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Intravascular distribution of zidovudine: role of plasma proteins and whole blood components

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Summary

Knowledge of drug protein-binding and blood cell partitioning may be important for evaluating the pharmacokinetic parameters of zidovudine, particularly because of its intracellular site of action and potential to induce side effects. Equilibrium dialysis studies of zidovudine were performed over 2 h to identify the extent and site of binding. Zidovudine was added to anticoagulated whole blood to study blood cell distribution over a 24 h period at 37°C and at 21°C. Concurrent plasma and whole blood samples were determined at various time-points and blood partitioning was determined by application of a mass balance equation. All samples were analyzed using radioimmunoassay. The free fraction of zidovudine at a concentration of 500 ng/ml (1.7 μM) was 0.77 ± 0.05 in plasma, 0.78 ± 0.03 in serum, 0.88 ± 0.03 in 4 g/dl albumin solution, and 1.0 in 100 mg/dl α1-acid glycoprotein solution. A free fraction of 0.72 ± 0.10 was observed in plasma from HIV-infected patients with zidovudine concentrations ranging from 16 to 91 ng/ml. Zidovudine equilibration between plasma and blood cells occurred rapidly, being complete within 10 min. After equilibrium was complete, the mean whole blood:plasma ratio was 0.86 ± 0.02 and 0.80 ± 0.04 ($P = 0.20$) and mean blood cell Partitioning ratio, [cell]/[plasma-free], was 0.85 ± 0.06 and 0.66 ± 0.14 ($P = 0.25$) for studies at 37°C and 21°C, respectively. The partitioning ratio was relatively consistent over the study period, suggesting no accumulation in blood cells. These results suggest that zidovudine binds to a small extent primarily to albumin. The free concentration equilibrates readily between blood cells and plasma independent of concentration and without signs of accumulation.

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Zidovudine; Pharmacokinetics; Protein-binding; Blood cell distribution

Introduction

Although only unbound, or free drug in plasma is pharmacologically active, therapeutic regimens and pharmacodynamic relationships are often based on the total drug concentration in plasma or serum. In addition to plasma proteins, erythrocytes are known to bind to certain drugs and influence their plasma concentrations. Also, the erythrocyte constitutes a large cell mass that may serve as a potential reservoir of drugs and may influence their pharmacokinetic behavior. It is well appreciated that plasma concentrations of cyclosporin A and ribavarin are influenced substantially due to their interactions with blood cells (Lemaire et al., 1982; Catlin et al., 1980).

The antiretroviral, zidovudine (3′-azido-3′-deoxythymidine) inhibits viral replication within infected lymphocytes (Mitsuya 1985). Zidovudine diffuses across lymphocyte cell membranes by non-facilitated diffusion, without evidence of saturability or concentration-dependence (Zimmerman et al., 1987). In lymphocytes and monocytes zidovudine is phosphorylated to the active moiety, zidovudine triphosphate (Furman et al., 1986). Thus, the free concentration of zidovudine is in a dynamic equilibrium between intra- and extracellular compartments. This equilibrium is influenced by the affinity of the drug for plasma and cellular components as well as the rate of systemic and intracellular metabolism.

It is well appreciated that substantial interpatient variability exists with regard to zidovudine pharmacokinetics. Data reported from clinical trials with zidovudine has identified highly variable peak serum concentrations, even when corrected for body weight (Blum et al., 1988; Klecker et al., 1987). Initial oral studies reported an estimated half-life of 1.1 h with a monoexponential decline. Other investigators have observed a prolonged pattern of zidovudine elimination suggestive of a multicompartmental distribution (Morse et al., 1990; Morse et al., 1992; Tartaglione et al., 1991). Earlier studies of zidovudine disposition noted a volume of distribution of 1.6 l/kg (Blum et al., 1988; Klecker et al., 1987), while more recent work utilizing a population pharmacokinetic analysis has found a distribution volume of 3 l/kg (Gitterman et al., 1990). Thus it is apparent that factors influencing zidovudine distribution remain to be defined.

Zidovudine is a weak acid ($pK_a = 9.2$) and at physiologic pH (7.4) is predominantly in a nonionized state. It is a very lipophilic compound due to the azido moiety, a factor which likely contributes to its large volume of distribution. Initial testing found zidovudine to be 34 to 38% bound to plasma proteins (Burroughs Wellcome Co.). Two animal studies have reported protein-binding of 15% in dogs (Krasny et al., 1986), and 20% in the rhesus monkey (Collins et al., 1988).

The purpose of this study was to investigate the factors that contribute to the distribution of zidovudine in the intravascular space; that is the distribution between the extracellular compartment (plasma) and intracellular sites (blood cells). Specifically, we examined plasma protein-binding and blood cell distribution of zidovudine.

Materials and Methods

Materials

Analytical grade zidovudine was supplied by Burroughs Welcome (Research Triangle Park, NC). Human serum albumin (A-3782; essentially fatty acid-free and globulin-free) and human α-1-acid glycoprotein (G-9885; purity 99%) were obtained from Sigma Chemical Company. Phosphate-buffered saline (0.1 M) was prepared in the laboratory, (K_2HPO_4 0.18%, Na_2HPO_4 0.95%) and the pH was adjusted to 7.4. ZDV-TRAC^{125}I radioimmunoassay kits were supplied by Incstar Corporation (Stillwater, MA).

Protein-binding determinations

Preliminary equilibration and stability studies. Preliminary equilibration and recovery studies indicated that a dialysis time of 2 h was adequate to attain equilibration in the dialysis system and that binding of zidovudine to the apparatus or membrane was not significant. Using high-performance liquid chromatography (Good et al., 1988), no degradation of zidovudine was evident when incubated at 21°, 37° and 56°C over a 24 h period (unpublished data). Freezing of zidovudine in plasma samples did not effect the degree of protein-binding.

Protein solution studies. Plasma was obtained from ten healthy volunteers into Vacutainer (Becton Dickinson, Rutherford, NJ) collection tubes containing ethylenediaminetetraacetic acid (EDTA). Total protein and albumin concentrations were measured by the Clinical Chemistry Laboratory at the Erie County Medical Center. Total protein concentration was 7.2 ± 0.5 g/dl (mean $\pm$ S.D.). Albumin concentration was 4.5 ± 0.3 g/dl (mean $\pm$ S.D.).

Protein-binding was determined by equilibrium dialysis, using plexiglas dialysis cells with a 400 microliter maximum capacity. The membrane employed was Spectrapor (Spectrum Medical Industries, LA, CA), with a molecular weight cut-off of 12 000 to 14 000 daltons. Zidovudine in protein solution was dialyzed against 0.1 M phosphate-buffered solution. Dialysis was performed at 37°C. The pH of the protein solution was adjusted to 7.4 using microliter quantities of 3 M HCl or NaOH. Protein-binding was determined in plasma at concentrations ranging from 500 to 3000 ng/ml (1.74 to 10.5 μM). Protein-binding was also determined in serum, 4 g/dl human serum albumin solution (HSA), and 100 mg/dl α-1-acid glycoprotein (AGP) solution at two concentrations of zidovudine; 1000 and 2000 ng/ml (3.5 and 7.0 μM). Plasma

and serum were collected from ten healthy volunteers (age 25 to 35) after informed consent was obtained. Serum was collected in Vacutainer blood collection tubes containing no anticoagulant. HSA and AGP were solubilized in phosphate buffer, pH 7.4. Equilibrium dialysis was also performed on plasma samples collected from HIV-infected patients on chronic zidovudine therapy. Plasma samples were obtained three h after a 100 mg oral dose. Additional binding studies in plasma included varying the pH from 7.0 to 7.8, determinations at 21°C, and studies of zidovudine in plasma that had been maintained for 3 h at 56°C (to stimulate the viral inactivation step which is routinely used prior to analyzing clinical specimens from HIV-infected patients).

Whole blood distribution

Preliminary stability studies. Preliminary studies were performed to verify the stability of red blood cells during the experimental period (data not shown). A sample of whole blood, collected in Vacutainer blood collection tubes containing heparin, was incubated in a water bath at 37°C under constant motion (10 rpm) for 24 h. Aliquots were removed at designated time-points, centrifuged and the plasma analyzed for potassium content. This procedure was repeated at 21°C. As both the concentration of potassium and visual inspection of the plasma suggested no hemolysis, blood cells were considered to be stable during the experimental period. Preliminary studies also indicated 95% recovery of zidovudine (data not shown).

Distribution studies. Whole blood was collected using Vacutainer blood collection tubes containing ethylenediaminetetraacetic acid (EDTA). Zidovudine was added to the whole blood to attain concentrations ranging from 900 to 1400 ng/ml (3.1 to 5.2 μM). The blood was vortexed for 30 s and then aliquoted into ten polypropylene tubes and placed in a water bath with constant rotation. To examine the time-course of distribution between plasma and blood cells, an aliquot was removed from the water bath at 0, 10, 20, and 30 min and at 1, 2, 4, 6, 12, and 24 h. From each aliquot, a sample of whole blood was withdrawn and the remaining blood was immediately centrifuged and the plasma supernatant was pipetted into a polypropylene tube. Hematocrit values were determined at 0, 6, 12 and 24 h using capillary tubes and a hematocrit centrifuge. A plasma sample containing zidovudine was also placed in the water bath concurrently with the experimental samples to control for degradation of zidovudine during the study period. The procedure was performed in triplicate (on different days) at temperatures of 37°C and 21°C. After the time sequence of equilibration was determined, additional studies were performed at decreasing concentration values of zidovudine. All samples were stored at −20°C until analyzed. Frozen whole blood samples underwent a freeze/thaw procedure three times to insure lysis of cells. Lysis was confirmed via microscopic examination.

Analysis

Analysis of samples. All samples were analyzed for zidovudine with a radioimmunoassay (ZDV-Trac, INCStar Co. Stillwater, MN). It is a competitive direct-equilibrium assay, utilizing an ^{125}I-labelled zidovudine derivative. Samples were diluted 21-fold with phosphate buffer. Then 200 μl of diluted samples, standards, and controls were incubated for 2 h in the presence of a rabbit anti-zidovudine antibody and the labelled tracer. This was followed by addition of a second antibody precipitating complex and a 30 min incubation at room temperature. Samples were centrifuged at $1000 \times g$ for 20 min to separate bound from unbound tracer. The unbound tracer was removed via decantation of the supernatant. Radioactivity in the remaining pellets was then counted using a gamma scintillation counter (Wallac LKB, 1272 Minigamma). Counts obtained are inversely proportional to the amount of zidovudine present. Data reduction was performed via a computer generated spline standard curve which plots percent bound/total bound versus concentration.

The standard curve of the assay ranged from 5000 ng/ml to 6.4 ng/ml. The sensitivity was approximately 0.3 ng/ml and there was no cross reactivity with zidovudine metabolites. Quality controls were 1000 (high) and 100 (low) ng/ml. The intraday coefficient of variation was 3.2% and 5.2%, and interday was 5.1% and 4.4% for high and low controls, respectively.

Since this assay is designed for plasma analysis, two modifications were necessary for whole blood analysis. First, the whole blood blank tubes which measure any non-specific binding of ^{125}I-labelled zidovudine were substituted for the plasma non-specific binding values. Whole blood (50 μl) was diluted in 1000 μl of sample diluent supplied with the assay kit. From this dilution, two portions of 200 μl were used for the non-specific binding tubes and treated according to standard assay procedure. Secondly, whole blood quality controls of 2000 ng/ml (high) and 500 ng/ml (low) were used with the whole blood assay. The coefficient of variation for these controls were 5.7% and 5.0% for intraday, and 5.9% and 3.3% for interday variation of high and low controls, respectively.

Calculations. Fraction of unbound drug (F_u) was determined using the equation:

$$F_u = \frac{C^*_B}{C^*_P}$$

where C^*_B is the zidovudine concentration in buffer after dialysis and C^*_P is the zidovudine concentration in the protein solution after dialysis (Behm and Wagner, 1979). The results are reported with respect to the concentration in the plasma compartment after dialysis. The protein binding data was corrected for any volume shift that occurred during the dialysis period using the equation:

$$Fu_{corr} = \frac{C_B^*}{[(C_P^* - C_B^*) \times R] + C_B^*}$$

where Fu_{corr} is the corrected fraction of drug bound, and R is the ratio of plasma volume after dialysis to the plasma volume before dialysis. (Boudinot and Jusko, 1984) The concentration of zidovudine in blood cells was calculated utilizing the mass balance equation:

$$C_{WB}V_{WB} = C_PV_P + C_{BC}V_{BC}$$

where C_{WB} is the drug concentration in whole blood, C_P is the drug concentration in plasma, and C_{BC} is the drug concentration in blood cells. The volume of whole blood (V_{WB}) is assumed to be one, the volume of blood cells (V_{BC}) is equal to the hematocrit and the volume of plasma (V_P) is (1-H) (Rowland and Tozer, 1989).

The ratio of the concentration in the blood cells to that unbound in plasma, a measure of the affinity of the drug for the blood cells and is represented by the partitioning coefficient (ρ). It was calculated by applying the whole blood distribution data to the equation:

$$p = \frac{(H-1) + (C_{WB}/C_P)}{F_u \times H}$$

where H is the hematocrit. (Rowland and Tozer, 1989).

Statistical. One-way analysis of variance (ANOVA) was used to compare the mean fraction bound of zidovudine in plasma, serum, albumin, and AGP. Tukey's test was employed for multiple comparisons. The Student's t-test was used to compare results of factors influencing binding and whole blood distribution (whole blood/plasma ratio and ρ). Simple linear regression was used to analyze the data for relationships. Significance was accepted at $P < 0.05$.

The study was approved by the State University of New York at Buffalo Investigational Review Board.

Results

Protein-binding studies

Plasma protein-binding results are presented in Table 1. The mean total recovery in protein-binding studies was 95%. The shift of volume was small (mean 20 μl; range 15–30 μl. This is most likely due to the short duration of dialysis of 2 h. Correcting for volume shift, the absolute difference in per cent bound was 0.9 to 1.6% higher. There was a gradual decrease in percent zidovudine bound in plasma over the concentration range studied (25.6% at

TABLE 1

Zidovudine protein-binding in plasma from normal volunteers[a]

Concentration[b] (ng/ml)	Fu	% Bound	% Bound corrected for volume shift
1650	0.82 (0.04)	17.6 (4.2)	18.4 (4.4)
1000	0.79 (0.04)	20.8 (3.7)	22.0 (4.0)
500	0.77 (0.05)	23.0 (5.1)	24.2 (5.5)
250	0.76 (0.09)*	24.1 (8.9)*	25.6 (9.5)*

[a]Results expressed as mean (± S.D.), $n = 10$ at each concentration.
[b]Zidovudine concentration post-dialysis.
*$P < 0.05$ vs. 1650 ng/ml (Tukey's test).

250 ng/ml to 18.4% at 1650 ng/ml). A statistically significant difference was found only between the lowest concentration, 250 ng/ml (0.9 μM), and the highest concentration, 1650 ng/ml (5.7 μM), $P<0.05$.

Protein-binding results for serum, HSA, and AGP are presented in Table 2. Volume shift was 4.0, 2.5 and 0% for serum, HSA, and AGP, respectively. The smaller volume shifts observed with albumin than with plasma or serum resulted in a minimal correction in the absolute per cent bound of approximately 0.3%. No binding to AGP was detected. There was no difference between the two concentrations studied with albumin and serum. Zidovudine-binding in serum was similar to that observed in plasma. Binding of zidovudine to albumin was significantly less than to plasma or serum at the concentrations studied ($P<0.05$). Considerable variability was observed among the volunteers in the binding of zidovudine to both plasma and serum, although total protein and albumin was normal in all volunteers. No correlation was detected between degree of protein-binding and concentration of total protein or albumin.

Plasma was obtained from seven HIV-infected patients. Demographic

TABLE 2

Protein-binding of zidovudine in serum, albumin and α1-acid glycoprotein[a]

	Concentration[b] (ng/ml)	Fu	% Bound	% Bound corrected for volume shift
Serum	1190	0.79 (0.08)	20.7 (7.6)	21.6 (7.7)
	600	0.78 (0.03)	21.8 (2.7)	22.7 (2.8)
Albumin	1170	0.89 (0.02)*	10.8 (2.4)*	11.0 (2.5)*
	640	0.88 (0.03)*	12.0 (2.8)*	12.3 (2.8)*
AGP	1050	1.01 (0.04)	0.0	n/a
	600	1.02 (0.11)	0.0	n/a

[a]Results expressed as mean (± S.D.), $n = 10$ at each concentration.
[b]Zidovudine concentration post-dialysis.
*$P < 0.05$ vs. serum (Tukey's test).
n/a not applicable.

TABLE 3
Demographic data for seven HIV-infected patients

	Mean	Range
Male/Female	5/2	
Age (years)	35	32–39
Weight (kg)	74	61–85
Height (cm)	167	150–173
Albumin (gm/dl)	4.6	4.2–4.8

characteristics are summarized in Table 3. All patients were asymptomatic with CD4 counts between 500 and 200 cells/mm^3. Plasma concentrations ranged from 16 to 91 ng/ml (mean 36 ng/ml). A free fraction of 0.72 ($\pm$0.10) and a corrected per cent bound of 29% ($\pm$10) was observed. This was consistent with the trend for increased binding of zidovudine at lower plasma concentrations. It also suggests that at earlier stages of the disease protein-binding is similar to normal healthy controls and zidovudine metabolites appear to have no effect on binding. Considerable variability was also observed in plasma protein-binding in these patients.

In studing the variables that can influence binding, no effect of temperature was observed; 22.0% ($\pm$4.0) at 37°C versus 25.8% ($\pm$7.5) at 21°C at a zidovudine concentration of 1000 ng/ml ($P=0.35$). Evaluation of pH over the range of 7.0 to 7.8 and effect of temperatures used for heat inactivation of clinical specimens appeared to have no effect on the binding of zidovudine in plasma.

Whole blood distribution studies. The whole blood to plasma ratio at each time-point for studies at 37°C and 21°C are illustrated in Figs. 1a and b, respectively. Examination of the time-course of distribution between plasma and blood cells revealed rapid equilibration of zidovudine. This was observed at both temperatures studied, although considerable variability between days was observed in the 21°C studies. The whole blood/plasma ratio and ρ remained relatively consistent over the 24 h period suggesting no accumulation of zidovudine in blood cells at either temperature.

The results of the distribution studies are summarized in Table 4. Values presented are the mean of the three studies at each temperature determined, using data from 12 and 24 h on each study day. The last 12 h were used to ensure complete equilibration. The mean whole blood/plasma ratio did not differ with respect to temperature; 0.86$\pm$0.02 and 0.80$\pm$0.04 for 37° and 21°C, respectively ($P=0.20$). The mean ρ was 0.85$\pm$0.06 and 0.66$\pm$0.14 at 37° and 21°C, respectively ($P=0.24$). Studies with decreasing concentrations of zidovudine from 500 ng/ml to 50 ng/ml resulted in similar values for the whole blood/plasma ratio (0.84$\pm$0.02) and for the ρ (0.79$\pm$0.08). Fig. 2 illustrates the measured concentration in the plasma relative to the measured concentration in whole blood at each concentration studied at 37°C. Clearly, a

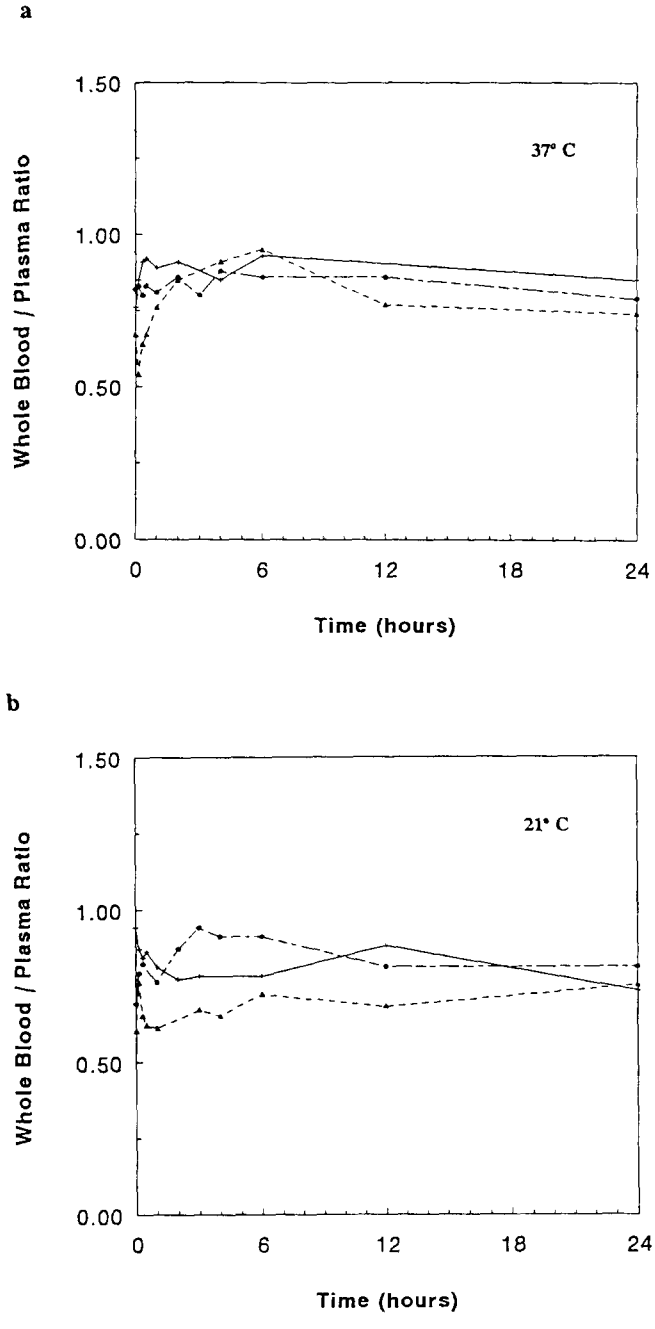

Fig. 1. Distribution of zidovudine in whole blood over the 24 h study period. The three studies performed at 37°C are represented in 1a and studies at 21°C in 1b. Each study was performed on different days in the same subject.

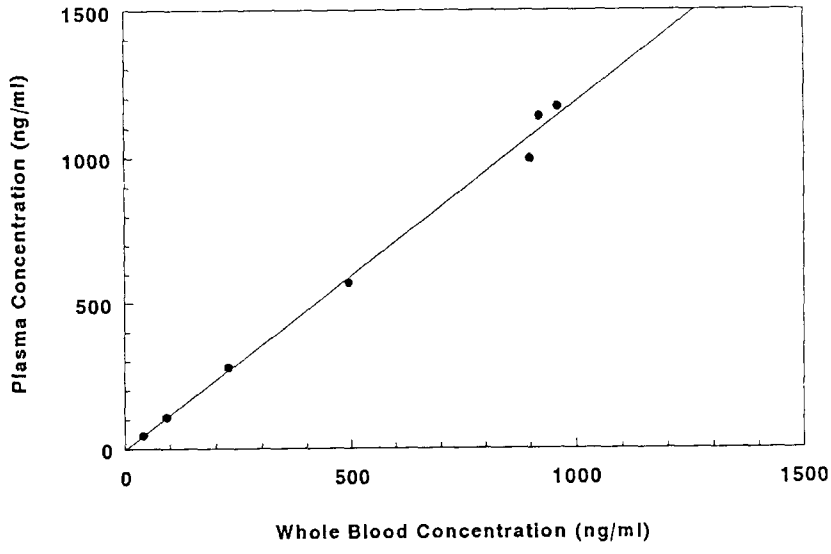

Fig. 2. Concentration of zidovudine in whole blood (ng/ml) relative to the concentration of zidovudine in plasma (ng/ml). The relationship, represented by the solid line, is described by: $y = 1.20x - 2.5$ ($r = 0.99$).

positive linear relationship was observed ($r = 0.99$), such that plasma concentrations increased proportionately to whole blood concentrations. This implies no saturation of zidovudine partitioning into blood cells. Also, the line passes through the origin, suggesting that the concentrations remain proportional even at very low concentrations. Thus accumulation is unlikely.

Discussion

Infection with HIV is associated with a progressive and relentless course

TABLE 4

Results of whole blood distribution studies[a]

	Whole blood (ng/ml)	Plasma (ng/ml)	Blood cells (ng/ml)[b]	Whole blood (plasma)	ρ
37°C					
mean	930	1100	630	0.86	0.85
S.D.	35	50	30	0.02	0.06
21°C					
mean	1100	1300	770	0.80	0.66
S.D.	260	290	280	0.04	0.14

[a]Mean of data from 12 to 24 h only, $n = 3$ at each temperature.
[b]Calculated via mass balance equation, mean H = 0.42.
ρ = partitioning coefficient.
S.D. = standard deviation.

toward the Acquired Immunodeficiency Syndrome with mortality the predominant endpoint. The disease eventually can affect most organ systems of the body (Weissman 1988). Also, the wasting syndrome that is so characteristic of the later stages of HIV infection causes extreme pathophysiologic changes in these patients primarily secondary to malnutrition. It is not uncommon for AIDS patients to develop anemia and hypoalbuminemia. Zidovudine remains the first line agent for treatment of HIV infection. Although zidovudine is routinely administered as a standard dose (i.e., 100 mg every 4 h), considerable variability has been observed in both the pharmacokinetics and pharmacodynamics of this agent (Blum et al., 1988; Klecker et al., 1987; Richman et al., 1987).

With the serious nature of the disease and the potential toxicities which limit chronic zidovudine therapy, it is imperative that we understand the facts which may influence the distribution of zidovudine. This is especially true in light of the intracellular mode of action of zidovudine. Zidovudine enters lymphocytes and is sequentially phosphorylated to the active form of the drug, zidovudine-triphosphate (Furman et al., 1986). Theoretically the free concentration of drug creates the primary diffusion gradient which promotes zidovudine entry into intracellular tissue sites and therefore distributional changes may influence the pharmacologic effect of zidovudine. Additionally, with evidence of the virus invading the central nervous system, it is important for antivirals to be able to gain access to this site (Ho et al., 1985).

An initial report found zidovudine to be 34% protein bound, although no zidovudine concentration or source of protein solution was described (Burroughs Welcome Co.). Our studies support these findings with protein-binding being relatively low, but dependent on concentration, with modestly increased binding at lower concentrations. As the range of concentrations studied is similar to concentrations observed clinically, interpatient variability in free fraction is conceivable. The degree of protein-binding was similar in plasma and serum suggesting that coagulation factors or platelets do not contribute to zidovudine-binding. As the drug is a weak acid, the lack of binding to AGP was not surprising. However, the low protein binding observed in albumin is interesting. Possible explanations for this observation is that zidovudine binds to other plasma proteins such as lipoproteins or immunoglobulins, or binding to albumin is modified in the presence of plasma.

Considerable interindividual variation was observed in the free fraction of zidovudine in both plasma and serum. As all the volunteers had albumin concentrations that were in the normal range, it is less likely that differences in albumin concentration would account for this variability. This variability was even more evident in the plasma samples obtained from HIV-infected patients. If binding to lipoproteins does occur, it is conceivable that the variability in binding may be attributed to variable levels of lipids in the subjects.

The high free fraction observed is consistent with reported saliva and cerebrospinal fluid concentrations of ZDV. Analysis of stimulated saliva found zidovudine concentrations to be $68 \pm 25\%$ of concurrent plasma concentrations

(Rolinski et al., 1990). In addition, zidovudine penetration into cerebrospinal fluid has been examined. 4 h after an oral dose, the concentrations in the cerebrospinal fluid was 50 to 60% of simultaneously measured plasma concentrations (Yarchoan et al., 1987). Also, the large volume of distribution and rapid elimination of zidovudine are also consistent with a low degree of protein-binding.

Blood cells can account for approximately 40% of blood volume and have been shown to be an important factor in the distribution of drugs. Therefore knowledge of how the blood concentration and plasma concentration are related is essential in interpreting pharmacokinetic data. The erythrocyte membrane, similar to that of other cells, consists of a lipid bilayer. Zidovudine's lipid solubility and essentially nonionized state favor diffusion into blood cells via a passive process. A previous in vitro study has suggested that zidovudine rapidly enters erythrocytes by nonfacilitated diffusion (Zimmerman et al., 1987). Our data support these findings of rapid, passive diffusion of zidovudine into blood cells with the blood cell concentration clearly increasing proportionally with increasing plasma concentrations. This unusual cell permeation property of zidovudine is attributed to the lipophilicity imparted to this nucleoside by the presence of the 3′-azido moiety which results in a large partition coefficient (1.26 in 1-octanol:0.1 M sodium phosphate, pH 7.0) (Zimmerman et al., 1987). In addition, Zimmerman et al. found the extent of zidovudine uptake is independent of temperature, which also supports a nonfacilitated process. We also observed no temperature dependence on the extent of blood cell uptake. Finally, distribution into blood cells is often a function of the concentration gradient for unbound drug in plasma. Our results are consistent with this concept in that the concentration in blood cells was similar to the free concentration in the plasma. In the distribution studies at 37°C, blood cell concentration was 81% of the free concentration in plasma.

The partition coefficient, ρ, for zidovudine was consistently less than one, suggesting that zidovudine equilibrates between blood cells and plasma water to a similar extent. As ρ remained consistent over time, accumulation in blood cells as is seen with ribavarin is unlikely (Catlin et al., 1980). Also, the ρ combined with the low binding of zidovudine to albumin is compatible with minimal binding to blood cells. However the possibility still exists that the rate of efflux of zidovudine may differ from that of influx, such that the blood cell site could potentially contribute to the prolonged elimination phase referred to above.

Recent studies (Gitterman et al., 1990; Tartaglione et al., 1990) have reported pharmacokinetic parameters inconsistent with those obtained in earlier studies (Blum et al., 1988; Klecker et al., 1987). A larger volume of distribution and lower serum concentrations were observed. This could have profound implications, as serum concentrations have been related to hematologic toxicity. These authors speculate that parameters may differ in patients who are at an earlier stage of disease, or not yet affected by the wasting syndrome. Whether protein-binding and blood cell distribution, or rather the

lack of, would contribute to the explanation of these observations is difficult to discern. Both of these factors could potentially be altered as a consequence of the disease or adverse effects of chronic treatment with zidovudine (Richman et al., 1987). Conceivably, a decrease in the hematocrit would decrease the volume of this site of distribution and an increase in the free concentration in plasma may have implications because of greater distribution of zidovudine into intracellular sites. Although clinically relevant changes would not generally be anticipated because of the low protein-binding and rapid equilibrium, the case may be different with zidovudine. Although the therapeutic range has yet to be defined for zidovudine, we do know that it is a narrow range. Available in vitro data indicates a concentration of 300 ng/ml is necessary for optimal antiviral effects (Mitsuya et al., 1986) and toxicities are observed at a concentration of 100 ng/ml (Johnson et al., 1988). Thus, modest changes in the concentration of zidovudine may be significant.

Whether whole blood would be superior for analyzing zidovudine concentrations in patients and establishing a therapeutic range and dosing regimens is intriguing. Certainly, studies to date have not established clear relationships utilizing plasma concentrations (Stretcher 1991). The tendency for less partitioning into blood cells at room temperature may be a potential source of variability, especially if the volume of this site is altered by changes in hematocrit. Intuitively, this potential source of error would be greatest in the patient at an earlier stage of disease, with normal hematocrit and protein concentrations. Further studies are underway to resolve this issue.

In conclusion, our data show that zidovudine does exhibit low protein-binding with albumin being the predominant protein. A second site is possibly involved. It appears that zidovudine partitions readily into blood cells at concentrations proportional to the free concentration in plasma, without evidence of accumulation. A change in constitution of blood components may influence zidovudine plasma concentrations, although the clinical relevance of this remains to be determined.

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