level of differentiation between Cambodian and Vietnamese populations (47).

The population pharmacokinetics of nevirapine was studied in a Cambodian HIV-infected population after long-term administration of nevirapine as backbone antiretroviral first-line therapy. The impressive efficacy of this antiretroviral drug regimen is in keeping with previous studies (7, 27). Such a positive virological outcome has already been pointed out in another Cambodian cohort with an efavirenz-based regimen (cART), as noted by Spire et al. (45). In the present study, most patients (99%) reported an adherence greater than or equal to 8 on a 10-point visual analog scale. It should be stressed that in both cohorts, antiretroviral therapy was provided free through Global Funds and NCHADS programs and that educational programs were implemented on a regular basis.

Although nevirapine is the antiretroviral drug of choice in low-income countries, little is known of between- and within-patient variabilities. Our data show that after more than 1 year, under steady-state conditions, intraindividual variability in trough nevirapine concentrations is quite low, in agreement with previous data as Nettles et al. indicated a within-patient variability of 25% in one patient who received nevirapine,
which is well below what has been reported for HIV protease inhibitors (19, 34). This is in keeping with nevirapine pharmacokinetic properties, with absolute bioavailability reported to be 90% after single-dose administration (26). The half-life at steady state is longer than the dosing interval in most patients despite autoinducing properties, which means that delaying drug intake or missing a dose will have little influence on steady-state concentrations. Intertatient variability is also quite low, most likely because absorption variability can be ruled out. Interestingly, Manosuthi et al. (30) recently reported that interpatient variability in the efavirenz group was 2.3-fold greater than in the nevirapine group, although these patients received concomitant use of rifampin, which could alter variability.

The estimation of nevirapine CL/F calculated at steady state in our population is in the range, albeit somewhat lower, of values in previous studies including different populations (2.95 to 3.35 liters/h) (11–13, 17, 33, 42, 50) and is roughly twice the apparent clearance reported after single-dose administration (21, 31), which clearly shows the importance of the autoinducing effect on either first-pass effect and bioavailability or total clearance. The 95% confidence interval for the apparent volume of distribution is large (111 to 446 liter), as the estimation error of this parameter is high. Therefore, comparison with other studies reporting somewhat lower values is difficult (21, 31). Intertatient variability in V/F and \( k_a \) could also not be estimated. This and the large standard error in V/F are related to the study design, since in most patients only one trough concentration was measured at each evaluation, giving mostly information on apparent clearance. This is one of the few studies demonstrating that \( CYP2B6 \ 516G>T \) genetic polymorphism and creatinine clearance affect nevirapine clearance but explains only 3.1% and 0.3% of the interpatient variabilities, respectively. Apparent clearance is decreased by 37% in homozygous patients carrying the loss-of-function allele compared with that in patients carrying the homozygous wild-type allele, which leads to increased half-lives estimated to be 52 h (range, 28 to 96 h) for patients with the GG genotype, 59 h (29 to 120 h) for those with the GT genotype, and 83 h (38 to 178 h) for those with the TT genotype. In 126 children, Saitoh et al. demonstrated a 30% decrease in nevirapine clearance in children with the TT genotype compared to that in children with the GG genotype (43). Similarly, higher nevirapine concentrations have been reported in patients with the \( CYP2B6 \ 516TT \) genotype (28, 36, 40), although the relationship is unclear after single-dose administration (9, 21). Such a discrepancy could be related to the autoinduction of \( CYP2B6 \) by repeated administration of nevirapine. Interestingly, genetic polymorphism was not found to affect the volume, ruling out a large inducing effect on bioavailability and first-pass effect. A relationship between nevirapine clearance and creatinine clearance was unexpected as nevirapine is eliminated mostly by biotransformations. Such a relationship was noted by Gandhi et al. (17) in a cohort of HIV-infected women, and they sug-

![FIG. 3. (a) Mean over the individual nevirapine clearance at the different occasions (M18, M36, and the extensive pharmacokinetic substudy) for each of the 152 patients with an informed \( CYP2B6 \ 516G>T \) genotype, sorted by genotype with the corresponding median (on a log scale). (b) Individual nevirapine clearance estimated at each occasion plotted versus the corresponding creatinine clearance observation. Data from each patient are connected by a segment. The solid line represents a regression spline (with y and x axes on a logarithmic scale). Patients with genotypes GG, GT, and TT for the \( CYP2B6 \ 516G>T \) polymorphism are represented with the symbols ∗, × and ○, respectively.](http://aac.asm.org/)
gested that the effect of uremic toxins on relevant hepatic transporters and metabolizing enzymes may explain the influence of renal insufficiency on nevirapine clearance. However, the clinical relevance of this phenomenon is small as the major changes were less than 20% from the mean. In agreement with others, no relationship between nevirapine clearance and weight was evidenced (11, 22).

No modification in nevirapine pharmacokinetics was seen in patients with liver disease (8, 11), and no relationship between ALAT and nevirapine concentrations was found in the present study.

This study has a number of limitations. First, plasma HIV RNA was not measured at inclusion in the cohort, as this parameter was not available in Cambodia when the ESTHER cohort was initiated. Therefore, no relationship between plasma HIV RNA decline and nevirapine exposure could be established. Treatment failure was seen only in a few antiretroviral-naïve patients at the first evaluation, which would have made such a relationship difficult to demonstrate. Second, patients who developed rashes and liver toxicity early after initiation of treatment were switched to efavirenz, so it cannot be shown whether the frequency of occurrence of these adverse events is dependent on the ABCB1, CYP3A45, or CYP2B6 loss-of-function allele. Third, it remains to be seen whether other infrequent variants contribute to the variability in nevirapine clearance.

Despite such limitations, this study demonstrates that 95% of the patients had sustained nevirapine exposure well above the 3,000-ng/ml threshold. Nevirapine clearance was shown to be affected by CYP2B6 516G→T genetic polymorphism and creatinine clearance, although this explained only part of the interpatient variability, which remains low compared to that for other antiretroviral drugs.

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