Effect of Dipyridamole on Zidovudine Pharmacokinetics and Short-Term Tolerance in Asymptomatic Human Immunodeficiency Virus-Infected Subjects

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Zidovudine delays the progression of infection and prolongs the survival of human immunodeficiency virus (HIV)-infected patients, but these benefits are limited by dose-related toxicity and the cost of the drug. Dipyridamole, in micromolar concentrations, acts synergistically with zidovudine, reducing the anti-HIV 95% inhibitory concentration of zidovudine 5- to 10-fold in vitro. We sought to establish a well-tolerated dose of dipyridamole for use in combination with zidovudine and to detect clinically significant pharmacokinetic interactions. Both objectives are essential for planning studies of the efficacy of the zidovudine-dipyridamole combination. Eleven asymptomatic HIV-infected subjects (median CD4+ cell count, 311 cells per mm³), 10 of whom had been on zidovudine at 500 mg/day for at least 6 months, were admitted to the study. Zidovudine pharmacokinetics were measured on day 1. Dipyridamole was then begun at 600 mg/day (subjects 1 to 3) or 450 mg/day (subjects 4 to 11), and zidovudine and dipyridamole pharmacokinetics were measured on day 5. All subjects given 600 mg of dipyridamole per day developed headache or nausea, or both. Six of eight subjects given dipyridamole at 450 mg/day developed headache or mild nausea that resolved after a median of 2 days. The area under the zidovudine concentration-time curve was not significantly different on day 1 in comparison with that on day 5 (P = 0.11). Symptoms were significantly correlated with the maximum zidovudine concentrations, which were achieved when dipyridamole was dosed concomitantly (p = 0.03). Total (free and protein-bound) dipyridamole trough concentrations were near those demonstrating synergy with zidovudine against HIV in vitro. Dipyridamole was highly protein bound, with a median free/total dipyridamole ratio of 0.7%; the percent free/total dipyridamole ratio was inversely correlated with alpha, acid glycoprotein concentrations (r² = 0.66). Results of the study indicate that adjustment of the zidovudine dose was not required to achieve equivalent zidovudine concentrations when zidovudine was administered in combination with dipyridamole at the doses studied. In the short study described here, the zidovudine-dipyridamole combination was well tolerated in asymptomatic HIV-infected subjects after the occurrence of mild transient symptoms.

Zidovudine has been shown to delay the clinical progression to AIDS or to improve the survival of patients with both early- and late-stage human immunodeficiency virus (HIV) infection (4, 5, 17). Zidovudine, however, does not permanently halt the progression of HIV disease, is subject to the development of resistance, and has frequent dose-limiting hematologic toxicity in patients with late-stage disease (9, 12). Additionally, although the use of zidovudine is cost-effective, it remains a significant financial burden for patients and the health care system (13). Accordingly, use of a combination of other drugs with zidovudine has been the focus of many clinical studies intended to improve the anti-HIV efficacy of zidovudine, reduce the emergence of resistance of HIV to zidovudine, limit zidovudine’s toxicity, or reduce its cost.

Dipyridamole has several attributes that recommend it for use in combination with zidovudine. Used alone, dipyridamole delays p24 antigen production after infection of human mono-
total daily doses of 200 to 300 mg commonly used on a chronic basis for other indications. In addition, it is very inexpensive (3).

As an initial step toward studies of the relative antiviral effect of the zidovudine-dipyridamole combination compared with the antiviral effect of zidovudine alone, we designed the present study to (i) define the maximum tolerated dose of dipyridamole (in combination with zidovudine) in an otherwise healthy HIV-infected population and (ii) explore the potential for zidovudine-dipyridamole drug interactions. Theoretically, dipyridamole could inhibit the metabolism of zidovudine (or vice versa) by competing for glucuronidation, the major clearance mechanism for both drugs (2, 10). Uridine uptake into cells might also be sufficiently inhibited by dipyridamole to limit the formation of UDP-glucuronide, which is necessary as a glucuronide precursor. A significant dipyridamole-mediated inhibition of zidovudine metabolism might require reduction of the zidovudine dose or dosing frequency to minimize the potential toxicity resulting from elevated zidovudine levels in vivo. Furthermore, zidovudine levels with and without dipyridamole coadministration must be known to fairly and accurately assess the zidovudine concentration-related antiviral effect in comparative studies of this drug combination.

MATERIALS AND METHODS

Subjects. Asymptomatic HIV-infected men from the U.S. Air Force HIV-infected cohort and currently on stable zidovudine regimens of 500 mg/day were recruited for the study. The subjects’ ages ranged from 23 to 44 years (median, 32 years), and they weighed from 73 to 93 kg (160 to 205 lbs.; median, 82 kg [181 lbs.]). Two subjects were black, and nine subjects were white. Their Walter Reed Classification System stages ranged from 2A (>400 CD4+ T cells, persistent lymphadenopathy, no opportunistic infections, no symptoms) to 5A (<400 CD4+ T cells, complete anergy, no opportunistic infections, no symptoms) with a CD4+ T-cell range from 181 to 605 cells per mm3 (median, 311 cells per mm3). Most of the subjects had been on zidovudine for 6 to 13 months without complications; one subject had been on zidovudine for only 3 days. All medications other than zidovudine were discontinued 72 h before the study; chronic suppressive doses of acyclovir were continued in one subject, however. Each subject signed an informed consent document, and the study was approved by a U.S. Department of Defense human subjects research review board as well as the local institutional review board, in accordance with Air Force Regulation 169-6.

All subjects were admitted to the hospital at least one night before the study began, baseline screening was repeated (day 0), and subjects were confined to the research unit for the 5-day duration of the study. An absolute fast was maintained 8 h before and 2 h after administration of the initial dose for the pharmacokinetic sampling periods on days 1 and 5. From the time of admission until the conclusion of the study, zidovudine was given to the subjects in a 100-mg dose by mouth every 4 h. On study day 1, 15 blood samples for pharmacokinetic analysis were collected over two 4-h dosing intervals at 0, 0.25, 0.5, 1.5, 2, 3, 4, 4.25, 4.5, 5.0, 5.5, 6, 7, and 8 h, coincident with and following the zidovudine dose given at approximately 0800 h. Zidovudine was also given at 1200 h. Food was allowed after 1000 h. At 1600 h on day 1, after the final blood sample for zidovudine level determination was collected, dipyridamole dosing was initiated at 100 or 75 mg by mouth every 4 h and was given concurrently with the zidovudine dose. An identical blood sampling schedule followed on day 5 beginning at 0800 h, by which time dipyridamole levels should have reached steady state. After the final sample was drawn at 1600 h on day 5, patients were discharged from the hospital. Blood for toxicity monitoring was drawn on days 3 and 5, and nondirected clinical assessments and vital signs were recorded every 4 h on day 1 through day 5. Single lots of both zidovudine (Burroughs-Wellcome) and dipyridamole (Boehringer-Ingelheim) were used throughout the study.

Three subjects who received each dose level starting at a dipyridamole dose of 600 mg/day and escalating by increments of 300 mg/day were scheduled to be studied, as long as moderate to severe toxicity (AIDS Clinical Trials Group Toxicity Scale grade 2 or greater) did not occur in two of three subjects at a dose level. Eight subjects were to be studied at the dose below that which caused moderate or greater toxicity in two of three subjects. On the basis of the variability of the area under the concentration-time curve (AUC) for zidovudine seen in earlier studies, these eight subjects would be sufficient to confidently (α = 0.05, β = 0.20) exclude a difference of 30% when comparing the zidovudine AUC with and without dipyridamole (8). The subjects’ symptoms were graded in severity from 1 to 10 by the patients themselves, and therapeutic interventions or withdrawal from the study were initiated at the patient’s request.

Zidovudine assay. Levels of zidovudine in serum were measured by using ZDV-Trac 125I radioimmunoassay kits (Incastar, Stillwater, Minn.).

Dipyridamole assay. Total and free dipyridamole concentrations were measured in human plasma by a high-pressure liquid chromatography method described previously (16) by using a Hitachi fluorescence detector with excitation and emission wavelengths of 285 and 485 nm, respectively. The Beckman Ultrasphere 5-μm column was eluted with methanol–5 mM Tris–HCl (85:15 [vol/vol]; pH 8.6) at 1 ml/min. Standards and quality control samples were prepared in human plasma. Standards were run in ascending order before the samples were run and in descending order after the clinical samples were run. Quality control samples were mixed randomly among the clinical samples. The total dipyridamole assay was linear over the 0.3- to 10-μM range, with a correlation coefficient of 0.999. Interday precision ranged from 10.6 to 11.4% and interday accuracy ranged from 3.8 to 4.9% on the basis of the quality assurance samples. Free dipyridamole levels were determined by using the Amicon Centrifree micropartition system at 37°C. The free dipyridamole assay was linear from 2.5 to 150 nM (r = 0.999), with an interday precision of 6.0% and an interday accuracy of 3.3%.

Alpha, acid glycoprotein assay. Alpha, acid glycoprotein levels in human plasma were determined by radial immunodiffusion on NOR-Partigen plates (Behring Diagnostics, Inc., Somerville, N.J.) according to the manufacturer’s protocol.

Pharmacokinetic analysis. The peak concentration of drug in serum (Cmax), time to Cmax (Tmax), and the trough concentration of drug in serum (Cmin) were observed from individual drug concentration-time data. The AUC was calculated for zidovudine and total dipyridamole by using the trapezoidal rule and was adjusted for dose per dosing interval. The zidovudine elimination half-life (t1/2α) was calculated as 0.693 divided by the slope of the linear least-squares regression line through the terminal linear phase of the semilogarithmic zidovudine concentration-time curve. These parameters were calculated for each patient and were summarized for each drug regimen (zidovudine alone and zidovudine plus dipyridamole) and dosing interval (0 to 4 and 4 to 8 h into the pharmacokinetic analysis period).

Statistical analysis. To quantify the effect of dipyridamole on zidovudine, a paired comparison was performed by using
the Wilcoxon rank sum test (because of the nonnormal distribution of the data), which compared the pharmacokinetic parameters for zidovudine alone with those for zidovudine plus dipyridamole. Paired comparisons of pharmacokinetic parameters in the 0- to 4-h with the 4- to 8-h dosing intervals for zidovudine (AUC) and dipyridamole (AUC and peak) were also tested for statistically significant differences by using the Wilcoxon rank sum test. All tests were two-sided, and a P value less than 0.05 was considered statistically significant. The paired t test was also performed for all of the comparisons described above, but in no case did the results differ in terms of statistical significance, and the results for these tests are generally not given. Spearman's correlation coefficient was used to test for correlations between parameters. Statistically significant correlations were fitted to a line by linear least-squares regression analysis, and the r² value is reported.

RESULTS

All three subjects given dipyridamole at 600 mg/day experienced moderate headaches (grade 1, not requiring analgesics) beginning on day 1 or 2 of the study. Two of the three subjects also had nausea on days 2 and 3, which prompted them to withdraw from the study on those days. The remaining patient completed the study, symptom free, on days 4 and 5. The total daily dose of dipyridamole was then reduced to 450 mg for the remainder of the study. At the reduced dose, six of eight subjects had mild headaches which did not require analgesia on the first or second day of dipyridamole treatment. The headaches resolved in 1 or 2 days in all but one patient. A headache occurred in four of these six patients while fasting on the morning of day 5. These headaches resolved soon after the patients drank a cup of coffee. Mild and transient nausea lasting less than 1 day occurred in three patients given dipyridamole at a total daily dose of 450 mg. A fourth subject vomited once on day 2 and had persistent mild nausea until dipyridamole was discontinued on day 5; this subject was also on acyclovir and was the only subject with persistent headaches. At both dose levels, the nausea and headaches usually, but not always, occurred in the second hour after zidovudine and dipyridamole dosing. At both doses, no significant changes from the baseline in either vital signs or routine clinical laboratory tests were noted. All of the pharmacokinetic data presented below were based on the 450-mg dipyridamole dose group.

In a comparison of the AUC for zidovudine with (day 1) and without (day 5) coadministration of dipyridamole (Table 1), no statistically significant differences were seen (P > 0.05 for both Wilcoxon and paired t tests). (On the basis of a two-sided type I error of 5%, we have 80% power to exclude a 30% difference in the 0- to 8-h zidovudine AUC on the basis of the variability observed in the study.) Similarly, there were no other statistically significant changes in the other zidovudine pharmacokinetic parameters, including t½,ZDV, Tmax, Cmax, and Cmin (Table 2), with the addition of a total daily dose of dipyridamole of 450 mg to the zidovudine regimen (P > 0.05 for all comparisons).

The peak concentration of free dipyridamole was 24.1 nM (interquartile range [IQR], 18.4 to 30.8 nM), with a calculated proportion of free/total dipyridamole of 0.7% (IQR, 0.6 to 0.9%). The free/total dipyridamole ratio correlated inversely (r² = 0.66) with the concentrations of alpha, acid glycoprotein.

In the 4- to 8-h collection interval, when food was allowed ad libitum, the variabilities in drug levels and pharmacokinetic parameters among the patients increased (Tables 1 to 3). The zidovudine Tmax and Cmin rose (significantly in the zidovudine alone regimen), while the Cmax fell (significantly in the combination regimen) (Table 2). The total dipyridamole peak

### Table 1. AUC for zidovudine with and without dipyridamole by time interval

<table>
<thead>
<tr>
<th>Subject</th>
<th>0-4 h interval</th>
<th>4-8 h interval</th>
<th>0-8 h interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>ZDV alone 620</td>
<td>ZDV + DPM 547</td>
<td>ZDV alone 544</td>
</tr>
<tr>
<td>E</td>
<td>ZDV alone 600</td>
<td>ZDV + DPM 707</td>
<td>ZDV alone 583</td>
</tr>
<tr>
<td>F</td>
<td>ZDV alone 429</td>
<td>ZDV + DPM 468</td>
<td>ZDV alone 338</td>
</tr>
<tr>
<td>G</td>
<td>ZDV alone 345</td>
<td>ZDV + DPM 460</td>
<td>ZDV alone 479</td>
</tr>
<tr>
<td>H</td>
<td>ZDV alone 397</td>
<td>ZDV + DPM 340</td>
<td>ZDV alone 451</td>
</tr>
<tr>
<td>I</td>
<td>ZDV alone 366</td>
<td>ZDV + DPM 327</td>
<td>ZDV alone 391</td>
</tr>
<tr>
<td>J</td>
<td>ZDV alone 904</td>
<td>ZDV + DPM 372</td>
<td>ZDV alone 430</td>
</tr>
<tr>
<td>K</td>
<td>ZDV alone 518</td>
<td>ZDV + DPM 338</td>
<td>ZDV alone 92</td>
</tr>
</tbody>
</table>

Median
Lower quartile 474 381
Upper quartile 416 359

### Table 2. Zidovudine pharmacokinetic parameters with and without dipyridamole by time interval

<table>
<thead>
<tr>
<th>Interval (h)</th>
<th>Regimen</th>
<th>AUC (ng·h/ml)</th>
<th>t½,ZDV (h)</th>
<th>Tmax (h)</th>
<th>Cmax (ng/ml)</th>
<th>Cmin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>ZDV alone</td>
<td>474 (381, 610)</td>
<td>0.84 (0.75, 0.93)</td>
<td>0.6 (0.5, 1.3)</td>
<td>334 (268, 650)</td>
<td>32 (18, 37)</td>
</tr>
<tr>
<td>4-8</td>
<td>ZDV alone</td>
<td>440 (364, 512)</td>
<td>0.95 (0.81, 0.99)</td>
<td>2.0 (1.1, 2.0)</td>
<td>208 (141, 332)</td>
<td>48 (38, 73)</td>
</tr>
</tbody>
</table>

*P = 0.05 versus the interval at 4 to 8 h for the same regimen.*
concentration (Table 3) in the initial 0- to 4-h period in which the initial two hours were fasting (3.0 µM; IQR, 2.8 to 3.3 µM) was greater than that in the 4- to 8-h period (2.2 µM; IQR, 1.9 to 2.5 µM), with a median decrease among the patients of 24% from the 0- to 4-hour period to the 4- to 8-h period (P < 0.02).

The total dipyridamole AUC (Table 3) was also higher during the 0- to 4-h period (7.9 µM · h/ml; IQR, 6.1 to 9.0 µM · h/ml) than during the 4- to 8-h period (6.0 µM · h/ml; IQR, 4.7 to 6.7 µM · h/ml) (P < 0.02). Among the subjects, the median decrease in AUC between these periods was 15% (IQR, 7 to 44%). The median C_{min} of total dipyridamole was 1.3 µM (IQR, 1.2 to 1.9 µM).

No significant correlations between changes in zidovudine pharmacokinetic parameters and the free or total dipyridamole AUCs or peak levels that were achieved could be found (data not shown). Symptom scores (quantified by intensity and duration) did, however, correlate significantly (P = 0.04) and linearly (r^2 = 0.80) with the zidovudine C_{max} observed in the 0- to 4-h interval, during which dipyridamole was also administered with zidovudine.

**DISCUSSION**

Results of the present study demonstrate no significant modulation of zidovudine kinetics induced by concomitant administration of dipyridamole at a total daily dose of 450 mg. Our calculated pharmacokinetic parameters for zidovudine were similar with and without the addition of dipyridamole. The values of the zidovudine pharmacokinetic parameters were also comparable to those published previously (2, 8). Even with the small sample size, we can exclude difference between the zidovudine AUC and the zidovudine plus dipyridamole AUC of as low as 30%. The inhibition of zidovudine glucoronidation by any of several mechanisms hypothesized to be induced by dipyridamole either was below our threshold of detection or was too erratic among our subjects to show a consistent pattern. It is notable, however, that although the zidovudine C_{max} did not increase statistically significantly with the addition of dipyridamole, it did correlate linearly with the degree and duration of symptoms that our subjects experienced. For the benefit of future clinical trials on the basis of the results of the present study, no adjustment in zidovudine dose in the zidovudine-dipyridamole combination should be necessary when the objective is to achieve zidovudine concentrations in serum similar to those achieved with zidovudine alone.

At 600-mg total daily doses of dipyridamole, the ambulatory, otherwise asymptomatic HIV-infected patients in our study encountered side effects that were intolerable. Studies of dipyridamole in combination with acivicin or methotrexate in cancer patients established a maximum tolerated dose of 23 mg/kg/72 h when the drugs were administered as a continuous infusion. This is the equivalent of a daily oral dose of 900 to 2000 mg (on the basis of a 70-kg subject and a dipyridamole bioavailability of from 27 to 59%) (10, 20, 21). Although those studies of patients with cancer used combinations of drugs with toxicities greater than that of zidovudine, their definition of the maximum tolerated dose was far more severe than our conservative criterion for stopping the dose escalation, given the composition of our intended outpatient population. Although headaches and mild nausea were seen at a total daily dipyridamole dose of 450 mg in six of eight patients, full resolution of both symptoms occurred by the fourth study day in all but one of the subjects. Because of the short 4-day duration of administration of the two-drug combination in the present study, no conclusions can be drawn regarding the long-term tolerance or safety of the combination regimen.

The difference in zidovudine and total dipyridamole levels seen in a comparison of the levels at the 0- to 4-h period with those at the 4- to 8-h period can best be explained by ad libitum food availability after the fast from 2400 to 1000 h on the days that blood was drawn for pharmacokinetic analysis. This effect was erratic, possibly because of the wide variation in uncontrolled food intake among our subjects during the period after the fast, which ended at 1000 h. It suggests that mildly higher peak and total (AUC) concentrations of both drugs could be achieved if the drugs were taken while the subjects fasted, although this may complicate compliance and provide only a marginal, highly variable gain.

C_{min} values of total dipyridamole, even with food allowed ad libitum (median, 1.3 µM), were several-fold higher than the 0.4 µM total dipyridamole concentrations that demonstrated a nearly 10-fold reduction in the zidovudine concentration required for 95% suppression of p24 antigen production in HIV-infected human monocyte/macrophage cells in vitro (14). It has been shown, however, that dipyridamole's inhibitory effect on nucleoside transport may depend on the free drug concentration rather than the total drug concentration in vitro (7). The free drug level may therefore be critical to the antiviral effect. The 20- to 30-nM free peak dipyridamole concentrations achieved in our subjects at tolerable doses were somewhat below the 60- to 80-nM free dipyridamole concentrations which potentized zidovudine's antiviral effects in vitro, at least in one cell system (14). However, the susceptibility of HIV to dipyridamole appears to vary with the state of cell differentiation, the virus inoculum size, and other parameters (14). Therefore, it is not easy to specify the lowest dose of dipyridamole that is effective in vitro.

Having established tolerability in the very short term and the absence of important pharmacokinetic interactions, further clinical studies must still be done to demonstrate both the long-term safety and synergistic anti-HIV activity of this drug combination in HIV-infected subjects.

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