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Pharmacokinetics of Zidovudine Phosphorylation in Peripheral Blood Mononuclear Cells from Patients Infected with Human Immunodeficiency Virus

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As part of an effort towards optimization of dosing of zidovudine (ZDV), formation and elimination of total phosphorylated ZDV (ZDVPt) in peripheral blood mononuclear cells were examined in 21 asymptomatic human immunodeficiency virus-infected patients during their first 24 weeks of therapy (AIDS Clinical Trials Group Protocol 161). Intracellular concentrations of ZDVPt were measured with a previously described and validated radioimmunoassay technique. Although ZDV phosphorylation occurred readily upon initiation of therapy, it declined with time; the area under the concentration-time curve (AUC) at week 4 (mean ± standard deviation, 3.41 ± 0.93 pmol · h/10^6 cells) was significantly greater than that at week 24 (2.19 ± 1.10 pmol · h/10^6 cells). Plasma ZDV AUC did not change with time and did not correlate with ZDVPt AUC. In dose-response experiments (20 to 100 mg orally), phosphorylation did not proportionally increase with increasing plasma ZDV concentrations. Similarly, compared with a single dose, two doses of ZDV over an 8-h period resulted in little ZDVPt increase in cells relative to increases in plasma ZDV concentrations. The half-life of intracellular ZDVPt was twice that of plasma ZDV (4 versus 2 h), suggesting that an every-8-h dosing regimen is justifiable. These findings suggest that metabolism of ZDV to its active intracellular forms may be saturable in some patients, is poorly correlated with plasma concentrations, and diminishes over time. These findings have implications for future development and management of anti-human immunodeficiency virus nucleoside therapy.

Zidovudine (ZDV) currently remains the recommended initial therapy for the treatment of human immunodeficiency virus (HIV) infection. Although effective in slowing the progression of HIV disease, the benefits of ZDV therapy appear to be transient, with patients eventually succumbing to opportunistic infections and/or neoplasms associated with immunosuppression. Its use is associated with several well-documented side effects, most notably anemia and neutropenia (17). The plasma pharmacokinetics of ZDV have been thoroughly studied (5, 12), but a therapeutic concentration range in plasma has not been established. Recommended dosing regimens still vary from country to country and are only weakly supported by pharmacokinetic data; the optimal dosing regimen remains to be determined. There has been one published report of a link between the occurrence of opportunistic infections and area under the concentration-time curve (AUC) in plasma (6), but more comprehensive studies have found little evidence of such relationships between concentrations in plasma or cerebrospinal fluid and clinical effects (4, 25).

A thymidine analog, ZDV is phosphorylated at the 5′ position by a series of cellular kinases after diffusion into the cell. The effectiveness of the drug is attributed to inhibition of HIV reverse transcriptase by ZDV 5′-triphosphate and by termination of the formation of DNA copies of the viral RNA genome as the triphosphate is incorporated into the growing chain (8). Similarly, toxicity is attributed to the inhibition of cellular DNA polymerases and termination of cellular DNA elongation and may be further complicated by inhibition of phosphorylation of endogenous thymidine (8) and inhibition of 3′ to 5′ exonuclease activity (3, 9) by accumulated concentrations of ZDV 5′-monophosphate. Therefore, unless plasma ZDV concentrations are observably related to intracellular concentrations of phosphorylated ZDV, determination of a relationship between concentrations in plasma and clinical effect is unlikely. A mechanism proposed to contribute to the eventual failure of ZDV and other nucleoside analog reverse transcriptase inhibitors is the possibility of decreased and potentially inadequate phosphorylation of these drugs at some sites of viral replication (10). Decreased phosphorylation could lead to the emergence of viral strains less susceptible to ZDV therapy.

We have previously described a radioimmunoassay (RIA) method for measurement of total phosphorylated ZDV (ZDVPt [the sum of ZDV 5′-mono-, di-, and triphosphates]) in peripheral blood mononuclear cells (PBMCs) from HIV-infected patients (22–24). While this assay does not allow for separate and distinct measurement of ZDV triphosphate, it does offer the considerable advantage of being clinically practical for large-scale pharmacokinetic studies: direct determinations can be performed with a patient's cells from a single 10-ml blood sample, the assay is specific for ZDV and is not subject to significant interference from endogenous compounds (such as thymidine), extensive sample preparation and cleanup are not necessary, and the assay uses a commercially prepared kit for ZDV determination, complete with premanufactured standards and quality-control samples. However, it

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should be recognized that, in the design of this study, no assumptions were made about the clinical utility of ZDVPt concentrations except that they might be more clinically significant than plasma ZDV concentrations. Subsequent analysis of data generated during this study and reported elsewhere did show that improvement in some surrogate markers of HIV disease is associated with an increased ability to phosphorylate ZDV (20).

This report describes results from AIDS Clinical Trials Group (ACTG) Protocol 161 (sponsored by the National Institute of Allergy and Infectious Diseases). ACTG Protocol 161 was a phase I investigation of the pharmacokinetics of total ZDV phosphorylation in PBMCs from asymptomatic HIV-infected patients. In addition to definition of basic pharmacokinetic parameters, the study sought to examine (i) the time required to reach maximum phosphorylation after initiation of therapy (i.e., whether slow induction of phosphorylating enzymes occurred), (ii) the comparative dose-concentration response for plasma ZDV and intracellular ZDVPt; and (iii) the relationship between plasma ZDV and intracellular ZDVPt pharmacokinetics. The study also investigated the effect that duration of therapy has on pharmacokinetics and thus the changing ability of PBMCs to phosphorylate ZDV over time. This information could lead to improved ZDV dosing regimens and/or individualized management of therapy, similar to how some nucleoside analogs are managed in the treatment of leukemia (13, 16).

MATERIALS AND METHODS

Study design. This study was an ambulatory-based pharmacokinetic study designed to investigate the pharmacokinetics of ZDV phosphorylation in PBMCs from previously untreated HIV-infected patients. The study was performed at the AIDS Treatment Center of the University of Cincinnati. Patients were initially assigned to one of two study arms for short-term study: a standard-dose arm, in which time to maximum ZDV phosphorylation was to be determined, and an escalating-dose arm, in which the response from weekly increasing dosages of ZDV was to be evaluated. Data from both arms were combined to calculate specific pharmacokinetic parameters and the effect of longer-term (24-week) therapy on ZDV phosphorylation. Figure 1 depicts the general study scheme.

In the standard-dose arm, patients were started on 100 mg of oral ZDV every 4 h while awake (500 mg/day). Phosphorylation and accumulation of ZDV were monitored hourly on days 1 and 14. Samples were drawn immediately predose (time zero) and hourly thereafter for an 8-h period, with a second dose administered at 4 h postintroduction of drug. Interim samplings (days 2, 4, and 8) were drawn as morning trough (immediately predose) and estimated peak (1.5 h postdose). For determination of AUC and other pharmacokinetic parameters on weeks 4 and 24, samples were drawn immediately before the first daily dose (time zero) and then at 1, 2, 4, 6, and 8 h postdose. No second dose was given at 4 h to allow decay of plasma ZDV and ZDVPt concentrations to be monitored for a full 8 h. Following a 48-h washout period to allow clearance of all forms of ZDV, patients were rechallenged with drug and samples were taken hourly (with a second dose at 4 h) to determine the extent of phosphorylation and accumulation of ZDVPt after prolonged therapy.

In the escalating-dose arm, patients were started on 20 mg of oral ZDV elixir while awake (100 mg/day). Each subsequent week, the dose was increased by 20 mg until the standard 100-mg (capsule) dose was reached on day 29. Weekly dose-response samplings were drawn as the morning trough and peak (1.5 h postdose) at the end of each week's dosing regimen (days 7, 14, 21, 28, and 35), with full AUC determinations at study weeks 8 and 28. Determinations at weeks 8 and 28 also included the 48-h washout and rechallenge as described for the standard-dose arm.

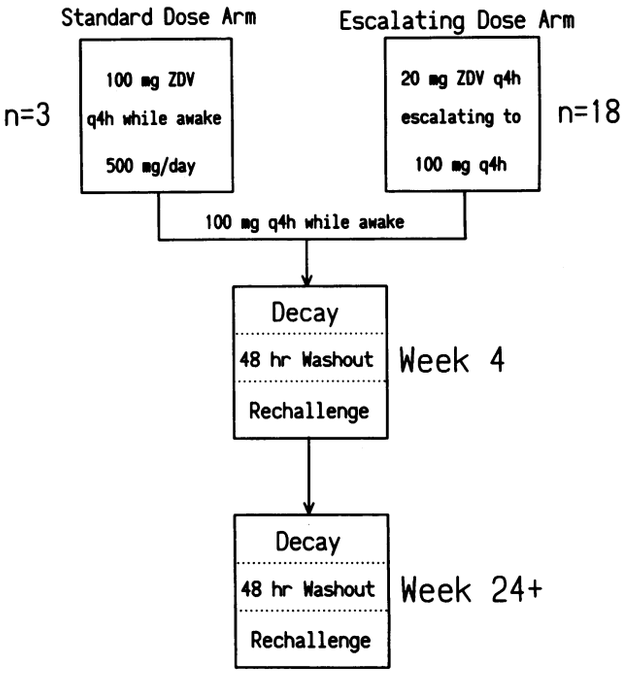

FIG. 1. Simplified scheme of ACTG Protocol 161. Weeks 4 and 24 of standard therapy correspond to escalating-dose study weeks 8 and 28, i.e., 4 weeks and 24 weeks after reaching standard therapy. q4h, every 4 h.

In order to reflect clinically relevant conditions, in which patients frequently dose themselves with food to minimize the emetic effects of ZDV therapy, patients were not required to fast prior to pharmacokinetic sampling at any time point in either study arm. All samples were drawn into evacuated 10-ml heparinized tubes and immediately transported to the laboratory for separation of PBMCs and plasma. For the standard-dose arm, clinical data were collected prior to entry and at weeks 4, 8, 16, and 24 and included the following: complete blood cell count, serum creatinine, total bilirubin, serum glutamic pyruvic transaminase-serum glutamic oxalacetic transaminase, alkaline phosphatase, and quantitation of $CD4^+$ and $CD8^+$ cells. Flow cytometry was performed by standard methods in an ACTG quality-controlled laboratory. Clinical data for the escalating-dose arm were collected as described for the standard-dose arm at entry and at weeks 4, 8, 20, and 28.

Patients. Thirty patients were enrolled in the study. To be eligible, patients had to be confirmed HIV positive, with a CD4 cell count between 200 and 500 cells per mm^3, 13 years or older, and with no prior history of treatment with ZDV. The following laboratory criteria also had to be met: hemoglobin, $\geq$11.0 g/dl; granulocyte count, $\geq$1,000/mm^3; leukocyte count, $\geq$2,500/mm^3; platelet count, $\geq$100,000/mm^3; serum creatinine, $<$1.5 times the upper normal limit; serum glutamic oxalacetic transaminase-serum glutamic pyruvic transaminase liver function tests, $<$5 times the upper normal limit; and Karnofsky Performance Score, $\geq$60. Women of childbearing potential

were required to have a negative pregnancy test within 7 days of entry. Patients who were unable to take oral medication reliably or who had malabsorption syndrome were not eligible to participate. Patients undergoing systemic therapy for active bacterial, fungal, or viral infections, licensed or investigational, were also ineligible, as were patients receiving probenecid or non-Food and Drug Administration-approved investigational drugs. Food and Drug Administration-approved antipneumocystis and antifungal prophylactic therapies were permitted. Concurrent medications that undergo extensive hepatic metabolism, such as isoniazid, rifampin, and phenytoin, were discouraged but not prohibited. All patients gave written informed consent, and the study was approved by the Institutional Review Board of the University of Cincinnati.

Drug assays. Isolation of PBMCs and subsequent assay of plasma ZDV and ZDVPt were performed as previously described (24). Briefly, PBMCs were isolated on a Ficoll gradient. The plasma layer was saved for determination of ZDV. Cells were washed with phosphate-buffered saline, and dye-excluding viable cells were counted under a light microscope. After decantation of buffer, cell pellets were stored at −20°C until extracted. Cells were extracted overnight at −20°C with 60% methanol. Extracts were decanted from cellular debris and then were evaporated to dryness. The resulting residue was reconstituted with 500 μl of 0.2 M Tris–1 mM $MgCl_2$ buffer (pH 9.5) and then split into two equal fractions. One of the fractions was treated with 10 μl of a 20-mg/ml bovine alkaline phosphatase solution (6 U of activity per mg) at 37°C for at least 1 h for removal of phosphate groups. Both fractions were stored at −20°C until simultaneously assayed for ZDV content with a commercial RIA (ZDV-Trac; INCSTAR Corporation, Stillwater, Minn.). Assays were performed according to kit instructions, except that all volumes were reduced by 50% and cell samples were not diluted prior to assay. Control samples from the kit manufacturer were included in each run. The concentration of ZDVPt equalled the difference in concentration between the enzyme-treated and untreated fractions. RIA concentrations (typically in the 0.5- to 2-ng/ml range) were converted to intracellular concentrations (picomoles per 10^6 cells) by correcting for sample volume (500 μl) and cell count. Intra- and interassay variability for this procedure is typically <10% for assay concentrations of >1 ng/ml and approximately 15% for concentrations of <1 ng/ml (19a, 23), with a lower limit of detection of 0.2 ng/ml. Recovery of ZDVPt from cell extracts is approximately 90% (24). Corresponding plasma ZDV concentrations were separately determined with the same RIA kit according to the manufacturer's recommendations.

Pharmacokinetic and statistical analysis. For calculation of pharmacokinetic parameters and effect of duration of therapy, data from both arms were combined by using the determinations performed at weeks 4 and 24 of standard therapy (corresponding to escalating-dose arm weeks 8 and 28). Pharmacokinetic parameters were estimated by noncompartmental methods. Concentrations from each full pharmacokinetic determination were plotted semilogarithmically as concentration versus time since dose. Peak concentration (C_{max}) and time to peak concentration (T_{max}) were the observed values. The terminal elimination phase was identified by visual inspection of each patient's plot, and the elimination rate constant (k_{el}) was estimated as the slope of the best-fit regression line of the terminal elimination phase (generally between 2 or 4 and 8 h). Terminal phase half-life ($t_{1/2}$) was calculated as $0.693/k_{el}$. Unless otherwise specified, AUCs were calculated from time of dose (time zero) through 8 h by using the trapezoidal rule and then were extrapolated to infinity by adding the amount C_{8h}/k_{el}, where C_{8h} is the measured plasma ZDV or intracellular ZDVPt concentration 8 h postdose. Statistical comparisons of pharmacokinetic parameters and clinical variables at different time points were made by paired nonparametric test; reported two-tailed P values are estimates based on a normal distribution. Linear regressions and statistical analyses were performed with the PC statistical package INSTAT (Graphpad Software, San Diego, Calif.).

RESULTS

Patients. Thirty patients were enrolled, and 21 were evaluable. Nine patients did not participate or were dropped early during the study because of self-withdrawal, noncompliance, or because they were inadvertently started on ZDV by their personal physician before their scheduled study starting date. The study population consisted of 16 men and 5 women (mean ± standard deviation age, 33.6 ± 7.8 years; range, 21 to 54 years). Fifteen were white, 5 were black, and 1 was Hispanic. Only three patients participated in the standard-dose arm, because after study of the first three subjects it was apparent that induction of phosphorylating enzymes was not a requirement for ZDV phosphorylation (see below), and thus we did not require additional data to calculate a mean time to maximum phosphorylation. Additionally, it was extremely difficult to recruit working patients into this arm because of the early and significant time commitments required. The remaining 18 patients (14 men, 4 women) therefore participated in the escalating-dose arm to improve the statistical quality of the dose-response data. All but one patient had complete pharmacokinetic determinations at week 4 of standard therapy, but data for three patients could not be determined at the final time point. For technical reasons, an additional three patients had pharmacokinetic data determined at time points greater than 24 weeks (two at week 32 and one at week 34) but were included in the final data analysis. None of the patients experienced clinical events requiring systemic therapy or use of drugs that undergo extensive hepatic metabolism during the study, nor were there incidents of ZDV toxicity requiring alteration of dose. Two subjects presented with mild hepatic dysfunction during the study, which did not require modification of the ZDV dose.

Pharmacokinetics. Mean pharmacokinetic parameters as determined at weeks 4 and ≥24 are shown in Table 1. Mean pharmacokinetic profiles from week 4 are shown in Fig. 2 (parameters in Table 1 were not calculated from these mean plots but represent the mean of individually determined patient values). Because sampling times were selected to optimize pharmacokinetic determination of intracellular ZDVPt while minimizing patient inconvenience and discomfort, plasma ZDV C_{max} is probably underestimated and T_{max} is probably overestimated since the first sampling time was 1 h postdose; previous plasma pharmacokinetic studies have demonstrated that in most patients plasma T_{max} is less than 1 h (5, 12). However, there was a delay in plasma T_{max} in about 33% of the full pharmacokinetic determinations that, in rare cases, was 4 h or longer. Peak intracellular concentrations of ZDVPt were generally reached about 1 h after peak plasma levels. The mean terminal phase intracellular ZDVPt $t_{1/2}$ was about twice the mean plasma ZDV $t_{1/2}$ of 2 h. Comparison of AUCs (Fig. 3) determined at all time points suggests that there is no readily discernible relationship between plasma ZDV and resultant intracellular ZDVPt concentrations.

The pharmacokinetic data demonstrate that the extent of phosphorylation of ZDV by PBMCs changes significantly with time in most patients. Figure 4 shows that, although the

TABLE 1. Pharmacokinetic parameters[a]

Time (no. of patients)	Intracellular ZDVPt parameter				Plasma ZDV parameter			
	AUC (pmol · h/10^6 cells)	$t_{1/2}$ (h)	C_{max} (pmol/10^6 cells)	T_{max} (h)	AUC (μmol · h/liter)	$t_{1/2}$ (h)	C_{max} (μM)	T_{max} (h)
Wk 4 (20)	3.29 ± 0.97	4.07 ± 1.13	0.48 ± 0.18	2.63 ± 1.36	2.65 ± 1.17	1.90 ± 0.73	1.25 ± 0.76	1.38 ± 0.74
Wk ≥24 (18)	2.16 ± 1.09[b]	3.96 ± 1.10	0.29 ± 0.18	2.94 ± 1.59	2.95 ± 1.53	2.05 ± 1.67	1.13 ± 0.50	1.77 ± 1.63

[a] Values are means ± standard deviations.
[b] Significantly lower than the week 4 AUC (P = 0.0035).

ZDVPt AUC was essentially equivalent to the plasma ZDV AUC upon initiation of therapy in patients from the standard-dose arm, there was increasing divergence with continued therapy. In all three patients enrolled in this arm, maximum phosphorylation occurred on day 1 of therapy. The ZDVPt AUC then declined to about 50% of baseline by week 4, although the plasma ZDV AUC actually increased. By week 24, the ZDVPt AUC averaged only 30% of baseline in these three patients. Because maximum phosphorylation was observed on day 1 of therapy in three consecutive, randomly recruited subjects, the data strongly suggested that induction of phosphorylation was not required and that slow accumulation of ZDVPt did not occur. Therefore, the standard-dose arm was closed after study of these three patients. Examination of data from the two dose experiments from all 21 study participants (as shown in Fig. 2B) confirmed that little accumulation of ZDVPt occurs after the first daily dose of ZDV, regardless of the duration of therapy and despite prior removal and clearance of the drug and intracellular metabolites. Additionally, a decline in intracellular ZDV phosphorylation with time occurred in virtually all patients: the ZDVPt AUC at week 24 or greater of therapy was significantly smaller than that at week 4 (P = 0.0035), although the plasma ZDV AUC was essentially unchanged (Table 1). Only one patient had a greater ZDVPt AUC at week 24 than at week 4.

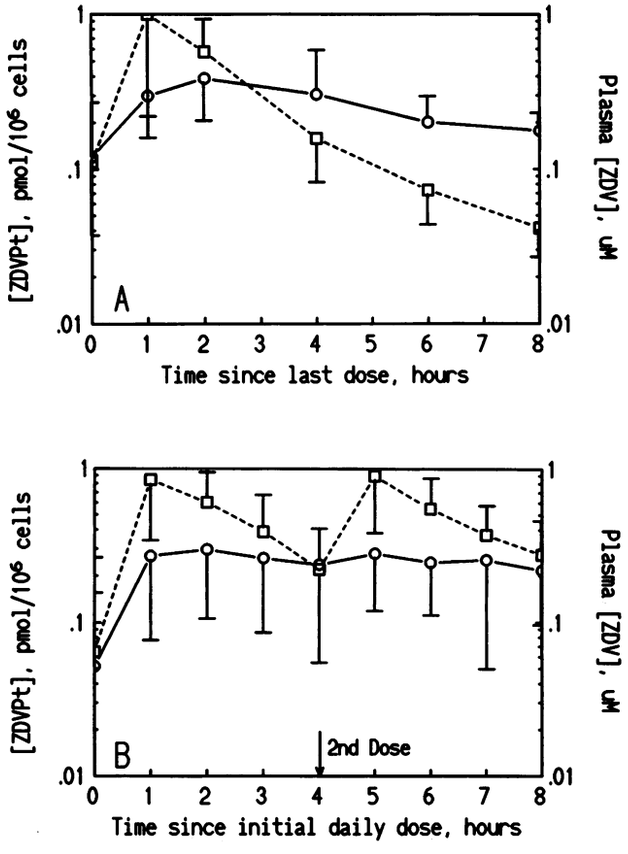

FIG. 2. Mean pharmacokinetic profiles at week 4 of standard therapy. Plots do not contain data from two patients who, for technical reasons, had samples drawn at times other than those prescribed by the study. Assuming a mean cell volume of 1 fl, picomole per 10^6 cells concentrations are approximately equivalent to micromolar concentrations. □, plasma ZDV; ○, ZDVPt. (A) Mean drug decay curves after a single 100-mg ZDV dose (n = 19 patients). (B) Mean drug levels during normal dosing. A 100-mg dose of ZDV was given at time zero and again at 4 h (ZDVPt, n = 18 patients; plasma ZDV, n = 16 patients). These mean plots were not used to calculate pharmacokinetic parameters; pharmacokinetic parameters were calculated from the individual patient profiles (not shown). Error bars represent standard deviations from the mean concentration.

Dose response. Over the dose range examined (20 to 100 mg every 4 h five times a day), the mean intracellular ZDVPt dose response was essentially linear (Fig. 5). However, there was considerable variability in dose response between patients and overlap in the concentration ranges for each dose; for individual patients, an increase in dose did not necessarily result in an increase in the measured ZDVPt concentration. The dose-response profile for plasma ZDV was similar but not identical to that of ZDVPt. Although the profile of mean concentration in plasma fits a linear dose-response model, the increase in concentration in plasma at 100 mg is somewhat suggestive of nonlinearity and may result from formulation differences be-

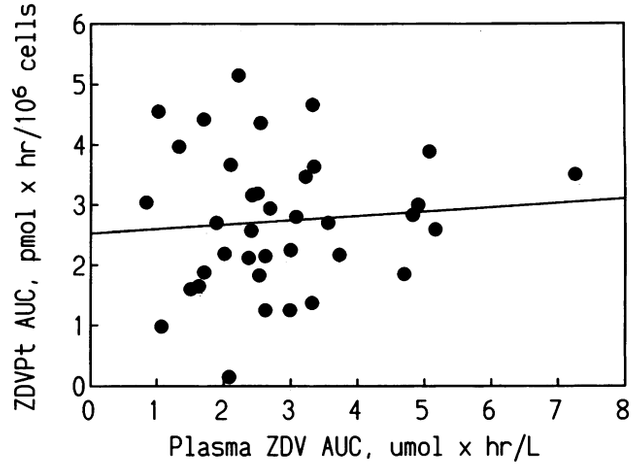

FIG. 3. Comparison of plasma and intracellular pharmacokinetics (n = 39; r^2 = 0.0072; no significant correlation). This figure shows AUC data determined at both weeks 4 and ≥24.

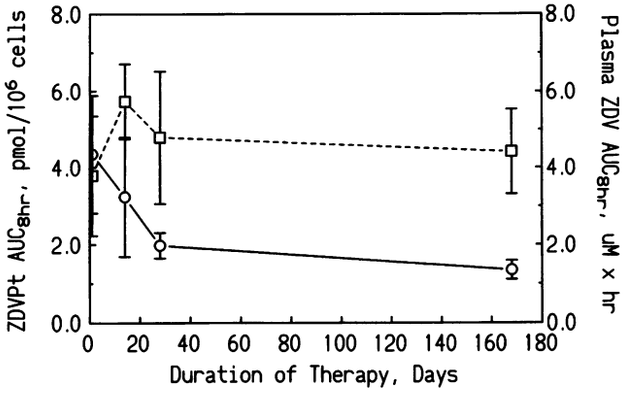

FIG. 4. Decline in zidovudine phosphorylation with duration of therapy in the standard-dose arm (n = 3). AUCs for this plot were determined over an 8-h period with dosing at 0 and 4 h, as in the accumulation experiments. □, plasma ZDV; ○, ZDVPt. Error bars represent standard deviations from the mean AUC.

tween the capsule used at the 100-mg dose and the elixir used at 20 to 80 mg. In contrast, the mean intracellular ZDVPt concentration at the same dose and capsule formulation increased linearly. A comparison between the mean concentration in plasma and the mean intracellular concentration at each dose (Fig. 6) shows their nonlinear relationship and suggests that a flattening of the plasma-intracellular concentration response curve occurs with increasing ZDV concentrations in plasma. For example, a 5-fold increase (20 to 100 mg) in dose resulted in a 6.2-fold increase in mean concentration in plasma but only a 2.0-fold increase in mean ZDVPt concentration. Individual plasma-intracellular concentration responses varied considerably between patients, as shown by the individual patient profiles in Fig. 6. Several patients showed little or no increase in ZDVPt concentrations with increasing ZDV concentrations in plasma, suggesting that in some patients the phosphorylation pathway may become saturated. A flattening of the plasma-intracellular concentration response curve is also suggested from the two-dose accumulation experiments, since mean intracellular ZDVPt concentrations increased little after introduction of a second 100-mg dose at 4 h, even though corresponding plasma ZDV concentrations increased signifi-

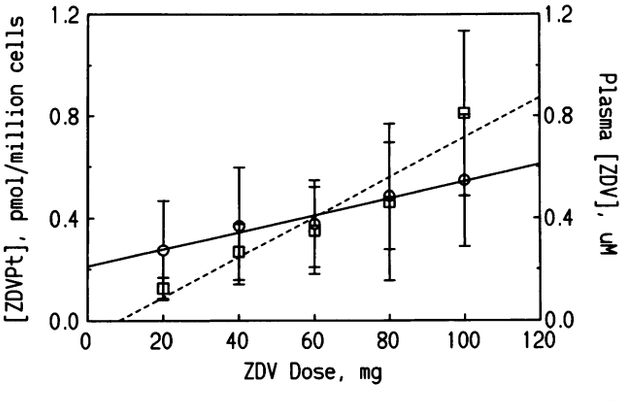

FIG. 5. Concentration response as a function of oral ZDV dose. □ (dashed line), plasma ZDV (r^2 = 0.91, P = 0.011); ○ (solid line), intracellular ZDVPt (r^2 = 0.96, P = 0.0035). Error bars indicate standard deviations from the mean concentration.

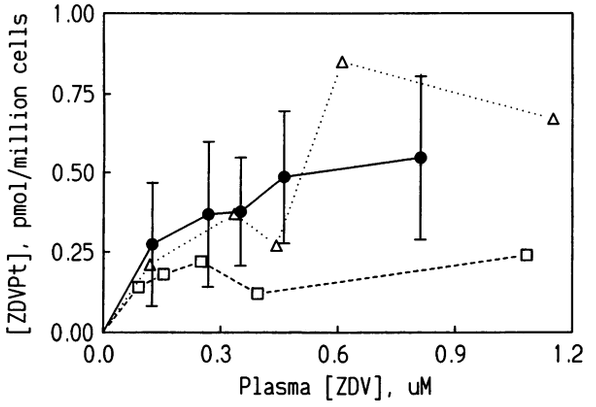

FIG. 6. Relationship between plasma ZDV and intracellular ZDVPt concentrations during escalating-dose experiments. Concentrations were measured 1.5 h after the time zero dose. ●, comparison of mean concentrations (n = 16). Error bars represent standard deviations from the mean intracellular concentration. Examples of individual extremes in concentration response are also shown: △, patient with an increased ability (versus the mean) to phosphorylate ZDV; □, patient with a decreased ability to phosphorylate ZDV. At the highest concentrations in plasma, note the approximately threefold difference in ZDVPt concentration between these patients despite nearly identical plasma ZDV concentrations.

cantly (Fig. 2B). Comparison of 8-h AUCs calculated from the mean plots shown in Fig. 2 shows a nearly 1.67-fold increase in plasma ZDV AUC from one versus two doses (2.42 versus 4.04 μmol · h/liter, respectively), while ZDVPt AUCs are essentially identical for one versus two doses (2.13 versus 1.98 pmol · h/10^6 cells, respectively).

Population differences. No significant differences in ZDV phosphorylation were observed between minority patients (n = 6) and the remainder of the study population. However, the mean ZDVPt AUC for female patients (n = 5) was significantly higher than that for male patients, even when corrected for differences in body weight. At week 4, the mean ± standard deviation female ZDVPt AUC was 4.29 ± 0.76 pmol · h/10^6 cells compared with 2.96 ± 0.80 pmol · h/10^6 cells for men (P = 0.0044). Additionally, the dose-response and two-dose phosphorylation patterns suggested that the flattening of the plasma-intracellular concentration response may be less pronounced in women than in men (not shown). Although preliminary and requiring further study because of the small population of women examined, these data suggest that women may phosphorylate ZDV differently than men.

DISCUSSION

We compared plasma ZDV pharmacokinetics and intracellular ZDVPt pharmacokinetics by examining the relationship between simultaneously determined AUCs. As previously suggested (22), no significant statistical correlation between plasma and intracellular AUCs was observed. Although it is possible that the observed correlation might be somewhat improved by using a sampling schedule more reflective of true peak plasma ZDV concentrations (i.e., prior to 1 h postdose), the interindividual variability in both plasma and intracellular pharmacokinetics makes it very unlikely that plasma ZDV concentrations can be used to predict intracellular concentrations of ZDVPt at any given time point with any degree of reliability. In a crude manner, the shape of the mean plasma-intracellular concentration response curve (Fig. 6) does sug-

gest that increased plasma ZDV concentrations result in increased ZDVPt concentrations. However, this relationship is complicated by apparent nonlinearity, possible saturation of phosphorylation, and intracellular metabolism that changes over time.

Pharmacokinetic parameters were similar to those previously described (5, 12), with a few exceptions. There were instances of delayed absorption of ZDV as evident from delays in plasma ZDV T_{max}. Some of these observed delays could have been due to drug administration following a fatty meal (14), since patients were not required to fast prior to their morning dose of drug. However, a few examples of extreme delay in plasma ZDV T_{max} were reproducible and could not readily be attributed to concurrent administration with a meal or drug known to interfere with ZDV absorption. Results confirm the intracellular $t_{1/2}$ of ZDVPt to be significantly longer than that of plasma ZDV as previously reported (22, 26). However, we also observed a terminal plasma ZDV $t_{1/2}$ longer than the 1 h commonly reported in the literature. This may be secondary to the longer interval of evaluation (8 versus typically 4 h) and/or the more sensitive assay technique employed. Others have similarly reported a prolonged plasma ZDV $t_{1/2}$ when pharmacokinetics were determined over a 24-h period (15). In the United States, a 4-h dosing interval is typically recommended and is based on a 1-h plasma ZDV $t_{1/2}$. Our plasma and intracellular $t_{1/2}$ data support an 8-h dosing interval, which has been used in some recent trials.

Slow induction of phosphorylating enzymes to reach maximum ZDV phosphorylation is apparently not required. In fact, total phosphorylation of ZDV was at a maximum on the first day of therapy in all three patients enrolled in the standard-dose arm. ZDVPt concentrations mirrored plasma ZDV concentrations on day 1, with increasing divergence with increasing duration of therapy. Given the small population examined, the probability that three randomly recruited subjects would have nearly identical patterns of phosphorylation over four distinct time points by chance is remote, particularly given the general variability in phosphorylation in the patient population as a whole. Therefore, we closed the standard-dose arm after study of these three patients to benefit the escalating-dose arm. Note that while the primary question of enzyme induction or maximum phosphorylation was satisfactorily answered after study of just three subjects, the calculated extent of the decrease in phosphorylation observed over the first 4 weeks of therapy in just three patients may not be reflective of the patient population as a whole. Additionally, it cannot be assumed from these data that the distribution of ZDV mono-, di-, and triphosphates is the same upon initiation of therapy as it is later in therapy.

We observed a decrease in intracellular ZDVPt AUC at week $\geq$24 compared with that at week 4 in virtually all patients. ZDVPt concentrations are an average obtained from a mixed population of PBMCs which may phosphorylate ZDV to different extents, probably secondary to their basic metabolic activity. We found little evidence that the gross composition of cell isolates changed significantly over the duration of the study. This suggests that the observed decrease in ZDVPt AUC is a result of a decreased ability of PBMCs to phosphorylate ZDV. Diminishing phosphorylation of ZDV with increasing duration of therapy has also recently been reported to occur in PBMCs isolated from children receiving long-term therapy (1). The mechanism of this change, the phosphorylated form or forms affected the most, and the resultant clinical implications must be resolved through further study; of particular interest is the potential relationship between diminishing phosphorylation and the emergence of ZDV-resistant viral strains.

The shape of the mean plasma-intracellular concentration response curve (Fig. 6) suggests that ZDV phosphorylation does not proportionally increase with higher concentrations in plasma and that in some patients phosphorylation may approach saturation. Although the shape of the curve may be slightly influenced by the 5-week duration of the study, the decrease in phosphorylation between each weekly time point should be small compared with the large percentage of increase in dose. This nonproportional response was also suggested by the relatively slight increase in mean intracellular ZDVPt concentration after the second dose of ZDV in the single-day accumulation experiments, which were not subject to the potential influence of duration of therapy. Saturation of phosphorylation has been suggested to occur in various leukocyte populations incubated with ZDV in vitro (2, 26). In a recent report describing specific measurement of ZDV triphosphate levels in PBMCs from six patients, a 4-fold increase in mean plasma ZDV concentration resulted in only a 1.7-fold increase in mean ZDV triphosphate concentration (18), similar to our observations for total phosphate. These findings are consistent with clinical observations regarding the lack of additional benefit of high (1,200 mg/day) versus low (600 mg/day) doses of ZDV (7). Given the variation in response after equivalent doses and/or concentrations in plasma, the saturation point probably varies between patients. Although the implications of these findings for optimizing individual therapeutic response require further study, potential saturation implies that some patients may benefit equally from doses lower than the current 100-mg standard (200 mg at some sites). Similarly, some patients might gain additional benefit from higher doses during later therapy if doses greater than 100 mg result in increased intracellular ZDVPt concentrations without an accompanying increase in toxicity.

Data from this study suggest that for asymptomatic, HIV-infected adults, 100 mg every 8 h (300 mg/day) may approximate the optimum initial dosing regimen. However, individually optimized use of a therapeutic agent requires the establishment of a therapeutic concentration range (a concentration range which is maximally beneficial and minimally toxic) for the active form or forms of the drug. Although we measured total phosphorylated ZDV rather than the active ZDV triphosphate, ZDV triphosphate concentrations may be related to ZDVPt concentrations when equilibrium between phosphorylated metabolites is achieved during chronic dosing, especially if total phosphorylation is at or near saturation. Although we cannot offer specific data on the relationship between total phosphorylated ZDV and ZDV triphosphate, it is apparent from other recently published studies that ZDVPt as measured by our method shares similar pharmacokinetic characteristics with the triphosphate as measured by completely different methods: i.e., concentrations apparently diminish with duration of therapy (1), do not increase proportionally with plasma ZDV concentrations (18), and have a longer $t_{1/2}$ than plasma ZDV (18). In contrast to methods reported for measurement of the individual metabolites (1, 11, 18, 19, 21), measurement of total phosphate requires far fewer labor-intensive separation and cleanup steps, allows for direct determination of metabolites in smaller samples of patient cells, and does not suffer from interferences from endogenous nucleosides. Therefore, measurement of ZDVPt can potentially be used in much larger study populations. Additional data generated from ACTG Protocol 161 indicate that ZDVPt AUC correlates with changes in surrogate markers associated with disease progression and ZDV toxicity (20). We therefore

speculate that monitoring of ZDVPt concentrations in PBMCs may allow individualization of therapy and/or suggest appropriate times to intervene with alternative or additional therapies before clinical events occur.

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