Enhanced Oral Bioavailability of DDI After Administration of 6-Cl-ddP, an Adenosine Deaminase-Activated Prodrug, to Chronically Catheterized Rats

Bradley D. Anderson,[1,2] Michael E. Morgan,[1] and Dharmendra Singhal[1]

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Purpose: 6-Cl-2',3'-dideoxypurine (6-Cl-ddP), an adenosine deaminase (ADA) activated prodrug of ddI, may be an effective antiretroviral agent for the treatment of AIDS dementia due to its ability to deliver increased concentrations of ddI to brain tissue. To examine the feasibility of administering this drug orally, the oral and hepatic portal bioavailabilities of 6-Cl-ddP were determined. In addition, the oral and portal bioavailabilities of ddI after administration of the prodrug were compared to those from administration of ddI itself. ***Methods.*** Pharmacokinetic and bioavailability studies were conducted in fully conscious, chronically catheterized rats in a randomized crossover design. Plasma ddI and 6-Cl-ddP concentration-time profiles were determined by HPLC. ***Results.*** 6-Cl-ddP has poor apparent oral bioavailability (7% ± 3%, n = 3) but high bioavailability after portal administration (97% ± 11%), suggesting either poor absorption or extensive gut wall metabolism. The appearance of >50% of the dose as ddI in the systemic circulation after an oral dose of 6-Cl-ddP rules out poor absorption of the prodrug, and confirms expectations of high ADA activity in the gastrointestinal tract. Gastric administration of 6-Cl-ddP resulted in a >10-fold increase in the oral bioavailability of ddI, from 3–7% to >50%, and a significant decrease in the variability in apparent bioavailability. ***Conclusions.*** These data indicate that lipophilic adenosine deaminase activated prodrugs of dideoxypurine nucleosides may have limited utility for improving CNS delivery after oral administration but may be useful in enhancing the oral bioavailability of highly polar and therefore poorly absorbed dideoxynucleosides.

KEY WORDS: oral; portal; bioavailability; adenosine deaminase; prodrugs; dideoxyinosine.

INTRODUCTION

Didanosine (2',3'-dideoxyinosine, ddI), the second antiretroviral agent approved by the Food and Drug Administration for the treatment of HIV infection, exhibits several delivery-related disadvantages, including low and highly variable bioavailability on oral administration and limited central nervous system (CNS) penetration. Initially, the low oral bioavailability of ddI observed in preclinical studies in animals (13% in mice (1); 8–11% in rats (2)) was thought to result from the susceptibility of ddI to acid hydrolysis (10% decomposition occurs in less than 2 minutes at a pH < 3 (3)). However, the mean bioavailability in patients when ddI was administered orally as a solution with antacid was only 41% ± 7% and 25% ± 5% when given as buffered tablets (4). Enteric coating yielded similar results. Moreover, variability in ddI's bioavailability has been a continuing concern (5, 6). Recent basic studies in animals suggest that the oral absorption of didanosine is membrane permeability-limited and intestinal-site dependent with absorption decreasing down the small intestine (7). Degradation in the stomach and metabolism by the contents of the lower intestinal tract appear to account for most of the presystemic first-pass elimination (8).

The ability of anti-HIV agents to penetrate the CNS has also become an important issue because of the prevalence of a progressive deterioration in mental function accompanying AIDS, referred to as AIDS dementia complex (9). The dideoxynucleoside reverse transcriptase inhibitors approved for AIDS therapy, 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyinosine (ddI), and 2'-3'-dideoxycytidine (ddC) exhibit variable CNS penetration. Both ddI and ddC are substantially less lipophilic than AZT and penetrate the CNS poorly (10, 11).

Studies in these laboratories have recently demonstrated that, in rats, the CNS delivery of ddI may be markedly enhanced via a series of adenosine deaminase (ADA)-activated 6-halo-dideoxypurine prodrugs (12, 13). At steady-state, the optimal candidate in this series, 6-Cl-2',3'-dideoxypurine (6-Cl-ddP) (Scheme I), provided> 10-fold higher brain parenchyma concentrations of ddI compared to ddI controls after intravenous infusions due to its increased lipophilicity and selective conversion to ddI in brain tissue. The attractiveness of this strategy, however, depends to some extent on whether or not ADA-activated prodrugs can be administered orally. Because 6-Cl-ddP is ≈ 30-fold more lipophilic than ddI, it may exhibit substantially improved bioavailability relative to ddI.

This study examines the feasibility of administering this prodrug orally. By determining the oral and hepatic portal bioavailability of 6-Cl-ddP and ddI after administration of either compound in a series of random cross-over studies using a chronically catheterized rat model (14), three questions were examined: (a) Is 6-Cl-ddP fully absorbed intact?; (b) Is the oral bioavailability of ddI enhanced by oral administration of 6-Cl-ddP?; and (c) What is the extent of liver first-pass metabolism of these compounds?

MATERIALS AND METHODS

Chemicals

ddI and 6-Cl-ddP were provided by the National Institute of Allergy and Infectious Diseases and were used without further purification. The preparation of 6-Cl-ddP has been described (15). All other chemicals were commercially available and were used as received.

Animals and Surgical Procedures

Male Sprague-Dawley rats were obtained from Sasco Laboratories (Omaha, NE), housed, and cared for at the Animal Resource Center, University of Utah, according to

[1] Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah 84112.
[2] To whom correspondence should be addressed.

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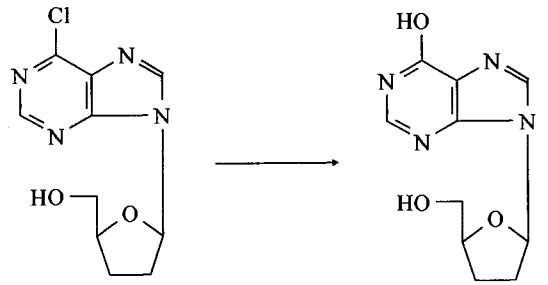

6-Cl-ddP ddI

Scheme I. Reaction scheme showing the adenosine deaminase catalyzed conversion of 6-Cl-ddP to ddI.

the USDA Animal Welfare Act and NIH publication #85-23, "Principles of Laboratory Animal Care" (revised 1985). On the morning of an experiment, animals were placed in individual cages and food and water were withdrawn for the duration of the drug infusion and sampling period. Blood specimens were taken at various times during and after drug administration using implanted cannulas as described below.

Animals were anesthetized with 10 mg/kg pentobarbital intraperitoneally and 70 mg/kg ketamine intramuscularly. Using aseptic technique, catheters were implanted into the abdominal aorta, inferior vena cava, and portal vein as described by Kimura and coworkers (14). Briefly, hair was removed from the abdomen and middle of the back and washed clean with 95% ethanol and sterilized with 1% betadine. After a vertical abdominal midline incision was made, three sterile heparin locks were routed subcutaneously through the skin above the lumbar vertebra into the abdominal cavity. The blood vessels were then cannulated, anchored in place with super glue, and attached to the heparin locks. The abdominal incision and heparin lock back entrance incision were closed with suture. Silicone glue was placed around the heparin locks forming a protective collar. The catheters were flushed daily with 0.3 ml of normal saline containing 500 U/ml heparin and 2.0 mg/ml ampicillin. Weights were taken daily following surgery. Rats were allowed a 5–7 day recovery period.

Experimental Designs

Four separate randomized crossover studies were conducted in four groups of animals at doses of 50 mg/kg, ddI equivalents. For group 1, a 2 × 6 crossover design (ddI intravenous vs. oral gavage) was utilized to determine the apparent oral bioavailability of ddI at a single dose. Group 2 animals were given the ddI intravenously as a 30 sec infusion and intraportally as either a 30 sec or 2 min infusion in a 3 × 4 crossover design to assess the role of first-pass liver metabolism. Group 3 rats were administered equimolar doses of either 6-Cl-ddP or ddI, both intravenously and by oral gavage in a 2 × 2 × 3 crossover design to obtain apparent bioavailabilities of both intact 6-Cl-ddP and ddI after prodrug administration. Group 4 animals were given 6-Cl-ddP intravenously and portally in a 2 × 3 crossover study to determine the portal bioavailability of 6-Cl-ddP and the intravenous and portal bioavailability of ddI after 6-Cl-ddP administration. Each treatment schedule was completed over a 7 to 10 day period. Animal weights, 301 ± 25 g, (mean ± SD, n = 16) did not change significantly over the study period.

Infusion and Sampling Protocol

On the day of an experiment solutions of ddI or 6-Cl-ddP (50 mg/kg (ddI equivalents) were infused intravenously (IV) or portally (P) or administered orally by gastric intubations (G) using a 16 g intubation needle attached to a 1.0 ml gas tight Hamilton syringe. Intravenous and intraportal infusions were generally at a rate of 4.0 ml/min for 30 sec. However, in group 2 animals, a second portal infusion rate (2 min @ 1 ml/min) was also explored. The infusion and oral intubation volumes were 2.0 ml and 1.0 ml, respectively. Oral dosing solutions were prepared in 0.9% NaCl at concentrations of ≈ 15 mg/ml and diluted with 0.9% NaCl for the infusion dose. Blood samples (0.25 ml) were collected from the abdominal aorta at various times over a 6 hour period with the first sampling time being at 30 sec in groups 1 and 3 and 2 min in groups 2 and 4. Sample aliquots were immediately centrifuged at 10,000 rpm for 1 min and a 0.1 ml aliquot of the resulting plasma was transferred into a microcentrifuge tube containing 1.0 ml of acetonitrile, vortexed, and stored at −20°C.

HPLC Analyses

ddI and 6-Cl-ddP blood concentrations were determined by reversed-phase HPLC with UV detection at 254 nm as described previously (13). Briefly, analyte resolution was achieved on a Supelcosil LC-18S 5μ column (4.6 mm i.d. by 25 cm) using a mobile phase consisting of either 4.5% methanol (ddI analyses) or 7% acetonitrile (6-Cl-ddP analyses) in phosphate buffer (pH 3.0; I = 0.02) containing 0.01% tetrabutylammonium ion. Under these conditions the retention volumes were 23 and 33 ml for ddI and 6-Cl-ddP, respectively. Recoveries from spiked plasma samples averaged 97% ± 5% for ddI and 101% ± 6% for 6-Cl-ddP.

Data Analyses

Pharmacokinetic parameters were determined by fitting the data for each experiment by non-linear least squares regression analysis using commercially available software (SCIENTIST, Micromath, Inc.). The mathematical model yielding the best fit was chosen using the computer generated Model Selection Criterion, which is a modified Akaike Information Criterion (16) that places a burden on the model having more parameters to not only have a better coefficient of determination but quantifies how much better it must be for the model to be deemed more appropriate. Areas under the concentration vs time profiles (AUCs) were initially determined by the linear trapezoidal method from zero to the last time point. Apparent fractions bioavailable (F) in each animal were calculated by the area ratio method ($F = AUC_{G \text{ or } P}/AUC_{IV}$) using these trapezoidal areas. Because trapezoidal area determinations were highly sensitive to the first sampling time, which varied between groups (0.5 min in groups 1 & 3; 2 min in groups 2 & 4), bioavailability deter-

minations were also obtained by comparison of computer fitted data. Non-compartmental pharmacokinetic parameters generated included steady-state volumes of distribution (V_{ss} = Dose*[AUMC/AUC^2 − ½T/AUC]), total clearance (CL = [Dose]/AUC), and mean residence time (MRT = AUMC/AUC − T/2) (17). Statistical analyses were performed using paired or unpaired Student's t tests. P values of less than 0.05 were considered statistically significant.

Models for ddI Pharmacokinetics After ddI Administration. Plasma concentrations (C) of ddI versus time, t, after intravenous or intraportal administration were analyzed using a triexponential (n = 3) mathematical model with zero-order input over 0.5 min having the following form:

$$C = k_o \sum_{l=1}^{n} C_l (e^{\lambda_l T} - 1) e^{-\lambda_l t} \quad (1)$$

where k_o is the rate of infusion, T is the infusion time, and the C_l's and λ_l's are fitted parameters. Plasma concentration-time profiles of ddI after oral gavage (Group 1) could be fit only by coupling a multiple term input function to the previously described ddI plasma disposition function. The mathematical form of the input function was obtained by assuming a two absorption-site (i.e., mixing tank) model as described by Dressman *et al.* (18), consisting of compartments connected in series by uni-directional, first-order transfer rate constants. Although this model was used solely for the purpose of constructing a smooth curve through the data for reliable AUC and AUMC determinations, it has some physiological basis, as regional differences have been demonstrated in the intestinal absorption of ddI (7).

Model for 6-Cl-ddP and ddI Pharmacokinetics After 6-Cl-ddP Administration. Plasma concentration-time profiles for the disappearance of 6-Cl-ddP after intravenous or intraportal administration were well fit by a biexponential equation having the form of Eq. 1. Various compartmental models were evaluated in attempting to identify the mathematical form of the equation which would fit the combined data for the disappearance of ddI after intravenous or intraportal infusions of ddI, the disappearance of 6-Cl-ddP after intravenous or intraportal infusions, and the kinetics of formation and subsequent disappearance of ddI after either intravenous or intraportal infusions of 6-Cl-ddP. The mathematical models employed in fitting the combined data (3 curves) assumed zero-order prodrug input (over 0.5 min) with 6-Cl-ddP exhibiting two-compartment kinetics, ddI exhibiting triexponential disposition kinetics, the formation of ddI occurring in either or both the plasma and tissue prodrug compartments, and parallel elimination of prodrug occurring in one or both prodrug compartments.

The plasma concentrations of 6-Cl-ddP following oral gavage were fit in the same way as the ddI data obtained after oral gavage. Again these fits were utilized solely for the purpose of constructing a smooth curve through the data and for reliable AUC and AUMC determinations. Since the fraction of intact 6-Cl-ddP reaching the systemic circulation from an oral dose was less than 10%, ddI formation after oral administration of the prodrug was assumed to occur presystemically (but after the rate-limiting step for oral absorption of 6-Cl-ddP).

RESULTS AND DISCUSSION

Pharmacokinetics and Bioavailability of ddI

Concentration-time curves of ddI after intravenous infusion and oral gavage, and after intravenous infusion and portal infusion (at two infusion rates) were determined in groups 1 and 2, respectively. Figure 1 shows semilogarithmic plots of the mean ddI plasma concentration-time curves during and after administration of 50 mg/kg in rats via 30 sec intravenous infusions (both group 1 & 2 data are superimposed), 30 sec intraportal infusion (group 2) or gastric gavage (group 1). Intravenously administered ddI was included in three of the four cross-over studies as a control. Concentration-time profiles from all three intravenous studies were superimposable so the results were pooled and best fit to a triexponential model (Eq. 1), as shown in Figure 1. The parameters generated are shown in Table I along with estimates of V_{ss}, CL, and MRT obtained from the model-based AUCs and AUMCs. These pharmacokinetic determinants are in close agreement with values reported in the literature for the range including the dose employed herein (2).

Trapezoidal areas under the plasma concentration-time curves (Table II) varied significantly between groups, despite the fact that the concentration-time curves were superimposable. These AUCs were quite sensitive to the time at which the first sample was collected because ddI is a high clearance drug with nearly half of its AUC accounted for within 2 min. The high plasma concentrations of ddI realized at the 0.5 min sampling time in groups 1 & 3 were not detected in group 2 animals as the first samples collected in these animals were at 2 min. These differences in trapezoidal AUCs between groups do not reflect real differences in the underlying data, but rather the erroneous linear extrapolation to t_0 from the first time-point inherent in the trapezoidal AUC method.

Errors in extrapolation to t_0 when using the trapezoidal method also account for the large differences in trapezoidal AUCs in comparing the 30 sec and 2 min portal infusions (Table II), since a larger fraction of the AUC was missed after a 30 sec infusion when the first sampling time was 2 min. When the ddI plasma concentration-time profiles resulting

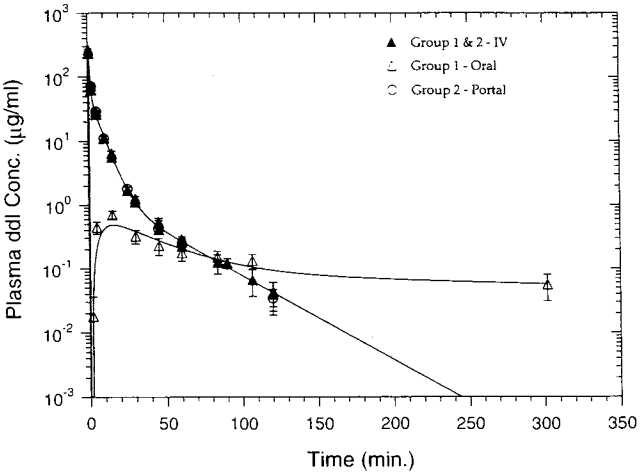

Fig. 1. Plasma concentrations of ddI after 30 sec intravenous infusion (▲), 30 sec intraportal infusion (○), or gastric (△) administration of ddI to rats. Curves represent computer fits.

Table I. Pharmacokinetic Parameters Describing the ddI or 6-Cl-ddP Plasma Concentration-Time Curves After 30 sec Intravenous Infusions of ddI or 6-Cl-ddp

Compound	ddI	6-Cl-ddP
V_{SS} (ml/kg)	490 ± 80[a]	760 ± 110[b]
CL (ml/min/kg)	79 ± 8[a]	150 ± 10[b]
MRT (min)	6.2 ± .6[a]	5.1 ± .5[b]
C_1 (kg/L)[c]	4.5 ± .4	2.2 ± .5
λ_1[c]	1.4 ± .1	1.1 ± 0.3
C_2 (kg/L)[c]	6.6 ± .3	4.4 ± .5
λ_2[c]	.159 ± 0.007	.142 ± 0.006
C_3 (kg/L)[c]	1.06 ± 0.07	—
λ_3[c]	.030 ± 0.001	—

[a] Determined from computer-fitted individual data, groups 1 & 3 (n = 9).
[b] Determined from computer-fitted individual data, group 3 (n = 3).
[c] Best-fit parameters according to Eq. 1.

from portal infusion of ddI at either infusion rate (30 sec @ 4 ml/min or 2 min @ 1 ml/min) were fit using the same parameters listed in Table I but with an additional multiplying factor, F, which allows for reduced bioavailability due to a first-pass effect, the computer generated AUCs (to last time point) and F values (Table III) indicated that the portal availability of ddI is 100%, independent of infusion rate over the range of infusion rates examined.

Plasma concentrations of ddI following oral ddI administration were determined in groups 1 & 3. Maximum concentrations of ddI in plasma were attained within 15 ± 7 min and averaged 0.61 ± 0.25 μg/ml. As illustrated in Figure 1, the terminal slope after an oral dose was shallow relative to that obtained after intravenous administration, indicating a "flip-flop" pharmacokinetic model with the decline in ddI concentration rate-limited by ddI absorption, with a prolonged terminal absorption half-life of 96 ± 64 min (obtained from averaging slopes of the mean concentration decline versus time data in groups 1 & 3). The rapid rise in ddI concentration to a peak in less than 15 min followed by an apparent biphasic decline and prolonged terminal phase could not be fit adequately by a simple equation containing 2 or 3 exponential terms. Rather, an equation derived from a "mixing tank" model comprised of two intestinal compartments connected in series with varying rate constants for absorption from each compartment, as described by Dressman *et al.* (18), was necessary. The curve superimposed on the oral absorption data in Figure 1 represents the best fit of this model to these data. Since the purpose of modeling these data was solely to obtain a smooth curve through the points for reliable AUC and AUMC determination, the mathematical equation and fitted parameters are not provided. Areas under the ddI concentration-time profiles estimated by trapezoidal integration are shown in Table II. Apparent bioavailabilities, calculated from computer generated or trapezoidal $AUC_{gastric}/AUC_{iv}$ ratios are listed in Table III. These ratios indicate that the bioavailability of orally administered ddI is 3–7% in rats.

The apparent oral bioavailability of ddI found in the present study is similar to that reported previously. The combined results from groups 1 and 3 gave values ranging from 1.8% to 9.6% (5.5% ± 2.98%, mean ± SD, n = 9) of a 50 mg/kg dose, similar to those of Ray *et al.* (2), who found a range of 1 to 9% at a 25 mg/kg dose and 8 to 11% at a 100 mg/kg dose. Their results and those reported herein are apparent rather than actual bioavailabilities, however, based on the assumption of linear pharmacokinetics. This laboratory (19) and others (20) have shown that the pharmacokinetics of ddI are dose dependent, exhibiting decreasing clearance with increasing dose. Thus, a comparison of iv and oral AUCs may require an adjustment for differences in clearance. In previous work by the authors, the clearances of ddI obtained from analyses of ddI plasma concentrations versus time during and after 2-hour infusions of ddI in rats at doses of 25 mg/kg, 65 mg/kg, and 250 mg/kg were 122, 76, and 38 ml/kg/min, respectively. Statistical significance could be shown only in the comparison of the lowest to the highest values, however. Wientjes and Au (21) used a concomitant intravenous dose of [^{3}H]ddI to determine the operative clearance after a 40 mg/kg oral dose of unlabeled ddI in rats. They found a mean CL of 115 ± 25 ml/min/kg and a mean % bioavailability of 16.5 ± 5.1%. The oral doses in the present study (50 mg/kg) were similar to those of Wientjes and Au. The plasma clearance value obtained from the ddI concentration-time profile after an intravenous infusion in the present study, 79 ± 8 ml/min/kg, is not statistically different from their value ($p > 0.05$). Thus, a correction of the ddI bioavailability reported herein for dose dependent clearance seems unwarranted. If such a correction were made, ddI's oral bioavailability would still remain below 10%, further justifying the assumption of linear kinetics.

The low and highly variable oral bioavailability of ddI is a significant clinical problem (4–6, 22). Among the factors to be considered in rationalizing the poor bioavailability of ddI are the acid lability of ddI (3), metabolism in the g.i. tract by purine nucleoside phosphorylase or other enzymes, first-pass liver metabolism, and the highly polar nature and, consequently, low intestinal permeability of ddI. Even though ddI is currently administered in combination with antacids to limit acid degradation, its oral bioavailability remains incomplete and highly variable with values reported in adults of 38 ± 15% (22) and < 5% to 89% in children (5), suggesting that the acid lability of ddI is not the principal problem. First-pass liver metabolism does not appear to account for the low oral bioavailability of ddI, but may be a partial contributor. The results in Table III show that there is no significant decline in AUC after portal administration of ddI compared to an intravenous dose, even at a 4-fold slower portal infusion rate. However, the portal blood concentrations during the first-pass of ddI were quite high in both portal infusion studies. Previous investigators have shown that the hepatic extraction of ddI is saturable both *in vivo* (8) and in the isolated perfused rat liver (2). Nevertheless, the maximum hepatic extraction which has been reported (for a 30 min infusion of a 12 mg/kg dose) is 23%, which would only partially account for the low oral bioavailability of ddI observed (8). Bramer *et al.* (8) concluded that, in addition to acid catalyzed decomposition in the stomach and saturable first-pass liver metabolism, metabolism by the contents in the lower intestinal tract appeared to be the major route of presystemic first-pass elimination of ddI.

The need to utilize a "mixing tank" absorption model as

Table II. Trapezoidal Areas Under the ddI or 6-Cl-ddP Plasma Concentration-Time Curves After Intravenous, Gastric, or Portal Administration of ddI or 6-Cl-ddP

Compound route	ddI (mean ± SD)			6-Cl-ddP (mean ± SD)			ddI from 6-Cl-ddP (mean ± SD)		
	IV	Gastric	Portal	IV	Gastric	Portal	IV	Gastric	Portal
Group 1 (n = 6)	740 ± 30	47 ± 24							
Group 2 (n = 4)	420 ± 70		450 ± 50[a] 550 ± 20[b]						
Group 3 (n = 3)	650 ± 70	26 ± 11		410 ± 80	29 ± 9		220 ± 20	330 ± 70	
Group 4 (n = 3)				290 ± 70		270 ± 9	310 ± 10		340 ± 30

[a] Infused over 30 sec. with first sample taken at 2 min.
[b] Infused over 2 min. with first sample taken at 2 min.

described by Dressman *et al.* (18) to adequately characterize the concentration-time profile of an oral dose of ddI is consistent with several studies which have shown that the intestinal absorption of ddI is regionally-dependent (6–8, 23, 24). Sinko *et al* (7) found that the oral absorption of ddI is permeability-limited and independent of water transport, with the absorption rate constant decreasing down the small intestine. These observations suggest that the bioavailability of ddI could be substantially improved via more lipophilic prodrugs. Indeed, recent studies (23) have demonstrated that modest increases in oral bioavailability can be achieved via hydrophobic ester formation.

Pharmacokinetics and Bioconversion of Intravenously or Intraportally Infused 6-Cl-ddP

Although several strategies have been reported that may have potential in increasing the CNS delivery of reverse transcriptase inhibitors effective in the treatment of AIDS, a series of lipophilic 6-halogenated congeners of 2′,3′-dideoxypurine nucleosides, which are activated by brain tissue adenosine deaminase, seem to be among the most promising candidates identified to-date (12, 13). The adenosine deaminase activity in brain tissue is relatively high both in rats (25) and in humans (26), making it an attractive target enzyme for CNS-directed prodrugs. Previous studies in these laboratories have shown, for example, that intravenous infusions of 6-Cl-ddP resulted in 10-fold increases in brain parenchymal concentrations of ddI relative to those obtained after a control infusion of ddI (13). A caveat of this approach, however, is the fact that among the highest adenosine deaminase activities are found in the stomach, duodenum, and jejunum (26). Thus, it seems unlikely that 6-Cl-ddP would have high oral bioavailability unless it were absorbed quite rapidly. In order to obtain baseline information to evaluate the potential oral bioavailability of 6-Cl-ddP, the pharmacokinetics and conversion of 6-Cl-ddP to ddI were first examined systemically after intravenous and intraportal administration.

The pharmacokinetics of intravenously administered 6-Cl-ddP and its conversion *in vivo* to ddI by adenosine deaminase were examined in both groups 3 & 4. Intraportal administration of 6-Cl-ddP was compared to intravenous administration in group 4. Figure 2 displays the profiles obtained in group 4 for 6-Cl-ddP administered both intraportally and intravenously, and the corresponding ddI concentration-time profiles. The 6-Cl-ddP intravenous data from group 3 were superimposable on the data obtained in group 4 animals (not shown). Nonlinear regression analysis of the pooled intravenous 6-Cl-ddP data indicated that a biexponential equation having the form shown in Eq. 1 provided the best fits. The parameters generated by this analysis are

Table III. Apparent Bioavailabilities (F) for ddI and 6-Cl-ddP After Administration of Either ddI or 6-Cl-ddP via Various Routes Using AUCs Obtained Either from Computer Fitting of Pooled Data or from the Trapezoidal Method

Admin. route	ddI		6-Cl-ddP		ddI from 6-Cl-ddP	
	Model calc'd F ± SD	Trapezoid F ± SD	Model calc'd F ± SD	Trapezoid F ± SD	Model calc'd F ± SD	Trapezoid F ± SD
IV	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.42 ± .11	0.41 ± .10
Portal (30 sec)	1.00 ± 0.02	1.08 ± .10	0.97 ± .11	0.97 ± .23	0.55 ± ND	0.52 ± .10
Portal (2 min)	1.01 ± 0.01	1.33 ± .18[a]				
Gastric	0.07 ± ND[b] 0.03 ± ND[c]	0.06 ± .03[b] 0.04 ± .02[c]	0.079 ± ND	0.07 ± .03	0.54 ± ND	0.51 ± .06

[a] Trapezoidal area method gave unreliable F values when 2 min portal infusion. AUCs were compared to 30 sec. intravenous infusion data in group 2 animals because a significant portion of the AUC was lost in the intravenous AUC when the first sampling time was at 2 minutes.
[b] Study 1 results.
[c] Study 3 results.

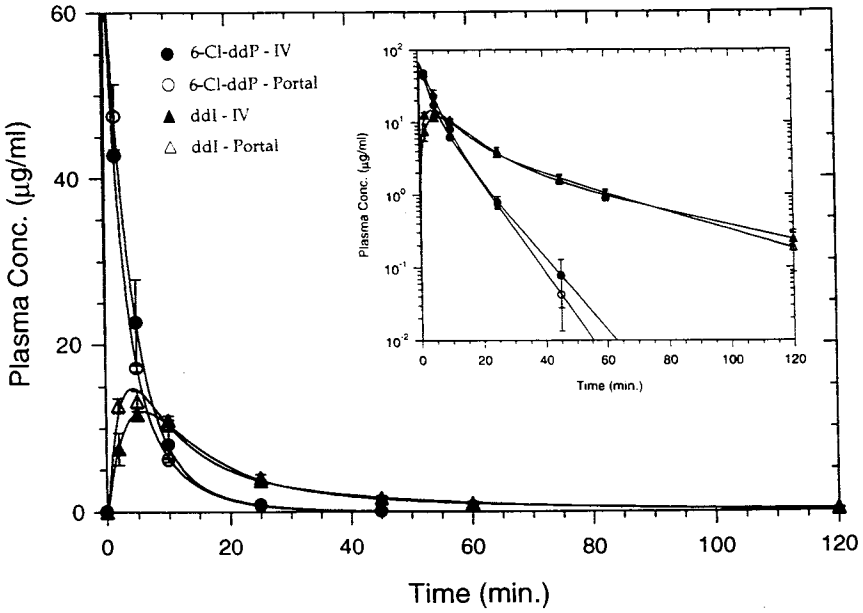

Fig. 2. Plasma concentrations of 6-Cl-ddP (○,●) or ddI (△,▲) after 30 sec intravenous (filled symbols) or portal (open symbols) administration of 6-Cl-ddP. Curves represent computer fits.

shown in Table I. In most respects, these values resemble those reported by this laboratory in a preliminary study of the pharmacokinetics of 6-Cl-ddP (13) but differ significantly in behavior after ≈ 45 min. The low residual plasma concentrations of 6-Cl-ddP detected after 45 min in the previous study may have been an artifact as the present study did not reproduce these results.

Trapezoidal areas under the concentration-time curves, shown in Table II, suggested an apparent difference in AUCs for the 6-Cl-ddP intravenous data in groups 3 & 4. As seen previously in the ddI trapezoidal AUC data, however, this is due to the sensitivity of the trapezoidal area determinations to the early sampling points for this rapidly cleared compound and the fact that 0.5 min samples were not collected in group 4 animals.

As shown in Figure 2, peak concentrations of 12.1 ± 0.6 μg/ml ddI were reached at 5 min after 30 sec intravenous infusions of 6-Cl-ddP. The bioavailability of ddI after intravenous administration of 6-Cl-ddP is 42 ± 11%, estimated from the combined data in groups 3 & 4 (Table III). Whereas the Vss and mean residence time for ddI after intravenous administration were found to be 490 ml/kg and 6.2 min, respectively, these parameters were 1390 ml/kg and 17.6 min, respectively, for ddI after iv administration of 6-Cl-ddP. An extended mean residence time for ddI formed from the prodrug is expected, as it is determined both by kinetic events governing ddI formation as well as those governing its elimination. The larger steady-state distribution volume for 6-Cl-ddP as compared to that of ddI (Table I) and the nearly 3-fold increase in Vss for ddI when it is formed from prodrug suggest that the prodrug may have greater tissue accessibility, consistent with its greater lipophilicity, and that either slow release of prodrug from the tissue followed by hydrolysis to ddI, slow hydrolysis of the prodrug within the tissue, or rapid prodrug hydrolysis within cells containing high activities of adenosine deaminase followed by slow transport of ddI from these cells may occur. The low bioavailability (< 50%) of ddI when it is produced from intravenous administration of 6-Cl-ddP suggests that the prodrug may undergo elimination in parallel with adenosine deaminase catalyzed hydrolysis. Alternatively, the prodrug may access cells containing high activities of ddI degrading enzymes (e.g., purine nucleoside phosphorylase) which are not readily entered by ddI itself. Once formed intracellularly, ddI may be sequestered in such cells to undergo sequential metabolism in parallel with its slow transport from the cell. Sequential metabolism of products formed *in situ* has previously been reported for the metabolism of gentisamide after its generation from salicylamide (27) and for the metabolism of nordiazepam generated *in situ* from diazepam (28).

Several compartmental models (not shown) were explored in an attempt to obtain mathematical equations to fit simultaneously the ddI, 6-Cl-ddP, and ddI from 6-Cl-ddP concentration-time profiles obtained in group 3 animals in order to test the above hypotheses. The models assumed two-compartments for 6-Cl-ddP, consistent with its biexponential kinetic behavior, and triexponential disposition of ddI, with ddI formation occurring in either the central or peripheral prodrug compartments, or both, and with parallel prodrug elimination occurring in either the central compartment, the peripheral compartment, or both. An alternative, sequential metabolism model was also explored by allowing ddI to accumulate in the peripheral prodrug compartment. The best fitting models, as judged by values of the Model Selection Criterion, required prodrug hydrolysis to ddI in both central and peripheral compartments. Also, concurrent non-ddI producing prodrug elimination was required, but models with parallel prodrug elimination from either or both central and peripheral compartments could not be distinguished. The sequential metabolism model provided the best

coefficient of determination but, since it contained an additional exponential term, had an inferior Model Selection Criterion value. The solid curves shown in Figure 2 reflect model generated concentration-time profiles.

Prodrug and ddI AUCs from portally administered 6-Cl-ddP were not significantly different from those obtained after intravenous dosing (group 4). However, absolute ddI bioavailabilities could not be directly determined in group 4 because an intravenous ddI reference curve was not generated in this group. Groups 3 & 4 were therefore pooled to obtain absolute bioavailability estimates (Table III).

Oral Bioavailability of 6-Cl-ddP and ddI After 6-Cl-ddP Administration

The apparent oral bioavailabilities of 6-Cl-ddP and ddI after oral administration of 6-Cl-ddP were examined in group 3. The trapezoidal AUCs are tabulated in Table II. Fractions bioavailable, F, obtained by comparing AUCs generated either by non-linear regression analyses or the linear trapezoidal method are shown in Table III. The apparent oral bioavailability of 6-Cl-ddP proved to be very low, ≈ 7%, similar to that of ddI, while its portal bioavailability is 97% ± 11%, suggesting either poor absorption or extensive gut wall metabolism.

Although the oral bioavailability of 6-Cl-ddP is low, the apparent ddI bioavailability after prodrug administration (> 50%) represents a >10-fold increase in bioavailability over that obtained after oral administration of ddI itself. A significant decrease in the variability in apparent bioavailability is also noted, from a coefficient of variation of 49% in animals given ddI to 12% in animals administered 6-Cl-ddP (Tables II & III). This large increase in systemic bioavailability of ddI to >50% of the dose when 6-Cl-ddP is given orally, levels similar to those obtained from an intravenous dose of prodrug, rules out poor prodrug absorption. Rather, these data, coupled with the low apparent bioavailability of 6-Cl-ddP itself, suggest that orally administered 6-Cl-ddP is relatively well absorbed but adenosine deaminase conversion of 6-Cl-ddP occurs prior to entry of the prodrug into the systemic circulation. Again, first-pass hepatic extraction does not appear to be important as there was no significant difference in the AUCS of 6-Cl-ddP given intravenously and portally, and the estimated bioavailabilities of ddI from portally and orally administered 6-Cl-ddP were similar (55% and 54%, respectively) and not significantly different from that obtained after intravenous administration of the prodrug. Additional studies at lower doses and/or lower infusion rates would be necessary to rule out possible effects of saturable hepatic metabolism, however.

Figure 3 compares the ddI plasma concentration-time profiles obtained in group 3 animals after intravenous doses of either ddI or 6-Cl-ddP and oral doses of either ddI or 6-Cl-ddP. Given the ≈ 30-fold greater lipophilicity of 6-Cl-ddP compared to ddI (29), it is somewhat surprising that the terminal slope of the ddI concentration-time profile after gastric administration of 6-Cl-ddP is so prolonged (67 ± 22 min), again suggestive of a "flip-flop" pharmacokinetic model with the decline in ddI concentration rate-limited by absorption. A "mixing tank" absorption model again provided the best fit the oral absorption data in Figure 3. The apparent ddI absorption half-life after oral administration of 6-Cl-ddP resembles the terminal absorption half-life for ddI administered gastrically (96 ± 64 min).

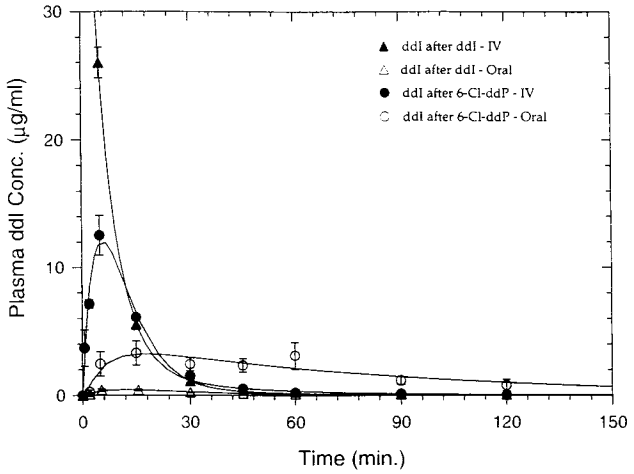

Fig. 3. Plasma concentrations of ddI after intravenous (filled symbols) or oral (open symbols) administration of ddI (△,▲) or 6-Cl-ddP (○,●). Curves represent best fits of the data.

The slow apparent ddI absorption kinetics after oral 6-Cl-ddP suggest that relatively rapid passage of the prodrug across the lumenal membrane of the gastrointestinal epithelium may be followed by adenosine deaminase catalyzed conversion of the prodrug in the enterocytes to ddI which then may undergo sequential intracellular metabolism (27, 28, 30) and, in parallel, slow release into the systemic circulation. Back-diffusion of ddI from the enterocyte into the intestinal lumen may also occur. Either sequential metabolism in the enterocyte or back-diffusion into the intestinal lumen would account for (a) the low systemic availability of 6-Cl-ddP; (b) the enhancement in ddI's oral bioavailability; and (c) the slow apparent absorption rate constant for ddI after 6-Cl-ddP administration when compared to ddI administration ($t_{1/2}$ = 67 min after 6-Cl-ddP; $t_{1/2}$ = 96 min after ddI) despite the 30-fold higher lipophilicity of 6-Cl-ddP.

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