Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals

Peter L. Anderson, Thomas N. Kakuda, Sagar Kawle and Courtney V. Fletcher

Objectives: Nucleoside analog reverse transcriptase inhibitors (NRTI) are used in virtually all anti-HIV regimens. Clinical response depends on the intracellular formation of the pharmacologically active triphosphate moiety. Our objective was to quantify the pharmacological characteristics of zidovudine and lamivudine triphosphate in HIV-infected individuals.

Methods: Peripheral blood mononuclear cells were obtained at multiple planned intervals from antiretroviral-naive adults participating in a study of zidovudine, lamivudine and indinavir, and triphosphate levels were determined by immunoassay and high-performance liquid chromatography/mass spectrometry. Plasma HIV-RNA, CD4 cell counts, and plasma drug concentrations were collected over 18 months. Data were analysed using non-parametric, regression and time-to-event methods.

Results: Thirty-three subjects were evaluated. The estimated half-lives of zidovudine and lamivudine triphosphate were 7 and 22 h, respectively. Triphosphate concentrations were elevated in individuals with low baseline CD4 cell counts. Triphosphate concentrations in women were higher than in men by 2.3 and 1.6-fold for zidovudine and lamivudine, respectively. Women reached an HIV-RNA level under 50 copies/ml twice as fast as men. Zidovudine triphosphate above 30 fmol/10⁶ cells was independently predictive of the time to under 50 copies/ml. Lamivudine triphosphate above 7017 fmol/10⁶ cells was independently predictive of a longer virological response. Indinavir concentrations were related to antiviral responses in univariate analyses.

Conclusion: Zidovudine and lamivudine triphosphate concentration thresholds were independently associated with the antiviral activity of zidovudine, lamivudine, and indinavir. The significantly elevated triphosphate concentrations in women and individuals with low baseline CD4 cell counts, groups that historically experience high rates of serious NRTI toxicities, provide a hypothesis for the pathogenesis of these events.

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From the Antiviral Pharmacology Laboratory, School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO, USA.
Correspondence and reprint requests to: Courtney V. Fletcher, PharmD, University of Colorado Health Sciences Center, School of Pharmacy, Box C-238, 4200 East Ninth Avenue, Denver, CO 80262, USA.
E-mail: courtney.fletcher@uchsc.edu
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Introduction

Nucleoside analog reverse transcriptase inhibitors (NRTI) are the current backbone of virtually all combination antiretroviral therapies for HIV infection [1,2]. NRTI are pro-drugs because the active moiety is the triphosphate anabolite formed intracellularly [3], which interferes with DNA synthesis thus eliciting anti-HIV activity via the inhibition of HIV reverse transcriptase [4], and presumably toxicity via the inhibition of mitochondrial DNA polymerase gamma [5]. Therefore, the most informed and rational use of these compounds fundamentally depends on a quantitative understanding of the clinical pharmacology of intracellular NRTI triphosphates.

The procedures needed to measure intracellular anabolites of NRTI are difficult and this has discouraged work in this area [6]. As a result, large gaps exist in our knowledge of the basic in-vivo pharmacological characteristics of NRTI triphosphates. These deficiencies are ill-advised, considering that the overall potency of most combination drug regimens depends on two NRTI plus only one other active agent [1,2], and because serious adverse events are increasingly attributed to NRTI, including painful peripheral neuropathy, fat maldistribution, and hepatomegaly/steatosis with lactic acidemia [7,8].

Epidemiological studies suggest that the pharmacological characteristics of NRTI may be significantly different among patients. For example, HIV-infected women were reported to experience a fourfold lower rate of disease progression than men during zidovudine monotherapy, and women experience exaggerated toxicities during NRTI therapy compared with men, including approximately fourfold more fat maldistribution, and disproportionately more lactic acidosis [9–13]. It has also been recognized that women respond differently to anti-cancer nucleoside analogs, for example, women are more prone to fluorouracil than men, independent of dose, body size, and age (P < 0.0001) [14]. To date, there are no scientific/mechanistic explanations for these findings.

The objective of this work was to characterize intracellular zidovudine triphosphate and lamivudine triphosphate concentrations in HIV-infected individuals, and to investigate associations between these concentrations, patient and disease characteristics, and anti-HIV activity.

Methods

Study design

Data were obtained from individuals enrolled in a prospective, randomized, open-label trial of standard dose versus concentration-controlled therapy with zidovudine, lamivudine, and indinavir. The complete study methods and primary results have been described previously [15,16]. Briefly, antiretroviral-naive individuals, 18–60 years of age, with plasma HIV-RNA levels of 5000 copies/ml or greater were eligible. Informed written consent was obtained before participation. Subjects randomly assigned to concentration-control therapy received an individualized regimen, based on the recipient’s pharmacokinetic parameters, to maintain predefined target plasma drug concentrations. The targets were steady-state average plasma concentrations of 170 ng/ml or greater for zidovudine and 400 ng/ml for lamivudine, and a trough concentration of 130 ng/ml or greater for indinavir. The study duration was 80 weeks, 52 weeks to the primary study endpoint and an optional 28 weeks of follow-up. Study visits were monthly.

Study evaluations

Subjects were asked to bring unused medications to each visit, and adherence was measured by medication counts, as previously described [15,16]. A complete blood count with differential, a routine blood chemistry panel, HIV-RNA (Roche Amplicor Ultrasensitive Assay; Roche Diagnostic Systems, Branchburg, NJ, USA), and CD4 lymphocyte counts were determined at regular intervals. All participants underwent 8 h plasma pharmacokinetic evaluations after observed simultaneous doses of study drugs at weeks 2, 28, and 56. At 2 h post-dose of each intensive study, and at variable but recorded times post-dose of each bi-monthly visit (weeks 8, 16, 24, 36, 44, 52, 64, 72, and 80), peripheral blood mononuclear cells (PBMC) were obtained from 15 ml of blood for the quantification of triphosphates.

Analytical procedures

PBMC were counted, extracted, and stored until assayed. A combined cartridge and competitive enzyme immunoassay (Sigma-Aldrich Corp., St Louis, MO, USA) was used to quantify zidovudine triphosphate, whereas a similar cartridge method with a liquid chromatography/mass spectrometry assay was used to quantify lamivudine triphosphate. We made minor modifications to our previous description of these methods [17]. A zidovudine–horseradish peroxidase conjugate that generated spectra at 490 nm after enzymatic reaction was used in place of a zidovudine radioisotope for the assay signal. A standard curve was generated from 10 to 2000 fmol/10⁶ cells, and quality controls were prepared in healthy volunteer PBMC extracts at 30, 100, and 300 fmol/10⁶ cells. The lower limit for the assay was 10 fmol/10⁶ cells, and all coefficients of variation for the quality controls were less than 20%. For lamivudine triphosphate, purified cell extract was reconstituted in high-performance liquid chromatography (HPLC) buffer, and applied to an
HPLC/APCI mass spectrometer system (Waters 2690; Waters Corporation, Milford, MA, USA; and Finnigan LCQ-Deca; ThermoQuest Corporation, San Jose, CA, USA, respectively). A standard curve was generated from 2730 to 54 500 fmol/10^6 cells and quality controls were similarly prepared at 5000, 15 000, and 45 000 fmol/10^6 cells. The lower limit of detection was 2730 fmol/10^6 cells and all quality control coefficients of variation were less than 10%. Plasma concentrations of indinavir, zidovudine, and lamivudine were quantified using validated HPLC procedures, as previously described [15].

Statistical methods

Apparent oral plasma clearances and steady-state average plasma concentrations were determined, as previously reported [15]. The indinavir trough concentration was the concentration observed at the end of the dose interval. The apparent half-lives of intracellular zidovudine triphosphate and lamivudine triphosphate were estimated in a pooled data approach by maximum likelihood regression. Mann–Whitney U tests were used when comparing a variable between two groups, and the Kruskal–Wallis test for more than two groups (StatView; SAS Institute Inc., Cary, NC, USA). Contingency tables were analysed using Fisher’s exact test. Linear regression and Spearman’s rank correlation coefficient tests were used to investigate associations between continuous variables, and multivariate linear regression was used to adjust for covariates. Time-to-event data were analysed in an intention-to-treat approach with Kaplan–Meier and Cox proportional hazards regression. Forward selection was used in stepwise Cox analyses, and only univariate predictors with \( P < 0.1 \) were considered. Predictors considered were treatment arm, plasma concentration targets, and data described in Table 1. Data are reported as median (interquartile range) unless stated otherwise.

Results

Data summary

Triphosphate data were obtained in 33 subjects. Cell extracts were divided to accommodate the different assays. Zidovudine triphosphate was quantified in 282 samples and lamivudine triphosphate was measured in 310 samples. In 28 lamivudine triphosphate samples, there was insufficient cell extract available to quantify zidovudine triphosphate, or zidovudine triphosphate was below the detection limit of the assay. Medication count data were available in 28 patients. The calculated fraction of prescribed doses taken was 0.97 (0.94–0.99) for zidovudine, 0.98 (0.93–0.99) for lamivudine, and 0.97 (0.93–0.99) for indinavir. There were no differences in adherence among the three drugs or between the two treatment groups, and when the average adherence was calculated for each subject, the overall rate was 0.97 (0.94–0.99).

Pharmacokinetics of intracellular zidovudine and lamivudine triphosphate

Fig. 1 depicts all zidovudine triphosphate and lamivudine triphosphate concentration time data with female and male data distinguished. As shown in Fig. 1a, the

Table 1. Patient and pharmacokinetic characteristics of zidovudine, lamivudine, and indinavir.

<table>
<thead>
<tr>
<th></th>
<th>Men (N = 29)</th>
<th>Women (N = 4)</th>
<th>Overall</th>
<th>( P ) (male vs female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36 (30–42)</td>
<td>33 (27–38)</td>
<td>36 (30–40)</td>
<td>0.47</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76 (56–112)</td>
<td>68 (52–84)</td>
<td>76 (52–112)</td>
<td>0.35</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>22</td>
<td>1</td>
<td>70% Caucasian</td>
<td>Not done</td>
</tr>
<tr>
<td>African descent</td>
<td>4</td>
<td>3</td>
<td>21% African descent</td>
<td>9% Other</td>
</tr>
<tr>
<td>Baseline HIV-RNA (log copies/ml)</td>
<td>4.6 (4.2–5.0)</td>
<td>4.6 (4.1–5.0)</td>
<td>4.6 (4.2–5.0)</td>
<td>0.87</td>
</tr>
<tr>
<td>Baseline CD4 cell count (cells/µl)</td>
<td>286 (162–505)</td>
<td>136 (76–204)</td>
<td>267 (147–493)</td>
<td>0.08</td>
</tr>
<tr>
<td>Zidovudine pharmacokinetic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zidovudine ( C_{\text{ss}} ) (ng/ml)</td>
<td>190 (164–216)</td>
<td>210 (185–230)</td>
<td>194 (175–222)</td>
<td>0.41</td>
</tr>
<tr>
<td>Zidovudine TP (fmol/10^6 cells)</td>
<td>46 (30–67)</td>
<td>106 (73–155)</td>
<td>51 (32–78) &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Zidovudine sample times (h post-dose)</td>
<td>2.8 (2.0–5.4)</td>
<td>3.4 (2.0–5.8)</td>
<td>3.3 (2.5–5.8)</td>
<td>0.68</td>
</tr>
<tr>
<td>Zidovudine TP half-life (h)</td>
<td>6 (5–7)</td>
<td>7 (3–10)</td>
<td>7 (5–9) Not done</td>
<td></td>
</tr>
<tr>
<td>Lamivudine pharmacokinetic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine ( C_{\text{ss}} ) (ng/ml)</td>
<td>473 (407–543)</td>
<td>589 (491–689)</td>
<td>480 (425–548)</td>
<td>0.12</td>
</tr>
<tr>
<td>Lamivudine TP (fmol/10^6 cells)</td>
<td>8096 (6481–9801)</td>
<td>12 619 (10 128–15 852)</td>
<td>8473 (6651–10761) &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Lamivudine sample times (h post-dose)</td>
<td>4.3 (2.7–8)</td>
<td>3.0 (2.0–5.2)</td>
<td>4.1 (2–7.5) 0.55</td>
<td></td>
</tr>
<tr>
<td>Lamivudine TP half-life (h)</td>
<td>29 (11–47)</td>
<td>32 (3–60)</td>
<td>22 (11–33) Not done</td>
<td></td>
</tr>
<tr>
<td>Indinavir pharmacokinetic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indinavir ( C_{\text{min}} ) (ng/ml)</td>
<td>130 (90–160)</td>
<td>120 (90–155)</td>
<td>130 (90–160)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

\( C_{\text{min}} \), Trough concentration; \( C_{\text{ss}} \), steady-state average plasma concentration; TP, triphosphate.

Data are reported as median (interquartile range), except triphosphate half-lives, which are the model-derived estimates (95% confidence interval).

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The apparent half-life for zidovudine triphosphate was 7 h, whereas the zidovudine plasma half-life was only 0.8 h (not shown). Fig. 1b displays all lamivudine triphosphate concentration time data. The apparent half-life was 22 h, whereas the plasma lamivudine half-life was 2.5 h (not shown). There was a strong linear relationship between the paired concentrations of lamivudine triphosphate and zidovudine triphosphate that was independent of times post-dose, the plasma concentrations of zidovudine and lamivudine, and baseline CD4 cell counts ($y = 7186 + 32x$, Spearman’s $r = 0.55$, $P < 0.0001$).

A direct relationship was also found between lamivudine triphosphate concentration data and paired lamivudine plasma data ($\mu g/ml$) that was independent of the time post-dose ($y = 7734 + 1933x$; $P < 0.0001$). No linear relationships were found between zidovudine triphosphate concentrations and zidovudine plasma concentrations, or the zidovudine dose. Neither triphosphate was found to change according to time on therapy, weight, age, indinavir concentrations, or medication counts.

### Intracellular triphosphate concentrations and sex

Demographic and pharmacological data, split by sex, are summarized in Table 1. There were no differences between women and men with regard to baseline disease characteristics or plasma indinavir, zidovudine, and lamivudine pharmacokinetics. In contrast, the zidovudine triphosphate and lamivudine triphosphate concentrations were significantly higher in women than men, as shown in Fig. 2. We calculated the median of all zidovudine triphosphates obtained in each individual, and found these median values were significantly higher in women compared with men (Mann–Whitney; $P = 0.003$). As depicted in Fig. 2a, when all zidovudine triphosphate concentrations were grouped by sex, the median value was 2.3-fold higher in the women than in the men, 106 fmol/10$^6$ (73–155) compared with 46 fmol/10$^6$ (30–67) cells, respectively ($P < 0.0001$). Similarly, the individual median lamivudine triphosphate concentrations were higher in female subjects than in men (Mann–Whitney; $P = 0.002$), and as shown in Fig. 2b, when all lamivudine triphosphate concentrations were grouped by sex the median value was 1.6-fold higher in women than in men, 12 619 versus 8096 fmol/10$^6$ cells, respectively ($P < 0.0001$).

As women had marginally lower baseline CD4 cell counts than men (Table 1), we compared individual median triphosphate concentrations in women with those in men, who had less than 250 CD4 cells/µl ($n = 11$). Compared with these men, women had twofold higher zidovudine triphosphate and 1.4-fold higher lamivudine triphosphate concentrations (both $P < 0.01$). These sex differences were also present in just the 2 h post-dose triphosphate concentrations, which were obtained after an observed dose administered during the intensive pharmacokinetic studies ($P < 0.05$). No difference in adherence was found between men and women for any drug or overall ($P > 0.25$).

Three out of four women were of African descent. We compared triphosphate concentrations in the 31 female samples and 38 male samples from subjects of African descent. Zidovudine triphosphate concentrations were significantly higher in these female samples compared with the male samples, 109 (71–156) versus 52 (30–73) fmol/10$^6$ cells, and also for lamivudine triphosphate, 11 903 (9924–14 506) versus 8309 (6643–10 036) fmol/10$^6$ cells, respectively (both $P < 0.0001$). No differences were found according to race/ethnicity (distribution in Table 1, Kruskal–Wallis; $P > 0.45$).
Relationships among triphosphate concentrations, sex, and anti-HIV response

Of all disease and demographic covariates, only sex and baseline HIV RNA were related, each independently, with time to less than 50 copies/ml of plasma HIV RNA in a stepwise Cox regression model ($P = 0.04$ and 0.03, respectively). Women reached less than 50 copies/ml faster than men (median 56 days versus 112 days, $P = 0.02$), as shown in Fig. 3. Linear regressions of the time to less than 50 copies/ml of plasma HIV RNA versus each individual’s median triphosphate levels demonstrated significant inverse relationships for both zidovudine and lamivudine triphosphates ($P < 0.04$). By inspecting these graphical relationships, response-discriminating triphosphate values were identified at the first quartile or 30 fmol/10⁶ cells for zidovudine triphosphate, and 7017 fmol/10⁶ cells for lamivudine triphosphate. These threshold values were considered as covariates in additional analyses. For the time to less than 50 copies/ml, a stepwise Cox regression was constructed that included the following univariate predictors: treatment arm ($P = 0.09$); sex ($P = 0.03$); baseline log HIV RNA ($P = 0.03$); and being above or below the zidovudine triphosphate ($P = 0.01$) and lamivudine triphosphate ($P = 0.02$) thresholds. Indinavir concentrations did not enter in the model ($P = 0.15$). Only zidovudine triphosphate concentrations above 30 fmol/10⁶ cells were associated with the time to less than 50 copies/ml (relative risk 1.3, $P = 0.02$) in this model. In a Kaplan–Meier analysis, those subjects with zidovudine triphosphate concentrations above 30 fmol/10⁶ cells had a significantly shorter time to reach less than 50 copies/ml compared with those who had lower concentrations (median of 84 versus 144 days, respectively; $P = 0.008$), as displayed in Fig. 4a.

The virological status ($< 50$ copies/ml) at 6 months and one year was investigated in subjects according to the triphosphate thresholds of 30 and 7017 fmol/10⁶ cells for zidovudine and lamivudine triphosphate, respectively. Of individuals with zidovudine triphosphate concentrations above 30 fmol/10⁶ cells, 91.7% had undetectable HIV-RNA levels at week 24, compared with 44.4% of those with lower zidovudine triphosphate concentrations ($P = 0.009$, Fisher’s exact test); at week 52 these values were 83.3 and 44.4%, respectively ($P = 0.07$). A total of 95.7% of individuals with

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time to less than 50 copies/ml ($P = 0.004$); indinavir target ($P = 0.03$); and concentrations above the triphosphate thresholds for zidovudine ($P = 0.02$) and lamivudine ($P = 0.002$). In this analysis, only the time to less than 50 copies/ml (relative risk 0.024, $P = 0.009$) and lamivudine triphosphate concentrations below 7017 fmol/10^6 cells (relative risk 2.2, $P = 0.009$) were associated with the time to viral rebound. Fig. 4b demonstrates a significantly shorter time to viral rebound greater than 50 copies/ml for subjects with lamivudine triphosphate concentrations below 7017 fmol/10^6 cells ($P < 0.0001$).

**Antiviral dynamics in the reduced cohort with medication count data**

In secondary analyses, antiviral dynamics were investigated in just the patients with medication count data. A stepwise Cox regression was generated for the time to viral rebound in these patients. The following univariate predictors were entered into the model: indinavir trough concentration ($P = 0.1$); lamivudine triphosphates below 7017 fmol/10^6 cells ($P = 0.009$); and combined drug medication counts ($P = 0.05$). In this model, only lamivudine triphosphate concentrations below 7017 fmol/10^6 cells were associated with the loss of virological control (relative risk 2.9, $P = 0.01$).

**Disease severity and triphosphate concentrations**

Both triphosphate concentrations were inversely associated with CD4 cell counts. At week 2, the 2 h zidovudine triphosphate and lamivudine triphosphate concentrations were inversely associated with the baseline CD4 cell level (Spearman’s $\rho = -0.57$, $P = 0.001$, and $-0.59$, $P = 0.0009$, respectively; Fig. 5), and also directly with the baseline log HIV-RNA level (Spearman’s $\rho = 0.35$, $P = 0.05$, and 0.38, $P = 0.03$, respectively). Multivariate regression showed that both the baseline CD4 cell count and log HIV-RNA level were independently associated with zidovudine triphosphate, whereas only the baseline CD4 cell count remained associated with lamivudine triphosphate concentrations ($P < 0.05$). This relationship was lost, however, when triphosphate concentrations at 6 months and one year were compared with the CD4 cell counts at these same later timepoints.

**Triphosphate concentrations and acute toxicities**

Therapy was well tolerated in this study [16]. Fourteen out of 33 patients had one or more grade I or greater hemoglobin, an absolute neutrophil count, or aspartate aminotransferase/alanine aminotransferase laboratory events. No differences were found in zidovudine triphosphate and lamivudine triphosphate concentrations in these individuals compared with those who did not experience a toxicity event ($P > 0.2$ for all comparisons).
Discussion

In this quantitative pharmacological analysis of zidovudine triphosphate and lamivudine triphosphate concentrations in HIV-infected individuals, we found that the intracellular half-life of zidovudine triphosphate was approximately 7 h and that of lamivudine triphosphate was 22 h. Women had 2.3-fold higher zidovudine triphosphate concentrations (\( P = 0.002 \)) and 1.6-fold higher lamivudine triphosphate concentrations (\( P = 0.003 \)) compared with men; plasma concentrations, however, were no different between the sexes. Patients with severe HIV disease, defined by low CD4 cell counts and high plasma HIV-RNA levels also had higher zidovudine triphosphate and lamivudine triphosphate concentrations, an effect most evident early in therapy and in individuals with fewer than 100 CD4 cells/\( \mu l \) at baseline (Fig. 5). We found strong relationships between zidovudine triphosphate and lamivudine triphosphate concentrations and the time to reach less than 50 copies/ml, the probability of having less than 50 copies/ml at 6 months and one year, and the time to rebound above 50 copies/ml once the viral nadir was achieved. In this study, female subjects reached undetectable levels of HIV RNA in half the time of the men. In other studies, women had a slower rate of disease progression on zidovudine monotherapy [9], and had higher incidences of NRTI-associated toxicities, such as fat maldistribution and lactic acidosis [9–13]. These sets of findings may be explained by higher NRTI triphosphate concentrations in women, and these new data also generate mechanistic hypotheses for sex differences in response to nucleoside analogs from other therapeutic classes, such as that shown for fluorouracil [14].

Many literature sources list a 3 h half-life for zidovudine triphosphate [1], a value obtained from in-vitro work [18]. In our in-vivo study, the zidovudine triphosphate half-life was estimated to be 7 h. This is consistent with the 10 h value reported by Rodriguez et al. [19]. The discrepancy between in-vitro and in-vivo data underscores major physiological differences between these systems, and illustrates that in-vitro-derived data should not be naively extended to patients. Our inability to detect relationships between the dose of zidovudine, plasma, and triphosphate concentrations, emphasizes the complexity of the in-vivo system, and we cannot conclude that zidovudine dose–plasma triphosphate relationships are absent. Indeed, the average zidovudine triphosphate concentration increased 125%, with a 130% increase in the average zidovudine dose. The lamivudine triphosphate half-life of 22 h is consistent with what others have reported in HIV-infected patients (16–32 h) [19,20]. The direct relationship between zidovudine triphosphate and lamivudine triphosphate confirms our previous finding [17], and may indicate that patients who experience sub-potent or toxic responses to one NRTI are predisposed to sub-potent or toxic responses to other NRTI.

The significant sex differences found with regard to triphosphate concentrations and the times to reach less than 50 copies/ml are new findings. There was no obvious biological explanation for a sex difference in triphosphate concentrations. No confounding effects from weight or baseline CD4 cell counts were found; the time post-dosing, adherence, and plasma zidovudine and lamivudine concentrations were no different between the sexes, and women were not taking birth control pills or any other medications except sulfas.
methoxazole/trimethoprim. There are limited data from other investigators that described possible NRTI phosphorylation differences between the sexes: Stretcher et al. [21] reported that women had 145% higher total intracellular zidovudine phosphate concentrations (sum of the mono, di, and triphosphates) compared with men ($P < 0.004$), although zidovudine triphosphate was not separately analysed. A recent letter described two to eightfold higher carbovir triphosphate (the active form of abacavir) levels in a single woman compared with four men [22]. In our study, the small number of women was a limitation; conversely, the multiple triphosphate measurements (average 10 per person) in each woman and man over several months strengthened these observations. Whereas these findings are preliminary, they are consistent with and provide explanations for clinical findings, and thereby provide sufficient motivation to conduct confirmatory investigations of intracellular NRTI triphosphate pharmacokinetics and pharmacodynamics in men and women, and to undertake similar investigations with nucleoside analogs among other therapeutic classes.

The associations between NRTI triphosphates and viral responses in this study are more comprehensive than what has been reported previously [17,23]. For example, the time to reach less than 50 copies/ml was strongly associated with zidovudine triphosphate concentrations above 30 fmol/10^6 cells. This virological characteristic has been shown to predict the duration of virological response [24]. We found that a sustained virological response was significantly associated with both a shorter time to reach less than 50 copies/ml and lamivudine triphosphate concentrations above 701 fmol/10^6 cells. These relationships between triphosphates and antiviral responses remained significant after adjustment for other univariate predictors, including baseline disease severity and indinavir concentrations. Prospective controlled studies will be needed to confirm the clinical significance of the triphosphate concentration thresholds elucidated in this work.

In secondary analyses with a reduced cohort, we investigated the effects of medication counts on antiviral responses. Importantly, 15% of the cohort were missing medication count data, and furthermore, medication counts have shown limited value as an adherence measure [25]. With these limitations acknowledged, we found that medication counts were a univariate predictor of virological failure, although medication counts were not significant in multivariate analyses with lamivudine triphosphate concentrations above or below 7017 fmol/10^6 cells included. Given that medication counts in our cohort were consistent and high (interquartile range 0.94–0.99), this univariate finding is concordant with other studies that identified a discriminatory adherence rate of greater than 95% for virological responses [26].

The pharmacodynamic findings in this work open several avenues for future research. For example, although we did not generate viral genotypes in individuals who failed therapy, low lamivudine triphosphate concentrations may predispose patients to the development of the main lamivudine resistance mutation, M184V [27]. Also, lamivudine triphosphate concentrations may be a surrogate for adherence, as the 22 h half-life would provide information from several previous doses. These possibilities merit future research efforts.

Other investigators have described higher NRTI phosphate concentrations in patients with low CD4 cell counts, or early versus later in therapy. Stretcher et al. [21] reported that maximal zidovudine phosphorylation, as measured by total phosphates, occurred at day one of therapy; total phosphates at week 24 were only 30% of this initial value. Hoggard et al. [28] and Barry et al. [29] described higher zidovudine phosphate levels (zidovudine triphosphates were not separately reported) in patients with fewer than 100 CD4 cells compared with others in their cohorts, but found no phosphorylation changes over time. An inverse relationship between low CD4 cell counts and triphosphate concentrations may seem counterintuitive because patients with low CD4 cell counts historically have poorer virological responses [1]. In this study, the higher triphosphate concentrations among patients with fewer than 100 CD4 cells decreased after the first weeks of therapy, and at later timepoints in therapy no relationship was found between triphosphates and CD4 cell counts. Biologically, higher NRTI phosphates may be formed in individuals with low CD4 cell counts, and early in therapy versus later, because of higher cellular activation associated with those characteristics [30,31]. In-vitro studies consistently demonstrated that cellular activation promotes higher NRTI phosphorylation, which has been shown for zidovudine, stavudine, and lamivudine [32]. High NRTI phosphate concentrations found in individuals with low CD4 cell counts provide a plausible explanation for the exaggerated NRTI-associated toxicities seen in patients with low CD4 cell counts, including lactic acidemia [12,33], fat maldistribution [34,35], peripheral neuropathy [8], and zidovudine-induced cytopenia [36].

We found no associations between the incidence of acute laboratory abnormalities and triphosphate concentrations. Ours was not a long-term study, however, nor did we collect data associated with long-term toxicity (for example dual-energy X-ray absorptiometry scans or serum lactates), which are clear limitations. Nevertheless, the new findings in this study and the epidemiological data that women and individuals with fewer than 100 CD4 cells experience exaggerated long-term NRTI-associated toxicity are compelling, and we suggest that these toxicities are more likely to develop.
in these patient groups because of a tendency to generate high intracellular NRTI triphosphate concentrations.

The treatment of HIV infection remains less than optimal, with sub-potent virological responses, pan-resistant viruses, and increasing reports of alarming therapy-related toxicities [2]. Most concentration-effect studies in HIV medicine have only considered protease inhibitors or non-nucleoside analog reverse transcriptase inhibitors. In this study, we showed that anti-HIV responses to a regimen of zidovudine, lamivudine, plus indinavir were significantly and independently associated with concentration thresholds for zidovudine triphosphate and lamivudine triphosphate, whereas indinavir concentrations and medication counts were associated with these anti-HIV responses in univariate analyses only. Therefore, clinical pharmacology studies of HIV therapy may need to be viewed in a different light, in which the antiviral contribution of the concomitant nucleoside analogs is more strongly considered. The new descriptions of sex and baseline CD4 cell-associated differences in these triphosphate concentrations provide a pharmacological basis to hypothesize a link between long-term NRTI-associated toxicities and high NRTI triphosphate levels. If established, this hypothesis also allows for the management/prevention of these toxicities if NRTI triphosphate concentrations are kept below a toxicity threshold. It is conceivable that women may need lower initial doses or less frequent dosing of NRTI than men to ensure equivalent long-term safety. Finally, patients with very low CD4 cell counts or high viral loads may be pharmacologically susceptible to nucleoside analog toxicity. This would provide one argument to start therapy when the disease is mild, which is in contrast to the current philosophy of withholding therapy as long as possible to avoid drug toxicities [1,2]. In summary, NRTI will remain core drugs for the treatment of HIV infection into the foreseeable future. To advance the state of HIV therapeutics, a high priority must be placed on efforts to understand the mechanisms of NRTI-associated virological and toxicological responses.

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