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Technical Note

Dose-Ranging Pharmacokinetics of Zidovudine (Azidothymidine) in the Rat

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INTRODUCTION

Zidovudine (ZDV), a nucleoside analogue of thymidine, has been shown to reduce considerably the mortality of patients with AIDS and AIDS-related complex (1). The pharmacokinetics of ZDV, in man, have been shown to be linear up to 7.5 mg/kg when given intravenously (2). After an iv dose, ZDV is cleared from the body primarily by metabolism to the ether glucuronide (~60% of the dose) and by excretion of the unchanged drug in the urine (~25% of the dose) (3). Although absorption of ZDV is thought to be complete when administered orally, the bioavailability of ZDV has been found to be only 60% because of the first-pass effect (2).

Toxicity data on ZDV indicate that the drug is toxic to the hematopoietic system, causing anemia and neutropenia (4). The toxicity of ZDV has been found to be dose limiting, dose dependent, and reversible (4). Moreover, an alarming interaction between ZDV and acetaminophen has been discovered. Concomitant therapy with acetaminophen significantly increases the risk of neutropenia (4). The mechanism of this interaction may be either pharmacokinetic or pharmacodynamic. In order to investigate pharmacokinetic interactions between ZDV and other drugs, a suitable animal model must first be identified. In this regard we have conducted baseline pharmacokinetic studies in the rat to ascertain whether or not this species is suitable for studying ZDV drug interactions.

EXPERIMENTAL

ZDV was administered intravenously, at three different dose levels (20, 40, and 60 mg/kg), in a randomized fashion to eight male Sprague Dawley rats (Charles River, 340–410 g) through a previously implanted (at least 24 hr before the study) jugular catheter. A washout period of at least 48 hr preceded each dose. The animals were placed in individual metabolic cages for 24 hr after each dose in order to obtain a complete urine collection. Food was withdrawn during the first 4 hr of the study, but water was supplied ad libitum. Blood samples (0.1–0.5 ml/sample but not >3 ml total) were collected through the cannula at 10, 15, 30, 60, 90, 120, 180, and 240 min following the dose. In order to obtain mainstream blood, about 0.2 ml of blood was first withdrawn in a syringe. Then, using a second syringe, the actual blood sample was collected. The first 0.2 ml of blood was then reinfused into the rat, followed by 0.2–0.5 ml of saline. Urine samples were collected in several fractions over 24 hr postadministration.

Plasma and urine ZDV concentrations were assayed by high-performance liquid chromatography using a procedure previously established in our laboratory (5). Briefly, plasma samples (10 μl–250 μl) were extracted, after addition of the internal standard (*p*-hydroxyphenobarbital), by gentle agitation for 20 min with 4 ml of ethyl acetate:diethyl ether (50:50 mixture). When the plasma concentrations were anticipated to fall outside of the calibration range, the samples were diluted in water prior to analysis. The organic phase was then evaporated under nitrogen in glass conical tubes. The residue was then reconstituted with the mobile phase [acetonitrile:0.025 *M* KH_2PO_4 (pH 2.2) (20:80), 350 μl] and about 300 μl of this reconstituted fluid was injected onto a C_{18} (Econosil, 10 μm) analytical column. The compounds were eluted at 1 ml/min and the effluent was monitored at 266 nm. Samples were reassayed, after dilution, when the

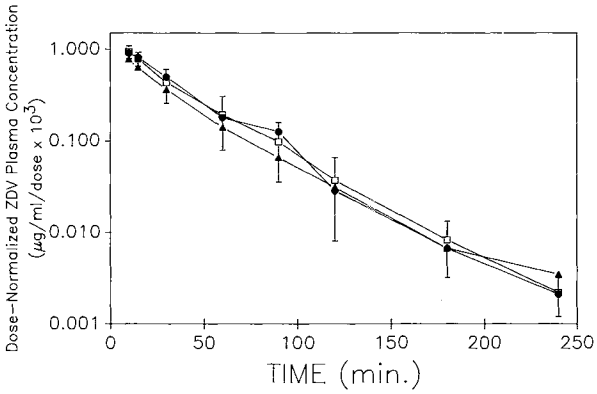

Fig. 1. Superposition of the dose-normalized mean plasma concentration–time profiles at 20-mg (▲), 40-mg (□), and 60-gm (●) doses indicates that the kinetics of ZDV are linear within this dose range. For clarity, the error (±SD) bars are drawn for the 40-mg/kg dose only.

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Table I. Mean (±SD) ZDV Total-Body Clearance (CL_p), Renal Clearance (CL_r), Steady-State Volume of Distribution (V_{ss}), and Mean Body Residence Time (MBRT) Do Not Change with the Size of the Dose Administered

	Dose of ZDV (iv) (mg/kg)			
	20	40	60	$\bar{X}$
CL_p (ml/min/kg)	28.7 (±3.9)	23.2 (±5.5)	23.2 (±5.4)	25.1 (±5.35)
CL_r (ml/min/kg)	20.2 (±5.6)	17.4 (±4.5)	18.2 (±4.6)	18.2 (±4.8)
V_{ss} (ml/kg)	1017.3 (±255.6)	804.4 (±334.6)	753.6 (±141.1)	864.6 (±272.4)
MBRT (min)	35.9 (±10.6)	35.1 (±11.5)	33.1 (±5.4)	34.8 (±8.8)

estimated ZDV concentrations fell outside of the calibration range. Sample contents were above the limit of assay sensitivity for all time points at all three dose levels.

Data Analysis. Area under plasma concentration–time curves (AUC) and area under moment curves (AUMC) were determined by the trapezoidal rule, with extrapolation to time infinity assuming first-order decay. The terminal elimination rate constant was estimated by fitting a log-linear model to the terminal portion (identified after plotting data) of the concentration time profile. The extrapolated areas for AUC and AUMC ranged from 0.1 to 4.5 and from 0.9 to 45.1%, respectively. Total-body clearance (CL_p), volume of distribution at steady state (V_{ss}), and mean body residence time (MBRT) were calculated as dose/AUC, dose · AUMC/$(AUC)^2$, and AUMC/AUC, respectively. Renal clearance (CL_r) was calculated as the ratio of the total amount excreted unchanged in the urine to AUC (from time zero to infinity). Although urine was collected in several fractions, renal clearance for each fraction was not computed since such analyses rapidly revealed that the animals did not completely empty their bladders at each collection. The effect of the size of the dose administered on these pharmacokinetic parameters was determined by analysis of variance.

RESULTS AND DISCUSSION

The superposition of the dose-normalized mean plasma concentration–time profiles indicates that the kinetics of ZDV are linear in the dose range of 20–60 mg/kg (Fig. 1). Although not readily apparent in these mean profiles, some of the plasma concentration time–profiles demonstrated biexponential decay, while others demonstrated monoexponential decay. The total body clearance (CL_p), renal clearance (CL_r), volume of distribution at steady state (V_{ss}), and mean body residence time (MBRT) did not change significantly ($p > 0.05$) with increasing ZDV dose (20 to 60 mg/kg) (Table I). However, when the individual data sets were examined, the total plasma clearance in one rat (No. 2) decreased by almost 50% with a change in dose from 20 to 40 mg/kg (Table II), indicating possible dose-dependent clearance of ZDV in this animal.

Table II. Individual ZDV Total-Body Clearance (CL_p; ml/min/kg) at Three Dose Levels After iv Administration

Dose (mg/kg)	Animal No.							
	1	2	3	4	5	6	7	8
20	24.4	27.7	ND[a]	29.3	26.1	35.7	28.8	ND
40	18.7	15.1	22.8	29.4	ND	27.7	ND	25.8
60	19.1	16.4	ND	30.0	25.0	ND	ND	25.2

[a] Not determined.

Contrary to findings in humans (3), when ZDV is administered intravenously, the rat excretes a majority (70–80%) of the dose unchanged in the urine. Thus, the renal clearance approximates the total-body clearance of the drug (Table I). The magnitude of the renal clearance, which is comparable to the average kidney plasma flow in this species (25.6 ml/min/kg) (6), indicates that ZDV is highly extracted by the kidney and that the drug is probably eliminated by filtration and secretion with little or no reabsorption. The MBRT of ZDV in the rat is about half that observed in humans (1.2 hr, calculated from mean data published in Ref. 2), reflecting the rapid clearance of this drug in the rat.

Although not usual, the experimental design used in this study was adopted in the belief that it would reduce the interanimal variability and therefore increase the power of detection of a change (if any) in the pharmacokinetics of ZDV with dose. Under this design, each rat should have received all three ZDV doses. However, due to catheter blockage, this goal was not achieved (as indicated by ND in Table II).

The lowest dose investigated was set at 20 mg/kg for several reasons. First, this dose allowed the assay of plasma samples (despite the small volumes available) for several half-lives and therefore yielded reliable pharmacokinetic parameters. Second, investigation of a lower dose was deemed unnecessary since the clearance of drug is linear over a wide dose range (20–60 mg/kg). These data, together with our findings that ZDV clearance approximates renal plasma flow, make it highly unlikely that the kinetics of ZDV are different at the lower doses.

Since the rat excretes ZDV primarily as unchanged drug in the urine, it is not a suitable animal model for hepatic metabolism studies. The rat is a good model for studying the effect of drugs which may interfere with the renal clearance of ZDV. The data generated here will also be useful in establishing dosing guidelines to investigate both the mechanisms of hematotoxicity of ZDV and the antiviral activity of ZDV in combination with other agents in the rat.

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