Relationship between Didanosine Exposure and Surrogate Marker Response in Human Immunodeficiency Virus-Infected Outpatients

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We used information available from routine clinic visits to characterize the pharmacokinetics of didanosine in 82 human immunodeficiency virus-infected patients. A total of 271 blood samples were collected for the measurement of didanosine concentrations in plasma (mean ± standard deviation [SD], 3.30 ± 2.21 samples/patient). Bayesian estimates of didanosine oral clearance (\(\text{CL}_{\text{oral}}\)) were obtained for these patients by the POSTHOC option within the NONMEM software package. Population priors from a previous NONMEM analysis of didanosine pharmacokinetics were used. The mean ± SD \(\text{CL}_{\text{oral}}\) was 132 ± 27.7 liters/h, which agrees reasonably well with estimates obtained from previous pharmacokinetic studies of didanosine. Estimates of individual didanosine exposure were then used to consider potential relationships between drug exposure and surrogate marker response over a 6-month period. No correlations were found between the didanosine area under the concentration-time curve from 0 to 6 months and the absolute CD4 cell count (\(r = 0.305; 0.1 < P < 0.2\)), weight response (\(r = 0.0857; P > 0.4\)), or percentage of CD4 lymphocytes (\(r = 0.0559; P > 0.4\)). Future efforts to characterize didanosine exposure in outpatients by random sampling methods should involve more directed efforts to limit residual variability in the data.

In 1991 didanosine became the second nucleoside analog to gain Food and Drug Administration approval for the treatment of human immunodeficiency virus (HIV) infection. Minimal hematologic toxicity and notable in vitro activity against zidovudine-resistant strains of HIV made didanosine an attractive alternative for patients who were intolerant of zidovudine or who were experiencing clinical or immunologic deterioration during zidovudine therapy. Early phase I trials with didanosine demonstrated potentially beneficial changes in weight, CD4 cell count, p24 antigen levels, and clinical signs or symptoms (2, 28).

The pharmacokinetics of didanosine have been studied in patients with AIDS and severe AIDS-related complex (14, 15, 17–20, 24, 31). Didanosine exhibits linear pharmacokinetic behavior after the administration of oral doses of 0.8 to 10.2 mg/kg of body weight. The elimination half-life after oral administration is approximately 1.4 h, with renal clearance accounting for up to 50% of total body clearance. Renal clearance values exceed the glomerular filtration rate, indicating renal tubular secretion. The intracellular half-life of the active form (2',3'-dideoxyadenosine-5'-triphosphate; dATP) of didanosine is at least 12 h (1), allowing for twice-daily dosing. The bioavailability of didanosine, an acid-labile drug, differs among the formulations studied and exhibits significant inter- and intrapatient variability.

Potential relationships between didanosine exposure and surrogate markers of antiretroviral efficacy have been investigated. Beltangady and coworkers (7) evaluated the relations between the average steady-state concentration in plasma (\(C_{\text{pss}}\)) of didanosine and CD4 cell counts, p24 antigenemia, and weight gain in patients with AIDS or severe AIDS-related complex participating in a phase I study. Median didanosine \(C_{\text{pss}}\) values among patients having favorable responses in CD4 cell counts, p24 antigenemia, and weight gain at week 12 of therapy were significantly higher than those among patients without such improvements. After corrections for baseline CD4 cell counts and prior zidovudine history with the use of a logistic regression model, didanosine \(C_{\text{pss}}\) values remained positively correlated with improvements in the surrogate markers studied.

Drusano et al. (11), in an earlier analysis of phase I data, defined didanosine exposure in terms of the steady-state area under the concentration-versus-time curve (AUC) when evaluating its relationship to average CD4 cell counts and p24 antigen concentrations during therapy. Increases in CD4 cell counts were determined to be independent of didanosine exposure and proportional to the baseline CD4 cell count. A reduction in circulating p24 antigen levels, however, was found to be related to both the single-dose and the cumulative didanosine AUC. Indeed, an increasing cumulative didanosine AUC appeared to be highly correlated with p24 antigen suppression.

The purpose of this investigation was to examine the feasibility of using observational data in a clinic setting to determine didanosine exposure and to further evaluate the relationship between didanosine exposure (defined as the cumulative didanosine AUC) and surrogate marker response in a group of HIV-infected outpatients.

MATERIALS AND METHODS

Patients. The criteria for inclusion into the pharmacokinetics portion of the study (Bayesian estimation of oral clearance \(\text{CL}_{\text{oral}}\)) included HIV infection...
and current treatment with didanosine. Patients receiving zidovudine in combination with didanosine were included in this analysis, because didanosine pharmacokinetics appear to be unaltered by zidovudine (5, 9, 23).

Inclusion criteria for the pharmacodynamic analysis were a minimum of 180 days of didanosine monotherapy (as initial didanosine exposure) and a minimum of three surrogate marker observations within the period of didanosine therapy. Those included a baseline determination which had been obtained within 60 days of the initiation of didanosine but not later than 11 days after therapy was begun. Eighty-two HIV-infected patients (76 males and 6 females) from the Immunology Clinic at Eric County Medical Center were included in the pharmacokinetics segment of the study. These individuals met the previously stated inclusion criteria, and adequate demographic and pharmacokinetic information about these patients was available from clinic records. For 22 of these patients evaluable surrogate marker data were available, and these data were included in the pharmacodynamic analysis. All patients gave written informed consent and were enrolled in the study from November 1991 through February 1994. After enrollment in the study, patients filled out a questionnaire during each visit to the clinic. Information obtained from the questionnaire included the date, time, and dose of didanosine taken most recently, as well as whether any doses had been missed in the 24 h prior to the clinic visit. In addition to dosing history, the questionnaire also provided information regarding the time of the patient’s last meal and the presence or absence of over-the-counter medication use. Also at each clinic visit, in addition to blood collected for routine laboratory tests, a whole-blood sample (approximately 5 ml) was collected into an EDTA-containing tube labeled with the patient’s identification number, the date and time that the sample was obtained, and the dose of didanosine taken by the patient. The blood sample was centrifuged at 2,000 rpm in a Marathon centrifuge for 15 min. The plasma layer was then transferred into a polypropylene test tube and frozen at −20°C until the sample was assayed. In a number of cases the whole-blood sample was not processed on the day of collection but was refrigerated until centrifugation. The patients’ clinic charts were reviewed to verify the dosing histories and to obtain demographic information, risk factors for HIV disease, and routine laboratory and surrogate marker data. Pertinent surrogate markers were considered to be the absolute CD4 cell count, the percentage of CD4 lymphocytes, and the patient’s weight.

Prior to assay, all samples were heat inactivated in a water bath at 30 min at 56°C. Plasma didanosine concentrations were determined by a radioimmunoassay method (10). Intra-assay variation (coefficient of variation) averaged 4.73% at 1 ng/ml and 2.99% at 30 ng/ml, while the interassay variation averaged 5.66% at 2.5 ng/ml, 6.89% at 5 ng/ml, and 10.6% at 30 ng/ml.

The plasma didanosine concentrations and dosing history information were input into a Lotus database and were later converted into a NONMEM-ready input file. Empiric Bayesian estimates of CLoral were obtained for each patient using the POSTHOC option within the NONMEM software package (6, 30, 30a).

The clearance estimates obtained from a prior multivariate analysis of factors influencing the pharmacokinetics of didanosine in a similar population of patients were taken to be the population priors (unpublished data). That analysis found clearance to be a linear function of weight and creatinine clearance. The mean population CLoral estimate, obtained by using one-compartment model with oral absorption, was 1.97 liters/h/kg.

Individual estimates of CLoral, along with didanosine daily dose information, were used to determine the didanosine 24-h AUC (AUC0–24) for each patient in the pharmacodynamics component of the study (AUC0–24 = daily dose/CLoral). The cumulative didanosine AUC throughout 6 months of therapy (AUC6) was calculated by using the dosing histories obtained from each patient’s clinic chart. Only two patients had dose changes during this 6-month period, and one patient experienced an interruption in therapy of more than 2 weeks.

Relationships between didanosine exposure (as measured by the AUC6) and surrogate marker response over the first 6 months of therapy were investigated. For 22 of the total number of patients at least three lymphocyte subset measurements were taken (for 18 of 22 patients at least three weight measurements were taken) for the pharmacodynamic analysis.

Graphs of each patient’s weight, CD4 count, and percent CD4 lymphocytes versus duration of didanosine therapy were constructed. The area under the surrogate marker-versus-duration of therapy curve was obtained with the LAGRAN program (27). The normalized AUC (NAUC) for the surrogate markers in each patient was calculated by dividing the LAGRAN-generated cumulative AUC from 0 to 6 months (AUC0–6) by the AUC which would have been observed if the surrogate marker value had remained at the baseline for 6 months (29). The cumulative NAUC from 0 to 6 months (NAUC0–6) of 1.1 indicated a 10% increase in the AUC over 6 months; an NAUC0–6 of 0.9 represented a 10% decrease in the AUC over the same period.

Graphs of normalized surrogate marker AUCs (NAUC0–6) versus the didanosine AUC0–6 were then constructed. Potential relationships were evaluated via simple linear regression and correlation analysis. A Student’s t test was used to determine if the observed correlation coefficients (r) were different from zero at a predetermined alpha error of 0.05.

## Results

### Pharmacokinetics

Data for 82 patients (76 males and 6 females) were included in the Bayesian estimation of CLoral (Table 1). Risk factors for HIV disease included homosexual activity (65% of patients), intravenous drug use (11% of patients), and both homosexual activity and intravenous drug use (6% of patients). Eighteen percent of the patients had either unidentified or other risk factors (e.g., heterosexual transmission). The mean ± standard deviation (SD) age at the time that each patient’s first blood sample was collected was 37.7 ± 8.29 years. The mean weight at that time was 73.3 ± 13.8 kg, and the mean serum creatinine level was 1.01 ± 0.191 mg/dl. None of the patients had a calculated creatinine clearance of less than 50 ml/min. The mean CD4 count at the initiation of

![FIG. 1. Didanosine (DDI) concentration versus times since last dose. The doses were normalized to 200 or 250 mg. Open circle, tablet; closed circle, sachet.](http://aac.asm.org/Downloaded from August 27, 2017 by guest)
didanosine AUC 0–6 shows that the variability in response is correlated that was observed (to 600 mg/liter). This was largely responsible for the poor DDI, didanosine.

Pharmacodynamics

Surrogate marker responses revealed significant variability within the most common dosing regimen of 400 mg of didanosine per day (200 mg twice daily). Normalized AUC0–6 values for CD4 count, percent CD4 lymphocytes, and weight were regressed against didanosine cumulative AUC0–6 (Fig. 3). The graph of the CD4 NAUC0–6 versus weight responses was relatively narrow, and individual responses did not appear to be correlated with didanosine exposure (r = 0.0857; P > 0.4). The percent CD4 lymphocyte response exhibited almost threefold variability and was not correlated with didanosine exposure (r = 0.0559; P > 0.4).

Twenty of the 22 patients in the surrogate marker analysis had received zidovudine prior to the initiation of didanosine therapy. None of these patients received zidovudine and didanosine concomitantly. The period between the initiation of zidovudine and the start of didanosine averaged 765 ± 283 days. CD4 NAUC0–6 did not appear to be correlated with the duration of prior zidovudine exposure (r = 0.295; 0.2 < P < 0.4). Interestingly, the largest responses were seen in patients who had at least 700 days of prior zidovudine therapy. This observation also held true for percent CD4 lymphocyte responses, which appeared to be less variable and which were poorly correlated with the duration of prior zidovudine exposure (r = 0.295; 0.2 < P < 0.4). Weight responses, although less variable, also were not correlated with the duration of prior zidovudine therapy (r = 0.150; P > 0.4).

FIG. 2. Variability of CLoral across dosing ranges. Data are for 82 patients. DDI, didanosine.

DISCUSSION

Pharmacokinetics. Our results indicate that a study design which uses the random collection of plasma didanosine concentrations during routine patient care may yield Bayesian estimates of CLoral which appear to be reasonable when differences in bioavailability among formulations are taken into consideration (14, 17–19, 24). Our analysis produced a mean CLoral estimate of 132 ± 27.7 liters/h (1.89 liters/h/kg for a 70-kg patient). However, largely unexplained variability in CLoral, coupled with a Bayesian estimate of CLoral that was very similar to the Bayesian estimated mean population value (132 versus 138 liters/h), forced us to consider the possibility that we had simply converged on the Bayesian estimated mean population value during our estimation. To address this possibility, we considered eliminating from the analysis patients from whom only one or two blood samples were collected during the study. It was felt that this might improve the accuracy and precision of the individual Bayesian CLoral estimates. However, we did not observe appreciable differences in the median CLoral among patients sampled with various degrees of frequency. Therefore, data for all patients were included in the final analysis.

Despite pharmacokinetic parameter estimates which appear to be reasonable, there may be shortcomings with the use of our observational data. We relied on accurate patient recall and compliance with a dosing history questionnaire. Since didanosine has a relatively short plasma half-life, a discrepancy between the reported time of administration of the last dose and the actual administration time of only an hour could sig-

<table>
<thead>
<tr>
<th>TABLE 2. Patient variables by CLoral categorya</th>
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<tbody>
<tr>
<td>CLoral category</td>
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<tr>
<td>Low (n = 21)</td>
</tr>
<tr>
<td>Medium (n = 41)</td>
</tr>
<tr>
<td>High (n = 20)</td>
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<td>P value</td>
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a Values are means (SDs). CLcr, creatinine clearance; IVDU, intravenous drug user.
Numerous factors potentially exerting an influence on the pharmacodynamics. We did not observe a relationship between our measure of response, CD4 NAUC_{0–6}, and didanosine AUC_{0–6} (r = 0.305; 0.1 < P < 0.2). When the CD4 response was measured as an average CD4 cell count during therapy, the correlation appeared even worse (r = 0.0771; P > 0.4). This is consistent with other reports and would suggest that CD4 responses are largely independent of the magnitude of didanosine exposure. Although this argument may not apply to an extreme example (i.e., little or no didanosine exposure), it appears to be reasonably valid for the usual dosing range.

Peak CD4 cell count responses usually occurred in the first 8 to 16 weeks of therapy. For 13 of 22 patients (59%) the CD4 cell count remained at or above the baseline CD4 cell count after 6 months of therapy with didanosine. Furthermore, for 14 of 22 patients (64%) the CD4 NAUC_{0–6} was at least 1; for another 3 patients the NAUC_{0–6} value was at least 0.9. These responses are comparable to those observed in another investigation in which some patients were switched to didanosine after a course of zidovudine (16).

Didanosine exposure was correlated with an increase in the CD4 cell count in another investigation (7). However, didanosine exposure was measured by the average C_{p24} and the period of assessment was only 12 weeks. Perhaps if we had limited our period of analysis to 3 months we could have found such a relationship. However, due to the lack of availability of CD4 cell count data for that time period for the calculation of CD4 NAUC_{0–6}, we selected 6 months. Those same investigators (7) also noted correlations between the didanosine C_{p24} and a decrease in the p24 antigen concentration as well as an increase in body weight. Drusano et al. (11) noted a sigmoidal relationship between p24 antigen suppression and the single-dose didanosine AUC. We did not have adequate p24 antigen data for consideration, and we were unable to find a relationship between the body weight NAUC_{0–6} and didanosine AUC_{0–6} (r = 0.0857; P > 0.4). Furthermore, no relationship between the percent CD4 lymphocyte NAUC_{0–6} (r = 0.0559; P > 0.4) and didanosine exposure was noted.

Kozal et al. (21) found a high frequency of zidovudine resistance mutations at codon 215 of reverse transcriptase in combination with didanosine mutations at codon 74 during 48 weeks of didanosine therapy preceded by zidovudine therapy. Other researchers have shown that this combination of mutations may confer an even greater degree of didanosine resistance than the codon 74 mutation by itself (12, 32). Hence, the isolates infecting patients with longer zidovudine exposures (and an increased prevalence of isolates with mutations at codon 215) may be more likely to develop more significant didanosine resistance. Despite these considerations, surrogate marker responses did not appear to correlate with previous zidovudine exposure in our patients. In fact, the greatest response in CD4 counts was seen in patients with a minimum of 700 days of previous zidovudine therapy.

Another consideration is the fact that we used the concentrations of didanosine in plasma as indirect measurements of drug exposure. Perhaps the intracellular concentrations of ddATP would allow estimates of drug exposure which would be positively correlated with surrogate marker responses. Since little or no information on intracellular ddATP concentrations or on the relationship between intracellular ddATP and plasma didanosine concentrations is available, we were left with plasma as the most feasible compartment in which to measure didanosine exposure.

Numerous factors potentially exerting an influence on the pharmacodynamics.
surrogate marker response highlight the imperfect nature of the surrogate markers that we investigated and make any clear relationship between didanosine exposure and response less likely. Changes in a surrogate marker over time should correlate with the risk of disease progression, and any effect of drug treatment on this risk of clinical progression should be explainable and predictable by its effect on the marker (22, 25). As has been previously demonstrated (8, 13, 33), CD4 counts prior to the initiation of therapy are reasonably strong predictors of disease progression. However, those studies note that the CD4 count, in particular, can explain only a portion of the favorable effects of zidovudine in delaying the progression of disease. In other words, zidovudine appears to convey to patients benefits which are independent of the effect of zidovudine on the CD4 count. Therefore, the CD4 count does not meet the strict definition of a complete surrogate marker (22, 25). Hence, it is possible that there does exist a relationship between drug exposure and a beneficial effect for patients: we are simply measuring suboptimal indices. Other markers of disease activity, such as plasma HIV RNA levels (which were not available at the time of this study), may be more highly correlated with didanosine exposure.

Conclusion. In summary, we believe that it is possible to obtain reasonable estimates of individual didanosine pharmacokinetic parameters in the course of routine patient care. However, concerted efforts to minimize residual variability in the data are essential. These estimates can be used to obtain measures of cumulative drug exposure for individual patients. Didanosine exposure, however, did not appear to be related to changes in CD4 counts, percent CD4 lymphocytes, or body weight over the initial 6 months of therapy. A multivariate analysis of these data including evaluation of nonlinear models is warranted. Other surrogate markers, such as viral RNA levels, should be investigated for their potential relationships with didanosine exposure.

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