Pergamon

Biochemical Pharmacology, Vol. 48, No. 11, pp. 2109–2116, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00405-6

TISSUE PHARMACOKINETICS OF 2′,3′-DIDEOXYINOSINE IN RATS

HYO-JEONG KUH KANG*, M. GUILLAUME WIENTJES*† and JESSIE L.-S. AU*†‡

*Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, and †Division of Urology, College of Medicine, The Ohio State University, Columbus, OH, U.S.A.

(*Received* 7 *April* 1994; *accepted* 20 *July* 1994)

Abstract—The present study examined in rats the concentration–time profiles of 2′,3′-dideoxyinosine (ddI) in pharmacologically relevant tissues, including organs where drug effects are desired (brain, lymph nodes, spleen), organs with known drug toxicity (pancreas), and major eliminating organs (liver, kidney). ddI was analyzed by high performance liquid chromatography and radioimmunoassay. In the liver, pancreas, spleen, brain and lymph nodes, the highest concentrations were reached between 4 and 7 min after drug administration. The concentrations subsequently declined in parallel with those in plasma, indicating that plasma and these tissues were in rapid equilibrium. The concentrations in these tissues were less or equal to that of plasma. In the kidney, the maximal concentration occurred at a later time of 14 min, after which concentrations also declined in parallel to those in plasma. The kidney concentrations were about 10-fold greater than plasma concentrations. The ratios of tissue-to-plasma concentrations and of areas under the tissue and plasma concentration–time profiles showed a 230- to 300-fold range with the rank order of kidney $\gg$ liver $\approx$ pancreas $>$ lymph nodes $>$ spleen $\gg$ brain, with respective values of 10.4, 1.09, 0.90, 0.75, 0.42, and 0.04. These data indicate no accumulation of ddI in brain, lymph nodes, spleen, pancreas and liver, and a significant accumulation in the kidney. The low tissue-to-plasma ratios in brain and spleen and a moderate ratio in lymph nodes indicate that further enhancement of the therapeutic effect of ddI requires improved drug delivery and entrapment in these tissues.

Key words: ddI; tissue pharmacokinetics; lymph nodes; brain; spleen; pancreas; liver; kidney; metabolism; HPLC; RIA

ddI§ is used to treat patients infected with HIV. The active anabolite of ddI, dideoxyadenosine triphosphate (ddATP), is incorporated into the viral DNA resulting in termination of chain elongation and, consequently, inhibition of viral DNA synthesis [1]. ddI blocks HIV replication at concentrations that are 10–20 times lower than those needed to inhibit cellular proliferation [2]. ddI has a relatively high therapeutic index compared with other dideoxynucleosides, and is less toxic to human bone marrow progenitor cells than 3′-azido-3′-deoxythymidine (AZT) *in vitro* [2, 3].

Fauci and co-workers [4] recently reported that lymph nodes harbor much more HIV than blood cells in the early-stage of the disease, and that active viral replication occurs in nodal tissues throughout the period of clinical latency when little or no active viral production can be detected in blood cells. Lymph nodes are filled with an intricate lattice of FDC. HIV particles are trapped on the villus processes of FDC that surround and are intimately associated with lymphocytes. As the disease progresses and the immune system collapses, the lymph node is disrupted. Degeneration or death of the FDC leads to a loss of their ability to trap the virus particles and the subsequent release of HIV into the bloodstream, resulting in plasma viremia. Involvement of brain tissue occurs as a late complication of HIV infection in the AIDS–dementia complex. There is evidence that HIV is present in the brain and cerebrospinal fluid of demented patients [5, 6]. Distribution of anti-HIV drugs into the brain, lymph nodes and other lymphoid tissues may play an important role in preventing and slowing the progress of the disease [7, 8].

Peripheral neuropathy and potentially lethal pancreatitis are the major dose-limiting toxicities in humans. ddI-induced pancreatitis is apparently dose-dependent and is often found in patients who receive ddI at the highest dose levels [9, 10]. Grady *et al.* [11] evaluated the effect of ddI on the pancreas in rats. These authors concluded that ddI had no direct toxic effect on the pancreas, based on the unaltered pancreatic tissue water content and morphology, subcellular enzyme distribution, and amylase secretion after chronic ddI administration. Nordback *et al.* [12] investigated the acute effect of ddI on isolated perfused canine pancreas preparations and found changes in acinar cell physiology and ultrastructure. However, the typical injury responses associated with most forms of pancreatitis, such as edema of the pancreas, increase in tissue weight, and increased

‡ Corresponding author: Jessie L.-S. Au, College of Pharmacy, 500 West 12th Av., Columbus, OH 43210. Tel. and FAX (614) 292-4244.

§ Abbreviations: ddI, 2′,3′-dideoxyinosine; HIV, human immunodeficiency virus; FDC, follicular dendritic cells; RIA, radioimmunoassay; AUC_{tissue} and AUC_{plasma}, area under the concentration–time curve in tissue and plasma, respectively; and C_{tissue} and C_{plasma}, concentration in tissue and plasma, respectively.

amylase activity in the perfusate, were not observed. The pathogenesis of ddI-induced pancreatitis remains unknown. Organ specific toxicity is often related to accumulation of drug and/or metabolites in specific tissues.

The present study examined in rats the ddI concentration–time profiles in pharmacologically relevant tissues, including organs where drug effects are desired (brain, lymph nodes, spleen), organs with known drug toxicity (pancreas), and major eliminating organs (liver, kidney). ddI in serum, plasma, and urine has been analyzed by HPLC and RIA [13–15,*]. The published methodologies were modified to analyze for ddI in tissues.

MATERIALS AND METHODS

Chemicals and reagents. ddI and ftorafur were obtained from the National Cancer Institute (Bethesda, MD). Reagent grade chemicals and HPLC grade solvents were purchased from the Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Cincinnati, OH). [Ribose-2′,3′-^{3}H]ddI (sp. act. 124 mCi/mg; Lot No. 7009-93) was provided by Research Triangle Institute (Research Triangle Park, NC) under contract with the National Institutes of Health. The purities of unlabeled and tritiated ddI, analyzed by HPLC, were >98.9 and 96%, respectively. Rabbit IgG, rabbit anti-ddI antiserum (Lot No. 053H8828), RIA assay buffer, and rabbit IgG immunoprecipitation reagent were purchased from the Sigma Chemical Co. The cross-reactivities of the anti-ddI antiserum for dideoxyadenosine and hypoxanthine were 0.54 and 0.002%, and were <0.001% for inosine, adenosine, uric acid and caffeine.* The cross-reactivity for ddATP is not known. Solvable tissue gel solubilizer and Atomlight scintillation fluid were purchased from DuPont Biotechnology Systems (Boston, MA).

Apparatus. The HPLC system consisted of a solvent delivery pump (model 510, Waters Associates, Milford, MA), an automated sample injector (WISP 710B, Waters Associates), a variable wavelength UV detector (Spectroflow 757, Kratos Analytical Instruments, Ramsey, NJ), and an integrator (HP3394A, Hewlett-Packard Co., Menlo Park, CA). The analytes were quantified by their UV absorbance at 254 nm. Liquid scintillation counting was performed on a Tri-Carb analyzer (model 1600TR, Packard Instrument Co., Meriden, CT).

Animal protocol. Female Fisher rats (Charles River, Wilmington, MA), 5 to 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 220 ± 20 g. One day before the study, rats were anesthetized with ether, and a permanent catheter (Silastic medical grade tubing, 0.020 in. i.d. and 0.037 in. o.d., Dow Corning Corp., Midland, MI) was implanted in the right jugular vein. On the day of study, the rats were lightly anesthetized with ether to facilitate restraining of the animal on a surgical board. About 30–40 min later, a drug solution was administered (100 mg/kg) intravenously (i.v.) via the jugular catheter. The dosing solutions, with a final concentration of 21–24 mg/mL, were prepared in saline and adjusted to pH 7.0 with 1 N HCl and 1 N NaOH solutions. The dose was administered over 2 min, between 9:00 and 11:00 a.m.

The arterial plasma and tissue concentration versus time profiles were obtained using one rat per time point. Rats were anesthetized with ether 3–5 min before the predetermined time. The abdomen was opened by midline incision. Arterial blood was withdrawn from the abdominal aorta at 1, 4, 7, 14, 21, 22, 36, 44, 52, 63, 76, 93, 109, 120, 150, and 179 min post-administration. After killing the rat by exsanguination, organs, including major lymph nodes (submaxillary, cervical, intestinal, cisternal, lumbar, and cordal lymph nodes), brain, spleen, pancreas, liver, and kidney were excised. The cisternal lymph nodes were collected near cisterna chyli. The order of collection was spleen, pancreas, kidney, liver, lymph nodes and brain. The removal of the first four tissues required about 1–7 min and the last two tissues another 8–10 min. Due to cessation of blood flow, drug redistribution among tissues during tissue removal was unlikely. After excision, tissues were blot-dried and weighed. Blood and tissue samples were kept on ice during processing to avoid drug degradation. Plasma and tissue specimens were frozen, using dry ice within 30 min of death, and stored at −20° until analysis.

Tissue metabolism. Rats, 5- to 6-months-old and weighing 208 ± 15 g, were used. Three to four animals were used per experiment, and all experiments were performed in triplicate. The animals were fasted overnight but allowed water *ad lib*. Rats were anesthetized with ether and euthanized by cervical dislocation. The spleen, pancreas, kidney, and brain were removed, and tissue homogenates (25%, w/v) were prepared in 10 mM sodium phosphate buffer containing 1.15% KCl as previously reported [16]. ddI at concentrations of 10, 30, 30, and 400 μg/mL was incubated with brain, spleen, pancreas, and kidney homogenates, respectively. The 1.88-mL incubation mixture contained 20% (w/v) tissue homogenate and 5 mM $MgCl_2$. Serial samples were collected over 60 min, and the degradation of ddI over time was determined.

Plasma and tissue extraction. Plasma samples were extracted as described previously [15]. In brief, 100 μL of plasma was mixed with 40 μL of an aqueous solution containing ftorafur (100 μg/mL) as the internal standard. The mixture was extracted by solid–liquid extraction using an SPE-C_{18} extraction column (Supelclean, Bellafonte, PA). Analysis of tissue samples required the following modifications in the sample clean-up procedures to separate ddI and ftorafur from interferences. Tissue samples were minced, frozen in liquid nitrogen, and homogenized in a mortar. A portion (0.2 g) of the tissue homogenate was mixed with ftorafur (50 μg/50 μL water) and 200 μL of water. Acetonitrile (5 mL) was added to precipitate the proteins. The acetonitrile/water layer was transferred and evaporated to dryness under nitrogen. The residue was reconstituted in

* Krogstad DJ, Styenglein KJ, Murray DM, Evland MR and Gluzman IY, Sensitive radioimmunoassay for dideoxyinosine (ddI). *Sixth International Conference on AIDS*, 1990.

1 mL of water and mixed with 2 mL of chloroform. The emulsion formed during vortexing was separated into water and chloroform layers by freezing in an acetone–dry ice bath and centrifugation. The lipid-containing chloroform layer was discarded, and the aqueous phase was extracted by solid–liquid extraction as described for plasma samples.

HPLC analysis. Plasma and tissue extracts were analyzed by two separate HPLC assays. The plasma extracts were analyzed using a reverse-phase μBondapak C_{18} column (Waters Associates) and an aqueous mobile phase containing 30 mM sodium acetate, 4% acetonitrile, and pH adjusted to 4.5 with acetic acid [15]. The elution volumes of ddI and ftorafur were 20 and 24 mL, respectively. The tissue extracts were analyzed using a stationary phase consisting of two different reverse-phase C_{18} columns placed in sequence, i.e. a Nova-Pak cartridge pressurized in a compression module (RCM, Waters Associates) followed by an analytical μBondapak column (Waters Associates). The aqueous mobile phase consisted of 30 mM sodium acetate, 4% acetonitrile, and 5 mM tetraethylammonium acetate. The pH was adjusted to 4.5 with acetic acid. The retention volumes of ddI and ftorafur under these conditions were 29 and 36 mL, respectively.

Rat plasma and tissue samples were spiked with known amounts of ddI (0.2 to 100 μg/mL or g) and ftorafur (25 μg/mL or g). The peak height ratios of ddI to ftorafur in these samples were used to construct the standard curves. The extraction recovery of ddI at a concentration of 5 μg/g was determined in brain, kidney and liver tissues (N = 3 for each tissue). The intra-day variability was determined using brain and kidney tissues; the ddI concentrations were 1 and 20 μg/g in brain (N = 3 at each of two concentrations) and 0.5 to 100 μg/g in kidney (N = 3 at each of eight concentrations). The inter-day variability on 3 separate days was determined in brain tissues at ddI concentrations of 1 and 20 μg/g (N = 3 at each of two concentrations).

Radioimmunoassay. The procedure for determination of ddI concentrations in filtered bovine serum provided by the Sigma Chemical Co. was modified to analyze ddI in plasma and tissue. Frozen tissue samples (0.1 g) were pulverized and mixed with 200 μL of water. The tissue homogenates and plasma samples (0.1 mL) were deproteinized by adding 1 mL of acetonitrile. The acetonitrile/water layer was transferred and dried under nitrogen and the residue reconstituted with 1 mL of water. One hundred microliters of the aqueous extract was added to a tube containing 0.008 μCi [^{3}H]ddI and 20 μg rabbit IgG in RIA buffer. One hundred microliters of ddI-antiserum was added and mixed by gentle vortexing. The mixture was incubated for 60 min at ambient temperature. One milliliter of IgG immunoprecipitation reagent was added to the mixture and gently mixed. After centrifugation, the supernatant was removed carefully, and the precipitate was dissolved in 0.6 mL of Solvable tissue gel solubilizer. An aliquot (0.5 mL) of the solution was mixed with 15 mL of Atomlight and analyzed by scintillation counting. Standard curves were prepared at a concentration range of 5 to 200 ng/mL or g for plasma or tissue. ddI concentrations were determined by the displacement of radioactivity from the precipitated antibodies. B_0 is the antibody-bound radioactivity in the absence of ddI. The bound radioactivity remaining in the presence of ddI is expressed as a percentage of B_0. The standard curve was constructed by plotting the logit of $\%B_0$ versus the logarithm of ddI concentrations. In the cases when the ddI concentration exceeded the upper limit of the standard curves, the extract was diluted with water and reassayed. There were no significant differences in standard curves made of various tissue extracts and of aqueous solutions. Therefore, standard curves were prepared using aqueous solutions. The intra-day variability was determined at ddI concentrations of 12.5, 25, 50, and 100 μg/mL or g (N = 3 at each concentration).

Paired plasma and tissue samples were analyzed using the same analysis method, i.e. HPLC or RIA. The HPLC assay was used to analyze the plasma and tissue samples that were expected to have concentrations higher than 200 ng/mL or g. Samples at later time points that were expected to have concentrations lower than 200 ng/mL or g were analyzed by RIA. To determine if HPLC and RIA provide comparable results, tissue samples containing 0.2 to 40 μg/g ddI were analyzed by both methods.

Data analysis. The plasma and tissue concentration–time data of ddI were analyzed using compartmental and noncompartmental methods. We previously showed that both methods give similar clearance values [17]. The α distribution phase and the β elimination phase half-lives, the area under the concentration–time curve (AUC) during and after the 2-min drug administration, and clearance were calculated according to standard procedures [18].

RESULTS

ddI analysis. Figure 1 shows a representative HPLC profile of kidney tissue extract, with baseline separation of ddI and the internal standard, ftorafur, from endogenous compounds. Similar results were found in brain, liver, spleen, and pancreas. The extraction recovery ranged from 77.7 to 79.1% in the kidney, liver and brain. Preliminary results showed that there were no significant differences among the various tissues. Hence, assay precision was determined using only kidney and brain tissues and the standard curve using only kidney tissue. The HPLC assay had a detection limit of 200 ng/g, and had a high precision as shown by the low coefficient of intra-day variation of <6.3% and a low coefficient of inter-day variation of <3.9%. The standard curve was linear over the concentration range of 0.2 to 100 μg/g with a coefficient of determination (r^2) of 0.9998.

The detection limit of the RIA assay in plasma and tissue extracts was 5 ng/mL or g. The standard curves were linear over the concentration range of 5 to 200 ng/mL or g with an r^2 of 0.997. Standard curves were prepared daily. The coefficients of intra-day variability for the RIA over a concentration range of 12.5 to 100 ng/mL or g varied from 1.3 to 5.0%.

Simultaneous analysis of extracts of pancreas, brain, liver, and kidney tissue samples containing

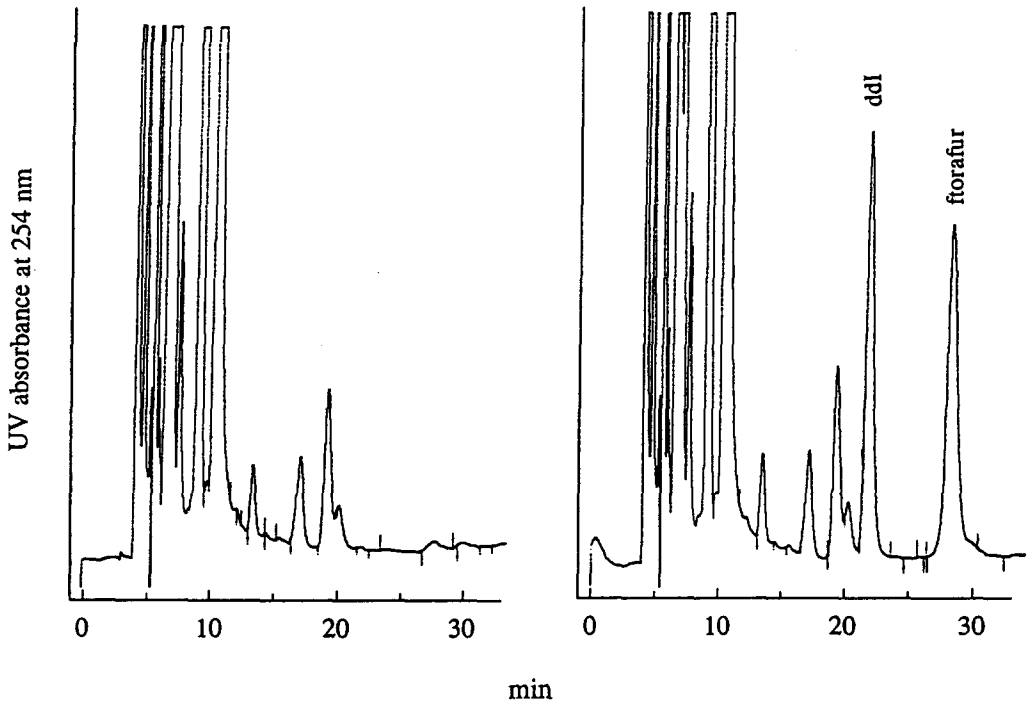

Fig. 1. Representative HPLC profiles. Left: a blank kidney sample. Right: a kidney sample spiked with 5 μg/g of ddI (retention time of 21.75 min) and 25 μg/g of the internal standard ftorafur (28.21 min).

0.2 to 40 μg/g of ddI by RIA and HPLC gave comparable results. A plot of the concentrations determined by RIA against those by HPLC gave a regression line of $y = 0.92x - 0.033$ with a $r^2 = 0.9801$.

Plasma and tissue concentration–time profiles. Figure 2 shows the concentration–time profiles of ddI in arterial plasma, liver, pancreas, lymph nodes, spleen, brain, and kidney after an i.v. injection of 100 mg/kg ddI over 2 min. Table 1 summarizes the pharmacokinetic parameters. The plasma concentration–time profile showed a biphasic decline. The ddI concentration–time profiles in all tissues showed an initial phase where the drug concentrations rapidly rose to the maximal values. This is consistent with a process of drug distribution from plasma to the organs. For the liver, pancreas, lymph nodes, spleen, and brain, the concentrations peaked between 4 and 7 min, and subsequently declined in parallel to those in plasma. The α- and β-phase half-lives in these five tissues were within 30 and 60% of those in plasma, respectively. The concentration–time profile in the kidney showed a different pattern from that in plasma and the other five tissues. First, the highest concentration, found at a later time of 14 min, was 20% higher than the concentration at 7 min. In contrast, in plasma and the other five tissues (liver, pancreas, lymph nodes, spleen, brain), the ddI concentrations declined by 33–56% from 7 to 14 min. The concentration decline in the kidney from 22 min onward was parallel to that of plasma. Second, the concentrations in the kidney were about 10-fold higher than the plasma concentrations, whereas the concentrations in other tissues were lower or equal to those in plasma.

Data for each time point were obtained using an individual animal. Inter-animal variation in drug disposition contributed to the fluctuation in the concentration–time profile. To minimize the effect of data fluctuation on data analysis, we compared the average tissue-to-plasma concentration ratios and the ratios of AUC_{tissue}: AUC_{plasma}. The kidney showed the highest ratios, and the brain showed the lowest ratios. The data in Table 1 show a 230-fold range in the average C_{tissue}: C_{plasma} ratios and a 300-fold range in the AUC_{tissue}: AUC_{plasma} ratios with the rank order of kidney $\gg$ liver $\approx$ pancreas $>$ lymph nodes $>$ spleen $\gg$ brain.

Tissue metabolism. Upon incubation with 20% spleen homogenates, the ddI concentration showed a semilogarithmic decline with a degradation half-life of 7 min. There were no changes in the ddI concentrations in the presence of pancreas, brain, and kidney homogenates for at least 60 min.

DISCUSSION

Several previous studies reported limited data on ddI distribution in rodent tissues [19–22]. The current study expanded on this previous work by using specific and more sensitive assays for ddI. In addition to the more commonly used HPLC that was used to analyze the higher concentrations, an RIA was used to analyze the low concentrations (<200 ng/mL).

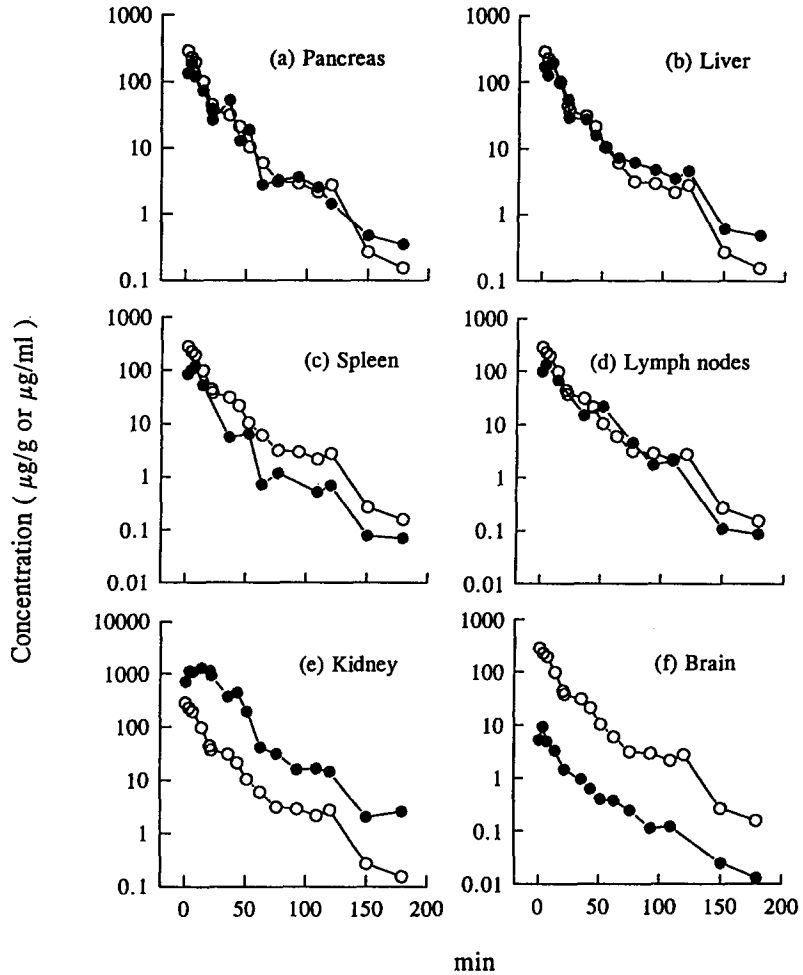

Fig. 2. Tissue distribution of ddI. Rats were given an i.v. injection of ddI (100 mg/kg) over 2 min. Each data point represents one rat. Symbols: (○) plasma; and (●) tissue. Time zero represents the end of the 2-min infusion.

Table 1. Tissue distribution parameters of ddI following i.v. bolus administration (100 mg/kg) in rats

Tissue	C_{max} ($\mu g \cdot g^{-1}$)	T_{max} (min)	$T_{1/2}\alpha$ (min)	$T_{1/2}\beta$ (min)	AUC* ($\mu g \cdot min \cdot g^{-1}$)	$C_{tissue}:C_{plasma}$ ratio†	$AUC_{tissue}:AUC_{plasma}$ ratio
Plasma	288‡	1	5.7	20.5	4776§	1.0	1.0
Liver	190	7	3.9	25.6	4121	1.32 ± 0.69	0.863
Pancreas	187	4	6.7	32.2	3654	1.04 ± 0.55	0.765
Lymph nodes	136	4	−‖	15.8	3215	0.82 ± 0.55	0.673
Spleen	120	7	5.8	28.9	2283	0.36 ± 0.17	0.478
Brain	9.25	4	5.0	24.2	147.7	0.05 ± 0.02	0.031
Kidney	1291	14	−‖	21.9	43758	11.7 ± 7.35	9.162

* AUC from time zero to time infinity including AUC during the 2-min dosing.

† Mean ± SD; average of all time points (N = 10–16).

‡ $\mu g \cdot mL^{-1}$.

§ $\mu g \cdot min \cdot mL^{-1}$. AUC from the first time point at 1 min post drug administration to infinity was 4118 $\mu g \cdot min \cdot mL^{-1}$, and the extrapolated AUC during 2-min infusion and from time zero (end of infusion) to 1 min was 343 and 315 $\mu g \cdot min \cdot mL^{-1}$, respectively.

‖ α Phase could not be accurately determined due to fluctuation in data.

We evaluated the tissues important for ddI effect (i.e., lymph nodes, spleen, and brain), toxicity (pancreas), and elimination (liver and kidney). None of the previous studies [19–22] evaluated the ddI distribution in lymph nodes. In addition, the present study evaluated the tissue distribution early after an i.v. bolus administration so that the rate of equilibration between plasma and individual tissues could be determined. Our results indicate that ddI was present in brain at much lower concentrations than in other organs and plasma. The ddI concentrations in spleen were approximately one-half of plasma concentrations, while pancreas, liver and lymph node concentrations were similar to or slightly lower than plasma concentrations. Drug equilibration between plasma and these tissues was rapid, with maximum concentrations occurring between 4 and 7 min. The only organ with substantial drug accumulation was the kidney, as shown by the more than 10-fold higher ratio of kidney-to-plasma concentrations and the later time to reach maximum tissue concentration.

We previously reported significant liver metabolism of ddI, with a half-life of 13 min in 20% liver homogenates [16]. The present study showed a degradation half-life of 7 min in 20% spleen homogenates. These data suggest that substantial ddI metabolism may occur during the 1–2 min and 5–7 min needed to collect the spleen and liver, respectively. Accordingly, it is possible that the measured ddI concentrations in spleen and liver are lower than the concentrations present at the time the animals were euthanized. On the other hand, the data showing no metabolism of ddI in pancreas and brain homogenates confirm that ddI was not accumulated in these organs.

In the present study, tissues were harvested after exsanguination, but no attempts were made to exhaustively remove residual blood. In rats, the volume of blood compared to the tissue weight is 2% in brain, 15% in spleen, 8% in kidney, and 13% in liver after exsanguination [23]. Accordingly, the maximum contamination due to residual blood would be about or less than 15%, and hence would not affect the tissue concentrations significantly.

A comparison of our results with the results of previous studies [19–22] shows striking differences, as summarized in Table 2. These differences are likely due to the assay methodologies used. The present study analyzed ddI by HPLC and RIA, which are specific for the unchanged drug. The limited data obtained by other investigators using specific assay methodology [21, 22] agree with our results. Studies using total radioactivity [19, 20] generally showed higher tissue-to-plasma concentration ratios at 4 hr, at which time the major sources of radioactivity are expected to be represented by inactive catabolites, as indicated by the following literature data. In T cells incubated with radiolabeled ddI or its precursor 2′,3′-dideoxyadenosine, only 1–2% of the radiolabeled intracellular metabolites was represented by ddATP and the remaining 98–99% was ADP and ATP, which were recycled from hypoxanthine [24, 25], suggesting that the major metabolic pathway of ddI is by catabolism to the inactive hypoxanthine and that the conversion of ddI to its active metabolite, ddATP, is a minor pathway. Radioactivity in the plasma and tissues after administration of [8-^{3}H]ddI or [8-^{14}C]ddI is represented by ddI and its catabolites [19, 20].

The ddI clearance found in the present study was 43% lower than that found in our previous study [17]. The present study used arterial blood samples, whereas the previous study used venous blood samples. A separate study in three rats showed that arterial concentrations were slightly lower than simultaneously obtained venous concentrations (data not shown), thus ruling out the arterial-venous concentration difference as the major contributing factor of the lower clearance. A difference in the experimental procedure between the two studies is the use of anesthesia. In the present study, animals were under ether anesthesia shortly before drug

Table 2. Tissue-to-plasma concentration ratios in rodents

Tissue	Present study* (HPLC/RIA)	Ray *et al.*† (Total ^{3}H)	Gallicano *et al.*‡ (Total ^{14}C)	Hoesterey *et al.*§ (HPLC)	El Dareer *et al.*‖ (HPLC)
Liver	1.09	1.74	18.3	NM¶	1.30
Pancreas	0.90	0.78	NM	NM	NM
Lymph nodes	0.75	NM	NM	NM	NM
Spleen	0.42	1.53	14.9	NM	NM
Brain	0.04	0.89	0.59	0.05	ND**
Kidney	10.43	2.25	12.6	NM	8.60

Data of the present study were compared with the literature data. The methods of analysis are indicated in parentheses.

* Average of C_{tissue}: C_{plasma} ratios and AUC_{plasma} : AUC_{tissue} ratios, after an i.v. bolus administration of ddI (100 mg/kg).

† Obtained after a 4-hr i.v. infusion of [^{3}H]ddI (60 mg/kg/hr) in rats [19].

‡ Four hours after an i.v. bolus administration of [^{14}C]ddI (50 mg/kg) in rats [20].

§ During i.v. infusions by varying rates in rats (12.4 to 125 mg/kg/hr) [21].

‖ Ratio at 60 min after an oral dose (452 mg/kg) of ddI in mice (calculated from data in Ref. 22).

¶ NM, not measured.

** ND, below limit of detection.

administration and were restrained during the experiment, whereas the previous study was done using awake and unrestrained rats. We speculate that ddI elimination was reduced due to the use of anesthesia [26, 27] and/or restraint.

To evaluate if the presence of urine in kidney tubules at the time of tissue harvest contributed to the 10-fold higher concentration, the following calculation was done. In rats, about 17% of an i.v. ddI dose is excreted in urine [17]. The renal clearance calculated using the clearance of 20.9 mL/min/kg found in the present study is therefore 3.6 mL/min/kg. The concentration of ddI in urine can be calculated as plasma concentration · renal clearance ÷ urine flow rate. The average urine flow rate in rats is 0.14 mL/min/kg [28]. The estimated ddI concentration in urine is approximately 26 times the plasma concentration. To account for the high kidney concentration, it would require a residual urine volume of 0.52 mL, or 39% of the average kidney weight of 1.35 g. In comparison, tubular urine volume accounts for 1% or less of total kidney volume [29]. Hence, the presence of a small volume of urine in the kidney may have contributed to but could not fully account for the high tissue concentration, thus confirming the affinity of ddI for kidney tissue.

The present study ruled out accumulation of ddI in the pancreas, the organ of major drug toxicity in humans. The absence of organ specific drug distribution and accumulation may be related to the absence of pancreatic toxicity in rats reported by Grady *et al.* [11] and Nordback *et al.* [12] during chronic or high dose treatment. Further studies are needed to identify a valid *in vivo* model for pancreatic toxicity and to elucidate the cause of ddI-induced pancreatitis. Maintenance of a high drug concentration level in the brain, lymph nodes and lymphoid tissues is needed to prevent or slow the progress of the disease [4, 5, 7]. Our data show a rapid equilibration of ddI between plasma and these tissues, but no tissue accumulation. The low to moderate tissue-to-plasma ratios in the brain, spleen, and lymph nodes indicate that further enhancement of the therapeutic effect of ddI requires improved drug delivery to and entrapment in these tissues.

Acknowledgements—This work was supported, in part, by research grants RO1 AI28757 and RO1 AI29133 from the National Institute of Allergy and Infectious Diseases, and a Research Career Development Award (KO4 CA01497) to J. L.-S. Au from the National Cancer Institute.

REFERENCES

1. Ahluwalia G, Cooney DA, Mitsuya H, Fridland A, Flora KP, Hao Z, Dala M, Broder S and Johns DG, Initial studies on the cellular pharmacology of 2',3'-dideoxyinosine, an inhibitor of HIV infectivity. *Biochem Pharmacol* **36**: 3797–3800, 1987.
2. Mitsuya H and Broder S, Inhibition of the *in vitro* infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides. *Proc Natl Acad Sci USA* **83**: 1911–1915, 1986.
3. McGowan JJ, Tomaszewski JE, Cradock J, Hoth D, Grieshaber CK, Broder S and Mitsuya H, Overview of the preclinical development of an antiretroviral drug 2',3'-dideoxyinosine. *Rev Infect Dis* **12** (Suppl 5): 513–521, 1990.
4. Pantaleo G, Graziosi G, Demarest JF, Butini L, Montroni M, Fox CH, Orenstein JM, Kotler DP and Fauci AS, HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* **363**: 355–358, 1993.
5. Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA and Fauci AS, Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* **223**: 1089–1093, 1986.
6. Shaw GM, Harper ME, Hahn BH, Epstein LG, Gajdusek DC, Price RW, Navia BA, Petto CK, O'Hara CJ, Groopman JE, Cho E-S, Oleske JM, Wong-Staal F and Gallo RC, HTLV-IV infection in brains of children and adults with AIDS encephalopathy. *Science* **227**: 177–181, 1985.
7. Morgan ME, Chi SC, Murakami K, Mitsuya H and Anderson BD, Central nervous system targeting of 2',3'-dideoxyinosine via adenosine deaminase-activated 6-halo-dideoxypurine prodrugs. *Antimicrob Agents Chemother* **36**: 2156–2165, 1992.
8. Cohen J, Keystone's blunt message: "It's the virus, stupid". *Science* **260**: 292–293, 1993.
9. Butler KM, Venzon D, Henry N, Husson RN, Mueller BU, Balis FM, Jacobsen F, Lewis LL and Pizzo PA, Pancreatitis in human immunodeficiency virus-infected children receiving dideoxyinosine. *Pediatrics* **91**: 747–751, 1993.
10. Seidlin M, Lambert JS, Dolin R and Valentine FT, Pancreatitis and pancreatic dysfunction in patients taking dideoxyinosine. *AIDS* **6**: 831–835, 1992.
11. Grady T, Saluja AK, Steer ML, Lerch MM, Modlin IM and Powers RE, *In vivo* and *in vitro* effect of the azidothymidine analog dideoxyinosine on the exocrine pancreas of the rat. *J Pharmacol Exp Ther* **262**: 445–449, 1992.
12. Nordback IH, Olson JL, Chaisson RE and Cameron JL, Acute effect of a nucleoside analog dideoxyinosine (ddI) on the pancreas. *J Surg Res* **53**: 610–614, 1992.
13. Ravasco RJ, Unadkat JD and Tsai C-C, A high performance liquid chromatography assay for dideoxyinosine in monkey plasma and urine. *J Pharm Sci* **81**: 690–691, 1992.
14. Ray G and Murrill E, Determination of 2',3'-dideoxyinosine in plasma by high performance liquid chromatography. *Anal Lett* **20**: 1815–1838, 1987.
15. Wientjes MG and Au JL-S, High-performance liquid chromatographic analysis of 2',3'-dideoxyinosine in biological samples. *J Chromatogr* **563**: 400–406, 1991.
16. Bramer SL, Au JL-S and Wientjes MG, Gastrointestinal and hepatic first pass elimination of 2',3'-dideoxyinosine in rats. *J Pharmacol Exp Ther* **265**: 731–738, 1993.
17. Wientjes MG, Mukherji E and Au JL-S, Nonlinear disposition of intravenous 2',3'-dideoxyinosine in rats. *Pharm Res* **9**: 1070–1075, 1992.
18. Rowland M and Tozer TN, *Clinical Pharmacokinetics, Concepts and Applications*. Lea & Febiger, Philadelphia, 1980.
19. Ray GF, Mason WP and Badr MZ, Pharmacokinetics of the anti-AIDS drug 2',3'-dideoxyinosine in the rat. *Drug Metab Dispos* **18**: 654–658, 1990.
20. Gallicano KD, Buttar HS and Moftatt JH, Effect of ciprofloxacin on the disposition of ^{14}C-dideoxyinosine-derived radioactivity in the rat. *Res Commun Chem Pathol Pharmacol* **80**: 163–174, 1993.
21. Hoesterey BL, Galinsky RE and Anderson BD, Dose dependence in the plasma pharmacokinetics and

uptake kinetics of 2′,3′-dideoxyinosine into brain and cerebrospinal fluid of rats. *Drug Metab Dispos* **19**: 907–912, 1991.
22. El Dareer SM, Tillery KF, Kalin JR and Hill DL, Disposition of 2′,3′-dideoxyadenosine and 2′,3′-dideoxyinosine in mice. *Invest New Drugs* **7**: 139–146, 1989.
23. Dellenback RJ and Muettieins GH, Red blood cell volume and distribution before and after bleed-out in the rat. *Am J Physiol* **198**: 1177–1180, 1960.
24. Cooney DA, Ahluwalia G, Mitsuya H, Fridland A, Johnson M, Hao Z, Balzarini J, Broder S and Johns DG, Initial studies on the cellular pharmacology of 2′,3′-dideoxyadenosine, an inhibitor of HTLV-III infectivity. *Biochem Pharmacol* **36**: 1765–1768, 1987.
25. Hao Z, Cooney DA, Hartman NR, Perno CF, Fridland A, DeVico AL, Sarngadharan MG, Broder S and Johns DG, Factors determining the activity of 2′,3′-dideoxynucleosides in suppressing human immunodeficiency virus *in vitro*. *Mol Pharmacol* **34**: 431–435, 1988.
26. Korten K and Van Dyke RA, Acute interaction of drugs–I. The effect of volatile anesthetics on the kinetics of aniline hydroxylase and aminopyrine demethylase in rat hepatic microsomes. *Biochem Pharmacol* **22**: 2105–2112, 1973.
27. Van Dyke RA and Rikans LE, Effect of the volatile anesthetics on aniline hydroxylase and aminopyrine demethylase. *Biochem Pharmacol* **19**: 1501–1502, 1970.
28. Davies B and Morris T, Physiological parameters in laboratory animals and humans. *Pharm Res* **10**: 1093–1095, 1993.
29. Weaver AN, McCarver CT and Swann HG, Distribution of blood in the functional kidney. *J Exp Med* **104**: 41–52, 1956.