Central Nervous System Targeting of 2',3'-Dideoxyinosine via Adenosine Deaminase-Activated 6-Halo-Dideoxypurine Prodrugs

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Received 31 January 1992/Accepted 17 July 1992

AIDS dementia complex is a neurologic disorder, characterized by increasingly severe cognitive, behavioral, and motor impairment, which is associated with human immunodeficiency virus (HIV) infection in the central nervous system (CNS). Many of the dideoxynucleosides effective systemically in the treatment of HIV infections, such as 2',3'-dideoxyinosine (ddI), exhibit limited penetration into the CNS and limited or variable effectiveness in reversing the symptoms of AIDS dementia. Thus, approaches for increasing the CNS uptake of ddI and other dideoxynucleosides are needed. The CNS uptake of a series of 6-halo-2',3'-dideoxypurine ribofuranosides (6-halo-ddPs) previously shown to be active against HIV because of their conversion to ddI through the action of adenosine deaminase was examined in rats. In vitro studies in rat blood and brain tissue homogenate suggested a favorable selectivity for bioconversion in brain tissue, but with bioconversion half-lives varying widely within the series. In vivo infusions of 6-chloro-ddP (6-Cl-ddP), 6-bromo-ddP (6-Br-ddP), and 6-iodo-ddP (6-I-ddP) resulted in significant increases (20- to 34-fold) in the ddI concentration ratios in brain parenchyma/plasma when compared with those after an infusion of ddI alone. Absolute concentrations of ddI in brain parenchyma were increased 10- and 4-fold, respectively, following 30-min infusions of 6-Cl-ddP or 6-Br-ddP, but were 2.4-fold lower after an infusion of 6-I-ddP relative to that after a control infusion of ddI. Detailed studies of the plasma pharmacokinetics, CNS uptake kinetics, and bioconversion of 6-Cl-ddP were conducted to compare in vivo transport and bioconversion parameters with those predicted from in vitro measurements and to rationalize the efficiency of CNS delivery of ddI from 6-Cl-ddP. The results show that increased lipophilicity alone does not ensure that a given prodrug will deliver higher levels of a parent compound to the CNS. Both the selectivity and absolute rate of bioconversion in the brain are important factors.

Because of the prevalence of a progressive deterioration in mental function in patients with AIDS, referred to as the AIDS dementia complex, and its association with human immunodeficiency virus (HIV) infection in the central nervous system (CNS) (24, 25, 31), the ability of anti-HIV agents to penetrate into the CNS has become an important issue in the development of therapy against AIDS. The reverse transcriptase inhibitor zidovudine (AZT) has been shown to exhibit relatively high CNS penetration in patients (17) and to reverse the neurological dysfunction characteristic of AIDS dementia complex in some patients (28, 29, 42). Unfortunately, its benefits are limited by its toxic effects, particularly myelosuppression (13, 15, 34, 43), and the emergence of AZT-resistant strains of HIV in patients receiving long-term AZT therapy has posed a serious concern (19, 33). Other 2',3'-dideoxynucleosides currently approved for the treatment of AIDS (2',3'-dideoxyinosine [ddI]) or in the late stages of clinical development (e.g., 2',3'-dideoxycytidine and others) are substantially less lipophilic than AZT and exhibit reduced CNS uptake (4, 12). In children, steady-state cerebrospinal fluid (CSF)/plasma concentration ratios of 0.24, 0.03, and 0.05 have been reported for AZT, 2',3'-dideoxycytidine, and ddI, respectively (8, 28). Cognitive improvement in children with symptomatic HIV infec-

tion appears to correlate with CSF/plasma concentration ratios, because AZT is most effective (28), ddI's effects are highly variable (9), and IQ scores continue to drop after the use of ddC alone (27). These data suggest that approaches for increasing the CNS penetration of dideoxynucleosides active against HIV may enhance their clinical utility for the treatment of AIDS dementia complex.

Shirasaka et al. (35, 36) have recently reported that a series of 6-halogenated congeners of 2',3'-dideoxypurine nucleosides, which are substrates for adenosine deaminase (ADA), have potent activity against HIV in vitro and may represent a class of lipophilic prodrugs of ddI and 2',3'-dideoxyguanosine. The potential of such agents to deliver active nucleoside to infected brain tissue is likely to be determined not only by their increased lipophilicity but also by the rate and selectivity of their bioconversion in the CNS relative to that in the peripheral circulation. Studies in our laboratories have shown, for example, that lipophilic 5'-aliphatic acid esters of ddI do not enhance the delivery of ddI to the brain because of the unfavorable ratio of esterase activity in brain tissue in comparison with that in plasma (3, 41). In contrast, ADA activity is relatively high in brain tissue both in animals (6) and in humans (37). In vitro studies (2) in our laboratories with 6-halo-dideoxypurine nucleosides (6-halo-ddPs) as substrates have shown that ADA activity in rat brain tissue homogenate exceeds its activity in blood by approximately 10-fold. Consistent with their increased li-

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FIG. 1. Chemical structures of 6-halo-ddPs. X indicates the locations of the halogen substituents F, Cl, Br, and I.

pophilicity and the favorable distribution of ADA, the 6-halo-ddPs, particularly 6-chloro-ddP (6-Cl-ddP), have been shown to enhance concentrations of ddI in vivo in the CNS of rats (2). A series of 2-amino-6-halo-ddPs has also been shown to penetrate into brain tissue in mice when administered intravenously (36). In this study, the CNS delivery of the 6-halo-ddPs shown in Fig. 1, and particularly 6-Cl-ddP, are examined in greater detail. The pharmacokinetics and kinetics of CNS uptake and bioconversion of 6-Cl-ddP were determined in rats, and compartmental models were developed to describe these processes quantitatively. The kinetic parameters obtained from the in vivo data are compared with related parameters from in vitro measurements to examine why, among the 6-halo-ddPs explored, 6-Cl-ddP is superior in its ability to enhance the delivery of ddI to the brain in rats.

MATERIALS AND METHODS

Chemicals. ddI was provided by the National Institute of Allergy and Infectious Diseases and was used as received. The preparation of the four 6-halo-ddPs tested in the present study—6-fluoro-ddP (6-F-ddP), 6-Cl-ddP, 6-bromo-ddP (6-Br-ddP), and 6-iodo-ddP (6-I-ddP)—has been described previously (23). All other compounds were commercially available and were used as received.

In vitro hydrolysis studies: determination of initial rates of formation of ddI from 6-halo-ddPs in rat blood and brain. Pooled blood and brain tissue from three to five rats were used for the in vitro hydrolysis studies. Blood was diluted to 5 to 25% (by weight) with phosphate buffer (pH 7.4; ionic strength [I] = 0.15). Brain tissue (stored frozen at -20°C prior to use) was diluted with an equal weight of buffer, homogenized, and diluted to 10% (by weight) in pH 7.4 buffer. Diluted blood or brain tissue homogenate was warmed to 37°C for 5 min prior to initiating the hydrolysis reactions. Reactions were initiated by the addition of a concentrated 6-halo-ddP solution. Initial rates of ddI formation in blood were monitored by high-pressure liquid chromatography (HPLC) at prodrug concentrations ranging from $\sim 2 \times 10^{-3}$ to 0.05 M. Final concentrations in 10% brain homogenate were 0.5 to 2 mM. Initial velocity-substrate concentration profiles were subjected to nonlinear regression analysis to obtain estimates of $V_{\max}$ and K_m in blood by using a computer program (MINSQ; MicroMath Inc., Salt Lake City, Utah). Apparent half-lives in blood and brain tissue were determined from estimated first-order hydrolysis

rate constants, which were generated from $V_{\max}/K_m$ ratios for each compound, or from first-order rate constants calculated from concentration-versus-time plots obtained at substrate concentrations well below the K_m .

The fraction of 6-halo-ddPs undergoing bioconversion to ddI in blood and brain tissue homogenate was estimated in separate experiments in which both prodrug (6-F-ddP or 6-Cl-ddP) disappearance and ddI formation were monitored versus time by HPLC. 6-F-ddP studies were conducted in 10% blood and 1% brain tissue (diluted with phosphate buffer [pH 7.4; I = 0.15]). 6-Cl-ddP reactions were performed in 100% blood and 5% brain homogenate. Initial 6-halo-ddP concentrations were 0.1 to 0.2 mM, well below the respective K_m values determined previously. Rate constants were calculated by computer analysis (MINSQ; MicroMath Inc.) assuming a parallel, first-order reaction model.

In vivo studies. (i) Preparation of animals and drug infusion solutions. Male Sprague-Dawley rats obtained from Sasco Laboratories (Omaha, Nebr.) were placed under ether anesthesia, and a cannula was implanted in the right jugular vein (39). The cannula was routed subcutaneously to the dorsal base of the neck and secured with a ligature that permitted intravenous (i.v.) drug infusions or i.v. serial sampling in awake rats. Animals recovered from surgery for a 1- or 2-day period. On the morning of the infusions, they were placed in individual metabolism cages, and food but not water was withdrawn for the duration of the study.

Infusion solutions of ddI, 6-Cl-ddP, and 6-Br-ddP were prepared in 0.9% saline at concentrations of ~ 20 mg/ml (ddI equivalents). Because of the limited water solubility of 6-I-ddP, infusion solutions of this compound were made in dimethyl sulfoxide (120.0 mg/ml).

(ii) Protocol for evaluation of CNS delivery of ddI after 30-min infusions of ddI or 6-halo-ddPs. Infusion studies (30 min) were performed in which two to six animals received each compound. Body weights on the day of the experiment were 336 ± 29 g (mean $\pm$ standard deviation [SD]). The target dose for the infusions was 125 mg of body weight per h (the actual dose was 119.3 ± 9.3 mg/kg/h; mean $\pm$ SD). ddI, 6-Cl-ddP, and 6-Br-ddP solutions were infused at a rate of 0.034 ml/min for 30 min. 6-I-ddP (in dimethyl sulfoxide) was infused at a rate of 0.0079 ml/min for 30 min. At the end of 30 min, animals were placed under light ether anesthesia for sample collection. Blood and CSF samples were withdrawn while continuing the infusion. Blood (0.25 ml) was drawn from the inferior vena cava in a heparinized syringe and centrifuged at $8,600 \times g$ for 1 min. Plasma (0.1 ml) was diluted with acetonitrile (0.5 ml), vortexed, and stored at -20°C . CSF samples were obtained by cisterna magna puncture by using a 24-gauge needle attached to silastic tubing as described by Waynforth (38). CSF samples were weighed and stored at -20°C . After withdrawal of CSF, the infusions were stopped and rats were immediately decapitated. Brains were dissected from the cranium within 1 to 2 min after decapitation, placed in a vial, frozen in a dry ice-ethanol mixture, and then stored at -20°C .

(iii) i.v. bolus plasma pharmacokinetics of ddI versus those of 6-Cl-ddP. Injections of drug (ddI or 6-Cl-ddP) were made over 2 min into the previously implanted jugular cannulas, and the cannulas were immediately flushed with 1.0 ml of heparinized saline. From each of four animals receiving an i.v. bolus dose of ddI (80 mg/kg), serial blood samples (0.25 ml) were collected at eight time points between 2 min and 1 h for analysis of the ddI concentration. Two animals were administered an i.v. bolus dose of 6-Cl-ddP (80 mg of ddI

equivalent per kg), and blood samples (0.25 ml) were withdrawn at times ranging from 2 min to 3 h after dosing for analysis of 6-Cl-ddP and ddI concentrations. Blood samples were centrifuged at $8,600 \times g$ for 1 min, and the plasma was processed and stored as described above.

(iv) Protocol for monitoring the kinetics of CNS uptake and bioconversion of 6-Cl-ddP during and after 1-h infusions. One-hour infusions of either ddI or 6-Cl-ddP were performed in a sufficient number of animals to obtain two to four determinations at seven time points over a 120-min period during and after the i.v. infusions. Body weights on the day of the experiment were 313 ± 18 g (mean $\pm$ SD). The target dose was 125 mg of ddI equivalents per kg/h (the actual dose was 129.5 ± 8.0 mg/kg/h). ddI and 6-Cl-ddP in saline were infused at a rate of 0.034 ml/min for 60 min. Samples were taken at 0, 30, and 60 min during the infusions and at 10, 20, 40, and 60 min after the infusions were terminated. Animals were anesthetized with ether prior to sample collection. Blood, CSF, and brain samples were collected and treated as described above, with the exception that blood was drawn from the abdominal aorta rather than the vena cava.

(v) Sample preparation. The blood and brain tissue homogenate samples (100 μ l) collected in the in vitro studies were diluted with acetonitrile (300 μ l), vortexed, and centrifuged; and then they were either further diluted with buffer or evaporated under nitrogen and reconstituted in buffer for HPLC analysis. Frozen plasma samples from the in vivo experiments were thawed, vortexed, and centrifuged ($340 \times g$ for 1 min); and the supernatant was collected in a 4.0-ml glass vial. The residue was treated with 0.1 ml of water and 0.5 ml of acetonitrile, vortexed, and centrifuged ($5,500 \times g$ for 3 min); and the supernatant was combined with the supernatant collected previously. The combined supernatants were evaporated to dryness at 35°C under a stream of nitrogen and were reconstituted with 0.5 ml of phosphate buffer (pH 7.4; $I = 0.02$). Frozen brains were slightly thawed, and one-half of each brain (~ 0.7 to 0.8 g) was weighed and placed into a 50-ml polyethylene conical tube. A 1.5-ml aliquot of water was added, and the samples were homogenized. The homogenizer probe was rinsed with 1 ml of water, and the rinse water was added to the homogenate solution. The samples were then vortexed with acetonitrile (12.5 ml) and centrifuged, and the supernatant was collected. The residue was resuspended by vortexing with 0.5 ml of water, and then the solution was mixed with 2 ml of acetonitrile and centrifuged. The supernatant was combined with that collected previously, and the liquid was evaporated to dryness under a stream of nitrogen at 35°C . The dried residue was reconstituted with 1 ml of phosphate buffer (pH 7.4; $I = 0.02$) and filtered (pore size, $0.45 \mu\text{m}$; Acrodisc CR; Gelman). CSF samples were diluted with phosphate buffer prior to analysis.

HPLC analyses. The concentrations of ddI and prodrugs in blood, CSF, and brain tissue were analyzed by HPLC with UV detection at 254 nm by using a modular HPLC system with reversed-phase columns and acetonitrile-water or methanol-water mobile phases. Polar modifiers such as tetrabutylammonium ion were added, and the pH of the mobile phase was adjusted with buffers (ammonium acetate or phosphate) as needed to optimize the resolution and improve the separation of ddI and the 6-halo-ddPs from tissue component peaks. Analyte identities and concentrations were determined by comparison of peak retention times and heights with those of external standards with known compositions. Optimal resolution was obtained by using a Supelcosil LC-18S 5μ column (4.6 mm [inner diameter] by 25

cm). A typical mobile phase for the analysis of 6-halo-ddPs in plasma, CSF, or brain tissue was 8% acetonitrile-phosphate buffer (pH 7.4; $I = 0.02$). Under these conditions, retention volumes for 6-F-ddP, 6-Cl-ddP, 6-Br-ddP, and 6-I-ddP were 21, 32, 40, and 56 ml, respectively. A typical mobile phase for the analysis of ddI in plasma and CSF was 4% methanol-0.01% tetrabutylammonium ion-phosphate buffer (pH 3.0; $I = 0.02$). Under these conditions, the retention volume of ddI was 19 ml. Brain tissue ddI concentrations were analyzed by using 2 to 3% methanol-0.02 M ammonium acetate (pH 3.2). The retention volume of ddI under these conditions was 51 ml. Generally, assays for ddI and the 6-halo-ddPs were linear over the concentration range of 5×10^{-7} to 1.0×10^{-4} M. Detection limits (three times the SD of the lowest standard) for ddI were 0.8 and 2.0 ng (100- μ l injection size, 8 and 20 ng of sample concentration per ml) under assay conditions for plasma or CSF and brain, respectively, and ~ 1 ng for the 6-halo-ddPs. Intra- and interassay coefficients of variation (mean $\pm$ SD) for ddI were $2.7\% \pm 2.1\%$ and $3.4\% \pm 2.0\%$, respectively, in plasma or CSF and $2.4\% \pm 1.5\%$ and $3.6\% \pm 1.6\%$, respectively, in brain tissue. Coefficients of variation for the 6-halo-ddPs ranged from $2.6\% \pm 1.7\%$ to $4.25\% \pm 1.2\%$. Recoveries of prodrug and ddI from spiked plasma samples averaged $90\% \pm 7\%$ and $103\% \pm 5\%$, respectively. Recoveries from spiked brain tissue samples averaged $83\% \pm 1\%$ and $93\% \pm 14\%$ for the 6-halo-ddPs and ddI, respectively.

Pharmacokinetic evaluation of 6-Cl-ddP and ddI. (i) Bolus pharmacokinetics. The concentrations of ddI and 6-Cl-ddP in plasma versus time resulting from bolus injections of either ddI or 6-Cl-ddP were fitted to mathematical equations that reflect the model depicted in Fig. 2 by using nonlinear regression analysis (MINSQ; MicroMath Inc.). The selection of the appropriate number of compartments for each compound was based on model selection criteria derived from Akaike's information criterion (1, 22, 40). Weighting factors of $1/C_{\text{plasma}}$ or $1/C_{\text{plasma}}^2$ (where C_{plasma} is concentration of drug in plasma) were used in the curve-fitting analyses, depending on which method gave the best visual fit of the data. Parameters describing ddI's pharmacokinetics were determined from the ddI concentration-time profiles generated after bolus injections of ddI. These parameters were then held constant in fitting the parameters that described the pharmacokinetics of 6-Cl-ddP and its bioconversion to ddI. The systemic clearances (CLs) of ddI and 6-Cl-ddP from plasma were calculated from areas under the respective concentration-time curves (AUCs) extrapolated to infinity ($\text{CL} = \text{dose}/\text{AUC}_{0-\infty}$). Area estimates were obtained from the pharmacokinetic parameters generated in separate regression analyses by a polyexponential curve-fitting computer program (RSTRIP; MicroMath Inc.) by using a triexponential equation for ddI and a biexponential equation for 6-Cl-ddP. The fraction (F) of prodrug converted to ddI (i.e., the apparent availability of ddI in plasma after prodrug administration) was estimated in the following two ways: by the AUC ratio method (where F is the ratio of the AUC for ddI after a bolus injection of 6-Cl-ddP to the AUC for ddI after a bolus injection of ddI) and by comparing the rate constant for prodrug hydrolysis (k_{hyd}) with the rate constant for prodrug elimination ($k_{\text{el}}^{\text{PD}}$) by other routes [$F = k_{\text{hyd}}/(k_{\text{hyd}} + k_{\text{el}}^{\text{PD}})$].

(ii) Plasma pharmacokinetics and kinetics of drug uptake into the CSF and brain during and after 60-min infusions. Plasma (arterial sampling) ddI and 6-Cl-ddP concentrations determined during and after 60-min infusions of either ddI or 6-Cl-ddP at 125 mg/kg/h (ddI equivalents) were analyzed in a

FIG. 2. Compartmental model used to describe the pharmacokinetics of ddI and/or 6-Cl-ddP in plasma after bolus administration of either ddI or 6-Cl-ddP.

manner similar to that in which data from bolus injections were analyzed. The data for the ddI concentrations in plasma from animals infused with ddI generated in this study were pooled with data reported previously (4, 16) to generate pharmacokinetic parameters for ddI applicable to the infusion rate that we used. A two-compartment model with elimination occurring from the central (plasma) compartment was adequate to describe the ddI concentrations in plasma from animals receiving infusions of ddI. These parameters were then held constant in fitting the 6-Cl-ddP and ddI concentration-time curves during and after an infusion of 6-Cl-ddP. A two-compartment model with both elimination and prodrug hydrolysis occurring in the central (plasma) compartment was used to fit the plasma 6-Cl-ddP and ddI concentrations with time. More complex models which assumed that hydrolysis could also occur in tissue, followed by diffusion of ddI back into the systemic circulation, were also explored, but they were found to yield no improvement in fit.

Concentration-time profiles of ddI in CSF during and after a 60-min i.v. infusion of ddI were subjected to simple compartmental analysis as described previously (4). The model, which is believed to be the first approximation of the more complex processes actually involved, accounts for the rate of uptake (t) of ddI into CSF ($d[\text{ddI}]_{\text{CSF}}/dt$) by a single apparent rate constant for entry (k_{in}) and a rate constant for efflux (k_{out}) as given by the following equation:

$$d[\text{ddI}]_{\text{CSF}}/dt = k_{\text{in}}^{\text{ddl}} [\text{ddI}]_{\text{plasma}} - k_{\text{out}}^{\text{ddl}} [\text{ddI}]_{\text{CSF}} \quad (1)$$

Integrated expressions of CSF concentration of ddI versus time were obtained by Laplace transforms as described previously (4), and ddI concentration-time profiles were

fitted by nonlinear regression analysis to obtain values for the parameters $k_{\text{in}}^{\text{ddl}}$ and $k_{\text{out}}^{\text{ddl}}$.

Concentration-time profiles of 6-Cl-ddP and ddI in CSF during and after 60-minute i.v. infusions of 6-Cl-ddP were treated similarly, but with an additional term for the apparent hydrolysis in the CSF ($k_{\text{hyd}}^{\text{CSF}}$). ADA activity in CSF is negligible. Therefore, $k_{\text{hyd}}^{\text{CSF}}$ is a hybrid rate constant which is physiologically meaningless. In the treatment described here, positive values of $k_{\text{hyd}}^{\text{CSF}}$ most likely reflect relatively rapid equilibration of drug between the CSF and surrounding brain tissue, with prodrug conversion actually occurring in the brain tissue. Equations 2 and 3 describe the rate of change in 6-Cl-ddP and ddI concentrations, respectively, in CSF with time:

$$d[\text{ddP}]_{\text{CSF}}/dt = k_{\text{in}}^{\text{PD}} [\text{ddP}]_{\text{plasma}} - k_{\text{out}}^{\text{PD}} [\text{ddP}]_{\text{CSF}} - k_{\text{hyd}}^{\text{CSF}} [\text{ddP}]_{\text{CSF}} \quad (2)$$

$$d[\text{ddI}]_{\text{CSF}}/dt = k_{\text{in}}^{\text{ddl}} [\text{ddI}]_{\text{plasma}} - k_{\text{out}}^{\text{ddl}} [\text{ddI}]_{\text{CSF}} + k_{\text{hyd}}^{\text{CSF}} (\text{MW}_{\text{ddl}}/\text{MW}_{\text{ddP}}) [\text{ddP}]_{\text{CSF}} \quad (3)$$

where the concentration in plasma terms in equations 2 and 3 were obtained from computer fits of plasma concentration-time profiles described above and MW_{ddl} and MW_{ddP} are molecular weights of ddI and the 6-halo-ddPs, respectively. Integrated expressions of 6-Cl-ddP and ddI versus time were obtained by the method of Laplace transforms; and concentration-time profiles were analyzed by nonlinear regression analysis to obtain estimates for $k_{\text{in}}^{\text{PD}}$, $k_{\text{out}}^{\text{PD}}$, and $k_{\text{hyd}}^{\text{CSF}}$. Parameter values for $k_{\text{in}}^{\text{ddl}}$ and $k_{\text{out}}^{\text{ddl}}$ obtained in the analysis of the CSF ddI concentration-time profile during

TABLE 1. Kinetic parameters for the ADA-catalyzed hydrolysis of 6-halo-ddPs in rat blood and brain homogenate at 37°C

Compound	Whole blood			$t_{1/2}$ (min) in brain homogenate ^a
	K_m (M)	V_{max} (M/min)	$t_{1/2}$ (min) ^a	
6-F-ddP	7.2×10^{-4}	1.4×10^{-4}	3.5	0.46
6-Cl-ddP	0.017	4.4×10^{-5}	270	19
6-Br-ddP	0.016	4.0×10^{-5}	280	20
6-I-ddP	ND ^b	ND	1,690	178

^a Apparent half-lives ($t_{1/2}$ s) in blood and brain tissue were determined from estimated first-order hydrolysis rate constants, which were generated from V_{max}/K_m ratios for each compound, or first-order rate constants calculated from concentration-versus-time plots obtained at substrate concentrations well below the K_m .

^b ND, not determined.

and after an i.v. infusion of ddI were assumed to apply also during and after the i.v. infusion of 6-Cl-ddP.

The treatment of concentrations of ddI in brain tissue versus time during and after ddI infusions were treated as described in an earlier report (4). The equations are similar to the CSF concentration-time relationships described above but contain an additional term for the vascular space contribution (V_s). Estimates of this regional blood volume vary from 1 to 4% of the tissue volume (26, 30).

Statistical analysis. Levels of statistical significance were assessed by using a two-tailed Student's *t* test for unpaired data. *P* values of less than 0.05 were judged to indicate significant differences.

RESULTS

In vitro bioconversion in blood and brain tissue. Enzyme kinetic constants for the hydrolysis of the 6-halo-ddPs in blood and brain tissue homogenate, which were determined from the initial rates of ddI formation as a function of prodrug concentration, are listed in Table 1. The K_m values in blood exceeded the substrate concentrations likely to be encountered in vivo, indicating that the first-order hydrolysis rate constants, which were obtained from the V_{max}/K_m ratios, are the relevant parameters for comparing substrate reactivities in vivo. Half-lives in blood and brain tissue homogenate generated from first-order hydrolysis rate constants are also listed in Table 1. These data demonstrate that ADA activity in brain tissue homogenates exceeds that in whole blood for this series of substrates by approximately 1 order of magnitude, regardless of substrate lability. Half-lives for bioconversion in brain tissue span a range of ~400-fold (from ~0.5 min for 6-F-ddP to ~3 h for 6-I-ddP).

The conversion of the 6-halo-ddPs to ddI was quantitative in brain tissue, but not in blood. In separate experiments in which we used 6-F-ddP and 6-Cl-ddP, we monitored prodrug disappearance as well as the appearance of ddI in both blood and brain tissue homogenates. Blood concentration-versus-time curves were well described mathematically by a parallel first-order kinetic model, with the rate constant k_1 reflecting the ADA-catalyzed conversion to ddI and the rate constant k_2 representing other unknown routes of prodrug decomposition. Table 2 summarizes the values obtained for these rate constants and also lists estimates of the fraction (*F*) of prodrug undergoing conversion to ddI [$F = k_1/(k_1 + k_2)$] in each tissue. The conversions of 6-halo-ddPs to ddI in blood were 54 and 28% for 6-F-ddP and 6-Cl-ddP, respectively.

In vivo comparison of the CNS targeting potential of 6-halo-ddPs. A preliminary assessment of the potential of 6-halo-

TABLE 2. First-order rate constants for the parallel pathways of decomposition of 6-Cl-ddP and 6-F-ddP in blood and brain tissue in vitro and estimates of the fraction of prodrug converted to ddI

Compound	Whole blood		<i>F</i>	Brain tissue		<i>F</i>
	k_1 (min ⁻¹)	k_2 (min ⁻¹)		k_1 (min ⁻¹)	k_2 (min ⁻¹)	
6-F-ddP	0.15	0.13	0.54	1.51	~0	1.00
6-Cl-ddP	3.0×10^{-3}	7.4×10^{-3}	0.28	0.032	~0	1.00

ddPs to penetrate into the CNS and undergo conversion to ddI in the CNS was obtained during 30-min i.v. infusions of the 6-halo-ddPs in rats at an infusion rate of 125 mg/kg/h (ddI equivalents). Concentrations of both intact prodrug and ddI in plasma (venous sampling), CSF, and brain tissue homogenate were determined by HPLC. The concentrations of intact prodrug in plasma, CSF, and brain parenchyma (corrected for an assumed vascular volume of 2%) were compared with the ddI concentration after a 30-min infusion of ddI at the same rate of infusion (Fig. 3). Animals receiving 6-F-ddP became lethargic and displayed labored breathing within 15 min after the start of an infusion and were near death at 30 min, when the infusions were terminated. Therefore, 6-F-ddP was not explored further in vivo. Whereas the concentrations of intact prodrug in plasma during 30-min infusions of prodrug were similar to the concentration of ddI in plasma after a 30-min infusion of ddI, prodrug concentrations in CSF and brain parenchyma were significantly higher ($P < 0.0005$, 0.025, and 0.005 for 6-Cl-ddP, 6-Br-ddP and 6-I-ddP, respectively, in CSF; $P < 0.005$, 0.025, and 0.01 for 6-Cl-ddP, 6-Br-ddP, and 6-I-ddP, respectively, in brain parenchyma). CSF/venous plasma concentration ratios for the intact 6-halo-ddPs were 0.64, 0.52, and 0.42 for 6-Cl-ddP, 6-Br-ddP, and 6-I-ddP, respectively, in contrast to a CSF/venous plasma concentration ratio of only 0.02 for ddI administered as the parent compound. Apparent brain parenchyma/plasma concentration ratios were 0.67, 0.40, and 0.48 for 6-Cl-ddP, 6-Br-ddP, and 6-I-ddP, respectively, in comparison with a corresponding ratio of only 0.02 for ddI administered as the parent drug.

Concentrations of ddI in plasma (venous samples), CSF, and brain parenchyma after 30-min infusions of the 6-halo-ddPs were compared with those obtained after a ddI infusion

FIG. 3. 6-Halo-ddP concentrations in venous plasma, brain parenchyma (Brain-P; corrected for vascular space), and CSF after 30-min infusions at a rate of 125 mg (ddI equivalents)/kg/h. Also shown for comparison are the concentrations of ddI in each of these tissues after a 30-min infusion of ddI at the same rate.

FIG. 4. Concentrations of ddI in venous plasma, brain parenchyma (Brain-P; corrected for vascular space), and CSF after 30-min i.v. infusions of ddI, 6-Cl-ddP, 6-Br-ddP, or 6-I-ddP in rats at 125 mg/kg/h (ddI equivalents). Inset, magnification of the concentrations in brain parenchyma and CSF.

at the same rate (Fig. 4). A dramatic shift in the distribution of ddI toward the CNS occurred following a 30-min infusion of 6-Cl-ddP or 6-Br-ddP, whereas 6-I-ddP infusions yielded lower concentrations of ddI in all tissues. In CSF, ddI concentrations were significantly higher after infusions of 6-Cl-ddP (4-fold higher; $P < 0.025$) and 6-Br-ddP (1.6-fold higher; $P < 0.025$) but were 3.8-fold lower after infusion of 6-I-ddP ($P < 0.001$) compared with the ddI concentration in controls. Brain tissue ddI concentrations (uncorrected for vascular space contributions) were also significantly increased after infusion of 6-Cl-ddP (fivefold higher; $P < 0.001$) and 6-Br-ddP (twofold higher; $P < 0.05$) but were reduced after infusion of 6-I-ddP (fourfold lower; $P < 0.025$). Correcting for the vascular space contribution to the apparent concentrations in brain tissue, which was estimated to be 2% in this study, allows estimation of the actual concentration of drug in brain parenchyma. Concentrations of ddI in brain parenchyma were increased 10- and 4-fold after infusions of 6-Cl-ddP and 6-Br-ddP, respectively, relative to those after an infusion of ddI. Concentrations of ddI in brain parenchyma were 2.4-fold lower after an infusion of 6-I-ddP relative to those after an infusion of ddI alone.

Plasma pharmacokinetics and bioconversion of 6-Cl-ddP: i.v. bolus studies. 6-Cl-ddP emerged from the single-point 30-min infusion studies as the most promising prodrug in the series from the standpoint of delivery of ddI to the CNS. Therefore, more extensive studies of its pharmacokinetics and CNS uptake kinetics were undertaken. The time course for the disappearance of 6-Cl-ddP and for the appearance of ddI in venous plasma after i.v. bolus injections (80 mg of ddI equivalents per kg) were monitored. These data were compared with the venous plasma concentration-versus-time profiles for ddI administered i.v. at the same dose to obtain estimates of the rate constant for systemic conversion of 6-Cl-ddP to ddI and the systemic bioavailability of 6-Cl-ddP, as measured by relative areas under the ddI concentration-versus-time curves.

Figure 5 shows a semilogarithmic plot of the ddI concentration-versus-time profile after an administration of an i.v. bolus of ddI (80 mg/kg) (Fig. 5a), a semilogarithmic plot of the 6-Cl-ddP concentration versus time after administration of prodrug at 80 mg/kg (ddI equivalents) (Fig. 5b), and a comparison of the ddI concentration-time curve resulting from bioconversion of 6-Cl-ddP in the systemic circulation to that from direct injection of ddI (Fig. 5c). These pharmaco-

FIG. 5. Plasma pharmacokinetic data from i.v. bolus (80 mg of ddI equivalents per kg) injections of ddI or 6-Cl-ddP. (a) Semilogarithmic plot of ddI concentration versus time after administration of ddI; (b) semilogarithmic plot of 6-Cl-ddP concentration versus time after administration of 6-Cl-ddP; (c) ddI concentration-versus-time profiles resulting from an injection of 6-Cl-ddP followed by systemic bioconversion (●) or from a direct injection of ddI (■).

kinetic data were fitted to mathematical equations, assuming the multicompartmental model shown in Fig. 2, to generate the parameters listed in Table 3. The apparent fraction of 6-Cl-ddP converted to ddI obtained from $AUC_{0-\infty}$ ratios was

TABLE 3. Pharmacokinetic parameters describing the ddI or 6-Cl-ddP plasma concentration-time curves after i.v. bolus injections of ddI or 6-Cl-ddP^a

Compound	V_c (ml/kg)	k_{12} (min ⁻¹)	k_{21} (min ⁻¹)	k_{13} (min ⁻¹)	k_{31} (min ⁻¹)	$t_{1/2\alpha}$ (min)
ddI	367 ± 20	0.0336 ± ND	0.0447 ± 0.0015	0.193 ± ND	0.379 ± 0.024	1.03 ± 0.096
6-Cl-ddP	1,050 ± 121	0.017 ± 0.0018	0.010 ± 0.002	NA	NA	5.33 ± 0.33

^a Injections contained 80 mg/kg (ddI equivalents). V_c , apparent volume of distribution in the central compartment; k_{12} , k_{21} , k_{13} , and k_{31} , see Fig. 2; $t_{1/2\alpha}$, half-life at the "shallow" distribution phase; $t_{1/2\beta}$, half-life at "deep" distribution phase; $t_{1/2\gamma}$, half-life at elimination phase; k_{el} , elimination rate constant; k_{hyd} , rate constant for prodrug hydrolysis; AUC_{6-Cl-ddP} and AUC_{ddI}, areas under the concentration-time curves for 6-Cl-ddP and ddI, respectively; CL_{plasma}, clearance from plasma; ND, not determined; NA, not applicable.

0.48, which was similar to the value of 0.53 obtained from the ratio of k_{hyd} to the sum of the rate constants for elimination ($k_{hyd} + k_{el}^{PD}$) of 6-Cl-ddP. The half-life for conversion of prodrug to ddI in the systemic circulation was ~12 min.

Plasma pharmacokinetics, CNS uptake kinetics, and bioconversion of 6-Cl-ddP during and after 60-min infusions. In the 30-min infusion studies and bolus pharmacokinetic studies described above, venous plasma samples were analyzed. A comparison of ddI concentrations in venous plasma and arterial plasma samples after a 30-min infusion at the same rate revealed a significantly lower apparent steady-state concentration of ddI in the venous samples (29 versus 50 µg/ml). This difference was confirmed in subsequent experiments in three rats in which both venous and arterial blood samples were withdrawn from each animal at the 30-min time point during an infusion of ddI. Concentrations in venous plasma (mean, 26.7 µg/ml) were significantly lower than concentrations in arterial plasma (mean, 45.0 µg/ml) ($P < 0.05$; paired t test).

Since the driving force for drug entry into the CNS during a jugular vein infusion is the arterial concentration of unbound drug, for studies of the CNS uptake kinetics of 6-Cl-ddP we used arterial sampling. Data from control ddI infusions conducted in parallel with the 6-Cl-ddP experiments were not significantly different from results published previously (16) (data not shown). Plasma ddI and 6-Cl-ddP concentration-time profiles obtained during and after 60-min i.v. infusions of 6-Cl-ddP are shown in Fig. 6. The solid lines in Fig. 6 reflect the best fits of these data according to the compartmental models described in Fig. 2. These model-calculated plasma concentration-time profiles were used in equations 2 and 3 to deconvolute the CSF concentration-versus-time profiles.

The concentrations of 6-Cl-ddP and ddI in CSF versus time during and after i.v. infusions of 6-Cl-ddP are shown in Fig. 7. Also shown in Fig. 7 are the ddI concentrations in CSF during and after i.v. infusions of ddI at the same rate. At steady state (60 min), the concentration of ddI in CSF was 4.5-fold higher when 6-Cl-ddP was administered. Nonlinear regression analysis of the CSF concentration-versus-time profiles in Fig. 7 according to the integrated forms of equations 1 to 3 yielded apparent rate constants for entry into and efflux from the CSF and the apparent rate constant for hydrolysis of 6-Cl-ddP in CSF. These rate constants are listed in Table 4.

Concentrations of ddI in brain tissue versus time were determined during and after a 60-min i.v. infusion of ddI and were compared with the prodrug and ddI concentrations obtained during i.v. infusions of 6-Cl-ddP. The uptake kinetics of ddI into brain tissue during and after 2-h ddI infusions have been published previously (4). The results of the present study, listed in Table 4, are similar, with the excep-

tion that the calculated vascular space contribution (V_s) in the present study was nearly twofold lower because of the more thorough rinsing of residual blood from the surface of extracted brain tissue. Prodrug and ddI concentrations in brain tissue were obtained at only two time points during the 6-Cl-ddP infusions. Prodrug concentrations in brain tissue were 15.3 ± 2.7 and 13.5 ± 4.3 µg/ml at 30 and 60 min, respectively, while ddI concentrations were 5.5 ± 0.7 and 7.6 ± 1.4 µg/ml, respectively, at these time points. With such limited data, estimates of the blood-brain barrier transport of 6-Cl-ddP, k_{in}^{PD} and k_{out}^{PD} , could not be individually estimated. The rate constant for hydrolysis of 6-Cl-ddP in brain tissue could be reliably determined, however. The apparent half-lives for the in vivo bioconversion of 6-Cl-ddP were similar in brain tissue and CSF (28 versus 16 min). Steady-state prodrug concentrations were not significantly different in CSF and brain tissue, whereas the ddI concentrations in brain tissue were nearly twofold higher than those in CSF at steady-state because of an apparent slower rate of efflux of ddI from brain tissue.

DISCUSSION

Several laboratories have attempted to increase the penetration of 2',3'-dideoxynucleosides into brain tissue. A series of lipophilic 5'-esters of ddI failed to enhance the delivery of ddI into the CNS of rats primarily because esterase activity in plasma far exceeds that in brain tissue (2, 3, 41). Several groups have prepared the dihydropyridine derivative of AZT on the basis of the dihydropyridine-pyridine redox

FIG. 6. Plasma 6-Cl-ddP (□) and ddI (■) concentration-versus-time profiles obtained during and after 60-min infusions of 6-Cl-ddP in rats at 125 mg (ddI equivalents) per kg per h. Solid lines reflect model-calculated concentrations in plasma.

TABLE 3—Continued

$t_{1/2\beta}$ (min)	$t_{1/2\gamma}$ (min)	k_{el} (min^{-1})	k_{hyd} (min^{-1})	$AUC_{6\text{-Cl-ddP}}$ ($\mu\text{g} \cdot \text{min}/\text{ml}$)	AUC_{ddI} ($\mu\text{g} \cdot \text{min}/\text{ml}$)	CL_{plasma} ($\text{ml}/\text{min}/\text{kg}$)
5.2 ± 0.11 81.5 ± 16.3	19.4 ± 0.52 NA	$0.189 \pm \text{ND}$ 0.0515 ± 0.007	NA 0.0595 ± 0.0067	NA 741	1.154 550	69.3 108

system initially developed by Bodor et al. (5), but the degrees of enhancement of delivery of AZT to CNS that have been reported are highly variable from laboratory to laboratory (7, 10, 20, 21).

Recent findings that a series of lipophilic 6-halo-ddPs exhibit activity against HIV in vitro (11, 18, 35, 36) and that their activities were completely abrogated in the presence of an ADA inhibitor (23, 35, 36) coupled with preliminary indications of enhanced CNS delivery via this approach in rats (2), suggest a promising new enzyme-based strategy for improving the delivery of dideoxynucleosides to the CNS. The activity of ADA in human brain tissue is relatively high (37), further supporting the potential of this approach for the treatment of patients with AIDS dementia complex.

Factors influencing the CNS targeting efficiency of 6-halo-ddPs. All four halogenated congeners exhibit substantially higher octanol/water partition coefficients than does ddI (35). Lipophilicity corresponds to the size of the halogen atom, with 6-F-ddP being ~18-fold, 6-Cl-ddP being 30-fold, 6-Br-ddP being 40-fold, and 6-I-ddP being ~60-fold more lipophilic than ddI. On the basis of their increased lipophilicities, the 6-halo-ddPs would be expected to enter the CNS much more readily than ddI. Consistent with this expectation, Fig. 3 shows that, unlike ddI, the 6-halo-ddP concentrations in CSF and brain tissue approached the concentrations in plasma (within a factor of 2). CSF/plasma and brain tissue/plasma ratios of the 6-halo-ddPs at steady state were >10-fold higher than the corresponding ratios obtained for ddI when ddI was administered as the parent drug.

Increased lipophilicity alone does not ensure that a given prodrug will deliver higher levels of a parent compound to

the CNS, however. Both the selectivity and the absolute rate of bioconversion at the site of action are also important factors. In rats, the enhanced delivery of ddI to CNS achieved via ADA-activated prodrugs is a consequence of the relatively high activity of ADA in brain tissue, as indicated in Table 1. In vitro hydrolysis experiments further indicated that the ADA activity toward these substrates in brain tissue exceeds that in blood by approximately 1 order of magnitude, suggesting the possibility for brain-selective bioconversion to ddI. The reactivities of the 6-halo-ddPs toward ADA are highly sensitive to the steric bulk of the halogen atom, with the estimated half-lives in brain tissue homogenate ranging from <0.5 min (6-F-ddP) to ~3 h (6-I-ddP). Simulations of the CNS delivery of ddI from the 6-halo-ddPs recently conducted by us (2) led to the conclusion that an optimum enhancement in the CNS delivery of ddI would occur as the bioconversion half-life in the target tissue is varied, with the optimum half-life lying between the experimentally observed values for 6-F-ddP and 6-Cl-ddP. Thus, simulations based on in vitro measurements predicted the following rank order in targeting of ddI to the CNS: 6-Cl-ddP $\geq$ 6-Br-ddP $\gg$ 6-I-ddP. (6-F-ddP was not included in the simulations because of its toxicity).

As predicted in the simulations and as demonstrated in Fig. 4, 6-Cl-ddP yielded dramatic increases in the steady-state concentration of ddI in both CSF and brain tissue, while 6-I-ddP resulted in very low levels of ddI in the CNS because of its resistance to ADA. Further in vivo pharmacokinetic studies of 6-Cl-ddP were conducted, in part, to determine the degree to which the hydrolysis parameters measured in vitro and the blood-brain barrier permeability increases predicted from the increased octanol/water partition coefficients (32) agreed with the apparent hydrolysis and uptake parameters measured in vivo.

Comparison of kinetic parameters measured in vivo with in vitro estimates. (i) **Prodrug bioconversion in the systemic circulation.** From the relative areas under the ddI plasma concentration-versus-time curves after an i.v. bolus injection of either 6-Cl-ddP or ddI (Fig. 5c), the fraction of 6-Cl-ddP converted to ddI in the systemic circulation was 0.48. A similar estimate (0.53) was obtained from the ratio of the rate constant for hydrolysis, k_{hyd} , to the sum of the rate constants for elimination of 6-Cl-ddP from plasma given in Table 3. In vitro hydrolysis studies in rat blood (Table 2) also indicated that the 6-halo-ddPs undergo decomposition by multiple routes in blood, with the fraction of 6-Cl-ddP hydrolyzing to ddI being only 0.28. The bioavailability of ddI from 6-Cl-ddP in plasma was somewhat higher than that indicated by the blood bioconversion studies, suggesting that the ADA activity in tissues into which prodrug may rapidly equilibrate from circulating blood contributes significantly to the rate of prodrug bioconversion in the systemic circulation. In agreement with this hypothesis, the k_{hyd} determined from curve fitting of the in vivo i.v. bolus data in Fig. 4 (Table 3) was 0.06 min^{-1} , which was ~20-fold larger than the first-order rate constant obtained in blood in vitro ($2.6 \times$

FIG. 7. Concentrations of 6-Cl-ddP ($\square$) and ddI ($\blacksquare$) in CSF versus time during and after 60-min i.v. infusions of 6-Cl-ddP in comparison with the concentrations of ddI ($\blacktriangle$) obtained in CSF during and after 60-min i.v. infusions of ddI in rats at 125 mg/kg/h (ddI equivalents). Solid lines reflect the concentration profiles generated from the parameters listed in Table 4.

TABLE 4. Kinetic parameters obtained from nonlinear regression analyses of 6-Cl-ddP and ddI concentrations in CSF and brain tissue versus time and after 1-h infusions of ddI in rats^a

Fluid or tissue	k_{in}^{ddI} (min ⁻¹)	k_{out}^{ddI} (min ⁻¹)	k_{in}^{PD} (min ⁻¹)	k_{out}^{PD} (min ⁻¹)	k_{hyd} (min ⁻¹)	V_s (%) ^b
CSF	0.002 ± ND ^c	0.125 ± ND	0.01 ± 0.023	0.177 ± 0.049	0.043 ± 0.090	0.0 ^d
Brain tissue	2.75 × 10 ⁻⁴ ± ND	0.038 ± ND	— ^e	—	0.025 ± 0.032	2.0

^a Infusions were at a rate of 125 mg/kg/h (ddI equivalents).^b V_s , vascular space contribution.^c ND, not determined.^d Concentrations in CSF contain no vascular component.^e —, insufficient data to obtain transport parameters.

10⁻³ min⁻¹). Computer fits of the 6-Cl-ddP i.v. infusion data (solid lines, Fig. 6) yielded an estimated k_{hyd} of 0.085 min⁻¹, which was similar to the result obtained from an i.v. bolus. These results imply that the highly favorable brain tissue/blood selectivity in ADA-catalyzed prodrug conversion seen in vitro was substantially diminished in vivo because of the much higher k_{hyd} in the systemic circulation than that estimated from blood hydrolysis data. Nevertheless, higher systemic bioconversion of prodrug had a minor impact on the efficiency of targeting of ddI to the brain because the steady-state concentration of prodrug in the circulation is determined to a large extent by other pathways for elimination of prodrug which do not involve ADA. Thus, a reduction of k_{hyd} to zero would have decreased the overall plasma elimination rate constant ($k_{el} + k_{hyd}$) by a factor of only 2, thereby doubling the steady-state concentration of 6-Cl-ddP in the circulation, which is the driving force for CNS delivery. Indeed, the steady-state concentration of 6-I-ddP in plasma, which is a poor substrate for ADA, was only twofold higher than that of 6-Cl-ddP (Fig. 3). These results indicate that the selectivity of bioconversion in the target tissue relative to that in the circulation is not as important as the ratio of target tissue bioconversion to the rate constant for overall elimination from the systemic circulation. This ratio, which was calculated to be 0.23, was still sufficiently large to result in enhanced CNS delivery of ddI from 6-Cl-ddP.

(ii) **Kinetics of uptake and bioconversion in CNS.** Estimates of the rate constants for both prodrug entry and efflux and the hydrolysis rate constants (Table 4) were obtained from an analysis of the 6-Cl-ddP and ddI concentration-versus-time data in CSF (Fig. 7) and in brain tissue (data not shown). Whereas the apparent rate constant for efflux of 6-Cl-ddP from CSF was only slightly higher than that of ddI, the k_{in} for 6-Cl-ddP was ~50-fold higher than k_{in}^{ddI} . This magnitude of increase in uptake rate is similar to the increase expected from the 30-fold higher octanol/water partition coefficient of 6-Cl-ddP relative to that of ddI. Thus, k_{in} appears to be highly sensitive to dideoxynucleoside lipophilicity, while k_{out} is much less sensitive, in agreement with previous suggestions that the efflux of dideoxynucleosides such as ddI may be governed by active rather than passive processes (4, 14).

The half-life for bioconversion of 6-Cl-ddP in the CNS generated from in vivo brain tissue concentration-versus-time data (28 min) agreed within experimental error to the value (19 min) determined in vitro by using brain tissue homogenates. Thus, the kinetic model used to describe the in vivo CSF and brain tissue concentration-versus-time data appears to yield parameter estimates which coincide with the values obtained from in vitro measurements.

These comparisons illustrate the value of in vitro physicochemical property measurements in predicting the poten-

tial of a given prodrug to enhance the delivery of a parent drug to a specific target site. The results also show that the efficiency of targeting is a complex function of several factors, including biomembrane transport properties, target tissue bioconversion rate, and the rate constants for a variety of competing conversion and elimination processes. The rational design of drug carriers for targeting must consider all of these factors to be successful.

ACKNOWLEDGMENT

This work was supported by National Institutes of Health contract NO1 AI 82680 from the National Institute of Allergy and Infectious Diseases.

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