Multiple-Dose Pharmacokinetics of Oral Zidovudine in Hemophilia Patients with Human Immunodeficiency Virus Infection

GENE D. MORSE,^{1,2*} AMY PORTMORE,^{2,3} JOHN OLSON,^{2,3} CHARLENE TAYLOR,^{1,2} CAROL PLANK,² AND RICHARD C. REICHMAN^{2,3}

Departments of Pharmacy and Medicine, State University of New York at Buffalo, Erie County Medical Center, Buffalo, New York 14215,¹ and AIDS Clinical Trial Unit, University of Rochester,² and Department of Medicine, University of Rochester School of Medicine and Dentistry,³ Rochester, New York 14627

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The disposition of zidovudine (ZDV) was examined during chronic oral dosing (300 mg every 4 h while awake) for 12 weeks in eight asymptomatic patients with hemophilia who were infected with the human immunodeficiency virus. Pharmacokinetic studies were conducted at the initiation of drug administration and after 6 and 12 weeks. Baseline liver function tests indicated normal values for bilirubin, albumin, and prothrombin time, while hepatic enzyme levels ranged from one to three times the normal levels. Initially, the mean peak ZDV concentration in plasma was 2,052 ng/ml with a range of 1,033 to 3,907 ng/ml, while during chronic dosing the peaks were $1,619 \pm 1,062$ ng/ml and $1,711 \pm 786$ ng/ml at weeks 6 and 12, respectively. ZDV concentrations at 4 h declined to 77 ± 53 ng/ml, 110 ± 43 ng/ml, and 101 ± 49 ng/ml at weeks 1, 6, and 12, respectively. Initially, the plasma concentration-versus-time decay in three patients was linear, with a mean half-life ($t_{1/2}$) of 1.3 ± 0.5 h, while five patients had detectable concentrations in plasma after 4 h with an apparent delayed terminal-phase $t_{1/2}$ of 4.8 ± 2.8 h. At week 6 the prolonged elimination pattern was noted in all patients (terminal $t_{1/2} = 4.1 \pm 2.0$ h). No correlation between hepatic enzyme levels and $t_{1/2}$ was noted. These findings suggest that ZDV may display a prolonged elimination phase during multiple dosing. Further studies utilizing a more sensitive assay may help to further define this later phase of ZDV elimination.

Zidovudine (ZDV) is the only antiviral agent currently licensed for treatment of symptomatic human immunodeficiency virus (HIV) infection (4). A pharmacologic consideration for the use of ZDV in hemophilia patients with HIV disease is the presence of chronic hepatitis among many of these patients and the potential for impaired drug metabolism (2, 3, 6, 8-10). A recent report on the disposition of a single oral dose of ZDV in patients with hemophilia described considerable variability with regard to the concentrations in plasma attained (12). The present study was conducted to extend these initial findings and to examine the pharmacokinetics of oral ZDV in asymptotically HIV-infected hemophilia patients during chronic oral administration.

MATERIALS AND METHODS

Eight stable hemophilia patients without cardiac, gastrointestinal, renal, or other hematologic disease were admitted to the study after informed consent was obtained. All patients had antibodies to HIV as determined by enzyme-linked immunosorbent assays and Western blot (immuno-blot) tests. Serum chemistries, complete blood counts, peripheral T-helper cell counts, and serum p24 antigen levels were determined throughout the study period. Patients were excluded if they were receiving any drugs which theoretically could have altered hepatic glucuronidation of ZDV. After a patient was admitted to the Clinical Research Center at the University of Rochester, an intravenous catheter was placed and patency was maintained with a dilute heparin solution for each pharmacokinetic study, the patient was maintained without food for 6 h before and 2 h after oral drug administration, and all subsequent doses of ZDV were discontinued until discharge. After the completion of a 24-h intravenous study, a single oral ZDV dose (300 mg) was

administered and blood samples were obtained at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h. Serum was obtained by centrifugation and stored at -70°C until assayed. The patients were then discharged and instructed to take 300 mg of ZDV (three 100-mg capsules) every 4 h while awake for a 12-week period. Pharmacokinetic studies were repeated after 6 and 12 weeks as described above.

ZDV was measured in plasma with a high-pressure liquid chromatography (HPLC) technique originally described by Good et al. (5). Plasma standards (5,000 to 38 ng/ml) and unknown ZDV concentrations were determined by calculation of the ratio of the corresponding peak area to the area of the internal standard. Linear regression was used to determine the unknown sample concentrations. High (4,000 ng/ml) and low (200 ng/ml) quality control samples for ZDV were analyzed with each set of patient samples. The intraday relative standard deviations were 7.3 and 8% for the high and low quality controls, respectively. The lower limit of ZDV detection was 38 ng/ml, and data presented in the Results section reflect only those time points at which the ZDV concentration was higher than the lower limit of detection. The interday relative standard deviation for the standard of 38 ng/ml was 10.2%. In addition, we participated in two quality control programs sponsored by Burroughs Wellcome Laboratories and the AIDS Clinical Trials Group.

The plasma concentration data were analyzed, using both a noncompartmental pharmacokinetic technique (14) and a multicompartmental, nonlinear regression analysis (NONLIN [11]). The following parameters were determined: area under the plasma concentration-versus-time curve (AUC); elimination rate constant, calculated as the slope of the terminal phase of the log-linear plasma concentration-versus-time profile; and oral clearance (CL_{oral}), obtained by dividing the dose by the AUC. Peak and 4-h concentrations, AUC, CL_{oral} , and half-life ($t_{1/2}$) parameters were statistically

* Corresponding author.

TABLE 1. Liver function tests for eight hemophilia patients receiving oral ZDV for 12 weeks

Patient	Aspartate transaminase (U/liter) at wk:			Alanine transaminase (U/liter) at wk:			Alkaline phosphatase (U/liter) at wk:			Bilirubin (g/dl) at wk:		
	1	6	12	1	6	12	1	6	12	1	6	12
1	59 ^a	52 ^a	57 ^a	56 ^a	43 ^a	43 ^a	139 ^a	114 ^a	109	0.5	0.4	0.8
2	50 ^a	29	44 ^a	41	37	48 ^a	88	84	91	0.8	0.9	0.5
3	41 ^a	27	35	31	10	15	135 ^a	105	96	0.4	0.4	0.6
4	50 ^a	49 ^a	40 ^a	93 ^a	80 ^a	76 ^a	59	63	64	0.8	0.4	0.6
5	86 ^a	143 ^a	853 ^a	57 ^a	136 ^a	419 ^a	162 ^a	173 ^a	161 ^a	0.3	0.4	0.7
6	220 ^a	122 ^a	106 ^a	120 ^a	77 ^a	109 ^a	139 ^a	112 ^a	121 ^a	0.5	0.7	0.5
7	71 ^a	121 ^a	69 ^a	61 ^a	116 ^a	41	105	87	98	0.4	0.6	0.4
8	104 ^a	106 ^a	99 ^a	81 ^a	109 ^a	64 ^a	109	121 ^a	115 ^a	0.5	0.5	0.6

^a Abnormal value. Normal ranges: aspartate transaminase, 10 to 36 U/liter; alanine transaminase, 9 to 41 U/liter; alkaline phosphatase, 40 to 110 U/liter; bilirubin, 0 to 1.4 g/dl.

evaluated with analysis of variance and the Student *t* test for repeated measures. In addition, because of the bimodal nature of the data, the harmonic mean was determined.

RESULTS

Patient characteristics. The mean age of the patients was 37 ± 10 years, and the mean weight was 74 ± 17 kg. All patients had serum chemistry values within normal limits. The mean serum creatinines were 0.8 ± 0.1 , 0.8 ± 0.1 , and 0.8 ± 0.1 mg/dl at weeks, 1, 6, and 12, respectively. The T4 levels were 203 ± 128 , 173 ± 55 , and 247 ± 126 mm³; p24 antigen levels were detectable only in patients 1 (8.7 pg/ml) and 3 (10.5 pg/ml) upon entry into the study. Individual values for aspartate transaminase, alanine transaminase, alkaline phosphatase, and bilirubin are summarized in Table 1. Patient 5 had elevated alkaline phosphatase levels upon entry. Protocol guidelines dictated that this patient be discontinued because of a persisting elevation of alkaline phosphatase at 6 weeks, since it could not be ruled out that ZDV may have been a contributing factor. Data from two other patients at 12 weeks were not available because of noncompliance with the study protocol.

Pharmacokinetics. ZDV was rapidly absorbed, with the peak concentration occurring at 0.5 to 1.0 h. The mean peak ZDV concentrations were $2,052 \pm 970$ ng/ml (week 1), $1,619 \pm 1,062$ ng/ml (week 6), and $1,711 \pm 786$ ng/ml (week 12) ($P > 0.05$). Concentrations in plasma declined in a linear fashion with mean 4-h concentrations of 77 ± 53 ng/ml (week 1), 110 ± 43 ng/ml (week 6), and 101 ± 49 ng/ml (week 12) ($P > 0.05$). Individual concentrations in plasma are presented in Table 2.

The mean AUCs were $3,216 \pm 1,722$, $2,756 \pm 712$, and $2,588 \pm 842$ ng · h/ml at weeks 1, 6, and 12, respectively. The segment of the total AUC for each study day that was extrapolated comprised 6.5, 13.6, and 9.8% of the total AUC, respectively. The mean CL_{oral}s were 1.98 ± 1.04 liters/min per 70 kg after the first oral dose, 1.94 ± 0.69 liters/min per 70 kg at week 6, and 1.90 ± 0.76 liters/min per 70 kg at week 12 ($P > 0.05$).

Visual inspection of the plasma concentration-versus-time profiles indicated a variable pattern of ZDV disposition. After the first oral dose, five of eight patients demonstrated delayed patterns of elimination after 4 h (mean terminal $t_{1/2}$, 4.8 ± 2.8 h), while three patients had monoexponential declines (mean $t_{1/2}$, 1.3 ± 0.5 h) with undetectable ZDV concentrations after 4 h (Fig. 1, top). At week 6, all patients had detectable ZDV concentrations after 4 h, but the number of measurable time points varied among patients (Fig. 1, middle). Of the five patients studied at 12 weeks, two reverted to linear decays (mean terminal $t_{1/2}$, 1.7 ± 0.2 h) and

three retained delayed elimination patterns (mean terminal $t_{1/2}$, 5.4 ± 2.7 h) (Fig. 1, bottom). Table 3 summarizes the individual pharmacokinetic parameters.

DISCUSSION

The pharmacokinetic data obtained from this group of hemophilia patients indicate that a fixed-dose ZDV regimen resulted in a variable AUC both within and between patients during chronic therapy. Initially, the peak ZDV concentration in plasma ranged from 1,033 ng/ml (dose, 4.8 mg/kg) in patient 6 to 3,907 ng/ml (dose, 4.6 mg/kg) in patient 2. The fact that these two patients received the same dose (even when corrected for body weight) illustrates a potential drawback to the use of a standardized ZDV-dosing regimen because variable pharmacokinetics may lead to differing ZDV plasma levels. In addition to variations among patients, peak ZDV concentrations within a patient were unpredictable during chronic dosing. As shown in patient 2, peak ZDV concentrations decreased from an initial value of 3,907 ng/ml to 886 ng/ml at week 6. On the other hand, patient 5 had a twofold increase in peak concentration from week 1 to week 6. The observation that there was a poor correlation between the weight-normalized dose and peak concentrations suggested that intra- or interindividual patterns of ZDV adsorption or first-pass metabolism have been responsible for variations among patients. These data contrast with the minimal intersubject pharmacokinetic variability that has been previ-

TABLE 2. ZDV concentrations in plasma during multiple dosing for a 12-week period

Patient	Dose (mg/kg)	ZDV concn (ng/ml) at indicated wk					
		Peak			4-h		
		1	6	12	1	6	12
1	3.2	1,086	1,013	1,839	71	103	78
2	4.6	3,907	886	NS ^a	71	138	NS
3	5.0	2,438	2,425	2,376	47	78	82
4	3.0	2,336	2,327	2,303	42	98	82
5	5.3	1,654	3,623	NS	54	96	NS
6	4.8	1,033	1,129	1,610	47	82	75
7	4.8	1,357	491	NS	79	80	NS
8	3.4	2,607	1,060	425	204	205	189
Mean	4.3	2,052 ^b	1,619 ^b	1,711 ^b	77 ^b	110 ^b	101 ^b
SD	0.9	970	1,062	786	53	43	49

^a NS, Not studied.

^b No significant difference by analysis of variance between weeks 1, 6, and 12.

FIG. 1. Plasma concentration-versus-time profiles for patients receiving oral ZDV administration (300 mg every 4 h while awake). Each symbol represents the same patient at weeks 1, 6, and 12. The last datum point in each curve represents the last concentration in plasma within the limit of detection for the HPLC method, despite the extensive 24-h period of sample collection.

ously reported for patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) (1, 7).

In addition to variable bioavailability, the distribution and elimination pattern of ZDV in our hemophilia patients was different from previous data obtained from AIDS or ARC patients. Klecker et al. (7) and Blum et al. (1) reported the disposition of ZDV in AIDS and ARC patients who received oral doses of 2 to 5 mg/kg every 4 h. In both reports, ZDV disposition followed a monoexponential decline during oral dosing and yielded a mean $t_{1/2}$ of 1.1 h. In the present study, three patients demonstrated similar linear patterns after the first oral dose, but five patients had measurable ZDV concentrations after 4 h. This pattern was also present in most patients after multiple dosing, suggesting that ZDV elimination from a deep tissue compartment was occurring. However, the data did not fit well to a nonlinear multicompartmental analysis, probably because of an inadequate number of measurable values of concentration in plasma. The observation of prolonged elimination phases in our hemophilia patients may be a unique characteristic of this population. On the other hand, it should be noted that the two studies cited above (1, 7) utilized a 4-h sampling protocol as well as a lower dose in some patients. The shorter sampling period would have prevented the investigators from describing the latter phase, and the lower dose may have yielded concentrations that were lower than the detection limit of the HPLC assay. With regard to the reproducibility of the lower ZDV concentrations in the present study, the values were reproduced by HPLC analysis on different days. The potential for assay interference has been assessed by the original investigators of this HPLC method (5). In addition, the exclusion of patients who were receiving other chronic medications

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TABLE 3. Pharmacokinetic parameters describing oral ZDV disposition during chronic administration to HIV-infected hemophilia patients

Patient	Dose (mg/kg)	wk 1			wk 6			wk 12		
		AUC (ng · h/ml)	CL _{oral} (liters/min per 70 kg)	t _{1/2} (h)	AUC (ng · h/ml)	CL _{oral} (liters/min per 70 kg)	t _{1/2} (h)	AUC (ng · h/ml)	CL _{oral} (liters/min per 70 kg)	t _{1/2} (h)
1	3.2	1,960	1.82	1.4	2,212	1.61	4.1	2,133	1.68	1.9
2	4.6	6,170	0.91	2.6	2,244	2.38	2.7	NS ^a	NS	NS
3	5.0	3,866	1.61	9.4	3,019	2.10	3.7	3,355	1.82	2.0
4	3.0	2,860	1.26	3.1	3,507	0.98	2.5	3,633	0.98	7.6
5	5.3	1,958	3.22	1.7	3,790	1.61	5.0	NS	NS	NS
6	4.8	1,942	2.87	0.8	2,168	2.59	2.4	1,789	3.08	1.5
7	4.8	1,672	3.36	3.3	1,897	2.97	8.6	NS	NS	NS
8	3.4	5,296	0.77	5.5	3,212	1.23	3.8	2,032	1.96	6.5
Mean	4.3	3,216 ^b	1.98 ^b	3.5 ^b /2.6 ^c	2,756 ^b	1.94 ^b	4.1 ^b /3.5 ^c	2,588 ^b	1.90 ^b	3.9 ^b /2.5 ^c
SD	0.9	1,722	1.04	2.8	712	0.69	2.0	842	0.76	2.9

^a NS, Not studied.^b No significant difference by analysis of variance between weeks 1, 6, and 12.^c Harmonic mean.

which might interfere with the HPLC analysis as well as the eventual return to baseline for all patient chromatograms after 12 h would argue against assay interference as an explanation for the observed ZDV concentrations after 4 h. On the basis of the known intracellular site of action for ZDV, it seems reasonable that the disposition of this antiviral agent would be described by a multicompartmental pattern during chronic dosing.

In summary, administration of ZDV to asymptotically HIV-infected hemophilia patients over a 12-week period produced a wide range of ZDV concentrations in plasma. A prolonged pattern of ZDV elimination was observed during multiple dosing, and a longer terminal $t_{1/2}$ than has been previously reported was noted. Clearly, the intracellular metabolism of ZDV to the active triphosphate moiety, as well as the cumulative nature of certain ZDV toxicities (13), makes interpretation of extracellular pharmacokinetic observations difficult. However, the intra- and interpatient variations observed in this group of HIV-infected hemophilia patients suggest that during long-term ZDV administration, not all patients receiving a similar dosage will receive an equivalent systemic exposure to ZDV. More studies are needed to correlate extracellular ZDV disposition with intracellular phosphorylation patterns and, ultimately, with an anti-HIV effect. In addition, the use of an assay with greater sensitivity (i.e., radioimmunoassay) may allow for a more accurate determination of ZDV elimination kinetics.

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