Nonlinear Disposition of Intravenous 2',3'-Dideoxyinosine in Rats

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The pharmacokinetics of 2',3'-dideoxyinosine (ddI) were examined in rats given intravenous doses of 8, 40, or 200 mg/kg. The concentrations of ddI in whole blood and plasma were identical. The concentration decline was multiexponential, with mean half-lives of 2 and 20 min for the first and second phases, respectively. At the highest dose, a slower third phase with a half-life of 56 min was observed. The total-body clearances were 99, 77, and 37 ml/min-kg for the 8, 40, and 200 mg/kg doses. The steady-state volume of distribution showed a trend for a decrease with increasing doses, but the difference was not statistically significant. Twenty-four-hour urinary recovery of unchanged drug for the three doses was similar at about 20%, suggesting that a major fraction of the dose was metabolized. Urinary excretion of ddI metabolite, hypoxanthine, accounted for less than 5% of the dose. Renal and metabolic clearances decreased with increased doses. ddI was metabolized in blood; the addition of inorganic phosphate, a cosubstrate in phosphorylase-mediated nucleoside catabolism, enhanced the degradation by about fourfold. In summary, these data indicate equal distribution of ddI in the extracellular and intracellular spaces in blood, its enzymatic degradation in blood, and nonlinear elimination kinetics.

KEY WORDS: nonlinear disposition; 2',3'-dideoxyinosine; anti-AIDS drug; dideoxynucleosides; pharmacokinetics.

INTRODUCTION

2',3'-Dideoxynucleosides (ddNS) are effective against human immunodeficiency virus (HIV) *in vitro*. The triphosphate nucleotides of ddNS inhibit the enzyme reverse transcriptase which is necessary for viral replication and infectivity (reviewed in Ref. 1). These ddNS, including 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine, 2',3'-dideoxyadenosine (ddA), and 2',3'-dideoxyinosine (ddI), are currently the most promising and vigorously pursued drugs for the treatment of patients afflicted with HIV (1). Because of the dihydroadenine-mediated nephrotoxicity (2) associated with ddA and because ddI is equally effective as ddA in *in vitro* tests (1), ddI is now used in place of ddA and is currently in phase II/III clinical trials. Dose-limiting toxicities for ddI are peripheral neuropathy and occasionally pancreatitis (3). ddI is used either alone or in combination with other agents such as AZT (4).

Limited pharmacokinetic data on ddI in patients and experimental animals are available (5–9). Some of these data were obtained after the administration of ddA, which is rapidly converted to ddI *in vivo*. Earlier studies in man using unbuffered ddI gave an oral bioavailability ranging from 6 to 50%. The low and variable bioavailability is due in part to the instability of ddI in acid (1). Later studies using buffered ddI gave an oral bioavailability of 38 ± 15% (3). Hence, a large fraction of the dose is unabsorbed and/or eliminated by first-pass metabolism. Data on the extent and saturability of gastrointestinal and hepatic metabolism, necessary to define the cause of variable oral bioavailability, are not available. Studies of potential pharmacokinetic interaction between ddI and other anti-AIDS drugs require suitable animal models and well-defined pharmacokinetics. This study was conducted in rats to examine the pharmacokinetics of ddI, the stability of ddI in blood, and the partitioning of ddI between the intracellular and the extracellular spaces in blood and to determine whether there are dose-dependent kinetics.

MATERIALS AND METHODS

Chemicals and Reagents. ddI was obtained from the National Cancer Institute (Bethesda, MD). 5-Deoxy-5-fluorouridine (dFUR), a gift from Hoffmann LaRoche Laboratories (Nutley, NJ), or N^6-methyl adenosine, purchased from Sigma (St. Louis, MO), was used as the internal standard. Reagent-grade chemicals and high-pressure liquid chromatographic (HPLC) solvents were purchased from Sigma and Fischer Scientific (Cincinnati, OH). HPLC analysis showed that ddI was >98.9% pure, and dFUR was >99.5% pure. All chemicals and reagents were used as received.

Stability of ddI in Blood. Freshly obtained blood from female Fischer rats was pooled and kept on ice. Heparin, to prevent clotting, and ddI in physiologic saline (100 μg/ml, pH adjusted to 7.0) were added. The final mixture contained 44.45 μg/ml ddI, 111 U/ml heparin, and 45% whole blood, pH 6.9. Identical samples were prepared in physiologic saline instead of whole blood to establish the extent of nonenzymatic degradation. A second set of experiments examined the effect of inorganic phosphate on the degradation rate; the phosphorylase enzymes use inorganic phosphate as a cosubstrate. Mixtures were prepared as described above, except ddI was dissolved in 200 m*M* phosphate buffer, pH 7.0. The final phosphate concentration was 89 m*M*. The ddI/blood or ddI/saline mixtures were incubated at 37°C in a shaking water bath. Duplicate samples were used for each time point. The pH of the blood and saline mixtures was unchanged after 4 hr of incubation. Four hundred microliters of water containing the internal standard (10 μg/ml) was added to each tube to lyse the blood cells completely. The lysis of blood cells, ascertained by microscopic examination, facilitated the solid phase extraction. The mixtures were centrifuged at 13,000*g* and 22°C for 1 min to remove the cell debris. The supernatant was transferred, extracted, and analyzed as described below. Similar experiments were performed with incubation at 4°C to define drug stability during storage.

Animal Protocol. Female Fischer rats, 5–6 months old, were housed in metabolic cages and had access to food and water ad lib. The pretreatment body weights of the rats were

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198 ± 7 g (mean ± SD; n = 16). One day before the study, rats were anesthetized with ether or Avertine (1 ml/100 g body weight; 3.1% amyl alcohol and 12.5 mg/ml tribromoethanol in water), and a permanent catheter was implanted in the right jugular vein. Intravenous dosing solutions of ddI were prepared in physiologic saline at concentrations of 4 to 30 mg/ml and adjusted to pH 7. Dose administration was between 8 and 10 AM. The administered volume was about 0.4 ml for the 8 and 40 mg/kg doses and about 1.3 ml for the 200 mg/kg dose. The larger volume for the higher dose was necessary because of the limited solubility of ddI in physiologic saline at pH 7.0. The 8 and 40 mg/kg doses were administered over 0.5 min, and the 200 mg/kg dose over 2 min. Serial blood samples were withdrawn through the venous catheter and kept on ice to avoid drug degradation. The plasma fractions were obtained by centrifugation at 13,000g and 22°C for 1 min. Silastic catheters were used for dose administration and sample collection. Binding of ddI to the catheter was ruled out by the following experiment. A sample of the dosing solution (30 mg/ml) was equilibrated with a catheter. After flushing with a 0.5-ml wash, a sample of 0.5 ml saline was withdrawn through the catheter. ddI was not detected in the sample.

Blood-to-Plasma Ratio. The partitioning of ddI into blood cells was studied in two rats. The rats were given an intravenous bolus dose of 200 mg/kg ddI, as described above. Serial blood samples were taken and split into two separate samples. Four hundred microliters of water containing the internal standard was added to one sample of 100 μl whole blood to lyse the blood cells. The cell debris-free supernatant obtained after centrifugation was transferred, extracted, and analyzed. The other sample was centrifuged to separate the blood cells and plasma, and 50 μl of the plasma fraction was analyzed.

Sample Analysis. Plasma samples were analyzed as described elsewhere (10). In brief, within 4 hr of sample collection, 50 to 100 μl plasma was mixed with 400 μl of aqueous solution containing an internal standard (10 μg/ml). The mixture was extracted by solid–liquid extraction using a C_{18} solid phase extraction column (Supelclean; Bellafonte, PA). The extracted samples were stored frozen. Extracts were analyzed by HPLC using a reverse-phase μBondapak C_{18} column (Waters Associates, Milford, MA) and an aqueous mobile phase containing 10 mM sodium phosphate buffer, pH 6.9, and 4% acetonitrile. The lower limit of detection was 0.1 μg/ml. One hundred microliters of the urine, diluted 10- to 100-fold, was mixed with 400 μl of the internal standard solution and analyzed after direct injection. One of the breakdown products of ddI is hypoxanthine (11). We monitored the appearance of hypoxanthine in the urine. The HPLC retention volume of hypoxanthine was 5 ml. Standard curves of ddI and hypoxanthine were constructed based on the ratios of peak heights of the drug to the internal standard.

Data Analysis. The plasma concentration–time data of ddI for individual animals were analyzed using noncompartmental and compartmental methods. For noncompartmental analysis, the area under the plasma concentration–time curve (AUC) from time zero to time infinity, the total-body clearance (CL), the volume of distribution at steady state ($V_{d_{ss}}$), and the mean residence time (MRT) were calculated according to standard procedures (12). Renal CL (CL_R) was calculated as {CL} multiplied by {fraction of dose excreted unchanged in urine, F_e}. F_e was determined from 24-hr urine collections. Nonrenal or metabolic CL (CL_M) was calculated as {CL} minus {CL_R}. In the compartmental analysis, a two- or three-compartment open model with elimination from the central compartment was computer-fitted to the data. The computer fitting used the NONLIN84 pharmacokinetic data analysis program (Metzler and Weiner, Statistical Consultants Inc., Lexington, KY) and/or the NLIN routine of SAS (Statistical Analysis Systems, Cary, NC). Both programs use a least-squares regression analysis algorithm to determine best-fit parameters and yielded essentially identical parameter estimates for well-fitted data sets. Because the half-life of the first phase was determined to be about 2 min, the time taken to administer the dose, i.e., 0.5 min for the 8 and 40 mg/kg doses and 2 min for the 200 mg/kg dose, was significant. Hence, we analyzed the data using either a bolus input or a zero-order input over 0.5 to 2 min and compared the results.

Statistical analysis was performed using the paired or unpaired Student's t test. Comparisons of pharmacokinetic parameters among three dose groups were performed using the analysis of variance. This was combined with repeated t tests to identify dose groups with different means. The data fits obtained using two- and three-compartment pharmacokinetic models were compared by the distribution of residuals (13), the F test (13), and the Akaike Information Criterion (14). P values of less than 0.05 were considered statistically significant.

RESULTS

Stability of ddI in Blood. Figure 1 compares the degradation of ddI in blood or physiologic saline at 37°C. ddI was stable in saline, with <4% loss in 4 hr. In comparison, ddI in 45% whole blood showed a first-order decline from 0.5 to 4 hr, with about 25% loss in 4 hr and a rate constant of 0.064 hr^{-1}. At a phosphate concentration of 89 mM, the first-order rate constant of ddI degradation in blood was enhanced to 0.247 hr^{-1}. In comparison, the decomposition of ddI in saline was not affected by phosphate concentration and remained at <4% over 4 hr at 89 mM phosphate (Fig. 1). In parallel studies, ddI was incubated with blood and saline at 4°C; less than 4% was degraded after 6 hr (data not shown).

Distribution of ddI into Blood Cells. Figure 2 compares the concentrations of ddI in blood and plasma in two rats given a bolus intravenous dose of 200 mg/kg ddI. The blood and plasma concentrations, over a concentration range of 1 to 400 μg/ml, were nearly superimposable and were not different when tested by paired t test. The ratios of blood-to-plasma concentrations from 1 to 120 min were 0.993 ± 0.115 (mean ± SD; n = 15) and were not distinguishable from unity.

Pharmacokinetics of ddI. Figure 3 shows the mean plasma concentration–time profile of ddI in rats given three different ddI doses, i.e., 8, 40, and 200 mg/kg, by bolus injection over 0.5 to 2 min. After the lower doses of 8 and 40 mg/kg, the decline of the concentrations was biexponential, the β half-life was about 20 min. In comparison, the half-life in dogs is 3-fold longer (7). The computer-predicted concentrations for a two-compartment body model, using either

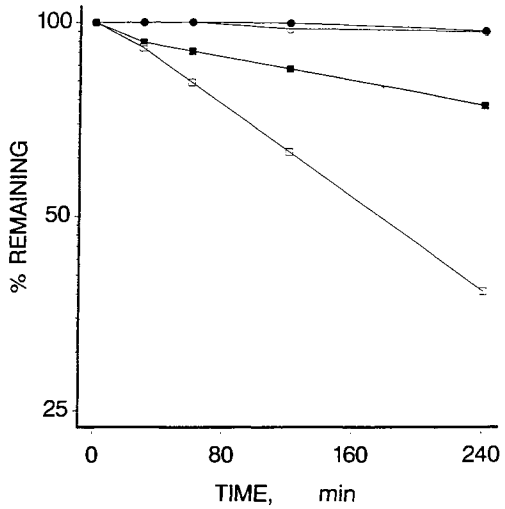

Fig. 1. Degradation of ddI in blood and effect of phosphate. Mixtures of ddI in 45% whole blood (squares) or physiologic saline (circles) were incubated at 37°C in a shaking water bath. All incubates contained 44.45 μg/ml ddI and 111 U/ml heparin, pH 7.0. Incubations were performed in the presence (open symbols) or absence (filled symbols) of 89 m*M* phosphate.

NONLIN84 or SAS, differed by <10% from the observed values with a random distribution of residuals. The two-compartment model gave an adequate fit for 9 of the 10 animals, based on the *F* test and the Akaike information criterion (13,14). The data for the remaining animal were best fitted with a three-compartment model. The computer-fitted parameters such as CL and V_d were within 5–10% of those obtained by noncompartmental analysis (data not shown). After the 200 mg/kg dose, the decline of concentrations yielded a better fit for five of six animals when analyzed by a three-compartment rather than a two-compartment model. The first and second phases had half-lives similar to those of the 8- and 40-mg doses. The additional third phase had a significantly longer half-life of about 56 min. Table I compares the pharmacokinetic parameters of ddI after the three doses. The CL of ddI decreased with increased dose, from 99 ml/min-kg at 8 mg/kg to 37 ml/min-kg at 200 mg/kg. Dose administration of ddI was over 0.5 to 2 min, a period relatively long compared to the α phase half-life of approximately 2 min. AUC estimates were 8 to 12% larger if calculated for a bolus input as compared to a short-term infusion. Pharmacokinetic parameters listed in Table I were calculated using the equation which describes a zero-order infusion input.

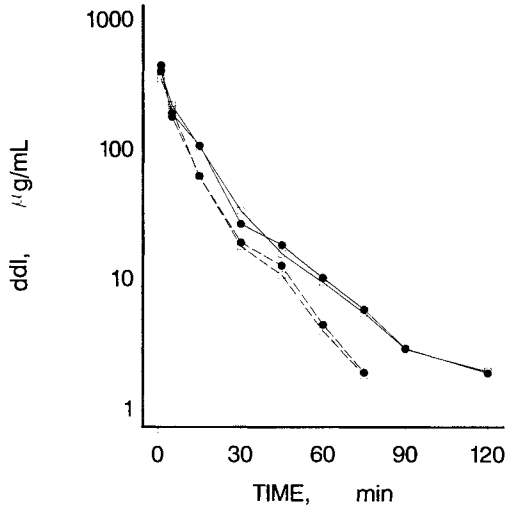

Fig. 2. Partition of ddI into blood cells. Two rats were given intravenous bolus doses of 200 mg/kg ddI. Serial blood samples were obtained and separated into two samples, i.e., 100 μl whole blood and 50 μl plasma. The cells in the whole-blood samples were lyzed by hypotonic shock. The ddI concentrations in blood (opened squares) and plasma (filled circles) were analyzed. Rat 1, solid lines; rat 2, dashed lines.

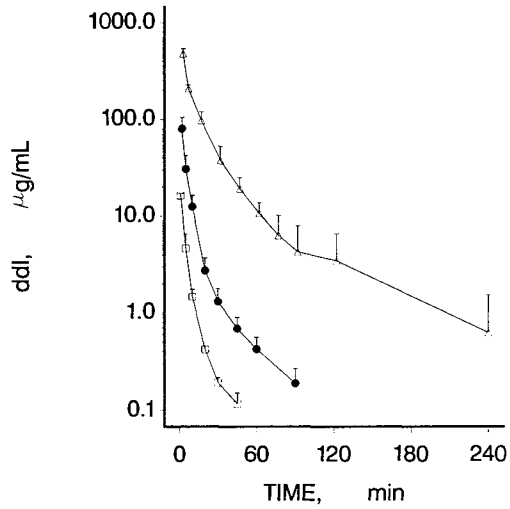

Fig. 3. Plasma concentration–time profiles of ddI in rats. Rats were given intravenous bolus doses of ddI, 8 mg/kg (squares, $n = 4$), 40 mg/kg (circles; $n = 6$), and 200 mg/kg (triangles; $n = 6$). Mean + SD.

Renal Excretion of ddI. The fractions of the ddI dose excreted in urine as unchanged drug over 24 hr for the 8, 40, and 200 mg/kg doses were 21 ± 12, 20 ± 5, and 17 ± 7%, respectively, and were not statistically different. The calculated renal CL decreased with increased doses (Table II). This decrease was statistically significant for the 40- and 200-mg dose group. The pretreatment urine samples showed the presence of hypoxanthine. The renal excretion of hypoxanthine, which is a known metabolite of ddI, was estimated based on the difference between the concentrations excreted before and those after ddI treatment. The excretion of hypoxanthine was estimated to be less than 5% of the dose.

DISCUSSION

Equal Distribution of ddI in Blood Cells and Plasma. The entry of ddI into blood cells including the virus-containing T lymphocytes is necessary for its therapeutic activity. Due to insignificant plasma protein binding [<6% (6)], total plasma concentrations equal unbound plasma concentrations. The equal ddI concentrations in blood and plasma indicate that ddI entered the blood cells and maintained an equilibrium between the intra- and the extracellular spaces. This, together with the lack of concentration-dependent cellular uptake over a 400-fold concentration range, suggests that ddI transport into cells is by diffusion.

Table I. Pharmacokinetic Parameters of ddI: Rats Were Given Intravenous Injections of ddI at a Dose of 8, 40, or 200 mg/kg[a,*]

Dose (mg/kg)	CL (ml/min-kg)	$V_{d_{ss}}$ (ml/kg)	α half-life (min)	β half-life (min)	γ half-life (min)	MRT (min)
8 (n = 4)	99.1 ± 12.2[b]	831 ± 131	1.87 ± 0.17	13.6 ± 5.6[b]	NA	8.03 ± 1.15[b]
40 (n = 6)	76.5 ± 27.5[b]	713 ± 264	2.30 ± 0.63	25.9 ± 8.1[c]	NA	9.23 ± 0.98[b]
200 (n = 6)	36.8 ± 4.8[c]	551 ± 160	1.71 ± 1.08	13.4 ± 2.3[b]	56 ± 19.2	15.2 ± 3.6[c]
P	0.0004	NS	NS	0.005	NA	0.0005

[a] Data are presented as mean ± SD. NA, not applicable. Statistical differences were analyzed by analysis of variance with repeated t tests to identify dose groups with different means. P is the level of significance from the ANOVA test. NS, not significant.

* For each parameter, the means followed by a superscript b are significantly different from those denoted by c. Means followed by the same superscript letter, b or c, are not significantly different from each other.

This is in agreement with its transport into lymphocytes by unfacilitated diffusion (1).

Metabolism in Blood. Purine phosphorylases catalyze the bidirectional reaction between purine nucleosides and bases, using inorganic phosphate as a cosubstrate. ddI is metabolized to hypoxanthine by the purine phosphorylases (11,15). These enzymes are distributed in various mammalian tissues including blood (16,17). Our data showing the enzymatic degradation of ddI in blood and its enhancement by inorganic phosphate support the role of blood phosphorylases in ddI degradation. We estimated the metabolic CL of ddI by blood and its contribution to the total-body CL. The calculated contribution of blood to the total-body CL of ddI is {first-order degradation rate constant in 100% whole blood} multiplied by {blood volume}. Using a rate constant of 0.143 hr^{-1} in 100% whole blood and a blood volume of 100 ml/kg body weight in rats, the calculated CL in whole blood was 0.238 ml/min-kg. This represents 0.6% of the total-body CL after a 200 mg/kg dose. Hence the metabolism in blood did not contribute significantly to ddI elimination. The metabolism of ddI in blood indicates the need of appropriate storage and processing of blood samples in order to avoid drug degradation during sample workup. Our data show that the enzymatic degradation of ddI is diminished at low temperatures. Storage of ddI samples on ice, followed by drug extraction on the same day, limited drug degradation to less than 4%.

Dose-Dependent Kinetics of ddI. Previous data on ddA, which rapidly converts to ddI *in vivo*, suggest nonlinear disposition of ddI in dogs (7) and mice (18). Our data showed that after ddI administration, the disposition of ddI in rats followed nonlinear kinetics and the CL decreased with increased concentrations. A possible cause of the higher CL of the 8- and 40-mg doses could be an underestimation of the AUC, due to the inability to measure a slow third phase. The γ-phase AUC accounted for 13% of the total AUC from the 200-mg dose. To estimate this potential error, we calculated the AUC for a third phase using the averaged γ-phase half-life derived from the 200 mg/kg data and the last measured concentration after the 8- and 40-mg doses (12). Inclusion of the projected third phase would, for the 8- and 40-mg/kg doses, increase the AUC by 8.7 and 3.6% and decrease the CL to 90.3 and 73.4 ml/min-kg. These CL values are more than twofold greater than the CL of the highest dose of 200 mg/kg. Hence the higher CL of the lower doses could not be due to neglecting the third phase. The dose-dependent CL indicates nonlinear disposition of ddI at higher concentrations. The CL of the 200 mg/kg dose is comparable to the reported CL of 42 ml/min-kg during intravenous infusion of 125 mg/hr-kg for 2 hr (calculated from data given in Ref. 6).

At the lower doses of 8 and 40 mg/kg, the decline in concentration was biexponential, with a β half-life of about 20 min. A triexponential disposition of ddI, with a terminal half-life of 56 min, was observed after the highest dose of 200

Table II. Urinary and Metabolic Clearances of ddI: 24 hr Urine Was Collected and Analyzed for ddI and Hypoxanthine[a]

Dose (mg/kg)	CL_R (ml/min-kg)	CL_M (ml/min-kg)	F_e(ddI) (% of dose)	Hypoxanthine excreted (% of dose)
8 (n = 3)[b]	20.8 ± 14.3	76.1 ± 12.9[c]	21.0 ± 12.5	<5
40 (n = 6)	13.5 ± 4.7	52.4 ± 6.8[d]	20.2 ± 5.0	<5
200 (n = 6)	7.37 ± 4.28	29.4 ± 5.9[e]	16.7 ± 6.9	<5
P	0.07[f]	0.0001	NS	NA

[a] The fraction of the dose excreted as unchanged ddI (F_e) was determined. The renal and metabolic CL of ddI were calculated. Statistical differences were analyzed by analysis of variance with repeated t test to identify dose groups with different means. P was the level of significance from the ANOVA test. NS, not significant. NA, not applicable.

[b] n = 3, excluding an outlier which produced <0.1 ml of urine and excreted <2% of the dose.

[c–e] Means with different superscript letters (c–e) were different from each other.

[f] Unpaired t tests comparing the 200 mg/kg dose group with the 8 and 40 mg/kg dose groups yielded P values of 0.06 and 0.05, respectively.

mg/kg. The third phase was observed only after administration of the highest dose but likely also occurred at the lower dose levels. In other ongoing studies where an intravenous bolus dose of radiolabeled ddI was administered concomitantly with unlabeled ddI given by other routes, a γ terminal phase with a half life of 40–50 min was detected for the radiolabeled ddI at a low total plasma concentration of 0.4 μg/ml (S. L. Bramer, M. G. Wientjes, and J. L-S. Au, unpublished results). Inhibition of viral replication requires the continuous presence of ddI and its active metabolites. A long half-life is important for the desired prolonged therapeutic effect. The third phase found in our study may be due to redistribution of unchanged drug and metabolites released from intracellular sites or accumulated in specific organ sites. The volume of distribution of ddI was close to the total-body water of 700 ml/kg in the rat (19). There was a trend of decreasing volumes after higher doses, but the differences between dose groups were not significant. A similar trend was observed for AZT (20).

Metabolic CL. About 80% of the ddI dose was eliminated by metabolism. There was a nearly 3-fold difference in plasma CL and metabolic CL_M at low and high plasma concentrations over the 25-fold dose range. Plasma protein binding of ddI is minimal (6) and cannot contribute to dose-dependent kinetics. The lower metabolic CL at high concentrations suggests saturable metabolism of the drug. Preliminary data from ongoing studies suggest a concentration-dependent hepatic drug extraction of 6 to 22% (S. L. Bramer, M. G. Wientjes, and J. L-S. Au, unpublished results).

The metabolic fate of ddI *in vivo* has not been reported. In cellular systems, ddI is metabolized by several enzymes. The activation to the reverse transcriptase inhibitor ddATP is quantitatively a minor pathway, while catabolism by purine nucleoside phosphorylases to the inactive hypoxanthine is the major metabolic pathway (11,21). Cleavage of the C–N glycosidic bond, as shown in cultured T cells (11,21), is also a likely first step in the metabolism of ddI *in vivo* (18). Phosphorolysis by purine nucleoside phosphorylases would result in the formation of hypoxanthine and dideoxyribose 1-phosphate (16). In mammals, hypoxanthine is partly reutilized for purine biosynthesis and partly oxidized at the C-2 and C-8 positions to form uric acid. Uric acid is the final excretion product in man but is further broken down to allantoin in the rat (22). In the present study, only small amounts of hypoxanthine were found in urine (less than 5% of the dose). It is possible that the conversion of hypoxanthine to uric acid and allantoin is rapid and complete. We did not analyze for these metabolites, partly because it is difficult to distinguish the metabolite pool from the endogenous pool.

In phase I studies, the intravenous dose used in man was between 1 and 16 mg/kg. The corresponding maximal plasma concentration was <20 μg/ml, which was 4- to 25-fold lower than the maximal concentrations in rats at 40 and 200 mg/kg doses. The lack of apparent nonlinear kinetics in man (8,9) may be due to the lower plasma drug concentration. Our finding of nonlinear ddI metabolism may have the following clinical implications. Clinically, ddI is given orally. The drug concentration presented to the first-pass organs (e.g., gastrointestinal mucosa and liver) are much greater than the plasma concentrations and are likely to be in the range of nonlinear disposition. Concentration-dependent metabolism may occur during drug absorption, which will result in variable oral bioavailability. The contributions of the intestinal wall and liver to the first-pass metabolism of ddI and the saturability of the enzyme systems need to be determined to estimate the quantitative importance of this phenomenon.

Renal CL. Urinary recovery of ddI accounted for approximately 20% of the 8 to 200 mg/kg doses. The renal CL ranged from 7.4 ml/min-kg for the highest dose to 20.8 ml/min-kg at the lowest dose. These values were greater than the literature value of 5.4 ml/min-kg for glomerular filtration in the rat (24), suggesting an excretory component to the renal CL of ddI. The renal CL decreased with increased dose and was more than two-fold slower at higher doses. This raises the possibility of a saturable excretion process. Saturable renal secretion has been reported for AZT (20).

In summary, these data indicate equal distribution of ddI in the extracellular and intracellular spaces in blood, its enzymatic degradation in blood, and nonlinear elimination kinetics.

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