Steady-State Pharmacokinetics of Abacavir in Plasma and Intracellular Carbovir Triphosphate following Administration of Abacavir at 600 Milligrams Once Daily and 300 Milligrams Twice Daily in Human Immunodeficiency Virus-Infected Subjects

Graeme Moyle, Marta Boffito, Carl Fletcher, Chris Higgs, Phillip E. Hay, Ivy H. Song, Yu Lou, Geoffrey J. Yuen, Sherene S. Min, and Elena M. Guerini

St. Stephen’s Centre, Chelsea and Westminster Hospital, London, United Kingdom; Genito-Urinary Medicine, St. Georges Hospital, London, United Kingdom; GlaxoSmithKline, Research Triangle Park, North Carolina; and GlaxoSmithKline, Stevenage, United Kingdom

Received 25 July 2008/Returned for modification 20 November 2008/Accepted 20 January 2009

Abacavir (ABC) is administered either at 600 mg once daily (ABC 600 mg QD) or 300 mg twice daily (ABC 300 mg BID) in anti-human immunodeficiency virus (anti-HIV) combination therapy. Although ABC plasma pharmacokinetics following each regimen has been well defined, no study has directly compared the regimens with respect to pharmacokinetics of ABC’s active intracellular anabolite, carbovir-triphosphate (CBV-TP). In an open-label, two-period, crossover study, 34 HIV-infected male and female subjects stabilized on antiretroviral regimens containing either ABC 600 mg QD or ABC 300 mg BID received their usual doses on days −1 and 1 and then switched regimens for days 2 to 11. Serial blood samples collected on days 1 and 11 were assayed for plasma ABC and intracellular CBV-TP concentrations using validated high-performance liquid chromatography–tandem mass spectrometry methods. Pharmacokinetic parameters were calculated using noncompartmental methods. Analysis of variance with a mixed-effect model was performed for treatment and gender comparisons. In 27 evaluable subjects, the regimens provided bioequivalent ABC daily areas under the concentration–time curve from 0 to 24 h (AUC\textsubscript{0–24}) and comparable CBV-TP concentrations at the end of the dosing interval (C\textsubscript{ss}). As expected, ABC QD resulted in 109% higher ABC maximum concentrations in plasma (C\textsubscript{max}) than did ABC BID. ABC QD also resulted in 32% higher CBV-TP AUC\textsubscript{0–24} and 99% higher CBV-TP C\textsubscript{max} than did ABC BID. Females had a 38% higher weight-adjusted ABC AUC\textsubscript{0–24} and 81% higher weight-adjusted CBV-TP AUC\textsubscript{0–24} than did males. Virolologic suppression was maintained during regimen switch, and no tolerability differences between regimens were observed. In conclusion, this study showed that ABC 600 mg QD and ABC 300 mg BID regimens led to similar intracellular CBV-TP C\textsubscript{ss} values, thus providing pharmacokinetic support for the interchangeability of these two regimens. Women had higher intracellular CBV-TP exposure than did men.

Simplification of antiretroviral treatment (ART) by changing twice-daily (BID) regimens to once-daily (QD) regimens has been shown to contribute to better adherence and patient satisfaction (1, 4, 5, 11, 12, 15, 16), although differences in treatment outcome have not been commonly observed (5, 11). The decision to choose a QD regimen over a BID one, or vice versa, depends on the clinical situation. QD dosing provides an especially practical regimen for subjects who require directly observed therapy, such as those who are incarcerated or in mental health facilities and those attending methadone clinics (10, 14). However, in subjects who are receiving ART drugs that are all administered BID, addition of a further antiretroviral drug with a BID regimen maintains dose symmetry and is easy to remember since all regimen components can be administered at the same time.

Abacavir (ABC) is a nucleoside reverse transcriptase inhibitor that is available both as a single-agent formulation and in fixed-dose combination formulations containing lamivudine (3TC) or 3TC-zidovudine (ZDV) to be used with drugs from other antiretroviral classes in combination ART. QD and BID ABC regimens have demonstrated similar efficacies as initial therapy backbones and in switch studies (5, 11). Switching from a BID regimen of ABC plus 3TC to QD ABC-3TC fixed-dose combination tablets has been shown to improve adherence without changing virologic efficacy or tolerability (11).

Since ABC is a nucleoside analog, its mode of action is to form carbovir-triphosphate (CBV-TP) through serial intracellular phosphorylation to exhibit antiviral activity. To date, no study has compared the QD and BID regimens with respect to intracellular CBV-TP pharmacokinetics (PKs). The estimated 12- to 20-h elimination half-life (t\textsubscript{1/2}) of CBV-TP is based on three small studies involving mostly male subjects (3, 6, 13). Two studies have shown gender differences in intracellular TP concentrations of 3TC and ZDV in human immunodeficiency virus (HIV)-infected subjects (2, 17). However, the effect of gender on CBV-TP concentrations has not been evaluated. The purpose of the present study was to compare the steadystate PKs of plasma ABC and intracellular CBV-TP by using a crossover design in subjects receiving ART regimens contain-
ằng ABC at either 600 mg QD or 300 mg BID (ABC 600 mg QD and ABC 300 mg BID, respectively) and to investigate gender differences in intracellular CBV-TP PKs.

MATERIALS AND METHODS

Study design. This was a phase I open-label, two-period, crossover, PK study conducted at a single center (St. Stephen’s Centre, Chelsea and Westminster Hospital, London, United Kingdom) from 5 September 2005 until 22 May 2006. Men or women aged 18 to 65 years with HIV type 1 (HIV-1) infection documented by HIV-1 antibody enzyme-linked immunosorbent assay and confirmed by Western blot detection of HIV-1 antibody were eligible for this study if they had the following characteristics at screening: undetectable viral load (<400 copies/ml), being on an ABC-containing regimen for ≥8 weeks, CD4+ count of ≥250 cells/μl, weight of 40 to 100 kg, body mass index of 19 to 29 kg/m², and willingness to temporarily switch their ABC schedule from QD to BID, or vice versa, for 11 days. Females were to have no childbearing potential or were to agree to protocol-specified methods of contraception, including a double-barrier method, complete abstinence from intercourse from 2 weeks prestudy to 2 weeks poststudy, or use of an intruterine device with a <1% failure rate.

Subjects. If they were eligible, subjects were randomized to receive either ABC 600 mg QD or ABC 300 mg BID. Intracellular PK assessments were performed at study days 1, 11, and 22. Subjects attended a follow-up visit 7 days after dosing; when subjects received an ABC BID-containing regimen, the study drug was stopped for 2 h after dosing; when subjects received an ABC QD regimen, the study drug was stopped for 10 days afterwards. On the days of PK sampling (day 1 and day 11), subjects were discharged on day 12 and were allowed to switch back to their original ABC regimen. Subjects attended a follow-up visit 7 days after dosing at follow-up. Adverse events were assessed intermittently throughout the study.

PK sample collection and processing. Blood samples (16 ml each) were collected in CPT tubes (two tubes, 8 ml per sample) at the time points specified (predose and 2, 4, 6, 8, 12, 16, and 24 h postdose) for measuring intracellular CBV-TP and plasma ABC on day 1 and day 11. In the morning on day 2, subjects had the last 24-hour PK sample on their current regimens collected; this was immediately followed by the ABC regimen switch. An additional 2-ml blood sample for plasma ABC only was collected in a blood collection tube with EDTA at 1 h postdose on day 1 and day 11.

The CPT tubes were centrifuged within 60 min of blood collection at controlled room temperature (18 to 25°C) in a horizontal rotor (swing-out bucket) for 20 min at a relative centrifugal force of 1,500 to 1,800. A 1-ml aliquot of plasma was collected from the tube, transferred to a 1.8-ml Nunc polystyrene storage tube, and stored at −80°C till assayed for ABC concentrations. The peripheral blood mononuclear cell (PBMC) layer was suspended in the remaining plasma, transferred from both CPT tubes to a single graduated 50-ml conical tube, and mixed with isotonic saline (0.9%) to achieve a total volume of 30 ml. A 500-μl aliquot was removed from the suspension for cell counting using a Kova slide by trained personnel following a validated standard procedure. Cell counting was performed in duplicate and was repeated until cell counts from duplicates were within 25% of each other. The total number of PBMC counts from each PBMC sample (PBMCCT) was calculated and reported in the unit of million cells. The volume of remaining suspension was recorded (accurate to 0.1 ml). At the suspension was centrifuged for 15 min at a relative centrifugal force of 400 to pellet the cells. The supernatant was aspirated and discarded (plasma and platelets), and the cell pellet was well mixed with 1 ml ice-cold 70% methanol solution to lyse and completely redissolve the cell pellet. The lysed PBMC extract was completely transferred to a 1.8-ml Nunc cryostorage vial and stored at −70°C until assayed for CBV-TP concentrations.

Bioanalysis. PBMC extract samples were analyzed for CBV-TP by Taylor (Princeton, NJ) using a validated analytical method based on anion exchange on Waters Accela QMA solid-phase extraction plates followed by enzymatic hydrolysis to CBV using alkaline phosphatase. The sample was cleaned up on a Varian C₁₈ solid-phase extraction plate and reconstituted with water followed by high-performance liquid chromatography–tandem mass spectrometry (MS/MS) analysis using a YMC octylsilica silanol AQ 2.0-× 50-mm column and positive-ion MS/MS using a TurboIonSpray (MDS Sciex [Mississauga, Canada]) interface and multiple reaction monitoring. This method had a lower limit of quantification of 0.05 μg/ml with a 500-μl aliquot of human PBMC extracts. Bias and precision were calculated using interpolated concentrations of quality control samples at three concentration levels over a calibration range of 0.05 to 10 ng/ml. The interassay precision (CV) and interassay bias were 5.91% and −1.87%, respectively, with CBV-TP at 0.15 ng/ml; 5.58% and −1.39%, respectively, with CBV-TP at 1 ng/ml; and 3.54% and −1.41%, respectively, with CBV-TP at 7.5 ng/ml.

Plasma samples were analyzed for ABC concentration by GlaxoSmithKline (Research Triangle Park, NC) by protein precipitation and high-performance liquid chromatography–MS/MS in the positive-ion TurboIonSpray mode. The calibration curve range for ABC in human plasma was 2.5 to 2,500 ng/ml using a 50-μl sample aliquot of human plasma.

For each analytical method, quality control samples, containing the relevant analytes at three different concentrations and stored with study samples, were analyzed with each batch of samples against separately prepared calibration standards.

PK analyses. CBV-TP concentrations (C), in fmol/million cells, were calculated based on the CBV-TP concentration in PBMC extracts reported in ng/ml and cell counts (PBMCCT) using the formula C (fmol/million cells) = C (ng/ml) × 10⁹/(molecular mass × PBMCCT/1 ml), where molecular mass is that of CBV-TP, 487.3 Da.

The following PK parameters for plasma ABC and intracellular CBV-TP were estimated using the noncompartmental Model 200 (for extracellular administration) of WinNonlin Professional Edition version 4.1 (Pharsight Corporation, Mountain View, CA) and actual elapsed time from dosing: AUC₀₋₂₄ is area under the drug concentration-time curve over a dosing interval and steady state (AUCₜ), Cᵣ is steady state, time of maximum concentration at steady state, Cₛ is steady state, and terminal t₁/₂ is AUCₜ for the BID regimen was calculated as 2 × AUCₜ. In addition, the ratio of CBV-TP AUC₀₋₂₄ and ABC AUC₀₋₂₄ (AUC_CBV/AUC_ABC) was calculated for each subject.
As female subjects enrolled in this study had lower body weights than did male subjects, in order to accurately evaluate the gender difference, weight-normalized (to a 70-kg person) PK parameters, including AUC0–24, Cmax, and C0, were estimated for plasma ABC and PBMC CBV-TP using the formula weight-normalized PK = PK × weight (in kg)/70 kg.

**Statistical analyses.** Summary statistics were provided for PK parameters by treatment and gender. Statistical analysis was performed to compare exposure differences in ABC and CBV-TP between ABC BID and ABC QD regimens and to assess gender effect. Analysis of variance, considering treatment arm, period, treatment, and gender as fixed effects and subject within treatment arm as a random effect, was performed using the SAE (version 8.2) mixed linear model procedure. The treatment-by-gender interaction was also included as a fixed effect in the model. Ratios of geometric least square means and associated 90% CIs were estimated for the key PK parameters: AUC0–24, Cmax, and C0 (both original and weight normalized) for intracellular CBV-TP and plasma ABC and AUC0–24/ABC, and AUC0–24/CBV. A linear regression, considering intracellular CBV-TP AUC0–24 as the response variable and plasma ABC AUC0–24 as the predictive variable, was also performed by treatment and overall to assess the relationship between exposures of plasma ABC and intracellular CBV-TP. The PK parameters were log transformed before the primary analyses, and treatment comparisons were expressed as ratios on the original scale. There was no formal statistical analysis of safety data.

**RESULTS**

**Study demographics.** Thirty-four subjects were enrolled into the study, of whom 33 were dosed and 29 completed the study. Due to enrollment difficulty, only 10 female subjects participated in this study. Five subjects were withdrawn for the following reasons: alcoholic intoxication impairing the subject’s ability to participate (one subject), positive urine test for drugs of abuse (two subjects), or abnormal alanine aminotransferase level in period 1 (two subjects).

Of the 33 subjects who were dosed, 23 (70%) were male and 10 (30%) were female. The mean age of the subjects was 45.1 years (range, 25 to 66 years). Most of the subjects (25 [76%]) were Caucasian, six (18%) were African, one (3%) was American Indian, and one (3%) was Asian. All subjects had undetectable HIV-1 RNA (<400 copies/ml), and the median CD4+ cell count was 657 cells/mm3 (range, 280 to 3,290 cells/mm3).

**PKs.** Twenty-seven (18 males and 9 females) of the 33 subjects completed both treatment study periods and were included in the PK analysis and treatment comparison. PK results and the statistical comparison of steady-state plasma ABC and CBV-TP PK parameters are presented in Tables 1 and 2.

**Intracellular CBV-TP.** Intracellular CBV-TP concentrations were much more stable over time than were plasma ABC concentrations (Fig. 1). However, considerable variability in intracellular CBV-TP concentrations was observed within and between subjects; therefore, it was difficult to accurately estimate t1/2 of intracellular CBV-TP for both regimens. The reportable t1/2 values averaged 14.1 h and ranged from 4.8 to 39 h. Intracellular CBV-TP exposures were higher from the ABC 600 mg QD regimen than from the ABC 300 mg BID regimen. ABC 600 mg QD provided CBV-TP AUC0–24 and Cmax values that were on average 32% and 99% greater, respectively, than those from the ABC 300 mg BID regimen (Table 1). CBV-TP C0 from the QD regimen was 18% higher than that from the BID regimen; however, such a difference was not statistically significant, as the 90% CI of the treatment ratio included 1 (Table 1). Intracellular CBV-TP exposures were higher in female subjects than in male subjects, with AUC0–24, Cmax, and C0 values 109%, 105%, and 113% greater, respectively, in females than in males. When the comparisons...
### Table 2: Steady-state plasma ABC PK parameters, by treatment and gender, and comparisons

<table>
<thead>
<tr>
<th>Plasma ABC PK parameter</th>
<th>Treatment A (ABC 300 mg BID regimen)</th>
<th>Treatment B (ABC 600 mg QD regimen)</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean (% CV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0–24} (h^2/g/ml)</td>
<td>7.90 (66)</td>
<td>6.74 (41)</td>
<td>6.88 (60)</td>
</tr>
<tr>
<td>C_{max} (µg/ml)</td>
<td>7.04 (41)</td>
<td>9.88 (48)</td>
<td>10.33 (58)</td>
</tr>
<tr>
<td>C_{AUC} (µg/ml)</td>
<td>7.88 (66)</td>
<td>9.49 (41)</td>
<td>10.33 (58)</td>
</tr>
<tr>
<td>Wt-normalized^c</td>
<td>0.008 (20)</td>
<td>0.013 (38)</td>
<td>0.010 (35)</td>
</tr>
<tr>
<td>AUC_{0–24}/wt</td>
<td>0.065 (34)</td>
<td>0.082 (34)</td>
<td>0.075 (36)</td>
</tr>
</tbody>
</table>

DISCUSSION

The currently approved dosing regimens for ABC are 300 mg BID and 600 mg QD, both given in combination with other antiretroviral drugs. The approval of the 600 mg QD ABC dosing regimen was based on the findings of a randomized clinical study, CNA30021 (12), which demonstrated that ABC 600 mg QD dosing was noninferior to ABC 300 mg BID dosing in combination with QD 3TC and efavirenz. Additionally, CNA10905, a PK study of intracellular CBV-TP, reported an...
intracellular CBV-TP $t_{1/2}$ of ~20 h, therefore supporting QD dosing of ABC (13). However, CNA10905 examined an ABC 300 mg BID dosing regimen only. Another PK study, COL101665, was conducted to characterize the PKs of intracellular CBV-TP following ABC 600 mg QD dosing (3). Unexpectedly low exposures of both intracellular CBV-TP and plasma ABC concentrations were observed. The lower plasma ABC exposure was inconsistent with all previously reported PK studies in adults. The reason for the low concentrations of CBV-TP and ABC in this study has not been established.

Our current study compared intracellular CBV-TP exposure between ABC 300 mg BID and ABC 600 mg QD dosing in a crossover design in the same set of subjects and investigated gender differences in intracellular CBV-TP PKs. We found that plasma ABC AUC$_{0-24}$ values were equivalent between the two regimens while intracellular CBV-TP AUC$_{0-24}$ from 600 mg QD was slightly (on average, 32%) higher than that following the 300 mg BID regimen. Most importantly, despite the lower plasma ABC $C_{\text{max}}$ after ABC 600 mg QD dosing, the intracellular CBV-TP $C_{\text{max}}$ values were similar between ABC 600 mg QD and ABC 300 mg BID; $C_{\text{max}}$ values of intracellular CBV-TP were higher for ABC 600 mg QD than for ABC 300 mg BID. Thus, the PK results support the clinical efficacy data that demonstrated that ABC 600 mg QD dosing was noninferior to 300 mg BID dosing (12).

Due to complexity in sample collection and processing or inherent variability in ABC anabolism, high intersubject variability in intracellular CBV-TP concentrations was observed in this study. The intersubject variability in CBV-TP exposure (AUC, $C_{\text{max}}$, and $C_{\text{min}}$) was greater than 50%. The intersubject variability in $t_{1/2}$ was even higher, which may have been, in part, a consequence of the short sampling period of only up to 24 h that prevented an accurate estimation of $t_{1/2}$. Intracellular CBV-TP PKs following ABC 300 mg BID or ABC 600 mg QD dosing have been reported in several studies (3, 6, 7, 9, 13). There was significant variation in intracellular CBV-TP concentrations among different studies, which is in part due to the different assays that were employed. Despite the variability, all studies demonstrated prolonged terminal $t_{1/2}$ of intracellular CBV-TP: >12 h by Harris et al. (6) and Kewn et al. (9), 18 h by Hawkins et al. (7), and 21 h by Piliero et al. (13). The observed prolonged terminal $t_{1/2}$ supported the ABC 600 mg QD dosing regimen. Intracellular CBV-TP concentrations following ABC 300 mg BID dosing seen in our study are consistent with those observed in CNA10905 (13), which used similar sample collection/processing procedures and the same bioanalytical assay.

An influence of gender on plasma ABC and intracellular CBV-TP was observed in our study. Female subjects had higher exposure than did male subjects, even after adjusting for body weight. The higher intracellular CBV-TP exposure in female subjects was correlated with higher plasma ABC exposure and higher extent of conversion from plasma ABC to intracellular CBV-TP. To date, one other PK study that involved one female and four males treated with ABC 600 mg QD reported that the female had PBMC CBV-TP concentra-

FIG. 1. Mean concentration-time profiles of plasma ABC (dashed lines) and intracellular CBV-TP (solid lines) following ABC 300 mg BID (circles) and ABC 600 mg QD (triangles). Error bars represent standard deviations.
tions two to eight times greater than those in the male subjects (6). However, a gender-related difference in ABC plasma PKs was not shown in the population PK studies by Weller et al. (18) or Jullien et al. (8). Pivotal clinical trials of ABC-containing regimens (CNA30021, CNA30024, and CNAB3002) that performed a statistical analysis of 48-week efficacy findings (proportion of subjects achieving HIV-1 RNA levels of ≤50 or ≤400 copies/ml) by gender did not find significant differences between responses in women and those in men, although generally fewer than 25% of the study populations were female (data on file, GlaxoSmithKline). Studies of other nucleoside reverse transcriptase inhibitors have reported gender-related differences in both PKs and treatment response. Anderson et al. (2) evaluated 33 antiretroviral-naïve adults, including four women, treated with a regimen of ZDV, 3TC, and indinavir and followed their treatment response and PBMC TP concentrations for over 18 months. Assessment of 310 ZDV-TP and 3TC-TP PBMC samples revealed that ZDV-TP and 3TC-TP concentrations in women were 2.3- to 1.6-fold higher than those in men (P < 0.001). Women achieved virologic suppression (HIV-1 RNA level of <50 copies/ml) twice as fast as did men. Another study, ACTG161 (17), also noted that even when data were corrected for differences in body weight, women (n = 5) on the same ZDV dosage regimen as that of men (n = 16) had a mean ZDV-total phosphate AUC that was 45% greater (P < 0.005). Again, all gender studies of PKs and pharmacodynamics have included a disproportionately smaller number of female subjects than of male subjects, and future studies should target equivalent numbers of subjects of each gender.

In conclusion, similar intracellular CBV-TP $C_{\text{AUC}}$ values were observed with ABC 600 mg QD and ABC 300 mg BID dose regimens in this study, providing PK support at the intracellular level for the interchangeability of these two regimens. A gender difference in intracellular phosphorylation of ABC was observed, with women showing higher intracellular CBV-TP exposure than that of men.

ACKNOWLEDGMENTS

This work was supported by GlaxoSmithKline. We thank GSK employees Andrew Preece for study coordination and Gary Pakes for his contribution to the preparation of the manuscript.

REFERENCES


