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Abacavir Pharmacokinetics During Chronic Therapy in HIV-1-Infected Adolescents and Young Adults

JW Sleasman¹, BL Robbins², SJ Cross³, JC Lindsey⁴, JM Kraimer⁵, BE Heckman⁶, HL Sprenger⁶, NB Tustin⁷, CH Rose³, PA Poston³, EF Neal³, GE Pakes⁸, M Nikanjam⁹, and EV Capparelli⁹

¹Division of Allergy, Immunology, and Rheumatology, Department of Pediatrics, University of South Florida/All Children's Hospital, St. Petersburg, Florida, USA

²Department of Pharmacy Practice, University of Nebraska Medical Center, Omaha, Nebraska, USA

³Pharmaceutical Sciences and Services, St. Jude Children's Research Hospital, Memphis, Tennessee, USA

⁴Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston, Massachusetts, USA

⁵Clinical Research and Biosciences, IMPAACT Operations Office, Silver Spring, Maryland, USA

⁶Frontier Science Technology and Research Foundation, Amherst, New York, USA

⁷Department of Pediatrics, Immunologic and Infectious Diseases, The Children's Hospital of Philadelphia, Abramson Research Center, Philadelphia, Pennsylvania, USA

⁸Infectious Diseases MDC-HIV, GlaxoSmithKline, Research Triangle Park, North Carolina, USA

⁹Department of Pediatrics, Pediatric Pharmacology Research Unit, University of California, San Diego, California, USA

Abstract

The pharmacokinetics of abacavir and its metabolites were investigated in 30 human immunodeficiency virus (HIV)-infected adolescents and young adults 13-25 years of age, equally divided into two groups: <18 years of age and ≥18 years of age. All the subjects received the recommended adult dose of 300 mg twice daily. The area under the plasma concentration-time curve (AUC) and half-life of abacavir did not differ significantly between the age groups or by gender or race, and there were only modest associations of age with apparent abacavir clearance and with volume of distribution. There were no significant correlations of carboxylate or glucuronide metabolite levels with age or gender, although glucuronide AUC was higher in Hispanic subjects than in African-American subjects. Zidovudine and lamivudine concentration profiles were also similar in the two age groups. A novel aspect of the study included an assessment of intracellular carbovir, zidovudine, and lamivudine triphosphate levels, and these were found to be similar in the two age-based groups. Overall, these findings suggest that current recommendations relating to adult dosages are appropriate for adolescents and young adults.

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Correspondence: EV Capparelli (ecapparelli@ucsd.edu).

CONFLICT OF INTEREST

G.E.P. is employed by and owns stock in GlaxoSmithKline. E.V.C. has served as a paid consultant to GlaxoSmithKline. The other authors declared no conflict of interest.

Abacavir, in combination with other antiretroviral agents, is currently available for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in children and adults, but there are only limited data regarding its use in adolescents and young adults.¹ Similar to other nucleoside analogs, its antiviral activity depends on intracellular metabolism to an active carbovir-triphosphate (carbovir-TP) moiety. Drug clearance occurs primarily through the hepatic pathways of carboxylation and glucuronidation, and the ratios of these metabolites to the parent compound closely correlate with abacavir clearance and area under the plasma concentration-time curve (AUC).²

The pharmacokinetic (PK) parameters of abacavir, including the AUC, apparent oral clearance, and half-life have previously been shown to differ significantly between children and adults.³⁻⁸ Clearance in children is approximately twice that in adults when normalized to body weight, with a correspondingly lower AUC at the same dose. This provides the rationale for the difference in currently recommended dose regimens of 300 mg (~4 mg/kg) twice daily for adults vs. 8 mg/kg twice daily for children.¹ However, the maximum recommended dose for children is 300 mg twice daily which, for children and adolescents >37.5 kg in body weight, results in per-kilogram doses that are progressively lower on the basis of body weight. In a single-dose pharmacokinetics study of abacavir in HIV-infected children and adolescents, the PK parameters of a single dose (8 mg/kg) in adolescents were more similar to the corresponding parameters in children than to those in adults, independent of Tanner stage.⁹ The changes in abacavir PK parameters that occur as adolescents transition to young adults have not been examined. Previous PK studies of abacavir in adults have been in subjects of average age 35-40 years. Thus, in addition to there being no PK data in adolescents receiving abacavir at a dose of 300 mg twice daily, there is also very limited PK information in young adults.¹⁰

Results

Characteristics of the study population

Table 1 shows demographics and HIV disease characteristics of the 30 subjects enrolled in the protocol, overall as well as by age stratum. Sixteen (53%) were male subjects and seventeen (57%) were African Americans, non-Hispanic. Weight-for-age- and-gender *z*-scores were significantly higher than those in the reference populations for both age strata, but height *z*-scores were significantly lower in the age stratum of <18 years.¹¹ Body mass index *z*-scores were significantly higher in the younger age stratum (data not shown). All subjects in the older age stratum were Tanner stage 5, but 9 of the 15 subjects in the younger age stratum had not achieved full sexual maturity. Serum creatinine was similar between the younger and older age groups with mean values of 0.64 and 0.74 mg/dl, respectively. The two groups were balanced with respect to CD4 T-cell counts, viral loads, and HIV disease stage. Overall, the cohorts had intact immunity, with median CD4 T-cell counts of 565 cells/mm³ and 27%, whereas four subjects (13%) had CD4 T-cell counts <200 cells/mm³. Over half of the subjects (52%) had viral loads of <400 copies/ml, and 57% were in the Centers for Disease Control and Prevention disease category A. Thirteen subjects had a history of at least one HIV-related-illness diagnosis at entry.

All the participants had received abacavir for at least 8 weeks, the majority (*n* = 24, 80%) of whom were receiving it as a co-formulation with zidovudine and lamivudine. Antiretroviral therapy consisted exclusively of nucleotide reverse transcriptase inhibitors (NRTIs) (including tenofovir) in 60% of the subjects, with 40% of the subjects also receiving either a protease inhibitor (PI) or non-NRTIs (NNRTIs), or both. Therapeutic regimens varied between the age groups, with a larger proportion of adolescents and adults (>18 years) being treated with NRTIs exclusively (12 of the 15 subjects, 80%) as compared to 6 of the 15 (40%) in the younger cohort.

Abacavir pharmacokinetics

Abacavir plasma PK parameters were well described using a one-compartment model with first-order absorption, including a lag time, in 16 subjects. These PK parameters are summarized in Table 2. There were no significant differences in abacavir PK parameters by gender or race based on Wilcoxon rank sum tests. AUC, peak plasma concentration ($C_{\max}$), and clearance (unadjusted and adjusted to weight and body surface area) were compared in the two age groups, and no differences were detected (Table 2 and Figure 1). Abacavir half-life was also similar, with median values of 1.34 h (subjects ≥ 18 years) and 1.22 h (subjects <18 years) ($P=0.317$). Spearman correlations of PK parameters with characteristics of the study population showed no statistically significant correlations of age with $C_{\max}$, AUC, or apparent clearance (unadjusted) of abacavir. However, the apparent volume of distribution ($r = -0.389$, $P=0.033$) and apparent clearance based on weight ($r = -0.379$, $P=0.038$) were correlated with age. There were no significant correlations of abacavir-carboxylate or glucuronide concentrations with age or gender. However, the abacavir-glucuronide AUC was higher in Hispanics than in African Americans, the median values being 8.76 and 7.09 $\mu\text{g}\cdot\text{h}/\text{ml}$, respectively ($P=0.044$). Abacavir AUC was also significantly correlated with the abacavir-carboxylate-to-abacavir AUC ratio ($r = -0.619$, $P<0.001$) and also with the abacavir-glucuronide-to-abacavir AUC ratio ($r = -0.543$, $P=0.001$).

Abacavir apparent clearance adjusted for body weight ($P=0.024$) and abacavir-carboxylate-to-abacavir AUC ratio ($P=0.020$) were higher in subjects with undetectable HIV-1 RNA at entry as compared to those with detectable level. Abacavir apparent clearance (unadjusted and adjusted both for weight and body surface area), volume of distribution, abacavir-carboxylate-to-abacavir AUC and abacavir-glucuronide-to-abacavir AUC ratios were higher in the five subjects who were also receiving NNRTIs, and their abacavir AUCs were lower. Higher abacavir-glucuronide-to-abacavir AUC ratios were seen in the nine subjects receiving PIs (data not shown).

Zidovudine and lamivudine pharmacokinetics

The zidovudine and lamivudine concentration profiles and AUCs were also similar in the two age groups (Table 2 and Figure 2). There were statistically significant correlations of plasma abacavir AUC with zidovudine ($r = 0.611$, $P<0.001$) and with lamivudine ($r = 0.432$, $P=0.030$) AUCs. Three subjects had plasma AUCs >1 SD below the corresponding mean value, with respect to two of their three NRTIs.

Abacavir, zidovudine, and lamivudine TP metabolite pharmacokinetics

Intracellular TP metabolite concentrations are summarized in Table 3. Carbovir-TP concentrations were above the limit of quantitation in all the samples. Three of the samples were below the limit of quantitation for the other NRTI-TPs, two zidovudine-TP predose (C_0) samples and one lamivudine-TP 4-h concentration (C_4). Overall, the C_4 s were higher than the C_0 concentrations, with median ratios of 1.89, 1.95, and 1.31 for carbovir-TP, zidovudine-TP, and lamivudine-TP, respectively (Figure 3). While there were no differences in these parameters between age cohorts or gender, there were strong correlations of the estimated AUCs for carbovir-TP with zidovudine-TP ($r = 0.821$, $P<0.0001$) and also with lamivudine-TP ($r = 0.858$, $P<0.001$).

Discussion

Administering the appropriate dosage of antiretroviral agents is essential for achieving optimal suppression of viral replication and preventing the emergence of resistance. Extrapolations of adult dosages are commonly utilized when prescribing for adolescents, even though the former are often based on PK data from adults over 30 years of age.¹² The

current recommended abacavir dose of 300 mg twice daily for adults has been extrapolated to adolescents, resulting in a situation in which those who weigh >37.5 kg receive a smaller mg/kg dose than is recommended in children. The degree of sexual maturity, body mass index, diet, and other physiologic factors impacting drug disposition vary widely among adolescents of similar ages and are likely to influence optimal abacavir dosage requirements in adolescents and young adults. A recent study of abacavir pharmacokinetics in HIV-infected children and adolescents indicates that abacavir clearance does not change significantly during puberty.² The apparent clearance of abacavir in adolescents was higher than would be expected in adults of similar size, and it did not correlate with weight, gender, or Tanner stage. This finding suggested that abacavir pharmacokinetics should be re-examined in adolescents and young adults.

Overall, this study found a modest, but significant, relationship between apparent abacavir clearance and volume of distribution (weight adjusted) and the age of the subject. This was in spite of the relatively large variability and narrow age range evaluated. However, when examined categorically by age strata, there were no significant differences in PK parameters. There were no age correlations or group differences in abacavir AUC or C_{max} . The study was balanced across age strata for gender, ethnicity, associated disease stage, viral load, and CD4 T-cell counts. The two groups were unbalanced with respect to the use of NNRTIs and PIs. A larger proportion of subjects in the younger age group received PI-containing regimens as compared to the older subjects, who were predominantly treated with NRTIs with or without an NNRTI. Given that some PIs have been found to modestly increase abacavir clearance, this may have contributed to this observed association with age. The removal of data relating to these subjects led to loss of power and statistical significance; however, the age trends remained. The sample size for this study was derived to detect an age-related difference in AUCs of at least 25%; the observed difference in the mean values in the two age groups (7.35 $\mu\text{g}\cdot\text{h}/\text{ml}$ vs. 7.61 $\mu\text{g}\cdot\text{h}/\text{ml}$), was much smaller. Given its wide variability, the mean apparent clearance in the younger cohort, of 13.03 ml/min/kg, was not sufficiently different from the older cohort value of 10.43 to reach statistical significance. Given that the AUCs were similar for the two age strata, the indications are that a single standardized dose can be used across both age strata.

Adherence to antiretroviral therapy is always a concern, especially in the adolescent population. Only 50% of the subjects had undetectable viral levels, suggesting that long-term adherence may have been suboptimal. However, a large proportion of the population were receiving antiretroviral therapy consisting solely of abacavir, zidovudine, and lamivudine, a combination that is known to be less effective at controlling viral replication than NNRTI- or PI-containing regimens, especially in the setting of high baseline viral loads.¹³ Potential nonadherence is also unlikely to have an undue influence on the PK results. The PK evaluations were performed around an observed dose, and all three compounds studied have short plasma half-lives, with little accumulation. In addition, all of the C_0 values for carbovir-TP and lamivudine-TP were above the limit of detection, indicating no gross nonadherence with the doses just before the PK evaluations.

A comparison of the results of this study with those of previous PK studies of abacavir in children, adolescents, and older adults provides a reference point for determining safe and effective therapy. Older adults receiving abacavir at a dose of 300 mg twice daily (~4 mg/kg) have been shown to have steady-state AUC in the range of 5-6 $\mu\text{g}\cdot\text{h}/\text{ml}$.^{6,12-14} In this study, the mean AUC among all adolescent and young adults receiving the same dose was 7.48 $\mu\text{g}\cdot\text{h}/\text{ml}$. Apparent clearance was also in the range previously reported in adults. The apparent clearance of 0.86 l/min, reported by McDowell *et al.*, translates to a weight-adjusted apparent oral clearance of 12.3 ml/min/kg for a 70-kg adult.¹² This is very consistent with the overall value from our study (11.7 ml/min/kg).

The AUC in this study was similar to the AUC of 8.4 $\mu\text{g}\cdot\text{h}/\text{ml}$ previously reported in children and adolescents (PACTG study P1018), but the dose in this study (300 mg twice daily) was lower than in P1018, in which the adolescents received 8 mg/kg (average 460 mg).² Accordingly, the average apparent clearance adjusted for weight among the younger cohort in this study was lower than for the seven adolescents aged 13-18 years in P1018 (13.0 and 16.7 ml/min/kg, respectively). The lower apparent clearance in this study was not associated with a slower half-life (1.23 h vs. 1.49 h). While the reason for the possible differences between the various study data with respect to clearance is multifactorial, a higher frequency of PI use in P1018 (80%) is likely to have contributed to the differences.

Abacavir metabolism takes place primarily through the hepatic pathways of carboxylation and glucuronidation, with minimal renal clearance. Evaluation of metabolite ratios can provide mechanistic insights regarding PK differences, helping to distinguish between absorption and metabolism processes. The abacavir-carboxylate-to-abacavir ratios as well as the abacavir-glucuronide-to-abacavir ratios were not different between the age cohorts. Overall, there was no significant impact of age or gender on metabolite levels. However, the study population contained a large proportion of minority subjects and showed higher abacavir-glucuronide AUC among Hispanics than among non-Hispanic African Americans. There are well-documented genetic polymorphisms of UDP-glucuronosyltransferase enzymes, including UGT2B7, that are known to play a role in abacavir metabolism. These polymorphisms impact the metabolite profile of abacavir^{15,16} and are likely to contribute to the interindividual variability among the subjects. They are possibly involved in the ethnicity effect observed in this study.¹⁷ Zidovudine is also predominantly metabolized by UGT2B7. It is therefore not surprising that there was a rather strong association between abacavir and zidovudine AUC. However, what was unexpected was the high correlation between abacavir and lamivudine AUC, given that lamivudine predominantly undergoes renal elimination. These linked exposures suggest that absorption may be an important determinant for intersubject variability. Also, it is potentially a matter for concern that subjects with low abacavir exposure are more likely to experience low zidovudine and lamivudine exposure and effects. This may contribute to the high rate of viral breakthrough in patients receiving abacavir, zidovudine, and lamivudine as initial antiretroviral therapy or when de-intensifying to triple nucleoside regimens following viral suppression.^{18,19}

Using the combined-cartridge liquid chromatography-tandem mass spectrometry assay, we were able to measure all of the TPs of the NRTIs in Trizivir in one injection with liquid chromatography-tandem mass spectrometry. While the procedure is thus somewhat simplified, it still remains complex and one not to be undertaken lightly. Still, this study has shown that the measurement of all three TPs is feasible. The average intracellular lamivudine-TP concentrations in this study of 3.73 pmol/ 10^6 cells predose and 5.38 pmol/ 10^6 cells at 4 h after the dose were also within the range of average concentrations reported in previous studies (1.7-8.6 pmol/ 10^6 cells).²⁰⁻²⁵ Similarly, the zidovudine-TP concentrations in this study, of 32.7 fmol/ 10^6 cells predose and 74.0 fmol/ 10^6 cells at 4 h after the dose, were within the range of 6.3-58 fmol/ 10^6 cells seen in previous studies.²⁰⁻²³ We did find a correlation between the concentrations of the three TP anabolites, possibly because of shared steps of metabolism, including nucleoside diphosphate kinase.²¹ However, we did not note any gender-related effects on zidovudine-TP or on lamivudine-TP as previously reported.²⁰ Despite the wide interindividual variability in carbovir-TP concentrations achieved, the ranges have been determined in patients responding to treatment (see above). Theoretically, assessment of steady-state carbovir-TP concentrations to determine whether they are within the reported ranges could allow assessment of a patient's adherence to the abacavir regimen. However, the carbovir-TP ranges can differ between laboratories due to differences in cell processing and assay methodologies used, preventing routine clinical use of these assays.

This study represents the first evaluation of intracellular carbovir-TP concentrations in an HIV-infected adolescent population. The adolescent group exhibited carbovir-TP concentrations and carbovir-TP AUCs—predose and 4 h after the dose—that were similar to those in the young adult group. The overall median carbovir-TP values predose and 4 h after the dose (28.0 and 38.0 fmol/10⁶ cells, respectively) were at the lower end of the range reported from previous studies (4-290 fmol/10⁶ cells).^{23,26-28} There was tremendous interpatient variability in carbovir-TP concentrations, the range of variability being as much as 60-fold in samples both predose and 4 h after the dose. The majority of the subjects had higher carbovir-TP concentrations in their samples taken 4 h after the dose (20% higher than predose concentrations in 83% (25 of the 30 subjects)). The median predose intracellular carbovir-TP concentration was more than double the *ex vitro* carbovir-TP reverse transcriptase K_i of 21 nmol/l (~10 fmol/10⁶ cells).²⁸ Moreover, the median carbovir-TP C_{ave} exceeded the K_i by approximately fourfold. This suggests that carbovir-TP exercises a substantial inhibitory effect on the incorporation of deoxyguanosine triphosphate into DNA by HIV-1 reverse transcriptase due to abacavir in these patients. In contrast to some reports, we found no apparent gender-related differences in carbovir-TP concentrations or plasma abacavir concentrations. There were also no significant correlations between plasma abacavir PK parameters and carbovir-TP concentrations. This is in contrast to the findings of Piliero *et al.*,²⁸ who observed a significant linear relationship between plasma abacavir AUC and carbovir-TP AUC.

Prior studies have indicated an average intracellular carbovir-TP half-life of between 12 and 20 h, allowing once-daily dosing.^{27,28} With only two samples available per patient, and the short (12-h) dose interval, this study was unable to estimate the half-life of carbovir-TP in individual subjects. However, the magnitude of carbovir-TP fluctuation during the dose interval, characterized by the C_4/C_0 ratio (median value 1.9), was consistent with a relatively short half-life. If the half-life were 20 h, one would expect a ratio of 1.3, or possibly lower if the 4-h concentrations were to miss the peak. Given that the “predose” sample for study purposes was collected after an unsupervised dose, nonadherence would have preferentially impacted the predose concentrations and inflated this ratio. Significant nonadherence in this population, although possible, is unlikely to have occurred, given that the lamivudine-TP ratio of 1.3 is close to the value expected as indicated by prior studies and lamivudine-TPs intracellular half-life. Whether the larger fluctuation in carbovir-TP concentrations is a characteristic of this study population or of adolescents in general requires further inquiry. This may have clinical relevance when using extended-dose intervals. Further study would also be needed to rule out the possibility of adolescents having a shorter carbovir-TP half-life than adults do.

In conclusion, this study revealed modest associations of age with abacavir clearance and with volume of distribution, but these did not reach statistical significance in age group comparisons. This study is the first comprehensive evaluation of these antiretroviral agents and their metabolites in adolescents and young adults. The abacavir pharmacokinetics parameters were highly variable among the subjects, and it is likely that genetics and concomitant medications contributed to this variability.²⁹ A novel aspect of this study was the assessment of intracellular levels of carbovir, zidovudine, and lamivudine-TP. Importantly, NRTI-TP concentrations were similar in the two age groups, while carbovir-TP concentration showed greater fluctuation than has been reported in other studies. Overall, plasma and intracellular carbovir-TP exposure were similar in this study population of adolescents and young adults and in accordance with previous reports in older individuals, thereby indicating that the current recommended dosage of 8 mg/kg up to a maximum of 300 mg twice daily is both safe and efficacious in adolescents.

METHODS

human subjects and study design

The study was an open-label, agestratified (<18 and ≥18 years), single-dose pharmacokinetics study of abacavir in 30 HIV-1-infected adolescents and young adults who had been receiving the currently recommended abacavir dose of 300 mg twice daily for at least 8 weeks before entry into the study, and were known to be adherent to their regimen (documented using the PACTG Adherence Module One questionnaire consisting of a 3-day recall of medications). The subjects were between 13 and 25 years of age, with a body weight >37.5 kg, confirmed HIV-1 infection, CD4 T-cell count >100 cells/ μ l, and HIV viral load <100,000 copies/ml. All the subjects were on a treatment regimen containing abacavir, either as a single agent or in combination formulations. Subjects with clinically significant diseases other than HIV infection and those who were pregnant were excluded from the study. The protocol was approved by the Institutional Review Board of each participating institution. All the subjects or their legal guardians provided written informed consent/assent for participation in the study.

The subjects received a single supervised abacavir dose of 300 mg in the morning. This was administered as either the commercially available oral tablet of abacavir or a combination tablet containing abacavir 300 mg, zidovudine 300 mg, and lamivudine 150 mg, in accordance with their existing antiretroviral treatment regimen. The subjects fasted for 1 h before and 2 h after the abacavir administration. PK blood samples were collected at predose and at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 h after the dose to estimate abacavir, zidovudine, and lamivudine concentrations. Additional blood samples were collected at predose and at 4 h after the dose to measure intracellular concentrations of carbovir-TP, zidovudine-TP, and lamivudine-TP in the mononuclear cells in the blood.

Determination of intracellular concentrations of drug and metabolite in blood

Blood samples were centrifuged within 2 h of collection, and the plasma was separated and stored at freezing temperatures for shipping to the core pharmacology laboratory site, where they were stored at -80 °C. Peripheral blood mononuclear cells from patients were isolated using heparinized blood cell preparation tubes (Becton Dickinson, Franklin Lakes, NJ) and extracted with methanol in accordance with the method previously described, with modifications.³⁰⁻³⁴ By using a liquid chromatography-mass spectrometry-based assay, we were able to reduce the required blood volume to a single 8-ml CPT tube. We also standardized the volume of methanol extraction buffer to 500 μ l per sample.

Plasma concentrations of abacavir and its metabolites, as well as of lamivudine and zidovudine, were determined using reverse-phase high-performance liquid chromatography assay with a lower limit of quantitation of 25 ng/ml. Plasma samples were assayed for abacavir and its metabolites by extracting 200- μ l aliquots, using a Waters HLB cartridge (Milford, MA). Samples were applied to a cartridge preconditioned with 3 ml of methanol followed by 3 ml of water. The sample was applied, rinsed with 1 ml of water, eluted with 1 ml of methanol, transferred to a fresh tube, and evaporated to dryness. The resultant pellets were suspended with 100 μ l of mobile phase A and 50 μ l phase B aliquot and subjected to gradient separation on a 150×4.6 mm², 3- μ m particle size, Phenomenex Luna C-8 reverse-phase column. The separation was performed at a flow rate of 0.75 ml/min and a temperature of 30 °C. Mobile phase A consisted of 25 mmol/l sodium acetate at pH 4.0, and mobile phase B consisted of 25 mmol/l sodium acetate and 50% acetonitrile at a final pH of 5.7. The separation gradient was a 10-min linear gradient from 3 to 8% B, a step up to 20% B, holding for 17 min, and a step up to 100% B for 2 min. Analytes were detected by ultraviolet spectrophotometry at 265 nm. The assay showed a good performance with the

coefficient of variation and error percentages being <15. The internal standard used was 2', 3'-dideoxyuridine (Aldrich Chemicals). This assay was also used for the quality assurance/quality control samples from the PACTG quality testing program, and was within the performance tolerance set by this program.

Intracellular concentrations of the TPs of zidovudine, lamivudine, and carbovir were determined using a combined cartridge liquid chromatography-tandem mass spectrometry assay developed by the investigators (B.L.R.) in the St. Jude Children's Research Hospital Pharmacology Laboratory.³⁰⁻³⁴ Briefly, peripheral blood mononuclear cell extracts were extracted with methanol, and the TPs isolated with anion exchange cartridges [¹³C] zidovudine internal standard was added, the samples dephosphorylated with acid phosphatase and then desalted with Waters OASIS HLB cartridges. The samples were evaporated to dryness and reconstituted with water, and the resultant carbovir, zidovudine, and lamivudine were analyzed using liquid chromatography-tandem mass spectrometry. The quantitation range of our assay corresponded to 0.1-11.0 pmol per million cells lamivudine-TP, 4-375 fmol zidovudine-TP per million cells, and 2-200 fmol carbovir-TP per million cells, extracted from 10 million cells. Performance was measured over 5 days at three concentrations each of lamivudine, carbovir, and zidovudine, corresponding to low, middle, and high portions of the concentration curves. The overall % coefficient of variation for lamivudine was 7.78%, with an error of 3.48%; the overall coefficient of variation for carbovir was 9.927%, with an error of 4%; and the overall coefficient of variation for zidovudine was 8.96%, with an error of 6.67%.

PK analysis

The PK data relating to abacavir were analyzed using both compartmental and noncompartmental methods. Plasma abacavir concentration data were analyzed using the ADAPT II, version 4.0 PK/pharmacodynamic analysis program (University of Southern California, Los Angeles, CA).³⁵ A one-compartment, zero-order absorption model was fitted to each patient's plasma abacavir concentration-time profile using weighted least squares estimation. The model estimated the volume of distribution (V_d/F) and the elimination rate constant (K_{el}). Apparent oral clearance was calculated as the product of V_d/F and K_{el} , and the elimination half-life was calculated as $0.693/K_{el}$. The overall goodness of fit was assessed for precision and bias by calculating the mean absolute error and mean error, presented as percentages of the values predicted, and the correlation coefficient of the model fit. Noncompartmental methods were used to determine AUC, C_{max} , time to maximal concentration, t and λ_z for abacavir, abacavir-glucuronide, abacavir-carboxylate, zidovudine, and lamivudine. C_{max} and time to maximal concentration were observed values of the concentration while λ_z was determined from the log-linear portion of the concentration profile. AUC over the sampling interval (AUC_{0-8}) was determined using the trapezoidal method. If C_8 or C_0 was above the limit of detection, AUC_{C8-inf} was estimated as C_8/λ_z , and AUC_{C0-inf} as C_0/λ_z . AUC_{0-inf} from a single dose was calculated as $AUC_{0-8} + AUC_{C0-inf} - AUC_{C8-inf}$. Given the very low intracellular concentrations, a population approach was used to estimate the population half-lives of the three NRTI-TPs, using the program NONMEM (version 6) (ICON Development Solutions, Ellicott City, MD).³⁶ The first-order conditional estimation subroutine, with interaction and a proportional residual error, was used. These models were successful in the covariance estimation step, had mean individual deviations (η -bar) not significantly different from 0, and (graphically) were found to be free of bias. No search was attempted for potential covariates. Typical parameter values, intersubject variabilities (61-93%), and model residual errors (37-42%) were used in generating empiric Bayesian *post hoc* NRTI-TP AUC estimates for individual subjects, where $AUC = \text{dose}/V_d \cdot K_{el}$. The time average NRTI-TP concentration (C_{ave}) was determined by dividing the NRTI-TP AUC by the dose interval (τ).

Statistical analysis

The results are summarized using medians and mean values $\pm$ SDs unless otherwise stated, to allow for comparisons with previously published data. Nonparametric (Wilcoxon rank sum) tests are used in comparing distributions by age group, gender, race/ethnicity, and HIV viral load (<400 copies/ml vs. 400 copies/ml). The sample size for the study was calculated based on the AUC from an 8 mg/kg-abacavir dose, as estimated in previous pediatric studies on the assumption that the variability is similar among populations.⁴⁻⁹ From the perspective of formal hypothesis testing, if the observed sample mean AUC among the adolescents and young adults enrolled in PACTG 1052 was lower than 75% of the observed clearance of 6 $\mu\text{g}\cdot\text{h}/\text{ml}$ seen in adults, then the null hypothesis would be rejected. In order to assess the potential impact of concomitant therapy, subjects receiving only NRTIs were compared with subjects who also received NNRTIs or PIs. Spearman correlations based on ranks are used for exploring potential associations among PK parameters and clinical characteristics.

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Figure 1.

Plasma concentrations of abacavir (ABC) and its metabolites, abacavir-glucuronide (ABC-G) and abacavir-carboxylate (ABC-C) by age cohort; age <18 years (group 1) and ≥18 years (group 2). Thin solid lines represent individual concentration profiles in the younger subjects and thin broken lines represent data pertaining to the older subjects. The thicker solid and broken lines represent the median concentrations for groups 1 and 2, respectively.

Figure 2.

Plasma concentrations of zidovudine (ZDV) and lamivudine (3TC) by age cohort, age <18 years (group 1) and ≥18 years (group 2). Thin solid lines represent individual concentration profiles in the younger subjects, and thin broken lines represent data relating to the older subjects. The thicker solid and broken lines represent the median concentrations for groups 1 and 2, respectively.

Figure 3.

Intracellular carbovir-triphosphate (CBV-TP) concentrations are shown during the intervals between doses. The age cohort <18 years of age is represented by circles, and the cohort ≥18 years is represented by triangles. The broken line represents the population-based concentration profile.

Table 1
Subject demographics

Characteristic	Total (n = 30)	<18 years (n = 15)	18 years (n = 15)
Age (years)			
Mean ± SD	18.7 ± 3.3	15.9 ± 1.3	21.5 ± 2.0
Range	13.7-24.3	13.7-17.6	18.5-24.3
Gender			
Male:female	16:14	8:7	8:7
Race/ethnicity			
Caucasian (%)	2 (7)	1 (7)	1 (7)
African American (%)	17 (57)	8 (53)	9 (60)
Hispanic (%)	11 (37)	6 (40)	5 (33)
Weight (kg)			
Mean ± SD	67.2 ± 19.5	62.8 ± 14.5	71.6 ± 23.2
Range	37.6-122.7	37.6-89.2	44.1-122.7
BSA (m ²)			
Mean ± SD	1.7 ± 0.29	1.7 ± 0.23	1.8 ± 0.33
Range	1.2-2.5	1.2-2.0	1.4-2.5
Creatinine (mg/dl)			
Mean ± SD	0.69 ± 0.14	0.64 ± 0.12	0.74 ± 0.14
CD4 T cells			
Mean ± SD	26.8 ± 8.6%	27.0 ± 9.1%	26.6 ± 8.4%
HIV viral load			
Median ± quartile ranges	<400 copies/ml (<400-1,490)	<400 copies/ml (<400-1,490)	490 copies/ml (<400-1,732)
PI-containing regimens	9	9	0
Abacavir dose (mg/kg)			
Mean ± SD	4.6 ± 1.45	4.9 ± 1.48	4.2 ± 1.36

BSA, body surface area; PI, protease inhibitor.

Table 2
Abacavir (ABC), zidovudine (ZDV), and lamivudine (3TC) pharmacokinetics mean (SD)/median

parameter	Total (n = 30)	<18 years (n = 15)	18 years (n = 15)	P value
ABC $C_{\max}$ (ng/ml)	2,864.27 (1,193.43)	2,788.53 (1,153.85)	2,940.00 (1,267.52)	0.918
	2,620.00	2,580.00	2,740.00	
ABC AUC ($\mu\text{g}\cdot\text{h}/\text{ml}$)	7.48 (2.63)	7.35 (2.90)	7.61 (2.43)	0.579
	6.72	7.01	6.59	
ABC CL/F (l/h)	45.44 (16.87)	47.47 (19.52)	43.40 (14.13)	0.607
	44.70	42.80	45.50	
ABC CL/F (ml/min/kg)	11.73 (4.84)	13.03 (5.31)	10.43 (4.09)	0.271
	10.60	9.80	12.10	
ABC CL/F (ml/min/m ²)	437.77 (168.51)	481.60 (192.62)	393.93 (132.66)	0.280
	390.50	382.00	399.00	
ABC V_d/F (l)	77.84 (38.28)	82.91 (43.96)	72.77 (32.37)	0.681
	71.55	67.10	73.50	
ABC V_d/F (l/kg)	1.19 (0.60)	1.35 (0.65)	1.04 (0.53)	0.193
	1.05	0.90	1.10	
ABC half-life (h)	1.19 (0.28)	1.23 (0.30)	1.14 (0.27)	0.317
	1.23	1.22	1.34	
ABC-G:ABC AUC ratio	1.32 (0.84)	1.55 (1.10)	1.09 (0.37)	0.271
	1.05	1.04	1.21	
ABC-C:ABC AUC ratio	0.63 (0.23)	0.64 (0.25)	0.62 (0.22)	0.821
	0.54	0.53	0.68	
ZDV AUC ($\mu\text{g}\cdot\text{h}/\text{ml}$)	1.87 (0.85)	1.69 (0.69)	2.00 (0.96)	0.437
	1.83 (n = 24)	1.84 (n = 10)	1.54 (n = 14)	
3TC AUC ($\mu\text{g}\cdot\text{h}/\text{ml}$)	6.83 (1.62)	7.08 (1.40)	6.64 (1.80)	0.404
	6.66 (n = 25)	6.53 (n = 11)	7.20 (n = 14)	

ABC-C, abacavir-carboxylate; ABC-G, abacavir-glucuronide; AUC, area under the plasma concentration-time curve; CL/F, apparent oral clearance; $C_{\max}$, peak plasma concentration; V_d/F , volume of distribution.

Table 3
Triphosphate (TP) pharmacokinetics mean (SD)/median

parameter	Total	<18 years	18 years	P value
Carbovir-TP	<i>n</i> = 30	<i>n</i> = 15	<i>n</i> = 15	
C_0 (fmol/10 ⁶ cells) ^a	33.97 (34.49)	33.53 (44.29)	34.37 (23.60)	0.240
	28.00	36.90	19.45	
C_4 h (fmol/10 ⁶ cells)	66.49 (75.86)	46.07 (38.98)	86.90 (97.51)	0.181
	37.95	60.00	28.80	
AUC (fmol/10 ⁶ cells)	631.83 (629.29)	526.80 (583.01)	736.87 (675.84)	0.289
	455.50	530.00	315.00	
Zidovudine-TP	<i>n</i> = 24	<i>n</i> = 10	<i>n</i> = 14	
C_0 (fmol/10 ⁶ cells) ^a	32.67 (24.22)	33.58 (26.49)	32.09 (23.67)	0.975
	24.50	23.80	24.50	
C_4 h (fmol/10 ⁶ cells)	74.02 (88.02)	52.56 (33.02)	89.34 (111.11)	0.404
	50.00	51.85	45.00	
AUC (fmol/10 ⁶ cells)	649.95 (573.03)	554.10 (351.59)	718.41 (695.17)	0.750
	487.90	563.50	441.95	
Lamivudine-TP	<i>n</i> = 25	<i>n</i> = 11	<i>n</i> = 14	
C_0 (pmol/10 ⁶ cells) ^a	3.73 (2.44)	3.61 (2.36)	3.81 (2.57)	0.839
	3.63	3.66	3.02	
C_4 h (pmol/10 ⁶ cells)	5.38 (4.12)	4.31 (2.70)	6.22 (4.90)	0.404
	4.29	4.68	4.26	
AUC (pmol/10 ⁶ cells)	52.28 (29.07)	47.89 (21.70)	55.74 (34.18)	0.725
	48.26	46.69	48.26	

^a C_0 values for one patient excluded as prior dose taken 24 h before testing rather than 12 h. AUC, area under the plasma concentration–time curve.