Quantitative Relationships between Zidovudine Exposure and Efficacy and Toxicity

G. L. DRUSANO, 1,* F. M. BALIS, 2 S. R. GITTERMAN, 3 AND P. A. PIZZO 2

Albany Medical College, Division of Clinical Pharmacology, Albany, New York 12208; Division of Infectious Diseases, University of Maryland Program of Clinical Pharmacology, Baltimore, Maryland; and Pediatric Branch, National Cancer Institute, Bethesda, Maryland.

Received 25 May 1993/Returned for modification 6 October 1993/Accepted 14 May 1994

We examined the relationship between the concentrations of zidovudine in plasma given by continuous intravenous infusion to human immunodeficiency virus-positive pediatric patients and a surrogate marker of outcome (measured by the increase in the number of CD4-positive T cells) as well as drug-mediated toxicity (change in granulocyte count). The return of CD4-positive T cells was most strongly related to the number of these cells present at the start of therapy. Drug concentration data added little explanatory power to this relationship, indicating that the effect of zidovudine was near maximal throughout the range of concentrations examined. The change in granulocyte count was significantly correlated with zidovudine concentration both from weeks 1 through 8 and from weeks 8 through 12. These findings imply that it may be wise to stratify phase I antiretrovirus drug trials for the entry level of CD4-positive T cells if pharmacodynamic relationships with this marker as the dependent variable are to be sought. Continued efforts need to be made to derive quantitative relationships between drug exposure and measures of both efficacy and toxicity so that the maximal amount of information is derived from small phase I studies.

Zidovudine has been demonstrated to have efficacy in the prolongation of survival in patients with AIDS (10) and has delayed disease progression from earlier-stage human immunodeficiency virus (HIV) disease (16). Unfortunately, zidovudine also has demonstrated toxicities, particularly affecting cells of the hematopoietic lineage (14). This drug will be the primary agent in use for patients with HIV disease for the immediate future. In order to obtain the best results, doses and schedules of administration should provide concentrations in plasma which are therapeutically optimal (or nearly so) without engendering undue toxicity. To accomplish this, quantitative relationships between plasma drug concentration and a marker of therapeutic outcome as well as the dose-limiting toxicity of the drug are required.

Because CD4-positive T cells may play a central role in the pathophysiology of HIV disease (2) and because small absolute numbers of these cells put a patient at risk for opportunistic infections, we chose to examine changes above the baseline in the numbers of these cells as a surrogate marker for efficacy. Likewise, because granulocytopenia is the most frequent dose-limiting toxicity, we examined the change in granulocyte counts as a function of zidovudine exposure.

MATERIALS AND METHODS

Pediatric patients known to be HIV infected were studied at the Clinical Center of the National Institutes of Health by investigators from the Pediatric Branch of the National Cancer Institute. Written informed consent was obtained according to institutional guidelines. The eligibility criteria and protocol for this phase I investigation have been published previously (13). Zidovudine was administered as a continuous intravenous infusion through a surgically placed catheter.

Plasma samples for determination of zidovudine concentra-

1* Corresponding author. Mailing address: Division of Clinical Pharmacology, Albany Medical College, Albany, New York 12208. Phone: (518) 262-6761. Fax: (518) 262-6794.

tions were obtained periodically from all patients at steady state (13). Plasma samples were assayed for the parent concentration of zidovudine by a validated high-pressure liquid chromatographic assay. Concentrations in plasma from multiple determinations were averaged in a weighted fashion (by length of time on a particular infusion schedule) for use in the mathematical analysis. CD4-positive T-cell counts were also time-averaged for inclusion in the analysis. Briefly, the area under the CD4-positive T-cell–time curve was determined by using the LAGRAN program of Rocci and Jusko (15). The area under the baseline CD4-positive T-cell count curve was determined and the two areas were subtracted. This value was divided by the number of weeks of observation, giving the average rise in the CD4-positive T-cell count over the period of observation.

Linear models were evaluated for the regressions. Also, because the effect of drug administration has often been shown to be effectively modeled by Hill’s equation (7), we examined its utility, as well as a variant, the sigmoid maximal observed effect (Emax) model, in describing the return of CD4-positive T cells as a function of zidovudine exposure.

The sigmoid Emax model takes the following form:

\[
\text{CD4 cell return} = \frac{\text{maxCD4 return} \cdot [\text{AZT}]^H}{[\text{AZT}]_{50}^H + [\text{AZT}]^H} \quad (1)
\]

where maxCD4 return is the identified maximal response brought about by drug exposure, [AZT]_{50} is the concentration of zidovudine inducing 50% of the maximal response, H is the sigmoidicity factor, which determines the “steepness” of the sigmoidal curve (this value is fixed at 1 in the classical Hill’s model), and [AZT] is the actual measured concentration of zidovudine (units of micromolar).

A combined model, incorporating two independent variables (starting CD4-positive T-cell count and zidovudine concentration), was also evaluated.
CD4 return = \(a \cdot \text{SCD4} + b + \frac{\max \text{CD4 return} \cdot (\text{AZT})^H}{[\text{AZT}]_{30}^H + [\text{AZT}]^H}\)  \(\text{(2)}\)

where SCD4 is the baseline CD4-positive T-cell count and \(a\) and \(b\) are linear constants. The other variables are as defined above.

All regression analyses were accomplished by using the ADAPT II package of programs of D'Argenio (4). The Nelder-Mead Simplex was the search algorithm.

To counteract any heteroscedasticity, a generalized least-squares (GLS) routine was used. In the GLS routine, both the structural model (see above) and the error model must be specified. We chose a traditional power variance model for the error model. In the initial stage of the GLS routine, parameter estimation was performed with unweighted (weight = 1), nonlinear, least-squares regression; this was followed by maximum likelihood estimation (stage II) of the variance model parameters. Reestimation of the structural model parameters was performed by using stage I parameters as the initial estimates and the variance model to provide the weights (stage III). This loop was repeated until all parameter estimates (both structural and variance models) ceased to change appreciably, ensuring a global minimum. Preliminary work showed that the search spaces for the more complex models were fraught with many false minima, which influenced our decision to use the GLS routine.

**RESULTS**

Figure 1 displays the relationship between zidovudine concentration and CD4-positive T-cell increase, as predicted from a sigmoid \(E_{\text{max}}\) model. The fit of the model to the data was not impressive nor statistically significant. Two patients had a far greater return of CD4-positive T cells than predicted by the model. In the same way, multiple patients displayed a far smaller return of cells than was predicted. Examination of the starting CD4-positive T-cell counts of these patients was enlightening; those whose values were overpredicted had very low CD4-positive T-cell counts, while those whose values were underpredicted had much higher initial counts. This led us to examine the relationship (using a linear model) between the initial CD4-positive T-cell count and the return of CD4-positive T cells after zidovudine exposure. There was a highly significant relationship between these two variables (Fig. 2), irrespective of the steady-state zidovudine concentration \((r = 0.738; P < 0.005)\).

Thus, the initial state of the patient's CD4-positive T cells was clouding the ability to determine the relationship between drug exposure and the return of CD4-positive T cells. We therefore examined the two-independent-variable model, the results of which are displayed in Fig. 3. Note that while this model may be more "physiologic" in that both variables (zidovudine concentration and starting CD4-positive T-cell count) that would be thought to influence the outcome (CD4-positive T-cell return) are included, the amount of variance explained was not statistically different from that of the single-independent-variable relationship of Fig. 2.

The clinical study described here was not designed to test the effectiveness of relatively low concentrations of zidovudine. Hence, little information is present for those concentrations, making optimal estimation of the parameters of the sigmoid \(E_{\text{max}}\) part of the model difficult. It is consistent, however, with having the effect of zidovudine administration near maximal at relatively low concentrations in plasma. This is supported by the relatively high correlation coefficient (and the consequent large percentage of the observed variance being explained) seen with only the linear part of the model (Fig. 2). This
FIG. 2. Relationship between initial numbers of CD4-positive T cells and their return after the institution of zidovudine therapy ($r = 0.738; P < 0.005$).

FIG. 3. Correlation between the predicted return of CD4-positive T cells and the observed return when two independent variables are included in the model (initial count of CD4-positive T cells and mean steady-state zidovudine concentration in plasma); the line of identity is displayed to provide a visual indication of the goodness of fit ($r = 0.740; P < 0.005$). This model does not explain significantly more variance than the simpler model displayed in Fig. 2. Note that zidovudine concentration can explain a maximal return of only 16 CD4-positive T cells. For a patient initiating zidovudine therapy with 200 CD4-positive T cells, the concentration of drug is responsible for a maximum of 22.5% of the return of these cells.
suggests that virtually all of the effect in raising the CD4 cell count is present at relatively low concentrations and that the number of CD4-positive T cells that the patient starts with controls the number of these cells which return.

The other important aspect of drug administration is the relationship between drug administration and dose-limiting toxicity. Figure 4 displays the relationship between zidovudine concentration and the change in granulocyte count in the first 8 weeks after the initiation of zidovudine therapy. Because of the non-zero y intercept, it is clear that low concentrations of zidovudine may actually mediate an increase in granulocyte count. We speculate that low concentrations effectively inhibit viral replication in bone marrow stem cells, allowing for the more efficient production of mature granulocytes. We further speculate that higher concentrations produce a direct toxic effect which overcomes the salutary effect seen at lower levels, resulting in a net decline. The x intercept of the line, which represents the point of equilibrium between these two proposed effects is 2.9 µM, a value in excellent agreement with the value of 3 µM found by Balis et al. (1), which represented the best value for discriminating between those who would or would not become granulocytopenic on zidovudine therapy.

There may be time dependency in the relationship between zidovudine exposure and the change in granulocyte count from the baseline. To evaluate this, we examined the relationship between zidovudine concentration and granulocyte count change from weeks 8 through 12 (Fig. 5). Fewer points are present in the plot in Fig. 5 because of patient dropout from the phase I protocol. The line in Fig. 5 is steeper than that in Fig. 4, with a larger y intercept, but with an x intercept (2.6 µM) similar to that seen in Fig. 4. If one reanalyzes the data from weeks 1 through 8 for only the patients contributing data to Fig. 5, the resulting regression relationship is not significantly different from the line displayed in Fig. 4 (data not shown). One may interpret this as showing a time dependency of the relationship, but one in which the balance between the salutary and toxic effects of zidovudine on granulocyte count remains the same equilibrium concentration (circa 3 µM).

**DISCUSSION**

The results of the present study may be important for the use of zidovudine in individual patients, but as importantly, they should also affect the design of phase I studies of future anti-HIV compounds.

Whether one takes the viewpoint that the two-independent-variable model is supportable on a biologic basis or that the second variable is insupportable on a statistical basis, the conclusions of the study do not change. If one uses only the starting CD4-positive T-cell count, the response of CD4 cells occurs irrespective of the zidovudine concentration. That is, the concentration-effect relationship for CD4-positive T-cell return reached its (near) maximum even at the lowest concentration observed in the present study. If one examines the two-independent-variable model, the concentration of zidovudine which mediates 90% of the maximal increase of CD4-positive T cells is approximately equivalent to that of the x intercept seen in the toxicity relationships (Fig. 4 and 5). These relationships imply that zidovudine can be administered at a dose that provides 90% of the maximal therapeutic response of the surrogate variable chosen (CD4-positive T-cell return) without engendering undue toxicity (decrement in granulocyte count). For the individual patient, these relationships provide a rationale for monitoring plasma zidovudine concentrations, so that drug doses can be altered to obtain drug concentrations in this therapeutic, nontoxic range (5). As predicted by the relationships derived above, lower doses of drug have recently been shown to be effective by two prospective clinical trials with zidovudine (3, 9) in which equivalent therapeutic outcomes were obtained when comparing high (1,500 mg/day) and
low (600 or 300 mg/day) doses. There is, however, a lower bound for zidovudine exposure that will produce a full CD4 cell return. Fischl et al. (8) demonstrated that administration of 50 mg of zidovudine every 8 h caused a significantly lower rise in the CD4 count than larger zidovudine doses.

If therapeutic and relatively nontoxic results of therapy can be obtained by simply using lower doses, one must ask if there is a compelling need to monitor the concentrations of zidovudine in plasma. Unfortunately, there are relatively large differences in the way in which individual patients clear zidovudine. The major pathway of elimination is hepatic glucuronidation, with a smaller contribution by renal elimination. We demonstrated (5a) a threefold range in the clearance of zidovudine from plasma in patients on hemodialysis or peritoneal dialysis. Because their renal function was so impaired that dialysis was required, the range of observed clearances was due to differences in hepatic glucuronidation. Drug clearances were also highly correlated with the clearance of indocyanine green dye, indicating that the drug is heptically extracted in a flow rate-dependent manner. The range of zidovudine clearances, then, is likely due to differences between patients in hepatic blood flow, which limits the amount of drug which can be metabolized by the liver. Because both renal impairment and hepatic disease are common among HIV-positive patients (particularly among patients with intravenous drug abuse as a risk factor), a broad range of drug clearances is to be expected, resulting in a broad range of concentrations in plasma for any given dose. This, together with the toxicity relationship, provides the reason for monitoring the concentrations of zidovudine achieved in an individual patient. These relationships were developed with continuous-infusion therapy. This is not practical or, perhaps, even desirable for clinical use. However, the ability to demonstrate such a relationship indicates that it may be possible to also derive such a relationship with more traditional, intermittent therapy.

As important, or more important, is the finding that surrogate marker response was linked to the initial CD4-positive T-cell count. This has implications for the design of phase I clinical antiretrovirus drug trials. Phase I trials of antiretroviral therapy have been modeled after those of oncolytic chemotherapy. The traditional focus of phase I investigations of oncolytic compounds has been the definition of the maximally tolerated dose of drug, under the assumption that the aim of drug therapy is to reduce the number of abnormal cells in the body to as close to zero as possible, which is accomplished by administration of the largest tolerable dose of drug. Therapy with antiretroviral agents (specifically, and especially, nucleoside analogs) is given chronically and has an aim different from that of oncolytic chemotherapy, that of protecting uninfected cells from HIV. This needs to be done over a long period of time, and thus should shift the focus of a phase I investigation to an examination of the best “therapeutic window,” in which a large percentage of the maximal therapeutic response is obtained without toxicity. This puts a premium on the delineation of the relationship between drug exposure and therapeutic response.

Certainly, classical oncolytic approaches to determination of drug doses for phase II and III trials resulted in far too high a dose of zidovudine, with the result that a very high percentage of patients were zidovudine intolerant (14). The same has been seen with another nucleoside analog, dideoxycytidine (12). Part of the reason for this is that the maximal therapeutic response (at least as judged by an increase in CD4-positive T lymphocytes) occurred at relatively low plasma nucleoside concentrations. All further dose escalations for defining a maximally tolerated dose only succeeded at adding toxicity.
without providing an increased therapeutic benefit. If one looks at clinical endpoints in an intent-to-treat analysis, the greater rate of drug intolerance seen with unnecessarily large doses means that a regimen will have a smaller observed effect than is possible with a smaller, more rationally derived dose. A clear example is the ACTG trial 116B/117, in which the larger dideoxynosine dose was significantly inferior to the smaller dideoxynosine dose, as was prospectively predicted from a similar analysis conducted by our group, but using the p24 level change as a marker of antiviral effect (6, 11).

As seen in the present study, if one has confounding variables, such as CD4-cell return being linked to the starting CD4-positive T-cell count, delineation of the true relationship between drug concentration and response is difficult. We have published other data developed with dideoxynosine which essentially recapitulate the findings of the present study. CD4-positive T-cell return was strongly linked to initial CD4-cell count (6). This implies that if we are to delineate the best relationships between drug exposure (nucleoside analogs and possibly others) and drug effect, attention must be paid to entry criteria, stratifying for the entry level of CD4-positive T cells, if this is to be used as the surrogate marker of efficacy in the pharmacodynamic analysis. Otherwise, improper conclusions can be drawn from the phase I evaluation, resulting in higher than necessary drug doses and unnecessary toxicity for patients, with too many people being declared intolerant of the agent in question. Also, serious consideration should be given to the use of virally derived markers of effect such as changes in p24 levels plasma viremia, or RNA PCR in the delineation of concentration-effect relationships. Simultaneous delineation of exposure-efficacy and toxicity relationships should be a part of all phase I antiretrovirus drug trials.

ACKNOWLEDGMENT
This study was supported in part by a grant from the National Aeronautics and Space Administration (NAG9-249).

REFERENCES