CONCISE COMMUNICATION

In Vivo Antagonism with Zidovudine plus Stavudine Combination Therapy

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Human immunodeficiency virus (HIV)-infected subjects receiving zidovudine were randomized either to add stavudine (d4T) or didanosine (ddI) to their current regimen or to switch to ddI or d4T monotherapy. After 16 weeks of therapy, the mean reduction in HIV RNA from baseline was 0.14 log10 copies/mL in patients receiving d4T or zidovudine plus d4T. In subjects receiving ddI or ddI plus zidovudine, reductions were 0.39 and 0.56 log10, respectively. CD4 cell counts remained stable or showed modest increases in all arms except the zidovudine plus d4T arm. Patients receiving zidovudine plus d4T showed progressive declines in CD4 cell counts with a median of 22 cells/mm3 below baseline by 16 weeks. Examination of intracellular levels of d4T-triphosphate in 6 subjects was consistent with previous in vitro studies demonstrating pharmacologic antagonism between zidovudine and d4T. Analysis of these data suggests that zidovudine and d4T should not be prescribed in combination and that ddI provides greater antiviral activity than d4T in zidovudine-treated patients.

The therapeutic utility of nucleoside reverse transcriptase inhibitor drug combinations for the treatment of human immunodeficiency virus (HIV) disease has often not been predicted by in vitro studies or clinical considerations. The combination of stavudine (d4T) and didanosine (ddI), initially thought to be contraindicated because of the overlapping toxicity profiles of the 2 drugs, was found not only to be safe but to provide more potent antiviral activity than either drug administered alone [1, 2].

The combination of zidovudine and d4T poses a theoretical risk because both drugs require intracellular phosphorylation by the cellular enzyme thymidine kinase. In vitro, zidovudine has >100-fold greater affinity for this enzyme than d4T, which potentially could result in antagonism when the drugs are combined. In vitro studies of the combination of zidovudine and d4T, however, produced conflicting results, providing a rationale for the in vivo evaluation of this drug combination [3–7].

The study presented here was designed to compare the virologic outcome of adding d4T or ddI to zidovudine or switching to ddI or d4T monotherapy in subjects already receiving zidovudine. Although the regimens tested in this study in 1995–1996 are no longer the standard of practice [8], nucleoside analogues remain a backbone component of combination antiretroviral therapy, and results of this study are useful in current designs of combination regimens for initial and salvage therapy.

Methods

Study patients. All study participants were infected with HIV type 1, were $\geqslant 12$ years of age, had a CD4 cell count of 300–600 cells/mm3, and had received $\geqslant 12$ weeks of zidovudine therapy. Additional entry criteria included serum creatinine $<1.5$ times the upper limit of normal, liver function tests $<5$ times the upper limit of normal, hemoglobin $>9$ g/dL for men and 8.5 g/dL for women, absolute neutrophil count $>1000$ cells/mm$^3$, and amylase $<1.5$ times the upper limit of normal. Subjects with a history of AIDS or with $>2$ weeks’ prior therapy with ddI, d4T, or lamivudine (3TC) were excluded.

Study design. This multicenter, partially blinded, randomized study compared the activity and safety of 4 antiretroviral regimens. Randomization was stratified by length of previous zalcitabine (ddC) use ($<14$ days’ experience vs. $>14$ days’ experience). When the protocol commenced in July 1995, the primary end point of the study was the change in CD4 cell count from baseline. The targeted accrual was 400 subjects, and the planned duration of the study was 48 weeks. On the basis of the recognition of the im-
portance of HIV RNA levels, in March 1996, the primary study end point was modified to include change in the plasma level of HIV RNA, and the sample size was reduced to 200 subjects. After an interim review in October 1996 the inferiority of therapy with zidovudine plus d4T compared with d4T, subjects in the arms of the study that contained d4T were unblinded in November 1997, and treatment with zidovudine was stopped in the arm of the study comprising patients who received zidovudine plus d4T. Subjects receiving zidovudine plus d4T were offered the opportunity to participate in a pharmacologic substudy to evaluate intracellular levels of zidovudine, d4T, and their metabolites before and after discontinuation of zidovudine.

**Treatment regimens.** Subjects received d4T (40 mg twice daily), zidovudine (300 mg twice daily) plus d4T, ddI (200 mg twice daily), or ddI plus zidovudine. Only zidovudine (or a matching placebo) was administered in a blinded fashion. Lower doses of ddI and d4T were administered to patients with lower body weights, as recommended in the package inserts.

**Assessments.** Baseline evaluations included a clinical assessment and blood tests for hematology, chemistry, and lymphocyte subsets. Plasma was collected and stored for batch HIV RNA testing. Subjects were evaluated at weeks 4, 8, 16, 24, 36, and 48 for signs and symptoms of drug toxicity or clinical evidence of HIV disease progression. Evaluations included targeted history and physical, blood tests for hematology and chemistry, and plasma collection for HIV RNA testing. HIV RNA tests were performed at a central laboratory on batched plasma from patients at the completion of the study with the 2.0 bDNA signal amplification assay as previously described [9].

**Statistical analysis.** The primary analysis of the study was the change in plasma levels of HIV RNA. The primary comparisons were d4T versus d4T plus zidovudine, ddI versus ddI plus zidovudine, ddI versus d4T, and zidovudine plus ddI versus zidovudine plus d4T. All analyses except for adverse events and toxicity used an intention-to-treat approach in which analysis was based on initial treatment assignment.

Short-term (week 16) and long-term (week 48) changes in viral load from baseline were evaluated on subjects who had detectable viral load at baseline. HIV RNA values measured after the baseline was established that were below detection were set at 500 copies/mL for the calculation of HIV RNA change from baseline. Treatment comparisons of changes in levels of HIV RNA were made with t tests and linear regression. Treatment differences in changes in CD4 cell counts were evaluated with Wilcoxon rank sum tests. Time-to-event survival distributions were compared with log-rank tests.

Assuming equal size in each treatment arm, a standard deviation of change of 0.6–0.80 log_{10} copies/mL, a loss to follow-up of 15%, and a significance level of .05, it was expected that a sample size of 50 subjects per arm would provide ≥80% power to detect a difference of 0.5 log_{10} copies/mL HIV RNA between the 2 treatment arms.

**Results**

**Study patients.** Between July 1995 and October 1996, 145 subjects were enrolled from 21 participating sites. One subject declined any treatment after randomization and was not included in further analyses. Study participants were predominately men (81%), white (59%), and had a median age of 37 years. Baseline characteristics were balanced among the study’s treatment arms (table 1). The study population had a median duration of prior zidovudine treatment of 135 weeks. The median baseline CD4 count was 401 cells/mm³, and the median HIV RNA level was 3.8 log_{10} copies/mL plasma.

The median time on study treatment for all participants was

<table>
<thead>
<tr>
<th>Variable</th>
<th>d4T (n = 37)</th>
<th>ZDV + d4T (n = 35)</th>
<th>ddI (n = 36)</th>
<th>ZDV + ddI (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, %</td>
<td>81</td>
<td>83</td>
<td>83</td>
<td>78</td>
</tr>
<tr>
<td>Median age, y</td>
<td>38</td>
<td>35</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Ethnicity, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>62</td>
<td>69</td>
<td>47</td>
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</tr>
<tr>
<td>African American</td>
<td>19</td>
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<td>36</td>
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<td>11</td>
<td>19</td>
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<td>0</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Current injection drug use, n</td>
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<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Previous ZDV use, median wk</td>
<td>156</td>
<td>184</td>
<td>101</td>
<td>111</td>
</tr>
<tr>
<td>Previous ddC use, %</td>
<td>14</td>
<td>11</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>HIV RNA, median log_{10} copies/mL</td>
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<td>3.9</td>
<td>3.8</td>
<td>3.8</td>
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<td>HIV RNA ≤500 copies/mL</td>
<td>18</td>
<td>9</td>
<td>13</td>
<td>6</td>
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<tr>
<td>Median CD4 cells/mm³</td>
<td>396</td>
<td>404</td>
<td>394</td>
<td>408</td>
</tr>
</tbody>
</table>

**NOTE.** d4T, stavudine; ZDV, zidovudine; ddI, didanosine; ddC, zalcitabine; HIV, human immunodeficiency virus.
41 weeks. Eleven percent of subjects prematurely discontinued treatment at the request of the subject or investigator; 5% of subjects discontinued for non–protocol-related toxicities; 4% sought alternative therapies; and 4% were lost to follow-up. There were no differences among treatment arms in the distribution of treatment modifications or study completion.

**HIV RNA levels.** HIV RNA levels were available for 128 subjects. Baseline samples were unavailable for 3 subjects, and 13 samples arrived at the central laboratory in unusable condition. Fifteen subjects had baseline HIV RNA levels ≥500 copies/mL. After 4 weeks of therapy, the mean change in log_{10} HIV RNA level was −0.27, −0.27, −0.44, and −0.77 for the d4T, zidovudine plus d4T, and ddI and zidovudine plus ddI arms, respectively (figure 1A). The mean change in HIV RNA at 16 weeks, the primary end point of the study, was −0.14 log_{10} copies/mL for both arms of the study that included d4T. The mean log changes were −0.39 log_{10} copies/mL for the ddI arm and −0.56 log_{10} for the zidovudine plus ddI arm. Within each of the ddI and d4T arms, there was no additional benefit to the addition of zidovudine, and overall the ddI arms exhibited greater mean reductions in HIV RNA levels ($P = .02$) compared with the d4T arms.

No subjects in the d4T plus zidovudine arm and only 2 (9%) subjects receiving d4T had <500 copies of HIV RNA/mL plasma at week 16. In the ddI arms, 26% and 39% of the ddI and zidovudine plus ddI arms, respectively, had measures below the limits of detection of the assay.

**CD4 cell count changes.** In all the arms of the study except for the zidovudine plus d4T arm, stable or modest increases in CD4 cell counts were observed (figure 1B). In contrast, the median CD4 cell counts decreased below baseline at every time examined in the zidovudine plus d4T arm. At week 16, the median CD4 cell count was 22 cells/mm$^3$ below baseline in this arm, and the week 16 median difference in CD4 cells was 39 between the d4T and zidovudine plus d4T arms ($P = .06$).

**Drug toxicity.** Seven subjects discontinued study medications because of drug toxicity. In the zidovudine plus d4T arm, 1 subject stopped because of elevations in liver enzymes. In the ddI arm, 1 subject discontinued the study for peripheral neuropathy, and 2 subjects discontinued the study for gastrointestinal toxicity. Two subjects in the zidovudine plus ddI arm discontinued study medications because of gastrointestinal toxicity, and 1 discontinued medication because of fatigue.

Signs and symptoms consistent with peripheral neuropathy were reported in 6 subjects receiving d4T, in 2 subjects receiving zidovudine plus d4T, in 2 subjects receiving ddI, and in 5 subjects receiving zidovudine plus ddI. All cases but 1 were graded as moderate in severity. In only 1 patient was neuropathy the reason for treatment discontinuation. Hematologic abnormalities were in general mild, although median white cell count decreased in the zidovudine-containing arms of the study, but not in the study’s monotherapy arms.

**AIDS- and HIV-related events.** One subject randomized to ddI and one subject randomized to d4T developed Kaposi’s sarcoma. Twenty-seven subjects reported a total of 31 HIV-related, non–AIDS-defining events, which were distributed equally among the study’s arms. The most common events reported were dermatitis (9 subjects) and bacterial infections (8 subjects). One subject in each arm developed herpes simplex virus disease.

**Pharmacology.** Six subjects receiving d4T participated in the pharmacokinetic substudy; all had detectable d4T in their serum. Two of the 6 subjects were also receiving zidovudine. Serum drug levels of both d4T and zidovudine were detectable in these 2 subjects. In subjects receiving d4T alone, the mean plasma level of d4T 1 h after dose was 281.8 ± 135.9 ng/mL. The mean plasma level of d4T was 401.6 ± 352.8 ng/mL in the 2 subjects receiving zidovudine plus d4T. The mean zidovudine plasma level in the patients receiving zidovudine plus d4T was 191.8 ± 118.8 ng/mL.

Measurements of intracellular d4T-triphosphate are consistent with the hypothesis of pharmacologic antagonism between zidovudine and d4T. Mean intracellular levels of d4T-triphosphate were 65 fmol/10$^6$ cells in the 4 subjects receiving d4T and 10 fmol/10$^6$ cells in the 2 subjects receiving zidovudine plus d4T. In subjects administered zidovudine plus d4T, intracellular zidovudine-monophosphate and zidovudine-triphosphate levels
were present at expected levels of 435 and 33 fmol/10^6 cells, respectively.

Discussion

The most important finding in this study was that the combination the d4T and zidovudine was inferior to d4T alone. The interim analysis demonstrated a significantly greater decline in CD4 cell count in the zidovudine and d4T arm compared with the d4T study arm. Although the difference in the change in CD4 cell counts was of borderline statistical significance in the final analysis, there was a consistent and steady decline in CD4 cell counts at all measured times in the patients receiving the combination compared with the patients receiving only d4T. Additionally, there was no significant decline in HIV RNA level from baseline in patients receiving the zidovudine plus d4T combination. Although this study included patients with extensive previous treatment with zidovudine, a trial in previously untreated patients similarly did not detect additive short-term activity between d4T and zidovudine [10]. Thus, there is no role for combination zidovudine plus d4T therapy in any patient population, and it should not be prescribed.

Pharmacologic antagonism, demonstrated previously in vitro with combinations of zidovudine plus d4T, is the most likely mechanism that explains why patients receiving these 2 drugs fared worse than patients receiving d4T monotherapy [3, 5]. The lower levels of d4T-triphosphate observed in the 2 subjects receiving zidovudine plus d4T in our study support the results from in vitro experiments. The greater affinity of thymidine kinase for zidovudine compared with d4T results in the preferential phosphorylation of zidovudine over d4T. In addition, in vitro studies suggest that zidovudine monophosphate competes with d4T monophosphate and reduces conversion to the triphosphate form [5]. It is not unexpected that patients who had a median of 135 weeks of previous zidovudine treatment, and in many cases who had detectable zidovudine resistance mutations at baseline, experienced little antiviral effect from this combination. Zidovudine was less likely effective in the presence of resistant virus, and d4T was not effective because the virus was not exposed to the active form of the drug. CD4 decline may have resulted from characteristic immune deterioration observed in patients receiving zidovudine monotherapy for >2 years [11].

These results illustrate the importance of the careful evaluation of drug combinations that appear antagonistic in vitro. Pharmacologic antagonism in vitro between zidovudine and ribavirin was reported over 10 years ago [12]. More recently, d4T and ribavirin were found to be antagonistic [5]. In contrast to the interaction between zidovudine and d4T, which depends on competition for thymidine kinase, the mechanism to explain antagonism between zidovudine or d4T and ribavirin is probably feedback inhibition of thymidine kinase by ribavirin-induced increases in deoxy-thymidine triphosphate. As ribavirin is prescribed for the treatment of hepatitis C [13] in HIV-infected patients, studies must evaluate whether antagonism between d4T and ribavirin occurs in vivo.

The pressure for clinicians treating HIV-infected patients to prescribe untested combinations of antiretroviral agents when patients are failing or intolerant of proven therapies cannot be overestimated. Before and during this study, zidovudine and d4T were being used together in treatment regimens, particularly among patients deteriorating on conventional therapy. With the wide number of antiretroviral agents available today, risks of untested regimens are multiplied. Pharmacologic interactions, particularly those mediated through effects on hepatic enzymes, can result in enhancement or diminution of effects of protease and nonnucleoside reverse-transcriptase inhibitors [14]. Discerning interactions among drugs requiring intracellular activation such as nucleoside and nucleotide reverse transcriptase inhibitors is even more complex because of the difficulty in measuring intracellular drug levels. Nevertheless, it remains crucial, for the optimal management of patients, that drugs with potential interactions are carefully studied to elucidate potential benefits or risks associated with combinations.

In conclusion, results of this study permit us to recommend against the use of d4T and zidovudine together in any antiretroviral combination regimen. Although ddI clearly exerted more antiretroviral effect in these zidovudine-experienced patients than d4T, we cannot make any conclusions regarding the optimal “sequence” of nucleoside reverse transcriptase inhibitors (NRTIs) in view of the many potential additive or synergistic NRTI combinations currently available. Future studies should consider both drug resistance and pharmacologic effects on the activity of NRTIs in designing regimens for previously treated patients.

Study Group Members

The following individuals and institutions participated in the performance of this trial: Catherine Sigmund Murch (Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL); Neel French (Weiss Memorial Hospital, Chicago, IL); Joseph Pulvirenti (Cook County Hospital, Chicago, IL); Jean Decker and Kristen Todd (Indiana University, Indianapolis); Gerianne Casey, Monica Pickthall, and Michael Boruk (University of Texas at Galveston); Allan Rodriguez, Ernesto Sceppella, Lilian Montoya, and Margaret A. Fischl (University of Miami, Coral Gables, FL); Pablo Tebas, Michael Royal, Michael Conklin, and William Powderly (Washington University School of Medicine, St. Louis, MO); Christine Fegan (University of California, San Diego); Michael Parra, Amy Fetzer, Judith Neidig, and Robert J. Fass (Ohio State University, Columbus); Laurie Andrews and Cindi Frank (Yale University, New Haven, CT); Deborah Neumann, Sharon Kohrs, Peter T. Frame, and Robert Draeger (University of Cincinnati, OH); Scott A. Smith
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References