Determination of Carrier-Mediated Transport of 2′,3′-Dideoxypurine Nucleosides in the Rat Ileum Using a Bidirectional Perfusion Technique

R. Tyler DeGraw[1] and Bradley D. Anderson[1,2]

Received May 30, 2003; accepted November 3, 2003

Purpose. Previous attempts to ascertain the role of uptake and efflux transporters in the oral absorption of anti-HIV dideoxypurine nucleosides have been inconclusive. A novel *in situ* intestinal perfusion technique with complete mesenteric arterial/venous cannulation was used to examine the asymmetry of ileal dideoxynucleoside permeability under near *in vivo* conditions.

Methods. Intestinal perfusions were performed in the rat ileum, with cannulation of the artery and vein immediately entering and leaving the segment. Urea and mannitol were used as passive permeability markers, and the directional transport of 2′,3′-dideoxyinosine (ddI), 2′-β-fluoro-2′,3′-dideoxyinosine (F-ddI), and 2′-β-fluoro-2′,3′-dideoxyadenosine (F-ddA) were examined.

Results. Urea and mannitol exhibited symmetric permeability ($P_{LtoB} = P_{BtoL}$), whereas F-ddI and ddI showed significantly higher permeability in blood-to-lumen transport ($P_{BtoL} > P_{LtoB}$). P_{BtoL} for F-ddA exceeded P_{LtoB}, but the difference did not reach significance at $p < 0.05$. P_{BtoL} for ddI was demonstrated to be saturable with increasing ddI concentrations, but P_{LtoB} was independent of ddI concentration. P_{BtoL} for ddI was shown to be dependent on sodium concentration and inhibited by probenecid.

Conclusions. Symmetric transport was demonstrated for urea and mannitol as expected for these passive permeability markers. F-ddI and ddI were shown to be preferentially transported from blood to lumen. The basolateral to luminal transport of ddI is saturable, inhibited by probenecid, and sodium ion dependent. These results are consistent with carrier-mediated uptake on the basolateral membrane.

KEY WORDS: ddI, transporters, intestinal absorption, dideoxynucleosides.

INTRODUCTION

2′,3′-Dideoxyinosine (ddI) is a reverse transcriptase inhibitor used extensively in the treatment of HIV infection. Because anti-HIV medications must be taken daily for the life of the patient, the development of orally bioavailable products is paramount to their utility. Although the oral delivery of ddI has been extensively studied, its limited oral bioavailability remains an enigma. Early clinical studies focused on the acid lability of ddI, which has a half-life of <1 min at pH 1 (1). As anticipated, the administration of ddI in buffer rather than saline improved the oral bioavailability of ddI in pentagastrin-treated dogs (to induce acid secretion) from 8% to 30% (2). In humans, ddI exhibits incomplete and erratic bioavailability even when administered with antacids with a mean fraction available of 43% and a range of 16–54% (3). Once-daily doses exhibit significant reductions in bioavailability in comparison to the same amount given twice daily (27% vs. 41%), suggesting the involvement of a saturable process (4). First-pass elimination by the liver, presumably by purine nucleoside phosphorylase (PNP) enzyme, may account for a portion of the low and variable absorption of ddI, with an extraction percentage estimated to be 22–23% in rats (5,6), but other factors are also believed to be important. Bramer *et al.* (6) concluded that presystemic metabolism in the intestinal wall does not contribute significantly to the first-pass elimination of ddI but suggested that degradation of unabsorbed drug by the intestinal flora in the lower intestinal tract may be involved. Consistent with this possibility, Sinko *et al.* have shown that the intestinal permeability of ddI, a relatively hydrophilic molecule, is low, occurring primarily via paracellular transport (7), and is regionally dependent, decreasing significantly in the terminal ileum and proximal ascending colon (8,9). Although these studies suggest that slow absorption coupled with degradation in the lower intestine may play a role, a significant component contributing to the low and variable oral bioavailability of ddI and other dideoxynucleosides remains to be conclusively identified.

Studies of ddI uptake in rat intestinal brush border membrane vesicles ruled out a significant carrier-mediated absorption component (7), and intestinal perfusion studies performed previously in these laboratories with F-ddA (120–240 μg/ml), ddI, and F-ddI (0.2 to 1.0 mg/ml) failed to detect any effect of concentration on permeability from lumen to blood (10,11). However, given the presence of both equilibrative (ENT) and sodium-dependent concentrative (CNT) nucleoside transporters in the intestine (12) and recent evidence that ddI is a substrate for some isoforms of CNT2 (13) and for human and rat ENT1 and ENT2 (14), an involvement of these transporters in the intestinal absorption of ddI would be a reasonable expectation. Transporters are known to play an important role in the disposition of ddI in other tissues, particularly in the brain, where a probenecid-sensitive efflux transporter has been implicated in the low steady-state concentrations of ddI present in brain tissue (15,16). However, to the authors' knowledge, the influence of probenecid on intestinal absorption of ddI has not been explored.

Recent reports of a significant drug interaction between orally administered ganciclovir, which also possesses low oral bioavailability, and ddI in HIV-positive patients are likewise difficult to rationalize based on current understanding of the intestinal absorption of ddI. Both Cimoch *et al.* (17) and Jung *et al.* (18) noted substantial increases (>100%) in the area under the ddI concentration–time curve and percentage of ddI excreted in urine when high oral doses of ganciclovir were administered either before or 2 h after oral doses of ddI. There was no significant effect of ganciclovir on the renal clearance of ddI, leading to the suggestion that ganciclovir

[1] Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40506.

[2] To whom correspondence should be addressed. (e-mail: bande2@email.uky.edu)

ABBREVIATIONS: ADA, adenosine deaminase; BBB, blood–brain barrier; CNT, sodium dependent concentrative nucleoside transporter; ddI, 2′,3′-dideoxyinosine; EHNA, (+)-*erythro*-9-(2-hydroxy-3-nonyl)adenine; ENT, equilibrative nucleoside transporter; F-ddI, 2′-β-fluoro-2′,3′-dideoxyinosine; F-ddA, 2′-β-fluoro-2′,3′-dideoxyadenosine; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; PNP, purine nucleoside phosphorylase.

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alters either ddI's intestinal absorption or metabolism. Cimoch *et al.* suggested, however, that because ddI is catabolized by PNP while ganciclovir is eliminated primarily by the kidney, ganciclovir should not affect ddI metabolism (17).

This study reexamines the involvement of carrier-mediated processes in the intestinal transport of ddI, 2′-β-fluoro-2′,3′-dideoxyinosine (F-ddI), an isostere of ddI that is both acid stable and resistant to PNP, and 2′-β-fluoro-2′,3′-dideoxyadenosine (F-ddA), an adenosine-deaminase (ADA)-activated prodrug of F-ddI, in the presence of an ADA inhibitor, using a novel bidirectional *in situ* intestinal perfusion model. Salphati *et al.* (19) have recently shown that drug absorption values obtained from a single-pass intestinal perfusion in rats are superior in predicting human absorption than cell-based assays. Previous studies in this laboratory examined the rat ileal uptake of various dideoxynucleosides using the single-pass intestinal-perfusion technique with mesenteric venous cannulation in order to simultaneously monitor luminal drug disappearance and appearance in the mesenteric blood along with intestinal bioconversion (10,11,20). This method was modified in the present study to allow either luminal or vascular drug perfusions, thus enabling the assessment of possible asymmetry in drug transport related to membrane transporters in an intestinal segment with its circulatory system intact. The effects of probenecid and sodium were explored to further characterize the transport.

MATERIALS AND METHOD

Materials

F-ddA, F-ddI, and (+)-EHNA [*erythro*-9-(2-hydroxy-3-nonyl)adenine] were provided by the National Cancer Institute (Bethesda, MD). ddI and ddA were obtained from the National Institute of Allergy and Infectious Diseases. [^{14}C]Mannitol and [^{14}C]urea were purchased from Moravek (Brea, CA) and American Radiolabeled Chemicals (St. Louis, MO), respectively. Oxyglobin was purchased from Butler (Dublin, OH). Ganciclovir was obtained from Sigma (St. Louis, MO). All these reagents were used as is with no further purification. All other chemicals were of analytic reagent grade.

Animals

Male Sprague-Dawley rats (300–350 g) (Harlan, Indianapolis, IN) were housed and cared for by the Division of Laboratory Animal Resources, University of Kentucky, in accordance with the United States Department of Agriculture Animal Welfare Act and the National Institutes of Health "Principles of Laboratory Animal Care."

Perfusion Studies

Carbon-14-labeled urea and mannitol were used as passive markers to measure baseline permeability, monitor the stability of the absorption barrier, and to validate the assumption that, for passive permeants, permeability coefficients should be the same in both arterial (B→L) and luminal (L→B) perfusions. F-ddI is an isostere of ddI, stabilized by the fluorine on the sugar ring. F-ddI and ddI have nearly identical physical properties, but F-ddI is stable at low pH and is resistant to PNP, which is the primary enzymatic degradation route for ddI (11,21). Studies of the transport and percentage recovery of both compounds were undertaken to determine whether or not intestinal metabolism of ddI plays a role in its overall absorption. F-ddA, a more lipophilic prodrug of F-ddI, was also examined in this study. Adenosine deaminase (ADA) is present in the intestinal epithelium and metabolizes F-ddA to F-ddI during its absorption (10). The ADA inhibitor *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) was used at a high concentration (>40 μg/ml) to eliminate the ADA-catalyzed bioconversion of F-ddA during its intestinal transport.

Four types of intestinal transport studies were performed as described below.

Standard Single-Pass Intestinal Perfusion with Mesenteric Venous Cannulation (Luminal Drug Perfusions, L→B)

The procedure for single-pass intestinal perfusions with mesenteric venous cannulation has been described previously (11). F-ddA, F-ddI, and ddI standard perfusions were performed previously at concentrations of 120 to 240 μg/ml for F-ddA and 200 to 1000 μg/ml for F-ddI and ddI, and these values were used for comparison with the present results (10). Briefly, the animal was placed under halothane anesthesia, and the jugular vein was cannulated. Blood donor animals were infused with 1000 U of heparin, and blood was withdrawn through the jugular cannula. For perfusion, a midline abdominal incision was made, and the ileum was exposed using the ileocecal junction as a reference point. A 6- to 15-cm ileal segment was isolated and cannulated such that all the blood flowing from the segment collected into one vein. The vein was cannulated and connected to a collection tube set to drip directly into acetonitrile to quench blood enzymes. Blood lost from the mesenteric vein was replaced with donor blood from another animal infused into the jugular vein. Compounds were perfused into the intestinal lumen in perfusion buffer [8.0 g/L $NaH_2PO_4 \cdot H_2O$ and 11.3 g/L Na_2SO_4, pH 7.4 (adjusted with NaOH) and 290–310 mOsm (adjusted with NaCl)] at 1.0 ml/min for the first minute to fill the segment, followed by 0.2 ml/min thereafter. Radioactive urea and mannitol were perfused at concentrations of 0.4 and 0.25 μCi/ml, respectively. Before the start of ddI/probenecid perfusions, probenecid was preinfused into the jugular for 60 min at 0.2 mg/min, and donor blood infused into the jugular vein during the experiment was spiked with 1.5 mg/ml probenecid to give a steady-state concentration in mesenteric blood >300 μg/ml. The luminal perfusion buffer contained 400 μg/ml probenecid and 350 μg/ml ddI. The effect of ganciclovir (1300 μg/ml) on ddI absorption was explored at a ddI concentration of 580 μg/ml. Perfusate and blood samples were collected continuously at 5-min intervals in preweighed vials. Samples analyzed by HPLC were immediately quenched with acetonitrile and stored at –20°C until analysis by HPLC. Radioactive samples were prepared for liquid scintillation counting immediately following each experiment.

Single-Pass Intestinal Perfusion with Mesenteric Arterial/Venous Cannulation

A diagram of the intestinal perfusion experiment with cannulation of the mesenteric artery and vein is shown in Fig.

1. With this technique, the permeant could be infused into either the vasculature or the intestinal lumen to monitor transport from blood to lumen (B→L) or lumen to blood (L→B). The procedures described in the previous section were followed to prepare the luminal segment and mesenteric venous cannula, and the flow rate for blood exiting the mesenteric vein was noted. Once blood flow was established from the vein, the superior mesenteric artery was exposed 4–5 cm proximal to the venous cannula. The artery was tied off, punctured, and cannulated with polyethylene tubing (PE-50). The tubing was threaded into the artery such that Oxyglobin or donor blood could be infused directly into the cannulated intestinal segment. The surrounding vasculature was ligated to insure isolation. Vascular perfusate (Oxyglobin or donor blood with or without permeant, maintained at 37°C throughout the experiment) was then perfused into the artery to achieve the exit flow rate noted previously. Luminal perfusion followed the procedure described for standard perfusions. Because PNP and ADA are also present in blood cells, Oxyglobin, a synthetic blood substitute approved by the FDA for the treatment of anemia in dogs, was used in most experiments as a vascular perfusion vehicle to eliminate possible metabolism in blood. Perfusate concentrations matched those used in standard perfusions, and samples were collected, stored, and analyzed as before.

Luminal Drug Perfusions (L→B)

Mesenteric vein/arterial cannulations with perfusion of drug into the lumen were used as a control to determine the effects of the technique on lumen-to-blood permeability. Perfusate concentrations were generally similar to those used in standard perfusions. The effect of ddI concentration was further examined over the concentration range of 200 to 2000 μg/ml.

Arterial Drug Perfusions (B→L)

The same surgical procedure was followed as above, but in this case the permeant was perfused arterially. Arterial perfusion solutions were prepared according to the requirements for each compound and were perfused into the mesenteric artery, with blank perfusion buffer perfused into the lumen. F-ddA, F-ddA/EHNA, F-ddI, and ddI were dissolved in a minimal amount of saline and added to the appropriate volume of Oxyglobin (or donor blood for some F-ddI experiments) to give the desired perfusate concentrations. F-ddA was perfused in the vasculature at concentrations similar to those used in standard perfusions (150–240 μg/ml). EHNA coperfusions used >80 μg/ml EHNA to completely eliminate metabolism by intestinal ADA. F-ddI was perfused in the vasculature at concentrations from 125 to 355 μg/ml, and ddI was perfused in the same range of concentrations used in previous perfusions (200 to 1000 μg/ml). Radioactive urea and mannitol were transferred to a serum vial, and the carrier solvent was evaporated under nitrogen. Oxyglobin was then added to give concentrations comparable to those used in standard perfusions. Probenecid coperfusions with ddI were also performed, with ~250 μg/ml ddI in the vasculature and from 35 to 1000 μg/ml probenecid in both luminal and vascular perfusate. The effects of sodium on ddI transport were examined by perfusing ddI arterially with a low-sodium buffer perfused into the lumen. The low-sodium buffer was prepared with potassium buffer salts to reduce the total sodium content from ~220 mM to <5 mM. Samples were collected, stored, and analyzed as before.

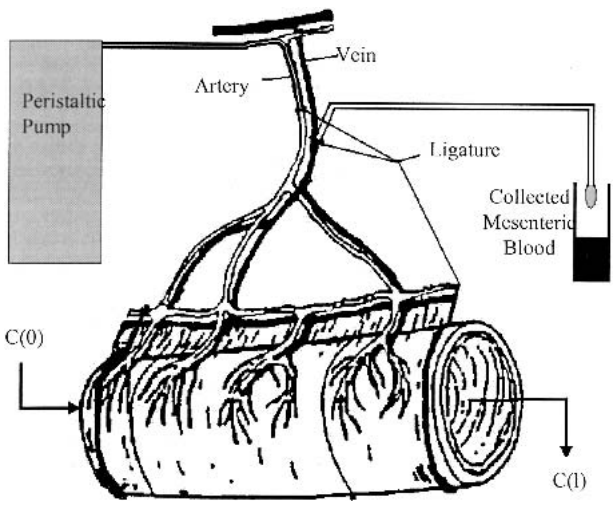

Fig. 1. Diagram of intestinal perfusion with cannulation of the mesenteric vein and artery.

Systemic Drug Infusions with Luminal Sampling (B→L)

These experiments were performed to verify results from the mesenteric arterial perfusions. The jugular vein was cannulated and attached to a syringe pump (Harvard Apparatus, Holliston MA). Radioactive urea and mannitol (2 μCi/ml and 0.5 μCi/ml, respectively), and ddI (20 mg/ml) were dissolved in saline and infused via the jugular cannula at 0.0206 ml/min for 3 h, with luminal sampling during the last hour of infusion. The abdominal aorta was cannulated before infusion, and arterial blood was collected in 100-μl aliquots over the course of the experiment, quenched with acetonitrile, and stored and analyzed with perfusate samples. After the 2-h preinfusion a 10-cm segment of the intestinal ileum was exposed and cannulated, following the procedure used in standard perfusions, with care to insure the mesenteric vein and artery were not disturbed. Blank perfusate buffer, perfused through the lumen at 0.2 ml/min, was collected in preweighed vials, weighed, quenched with acetonitrile, and stored at –20°C until analysis by HPLC or liquid scintillation counting. Probenecid was also added to some ddI infusion solutions to monitor the effects of this common inhibitor of organic anion transporters. The probenecid infusion rate was chosen to give a blood probenecid concentration during the luminal sampling period of >300 μg/ml (22,23). The luminal perfusate buffer also contained 400 μg/ml of probenecid in these experiments in an attempt to insure maximum transport inhibition, based on the inhibition of ddI cerebrospinal fluid efflux (15,16). Although this technique is useful in validating the mesenteric arterial/venous perfusion method, infusion to steady state requires a substantial amount of compound, and thus could not be used for regular drug screening.

Sample Preparation and Analysis

Samples to be analyzed by HPLC were thawed, vortexed, and allowed to settle. One milliliter of supernatant was re-

moved, dried, and reconstituted just before injection on the HPLC with phosphate buffer.

F-ddA, F-ddI, ddI, and EHNA were analyzed as described previously (10). Probenecid was separated on a Supelcosil ABZ+PLUS column (5 μm, 4.6 mm i.d. × 25 cm) with a mobile phase consisting of 20% acetonitrile and 80% phosphate buffer (ionic strength 0.02, pH 6.0) and quantified by UV detection at 254 nm. Under these conditions, probenecid had a retention volume of 9 ml.

Radioactive samples were prepared using the procedure published by Packard Biosciences (Groningen, Netherlands) for Solvable Tissue Solubilizer and Formula 989 scintillation cocktail. Samples were analyzed on a Beckman LS 6500 Multi-Purpose Scintillation Counter (Fullerton, CA).

Data Analyses

Permeability coefficients were determined using the following equations (11,24).

$$P_{\text{Appearance}} = \frac{(\Delta M/\Delta t)}{2\,\pi r l\,\langle C\rangle} \tag{1}$$

$$\langle C\rangle = \frac{C\,(0) - C\,(l)}{\ln\left(\dfrac{C\,(0)}{C\,(l)}\right)} \tag{2}$$

where $\Delta M/\Delta t$ is the steady-state flux of drug across the membrane (i.e., appearance in blood during luminal perfusions or appearance in the lumen during arterial perfusions), r is the intestinal radius (0.2 cm in the rat), l is the length of the intestinal segment, $<C>$ is log mean drug concentration perfused in the segment, given in equation 2, $C(0)$ is the initial perfusate concentration entering the segment, and $C(l)$ is the concentration of perfusate at the end of the segment (concentration at length l). The ratio of $C(l)/C(0)$ represents the fraction of drug remaining in the lumen after an absorption length l. The surface area of absorption is approximated in the denominator by the surface area of a right cylinder ($2\pi rl$). Using these equations to calculate permeability coefficients from both luminal and arterial perfusions assumes that the effective membrane surface area is the same in both directions.

RESULTS

The permeability coefficients generated in these experiments are listed in Table I. For all perfusions, the receiver concentration was <10% of the donor concentration, insuring that sink conditions existed throughout the experiment. Donor concentrations in both blood and lumen did not drop below 80% of the initial concentration in both luminal and arterial perfusions. Urea and mannitol permeability coefficients were shown to be independent of the perfusion method used ($p > 0.05$). F-ddA blood-to-lumen permeability showed a two-fold increase over lumen-to-blood permeability, but the increase was not statistically significant.

Total permeant recoveries are also listed in Table I. Recoveries were determined from the average flux of drug exiting the segment in both blood and lumen divided by the average flux into the segment. Values listed in Table I are average ± standard deviation; 95% confidence intervals were also calculated to determine if recovery was less than 100%. As indicated by footnote *k* in the table, all urea values, mannitol blood-to-lumen vascular perfusion, ddI standard perfusion, and ddI and F-ddA lumen-to-blood vascular perfusions had confidence intervals indicating less than 100% recovery. Comparison of the overall percentage recovery data for ddI and F-ddI revealed no significant differences, suggesting that PNP metabolism of ddI during absorption was negligible in these experiments.

F-ddA and ddI exhibited no degradation in Oxyglobin after incubation at 37°C for 2 h. F-ddI showed no degradation in Oxyglobin and whole rat blood after incubation at 37°C for 5 h.

Figures 2 and 3 display normalized average data from perfusions of ddI in the lumen and F-ddI in the vasculature, respectively. During perfusions, appearance of the permeant (mass/time) on the receiver side of the membrane was measured in 5-min intervals. These values were normalized to the absorption surface area and donor concentration for each individual experiment using equation 1 to obtain permeability coefficients. These permeability coefficients for each time point were then averaged to generate the data shown in Figs. 2 and 3. The lumen-to-blood permeability coefficients for F-ddI and ddI appeared to increase modestly in control experiments with the addition of vascular (arterial) cannulation, and the difference for ddI was statistically significant. The reason for this increase is currently unknown. An apparent increase was also observed for F-ddI and the passive permeability marker mannitol, but these were not statistically significant, and the permeability coefficients for urea and F-ddA (with complete ADA inhibition) were not affected by the arterial cannulation. All F-ddI perfusions were performed with donor rat blood in the vasculature, whereas ddI perfusions were performed with either Oxyglobin or donor blood. No difference was seen in ddI permeability coefficients whether the vasculature contained blood or Oxyglobin. The lumen-to-blood permeability coefficient for ddI increased from $(1.4 \pm 0.4) \times 10^{-6}$ cm/s during standard perfusions to $(3.3 \pm 0.8) \times 10^{-6}$ cm/s ($n = 7, 5$ with Oxyglobin and 2 with donor rat blood in vasculature), with a similar increase for F-ddI. All comparisons of ddI and F-ddI directional transport used the higher permeability coefficients for lumen-to-blood transport.

At low concentrations (<330 μg/ml), F-ddI and ddI showed statistically significant asymmetric permeability, with increased blood-to-lumen transport. F-ddI and ddI permeability coefficients increased by over four- and over eight-fold, respectively, compared to lumen-to-blood permeability. The asymmetry of ddI transport was further verified with systemic infusions. Although the lumen-to-blood permeability coefficient for ddI did not vary with ddI concentration, the blood-to-lumen permeability coefficient for ddI was saturable, approaching the value obtained in the lumen-to-blood direction as the vascular ddI concentration was increased to 1000 μg/ml (Fig. 4). The solid lines in Fig. 4 represent simultaneous fits of the curves with an assumed bidirectional passive permeability coefficient of 2.8×10^{-6} cm/s and a saturable component operating in the blood-to-lumen direction with $K_m << [ddI]$.

Probenecid had no effect on ddI lumen-to-blood absorption during standard perfusions but led to an over six-fold decrease in ddI blood-to-lumen permeability coefficient when included in vascular perfusions. This large decrease did not vary with probenecid concentration over the probenecid concentration range used in these experiments (35–1000 μg/ml). Perfusion of ddI in the vasculature with a low-sodium buffer

Table I. Permeability Coefficients for Several Compounds from Each of the Four Intestinal Transport Experiments

		Urea		Mannitol		ddI		ddI with Probenecid		F-ddI		F-ddA (with high EHNA)	
Experiment	Direction	P_{app}	% Recov	P_{app}	% Recov	P_{app}	% Recov	P_{app}	% Recov	P_{app}	% Recov	P_{app}	% Recov
Standard perfusion: drug in lumen	L → B	22 ± 5[b]	97.3 ± 1.6[k]	1.6 ± 0.6	96.4 ± 1.5	1.4 ± 0.4[e]	98 ± 1.0[k]	1.7 ± 0.5	101.7 ± 2.4	1.1 ± 0.4[b,d]	97.2 ± 3.4	8 ± 1[a,d]	96.7 ± 3.4
Vascular perfusion: drug in lumen	L → B	16 ± 4	94.3 ± 1.4[k]	2.8 ± 1.2	92.7 ± 3.2	3.3 ± 0.8[c,f,h,i]	92.1 ± 5.5[k]			3.3 ± 1.1	94.4 ± 2.5	8 ± 3	91.1 ± 2.1[k]
Vascular perfusion: drug in oxyglobin or blood	B → L	22 ± 6[a]	90.3 ± 5.8[k]	1.3 ± 0.2	96.7 ± 0.4[k]	27 ± 2[f,g]	101 ± 5	4.3 ± 0.7[e,l]	90.0 ± 5.7[k]	14.7 ± 1.4[b,f,g]	91.8 ± 5.3	15 ± 4	93.4 ± 8.9
Systemic infusion: drug in blood	B → L	20 ± 4[b]		2 ± 1		19 ± 5[f,g]		7 ± 2[j]					

Values are mean ± S.D.; n = 3 unless otherwise noted; P_{app} units are cm/s × 10^{-6}. Values shown for vascular ddI and F-ddI B → L perfusions were at donor concentrations <330 μg/ml. Systemic infusions with or without probenecid yielded mean steady-state ddI blood concentrations of 67 and 28 μg/ml, respectively.

[a] n = 2.

[b] n = 4.

[c] n = 7, 5 with Oxyglobin, 2 with donor blood.

[d] Reference 10.

[e] n = 5.

[f] Statistically different from standard perfusion value ($p < 0.05$).

[g] Statistically different from vascular perfusion in lumen value ($p < 0.05$).

[h] Statistically different from vascular perfusion in vasculature value ($p < 0.05$).

[i] Statistically different from systemic infusion value ($p < 0.05$).

[j] Statistically different from ddI standard perfusion and blood-to-lumen values without probenecid ($p < 0.05$).

[k] 95% confidence limit does not incude 100%.

[l] Perfusates contained 35–1000 μg/ml probenecid. There was no change in ddI permeability within this probenecid concentration range.

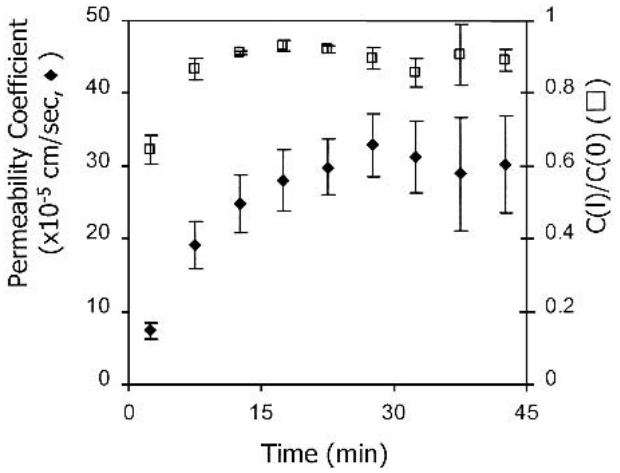

Fig. 2. Normalized average data from ddI lumen to blood perfusions with vascular cannulation. Appearance of ddI in the vasculature was normalized using equation 1 to calculate a permeability coefficient for each individual time point, and then time points were averaged over all experiments. Data shown are means ± SEM (n = 7: 5 with Oxyglobin and 2 with donor blood). ♦, Appearance of ddI in mesenteric blood, left axis. □, Ratio of ddI remaining in the lumen, right axis.

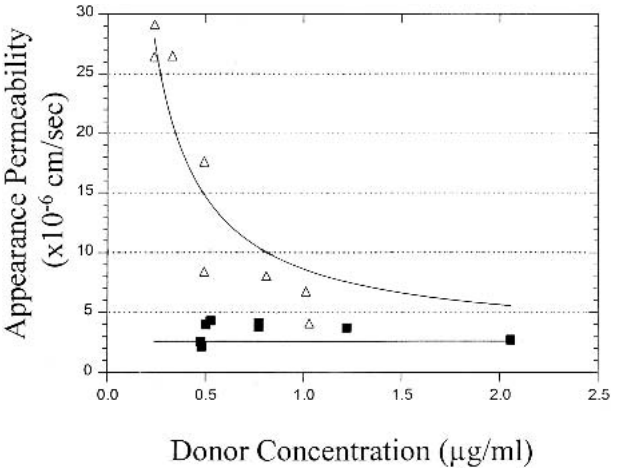

Fig. 4. The effect of increasing donor ddI concentration in either the lumen (■) or vasculature (♦) on ddI appearance permeability coefficient.

in the lumen also caused a twofold decrease in ddI blood-to-lumen permeability, with a permeability coefficient of (13 ± 3) $\times 10^{-6}$ cm/s (n = 2, mean ± SD). The addition of probenecid and the change in luminal sodium had no effect on net water flux based on fluid flow. Co-administration of a relatively high concentration of ganciclovir (>2 times the concentration of ddI used) had no effect on ddI lumen-to-blood permeability, yielding a value of (1.4 ± 0.3) $\times 10^{-6}$ cm/s (n = 2) in standard perfusions (without arterial cannulation).

DISCUSSION

The agreement of permeability coefficients for the paracellular transport markers urea and mannitol from each of the four types of intestinal transport experiments indicates that arterial cannulation and perfusion of Oxyglobin into the intestinal vasculature does not damage the intestinal passive permeability barrier. The agreement of F-ddA lumen-to-blood vascular cannulation permeability coefficients with standard perfusion values (with complete ADA inhibition) gives a strong indication that the transcellular absorption barrier also remains intact during the experiment. The symmetric transport of the passive permeability markers urea and mannitol also suggests that this technique can be used to explore possible asymmetric transport across the intestinal epithelium when transporters are present. Although *in vitro* methods are generally used to characterize the transport asymmetry resulting from the action of membrane transporters, intestinal perfusion with vascular cannulation allows one to directly monitor the entire absorption process and provides a simple way to determine the nature and extent of directional transport as well as metabolism in the intestine under near *in vivo* conditions.

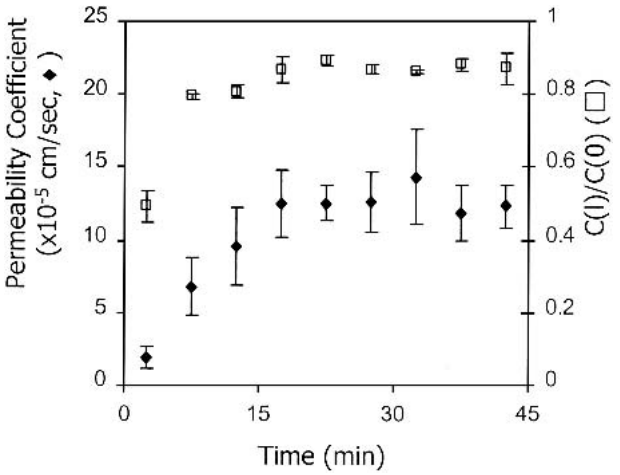

Fig. 3. Normalized average data from F-ddI blood-to-lumen perfusions with vascular cannulation with F-ddI perfused into the vasculature in donor blood. Appearance of F-ddI in the lumen was normalized using equation 1 to calculate a permeability coefficient for each individual time point, and then time points were averaged over all experiments. Data shown are means ± SEM (n = 4). ♦, Appearance of F-ddI in the intestinal lumen, left axis; □, ratio of F-ddI remaining in perfused blood, right axis.

Although carrier-mediated transport of ddI in the brain has been well documented (15,16), this is the first convincing evidence of asymmetric ddI transport in the intestine. Sinko *et al.* examined ddI transport in both rat and rabbit brush border membrane vesicles and saw no concentration dependence from 1 μM to 50 mM, indicating that, at least in the intestinal brush border, there is no evidence for active transport of ddI (7). Similarly, as shown in Fig. 4, large changes in luminal concentrations of ddI and inclusion of probenecid during standard perfusions had no effect on ddI absorption during standard perfusions. In addition, ganciclovir had no effect on the lumen-to-blood absorption of ddI, indicating that the increases reported in ddI bioavailability with ganciclovir coadministration are not caused by effects on L→B transport.

If the transporter or transporters producing the asymmetry in ddI intestinal absorption are primarily located on the basolateral membrane, the values obtained using the above methods would be relatively insensitive to concentration changes at the apical membrane. In normal intestinal perfusions, a luminal ddI concentration of 1.5 mM produces a mesenteric blood concentration of only 5 μM, so a transporter

located on the basolateral membrane would be exposed to a negligible change in ddI concentration over a very wide range of luminal concentrations. However, Fig. 4 also illustrates that increasing ddI concentrations in the vasculature (i.e., at the basolateral membrane) caused a concentration-dependent (i.e., saturable) decline in blood-to-lumen permeability coefficients with the values approaching those in the lumen-to-blood direction at ddI concentrations of 1000 μg/ml. Coadministration of probenecid caused a similar decrease in luminal appearance in blood-to-lumen perfusions, suggesting that the probenecid-sensitive carrier involved in ddI intestinal transport is on the basolateral membrane.

Several transporters in the intestinal tract may influence the absorption of dideoxynucleosides. Although probenecid-sensitive transport has been noted in the rat blood–brain barrier, the transporter or transporters involved have not been determined. The transporters involved in CNS penetration may also be different from those involved in intestinal transport. Members of both OAT and MRP transporter families are known to be sensitive to probenecid and to have some nucleoside-carrying properties (25,26). Many of these transporters are also specific to the basal membrane, and one or more of them may account for the ddI intestinal transport observations reported herein.

Organic anion transporters are most well characterized in the blood–brain barrier and in the kidney and liver, where they are extensively involved in drug clearance (25). Recently, OAT1 has been detected in human ileal tissue by semiquantitative PCR (27). The membrane localization of the OAT transporters depends on the tissue examined. OAT2 and OAT4 show no preference for the apical or basolateral membrane in the kidney or choroid plexus, whereas OAT1 and OAT3 are expressed preferentially on the basal membrane in kidney and the apical membrane in the choroid plexus (28).

Organic anion transporters have broad substrate specificity and have shown affinity for antiviral nucleoside analogues. OAT1 purified from rat kidney and expressed in *Xenopus laevis* oocytes showed reduced transport of *p*-aminohippurate when coincubated with 1 mM ddI, indicating that ddI is a substrate for rat OAT1 (29). Additionally, both OAT1 and OAT3 have shown affinity for other nucleoside analogues, such as zidovudine and acyclovir (29,30). Sugiyama *et al.* measured the sensitivity of these two transporters to probenecid and determined K_i values of 31 and 20 μM for OAT1 and OAT3, respectively (31). The >1 mM (>300 μg/ml) probenecid concentrations used in these experiments are almost certainly high enough to eliminate the effects of an OAT transporter in the intestine. OATs are very likely involved in the BBB efflux of ddI, and recent evidence for the presence of OAT1 in human ileal tissue leaves open the possibility of their involvement in ddI and F-ddI intestinal transport.

Although OATs are extensively involved in drug clearance, MRP transporters are primarily involved in cell function and protection and are therefore present in many tissues. Specifically, the RNA expression of MRPs 1, 2, 3, and 5 has been confirmed in the intestine, and MRPs 4 and 6 may be present as well (26). MRP transporters are expressed on both sides of the intestinal epithelium, with MRP2 preferentially expressed on the apical membrane and MRPs 1, 3, and 5 on the basolateral membrane (32). It does not appear that ddI has been examined as a substrate of the MRP transporters, but other nucleoside analogues, including zidovudine and 9-(2-phosphonylmethoxyethyl)-adenine, have been shown to be substrates (26).

The probenecid-sensitive component of ddI intestinal transport is most likely OAT1, OAT3, or one of the basal MRPs (MRPs 1, 3, and 5), but exactly which transporter or transporters is involved is difficult to establish. The high probenecid concentration used in these experiments should have been sufficient to eliminate activity from any of the above transporters, based on ddI CNS transport, (16) but probenecid did not reduce the blood-to-lumen permeability of ddI to symmetry. Thus, other transporters not susceptible to probenecid may also play a role. The presence of nucleoside transporters in the intestine is well established, making them likely candidates.

The nucleoside transporter family consists of the equilibrative sodium (Na^+)-independent transporters (ENT1 and ENT2) and the concentrative Na^+-dependent transporters (CNT1, CNT2, and CNT3). Nucleosides are essential to DNA production in new cell growth, and the nucleoside salvage system is extensive. Both equilibrative and concentrative Na^+-dependent nucleoside transporters have been found in the intestinal epithelium in many species, including humans, mice, and rats (33–35). In the intestinal epithelium, the equilibrative transporters are thought to be on the basolateral membrane, and the presence of Na^+-dependent transporters on the apical membrane was confirmed in brush border membrane vesicle studies (12,33,36).

Transporters on the basolateral membrane are the most likely source for the asymmetry in ddI transport, so the equilibrative nucleoside transporters may be involved. The ENT transporters act to facilitate diffusion of nucleosides into the cell as part of the nucleoside salvage process. With some exceptions, ENT1 is selective for pyrimidines whereas ENT2 is selective for purines. Thus, ddI is a much better substrate for ENT2 (12). Yao *et al.* showed a significant increase in ddI accumulation in *Xenopus* oocytes, expressing both human and rat ENT2 transporters with a ddI donor concentration of just 20 μM, but showed very little increase with expression of ENT1 (14). Both equilibrative and concentrative nucleoside transporters are sensitive to removal of hydroxyl groups from the nucleoside sugar ring. Particularly, the 3′-hydroxyl significantly reduces substrate affinity for all of these transporters (12,37).

The decrease in ddI blood-to-lumen transport with a decrease in luminal sodium indicates an effect on a sodium-dependent transporter. The sodium-dependent concentrative nucleoside transporter, CNT2, which is present in the rat intestine, has been shown to transport ddI at the concentrations used in these experiments (14,36,37). However, the CNT transporter, a component of the nucleoside salvage system, transports nucleosides across the apical membrane into the epithelial cells. During normal absorption, CNT2 would facilitate the transport of ddI from lumen to blood, so reducing luminal sodium would be expected to increase blood-to-lumen transport, not decrease it. The decrease in blood-to-lumen transport seen here with a decrease in sodium does not appear to be consistent with expectations for an interaction with CNT2.

The organic anion transporters OAT1 and OAT3 are indirectly coupled to the sodium gradient and may be respon-

sible for the sodium dependence (38,39). These transporters are organic anion/dicarboxylate exchangers, which couple with the sodium/dicarboxylate exchanger, leading to sodium dependence for carrier-mediated organic anion transport. The combination of probenecid and sodium data give further weight to the argument for the involvement of OAT1 and/or OAT3 in the intestinal permeability of ddI from the blood to lumen.

F-ddI exhibited a lower blood-to-lumen permeability compared to ddI, with a more than four-fold increase in relation to its lumen-to-blood transport. F-ddI and ddI have nearly identical physical properties and are most likely transported by the same carriers in the intestine. The lower blood-to-lumen permeability of F-ddI may result from a decreased binding affinity to the carrier with addition of fluorine to the sugar ring. Removal of the 2′-hydroxyl group from inosine resulted in a significant loss in affinity to the nucleoside salvage transporters (37), and the addition of fluorine at this site may cause an additional loss in affinity.

Although there was a slight increase in F-ddA blood-to-lumen transport compared to normal absorptive lumen-to-blood transport, the difference was not statistically significant, indicating that F-ddA intestinal transport is symmetric and probably not significantly influenced by transporters. This may be because of the higher contribution of passive (transcellular) permeation for F-ddA with its higher lipophilicity.

Although evidence has been presented in this paper to support the presence of a carrier-mediated process influencing the B→L transport of the dideoxynucleosides ddI and F-ddI, the impact of this transport process on oral delivery may be minimal, as suggested by the lack of effect of probenecid and ddI concentration on the L→B permeability of ddI. However, it is important to bear in mind that sink conditions were maintained on the receiver side in these perfusion experiments and under such conditions a basolateral uptake transporter would have no influence on permeability in the L→B direction. The situation may be quite different in patients whose blood concentrations cannot be assumed to be negligible. Further work will be needed to determine specifically which transporter(s) are involved and whether or not their effects are clinically relevant.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health (RO1 NS39178). Tyler DeGraw received additional support through an AFPE Pre-Doctoral Fellowship, a PhRMA Advanced Pre-Doctoral Fellowship, and a University of Kentucky Dissertation Year Fellowship.

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