STEADY-STATE PHARMACOKINETICS OF PLASMA ABACAVIR AND INTRACELLULAR CARBOVIR TRIPHOSPHATE FOLLOWING ABACAVIR 600mg ONCE DAILY AND 300mg TWICE DAILY IN HIV-INFECTED SUBJECTS

Graeme Moyle, MBBS, MD¹, Marta Boffito, MD, PhD¹, Carl Fletcher, RN¹, Chris Higgs RN¹, Phillip E. Hay, MBBS², Ivy H. Song, PhD³*, Yu Lou, MS³, Geoffrey J. Yuen, PharmD³,†, Sherene S. Min, MD, MPH³, Elena M. Guerini⁴

¹St. Stephen’s Centre, Chelsea and Westminster Hospital, London, United Kingdom, ²Genito-Urinary Medicine, St Georges Hospital, London, United Kingdom, ³GlaxoSmithKline, Research Triangle Park, North Carolina, United States, and ⁴GlaxoSmithKline, Stevenage, United Kingdom

*Corresponding author. mailing address: 17-2229.2B, 5 Moore Drive, Research Triangle Park, North Carolina 27709, United States. Phone: (01)-919-4837197. email: ivy.h.song@gsk.com.

†Note: currently employed by Gilead Science, Durham, North Carolina, United States.
ABSTRACT

Abacavir (ABC) is administered either as 600 mg once daily (QD) or 300 mg twice daily (BID) in 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 300 mg BID received their usual doses on days -1 and 1, 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 HPLC-MS/MS methods. Pharmacokinetic parameters were calculated using non-compartmental methods. Analysis of variance with mixed-effect model was performed for treatment and gender comparisons.

In 27 evaluable subjects, the regimens provided bioequivalent ABC daily AUC$_{0-24}$ and comparable CBV-TP $C_t$. As expected, ABC QD resulted in 109% higher ABC $C_{max}$ compared to BID. ABC QD also resulted in 32% higher CBV-TP AUC$_{0-24}$, and 99% higher CBV-TP $C_{max}$ than BID. Females had a 38% higher weight-adjusted ABC AUC$_{0-24}$ and 81% higher weight-adjusted CBV-TP AUC$_{0-24}$ than males. Virologic 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 300 mg BID regimens led to similar intracellular CBV-TP $C_t$, thus providing pharmacokinetic support for the inter-changeability of these two regimens. Women had higher intracellular CBV-TP exposure than men.

Key Words: Pharmacokinetics, intracellular concentrations, abacavir, carbovir triphosphate
INTRODUCTION

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 dosed at the same time.

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

As a nucleoside analog, the mode of action of abacavir 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. The estimated 12~20 hours elimination half-life of CBV-TP is based on three small studies involving mostly male subjects (3, 6, 13). Two studies have shown gender differences in intracellular triphosphate concentrations of the lamivudine and zidovudine in 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 steady-state pharmacokinetics of plasma ABC and intracellular CBV-TP
using a crossover design in subjects receiving ART regimens containing either ABC
600 mg QD or 300 mg BID, and to investigate gender differences in intracellular CBV-TP
pharmacokinetics.

METHODS

Study Design

This was a phase I open-label, two-period, crossover, pharmacokinetic study
conducted at a single centre (St. Stephen’s Centre, Chelsea and Westminster Hospital,
London UK) from September 5, 2005 until May 22, 2006. Men or women aged 18–
65 years with HIV-1 infection documented by HIV-1 antibody enzyme-linked
immunosorbent assay (ELISA) 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), on an ABC-containing regimen for ≥8 weeks,
CD4+ count ≥250 cells/mm³, weighed 40-100 kg, had a body mass index of 19-29 kg/m²,
and were willing to temporarily switch their ABC schedule from QD to BID, or vice versa,
for 11 days. Females were to be of non-childbearing potential or were to agree to
protocol-specified methods of contraception, including double-barrier method, complete
abstinence from intercourse from 2 weeks pre- to 2 weeks post-study, or use of an
intrauterine device associated with <1% failure rate.

Subjects were excluded if they were receiving tenofovir, hydroxyurea,
mycophenolate, or ribavirin; were in another experimental drug trial within 30 days of
screening; regularly consumed >83 mL of a 12% strength alcoholic beverage per day; had
a medical condition that interfered with the absorption, distribution, metabolism or
excretion of drugs; regularly took drugs of abuse; had liver enzyme tests >3 times the
upper limit of normal or bilirubin >2 times the upper limit of normal; had a haemoglobin <12
g/dL or platelet count <50,000/μL; pregnant or nursing; positive for HCV antibody or
hepatitis B surface antigen test; or had previous history of suspected ABC hypersensitivity reaction. All subjects provided written informed consent to participate in the study. The study protocol was approved by the Ethics Committee at the study site.

The primary objective of this study was to compare CBV-TP exposure (AUC$_{0-24}$, C$_{\text{max}}$, and C$_{\tau}$) from ABC 600mg QD and 300mg BID. The secondary objective included assessing gender difference in pharmacokinetics of plasma ABC and intracellular CBV-TP and assessing the safety and tolerability of dosing with ABC 300 mg BID and 600 mg QD during the study period.

A sample size of at least 24 evaluable subjects was deemed necessary based on experience with an earlier CBV-TP study, CNA10905, which had investigated the steady-state pharmacokinetics of intracellular CBV-TP in HIV-infected subjects on an ABC 300 mg BID regimen (13). In that study, inter-subject coefficients of variation (%CV) of CBV-TP pharmacokinetic parameters were between 62% and 73%. Assuming intra-subject variability was smaller than inter-subject variability and that the intra-subject %CV was at 40%, a two one-sided test at $\alpha=0.05$ for each side and sample size of 24, it was estimated that the width of 90% CI for the treatment ratio was within 20% of the point estimates for the pharmacokinetic parameters AUC and C$_{\text{max}}$. Due to the potential for subject withdrawal and the complexity of sample processing and possibility that some samples may have been unusable, 30 subjects were to be enrolled. If the intra-subject %CV was at 55%, and the sample size was 24 evaluable subjects, it was estimated that the lower and upper bounds of the 90% CI would be within 28% of the point estimate for AUC and C$_{\text{max}}$. It was planned to enroll at least 12 women to assess gender difference.

**Study Drug Dosing**

Subjects were screened between 30 and 8 days prior to the first study day. In Period 1 (Day -1 to Day 1), subjects continued their usual ABC regimen. In Period 2 (Day 2 to Day 12), subjects stabilized on ABC 300mg BID were switched to ABC 600mg QD
and those on ABC 600mg QD were switched to ABC 300mg BID from Days 2-11. Subjects continued dosing all other antiretroviral agents. Subjects were discharged on Day 12 and were allowed to switch back to their original ABC regimen. Subjects attended a follow-up visit 7-10 days afterwards. On the days of pharmacokinetic sampling (Day 1 and Day 11), subjects were fasted for at least 10 hours prior to ABC dose, the doses of ABC were taken under the supervision of clinic staff, and then subjects were fasted for 2 hours after dosing; when subjects received ABC BID-containing regimen, the evening dose of ABC was skipped on Day 1 or Day 11 to better characterize the pharmacokinetics of intracellular CBV-TP.

**Efficacy/Safety Assessment**

Blood or urine samples for laboratory safety assessments (clinical chemistry, hematology, immunology, urinalysis, and pregnancy test) were taken on Day -1, Day 5, Day 10, and at follow-up. On Day -1, Day 10 and at follow-up, blood samples (approximately 14 mL total) were also collected for measurement of viral load by HIV-1 MONITOR Version 1.0 polymerase chain reaction (PCR) assay (lower limit of quantitation [LLOQ], 400 copies/mL) (Roche, Nutley, New Jersey) and of CD4+ lymphocyte cell count by flow cytometry. Vital signs were collected on Day 2 and Day 11. Samples for viral genotyping were collected on Day 2, Day 12, and at follow-up. Adverse events were assessed intermittently throughout the study.

**Pharmacokinetic Sample Collection and Processing**

Blood samples (16 mL each) were collected in CPT tubes (2 x 8 mL per sample) at the time points specified (pre-dose, 2, 4, 6, 8, 12, 16 and 24 hours post-dose) 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 pharmacokinetic sample on his or her current regimen collected immediately followed by ABC regimen switch. An additional 2-mL blood sample
for plasma ABC only was collected in blood collection tube with EDTA at 1 hour post-dose on Day 1 and Day 11.

The CPT tubes were centrifuged within 60 minutes of blood collection at controlled room temperature (18-25°C) in a horizontal rotor (swing out bucket) for 20 minutes at 1500 to 1800 RCF (Relative Centrifugal Force). A 1-mL aliquot of plasma was collected from the tube and transferred to a 1.8 mL Nunc polypropylene storage tube and stored at ≤-20°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 30mL. A 500-µL aliquot was removed from the suspension for cell counting using a KOVA slide by trained personals following validated standard procedure. Cell counting was performed in duplicate and was repeated until cell counts from duplicate were within 25% of each other. The total number of PBMC cell 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) and the suspension was centrifuged for 15 minutes at 400 RCF to pellet the cells. The supernatant was aspirated and discarded (plasma and platelets) and the cell pellet was well-mixed with 1mL ice cold 70% methanol solution to lyse and completely re-dissolve cell pellet. The lysed PBMC cell 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 analysed for CBV-TP by Taylor Technology (Princeton, NJ, USA) using a validated analytical method based on anion exchange on Waters Accell™ QMA solid-phase extraction plates followed by enzymatic hydrolysis to carbovir using alkaline phosphatase. The sample was cleaned up on a Varian C18 solid-phase extraction plate and reconstituted with water followed by HPLC/MS/MS analysis.
using an YMC ODS AQ 2.0 X 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 ng/mL using 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 0.05-10 ng/mL. The inter-assay precision (% coefficient of variation) and inter-assay bias were 5.91% and -1.87%, respectively, with CBV-TP 0.15 ng/mL, 5.58% and -1.39%, respectively, with 1 ng/mL, and 3.54% and -1.41%, respectively, with 7.5 ng/mL.

Plasma samples were analyzed for ABC concentration by GlaxoSmithKline (RTP, NC, USA) by protein precipitation and high performance liquid chromatography (HPLC-MS/MS) in the positive ion turboionspray mode. The calibration curve range for ABC in human plasma was 2.5 to 2500 ng/mL using a 50-µL sample aliquot of human plasma.

For each analytical method, QC 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.

**Pharmacokinetic Analyses**

CBV-TP concentrations (C) in fmol/million cells, were calculated based on the CBV-TP concentration in PBMC extracts reported in ng/mL unit and cell counts (PBMCCT) using the following formula:

\[
C \text{ (fmol/million cells)} = C \text{ (ng/mL)} \times 10^6/(MW \times \text{PBMCCT/1 mL}),
\]

where MW is the molecular weight of CBV-TP, 487.3 daltons.

The following pharmacokinetic parameters for plasma ABC and intracellular CBV-TP were estimated using the non-compartmental Model 200 (for extravascular administration) of WinNonlin Professional Edition version 4.1 (Pharsight Corporation, Mountain View, CA, USA) and actual elapsed time from dosing: daily (24 h) area under the drug concentration-time curve at steady-state (AUC_{0-24}), area under the drug
concentration-time curve over a dosing interval at steady-state (AUC_{0–τ}), maximum concentration at steady state (C_{max}), time of maximum concentration at steady-state (t_{max}), concentration at the end of dosing interval at steady state (C_{τ}), and terminal half-life (t_{1/2}).

AUC_{0–24} for the BID regimen was calculated as 2 x AUC_{0–τ}. In addition, the ratio of CBV-TP AUC_{0–24} and ABC AUC_{0–24} (AUC_{CBV}/AUC_{ABC}) was calculated for each subject.

As female subjects enrolled in this study had lower body weight than male subjects, to accurately evaluate the gender difference, weight-normalized (to a 70-kg person) pharmacokinetic (PK) parameters, including AUC_{0–24}, C_{max}, and C_{τ}, were estimated for plasma ABC and PBMC CBV-TP using the following formula:

\[
\text{Weight-normalized (WN) PK} = \frac{\text{PK} \times \text{weight (in kg)}}{70 \text{ kg}}
\]

### Statistical Analyses

Summary statistics were provided for pharmacokinetic parameters by treatment and gender. Statistical analysis was performed to compare exposure difference in ABC and CBV-TP between ABC BID and ABC QD regimens and to assess gender effect. Analysis of variance (ANOVA), considering treatment arm, period, treatment and gender as fixed effects, subject within treatment arm as a random effect, was performed using SAS (Version 8.2) Mixed Linear Models procedure. The treatment by gender interaction was also included as a fixed effect in the model. Ratios of geometric least squares (GLS) means and associated 90% confidence intervals (CI) were estimated for the key pharmacokinetic parameters: AUC_{0–24}, C_{max}, and C_{τ} (both original and weight-normalized) for intracellular CBV-TP and plasma ABC and AUC_{CBV}/AUC_{ABC}. A linear regression analysis, considering intracellular CBV-TP AUC_{0–24} as the response variable, and plasma ABC AUC_{0–24} 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 pharmacokinetic 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 (1 subject), positive urine test for drugs of abuse (2 subjects), or abnormal alanine aminotransferase in Period 1 (2 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, 25-66 years). Most of the subjects (25 [76%]) were Caucasian, 6 (18%) were African, 1 (3%) was American Indian, and 1 (3%) was Asian. All subjects had undetectable HIV-1 RNA (<400 copies/mL), and the median CD4+ cell count was 657 cells/mm$^3$ (range, 280-3,290 cells/mm$^3$).

Pharmacokinetics

Twenty-seven (18 males and 9 females) of the 33 subjects completed both treatment study periods and were included in the pharmacokinetic analysis and treatment comparison. Pharmacokinetic 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 plasma ABC concentrations (Fig. 1). However, large variability in intracellular CBV-TP concentrations was observed between and within subjects; therefore, it was difficult to accurately estimate half-life ($t_{1/2}$) of intracellular CBV-TP for both regimens. The reportable $t_{1/2}$ values averaged at 14.1 hours and ranged from 4.8 to 39 hours. Intracellular CBV-TP exposures were higher from the ABC 600 mg QD regimen than the ABC 300 mg BID
regimen. ABC 600 mg QD provided CBV-TP AUC_{0-24} and C_{max} that were on average 32% and 99% greater, respectively, than those from the ABC 300 mg BID regimen (Table 1). CBV-TP C_{t} from the QD regimen was 18% higher than the BID regimen, however such 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 AUC_{0-24}, C_{max}, and C_{t} 109%, 105%, and 113% greater, respectively, in females than males. When the comparisons were performed based on weight-normalized pharmacokinetic parameters, AUC_{0-24}, C_{max}, and C_{t} in females were 81%, 78%, and 85% of those in males. AUC_{CBV}/AUC_{ABC} in females were on average 23% higher than males, however, such difference was not statistically significant as 90% CI included 1 (Table 1).

**Plasma ABC**

Plasma ABC concentrations changed more dramatically than intracellular CBV-TP concentration with estimated half-life averaged at 3-4 hours (Fig. 1). Due to short plasma half-life of ABC, plasma ABC C_{t} values were below the quantification limit for several subjects, especially during the QD treatment period.

Plasma ABC AUC_{0-24} was equivalent between the ABC 600mg QD and 300mg BID regimens, while the ABC C_{max} was 109% higher and C_{t} 62.6% lower with ABC 600mg QD than 300mg BID (Table 2). Plasma ABC exposures were also higher in female subjects than male subjects. Females had plasma ABC AUC_{0-24}, C_{max}, and C_{t} 60%, 43% and 84% greater than males, respectively. When pharmacokinetic parameters were adjusted by weight, plasma AUC_{0-24}, C_{max}, and C_{t} remained 38%, 24% and 60% greater in females than males, respectively (Table 2).

**Relationship between plasma ABC and intracellular CBV-TP exposure**

Intracellular CBV-TP AUC_{0-24} was significantly correlated with plasma ABC AUC_{0-24} (Fig. 2). However, when gender was included in the regression model, no significant
association between plasma ABC and intracellular CBV-TP AUC$_{0-24}$ was detected, and gender had become a significant predictor for the CBV-TP exposure, where females had a significant higher CBV-TP exposure than males. The association between plasma ABC AUC$_{0-24}$ and intracellular CBV-TP AUC$_{0-24}$ was also evaluated separately in male and female subjects and the results showed no significant association in either males or females.

**Efficacy/Safety**

Both treatment arms maintained similar percentages of subjects with undetectable HIV-1 RNA (<40 copies/mL) at screening and follow-up. The median CD4 cell count, 655/mm$^3$ and 660/mm$^3$ at screening in subjects on ABC 300 mg BID and on ABC 600 mg QD, respectively, remained stable during the course of the study, being 580 and 680/mm$^3$ in these arms, respectively, at follow-up.

Abacavir was equally well tolerated in the subjects on the ABC 600 mg QD and 300 mg BID arms, with same number (n=4, 12%) of subjects reporting any treatment-emergent AEs. No subjects died or reported serious adverse events, no subjects were withdrawn due to AEs, and no cases of ABC-associated hypersensitivity syndrome were reported when subjects switched frequency of ABC dosing. No vital sign or laboratory trends were noted for either treatment arm.

**DISCUSSION**

The currently approved dosing regimens for ABC are 300 mg BID or 600 mg QD, each 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 non-inferior to ABC 300 mg BID dosing in combination with QD 3TC and efavirenz. Additionally, CNA10905, a pharmacokinetic study of intracellular CBV-TP, reported an intracellular CBV-TP half-life of
~20 hours, therefore supporting QD dosing of ABC (13). However, CNA10905 examined ABC 300 mg BID dosing regimen only. Another pharmacokinetic study, COL101665, was conducted to characterise the pharmacokinetics 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 pharmacokinetic 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 600 mg QD dosing in a crossover design in the same set of subjects and investigated gender differences in intracellular CBV-TP pharmacokinetics. We found that plasma ABC AUC\(_{0-24}\) was equivalent between the two regimens while intracellular CBV-TP AUC\(_{0-24}\) from 600mg QD was slightly (on average, 32\%) higher than following the 300mg BID regimen. Most importantly, despite the lower plasma ABC C\(_\tau\) after ABC 600 mg QD dosing, the intracellular CBV-TP C\(_\tau\) was similar between ABC 600 mg QD and 300 mg BID; Cmax of intracellular CBV-TP were higher for ABC 600 mg QD compared to 300 mg BID. Thus, the pharmacokinetic results support the clinical efficacy data that demonstrated that ABC 600 mg QD dosing was non-inferior to 300 mg BID dosing (12).

Due to complexity in sample collection and processing or inherent variability in ABC anabolism, large inter-subject variability in intracellular CBV-TP concentrations was observed in this study. The inter-subject variability in CBV-TP exposure (AUC, C\(_{\text{max}}\) and C\(_\tau\)) was greater than 50\%. The inter-subject variability in t\(_{\text{1/2}}\) was even larger, which may have been, in part, a consequence of the short sampling period of only up to 24 hours that prevented an accurate estimation of t\(_{\text{1/2}}\). Intracellular CBV-TP pharmacokinetics following ABC 300 mg BID or 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\(_{\text{1/2}}\) of intracellular CBV-TP: >12 hours.
hours by Harris (6) and Kewn (9), 18 hours by Hawkins (7), and 21 hours by Piliero (13). The observed prolonged terminal $t_{1/2}$ supported the ABC 600mg QD dose 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 procedure and 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 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 pharmacokinetic study that involved 1 female and 4 males treated with ABC 600 mg QD reported that the female had PBMC CBV-TP concentrations 2 to 8 times greater than those in the male subjects (6). However, a gender-related difference in ABC plasma pharmacokinetics was not shown in the population pharmacokinetic studies of 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 $\leq$ 50 or $\leq$ 400 copies/mL) by gender did not find significant differences in response in women compared to men, although generally fewer than 25% of the study populations were female (Data on file, GlaxoSmithKline). Studies of other NRTIs have reported gender-related differences in both pharmacokinetics and treatment response. Anderson et al (2) evaluated 33 antiretroviral-naïve adults, including 4 women, treated with a regimen of ZDV, 3TC, and indinavir and followed their treatment response and PBMC triphosphate 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 greater than those in men ($P<0.001$). Women achieved virologic suppression (HIV-1 RNA <50 copies/mL) twice as fast as men. Another study, ACTG161 (17), also noted that even when corrected for differences in body weight, women (n=5) on the same ZDV dosage regimen as men (n=16) had a mean ZDV-total phosphate AUC that
was 45% greater (P<0.005). Again, all gender studies of the pharmacokinetic and pharmacodynamics have included a disproportionately smaller number of female subjects than male subjects, and future studies should target equivalent numbers of subjects of each gender.

In conclusion, similar intracellular CBV-TP C\textsubscript{T} were observed with ABC 600mg QD dose regimen compared to 300mg BID dose regimens in this study, providing pharmacokinetic 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 men.

ACKNOWLEDGMENT

This work was supported by GlaxoSmithKline. The authors would like to thank GSK employees Andrew Preece for study coordination and Gary Pakes for his contribution to the preparation of this manuscript.
REFERENCES


Table 1. Steady-State Intracellular CBV-TP Pharmacokinetic Parameters (Geometric Mean [%CV]), by Treatment and Gender, and Comparison

<table>
<thead>
<tr>
<th>Intracellular CBV-TP Pharmacokinetic Parameter</th>
<th>Treatment A (ABC 300mg BID Regimen)</th>
<th>Treatment B (ABC 600mg QD Regimen)</th>
<th>GLS Mean Ratio (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects (N=27)</td>
<td>Male (N=18)</td>
<td>Female (N=9)</td>
<td></td>
</tr>
<tr>
<td>Original Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–24&lt;/sub&gt; (h.fmol/mill cells)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>814 (64)</td>
<td>688 (53)</td>
<td>1138 (72)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (fmol/mill cells)</td>
<td>58.5 (54)</td>
<td>49.3 (49)</td>
<td>82.5 (42)</td>
</tr>
<tr>
<td>C&lt;sub&gt;τ&lt;/sub&gt; (fmol/mill cells)</td>
<td>23.5 (102)</td>
<td>18.7 (98)</td>
<td>37.2 (84)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;CBV/AUC&lt;sub&gt;ABC&lt;/sub&gt;&lt;/sub&gt;</td>
<td>103 (66)</td>
<td>102 (69)</td>
<td>105 (66)</td>
</tr>
<tr>
<td>Weight Normalized&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–24&lt;/sub&gt; (h.fmol/mill cells)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>811 (61)</td>
<td>719 (46)</td>
<td>1034 (82)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (fmol/mill cells)</td>
<td>58.3 (49)</td>
<td>51.4 (43)</td>
<td>75.0 (50)</td>
</tr>
<tr>
<td>C&lt;sub&gt;τ&lt;/sub&gt; (fmol/mill cells)</td>
<td>23.4 (98)</td>
<td>19.5 (91)</td>
<td>33.8 (99)</td>
</tr>
</tbody>
</table>

Abbreviations: ABC, abacavir; AUC<sub>0–24</sub>, area under the plasma concentration-time curve over 24 hours postdose; BID, twice daily; CBV-TP, carbovir triphosphate; CI, confidence intervals; C<sub>max</sub>, maximum intracellular concentration; C<sub>τ</sub>, intracellular concentration at end of dosing interval; CV, coefficient of variation; GLS, geometric least squares; QD, once daily. AUC<sub>CBV/AUC<sub>ABC</sub></sub> is the ratio CBV-TP AUC<sub>0–24</sub> and ABC AUC<sub>0–24</sub>, with unit of (fmol*h/million cells)/(ug*h/mL).

1. AUC<sub>0–24</sub> for BID regimen was calculated as 2xAUC(0–τ).
2. Weight-normalized pharmacokinetic parameters were calculated as PK*weight(kg)/70(kg).
3. Average of treatments A and B for each subject was used.
Table 2. Steady-State Plasma Abacavir Pharmacokinetic Parameters (Geometric Mean [%CV]), by Treatment and Gender, and Comparisons

<table>
<thead>
<tr>
<th>Plasma Abacavir Pharmacokinetic Parameter</th>
<th>Treatment A (ABC 300mg BID Regimen)</th>
<th>Treatment B (ABC 600mg QD Regimen)</th>
<th>GLS Mean Ratio (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Subjects (N=27)</td>
<td>Male (N=18)</td>
<td>Female (N=9)</td>
</tr>
<tr>
<td>Original Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC0–24 (h.ug/mL)¹</td>
<td>7.90 (46)</td>
<td>6.74 (41)</td>
<td>10.9 (35)</td>
</tr>
<tr>
<td>Cmax (ug/mL)</td>
<td>1.84 (40)</td>
<td>1.59 (34)</td>
<td>2.48 (32)</td>
</tr>
<tr>
<td>Cτ (ug/mL)</td>
<td>0.018 (105)</td>
<td>0.015 (106)</td>
<td>0.026 (89)</td>
</tr>
<tr>
<td>Weight Normalized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC0–24 (h.ug/mL)¹</td>
<td>7.88 (46)</td>
<td>7.04 (41)</td>
<td>9.88 (48)</td>
</tr>
<tr>
<td>Cmax (ug/mL)</td>
<td>1.84 (41)</td>
<td>1.66 (43)</td>
<td>2.25 (43)</td>
</tr>
<tr>
<td>Cτ (ug/mL)</td>
<td>0.018 (101)</td>
<td>0.016 (98)</td>
<td>0.024 (103)</td>
</tr>
</tbody>
</table>

Abbreviations: AUC0–24, area under the plasma concentration-time curve over 24 hours postdose; BID, twice daily; CI, confidence intervals; Cmax, maximum concentration; Cτ, concentration at end of dosing interval; CV, coefficient of variation; GLS, geometric least squares; QD, once daily.

1. AUC0–24 for BID regimen was calculated as 2xAUC(0–τ).
2. Weight-adjusted pharmacokinetic parameters were calculated as PK*weight(kg)/70(kg).
6. Average of treatments A and B for each subject was used.
Fig 1. Mean concentration-time profiles of plasma ABC (dashed lines) and intracellular CBV-TP (solid lines) following ABC 300 mg BID (circles) and 600 mg QD (triangles). Error bars represent standard deviation.
Fig 2. Relationship between plasma ABC AUC$_{0-24}$ and intracellular CBV-TP AUC$_{0-24}$.

Data from both ABC 600mg QD and 300mg BID treatments are included. Data from both females (solid circles) and males (open triangles) are presented. Significant correlation between plasma ABC and intracellular CBV-TP AUC$_{0-24}$ was found when using all data ($p<0.01$ based on regression of log-transformed data); however, when gender was included in the regression model, no significant association was detected.