

Pharmacokinetics of Subcutaneous Azidothymidine in Rhesus Monkeys

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The pharmacokinetics of subcutaneous bolus and continuous infusion azidothymidine (AZT) was studied in rhesus monkeys. Three animals received 100 mg/m² as a bolus injection both intravenously and subcutaneously, with the order of administration randomly determined. Two animals received a continuous subcutaneous infusion of 25 mg/m² per h for 12 or 24 h. AZT was measured in plasma by a reverse-phase high-pressure liquid chromatographic assay. Following intravenous bolus administration, AZT elimination was rapid, with a mean half-life of 1.2 h and a mean clearance of 318 ml/min per m² (range, 200 to 441 ml/min per m²). The bolus subcutaneous dose was rapidly (time to peak concentration, 15 to 30 min) and nearly completely (fraction absorbed, 92%) absorbed without evidence of local tissue toxicity. With continuous subcutaneous infusion of AZT, the steady state was attained within 4 h and steady-state concentrations in plasma in the two animals exceeded 3.0 μmol/liter. No local tissue toxicity was observed at the infusion site. The subcutaneous route may be a practical alternative to intravenous administration of AZT and deserves further clinical study.

The nucleoside analog azidothymidine (AZT; zidovudine) is a reverse transcriptase inhibitor that causes termination of viral DNA chain elongation. AZT has been shown to block the infectivity and cytopathic effects of human immunodeficiency virus (HIV) in vitro (6), and in a placebo-controlled clinical trial, AZT significantly lowered both the mortality rate and the frequency of opportunistic infections in patients with symptomatic HIV infection (2). Although therapeutic drug concentrations in plasma have not been defined, results of in vitro pharmacodynamic studies indicate that continuous exposure to AZT concentrations of 1 μmol/liter are required to optimally suppress HIV infection (6).

Because of a pharmacokinetic profile that includes rapid elimination and a short half-life, AZT has been administered intravenously and orally on a schedule of once every 4 h in an attempt to maintain a minimum target concentration in plasma of 1 μmol/liter (5; F. M. Balis, P. A. Pizzo, J. Eddy, C. Wilfert, R. McKinney, G. Scott, R. F. Murphy, P. F. Jarosinski, J. Falloon, and D. G. Poplack, *J. Pediatr.*, in press). More recently, a continuous intravenous infusion schedule, capable of maintaining micromolar AZT concentrations in plasma with a considerably lower total daily dose, was demonstrated to be efficacious (1a, 8). The clinical and pharmacokinetic advantages of the continuous infusion approach suggest that other practical methods of providing continuous exposure to AZT should be developed. In the present study, the subcutaneous route of administration of AZT was investigated in rhesus monkeys. The absolute bioavailability was determined after a bolus dose, and the feasibility of continuous subcutaneous infusion was examined.

MATERIALS AND METHODS

Drug formulation and administration. AZT (Retrovir) was supplied by the Burroughs Wellcome Co. (Research Triangle Park, N.C.) as a sterile, filtered aqueous solution at a concentration of 20 mg/ml. A bolus dose of 100 mg/m² was injected intravenously and subcutaneously at least 2 weeks apart, with the order of administration randomly deter-

mined. An infusion dose of 25 mg/m² per h was administered subcutaneously over 12 or 24 h with a computerized drug delivery pump (model 5500; Deltec Systems, St. Paul, Minn.).

Animals. Four adult male rhesus monkeys (*Macaca mulatta*) ranging in weight from 7.3 to 10.7 kg (median weight, 8.5 kg) were studied. The animals were housed individually and received water and food ad libitum. Three animals were treated with a bolus injection of AZT by two routes, subcutaneous and intravenous. In addition, one animal received a 12-h subcutaneous infusion of AZT and a second animal received a 24-h subcutaneous infusion of AZT. Subcutaneous infusions were administered at a single site on the back. Heparinized blood samples were drawn from a saphenous or femoral venous catheter (contralateral to the site of injection in the case of the intravenous doses) prior to the dose and at 5, 15, 30, 45, and 60 min and 2, 3, 4, 6, and 8 h after the dose. With the subcutaneous infusions, blood samples were obtained prior to the infusion and at 1, 2, 4, 6, 8, 12, and 24 h during the infusion. Plasma was separated immediately by centrifugation and frozen at -20°C until it was assayed.

Local toxicity of subcutaneous injection or infusion of AZT was assessed by careful examination of the site for the 2 weeks following administration of the dose.

Sample analysis. The AZT concentration in plasma was measured by a previously described reverse-phase high-pressure liquid chromatographic assay (5).

Pharmacokinetic calculations. The area under the plasma concentration-time curve (AUC) was derived by using the linear trapezoidal rule and extrapolated to infinity by using the elimination rate constant (3) derived from nonlinear regression analysis of the data with reciprocal weighting by using MLAB. The absolute bioavailability (*F*) of the 100-mg/m² subcutaneous bolus dose was calculated from the AUC by the following equation: $F = (AUC_{s.c.} \cdot dose_{i.v.}) / (AUC_{i.v.} \cdot dose_{s.c.})$, where $AUC_{s.c.}$ and $dose_{s.c.}$ are the AUC and dose after subcutaneous administration, respectively, and $AUC_{i.v.}$ and $dose_{i.v.}$ are the AUC and dose after intravenous administration, respectively. Clearance following the intravenous bolus dose was calculated by dividing the dose by the $AUC_{i.v.}$ and the volume of distribution at steady

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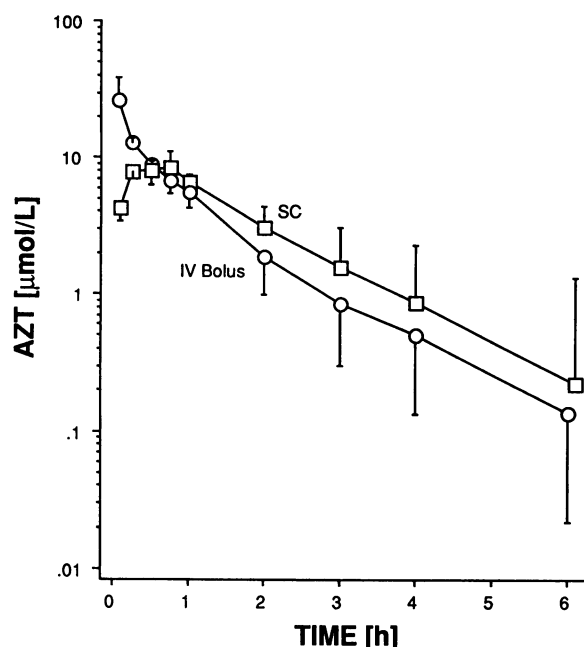


FIG. 1. Disappearance of AZT from plasma following intravenous (IV) bolus (○) and subcutaneous (SC) bolus (□) injection of 100 mg/m². Points and error bars represent the geometric means and standard deviations of data from three rhesus monkeys.

state was calculated from the area under the moment curve (7).

RESULTS

Figure 1 shows the plasma disappearance curves for equal doses of AZT administered both intravenously and subcutaneously to three animals. When administered subcutaneously, AZT was rapidly absorbed and the plasma concentrations were approximately those found following an intravenous dose. The AZT concentration in plasma fell to 1 μmol/liter by 3 h after the intravenous dose and by 4 h after the subcutaneous dose. The subcutaneous injection site was closely observed for 2 weeks after the dose, and no local toxicity was noted.

Table 1 lists the pharmacokinetic parameters derived from the AZT concentration in plasma-time data following intravenous bolus and subcutaneous bolus administrations. The elimination of AZT was rapid, with a terminal half-life of 1.2 h and a clearance of 318 ml/min per m². Absorption of the

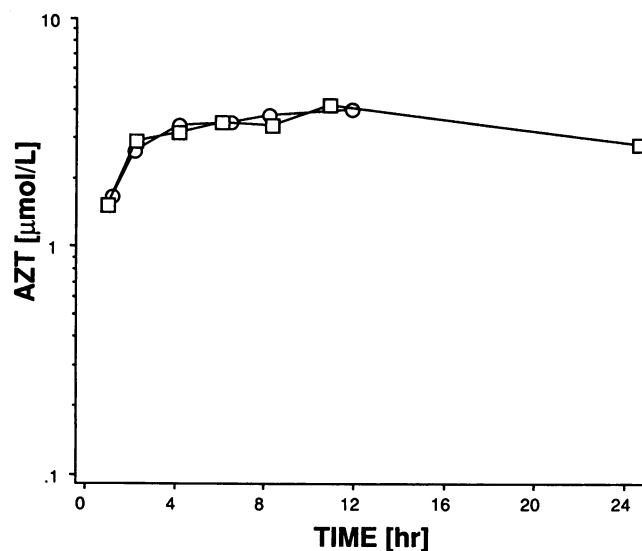


FIG. 2. Concentration of AZT in plasma during a continuous subcutaneous infusion of 25 mg/m² per h for 12 h in one animal (○) and 24 h in a second animal (□).

subcutaneous dose was rapid and nearly complete. The AZT concentration in plasma peaked at between 15 and 30 min, and on average, over 90% of the subcutaneous dose was absorbed.

Plasma concentrations achieved with a continuous subcutaneous infusion of AZT in two animals are shown in Fig. 2. The steady state was achieved within 4 h, and the mean steady-state concentrations of AZT for an infusion rate of 25 mg/m² per h were 3.46 and 3.71 μmol/liter in the two animals. In the single animal studied following both an intravenous bolus dose and a continuous subcutaneous infusion (animal 681P), the predicted steady-state concentration in plasma based on clearance following the bolus dose was 3.53 μM, and the measured value was 3.46 μM. As with the bolus dose, there was no evidence of local toxicity at the subcutaneous infusion site in either animal for 2 weeks following the infusion.

DISCUSSION

Subcutaneous administration of AZT appears to be a feasible alternative to intravenous administration. In our

TABLE 1. Pharmacokinetic parameters of AZT following intravenous bolus and subcutaneous bolus injection of 100 mg/m² in rhesus monkeys

Monkey	Intravenous administration ^a				Subcutaneous administration ^b		
	AUC _{i.v.} (μmol · h/liter)	t _{1/2} (h)	Clearance (ml/min per m ²)	V _{ss} (liter/m ²)	AUC _{s.c.} (μmol · h/liter)	Peak level (μmol/liter)	F (%)
681P	14.2	0.8	441	20.2	15.7	10.8	111
U726	19.9	0.8	314	13.7	19.6	8.70	98
98D	31.3	1.9	200	26.8	20.9	7.33	67
Mean ± SD	21.8 ± 8.7	1.2 ± 0.6	318 ± 121	20.2 ± 6.6	18.7 ± 2.7	8.94 ± 1.75	92 ± 23

^a AZT was administered at 100 mg/m². Abbreviations: AUC, area under the curve; i.v., intravenous; t_{1/2}, postdistributive half-life; V_{ss}, volume of distribution at steady state.

^b AZT was administered at 100 mg/m². Abbreviations: s.c., subcutaneous; F, fraction of the subcutaneous dose absorbed.

model there was good tissue tolerance and AZT absorption was rapid and nearly complete. Previous studies of subcutaneous methotrexate administration demonstrated that this model is predictive of the disposition of subcutaneously administered drugs in humans (1).

The variability in pharmacokinetic parameters exemplified by the twofold range in plasma clearance (Table 1) in three animals is consistent with the variability in AZT disposition observed in humans (1a; Balis et al., *J. Pediatr.*, in press). Variability in the bioavailability of the subcutaneous dose (67 to 111%) may also be, in part, related to intra-animal variation in the clearance rate, which is assumed to be constant when calculating absolute bioavailability. This degree of variability suggests that there is a role for therapeutic drug monitoring in patients treated by subcutaneous injection or infusion of AZT, in order to achieve desired concentrations in plasma (1a).

AZT only suppresses HIV infection. Human lymphoid cell lines infected with HIV *in vitro* require continuous exposure to the drug to block the cytopathic effects of the virus (6), and if placed in drug-free medium after prolonged exposure to AZT, the cells rapidly succumb to the virus. Because of the rapid elimination of AZT *in vivo*, it is difficult to maintain continuous exposure to virostatic concentrations of the drug on an intermittent schedule (5; Balis, et al., *J. Pediatr.*, in press). Results of the *in vitro* pharmacodynamic studies and the pharmacokinetic profile of AZT suggest that administration of the drug by continuous infusion may be the ideal schedule. A clinical trial (8) and a pharmacokinetic study (1a) of AZT administered by continuous intravenous infusion in children has shown that this schedule can produce responses (including dramatic improvement in patients with the acquired immune deficiency syndrome-dementia complex). There is a considerable pharmacokinetic advantage for this approach, which maintains micromolar AZT concentrations in plasma for prolonged periods at a lower dose.

Subcutaneous infusion of AZT may be a practical alternative to continuous intravenous infusion via a central venous catheter. The availability of lightweight, microprocessor-based portable infusion pumps allows for the outpatient administration of long-term subcutaneous and intravenous infusions with minimal disruption in daily schedules; this may actually be more convenient and have better compliance than an around-the-clock, every 4- to 6-h administration schedule. Analgesics and specific agents such as deferoxamine have been successfully administered subcutaneously via portable infusion pumps in chronically ill patients (1b, 4).

In the primate model, we were able to achieve steady-state

concentrations in plasma of greater than 3 $\mu\text{mol/liter}$, a concentration that produced unacceptable toxicities in a majority of patients in the continuous infusion study (1a). Thus, it should be possible to maintain therapeutic concentrations of AZT by continuous subcutaneous infusion. Clinical studies of this approach are indicated.

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