Joint Population Pharmacokinetic Analysis of Zidovudine, Lamivudine, and Their Active Intracellular Metabolites in HIV Patients

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Received 27 October 2010/Returned for modification 16 January 2011/Accepted 30 April 2011

The population pharmacokinetic parameters of zidovudine (AZT), lamivudine (3TC), and their active intracellular metabolites in 75 naive HIV-infected patients receiving an oral combination of AZT and 3TC twice daily as part of their multitherapy treatment in the COPHAR2-ANRS 111 trial are described. Four blood samples per patient were taken after 2 weeks of treatment to measure drug concentrations at steady state. Plasma AZT and 3TC concentrations were measured in 73 patients, and among those, 62 patients had measurable intracellular AZT-TP and 3TC-TP concentrations. For each drug, a joint population pharmacokinetic model was developed and we investigated the influence of different covariates. We then studied correlations between the mean plasma and intracellular concentrations of each drug. A one-compartment model with first-order absorption and elimination best described the plasma AZT concentration, with an additional compartment for intracellular AZT-TP. A similar model but with zero-order absorption was found to adequately describe concentrations of 3TC and its metabolite 3TC-TP. The half-lives of AZT and 3TC were 0.81 h (94.8%) and 2.97 h (39.2%), respectively, whereas the intracellular half-lives of AZT-TP and 3TC-TP were 10.73 h (69%) and 21.16 h (44%), respectively. We found particularly a gender effect on the apparent bioavailability of AZT, as well as on the mean plasma and intracellular concentrations of AZT, which were significantly higher in females than in males. Relationships between mean plasma drug and intracellular metabolite concentrations were also highlighted both for AZT and for 3TC. Simulation with the model of plasma and intracellular concentrations for once-versus twice-daily regimens suggested that a daily dosing regimen with double doses could be appropriate.

Zidovudine (AZT) and lamivudine (3TC) are common antiretroviral drugs for the treatment of human immunodeficiency virus (HIV) infection, which causes AIDS. AZT and 3TC are nucleoside reverse transcriptase inhibitors (NRTIs) that are often used in highly active antiretroviral therapy (HAART) along with one protease inhibitor (PI) boosted with ritonavir in general or along with a non-NRTI. The 2009 recommendations of the World Health Organization recommended the use of AZT as a preferred first-line therapy option, a less toxic alternative to the use of stavudine (38). As AZT and 3TC are frequently prescribed together, they are combined in one tablet (Combivir).

All NRTIs undergo a series of three sequential phosphorylation reactions producing monophosphates, diphosphates, and then triphosphates (TP) within the cell. AZT and 3TC are thus metabolized intracellularly to their active metabolites (AZT-TP and 3TC-TP, respectively), which block DNA synthesis by reverse transcriptase inhibition and chain termination. These active moieties are the determinants of the efficacy and toxicity of AZT and 3TC. Several studies have demonstrated that the antiviral activity of NRTIs does not always correlate with plasma concentrations of the parent nucleoside but rather with the intracellular concentration of their metabolites (1, 3, 15, 17, 25, 33). The review performed by Bazzoli et al. (6) reports these findings for NRTIs, as well as for other antiretroviral drugs, in more detail.

Adequately assaying the intracellular concentrations of antiretroviral drugs is still a major technical challenge, together with the isolation and counting of peripheral blood mononuclear cells (PBMCs) (6). That is why most studies with intracellular measurements of AZT-TP and/or 3TC-TP in patients have often been carried out with relatively few patients, with observations mostly at single time points. Even if population approaches seem to be more adequate to analyze sparse pharmacokinetic data, no population study based on the measurement of intracellular AZT-TP and 3TC-TP has yet been performed; only population pharmacokinetics (PK) analyses of plasma have been published.

The COPHAR2-ANRS 111 trial is a multicenter, noncomparative pilot trial of early therapeutic drug monitoring in HIV-positive patients naïve for PI-containing HAART (16). Patients received a combination of two NRTIs plus one PI as antiretroviral therapy. Population PK analyses of the data from the nelfinavir group and the ritonavir-boosted indinavir group obtained in this trial were performed to evaluate the impact of genetic polymorphisms on nelfinavir and indinavir pharmaco-
kinetics (PK) and the link between concentrations and short-term efficacy (10, 21). A substudy of the COPHAR2-ANRS111 trial consisted of measuring plasma and intracellular concentrations of AZT and 3TC in patients whose treatment contained AZT and 3TC as NRTIs with a PI.

In the present study, we focused on simultaneous population analyses of concentration data for AZT and 3TC and their respective intracellular active metabolites through the development of joint population models. Integrated modeling of parent-metabolite PK provides reliable estimates of metabolite parameters and their intersubject variability. We then investigated the influence of different covariates on the pharmacokinetic parameters of both of these models. Last, we explored the relationships between the mean plasma concentrations of the two drugs, as well as those between the mean intracellular concentrations of the two metabolites. We also studied the correlations between the plasma and intracellular concentrations of the two drugs and with the final covariates found for each model, respectively.

Materials and Methods

Patients and study design. The objective of the COPHAR2-ANRS 111 trial was to assess the benefit of pharmacological advice based on trough plasma concentrations of PIs (details can be found in reference 16). Patients initiated a HAART containing a combination of at least three drugs: one PI (indinavir/ritonavir, lopinavir/ritonavir, or nelfinavir) plus two NRTIs (AZT at 300 mg and 3TC at 150 mg [Combivir] twice a day [b.i.d.] or stavudine at 40 mg and 3TC at 150 mg [b.i.d.]). A substudy of the clinical trial has been performed to establish the PK of AZT and 3TC and of their intracellular moieties in patients receiving this dual combination.

To be eligible, patients had to be HIV infected, be over 18 years of age, have had no previous treatment with a PI regimen, have a plasma HIV RNA level above 1,000 copies/ml, be naive to antiviral treatment or else have a genotypic resistance test not indicating more than two major mutations. The main noninclusion criteria were concomitant use of drugs interacting with NRTIs and PIs; treatment of HIV infection with interleukin-2, alpha interferon, or vaccine; noninclusion criteria were concomitant use of drugs interacting with NRTIs and PIs; treatment of HIV infection with interleukin-2, alpha interferon, or vaccine; and approved the study protocol. All participants provided written informed consent. No dose adaptation was performed from week 0 and to week 4. After 2 weeks of treatment (W2), four blood samples per patient were taken to measure the plasma concentration at steady state before and at 1, 3, and 6 h after drug administration. This design was developed for the study of PI PK, and AZT and 3TC concentrations were assessed in the samples taken. Regarding intracellular measurements, patients receiving AZT and 3TC were sampled before and at 3 h after drug intake; additional blood samples were also collected at 1 and 6 h for patients in the nelfinavir group. Adherence was evaluated at W2 by means of a validated autoquestionnaire (11), and patients were classified as either adherent when reporting no shift in their treatment schedule during the last 4 days and nonadherent otherwise.

Analytical method. Plasma drug concentrations were centralized and measured at the end of the study in the department of clinical pharmacology of the Cochin Hospital, Paris, France, by a specific high-performance liquid chromatography method and reported in mg/liter. The lower limit of quantification (LOQ) was 0.05 mg/liter for AZT and 0.02 mg/liter for 3TC.

Intracellular concentrations of NRTI triphosphate metabolites were measured in the isolated PBMCs by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (7, 13) at the Pharmacology and Immunoanalysis Unit of the CEA, Gif sur Yvette, France. Isolation of PBMCs is the first step before analysis of the intracellular concentrations of NRTIs. PBMCs were isolated either using conventional Ficoll gradient centrifugation or using cell preparation tubes (Rection Dickinson). Cells were washed three times with a saline solution at +4°C and stored at −80°C until extracted. Then, the intracellular drug concentrations were extracted from cells using a Tris HCl (0.05 M, pH 7.4)-methanol (30:70 [vol/vol]) buffer (stored at −20°C). Cells were manually lysed (by scraping and vortexing). The supernatants were transferred and evaporated until a volume of 120 μl was obtained; 40 μl was then injected into the LC-MS/MS system. The chromatographic step was performed on a C18 reversed-phase column with a mobile phase containing acetonitrile and a buffer of ammonium formate and 1.5-dimethylhydrazine as a volatile counterion. Detection was achieved by MS/MS after electrospray ionization in the negative mode. All values were then divided by the number of PBMCs to obtain the concentration in fmol/10^6 cells.

For the present study, intracellular concentrations in fmol/liter were transformed in mg/liter considering that 1 mol of AZT-TP or 3TC-TP is equal to 507 or 469 g, respectively, and the volume of a PBMC is approximately 0.2 pl (11, 32).

There is no standard methodology to set a LOQ for these measurements since it would depend both on the quantification of the intracellular compound and on the PBMC count.

Population pharmacokinetic modeling. Two joint population pharmacokinetic models were developed in order to simultaneously describe the PK of AZT and 3TC and their active metabolites, respectively. Model fitting and estimation of the population parameters were performed using the SAEM algorithm for nonlinear mixed-effect models implemented in the MONOLIX software version 2.3 (http://www.monolix.org/). Plasma and intracellular drug concentrations were assumed to be at steady state with a dosing interval of 12 h. The trough concentration was that measured the day before drug intake, and the delay since the last dose intake was used.

As suggested by previous analysis, we used a one-compartment model with first-order absorption and elimination to describe plasma AZT concentrations (29) (Fig. 1). A compartment for AZT-TP was added, which was the link to the AZT compartment with a first-order metabolism rate constant k_em as it was assumed that there is no first-pass effect. The parameters of this model are bioavailability (F), the volumes of distribution of AZT and its metabolite AZT-TP (V_a and V_m, respectively), the first-order absorption rate constant of AZT; Tk0, zero-order absorption duration for 3TC; k_em first-order metabolism rate constant; k_e first-order elimination rate constant of the parent drug; V, volume of distribution of the parent drug; V_m volume of distribution of the metabolite; k_em first-order elimination rate constant of the metabolite.

![FIG. 1. Schematic representation of joint pharmacokinetic compartment models for the simultaneous prediction of plasma AZT and 3TC concentrations and intracellular concentrations of their active metabolites. AZT (or 3TC) in compartment 1 underwent absorption in compartment 2. D, dose; F, bioavailability of the parent drug; k_a absorption rate constant of AZT; Tk0, zero-order absorption duration for 3TC; k_em first-order metabolism rate constant; k_e first-order elimination rate constant of the parent drug; V, volume of distribution of the parent drug; V_m volume of distribution of the metabolite; k_em first-order elimination rate constant of the metabolite.](http://www.monolix.org/).
where $C_{3TC}$ is the plasma concentration of 3TC, $C_{7TC-TP}$ is the intracellular concentration of the metabolite 3TC-TP, and $k = k_{3TC} = k_{3TC-TP}$.

These models were reparameterized using the apparent clearance (CL) of NRTIs [CL/F = $k(V/F)$] and that of their metabolites [CL/F = $k_{mm}(V/F_{mm})$] from plasma. Since AZT and 3TC are orally administered, the $k_{3TC}$ of AZT, the $k_{3TC}$ of 3TC, $C_{7TC}$, $C_{7TC-TP}$, and $V/F$ were identifiable. Concerning metabolites, since no urinary concentrations were available, only the parameters $C_{7TC}$, $k_{3TC}$, and $V/F_{mm}$ were identifiable (21, 29). We could thus determine $k_{mm}$ as the ratio of both previous parameters and also the half-lives of the parent drugs and metabolites.

We then computed the intersubject variabilities of these derived parameters as the sum of the intersubject variability of the composing parameter, as there was no covariance between random effects. We also deduced the relative standard error (RSE) of $k_{mm}$ using the delta method. More complex models in terms of the number of compartments and routes of administration of the parent drug were tested, whereas no saturable metabolism or any such more complex phenomena were evaluated due to identification issues of the model.

For each joint model, an exponential model was used for intersubject variability, where random effects were assumed to follow a normal distribution with zero mean and diagonal variance matrix. Since the NONLINIX software handles left censored data, plasma AZT and 3TC concentrations below the LOQ were taken into account in the estimation step using the left censored LOQ (31). However, because of the complex assay techniques used for the measurement of intracellular AZT-TP and 3TC-TP, the data undetectable or below the quantifiable limit were omitted. Several residual-error models for each drug and for each moiety (i.e., additive, proportional, and combined) were investigated based on the Bayesian information criterion (BIC) (9, 23). We then built a model with random effects on all pharmacokinetic parameters. Model choice was based on the BIC and standard goodness-of-fit plots (observed concentrations versus predicted concentrations and population and individual weighted residuals versus predicted concentrations and versus time) for each drug and each moiety. For evaluation of the basic joint model, we performed a visual predictive check (VPC) with 5,000 simulations to evaluate the basic model for each drug and its metabolite.

The effects of the following covariates were then evaluated: age, body mass index (BMI), body weight, creatinine clearance, and albumin as continuous variables and coadministered PI (indinavir/ritonavir/lopinavir), ritonavir intake, coinfection with hepatitis B or C virus (HBV/HCV), adherence as previously defined, gender, and CDC classification of HIV infection. The continuous variables were centered to the median for convenience of model interpretation. Missing continuous covariates were replaced with the median, and patients with missing discrete covariates were eliminated from the corresponding analysis. The effects of covariates on the empirical Bayes estimates of each individual pharmacokinetic parameter from the basic model were tested with the Wilcoxon nonparametric test for categorical variables and the Spearman nonparametric test for continuous variables. The population covariate model was built with the covariates which were found to have an effect in this first step with a $P$ value of $< 0.05$. A forward selection of these covariates for the population model was performed by use of the likelihood ratio test (LRT) with a significance threshold at $P < 0.05$. For each nested model, the LRT can be applied by computing the log likelihood by importance sampling using a Monte Carlo size of 20,000. From this ascending method, a backward elimination procedure was used to keep only significant covariates ($P < 0.05$) in the final model and to assess their $P$ values by use of the LRT.

Relationships between plasma, intracellular AZT and 3TC concentrations, and covariates. Using joint models, the mean plasma drug and intracellular metabolite concentrations at steady state are given by $C_{mean} = \langle Dose/\gamma \rangle (CL/F)$ and $C_{mean,intra} = \langle Dose/\gamma \rangle (CL/F)/(1/(CL/F/k_{mm}))$, respectively. The mean metabolic ratio (MMR) is therefore expressed as follows: $MMR = C_{mean,intra}/C_{mean} = (V/F)/(CL/F/k_{mm})$.

### RESULTS

**Patients.** Seventy-five patients (47 men, 28 women) of the 115 patients in the COPENH2-ANRS 111 trial received the combination of AZT and 3TC. Among them, 27, 23, and 25 patients were, respectively, treated with the PIs indinavir, nelfinavir, and lopinavir. For two patients, plasma samples were missing. We therefore obtained plasma pharmacokinetic data for AZT and 3TC from 73 patients. Table 1 describes the main characteristics of the 73 patients included in the population analysis. We obtained a total of 280 plasma AZT concentrations and 287 plasma 3TC concentrations (a median of four samples per patient for AZT and 3TC). Among these 73 patients, only 62 had intracellular concentrations of both AZT-TP and 3TC-TP, 11 patients with four sampling times and the other patients with three, two, or one sample (a median of two samples per patient for AZT-TP and 3TC-TP). Joint population modeling of AZT and intracellular AZT-TP. Among the 280 samples, 6% were below the LOQ for AZT in plasma. Figure 2A displays AZT and AZT-TP concentrations versus time. The concentrations of both compounds were adequately described by a one-compartment model with first-order absorption and elimination for AZT with an additional compartment for AZT-TP. Indeed, more complex models did not improve the results. Residual variabilities of AZT and
AZT-TP were best depicted by an additive and a proportional error model, respectively. The available data were not sufficient to estimate $k_a$, and we had to fix the value of $k_a$ to 2.86 h$^{-1}$, which was obtained by Panhard et al. (29) in the population analysis of plasma AZT and 3TC concentrations originally from the COPHAR 1-ANRS 102 trial. Indeed, in that trial, an early sampling time (0.5 h) after drug administration was used. No sensitivity analysis was performed for this parameter, as very little information about the absorption phase is available. Results showed no significant improvement when the intersubject variability of $V_m/(Fk_m)$ was added, so that no variability was assumed for that parameter. The population parameter estimates and their RSEs are displayed in Table 2. The residual variability for AZT-TP was 45%. The RSE for all parameters were correct. We derived a value of the secondary parameter $k_{em}$ of 0.063 h$^{-1}$ with an intersubject variability equal to that of $CL_m/(Fk_m)$. The mean half-lives of AZT and AZT-TP were 0.81 and 10.73 h, respectively. The estimated intersubject variabilities, 94.8 and 58.4%, respectively, were large for both mean half-lives. VPCs of AZT and AZT-TP are displayed in Fig. 2A, and they show good evidence of the adequacy of the models.

Significant effects of adherence on $V/F$ ($P = 0.021$) and of gender on $CL/F$ ($P = 0.022$) were found. The population pa-
rarameters of this final model are given in Table 2. An increase of 33% in the apparent CL of AZT was observed for males. The apparent volume of distribution of AZT was increased by 55% for nonadherent patients.

**Joint population modeling of 3TC and intracellular 3TC-TP.** Three patients had plasma 3TC concentrations at 12 h below the LOQ. Figure 2B shows 3TC and 3TC-TP concentrations versus time. A model similar to that for AZT and AZT-TP, but with zero-order absorption, best depicted the concentrations of 3TC and its metabolite 3TC-TP. Tk0 was estimated to be 1.25 h. No variability in V00 was estimated. Tk0 was 1.25 h. The best error models were an additive error model for 3TC and a proportional error model for 3TC-TP. Table 3 shows the population parameter estimates and their RSE in percent. Parameters are well estimated with an RSE lower than 20%, except for V00(FKm) (47.6%). Important intersubject variabilities were estimated for Tk0 (74%) and CLm(FKm) (47.5%). The residual variability for 3TC-TP was 24.5%. We obtained a k0em of 0.027 h−1 with an intersubject variability of 47.5%. 3TC and 3TC-TP have mean half-lives of 2.97 and 21.16 h with intersubject variabilities of 39.2 and 47.5%, respectively. VPCs are given in Fig. 2B for the joint 3TC–3TC-TP model, and they show good evidence of the adequacy of the models.

Five covariates were included in the final model. We found a significant effect of ritonavir on Tk0 (P = 0.04). The apparent CL (CL/F) of 3TC was influenced significantly by age (P = 0.001), body weight (P = 0.007), and adherence (P = 0.02). An effect of gender on the apparent volume (V/F) of 3TC (P = 0.001) was also found. The duration of absorption of 3TC for a patient taking ritonavir, i.e., treated with lopinavir or indinavir as a PI, was increased by 121%. The apparent volume of distribution of 3TC was increased by 23% in male patients. With each increase of 10 years in age from the median (34 years), the apparent CL of 3TC decreased by 9%, whereas the latter increased by 7% with each body weight increase of 10 kg from the median (68 kg). A decrease of 15% of the apparent CL was observed for nonadherent patients.

**Relationships between plasma, intracellular AZT and 3TC concentrations, and covariates.** The median AZT and 3TC mean concentrations in plasma were 0.11 (IQR, 0.1 to 0.18) and 0.51 (IQR, 0.4 to 0.7) mg/liter for the 62 subjects in whom both plasma and intracellular concentrations of AZT and 3TC were available. The median mean intracellular concentration was 0.17 (IQR, 0.11 to 0.24) mg/liter for AZT-TP and 58.1 (IQR, 43.7 to 75.8) mg/liter for 3TC-TP. A significant relationship was also found between the mean plasma AZT and 3TC concentrations (r = 0.30, P = 0.016), as well as between the mean intracellular concentrations (r = 0.56, P < 0.001) (see Fig. 4). A significant relationship was also found between the mean AZT concentrations in plasma and the mean intracellular concentrations of AZT-TP (r = 0.35, P = 0.006) and also between those of 3TC and 3TC-TP (r = 0.48, P < 0.001) (Fig. 3).

The median MMRs were 0.66 (IQR, 0.46 to 1.16) for AZT and AZT-TP and 51.4 (IQR, 38.6 to 68.7) for 3TC and 3TC-TP. No relationship between the MMR of AZT and the MMR of 3TC, which was higher, was found.

Regarding the correlation with the covariates included in the final joint models, we found only a correlation between the mean plasma concentration of AZT and the mean intracellular concentration of AZT-TP with the gender covariate (P = 0.027 and P = 0.039, respectively) (Fig. 4). The median AZT mean concentration in plasma was 0.16 (IQR, 0.10 to 0.21) mg/liter for female patients (n = 23) and 0.10 (IQR, 0.07 to 0.15) mg/liter for male patients (n = 29). Similarly, the median AZT-TP mean concentration was higher in female patients than in men; when all mean AZT-TP concentrations were grouped by gender, the median value was 0.19 (IQR, 0.14 to

### Table 2. Population pharmacokinetic parameters of AZT and AZT-TP for the basic and final models

<table>
<thead>
<tr>
<th>Compound and parameter</th>
<th>Basic model</th>
<th>Final model</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>RSE (%)</td>
</tr>
<tr>
<td>AZT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k0 (h−1)</td>
<td>2.86 (fixed)</td>
<td>2.86 (fixed)</td>
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<tr>
<td>CL/F (liters/h)</td>
<td>200</td>
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<tr>
<td>βCL/F</td>
<td>0.28</td>
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<td>V/F (liters)</td>
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<tr>
<td>βV/F</td>
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<td>αCL (%)</td>
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<tr>
<td>αFR (%)</td>
<td>77.7</td>
<td>10.8</td>
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<tr>
<td>σAZT (mg/liter)</td>
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<td>33.6</td>
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<tr>
<td>AZT-TP</td>
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<td></td>
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<tr>
<td>CLm/FKm/FKm (liters)</td>
<td>322</td>
<td>10.1</td>
</tr>
<tr>
<td>V00/FKm/FKm (liters)</td>
<td>5.14 × 103</td>
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<tr>
<td>k0em (h−1)</td>
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<td>αCL/FKm/FKm (%)</td>
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<td>σAZT-TP (%)</td>
<td>45.4</td>
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### Table 3. Population pharmacokinetic parameters of 3TC and 3TC-TP for the basic and final models

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<tr>
<th>Compound and parameter</th>
<th>Basic model</th>
<th>Final model</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>RSE (%)</td>
</tr>
<tr>
<td>3TC</td>
<td></td>
<td></td>
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<tr>
<td>k0 (h−1)</td>
<td>1.25</td>
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<td>CL/F (liters/h)</td>
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<td>βCL/F</td>
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<td>βV/F</td>
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<tr>
<td>V/F (liters)</td>
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<td>βV/F</td>
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<td>αCL (%)</td>
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<td>αFR (%)</td>
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<tr>
<td>σ3TC (mg/liter)</td>
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<td>13.5</td>
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<tr>
<td>3TC-TP</td>
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<tr>
<td>CLm/FKm/FKm (liters)</td>
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<td>6.8</td>
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<td>V00/FKm/FKm (liters)</td>
<td>63.73</td>
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<td>k0em (h−1)</td>
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<tr>
<td>αCL/FKm/FKm (%)</td>
<td>47.5</td>
<td>11.2</td>
</tr>
<tr>
<td>σ3TC-TP (%)</td>
<td>24.5</td>
<td>7.7</td>
</tr>
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a RSE is the standard error of the estimate divided by the estimate and multiplied by 100. α, coefficient of variation for intersubject variability. σ, parameters of error model.

b Derived secondary parameter.
0.26) mg/liter for females and 0.14 (IQR, 0.08 to 0.23) mg/liter for males. No other significant correlations were found.

**DISCUSSION**

For the first time, joint models were developed for the analysis of plasma AZT and 3TC concentrations and the intracellular concentrations of their respective active metabolites, AZT-TP and 3TC-TP. Concentrations of AZT and of AZT-TP were satisfactorily described by a one-compartment model with first-order absorption and elimination for AZT, with an additional compartment for AZT-TP linked with a first-order rate constant. Regarding 3TC and 3TC-TP, the same compartment model but with a zero-order absorption fit the data adequately. The design used in the COPHAR2-ANRS 111 trial was originally planned to study the PK of PIs. Due to the absence of any sampling times during the absorption phase, we could not estimate the parameter $k_a$ of AZT, which has thus been fixed to 2.86 h$^{-1}$. This value was obtained by Panhard et al. (29) in the COPHAR1-ANRS 102 trial where the design included a sample in the absorption phase. We found apparent CL values of 200 and 22 liters h$^{-1}$ for AZT and 3TC, with estimated intersubject variabilities of 54 and 31%, respectively. The mean half-lives of AZT and 3TC were 0.8 and 2.9 h, respectively, and intersubject variabilities of 94.8 and 39.2% were derived. Values of the parameters obtained in the present study for plasma AZT and 3TC were within the range of those estimated previously. For instance, regarding half-life values in HIV patients, they are consistent with previously reported values: 1.2 to 4 h for AZT (14, 33) and 3.8 h for 3TC (14).

The PK of the metabolites AZT-TP and 3TC-TP have already been studied by using only usual statistics due to a lack of the information needed to build an adequate intracellular model. The use of a joint population model is optimum to obtain accurate pharmacokinetic parameters for metabolites instead of sequential analysis of each compound and can deal with sparse measurements. For AZT-TP and 3TC-TP, large intersubject variabilities of 58.4 and 47.5% were found for $CL_{int}(F_{int})$. The mean intracellular half-lives of AZT-TP and 3TC-TP are 10.7 and 21.2 h, with intersubject variabilities of 58.4 and 47.5%, respectively. Published data on HIV-infected patients show that the reported intracellular half-lives are rather variable. They are between 2 and 25 h for AZT-TP (4, 14, 18, 33) and between 10 and 22 h for 3TC-TP (1, 14, 26).
The differences between the reported intracellular half-lives can be explained by several factors. Most published studies were based on single measurements done at various times during a dosing interval, which can lead to large fluctuations in measurements. Then, quantification of intracellular concentrations is technically and analytically challenging, and there is still the need for a standardized method (6). As in most studies, we found that the intracellular active metabolites AZT-TP and 3TC-TP persist within cells longer than in plasma, indicating that once-daily dosing is more appropriate for the NRTI than twice-daily dosing (Fig. 5).

To develop these joint models, for plasma drug concentrations, we have included plasma concentration measurements below the LOQ and taken into account the left censoring in the MONOLIX software. Samson et al. (31) have shown that this proposed method is less biased than the usual methods of handling such data, i.e., deletion of all censored data points or imputation of LOQ/2 to the first point below the limit and deletion of the following points. However, for AZT-TP and 3TC-TP concentrations, the problem is different. Indeed, due to the mixture of two different assay techniques, it is not possible to define an upper limit like an “LOQ” for these data. That is why, for the estimation step, we omitted the intracellular data when they were undetectable or under the LOQ for one of the assay technique used. Regarding the mixture of the two different assay techniques, there is one for cell counting and one for measuring intracellular metabolite concentrations. Since the number of cells normalizes the intracellular metabolite concentration, cell counting is a critical step in the process of the intracellular assay. In the present study, cell counting was performed according to a validated biochemical assay (8) and the concentration was expressed as the amount per 10^6 cells and had been converted to the amount per volume unit on the assumption that the PBMC volume is 0.2 pl in order to compare intracellular and plasma concentrations. Another often-used value for the PBMC volume is 0.4 pl (19). Nevertheless, this may be questionable, as it varies according to the state of the cells (quiescent or stimulated) or to the nature of the cells (cell volume of a human lymphoblast, 2.1 pl) (36). Even if this point is largely discussed, the PBMC volume is only a scale factor and thus does not modify our results.

A major aim of population PK is to determine which measurable factors can cause changes in the dose-concentration relationship. We found an increase of 55% in the apparent volume of distribution for nonadherent patients. Similarly, adherence was shown to decrease the apparent CL of 3TC by 15% for nonadherent patients. We thus found a systemic correlation between the adherence effect and the apparent CL (CL/F) and the apparent volume of distribution (V/F). We assumed that the adherence factor might affect the PK of the drugs by means of bioavailability. Adherence is the major component of clinical variability in the response to drug treatment; low adherence to antiretroviral drug regimens may lead to suboptimal therapy (20) and the emergence of a resistant virus. We found an increase in the duration of 3TC absorption in patients taking ritonavir. Pharmacokinetic interaction studies with ritonavir have not been reported for 3TC. A hypothesis for the rise in the duration of absorption could be that patients receiving ritonavir were fed because 3TC absorption has been shown to be slower in fed than in fasted patients, which could be in agreement with the increased in \( T_{\text{K0}} \) (2, 27). We cannot confirm this hypothesis on the basis of our data because only patients receiving nelfinavir get oral and written dietary guidelines. Panhard et al. (29) found an increase in the duration of absorption in patients receiving nelfinavir and also an effect of age and body weight on this parameter. In the present study, we observed a slight but opposite influence of age and body...
weight on the apparent CL of 3TC, as found in the study of Moore et al. (28).

The effect of gender on the apparent volume of 3TC was pronounced, as well as that on the apparent CL of AZT. Furthermore, we found a significant correlation between the gender covariate and the mean plasma concentration of AZT, as well as the mean intracellular concentration of AZT-TP, pointing out the clinical relevance of the gender effect. Women had higher AZT and AZT-TP concentrations than men. This is in agreement with the effect found on the apparent CL of AZT, which is 33% higher for males, eliminating AZT quickly and as a result reducing AZT-TP concentrations. Anderson et al. (1) found a similar gender effect on intracellular concentrations of AZT-TP and 3TC-TP opposite to the one found by Aweeka et al. (4) for AZT-TP. This discrepancy may be explained by the fact that in the latter study, 20 women were enrolled (compared with only 4 women in the former) and various AZT doses were permitted. Stretcher et al. (34) provided gender-related differences in AZT-TP using area-under-the-curve estimates in 16 men and five women. The findings indicated a greater exposure of women. Current evidence, though limited, suggested the existence of a gender disparity and thus provided motivation to conduct further investigations of intracellular AZT PK and pharmacodynamics in men and women, a group for which historically experience showed high rates of serious toxicity (37).

The mean metabolic ratio of 3TC (median, 51.4) is much larger than the mean metabolic ratio of AZT (median, 0.66). The intersubject variability of MMR was 96.8% for AZT, and that for 3TC was 32.8%, which is far lower than that for AZT. 3TC-TP concentrations tended to be higher than AZT-TP concentrations, whereas the median mean plasma concentrations of both parent drugs are in the same range. Using descriptive statistics, Moore et al. (25) found mean intracellular and plasma concentrations ratios of 0.05 and 3% for AZT and 3TC, respectively. If we compare the MMRs of 3TC and AZT by calculating the ratio of the MMR of AZT to the MMR of 3TC, they are in the same range (0.016 and 0.013 in the study of Moore et al. and in this study, respectively).

In this study, including patients taking the dual-drug combination of AZT and 3TC, we found a statistically significant correlation between mean plasma concentrations of AZT and 3TC and also between mean intracellular concentrations of AZT-TP and 3TC-TP. Relationships between mean plasma and intracellular concentrations were also found for both AZT and 3TC. Although significant, the correlations are low (AZT, $r^2 = 0.12$; 3TC, $r^2 = 0.23$). Identical results were found by Hoggard et al. (22) for AZT, by Fletcher et al. (17) for 3TC, and by Durand-Gasselin et al. (15) for both drugs. Several published studies have also tested these correlations without significant results for AZT and AZT-TP (5, 14, 17, 30, 34, 35), but they were of limited size. The new findings on the relationship between the plasma and intracellular concentrations of the two drugs suggest the use of plasma drug concentrations for monitoring of AZT and 3TC treatment. In addition, plasma concentrations need a simpler assay technique than intracellular concentrations. The large difference in intracellular triphosphates between AZT and 3TC (i.e., very different MMR values) demonstrates that there are substantial differences in the extent of conversion of these antiviral agents, especially for cellular transport and metabolism. A further step will be to study relationships between intracellular concentrations and antiviral response through modeling (24) to really study the potential of intracellular concentrations as a therapeutic determinant of the efficacy or toxicity of antiretroviral therapy, as observed in previous studies (1, 3, 17, 25, 33).

In conclusion, a joint pharmacokinetic model was developed for AZT and its active metabolite AZT-TP, as well as for 3TC and 3TC-TP, that allowed accurate estimation of metabolite parameters but with a large interpatient variability. A very long half-life is observed for AZT-TP and 3TC-TP, supporting a once-daily regimen of both AZT and 3TC. The clinical relevance of the gender effect on AZT PK may have implications for drug efficacy and toxicity in men and women. Lastly, the correlation found between plasma and intracellular concentrations of both AZT and 3TC, even if it is limited, supports the necessity to measure intracellular concentrations for antiviral activity.

ACKNOWLEDGMENTS

We thank the study participants, the participating clinicians, and the Agence de Recherche Nationale sur le SIDA for financial support.

REFERENCES

17. Fletcher, C. V., et al. 2000. Zidovudine triphosphate and lamivudine triphos-


