A Multiple Drug Interaction Study of Stavudine with Agents for Opportunistic Infections in Human Immunodeficiency Virus-Infected Patients

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The effects of multiple opportunistic infection medications on stavudine pharmacokinetics were evaluated. Ten patients with CD4 counts of less than 200 cells/mm³ received stavudine (40 mg twice daily) in combination with one to three other drugs used to treat opportunistic infections. Serial blood samples for stavudine concentrations were collected after 1 week of therapy on each regimen and assayed for stavudine by using a validated high-pressure liquid chromatography method. Although the maximum concentration of drug in serum was significantly decreased when the drug was given in combination with three opportunistic infection medications, the area under the concentration-time curve did not significantly differ across various treatment regimens. Stavudine exposure was not significantly altered by multiple concomitant medications. Side effects were minor throughout the 3-month study period. The tolerability of stavudine, combined with its lack of drug interactions, makes it an attractive agent for use as part of a combination regimen.

Combination therapy has become the standard of care for patients infected with human immunodeficiency virus (HIV). In addition to antiretroviral regimens with at least three drugs, patients with late-stage disease often take prophylactic therapy for opportunistic infections (OIs). Thus, the complex medication regimens used by HIV-infected patients result in the potential for numerous drug interactions.

Various drug interaction studies have been performed in HIV-infected patients (10). A major limitation of these studies is that they often examine combinations of only two agents. In addition, many drug interaction studies are performed in HIV-negative volunteers to reduce the variability due to concomitant medications and disease states. Since HIV-infected patients commonly receive multiple therapies and may have altered organ functions, these studies cannot necessarily be extrapolated to actual clinical situations.

Stavudine (d4T) has become a widely used nucleoside analog as part of a combination regimen, due to its twice daily dosing, tolerability, cerebrospinal fluid penetration, and effectiveness in clinical trials (4, 7, 15). Stavudine elimination involves both renal clearance and hepatic metabolism (14). Approximately 40% of a dose is excreted in the urine, unchanged by both glomerular filtration and renal tubular secretion. The majority of elimination is attributed to a nonrenal component, although the exact metabolic pathway is unknown. Studies in nonhuman primates suggest that stavudine is converted to thymine with subsequent conversion to β-aminoisobutyric acid (1). There are limited data on drug interactions between stavudine and anti-infectives used to prevent and treat opportunistic infections (14). Furthermore, most drug interaction studies do not address how multiple concomitant medications affect the pharmacokinetics of antiretrovirals. The objective of the study was to characterize the pharmacokinetics of stavudine in the presence and absence of multiple medications commonly used for opportunistic infections. The study was designed to more closely simulate medication usage in clinical practice and assess potential interactions.

MATERIALS AND METHODS

The study was initiated in 1995, shortly after the release of stavudine and at a time when monotherapy was still considered an acceptable option for HIV therapy. The design was that of a sequential, eight-part, multiple-dose, nonblinded, randomized study to evaluate potential interactions between stavudine and the common OI medications fluconazole, rifabutin, and clarithromycin. Adult HIV-infected patients with a CD4 count of less than 200 cells/mm³ were eligible for the study. Patients were required to receive Pneumocystis carinii prophylaxis with either trimethoprim-sulfamethoxazole (TMP-SMX) or aerosolized pentamidine in commonly recommended doses. Exclusion criteria included a life expectancy of less than 3 months, a history of significant allergy to any study medications, a history of peripheral neuropathy or uveitis, laboratory values outside of protocol guidelines, persistent diarrhea or malabsorption, a positive urine pregnancy test, the use of experimental drugs within 14 days of starting the study, an abnormal chest X-ray, and the use of drugs or alcohol which could impair safety and compliance.

A sample study schedule for a patient is given in Table 1. Each patient received stavudine, given orally at a dose of 40 mg twice daily (for patients weighing less than 60 kg, 30 mg twice daily). Patients continued to receive stavudine for the duration of the study. After receiving this regimen for 1 week, serial blood samples were collected at time zero (predose) and at 0.5, 1, 2, 4, and 8 h after dosing. Patients were then randomized to receive therapy with either clarithromycin (500 mg twice daily), fluconazole (200 mg once daily), or rifabutin (300 mg once daily). They continued on the two-drug regimen for 1 week, at which time serial blood samples were again drawn. The OI drug was then discontinued, and patients received only stavudine for a 1-week washout period. The schedule continued with washout periods, so that each patient received the other two OI medications until they had received each drug as monotherapy with stavudine, and serial blood samples were collected at the end of each treatment week. Following the monotherapy study, patients were then randomized to receive two drugs in combination with stavudine for 1 week followed by serial sampling. A week-long period then began, during which one OI drug was discontinued and another was initiated. After 1 week, serial blood samples were again obtained. The process continued so that each patient received all possible combinations of two drugs with stavudine. Patients were then started on the third OI medication, so that they received stavudine with all three OI drugs. After 1 week of therapy,
Serial blood samples were collected. At the conclusion of the trial, each patient had received stavudine with all combinations of one, two, or three concomitant medications. Doses for pharmacokinetic sampling were administered in the presence of the investigators. Patients were allowed to take medications with food.

Immunologic (CD4 and CD8 counts) and viral (branched DNA) parameters were determined pre- and poststudy at SAIC Laboratories, Frederick, Md. Laboratory tests, including a hematology profile, serum chemistry, and urinalysis, were determined pre- and poststudy at SAIC Laboratories, Frederick, Md. Laboratory tests, including a hematology profile, serum chemistry, and urinalysis, were performed at each study visit at the NIH Clinical Center.

Analytical. Stavudine concentrations in plasma were determined by using a validated, reversed-phase high-pressure liquid chromatography method. Seven standards (0.1 to 2 μM concentrations) for stavudine were analyzed in duplicate for each assay. All standards were prepared in human plasma. Following the addition of an internal standard to 200 μl of the sample, solid-phase extraction was accomplished with 3M Empore C18 cartridges. A volume (50 μl) of the clean extract was injected onto a Waters Spherisorb C8 HPLC column at a flow rate of 1 ml/min. The mobile phase consisted of 5% acetonitrile and 95% 50 mM sodium phosphate buffer, pH 6.7. The retention time for stavudine was approximately 6 min; detection was at an optical density of 266 nm. The assay validation was performed with analysis of variance for triplicate quality controls (0.2, 1, and 5 μM concentrations) assayed on five separate days. Within-day and total coefficients of variation were all under 10%. The accuracy for stavudine ranged from 97.6 to 99.4% (3).

Adverse effects. Toxicity to medications was graded from I to IV according to standard AIDS Clinical Trials Group criteria (2).

Pharmacokinetics. The pharmacokinetic parameters of stavudine were determined by noncompartmental methods. The area under the concentration-time curve (AUC) from time zero to 8 h after dosing was determined by a standard trapezoidal estimation. The maximum concentration of drug in serum (Cmax) and time to Cmax (Tmax) were determined directly from concentration-time profiles.

Statistical methods. A mixed-model analysis of variance was used to analyze the data with the PROCMIXED software (SAS Institute, Cary, N.C.). The model specified each drug combination as a fixed effect and the patients as random effects. Means of the AUC, Cmax, and Tmax for each drug combination were estimated from the statistical model. In addition, contrasts were constructed to test whether the single OI drugs differed from one another and whether the pairs of agents differed from one another. Bonferroni’s corrections were used when appropriate to adjust for multiple statistical testing. The data were also examined to evaluate if the pharmacokinetic parameters exhibited a monotonic trend over the study period.

Adherence. Adherence to the drug regimen was facilitated by detailed patient calendars and dosing charts, whereby patients could document and check off each dose taken. Adherence was assessed by patient interview and review of dosing calendars.

RESULTS

Ten patients (nine males and one female) enrolled in and completed the study. At baseline, the mean CD4 count was 62 cells/mm³ (range, 9 to 143 cells/mm³) and the mean viral burden was 233,727 RNA copies/ml (range, <10,000 to 608,000 copies/ml). There were no significant changes in either parameter over the course of the study.

Medications were well tolerated throughout the study. One patient developed a grade III neutropenia due to TMP-SMX. One patient also developed peripheral neuropathy, likely related to stavudine. This patient had been receiving stavudine prior to starting the study. Minor side effects included taste alterations (four patients), gastrointestinal complaints (three patients), and rash (one patient) during treatment with clarithromycin and stavudine. One patient receiving rifabutin and stavudine developed a rash, and one patient receiving fluconazole, rifabutin, and stavudine had a grade II increase in liver function tests.

Figure 1 depicts the mean stavudine concentration-time profiles during each phase of the study. In general, stavudine concentrations peaked at 1 h and declined to nearly undetectable levels by 8 h after dosing. As shown, plots of stavudine alone and in combination with anti-infective drugs were similar in terms of absorption and elimination, although there was variability in Cmax. AUCs for each treatment regimen are shown in Table 2. The AUC did not significantly differ across treatment regimens. The greatest difference in AUC between stavudine alone and any other regimen was an 18% decrease when stavudine was given with rifabutin and clarithromycin.

The mean stavudine Cmax ranged from 422 to 654 ng/ml across all treatments. Cmax was significantly decreased (35%) when stavudine was given in combination with all three OI drugs compared to stavudine alone (P = 0.005). Tmax did not significantly differ between groups.

DISCUSSION

Drug interactions have become an important part of the management of HIV-infected patients. Many antiretroviral drugs may have their concentrations altered by other medications through a variety of mechanisms including changes in hepatic metabolism, gastric pH effects, chelation, or inhibition of renal clearance (10). Although there are increasing data available on drug interactions, many of the available studies examine only two-drug combinations, which do not reflect the types of regimens most commonly used by patients.

Stavudine has become a widely prescribed antiretroviral agent due to its tolerability and twice daily dosing schedule. However, there is limited information concerning its potential to cause drug interactions. The only available trial examining stavudine drug interactions was a pharmacokinetic study with didanosine in 10 patients (19). The results of this study showed that neither drug had alterations in absorption or clearance in the presence of the other. However, these data probably do not represent the average HIV-infected patient in a typical clinical situation.

Stavudine is not metabolized via cytochrome P-450, and thus, significant interactions were not expected via alterations in drug metabolism. However, this study did provide an important insight into drug absorption when patients receive a large number of drugs and pills per day. This study suggests that HIV-infected patients adequately absorb stavudine when it is administered with three other concomitant medications (or four drugs in patients receiving TMP-SMX), even in patients with advanced disease who have low CD4 counts.

Other studies have shown that drug absorption in HIV-infected patients may be reduced by the concomitant administration of multiple medications, especially in patients with tuberculosis. Sahai and colleagues recently examined the absorption of antitubercular medications in patients with AIDS (16). Compared to results for a group of healthy volunteers,
the AUCs for pyrazinamide and rifampin were reduced in HIV-infected patients by 24 and 32%, respectively. Patients with late-stage HIV infection also demonstrated a significant trend in reduced drug exposure for pyrazinamide. Peloquin et al. described a similar scenario in HIV-infected patients with tuberculosis (9). Blood samples collected 2 h after dosing showed decreased absorption for at least one drug in over 80% of patients, compared to established normal ranges.

**TABLE 2.** Comparison of stavudine pharmacokinetic parameters across treatment groups

<table>
<thead>
<tr>
<th>Parameter&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S</th>
<th>SC</th>
<th>SF</th>
<th>SR</th>
<th>SRC</th>
<th>SFR</th>
<th>SCF</th>
<th>SCFR</th>
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<tbody>
<tr>
<td><strong>AUC</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.570</td>
<td>1.446</td>
<td>1.545</td>
<td>1.352</td>
<td>1.306</td>
<td>1.326</td>
<td>1.407</td>
<td>1.392</td>
</tr>
<tr>
<td>SD</td>
<td>442</td>
<td>415</td>
<td>462</td>
<td>366</td>
<td>272</td>
<td>258</td>
<td>342</td>
<td>384</td>
</tr>
<tr>
<td>Range</td>
<td>1,002–2,341</td>
<td>801–2,239</td>
<td>833–2,462</td>
<td>896–2,208</td>
<td>817–1,734</td>
<td>964–1,677</td>
<td>757–2,049</td>
<td>946–2,176</td>
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<tr>
<td><strong>C&lt;sub&gt;max&lt;/sub&gt;</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Mean</td>
<td>849</td>
<td>718</td>
<td>757</td>
<td>588</td>
<td>636</td>
<td>650</td>
<td>612</td>
<td>571</td>
</tr>
<tr>
<td>SD</td>
<td>270</td>
<td>257</td>
<td>280</td>
<td>187</td>
<td>142</td>
<td>284</td>
<td>196</td>
<td>194</td>
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<tr>
<td>Range</td>
<td>433–1,260</td>
<td>435–1,276</td>
<td>426–1,110</td>
<td>233–798</td>
<td>444–836</td>
<td>321–1,256</td>
<td>350–989</td>
<td>341–935</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;min&lt;/sub&gt;</strong></td>
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<tr>
<td>Mean</td>
<td>5.2</td>
<td>3.1</td>
<td>9.1</td>
<td>10.7</td>
<td>6.5</td>
<td>6.0</td>
<td>4.5</td>
<td>9.5</td>
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<tr>
<td>SD</td>
<td>8.4</td>
<td>6.3</td>
<td>10.2</td>
<td>14.8</td>
<td>9.5</td>
<td>9.9</td>
<td>8.8</td>
<td>11.1</td>
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<tr>
<td>Range</td>
<td>ND–19.3</td>
<td>ND–15.2</td>
<td>ND–23.8</td>
<td>ND–42.1</td>
<td>ND–27.6</td>
<td>ND–23.8</td>
<td>ND–23.1</td>
<td>ND–28.2</td>
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<tr>
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<tr>
<td>Mean</td>
<td>0.8</td>
<td>1.1</td>
<td>0.9</td>
<td>1.1</td>
<td>1.1</td>
<td>1.2</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>SD</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Range</td>
<td>0.5–2</td>
<td>0.5–2</td>
<td>0.5–2</td>
<td>0.5–2</td>
<td>0.5–2</td>
<td>1–2</td>
<td>0.5–2</td>
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</table>

<sup>a</sup> AUCs are reported in nanograms times hour per milliliter. C<sub>max</sub> and minimum concentration (C<sub>min</sub>) are reported in nanograms per milliliter. T<sub>max</sub> is reported in hours.

<sup>b</sup> Various combinations of stavudine (S), clarithromycin (C), fluconazole (F), and rifabutin (R). ND, not detectable.
Other drug combinations in HIV-infected patients have demonstrated reduced absorption when drugs are given concomitantly. Polis and colleagues reported that zidovudine $C_{\text{max}}$ and AUC were significantly decreased by concomitant administration of clarithromycin (11). The specific mechanism is unknown, although it did not appear to involve chelation. The clinical significance of these interactions is unclear, since intracellular concentrations are likely to be more clinically relevant than samples from peripheral blood.

A variety of conditions may explain the poor absorption in HIV-infected patients, including Candida colonization, diarrheal infections, and gastric achlorhydria and/or atrophy (6, 22). Despite these potential problems, stavudine was well absorbed in this study of HIV-infected patients with mean CD4 counts of 60 cells/mm$^3$ who received two-, three-, and four-drug combinations.

Although pharmacokinetic interactions appear not to be clinically significant, clinicians need to be aware of potential pharmacodynamic interactions with stavudine. Preliminary results from AIDS Clinical Trials Group 290 reported that CD4 counts were decreased in patients receiving the combination of stavudine and zidovudine, compared to the other nucleoside combinations (8). The decrease is thought to involve the ability of zidovudine to significantly decrease stavudine intracellular phosphorylation (5). Recently, clinical data also suggest that patients receiving long-term zidovudine therapy may have low levels of intracellular phosphorylation of stavudine (20). There is also the possibility for increased toxicity when stavudine is administered with drugs that share its side-effect profile. However, clinical trials have not shown that the combination of stavudine and didanosine has led to an increased incidence of peripheral neuropathy (12, 13).

Stavudine has been shown to be well tolerated in clinical trials (17) and during this 8-week study. Although one patient did develop peripheral neuropathy thought to be due to stavudine, that patient had been receiving stavudine prior to entry into the study. There were no reported subjective complaints associated with stavudine therapy. Subjective adverse effects such as nausea, malaise, and fatigue are commonly seen in patients receiving zidovudine. A clinical trial comparing stavudine and zidovudine monotherapy demonstrated a higher incidence of nausea and vomiting in patients receiving zidovudine (21). As these side effects may be dose limiting and may affect a patient’s quality of life, the tolerability of stavudine combined with its lack of drug interactions makes it a practical alternative to zidovudine as part of a combination regimen.

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