Pharmacokinetic Modeling of Plasma and Intracellular Concentrations of Raltegravir in Healthy Volunteers

Lingzhi Wang, Gaik Hong Soon, Kok-Yong Seng, Jun Li, Edmund Leç, Eu-Leong Yong, Boon-Cher Goh, Charles Flexner, and Lawrence Lee

Cancer Science Institute of Singapore, National University of Singapore, 28 Medical Drive, Singapore 117456; Departments of Medicine, Pharmacology, and Obstetrics and Gynaecology; Yong Loo Lin School of Medicine, National University of Singapore, National University Health System, Singapore; Bioengineering Laboratory, Defence Medical & Environmental Research Institute DSO National Laboratories, 27 Medical Drive, Singapore 117510; and Department of Medicine, Johns Hopkins University, 600 N. Wolfe Street, Osler 527, Baltimore, Maryland 21287

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Raltegravir (RAL) is the first HIV integrase inhibitor licensed by the FDA in September 2007 (2). RAL selectively targets HIV integrase while not interfering with normal cellular function (16) and demonstrates potent antiviral activity in vitro and in vivo (8). Metabolism of RAL is dependent on the activity of UDP-glucuronosyltransferase 1A1 (UGT1A1) (11). These facts suggested that RAL has a low propensity of drug-drug interactions. Nevertheless, there is high variability in the systemic exposure of RAL in human subjects, especially in the concentration 12 h after the dose was given (C_{max}) with coefficients of variation of 212 and 122% for intersubject and intrasubject variability, respectively (14). This variability can be caused by several factors such as food intake (14), gastric pH (9), and genetic polymorphisms, although the effects of UGT1A1 polymorphisms are such as food intake (14), gastric pH (9), and genetic polymorphisms, although the effects of UGT1A1 polymorphisms are likely to be modest (22). So far, no strong pharmacokinetic (PK)-pharmacodynamic relationship has been defined despite this considerable PK variability, and RAL remains highly efficacious in most patients. Our hypothesis was that the consistent efficacy of RAL is due to the accumulation of RAL in lymphocytes. This could allow less frequent dosing of raltegravir. However, limited information is available on the intracellular PK of RAL, especially beyond 12 h postdose. This spurred us to investigate the PK profile of RAL in peripheral blood mononuclear cells (PBMCs), the target site of action. We performed a pilot study to model the association of the plasma and intracellular RAL concentrations for 48 h after a single 400-mg RAL dose by determining the time course and half-life of intracellular RAL and modeling the intracellular accumulation of RAL after a single dose.

MATERIALS AND METHODS

Subjects. We enrolled six healthy male subjects between 21 and 65 years of age. Other inclusion criteria included smoking less than 10 cigarettes per day and laboratory values fulfilling the following criteria: hemoglobin level of >10.9 g/dl, platelet count of ≥125,000/mm³, creatinine clearance of ≥60 ml/min, and lipase or pancreatic amylase level of <1.1× upper limit of normal. We excluded any significant acute or chronic medical illness that would interfere with the conduct or interpretation of the study. Exclusion criteria included any major surgery within 8 weeks of enrollment in the study and any gastrointestinal surgery that could impact absorption and donation of blood or plasma within 60 days of screening. Subjects who were taking any concomitant medication, including investigational, prescription, and any over-the-counter drugs and dietary supple-
ments, with the exceptions of aspirin, acetaminophen, chlorpheniramine, and multivitamins, were excluded. The study protocol was reviewed and approved by the Institutional Review Board, National Healthcare Group, Singapore. Written informed consent was obtained from all volunteers. The study was conducted in accordance with the guidelines on good clinical practice and with the ethical standards for human experimentation established by the Declaration of Helsinki.

Study design. We performed a single-dose PK evaluation of raltegravir (RAL) in plasma and peripheral blood mononuclear cells (PBMCs). Healthy volunteers were admitted to the clinical trials research unit at about 9 p.m. on day 0. On day 1, 400 mg of RAL given orally was administered at 8 a.m. with water. Blood was sampled for 12 h after the dose was given. Subjects returned on days 2 and 3 for the 24- and 48-h blood sample collection. Volunteers were given 400 mg of RAL while fasting. Paired blood samples for plasma and PBMCs were collected predose (0 h) and 4, 8, 12, 24, and 48 h postdose. Plasma samples were also collected 0.5, 1, 1.5, 2, 3, 5, 6, and 10 h postdose.

Bioanalytical methods. Serial blood samples were collected from the subjects for quantification of RAL using a well-validated liquid chromatography-tandem mass spectrometric (LC-MS/MS) method (20). Plasma samples were collected and placed in heparin tubes, while PBMC samples were collected and placed in cell preparation (CPT) tubes. The PBMCs were washed 3 times with cold phosphate-buffered saline (PBS) and counted in a hemocytometer. Concentrations were calculated assuming a cell volume of 0.4 pl. Deuterated RAL (RAL-d3) was used as the internal standard. All reference standards were purchased from Toronto Research Chemicals Inc. (Canada).

The LC-MS/MS system consisted of an Agilent 1100 binary pump equipped with an Agilent 1100 autosampler injector with a 100-μl loop and a column oven (Agilent Technologies, Waldbronn, Germany) set at 24°C and an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Ontario, Canada). Chromatographic separations were achieved on a ZORBAX Eclipse XDB-C8 (Agilent) (50 mm by 2.1 mm; 5 μm) with gradient elution. Mobile-phase solvent A was water containing 0.05% formic acid (analytical reagent; Sigma), and solvent B was acetonitrile (high-performance liquid chromatography [HPLC] grade) (Merck) containing 0.05% formic acid. The initial mobile-phase composition of 85% solvent A was maintained for 0.2 min and then decreased linearly to 10% from 0.2 to 4 min and maintained till 4.9 min. Next, solvent A was quickly reverted to 85% within 0.1 min. The run time was 9 min at a constant flow rate of 0.4 mL/min.

The mass spectrometer was operated in positive ion mode calibrated by polypropylene glycol. The purified plasma/cell samples were analyzed by tandem MS using the ion spray needle at 5,500 V, and the dwell time per channel was 200 ns for data collection. The optimum temperature was set at 450°C. The mass transitions of RAL and RAL-d3 were m/z 445→361 and m/z 446→364, respectively.

Both plasma and PBMC samples were prepared through protein precipitation. The linear calibration range of RAL was 2 to 2,000 nM in plasma and 0.225 to 22.5 nM in PBMC lysate. The accuracies ranged from 99.4% to 108.0% in plasma and from 97.9% to 101.4% in PBMCs. The precision over the quantification range was less than 2.5% and 4.8% in plasma and PBMCs, respectively.

RESULTS

Study population and safety. Six male subjects were included in the study. Their median weight was 67.4 kg (range, 56.5 to 87.1 kg), and their median age was 33.5 years (range, 31 to 36 years). No significant adverse events were noted.

Plasma and intracellular concentrations of RAL. The concentrations of raltegravir (RAL) in plasma and PBMCs were quantified and presented in Fig. 2. All plasma samples collected within 48 h of the dose could be quantified, but most of the intracellular samples at the last time point could not be measured using our validated LC-MS/MS method. The plasma geometric mean (GM) C12 (concentration 12 h after the dose was given) of RAL was 338.7 nmol/liter (95% confidence interval, 163.1 to 1,030.4) with 6.3-fold variation and PBMC intracellular GM C12 of RAL was 59.6 nmol/liter (30.4 to 223.3) with 7.3-fold variation.

Pharmacokinetic parameters. Using noncompartmental analyses, the GM (95% confidence interval) plasma RAL Cmax (AUC0–12) and area under the concentration-time curve from 0 h to infinity (AUC0–∞) were 2,246 (1,175 to 4,294) nM·h, 10,776 (5,770 to 20,126) nM·h, and 13,119 (7,235 to 23,788) nM·h, respectively. The apparent plasma RAL half-life was at least 3.84 for 1 degree of freedom. The covariance option in NONMEM was used to calculate estimate precisions, expressed as relative standard error (RSE).

Plasma drug pharmacokinetics was described using a one-compartment model, with first-order absorption and elimination rates. Interindividual and residual unexplained variability were estimated with an exponential error model and correlation between individual random effects of apparent volume of distribution/bioavailability (V/F) and apparent oral clearance/bioavailability (CL/F) was estimated. Following the procedure reported by Kappelhoff et al. (10), to describe the gradual and variable onset of oral drug absorption, a chain of transition compartments between the depot and central compartment was tested. The optimal number of transition compartments was investigated and the mean absorption time (MAT) was calculated using the formula MAT = (n + 1)k0, in which n represents the number of transition compartments and k0 represents the transition rate constant. The basic pharmacokinetic model as used for RAL is schematically depicted in Fig. 1.

The intracellular accumulation or partitioning ratio for RAL was estimated by using the NONMEM program and the following formula: CIC,obs = ACR × Cplasma,obs × e^(εIC,obs) where the observed intracellular concentration (CIC,obs) was predicted from the accumulation ratio (ACR), estimated plasma concentration (Cplasma,obs), and the individual error εIC.

Sample size and statistical analyses. A sample size of 6 subjects would provide 90% power to detect a correlation coefficient of 0.75 between plasma and intracellular raltegravir with a type 1 error of 5%. Correlations and graphs were performed using GraphPad Prism. Statistical analyses were performed using Stata version 10 (Stata Inc., College Station, TX).

FIG. 1. Basic population pharmacokinetic model for raltegravir.
The intracellular RAL \( C_{\text{max}} \), AUC\(_{0–12} \), and AUC\(_{0–24} \) were 383 (114 to 1,281) nM, 2,073 (683 to 6,290) M \( \cdot \) h, and 2,435 (808 to 7,337) nM \( \cdot \) h. The apparent intracellular RAL half-life was 4.5 (3.3 to 6.0) h. The intracellular RAL concentration was 24.0% (6.7 to 58.1%) of plasma concentrations.

The estimated population pharmacokinetic parameters using NONMEM are shown in Table 1. Observed interindividual variability in RAL plasma pharmacokinetics was high. Two transition compartments were adequate to describe the absorption process. The goodness-of-fit plots obtained from the final population pharmacokinetic model are shown in Fig. 3. Fig. 3A presents the observed versus population predicted concentrations. The data points in the low-to-mid regions were uniformly spread around the line of unity with moderate underprediction in the higher region. Examining Fig. 3B with observed versus individual predicted concentrations, those in the low-to-mid regions were closer to the line of unity compared to those in the higher region. The visual predictive checks (VPC), based on 500 concentration-time profiles simulated using parameter estimates of the final model, revealed that the final model predictions were in reasonable agreement with the observed concentrations. In VPC (Fig. 4), measured concentrations were overlaid with the 95% prediction intervals of model-predicted concentrations, stratified by plasma and intracellular RAL concentrations. Of the available observed concentrations, 97% were within the prediction interval. Overall, the results indicate that the study data were adequately described by the final model.

### Association between plasma concentration and intracellular RAL.

Concentration-time curves for intracellular and plasma RAL were roughly parallel, with intracellular/plasma ratios remaining stable for each patient without significant time-related trends over 48 h. Significant correlation was found between intracellular and plasma RAL concentrations \((r = 0.85;\)

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**TABLE 1. Population pharmacokinetic estimates of raltegravir after a single dose of 400 mg**

<table>
<thead>
<tr>
<th>Model parameter(^b) (unit)</th>
<th>Value for parameter</th>
<th>Population estimate</th>
<th>RSE(^c) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of transition compartments</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean absorption time (MAT) ( \text{h} )</td>
<td>0.887</td>
<td>27.6</td>
<td></td>
</tr>
<tr>
<td>Clearance/bioavailability ( CL/F ) ( \text{liter/h} )</td>
<td>39.1</td>
<td>20.8</td>
<td></td>
</tr>
<tr>
<td>Volume distribution/bioavailability ( V/F ) ( \text{liter} )</td>
<td>272</td>
<td>21.8</td>
<td></td>
</tr>
<tr>
<td>Accumulation ratio ( % )</td>
<td>11.2</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Random effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interindividually variability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIV in MAT ( % )</td>
<td>66.2</td>
<td>52.5</td>
<td></td>
</tr>
<tr>
<td>IIV in CL/F ( % )</td>
<td>56.4</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>IIV in ( V/F ) ( % )</td>
<td>56.7</td>
<td>24.6</td>
<td></td>
</tr>
<tr>
<td>IIV in accumulation ratio ( % )</td>
<td>104</td>
<td>55.6</td>
<td></td>
</tr>
<tr>
<td>Correlation between ( CL/F ) and ( V/F )</td>
<td>0.559</td>
<td>24.6</td>
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<tr>
<td>Residual error</td>
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<tr>
<td>Exponential error of plasma concen ( % )</td>
<td>86.7</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Exponential error of intracellular concen ( % )</td>
<td>64.9</td>
<td>51.1</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The pharmacokinetics were monitored for 48 h.

\(^b\) IIV, interindividual variability.

\(^c\) RSE, relative standard error (standard error divided by population estimate \( \times 100 \); for random-effect parameters, RSE is related to the corresponding variance scale).

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**Fig. 2.** Mean intracellular and plasma concentrations of raltegravir after a single dose of 400 mg. The concentration of raltegravir (nM) is shown on the y axis, and time (in hours) is shown on the x axis. IC\(50\) denotes the top end of the inhibitory concentration for 50% of HIV viruses (7 nM).

**Fig. 3.** Goodness-of-fit plots. (A) Observed versus population predictions and (B) observed versus individual predictions.
The mean ratio of intracellular to plasma RAL concentrations was 24.0% (range, 6.7 to 58.1%). Using NONMEM population PK modeling, the intracellular partitioning ratio was estimated to be 11.2% (35% relative standard error [RSE] estimate precision). There was a high interindividual variability (100%).

**DISCUSSION**

RAL concentrations in plasma and PBMCs were modeled simultaneously in this study. Even for fasting healthy volunteers, plasma and intracellular RAL concentrations are highly variable. $C_{\text{12}}$, an important pharmacokinetic parameter for anti-HIV agents given twice daily, showed significant variability in both plasma and intracellular concentrations, with 6.3- and 7.3-fold changes between the highest and lowest concentrations, respectively. Similar results were also observed in other major pharmacokinetic parameters, namely, $C_{\text{max}}$, $AUC_{0-12}$, and $AUC_{0-\infty}$, with the differences between the largest and smallest values being 3.7-, 5.9-, and 3.3-fold, respectively. This large variability in plasma RAL was also evidenced in previous studies (14). However, even larger variation was observed for $C_{\text{max}}$, $AUC_{0-12}$, and $AUC_{0-\infty}$ in PBMCs with the differences between the largest and smallest values of 11.2-, 9.2-, and 9.1-fold changes, respectively.

The final population pharmacokinetic model was a one-compartment model with two catenary gastrointestinal transit absorption compartments. One- and two-compartment models with components to describe the delayed and variable absorption of RAL were tested as initial structural models. Our attempts to fit the data to a two-compartment model were not successful, most likely due to little data collected during the $\beta$-elimination phase and the delayed and variable absorption of RAL. This finding notwithstanding, the apparent biphasic disposition in RAL concentration-time profiles in Fig. 2 should be further investigated during model selection using data from larger clinical trials. In our study, the one-compartment model was stable and adequately described the RAL disposition kinetics. In contrast to our results, a previous population pharmacokinetic analysis of RAL in 145 HIV patients and 19 healthy subjects indicated a preference for a two-compartment model over a one-compartment model (1). That study reported high interindividual variability (77 to 100%) and differences in mean population estimates for $CL/F$, peripheral volume of distribution, and absorption rate constant for HIV patients and healthy subjects. On the other hand, at least two studies reported that a one-compartment model provided an adequate data fit. Our healthy subjects had lower population mean ($%RSE$) $CL/F$, 39.1 (20.8%) liters/h compared to 264 (23%) and 191 (16%) liters/h in the study of Dickinson et al. (4) and Ter Heine et al. (19), respectively. For $V/F$, our study yielded 272 (21.8%) liters in comparison with 820 (25.5%) (4) and 76 (8%) liters (19). However, differences in sample sizes, subject type (healthy subjects or HIV patients), and dosage forms of RAL could have accounted for the variability in parameter estimates. Similar to these other studies, moderate to high interindividual (36 to 60%) and residual (46 to 68%) variabilities in RAL pharmacokinetics were observed, which likely contributed to the relatively high standard errors of the parameters. This is not unexpected, given the variable onset of absorption of RAL. Large pharmacokinetic studies are required to further characterize the pharmacokinetics of RAL before the population pharmacokinetic model could be applied in a clinical setting.

Intracellular RAL concentrations were low compared to other anti-HIV drugs like darunavir, etravirine, and ritonavir.
The intracellular-to-plasma ratios for etravirine, darunavir, and ritonavir were 12.9, 1.32, and 7.72, respectively (19). The intracellular-to-plasma ratios for RAL were only 0.11 to 0.24 in our study using two different methods of estimation. This result is consistent with other studies of HIV patients on standard doses (19) and an 800-mg once daily (15) dose of RAL. This result is also consistent with simple diffusion of unbound RAL into cells, based on protein binding of 83% in plasma (14).

Intracellular pharmacokinetics are usually more variable than plasma pharmacokinetics. The reasons for this greater variability include the fact that PBMC isolation and purification are tedious processes, even if commercially available devices for cell separation, such as CPT tubes, are used.

Washing with cold PBS is necessary to remove the residue of extracellular drug, in this case raltegravir. However, the intracellular raltegravir signal may be significantly reduced by multiple washings, as evidenced by recent data from Watson et al. (21). PBMC isolation and purification can be affected by many factors, including cell isolation technique, temperature, the number of washes, and the technician’s level of experience. During our experiment, we followed a strict protocol to minimize such variation. A potential limitation to all the intracellular drug analyses is that the loss of drug during sample processing cannot be estimated with great precision. This issue will need further investigation using well-designed protocols and a gold standard for intracellular concentration measurement, which currently does not exist.

Even though the intracellular RAL concentration was low, the intracellular RAL concentration 12 h postdose was a few-fold higher than the 50% inhibitory concentration (IC50) range of 2 to 7 nmol/liter of purified HIV-1 integrase by RAL (3). Our results may explain the equal antiviral potency over a wide dose range of 100 to 600 mg twice daily (13). This could help explain why RAL given twice daily consistently demonstrated a satisfactory pharmacodynamic effect on anti-HIV-1 under highly variable plasma concentrations in patients (6). On the other hand, the low intracellular RAL concentrations we observed at 24 h may explain the inferiority of 800 mg given once daily versus the 400 mg twice-daily dose in clinical trials (5). The results of this study would further reinforce the caution about using once-daily doses of the current formulation of RAL.

Several other factors (food intake, drug interaction, gastric pH, and emptying time) may also account for the variability of plasma and intracellular RAL concentrations. The effect of food and drug interaction can be excluded in our well-controlled clinical trial. However, variability in gastric pH remains. In this study, a secondary peak was observed in several individuals, suggesting possible enterohepatic circulation due to extensive glucuronidation of RAL. Furthermore, a secondary peak for one subject was much higher than for the other subjects. This phenomenon could be caused by the combined effect of delayed absorption in the gastrointestinal tract and enterohepatic circulation (19). The delayed absorption may be due to individual differences in gastric emptying time. RAL oral absorption is highly sensitive to pH changes with better absorption in an environment with higher pH (9). For those with shorter emptying time, the maximum absorption would appear in an earlier phase with a time to maximum concentration of drug in plasma (Tmax) of 1 to 3 h. In other subjects, RAL intestinal absorption might be delayed, resulting in a later Tmax of around 8 h. Similar temporal trends were found for intracellular RAL, suggesting rapid cellular uptake of RAL. Although a significant difference existed between plasma and intracellular RAL, there was a good correlation of plasma and intracellular parameters (Fig. 4). Modeling also showed that a simple diffusion barrier model for plasma to intracellular RAL fitted the data reasonably well. This suggests that there is little intracellular accumulation or persistence of RAL in PBMCs.

The higher variability of intracellular RAL levels compared to plasma RAL levels should be investigated further. Methodological issues could contribute to variability in sample processing, cell counting, and cell size (17). Variability could also be contributed by differential expression and function of transporters on PBMCs.

Since the pharmacokinetic profiles of HIV-infected patients were similar to those of healthy volunteers (7), our findings should also apply to HIV patients.

This study had several important limitations. First, the sample size was small. However, we believe the rich sampling strategies partially compensated for this, as evidenced by the success of the population pharmacokinetic modeling. Second, there was a lack of sampling after the 24-h time point, which made it difficult to estimate the terminal half-life accurately. However, the measurable plasma concentrations at the 48-h time point still allowed us to obtain an estimate of kα based on at least 2 time points in all subjects. Third, we could not detect intracellular concentrations in most of the subjects at 48 h and therefore could not get an accurate terminal half-life for intracellular raltegravir. However, the lower limit of quantitation for intracellular raltegravir was within the IC50 range, and therefore, we are confident that we did not miss measuring clinically relevant intracellular raltegravir concentrations.

Conclusions. In this pharmacokinetic study, there was no intracellular trapping or persistence of RAL in PBMCs. Intracellular concentrations of RAL above the IC50 were achieved at 12 h postdose but not at 24 h. Even though intracellular RAL concentrations were much lower than plasma RAL concentrations, they were 5 times higher than the IC50 range at 12 h. Intracellular RAL and its pharmacokinetics in PBMCs can be reasonably predicted from plasma data, suggesting that plasma RAL can be used as a surrogate marker of intracellular RAL in future studies. Last, although we successfully developed a population pharmacokinetic model that accounts for the variable and delayed absorption, the residual errors for plasma and intracellular concentrations remained high. Our population pharmacokinetic model should be further tested and refined using data collected from larger cohorts. This will also help to elucidate covariates influencing the plasma and intracellular pharmacokinetics of RAL.

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