Pharmacokinetics of Oral 2',3'-Dideoxyinosine in Rats

M. Guillaume Wientjes[1,3] and Jessie L.-S. Au[2]

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INTRODUCTION

2',3'-Dideoxyinosine (ddI)[4] is an effective agent in the treatment of patients with acquired immunodeficiency syndrome (1). *In vitro* studies have shown that ddI is converted intracellularly to 2',3'-dideoxyadenosine triphosphate, which is a potent inhibitor of the reverse transcriptase needed for viral replication (2,3). Drug treatment aims to inhibit viral replication; it does not eliminate the virus and lifelong maintenance therapy is required to suppress the viral activity. The oral route is used in the current clinical trials and will be the preferred route for patient treatment. The oral bioavailability of unbuffered ddI is low and highly variable, ranging from 6 to 50% (4). ddI is unstable at low pH's with half-lives of <1 and 14 min at pH 1 and 2.3 (5). It has been suggested that drug degradation in the acidic environment of the stomach contributes to the low and variable oral bioavailability. To reduce the degradation, ddI is currently administered in combination with antacids. However, the bioavailability remains variable and incomplete, with values of 38 ± 15% (mean ± SD) in adults (6) and <5 to 89% in children (7). This suggests factors other than acid instability have contributed to the low bioavailability. A recent study showed a low oral bioavailability (1–11%) of ddI in rats and that pretreatment with oral sodium bicarbonate did not enhance the absolute bioavailability; however, a comparison of the urinary excretion data of the intravenous and oral doses in that publication (8) suggests an oral bioavailability of 24–30%. The cause of the >3-fold difference in bioavailability calculated using the plasma and urine data is not apparent. One possible cause is that the systemic plasma clearance used in the bioavailability calculation was derived from data of an intravenous dose, with the assumption of linear kinetics and constant clearance of ddI by intravenous and oral routes. This assumption is incorrect, based on the nonlinear disposition kinetics of ddI shown in the above publication (8) and in our study (9) and may have led to erroneous bioavailability estimates. The present study is to assess the absolute oral bioavailability using concomitant administration of an intravenous dose of [^{3}H]ddI (to determine clearance) and an oral dose of unlabeled ddI. This may clarify the conflicting results in the previous publication (8). Our goal is to determine whether the rat is a suitable animal model to examine the presystemic elimination of oral ddI. The pharmacokinetics and bioavailability and the absorption rate–time profiles of oral ddI in rats were investigated.

MATERIALS AND METHODS

Chemicals. ddI (lot 234-B-1), [8-^{3}H]ddI (lot 5549-117; sp act, 11.0 μCi/μg), [2',3'-^{3}H]ddI (lot 5979-83; sp act, 124 μCi/μg), and ftorafur [N^1-(2-tetrahydrofuranyl)-5-fluorouracil] were obtained from the National Cancer Institute (Bethesda, MD). 5'-Deoxy-5-fluorouridine was a gift from Hoffmann LaRoche Laboratories (Nutley, NJ). HPLC analysis showed that ddI, 5'-deoxy-5-fluorouridine, and ftorafur were >98% pure. Reagent-grade chemicals and high-pressure liquid chromatographic (HPLC) solvents were purchased from Sigma and Fischer Scientific (Cincinnati, OH). Ecolite scintillation cocktail was obtained from ICN Biochemicals (Costa Mesa, CA). All chemicals and reagents were used as received.

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 185 ± 24 g (mean ± SD, n = 7). One day before the study, a rat was anesthetized under ether or Avertine (1 ml/100 g body weight; 3.1% amyl alcohol and 12.5 mg/ml tribromoethanol in water). A permanent catheter was implanted in the right jugular vein of the anesthetized rat. After a recovery period of at least 16 hr, the rats received by oral gavage a dose of ddI of 40 mg/kg. This was followed 5 min later by an intravenous tracer dose of [^{3}H]ddI, 20 μCi or about 0.2 to 2 μg in saline, pH 7.0. Depending on the availability of the chemical, either the [8-^{3}H]ddI or the [2',3'-^{3}H]ddI was used. The oral dosing solution contained ddI at 16 mg/ml in saline. The pH of the dosing solution was adjusted to 7.0 with NaOH. The dosing solution was stored frozen. Under these conditions, less than 2.5% of ddI decomposed over a period of 1 month. Dose administration was between 8 and 10 AM. The intravenous dose was administered over 30 sec. Serial blood samples (0.25 ml) were withdrawn through the venous catheter and kept on ice to avoid drug degradation. We previously found that ddI was enzymatically degraded in whole blood (9). Plasma was obtained by centrifugation at 13,000g and 22°C for 1 min. Samples were stored at −20°C until analysis.

Sample Analysis. Plasma samples were analyzed as described elsewhere (10). In brief, 50 to 100 μl of plasma was mixed with 400 μl of 100 mM phosphate buffer, pH 6.9 containing the internal standard 5'-deoxy-5-fluorouridine (10 μg/ml) or ftorafur (2 μg/ml). 5'-Deoxy-5-fluorouridine was used in the first half of the study, and ftorafur in the second half. The change to ftorafur was due to the higher extraction yield of ftorafur (~90%) compared to 5'-deoxy-5-fluorouridine

[1] Department of Surgery, The Ohio State University, Columbus, Ohio 43210.
[2] College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.
[3] To whom correspondence should be addressed at Suite 302, Comprehensive Cancer Center, 410 West 12th Avenue, Columbus, Ohio 43210-1228.
[4] Abbreviations used: ddI, 2',3'-dideoxyinosine; HPLC, high-pressure liquid chromatography; F, oral bioavailability; AUC, area under plasma concentration–time curve; $V_{d_{ss}}$, volume of distribution at steady state; $F_{e_{iv}}$ and $F_{e_{oral}}$, fractions of intravenous and oral doses excreted in urine.

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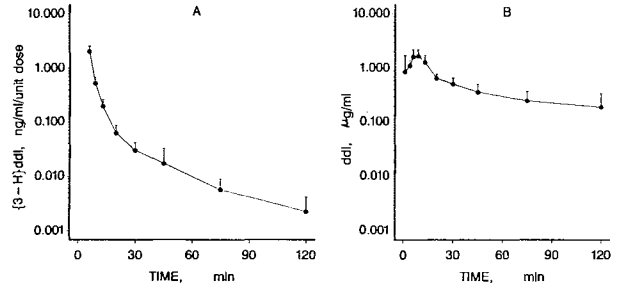

Fig. 1. ddI plasma concentration–time profiles after concomitant oral and intravenous administration in rats. Rats were given an oral dose of 40 mg/kg unlabeled ddI by gavage and, 5 min later, an intravenous bolus injection of [^{3}H]ddI (20 μCi, about 0.2 to 2 μg) over 30 sec. (A) Plasma concentrations of [^{3}H]ddI after intravenous administration. To correct for the different specific activities of ddI, concentrations as expressed as nanograms per milliliter, normalized for a dose of 1 μg/kg. (B) Unlabeled ddI after oral administration. The time scale is the time after the oral dose administration. Mean + 1 SD (n = 7).

(~40%). The mixture was placed onto a C_{18} solid phase extraction column (Supelclean, Bellafonte, PA) preconditioned with two 1-ml fractions of methanol and two 1-ml fractions of 100 m*M* sodium phosphate buffer, pH 6.9. Two fractions of 1 ml of 100 m*M* sodium phosphate buffer, pH 6.9, were passed through the Supelclean column to remove polar interferences. The sample was then eluted with 75:25 (v/v) of methanol and 5 m*M* sodium phosphate buffer of pH 6.9. The eluent was dried under nitrogen to a final volume of about 0.2 ml. Extracts were analyzed by HPLC using a reverse-phase μBondapak C_{18} column (Waters Associates, Milford, MA) and an aqueous mobile phase containing 10 m*M* sodium phosphate buffer of pH 6.9 and 4% acetonitrile. The HPLC equipment was as described previously (9). For analysis of [^{3}H]ddI concentrations, HPLC-eluting fractions were collected, and radioactivity was determined by liquid scintillation counting using a Beckman (Irvine, CA) Model LS8100 counter. The counting efficiency for ^{3}H was 40%. Plasma concentrations of [^{3}H]ddI were less than 0.1% of the total ddI concentrations at all times. Hence, the total ddI concentrations measured by UV absorbance were not corrected for [^{3}H]ddI concentrations. The standard curves for [^{3}H]ddI quantitation were established from the ratios of the ddI radioactivity to the UV absorbance of the internal standard. Urine samples were diluted 10- to 100-fold. One hundred microliters of the diluted urine was mixed with 400 μl of the internal standard solution and analyzed by HPLC without extraction. The assay sensitivity limit was 100 ng/ml in plasma and in urine.

Data Analysis. The plasma concentration–time data of ddI were analyzed using noncompartmental and model-dependent methods. In the noncompartmental analysis, the area under the drug concentration–time curve (AUC) was calculated by the trapezoidal rule. The plasma clearance of ddI was calculated after intravenous administration as {dose} divided by {AUC} (11), and the volume of distribution at steady state ($V_{d_{ss}}$) by the method of Benet and Galeazzi (12). Renal clearance was calculated as {amount of ddI excreted unchanged in urine} divided by {plasma AUC}. In the model-dependent analysis, the data were computer-fitted to a two-compartment open model with elimination from the central compartment. Model-dependent analysis was used to estimate values not easily obtained by noncompartmental methods, i.e., the AUC from the time of dose administration to the time that the first plasma sample was taken (1 min), and the apparent disposition half-lives. The computer program used for the data fitting was the NLIN routine of SAS (Statistical Analysis Systems, Cary, NC). This program uses the Gauss-Newton least-squares regression analysis algorithm to determine best-fit parameters (13). A weighting function which minimizes the differences between the logarithms of the observed and those of the calculated concentrations was used. Because the half-life of the first phase was determined to be about 2 min, the time taken to administer the intravenous dose (30 sec) was significant. Hence, we analyzed the data using a zero-order input over 30 sec. Analyses of the concentration–time data after oral administration with a one-compartment and a two-compartment model were compared using the Akaike information criterion (14). The one-compartment analysis was found to be the better model and was used. The rate of drug absorption was analyzed by deconvolution for data with unequal spacing between sampling points (15). The bioavailability was determined from the plasma data according to Eq. (1) and from the urine data according to Eq. (2). $F_{e_{iv}}$ and $F_{e_{oral}}$ are the fractions of the

Table I. Pharmacokinetic Parameters for ddI After Intravenous Administration[a]

Rat	Body weight (g)	Clearance (ml/min/kg)	Renal clearance (ml/min/kg)	$V_{d_{ss}}$ (L/kg)	$t_{1/2}^{\alpha}$ (min)	$t_{1/2}^{\beta}$ (min)
1	158	123.9	17.2	2.32	2.65	48.76
2	169	116.8	7.31	1.81	2.64	28.72
3	202	154.5	27.3	1.69	1.59	17.84
4	203	81.8	55.5	1.39	1.64	22.20
5	183	93.8	60.1	1.81	5.31	41.66
6	220	102.4	18.3	1.23	1.98	14.83
7	161	134.9	46.0	1.33	1.57	11.14
Mean	185	115.4	33.1	1.66	2.49	26.45
SD	24	25.0	20.7	0.38	1.33	14.12

[a] Rats received a dose of 40 mg/kg by oral gavage and, 5 min later, an intravenous bolus dose of [^{3}H]ddI (20 μCi, about 0.2 to 2 μg). The plasma concentration–time data of [^{3}H]ddI were analyzed by model-dependent methods to obtain half-lives, and model-independent methods to obtain the other parameters. $V_{d_{ss}}$ is volume of distribution at steady state.

intravenous and oral doses excreted unchanged in urine in 24 hr.

$$\text{Oral bioavailability} = \frac{\text{AUC}_{\text{oral}} \times \text{Clearance}_{\text{intravenous}}}{\text{Dose}_{\text{oral}}} \quad (1)$$

$$\text{Oral bioavailability} = \frac{F_{e_{\text{oral}}}}{F_{e_{\text{iv}}}} \quad (2)$$

RESULTS

Pharmacokinetics of the Intravenous Dose. We found previously that the elimination of ddI was concentration dependent (9). Therefore, it was necessary to obtain the drug clearance during the disposition of the oral dose, in order to calculate the absolute oral bioavailability. The absorption of orally administered ddI was studied in rats that received a concomitant intravenous tracer dose of [^{3}H]ddI. The clearance of the [^{3}H]ddI dose was used to calculate the oral bioavailability. Plasma concentrations of the intravenously injected [^{3}H]ddI declined rapidly, with an initial half-life of 2.5 min and a terminal half-life of 26 min (Fig. 1). The plasma clearance was 115 ± 25 ml/min/kg (Table I). In earlier and ongoing studies (9; S. L. Bramer, G. W. Wientjes, and J. L.-S. Au, unpublished results) to compare the disposition of ddI over a 25-fold dose range, we determined that the clearance after the lowest dose of 8 mg/kg was 99 ± 12 ml/min/kg, similar to the clearance obtained in the present study. These studies also showed two early phases of concentration decline, with half-lives of 1 and 5 min, as compared to one initial phase in the present study. The difference is likely due to the greater number of data points obtained during the first 15 min in the previous studies, which permitted the detection of the two phases.

Pharmacokinetics of the Oral Dose. The plasma concentrations of the orally administered ddI reached their maxima of 1.8 ± 0.5 μg/ml in less than 10 min. The half-life after the oral dose was longer than after intravenous administration, indicating a "flip-flop" pharmacokinetic model, where the disposition of ddI was rate-limited by its absorption (Fig. 1, Table II). Deconvolution analysis of the drug absorption profile showed that the rate of systemic drug absorption reached its apex at 3.5 ± 1.6 min (Fig. 2, Table III). After

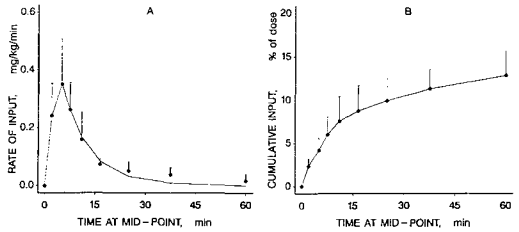

Fig. 2. Absorption profile of ddI as determined by deconvolution. Experimental conditions are as in Fig. 1. (A) Rate of systemic drug input versus the midpoint of the sampling intervals. The solid line is the computer-fitted curve for a drug input function that increases to a maximum value with a subsequent monoexponential decline. (B) Cumulative absorption of ddI as percentage of the administered dose.

that, the rate of drug input declined log-linearly, with a half-life of 6.1 ± 3.0 min. A slow but significant rate of drug input was maintained for approximately 60 min in most rats. Absorption of 50% of the bioavailable dose was complete at 16 ± 9 min.

The oral bioavailability of ddI, calculated using the plasma data and Eq. (1), was 16.5 ± 5.1% (Table II). Renal excretion of ddI was variable and accounted for 4.1 ± 2.7% of the oral dose and 32 ± 25% of the intravenous dose. The oral bioavailability, estimated from the renal excretion data and Eq. (2), was 14.9 ± 5.4% (Table II). The measurement of bioavailability based on renal excretion data is an indirect determination and is presented for confirmation of the plasma data. The oral bioavailability of ddI in rats is similar to 8–11% bioavailability of a 100 mg/kg dose obtained in male rats as reported by other investigators (8).

DISCUSSION

Data from this study show that (a) ddI was absorbed rapidly from the upper gastrointestinal tract of rats, (b) the absorption was incomplete and variable, and (c) the oral bioavailability of unbuffered ddI solution was <20%. Hence, over 80% of the dose was either not absorbed or eliminated by enzymatic or nonenzymatic degradation. In view of the rapid degradation of ddI in the pH range of 1–3 generally found in the stomach (16), acid-catalyzed hydrolysis is a

Table II. Pharmacokinetic Parameters of ddI After Oral Administration[a]

Rat	C_{max} (μg/ml)	t_{max} (min)	AUC (μg-min/ml)	$t_{1/2}$ (min)	F from plasma data (%)	$F_{e_{iv}}$ (%)	$F_{e_{oral}}$ (%)	F from renal data (%)
1	1.52	9.00	51.1	30.6	15.8	13.9	2.41	17.4
2	2.13	9.00	78.5	36.0	22.9	6.26	1.28	20.5
3	2.69	6.00	46.1	6.70	17.8	17.7	2.47	13.9
4	1.48	9.00	67.6	34.7	11.6	67.8	7.21	10.6
5	0.98	9.00	43.0	30.7	8.40	64.1	5.46	8.52
6	1.72	9.00	68.4	27.4	17.5	17.9	1.92	10.7
7	1.79	6.00	62.9	100.9	21.2	34.1	7.71	22.6
Mean	1.76	8.14	59.7	38.1	16.5	31.7	4.07	14.9
SD	0.54	1.46	13.2	29.4	5.1	24.9	2.67	5.4

[a] Rats received a dose of 40 mg/kg by oral gavage and, 5 min later, an intravenous bolus dose of [^{3}H]ddI (20 μCi, about 0.2 to 2 μg). The plasma concentration–time data of ddI were analyzed by model-independent methods. F was calculated using plasma data with Eq. (1) or using urine data with Eq. (2). C_{max} is the maximal plasma concentration occurring at time t_{max}.

Table III. Deconvolution Analysis of ddI Absorption After an Oral Solution[a]

Rat	Max input rate (mg/kg/min)	$t_{1/2}$ (min)	t_{max} (min)	$t_{50\%}$ (min)
1	0.40	5.35	3.48	11.7
2	0.49	4.76	3.96	16.4
3	0.69	5.38	4.54	6.6
4	0.21	12.85	3.50	14.3
5	0.49	5.59	<2.00[b]	8.9
6	0.33	5.00	4.74	24.6
7	0.41	4.08	4.10	30.9
Mean	0.43	6.14	3.47	16.2
SD	0.15	3.00	1.60	8.7

[a] The rate of systemic drug input was analyzed by deconvolution for data with unequal spacing between sampling points. t_{max} is the time for the input rate to reach the maximal value. The input rate subsequently declined log-linearly with the half-life, $t_{1/2}$. $t_{50\%}$ is the time needed for 50% of the bioavailable dose to be absorbed.

[b] The time of maximal input was before the first midtime point of 2 min and could not be estimated by data fitting. The data point was included in the calculation of the mean value as $t_{max} = 0$.

likely cause for limited drug bioavailability. In addition, ddI is a substrate for purine phosphorylases (17–19), which catalyze the breakdown of inosine nucleosides to hypoxanthine and the phosphorylated sugar moiety (20). Purine phosphorylases are found in the intestines, liver, blood, and other tissues. Phosphorylases are also present in bacteria. Enzymatic degradation of ddI during the absorption process can take place in the intestinal wall and in the liver, as well as the intestinal flora. We found that ddI was metabolized by rat intestinal content, with an initial half-life of 7 hr in a 14% (w/v) solution of fecal material (S. L. Bramer, M. G. Wientjes, and J. L.-S. Au, unpublished results). Other presystemic sites of drug degradation are presently unknown and need to be investigated to aid the development of administration forms with greater bioavailability. Increasing the bioavailability to or near 100% will also reduce the intersubject variability and may improve the therapeutic management of patients. The present study shows that the low and variable bioavailability of ddI in rats is qualitatively similar to the situation in humans. The data suggest that the rat may be a suitable animal model for studying the factors contributing to the extensive and variable presystemic elimination of ddI and for evaluating experimental approaches to improve its systemic bioavailability. Studies are ongoing to examine the relative significance of the gastrointestinal and hepatic first-pass elimination of ddI.

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