*Antiviral Research*, 11 (1989) 57–66
Elsevier



AVR 00336

# Pharmacokinetics of orally administered zidovudine among patients with hemophilia and asymptomatic human immunodeficiency virus (HIV) infection

Gene D. Morse,[1,2] John Olson,[1,3,4] Amy Portmore,[4] Charlene Taylor,[1] Carol Plank[1,4] and Richard C. Reichman[1,4]

[1]*University of Rochester AIDS Clinical Trial Group,* [2]*Department of Pharmacy, State University of New York at Buffalo, Erie County Medical Center, Buffalo, New York,* [3]*Rochester Hemophilia Center and* [4]*Infectious Diseases and Hematology Units, Department of Medicine; University of Rochester School of Medicine, Rochester, New York, U.S.A.*

(Received 20 July 1988; accepted 9 December 1988)

**Summary**

Zidovudine (formerly azidothymidine, AZT) is used to treat certain patients infected with the human immunodeficiency virus (HIV). However, the clinical use of zidovudine (ZDV) in hemophilia patients may be complicated by the high incidence of chronic hepatitis in this patient population. To examine the pharmacokinetics of ZDV eight asymptomatic HIV-infected hemophilia patients received a single oral dose (300 mg). ZDV and its glucuronide metabolite (GZDV) were measured in serum by HPLC. ZDV was rapidly absorbed with a wide range of peak serum concentrations (2052 ± 970 ng/ml) at 0.5 h. Peak GZDV serum concentrations were 4751 ± 2269 ng/ml at 1 h. Both ZDV and GZDV declined in a biexponential manner over 4 h. After 4 h, the ZDV serum concentration decay in three patients continued a log-linear decline, while five patients demonstrated a tri-exponential curve which had a mean terminal elimination half-life of 4.8 ± 2.8 h. No relationship between ZDV or GZDV kinetics and the degree of hepatic enzyme elevation was observed. Although a therapeutic window for ZDV has yet to be described, the wide range of serum concentrations that result from a standard dose suggests that clinical monitoring of ZDV levels may be of value in certain

*Correspondence to*: Gene D. Morse, State University of New York at Buffalo, Erie County Medical Center, 462 Grider Street, Buffalo, NY 14215, U.S.A.

patients. In addition, the prolonged elimination half-life of ZDV in the present study may provide a rationale for less frequent dosing in certain patients.

Zidovudine; Hemophilia; Human immunodeficiency virus infection

## Introduction

Zidovudine (formerly azidothymidine, AZT) is often administered to patients who exhibit clinical manifestations of infection with the human immunodeficiency virus (HIV). Many patients with hemophilia are infected with HIV as a result of contaminated factor VIII administration prior to 1985. Because of the high incidence of chronic non-A, non-B hepatitis in this population (Mannucci et al., 1975), and the dependence of zidovudine on hepatic biotransformation, the potential for decreased drug metabolism and increased toxicity in hemophilia patients is an important concern. Thus, optimal use of zidovudine for the treatment of hemophilia patients who develop the acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) requires that the pharmacokinetics of this agent be examined in this patient population. The present study was conducted to examine the disposition of zidovudine (ZDV) and its glucuronide metabolite (GZDV) following oral administration to asymptomatically HIV-infected patients with hemophilia.

## Methods

*Patients*

Patients with hemophilia were admitted to the study after written informed consent was obtained. All patients were infected with HIV as determined by two consecutively positive ELISA tests and a positive confirmatory Western blot test. No patient had severe cardiopulmonary, gastrointestinal or renal disease and none were symptomatic from their HIV infection. In addition, patients were not receiving any chronic medications suspected of interfering with zidovudine metabolism. Baseline hematologic, chemistry and clotting studies were performed prior to drug administration.

*Kinetic studies*

Patients were admitted to the Clinical Research Center at the University of Rochester and were kept n.p.o. prior to receiving ZDV. An indwelling venous catheter was placed prior to initiation of the study, and a blood sample (5 ml) was obtained prior to zidovudine administration. Zidovudine (3 × 100 mg capsules) was administered orally with 8 oz. of water and additional blood samples were obtained at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h. Serum was obtained by centrifugation and was stored at −70°C until assayed.

*Zidovudine analysis*

ZDV and GZDV were measured by utilizing the method of Good and Reynolds (1987). Serum samples were heat-inactivated for 5 h at 56°C to eliminate retroviral activity (McDougal et al., 1985; Resnick et al., 1986). Stability studies of ZDV and GZDV were conducted which indicated that no degradation occurred during the inactivation period. To 0.5 ml of patient sample or serum standard [0.1–20 μM (39–5000 ng/ml) of analytical grade ZDV; 0.2–40 μM (149–19000 ng/ml) of GZDV], 0.1 ml of internal standard (BW-A22U, Burroughs Wellcome Labs, North Carolina) was added. The sample was then allowed to stand for 15 min to attain equilibrium.

ZDV and GZDV were extracted from serum with a liquid-solid extraction phase technique. Three-milliliter extraction columns (C-18, Analytichem International, Harbor City, CA) were conditioned with one reservoir volume of methanol, followed by two volumes of phosphate buffered saline (PBS). Vacuum was adjusted to 4–7 inches of Hg and controlled with a plastic stopcock. Serum samples were then transferred to the individual extraction columns and washed with 1 ml PBS. The column was then dried for 5 min under full vacuum. ZDV and GZDV were eluted with two 1 ml rinses with methanol and evaporated to dryness under nitrogen in a water bath at 37°C. Samples were reconstituted with 200 μl of 15% acetonitrile in water and injected (50–150 μl) onto the HPLC system.

The HPLC system consisted of a Varian 5000 pump, (Varian Laboratories, Walnut Creek, CA), a Waters variable wavelength detector and 710B automatic injector (Waters Associates, Milford, MA). The composition of the mobile phase was adjusted with a gradient elution program which consisted of ammonium phosphate buffer:acetonitrile (90:10, volume/volume) × 26 min, a linear change to 30% acetonitrile × 0.3 min, 30% acetonitrile × 9 min, then a linear change back to ammonium phosphate buffer:acetonitrile (90:10) over 3 min which was then continued × 15 min. The mobile phase was run at 1.3 ml/min and ZDV and GZDV were detected at 267 nm.

ZDV and GZDV 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 and low quality control samples for ZDV and GZDV were analyzed with each set of patient samples and yielded an intraday and interday relative standard deviation of $< 7\%$. In addition, we participated in a quality control program sponsored by Burroughs Wellcome Laboratories in which unknown samples were sent to our laboratory for analysis. Determinations of the unknown concentrations revealed $< 8\%$ error.

*Pharmacokinetic analysis*

The area under the serum concentration versus time curve (AUC) and the area under the moment curve (AUMC) were determined by polynomial interpolation with the LAGRAN computer program (Rocci and Jusko, 1983). The serum concentration data were graphed on a log-linear plot and the terminal elimination phase was determined by visual inspection of the curve. The apparent oral clearance ($Cl_{oral}$) was calculated by Dose/AUC, where dose was equal to 300 mg. The elim-

ination phase half-life was calculated by $0.693/\lambda_Z$, where $\lambda_Z$ was the slope of the terminal elimination phase. The mean residence time was calculated as AUMC/AUC.

## Results

Demographic characteristics of the patients are summarized in Table 1. All patients had clotting studies consistent with their disease and normal hematologic tests. Patients had a wide range of mild hepatic enzyme elevations. Other routine chemical studies were normal, including indicators of severe liver dysfunction (bilirubin, prothrombin time, albumin) (Table 2). The mean bilirubin was 0.5 ± 0.2 g/dl and the mean prothrombin time was 11.7/11.0 s, while the mean serum albumin was 4.1 ± 0.5 g/dl. The mean total number of peripheral T4 cells was 203 ± 128/mm$^3$.

Following oral administration, zidovudine was rapidly absorbed with a mean peak serum concentration of 2151 ± 898 ng/ml at 0.5 h (Table 3). The serum concentration then declined in a bi-exponential fashion during the first 4 h in all patients. After 4 h, three patients continued a log-linear decline until the concentrations were below sensitivity. However, in five patients a third exponential phase was apparent which yielded measurable concentrations until 6–10 h. Fig. 1 illustrates the two different patterns of zidovudine disposition which were noted. The mean zidovudine concentration was 77 ng/ml at 4 h, and ranged from 35–204 ng/ml. GZDV serum concentrations peaked at 1–2 h with a mean concentration of 4751 ± 2269 ng/ml. The mean GZDV concentration was 244 ± 185 ng/ml at 4 h. The metabolite disposition profile followed a similar pattern to that of the parent compound. However, the ratio of GZDV to ZDV over the study period was variable among the patients (Fig. 2), and patients with a higher ratio tended to have a lower ZDV

TABLE 1

Demographic data for eight hemophilia patients receiving oral zidovudine

| Patient | Sex | Age (yr) | Weight (kg) | Scr (mg/dl) | T4 level (No./mm$^3$) |
|---|---|---|---|---|---|
| 1 | M | 43 | 94 | 1.0 | 145 |
| 2 | M | 27 | 65 | 0.8 | 221 |
| 3 | M | 46 | 60 | 0.7 | 176 |
| 4 | M | 25 | 100 | 0.8 | 502 |
| 5 | M | 31 | 57 | 0.6 | 103 |
| 6 | M | 34 | 63 | 0.6 | 188 |
| 7 | M | 35 | 63 | 0.8 | 103 |
| 8 | M | 33 | 88 | 0.9 | 189 |
| | Mean | 37 | 74 | 0.8 | 203 |
| | SD | 10 | 17 | 0.1 | 128 |

TABLE 2

Liver function tests for eight hemophilia patients receiving oral zidovudine

| Patient | AST (u/l) | ALT (u/l) | Alk Phos (u/l) | GGT (u/l) | Bilirubin (g/dl) | Prothrombin time (s) | Albumin (g/dl) |
|---|---|---|---|---|---|---|---|
| 1 | 59* | 56* | 139* | 34 | 0.5 | 12.4/11.0 | 4.1 |
| 2 | 50* | 41 | 88 | 21 | 0.8 | 10.5/11.0 | 4.2 |
| 3 | 41* | 31 | 135* | 27 | 0.4 | 11.4/11.0 | 3.4* |
| 4 | 50* | 93* | 59 | 14 | 0.8 | 14.2/11.0 | 3.9 |
| 5 | 86* | 57* | 162* | 500* | 0.3 | 11.1/11.0 | 3.9 |
| 6 | 220* | 120* | 139* | 46* | 0.5 | 11.5/11.0 | 4.6 |
| 7 | 71* | 61* | 105 | 56* | 0.4 | 10.6/11.0 | 5.0 |
| 8 | 104* | 81* | 109 | 35 | 0.5 | 11.5/11.0 | 3.7 |
| Mean | 85 | 68 | 117 | 92 | 0.5 | 11.7/11.0 | 4.1 |
| SD | 58 | 29 | 33 | 166 | 0.2 | 1.2/0.0 | 0.5 |

Normal range: AST, 10–36 u/l; ALT, 9–41 u/l; GGT, 6–28 u/l (male), 4–18 u/l (female); Alk Phos, 40–110 u/l; Bilirubin, 0–1.4 g/dl; Albumin, 3.5–4.6 g/dl; Prothrombin time, 9.5–12.5 s.
*Indicates an abnormal value.

AUC. This observation is consistent with the wide range of $Cl_{oral}$ values that were noted.

As a result of the two different patterns of disposition, the pharmacokinetic parameters describing ZDV and GZDV varied considerably. The mean zidovudine AUC was 3201 ± 1694 ng h/ml which then normalized to the dose yielded oral clearance values that ranged from 11 to 48 ml/min/kg. The mean terminal phase serum half-life in the patients with a bi-exponential decay was 1.3 ± 0.5 h, while in the patients with a tri-exponential profile the mean half-life was 4.8 ± 2.8 h. It should be noted that in three of the five patients with a tri-exponential profile the terminal elimination phase half-life was calculated from only two data points. Al-

TABLE 3

Zidovudine and zidovudine-glucuronide serum concentrations (ng/ml) following a single 300 mg oral dose to eight HIV-infected patients with hemophilia

| Patient | Dose (mg/kg) | Peak | | 4 h | |
|---|---|---|---|---|---|
| | | ZDV | GZDV | ZDV | GZDV |
| 1 | 3.2 | 1086 | 4795 | 71 | 566 |
| 2 | 4.6 | 3907 | 7282 | 71 | 121 |
| 3 | 5.0 | 2438 | 2333 | 47 | 104 |
| 4 | 3.0 | 2336 | 4219 | 42 | 78 |
| 5 | 5.3 | 1654 | 7103 | 54 | 193 |
| 6 | 4.8 | 1033 | 3383 | 47 | 138 |
| 7 | 4.8 | 1357 | 7268 | 79 | 264 |
| 8 | 3.4 | 2607 | 1625 | 204 | 487 |
| Mean | 4.3 | 2052 | 4751 | 77 | 244 |
| SD | 0.9 | 970 | 2269 | 53 | 185 |

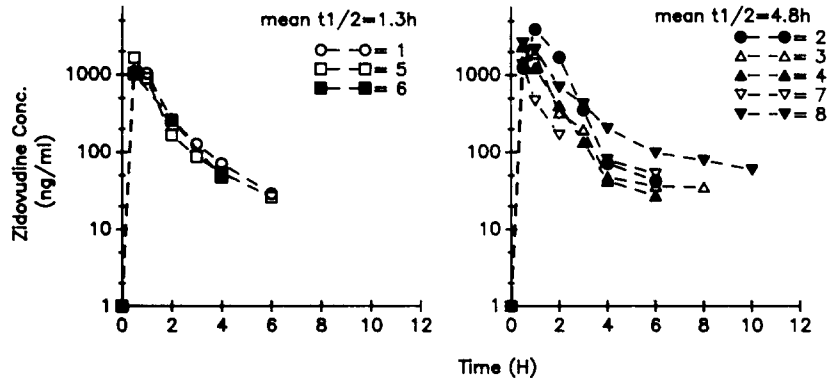


Fig. 1. Serum concentration versus time profiles following oral zidovudine administration. In some patients a bi-exponential pattern was observed (left) and in others a tri-exponential pattern (right).

though these values were at the lower end of the standard curve, the interday coefficient of variation for the 39 ng/ml standard was 7%. Therefore we interpreted these data as a true terminal elimination phase.

## Discussion

Two important considerations for ZDV therapy arise from these data obtained in the asymptomatic HIV-infected patients with hemophilia. As indicated in Table 3, there was a wide range of peak concentrations obtained following the administration of a standard 300 mg dose to all subjects. This observation may have resulted from interindividual differences in absorption or variable first-pass metabolism of ZDV. The only previously reported study of oral ZDV disposition utilized a solution of drug in most of their patients, and therefore a comparison with the

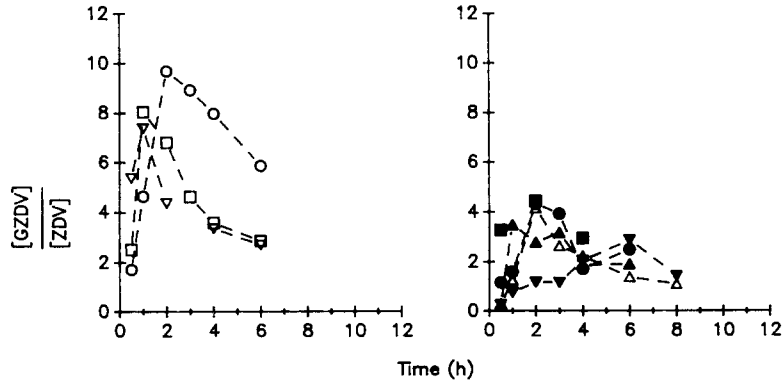


Fig. 2. The ratio of GZDV to ZDV following oral ZDV administration. The patients were divided into those with a maximum pealc ratio > 5 (left) or < 5 (right).

TABLE 4

Pharmacokinetic parameters for zidovudine and zidovudine-glucuronide following oral administration

| | Zidovudine | Zidovudine-glucuronide |
|---|---|---|
| AUC (ng/ml/h) | 3201 ± 1694 | 8097 ± 3850 |
| $Cl_{oral}$ (ml/min/kg) | 28 ± 15 | NC |
| Elimination phase half-life* (h) | | |
| Bi-exponential | 1.3 ± 0.5 | 1.2 ± 0.5 |
| Tri-exponential | 4.8 ± 2.8 | 5.2 ± 3.7 |
| Elimination rate constant* ($h^{-1}$) | | |
| Bi-exponential | 0.6 ± 0.2 | 0.7 ± 0.3 |
| Tri-exponential | 0.2 ± 0.1 | 0.2 ± 0.1 |
| Mean residence time (h) | 2.1 ± 1.0 | NC |

*Patients are divided into two groups based on the nature of the serum concentration versus time profile.
NC, not calculated.

results of the present study, in which capsules were administered, may not be appropriate. On the other hand, genetic variation in glucuronyltransferase activity in conjunction with the presence of mild hepatic inflammation may have contributed to the wide range of apparent oral clearance values (Table 4). Previous investigations of drug disposition during acute hepatitis have demonstrated a decreased clearance of hexobarbital (Breimer et al., 1975) and antipyrine (Burnett et al., 1976) but no change in distribution volume. As in the present study, these reports also found that there was little correlation between the degree of enzyme elevation and drug clearance. An additional consideration is that the use of a standard dose which was not individualized to body weight could have contributed to the wide range of peak plasma concentrations. However, inspection of Table 3 indicates that there was no relationship between the weight-adjusted dose of ZDV and either the peak or 4 h serum concentration.

It is also noteworthy that five of the eight patients in this study demonstrated a prolonged terminal elimination phase with a mean half-life of 4.8 h. A half-life of this duration is substantially longer than the 1 h value previously reported in AIDS and ARC patients (Klecker et al., 1987). A possible explanation for this discrepancy is that the latter study reported their data in patients that were receiving ZDV every 4 h. This would have prevented any description of the later elimination phase which occurred after 4 h in our study. Alternatively, the dosage of 300 mg may have provided a higher body load, which in turn yielded plasma concentrations during the 4–8 h period that were within the sensitivity range for the assay.

Zidovudine is currently the antiviral of choice for patients with severe manifestations of HIV infection (Fischl et al., 1987). However, limited data have been published which describe the human pharmacokinetics of this antiviral agent. Klecker et al. (1987) reported a bi-exponential decay profile with a terminal phase

serum half-life of ~ 1 h. Based on these and other unpublished data obtained in humans, ZDV was prescribed every 4 h in the initial clinical studies which demonstrated beneficial clinical and antiviral effects (Fischl et al., 1987). However, dose-limiting adverse effects (anemia, neutropenia) have made this drug difficult to administer chronically to many patients, and often necessitates a decrease in dose and/or prolongation of the dosing interval (Richman et al., 1987).

The use of zidovudine to treat HIV infections in patients with hemophilia may be further complicated by the concurrent presence of liver dysfunction which is commonly present in these patients. Although clinical manifestations of hepatitis are uncommon, there is a relatively high incidence of elevated serum transaminase levels in this population (Mannucci et al., 1975). Indeed, in the present study a majority of the patients had abnormal AST, ALT and alkaline phosphatase levels. Despite the presence of elevated enzymes, the primary indicators of chronic liver function (bilirubin, albumin, prothrombin time) were normal. Previous investigations which obtained a liver biopsy have found that from 63–95% of patients with elevated enzymes also have histologic findings that are consistent with chronic active liver disease (Schimpf et al., 1977). The question then arises as to whether a defect in drug conjugation may exist in these patients despite what appears to be normal metabolic capacity as suggested by indicators of chronic hepatic function.

In summary, the present study has demonstrated a longer elimination pattern of ZDV than has been described previously. Whether this observation is unique to hemophilia patients, patients with elevated liver enzymes, or if it exists in AIDS or ARC patients, or asymptomatically-infected patients without hemophilia with normal liver function tests, requires further investigation in each of these groups. If our observation is confirmed by studies in these other patient populations, it would suggest that a dosage interval considerably longer than 4 hrs would be appropriate for ZDV treatment of HIV infection. A longer dosage interval might result in equivalent drug efficacy while minimizing tissue accumulation and hematologic toxicity. Finally, the serum concentrations that result from a standard dose were highly variable and therefore, the clinical use of serum level monitoring during future studies in hemophilia patients may be useful to adjust the maintenance dosing interval in order to minimize drug accumulation and possibly ZDV toxicity.

**Acknowledgements**

Supported by contract AI-62551 from the National Institute of Allergy and Infectious Diseases, NIH; and Public Health Service research grant RR-00044 from the General Clinical Research Centers Branch, Division of Research Resources, NIH, Bethesda, MD.

The preparation of the manuscript by Kris Oldfield is appreciated.

## References


Biggs, R. (1978) Complications of treatment. In: R. Biggs, (Ed.), The Treatment of Haemophilia A and B and von Willebrand's Disease, pp. 181–202, Blackwell Publications, Oxford.

Breimer, D.D., Zilly, W. and Richter, E. (1975) Pharmacokinetics of hexobarbital in acute hepatitis and after apparent recovery. Clin. Pharmacol. Ther. 18, 433–440.

Burnett, D.A., Burak, A.J., Tuma, D.J. and Sorrell, M.F. (1976) Altered elimination of antipyrine in patients with acute viral hepatitis. Gut 17, 341–344.

Fischl, M.A., Richman, D.D., Grieco, M.H. et al. (1987) The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. N. Engl. J. Med. 317, 185–191.

Good, S. and Reynolds, D.J. (1987) Simultaneous quantitation of the anti-HIV agent, 3′-azido-3′-deoxythymidine (Retrovir) and its glucuronide in human serum by high performance liquid chromatography. (Abstract 377) In: Abstracts of the Twenty-Seventh Interscience Conference on Antimicrobial Agents and Chemotherapy, p. 162. American Society of Microbiology, Washington, D.C.

Klecker, R.W., Collins, J.M., Yarchoan, R. et al. (1987) Plasma and cerebrospinal fluid pharmacokinetics of 3′-azido-3′ deoxythymidine: a novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. Clin. Pharmacol. Ther. 41, 407–412.

Mannucci, P.M., Capitanio, A., Del Ninno, E. et al. (1975) Asymptomatic liver disease in hemophiliacs. J. Clin. Pathol. 28, 620–624.

McDougal, J.S., Martin, L.S., Cort, S.P., Mozen, M., Heldebrant, C.M. and Evatt, B.L. (1985) Thermal inactivation of the acquired immunodeficiency syndrome virus, human T lymphotropic virus-III/lymphadenopathy-associated virus, with special reference to antihemophilic factor. J. Clin. Invest. 76, 875–877.

Resnick, L., Veren, K., Zaki Salahuddin, S., Tondreau, S., Markham, P.O. (1986) Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. J. Am. Med. Assoc. 255, 1887–1891.

Richman, D.D., Fischl, M.A., Grieco, M.H. et al. (1987) The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. N. Engl. J. Med. 317, 192–197.

Rocci, M.L. and Jusko, W.J. (1983) LAGRAN program for area and moments in pharmacokinetic analysis. Comput. Programs Biomed. 16, 203–216.

Schimpf, K.L., Zimmerman, K., Rudel, J. et al. (1977) Result of liver biopsies, rate of icteric hepatitis and frequency of anti-$HB_s$ and $Hb_s$-antigen in patients of the Heidelberg hemophilia center. Thromb. Haemost. 38, 340.