Population Pharmacokinetic Analysis and Pharmacogenetics of Raltegravir in HIV-Positive and Healthy Individuals

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The objectives of this study were to characterize raltegravir (RAL) population pharmacokinetics in HIV-positive (HIV+ ) and healthy individuals, identify influential factors, and search for new candidate genes involved in UDP glucuronosyltransferase (UGT)-mediated glucuronidation. The pharmacokinetic analysis was performed with NONMEM. Genetic association analysis was performed with PLINK using the relative bioavailability as the phenotype. Simulations were performed to compare once- and twice-daily regimens. A 2-compartment model with first-order absorption adequately described the data. Atazanavir, gender, and bilirubin levels influenced RAL relative bioavailability, which was 30% lower in HIV+ than in healthy individuals. UGT1A9*3 was the only genetic variant possibly influencing RAL pharmacokinetics. The majority of RAL pharmacokinetic variability remains unexplained by genetic and nongenetic factors. Owing to the very large variability, trough drug levels might be very low under the standard dosing regimen, raising the question of a potential relevance of therapeutic drug monitoring of RAL in some situations.

Raltegravir (RAL) is the first integrase inhibitor approved by the FDA that acts by inhibiting the covalent integration of viral DNA into the host genome (8, 32). It is widely used in the treatment of infection with resistant HIV strains and is increasingly prescribed to treatment-naïve patients (11, 44).

RAL absorption is rapid and is affected by food intake (5) as well as by coadministered medications that affect gastric pH (3, 8, 10). It is highly bound to plasma proteins (83%) (8) and eliminated primarily by UDP glucuronosyltransferase 1A1 (UGT1A1), with minor contributions from UGT1A3 and UGT1A9 (22). Although genetic variation in UGT isozymes may therefore affect RAL exposure, two studies performed with healthy volunteers failed to demonstrate an effect of the well-known decrease function allele UGT1A1*28 on RAL metabolism, suggesting that other UGT isozymes might be involved (29, 48). RAL is generally well tolerated, with only 3% treatment discontinuation due to adverse effects (8, 40). The most common of these are fatigue, insomnia, and grade 2 to 4 liver function abnormalities, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin elevations (27).

The main objectives of this study were to characterize the RAL pharmacokinetic profile and variability in HIV-positive (HIV+ ) individuals and in healthy volunteers and to identify potential influencing factors. In addition, our genotyping strategy was comprehensive for the identification of new candidate genes for UGT-mediated glucurononidation of RAL. Finally, simulations were performed in order to compare concentrations at trough (C_{min}) after administration of 400 mg twice daily (BID) versus 800 mg once daily (QD).

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MATERIALS AND METHODS

Study design and population. RAL plasma levels in 145 HIV-infected individuals enrolled in the Swiss HIV Cohort Study (SHCS) were measured as part of a routine therapeutic drug monitoring program between October 2007 and November 2009 using a validated method previously reported (15). A median of 1 sample per patient (range, 1 to 14) was collected and drawn between 0.5 and 24.3 h after last drug intake under steady-state conditions. Administered doses were either 400 mg BID (n = 137) or 800 mg QD (n = 8). In addition, RAL plasma concentrations were obtained from 2 clinical trials. The first trial included 19 healthy volunteers enrolled in an open-label crossover pharmacokinetic interaction study of RAL with and without atazanavir (ATV) (29). Treatment arms were RAL at 400 mg BID alone or 400 mg plus ATV at 400 mg once daily. In both arms, participants were instructed to take medication at least 1 h before or 2 h after eating, and the actual time of drug intake was recorded both by electronic recording (Medication Event Monitoring System [MEMS]) and in a medication log. After 7 days of treatment, RAL con-

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centrations were measured at predose and 1, 2, 4, 8, 12, and 24 h after the last drug intake. The second trial involved 10 HIV-infected individuals who took part in a study aiming at determining overall RAL cellular disposition (16). RAL was administered at 400 mg BD just after a standardized breakfast, and steady-state levels were collected at predose and 1, 3, 6, 8, and 12 h after drug intake. Both studies had been approved by the local ethics committees, and all patients gave written informed consent to participate. The discovery population for genetic analysis was composed of 136 HIV-infected individuals from the SHCS. The replication data set was composed of 219 HIV-infected individuals (119 from the SHCS, 43 from the St. Stephen’s Centre, and 57 from the Liverpool Therapeutic Drug Monitoring cohort) and 19 healthy volunteers (29). All individuals signed the informed consent for genetic testing.

**Genotyping.** Genotyping was performed with a 127-plex customized Veracode array (Illumina). We included 102 single nucleotide polymorphisms (SNPs) in 3 groups of genes (UGT1A, UGT2B, and nuclear receptors) presumably involved in RAL metabolism. The tagging SNPs (tSNPs) \( (n = 51) \) were selected using HapMap phase III data (release 24) (www.hapmap.org) with Tagger software (13) to capture SNPs with minor allelic frequencies of >5% in the HapMap CEU population, known for a mean maximum pairwise \( r^2 \) of 0.80 between genotyped and not genotyped SNPs. The tSNPs covered the genome region of the RefSeq longest transcript plus 5 kb at the 3’ and 5’ untranslated regions (UTRs) in the HapMap Genome Browser (www.hapmap.org). Haplotype tagging SNPs (htSNPs) \( (n = 8) \) were selected to better cover the allelic diversity of the locus UGT1A (26). SNPs with proven functional effect \( (n = 43) \) were selected from the literature. The complete list of genes and SNPs included in the array is shown in the Table S1 in the supplemental material. SNPs that failed to be genotyped by the array \( (rs7643645, rs2741049, rs11692021, rs17671289, rs7574296, \) and \( rs1042640) \) were genotyped by commercially available TaqMan allelic discrimination (Applied Biosystems, Foster City, CA). SNPs that were not included in the array due to technical limitations were genotyped either by direct sequencing \( (rs3821242, rs6431625, \) and \( rs8175347) \) or by TaqMan allelic discrimination \( (rs1902023) \). UGT2B17 gene deletion was investigated with a previously published PCR strategy (26). For replication, the candidate SNP rs72551330 was genotyped using TaqMan allelic discrimination. Primers and probes are shown in Table S2 in the supplemental material.

**Population pharmacokinetic model.** (i) Basic model. One-, two-, and three-compartment models with first-order absorption from the gastrointestinal tract were compared based on the data obtained from the 19 healthy volunteers with rich plasma sampling, which provided an initial estimate for the complete analysis. The estimated parameters were apparent clearance (CL), apparent volume of distribution of the central compartment \( (V_1) \), apparent volume of distribution of the peripheral compartment \( (V_2) \), and intercompartmental clearance \( (Q) \). A relative bioavailability for the HIV+ data set was allowed in the model to capture observed differences between HIV+ \( (F_{\text{HIV}^+}) \) and healthy \( (F_{\text{HIV}^-}) \) individuals, with \( F_{\text{HIV}^-} \) fixed to 1. (ii) Statistical model. Proportional errors following a log-normal distribution were assumed for the description of between-subject variability (BSV) in the pharmacokinetic parameters, with mean 0 and variance \( \Omega \). A proportional error model with a mean of zero and a variance of \( \sigma^2 \) was used as well to describe the intrapatient (residual) variability. (iii) Covariate model. Potentially influential covariates were incorporated sequentially into the pharmacokinetic model. The typical values of the pharmacokinetic parameters were modeled to depend linearly on a covariate \( \lambda \) (centered on the mean for continuous covariates, e.g., 30 \( \mu \)mol/liter for total bilirubin levels, with categorical covariates being coded as indicator variables into 0 or 1 [43]). The available demographic covariates were sex, age, body weight, and ethnicity; few comedICATIONS were recorded, and these comprised principally other antiretroviral drugs and some medications presumed to influence RAL exposure. Laboratory tests of hepatic function (bilirubin, AST, and ALT) were also analyzed.

**Parameter estimation and selection.** Data analysis was performed with NONMEM (version 7.1, NM-TRAN version II) by means of the first-order conditional estimation method with interaction. As a goodness-of-fit statistic, NONMEM uses an objective function (OF), which is approximately equal to minus twice the logarithm of the maximum likelihood. The likelihood ratio test, based on the reduction in objective function \( (\Delta \text{OF}) \), was used to compare two models. A \( \Delta \text{OF} \) (minus twice the log likelihood, approximately \( \chi^2 \) distributed) of 3.84 for each additional parameter was used to determine statistical significance \( (P < 0.05, \) two sided) between two models. Covariate analysis comprised forward selection of influential factors followed by backward deletion. Model assessment was based on diagnostic plots (goodness-of-fit plots and visual predictive checks), along with the measure of the standard errors, the correlation matrix of parameter estimates, and the size of residual errors.

**Model validation.** The reliability of the analysis results was checked by the bootstrap resampling procedure with replacement on 200 replicates. The median and 95% confidence interval for each parameter were compared with those estimated from the original data set. The statistical analysis was performed using Perl-speaks-NONMEM version 3.2.4 (http://psn.sourceforge.net). In addition, simulations based on the final pharmacokinetic estimates were performed with NONMEM using 1,000 individuals to calculate 90% prediction intervals (PIs). The concentrations encompassing the range from the 5th to 95th percentiles at each time point were retrieved in order to construct the intervals. The values were generated using GraphPad Prism (version 4.00 for Windows; GraphPad Software, San Diego, CA).

An external model validation was performed as well on RAL sparse concentrations collected from a new cohort of 53 HIV+ individuals between December 2009 and November 2010. The final population parameter and variance estimates were used to calculate concentration predictions for the validation data set. Empirical individual Bayesian posterior estimates of the pharmacokinetic parameters were retrieved, based on the observed concentrations and the maximum-likelihood parameter estimates and variances obtained in the final model (by means of the NONMEM option MAXEVAL = 0). The predictions of the final model were compared graphically to actual RAL observations. The mean prediction error and root mean square prediction errors were calculated to derive bias and precision by comparing population predictions and individual predictions versus observed concentrations.

**Simulations.** Simulations were performed based on the average pharmacokinetic parameters and variability obtained from the final model for 1,000 individuals, using RAL at 400 mg BD or 800 mg QD as well as RAL at 400 mg BD associated with ATV. The mean and 90% prediction interval of concentrations at trough (i.e., at 12 and 24 h) were derived for both regimens.

**Genetic association analyses.** Genetic association analysis in the discovery population was performed with PLINK (33) using the individual \( a \) posteriori relative bioavailability estimates \( (F_{\text{HIV}^+}, \) post) as the phenotype. We explored 3 different models of inheritance: additive, recessive, and dominant. A genetic score was built up, assigning a value of 0 to a fully functional allele, +1 to a decrease/loss-of-function allele, and −1 to a gain-of-function allele. UGT1A haplotypes were constructed using PHASE, version 2.1 (University of Washington, Seattle, WA) (37, 38). The haplotype phylogenetic tree was constructed with MEGA 4.0 software (39) using maximum-parsimony methods. Epistatic interaction signals including combinations of 2, 3, 4, or 5 SNPs and other covariates (glomerular filtration rate [GFR], age, ATV, etravirine [ETV], tenofovir [TDF], and proton pump inhibitors [PPIs]) were evaluated using the generalized multifactor dimensionality reduction method (GMDR) (25).

**RESULTS**

**Population pharmacokinetic analysis.** A total of 544 RAL concentrations were used for the analysis, of which 335 values were obtained from 145 HIV+ patients and 209 from 19 healthy individuals. Plasma concentrations ranged between 4 and 10,192...
ng/ml. Demographic characteristics of the HIV\textsuperscript{+} population are summarized in Table 1, and the description of the population of healthy volunteers (HIV\textsuperscript{−}) is as reported by Neely et al. (7, 29).

First, the analysis of HIV\textsuperscript{−} rich data was performed. The data were best described by means of a 2-compartment model with first-order absorption from the gastrointestinal tract, which fitted the data more appropriately than a 1-compartment model (ΔOF = −172; P < 0.001). The addition of a third compartment did not significantly improve the fit (ΔOF = −5; P = 0.08). Different absorption models were tested, using zero-order absorption, sequential independent zero- and first-order absorption, sequential linked zero- and first-order absorption (minimization was not attained), parallel first- and zero-order absorption, and finally 2 parallel first-order transfer processes, which failed to achieve a better description of the absorption profile than the first-order absorption model (ΔOF > 10). The assignment of between-subject variability (BSV) to clearance (CL) (ΔOF = −302; P < 0.001), volume of distribution of the central compartment (V\textsubscript{1}) (ΔOF = −73; P < 0.001), and absorption rate constant (k\textsubscript{a}) (ΔOF = −11; P < 0.01) improved the description of the data. No BSV on the peripheral compartment (V\textsubscript{2}) or the intercompartmental clearance (Q) was observed (ΔOF = −0.0).

Further analyses combining both rich and sparse data were performed, while initially allowing different kinetic parameters to be estimated in both populations. Since major differences (40%) in CL, V\textsubscript{1}, and k\textsubscript{a} were observed between HIV\textsuperscript{+} and HIV\textsuperscript{−} individuals, a relative bioavailability component (F\textsubscript{HIV\textsuperscript{−}}) was introduced in the model, while fixing bioavailability to 1 for healthy volunteers (F\textsubscript{HIV\textsuperscript{−}} = 1). This model resulted in a significant improvement of the fit (ΔOF = −75; P < 0.001). Two distinct k\textsubscript{a} parameters were also kept in the model, since a difference in k\textsubscript{a} was observed between HIV\textsuperscript{+} and HIV\textsuperscript{−} rich data and confirmed in the analysis including the entire population. A reduction to a single value significantly worsened the fit (ΔOF > 6.6). As previously observed, significant BSV was estimated on CL, V\textsubscript{1}, and k\textsubscript{a} (ΔOF = −960.4; P < 0.001). In addition, assigning variability on both F\textsubscript{HIV\textsuperscript{−}} and F\textsubscript{HIV\textsuperscript{+}} improved the model fit (ΔOF = −44; P < 0.001). Since the magnitudes of the BSV on the bioavailability and on the absorption rate constant were similar for both groups, their variance was constrained to be the same. With the assignment of BSV on relative bioavailability, the BSV on CL decreased from 72% to 19% (coefficient of variation [CV]) and did not remain statistically relevant. The use of two distinct proportional components to describe residual error according to the population (HIV\textsuperscript{+} and HIV\textsuperscript{−} individuals) slightly improved the fit (ΔOF = −12; P < 0.01). The final pharmacokinetic parameters and variability (CV) without covariates were as follows: CL = 64 liters·h\textsuperscript{−1}, V\textsubscript{1} = 138 liters (90.8%), V\textsubscript{2} = 143 liters, Q = 9.3 liters·h\textsuperscript{−1}, k\textsubscript{a} = 0.65 h\textsuperscript{−1} and 0.24 h\textsuperscript{−1} (97.3%) in HIV\textsuperscript{−} and HIV\textsuperscript{+} individuals, respectively, and F\textsubscript{HIV\textsuperscript{+}} = 0.57 (90.6%).

Among the demographic factors tested in the model (age, gender, body weight, and ethnicity), we observed a 65% higher relative bioavailability in females than in males, which explained 43% of the variability in bioavailability (ΔOF = −6.4; P = 0.01). Ethnicity showed some impact on V\textsubscript{1} (ΔOF = −6.5; P = 0.01), suggesting a 60% lower V\textsubscript{1} for Caucasians than for other ethnicities, which explained approximately 3.8% of the variability in V\textsubscript{1}. No further influences of body weight and age were observed. The assignment of comedinations in the models revealed that only ATV increased RAL relative bioavailability, by 39% (ΔOF = −7.9; P < 0.01). The inclusion of acid-reducing agents, including antacids or proton pump inhibitors, yielded a 66% lower absorption rate constant, but the effect did not reach statistical significance (ΔOF = −3.5; P = 0.06). EFV coadministration resulted in 20% lower RAL bioavailability, but this effect also was not significant (ΔOF = −0.5; P = 0.5). The evaluation of the impact of AST, ALT, and bilirubin, as markers of hepatic dysfunction, on RAL pharmacokinetics showed that the RAL F\textsubscript{HIV\textsuperscript{−}} increased linearly with increased total bilirubin levels (ΔOF = −6; P < 0.01). A 30% increase in drug levels is expected in case of grade 1 hyperbilirubinemia (total bilirubin higher than 30 μmol/liter). No further

TABLE 1 Demographic characteristics of the HIV\textsuperscript{+} population

<table>
<thead>
<tr>
<th>Characteristic\textsuperscript{a}</th>
<th>Value</th>
<th>% of study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (no.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>114</td>
<td>78.6</td>
</tr>
<tr>
<td>Female</td>
<td>31</td>
<td>21.4</td>
</tr>
<tr>
<td>Median age, yr (range)</td>
<td>48.5 (18–72)</td>
<td></td>
</tr>
<tr>
<td>Median body wt, kg (range)</td>
<td>70 (45–114)</td>
<td></td>
</tr>
<tr>
<td>Median height, cm (range)</td>
<td>175 (152–194)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity (no.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>132</td>
<td>91.0</td>
</tr>
<tr>
<td>Black</td>
<td>10</td>
<td>6.9</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Coadministered drugs (no.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ritonavir</td>
<td>72</td>
<td>49.7</td>
</tr>
<tr>
<td>Lopinavir</td>
<td>21</td>
<td>14.5</td>
</tr>
<tr>
<td>Atazanavir</td>
<td>11</td>
<td>7.6</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>Darunavir</td>
<td>73</td>
<td>50.3</td>
</tr>
<tr>
<td>NRTIs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Didanosine</td>
<td>8</td>
<td>5.5</td>
</tr>
<tr>
<td>Abacavir</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>75</td>
<td>51.7</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>28</td>
<td>19.3</td>
</tr>
<tr>
<td>Emtricitabine</td>
<td>37</td>
<td>25.5</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>20</td>
<td>13.8</td>
</tr>
<tr>
<td>Gastric acid-reducing agents</td>
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<td></td>
</tr>
<tr>
<td>Antacids</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>5</td>
<td>3.5</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>5</td>
<td>3.5</td>
</tr>
<tr>
<td>Median AST concn (U/liter) (Range)</td>
<td>30 (10–472)</td>
<td></td>
</tr>
<tr>
<td>Median ALT concn (U/liter) (range)</td>
<td>29 (10–323)</td>
<td></td>
</tr>
<tr>
<td>Median total bilirubin concn (μmol/liter) (range)</td>
<td>12 (5–91)</td>
<td></td>
</tr>
<tr>
<td>Median CD4\textsuperscript{+} cell count, cells/mm\textsuperscript{3} (range)</td>
<td>335 (29–968)</td>
<td></td>
</tr>
<tr>
<td>Median HIV RNA level, copies/ml (range)</td>
<td>130 (0–277,000)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors.
influences of AST and ALT were observed (ΔOF = −0.0). Multivariate analyses and backward deletion confirmed that gender, ATV coadministration, and bilirubin levels significantly influenced $F_{\text{HIV}^+}$, as well as ethnicity influencing $V_1$ (ΔOF = −25.9 and $P < 0.001$ in comparison to the model without any covariates). The final average pharmacokinetic parameters and BSVs are presented in Table 2. Absorption half-lives ($t_{1/2}$) were 1 h and 3.4 h in HIV− and HIV+ individuals, respectively; distribution and terminal $t_{1/2}$ were 1.9 h and 10.9 h, respectively; and the volume of distribution at steady state ($V_{ss}$) was 368.4 liters. Plots of concentrations versus time with population predictions and the 95% prediction interval are presented in Fig. 1. Goodness-of-fit plots of population and individual predictions versus the observations are presented in Fig. S1 in the supplemental material.

**Model Validation.** The medians with 95% confidence interval (CI) parameter estimates obtained from the bootstrap analysis are presented in Table 2. The parameter estimates of the final population pharmacokinetic model lay within the 95% CI of the bootstrap results, suggesting that the model was acceptable. The external validation pharmacokinetic model lay within the 95% CI of the bootstrap results, suggesting that the model was acceptable.

### Table 2: Final population and bootstrap resampling estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Final population pharmacokinetic parameters</th>
<th>Bootstrap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE (%)</td>
</tr>
<tr>
<td>$CL/F$ (liters/h)</td>
<td>60.2</td>
<td>44.3</td>
</tr>
<tr>
<td>$V_1/F$ (liters)</td>
<td>223</td>
<td>52.6</td>
</tr>
<tr>
<td>$\theta_{\text{race}}$</td>
<td>−0.59</td>
<td>30.7</td>
</tr>
<tr>
<td>$V_2/F$ (liters)</td>
<td>113</td>
<td>41.0</td>
</tr>
<tr>
<td>$k_\text{a}$ (h⁻¹)</td>
<td>HIV</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>HIV+</td>
<td>0.21</td>
</tr>
<tr>
<td>$Q/F$ (liters/h)</td>
<td>8.5</td>
<td>54.1</td>
</tr>
<tr>
<td>$F_{\text{HIV}^+}$</td>
<td>1 (fixed)</td>
<td>1 (fixed)</td>
</tr>
<tr>
<td>$\theta_{\text{ATV}}$</td>
<td>0.75</td>
<td>40.4</td>
</tr>
<tr>
<td></td>
<td>0.39</td>
<td>17.5</td>
</tr>
<tr>
<td>$\theta_{\text{female}}$</td>
<td>0.55</td>
<td>52.4</td>
</tr>
<tr>
<td>$\theta_{\text{bilirubin}}$</td>
<td>0.36</td>
<td>47.9</td>
</tr>
<tr>
<td>$\omega^{d}$ (CV, %)</td>
<td>$V_1/F$</td>
<td>87.4</td>
</tr>
<tr>
<td></td>
<td>$k_\text{a}$</td>
<td>94.2</td>
</tr>
<tr>
<td></td>
<td>$F$</td>
<td>86.7</td>
</tr>
<tr>
<td>$\sigma^{d}$ (CV, %)</td>
<td>HIV−</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td>HIV+</td>
<td>60.0</td>
</tr>
</tbody>
</table>

* Final model: $F = \theta_1 \cdot (1 + \theta_{\text{bilirubin}} \cdot FBIL) \cdot (1 + \theta_{\text{female}} \cdot \text{sex}) \cdot (1 + \theta_{\text{ATV}} \cdot \text{ATV})$, where FBIL = (mean bilirubin concentration − mean bilirubin concentration)/(mean bilirubin concentration = 30 μmol·liter⁻¹).

* $CL/F$, mean apparent clearance; $V_1/F$, mean apparent volume of distribution of the central compartment; $V_2/F$, mean apparent volume of distribution of the peripheral compartment; $k_\text{a}$, mean absorption rate constant; $Q/F$, mean apparent intercompartmental clearance; $F_{\text{HIV}^+}$, bioavailability in the HIV+ population; $F_{\text{HIV}−}$, bioavailability in the healthy volunteers.

* Difference = (bootstrap median value − typical value from final model)/bootstrap median value.

* Estimate of variability is expressed as CV (%) on October 23, 2017 by guest http://aac.asm.org/ Downloaded from Antimicrobial Agents and Chemotherapy

![FIG 1: RAL concentrations (circles) versus time standardized for a 400-mg BID dosing in HIV− and HIV+ individuals, with population predictions (solid line) and the 95% prediction interval (dashed lines).](http://aac.asm.org/.../1/11001/H9258/631-148.9)
nal model validation based on 70 new RAL concentration data showed nonsignificant biases of $-23\%$ (95% CI, $-43.6\%$ to $2.6\%$) for population predictions and $-10\%$ (95% CI, $22.9\%$ to $0.7\%$) for individual predictions. The precisions of population and individual predictions amounted to 266% and 66%, respectively. Plots of population and individual predictions versus observations for the validation data set are presented in Fig. S2 in the supplemental material.

Concentration-genetic association analysis. Among the 102 single nucleotide polymorphisms (SNPs) assessed, 14 failed quality control criteria for genotyping and 6 were genotyped by other techniques (see Materials and Methods). Four SNPs and one gene deletion were not originally included in the array and were therefore genotyped using other techniques. One SNP (rs4860305) that was not in Hardy-Weinberg equilibrium and 2 SNPs (rs7439366 and rs1800961) that were monomorphic were excluded from analysis. Ninety-six SNPs in total were tested, using the individual a posteriori estimates of RAL $F_{\text{HIV}}$ as the pharmacokinetic phenotype. Assuming a dominant model of inheritance, one SNP (rs72551330, a marker of $UGT1A9^*3$) reached study-wide significance (beta, $+1.78$; $P = 4.18 \times 10^{-4}$) (Fig. 2A). However, the effect of this SNP on RAL $F_{\text{HIV}}$ was due to a single individual who was homozygous for this rare allele and exhibited very high RAL bioavailability (Fig. 2B). This individual had consistently high RAL plasma concentrations even though the dose was reduced over time (from 400 mg twice daily to 200 mg once daily). With both other models of inheritance, none of the SNPs reached study-wide significance.

We attempted to validate this signal by genotyping rs72551330 in a replication data set of 219 HIV-infected individuals and 19 healthy volunteers. However, none of them were homozygous for the rare allele. Since this SNP is localized in $UGT1A$, we performed a more detailed analysis of this locus. A genetic score with the 10 proven functional alleles in the locus $UGT1A$ was not significantly associated with the phenotype (see Fig. S3 in the supplemental material) ($P = 0.097$ by the Kruskal-Wallis test). Haplotype analysis using the 47 SNPs from the $UGT1A$ locus revealed 95 different haplotypes. We built up a phylogenetic tree with the 95 haplotypes.
together with the ancestral haplotype of the chimpanzee, and we
grouped them according to similarity in 34 stems to reduce the
number of haplotypes. However, none of them was found to in-
fluence \( F_{HIV} \) (\( P = 0.308 \) by the Kruskal-Wallis test) (see Fig.
S4 in the supplemental material). Finally, we investigated the pres-
ence of epistatic interactions between the 14 SNPs with proven
functional effect in the \( UGT1A \) and \( UGT2B \) genes using the gen-
eralized multifactor dimensionality reduction (GMDR) method
\( (25) \). Different epistatic models were investigated (combinations
of 2, 3, 4, or 5 SNPs) using the RAL \( F_{HIV} \) adjusted for discrete
(presence of ATV, ETV, TDF, and PPIs as concomitant medica-
tions) and quantitative (age and GFR) covariates as dependent
phenotypic traits. We did not identify any significant \( (P < 0.05) \)
epistatic interaction signals.

Simulations. Model-based simulations of RAL at 400 mg BID
yielded a median concentration at trough of 124 ng/ml (95% pre-
diction interval [PI], 10 to 1,380 ng/ml). Simulations of RAL at
800 mg QD yielded a median \( C_{\text{min}} \) of 52 ng/ml (95% PI, 4 to 817
ng/ml). The simulation of RAL at 400 mg BID associated with
ATV yielded a \( C_{\text{min}} \) of 171 ng/ml (95% PI, 14 to 1,783 ng/ml).
Taking the protein-adjusted 95% inhibitory concentration (IC\text{50})
of 15 ng/ml (33 nM) for naïve patients as a cutoff value for the
target \( C_{\text{min}} \). 5% of the patients were predicted to present concen-
trations below this level when receiving 400 mg BID, compared to
15% of those receiving 800 mg QD. Plots of the average concen-
trations with 95% PIs are presented for the 400-mg BID and
800-mg QD regimens in Fig. 3.

**DISCUSSION**

This study presents the first population pharmacokinetic analysis
of RAL in both HIV\(^+\) individuals and healthy volunteers. The
results show that RAL has a high apparent clearance and is widely
distributed, with values for clearance, volume of distribution, and
half-life comparable with published data \( (7, 20, 41) \). The high
apparent clearance suggests that RAL is a high-extraction drug,
subject to significant first-pass metabolism. It was therefore as-
tained that differences in the kinetics between HIV\(^+\) and HIV\(^-\)
individuals would depend mainly on variations in oral bioavail-
ability. As expected, a very large interindividual variability on RAL
bioavailability was observed. The 25% lower relative bioavailability
in HIV\(^+\) individuals than in healthy volunteers might be rel-
lated to adherence issues, since HIV\(^-\) individuals were studied
under more standardized conditions of drug intake and food status.
We cannot, however, exclude the possibility that this differ-
ence is related to the HIV disease, as gastrointestinal or malab-
sorption problems have been reported in HIV\(^+\) individuals \( (8, 18,
20) \). The RAL absorption half-life was very variable, which is con-
sistent with previously reported data \( (2, 9, 27, 46) \). The slower
absorption half-life of RAL in HIV\(^+\) individuals could be attrib-
uted to HIV-related motor gastrointestinal abnormalities or de-
layed gastric emptying, but it seems to have a modest impact on
time to peak (data not shown) \( (23, 30) \). It might also be the con-
sequence of a difference in food intake between the study popula-
tions, since meal type has been shown to explain part of the vari-
bility in RAL absorption \( (6) \). The important variability observed
in the absorption profile in both HIV\(^+\) and healthy subjects under
standardized feeding condition indicates, however, that factors
other than food intake state might be involved. The lack of infor-
mation on factors with a potential effect on RAL absorption limits
the interpretation of the results.

In line with previously reported data \( (10, 49) \), we observed an
approximately 40% increase in RAL bioavailability induced by
ATV coadministration, as a consequence of ATV-mediated
UGT1A1 inhibition \( (10, 17, 29) \). The small and nonsignificant
20% decrease in RAL bioavailability induced by EFV is similar to
previous observations \( (19, 49) \). The lack of statistical significance
is probably due to both the limited number of patients \( (n = 14) \)
exposed to the association and the modest influence of EFV. RAL
is commonly used with tenofovir, which has been shown to mod-
estly increase RAL exposure \( (47) \), and with etravirine, which is
known to induce UGT1A1, resulting in a slight decrease in RAL
concentrations \( (1) \). However, no significant effects were found in
this study for either drug.

In contrast to other reports \( (20) \), we found a 65% higher RAL
exposure in female than in male patients. Sex-related pharma-
cookinetic disparities have been reported for other antiretroviral
drugs \( (34, 42) \). There are many potential reasons for gender dif-
fferences in RAL pharmacokinetics, such as differences in gastric
pH, which is higher in females \( (36) \), lower hepatic expression of
ABCB1 P-glycoprotein in females \( (31) \), and lower hepatic blood
flow and consequently lower hepatic metabolic capacity \( (31, 36) \),
which could partly explain our findings. We observed a 60% lower
volume of distribution in Caucasian patients compared to pa-
tients of other ethnicities, who were mostly black patients. Ethnic

**FIG 3** Model-based simulations of RAL at 400 mg BID (A) and 800 mg QD (B) in HIV\(^+\) individuals. Average RAL concentrations (solid line) and 95% prediction intervals (dashed lines) are shown.
differences in drug distribution have been reported with many drugs, which were attributed mainly to differences in protein binding and in particular in binding to alpha1-acid glycoproteins (AAG) (orosomucoid) (21). RAL, formulated as a potassium salt (27), might preferentially bind to AAG, although this has not been formally demonstrated. An ethnicity-based difference in AAG levels could thus explain the difference in the distribution of RAL (21). The clinical significance of this finding is still not clear and needs more investigation with larger patient cohorts.

A clear relationship between RAL exposure and treatment outcome has not been formally reported (35). However, some recent evidence suggests a possible role of drug concentration, in addition to virological parameters, in the efficacy and induction of virological resistance to RAL (12, 14). In accordance with results of the QD Merck study (35), our simulations of 400 mg BID compared to 800 mg QD indicate that a higher percentage of patients would exhibit Cmin under the protein-adjusted IC50 with the 800-mg QD regimen than with 400 mg BID, which might put patients at risk of virological failure, in particular those with a high viral load, as reported by Eron et al. (14). Considering the very large BSV variability encountered, some patients might also exhibit very low RAL concentrations with the standard 400-mg BID regimen, which suggests that therapeutic drug monitoring of this drug could be relevant in some situations. However, the rather high intrapatient variability might limit its effectiveness. Based on some evidence about pharmacokinetic and pharmacodynamic relationships (12, 14) and on the good correlation between RAL intracellular concentrations and plasma concentrations (16, 28), there is a need to further explore the relationship between efficacy and pharmacokinetic exposure and the potential role of concentration monitoring. Better-standardized studies based upon more extensive sampling on a better-defined population would circumvent some of the limitations inherent to observational studies.

Genetic variations in UGT isoenzymes and nuclear receptors were not significantly associated with RAL exposure, except for the UGT1A9*3, which needs further confirmation. UGT1A9*3 is a loss-of-function allele that is substrate specific. It has been associated with decreased glucuronidation activity of irinotecan and mycophenolic acid but not flavopiridol (4, 24, 45). Studies using RAL metabolite profiles as the phenotype may represent a better alternative to identify genetic variants influencing RAL exposure.

In conclusion, the RAL pharmacokinetic profile is characterized by high interpatient and residual variability. ATV, gender, and hyperbilirubinemia appear to affect RAL bioavailability, whereas ethnicity affects the volume of distribution. Except possibly for UGT1A9*3, no genetic polymorphisms was found to explain the large RAL pharmacokinetic variability. Owing to this very large variability, drug concentrations may be very low under the standard dosage regimen of 400 mg BID and further decreased with 800 mg QD, suggesting that therapeutic drug monitoring of RAL could yet be relevant in some situations. Further studies focusing on concentration-response relationships should be performed to better define target plasma concentrations.

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