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Site-specific oral absorption of didanosine: in situ characterization and correlation with extent of absorption in vivo

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Abstract

Didanosine is a purine nucleoside analogue approved for the treatment of human immunodeficiency virus (HIV) infection. The permeability of didanosine was characterized in three intestinal regions, the proximal jejunum, mid-small intestine and distal ileum, in rats using an in situ steady-state single pass intestinal perfusion (SPIP) technique. Intestinal perfusions were performed with didanosine (0.01 mM) at pH 6.5 using an iso-osmotic Mes buffer. Permeability results were calculated using a modified boundary layer analysis and the mean, dimensionless wall permeabilities (P_w^*) of didanosine were 0.26 ± 0.03, 0.17 ± 0.01 and 0.09 ± 0.01 (mean $\pm$ SE) in the proximal jejunal, mid-small intestinal and distal ileal regions, respectively. The net water flux was $-0.38\%/\text{cm}$ in all three intestinal locations, indicating water absorption. The regional differences in permeability were significant whereas the differences in water transport were not. Based on the water transport and permeability results, it appears that intestinal permeability differences were not due to solvent drag suggesting that differences in the absorptive surface area or paracellular pore size and density may account for the decrease in the lower small intestinal permeability of didanosine. The mean P_w^* was then used in a previously developed theoretical model that correlates intestinal permeability and scaling factors with extent of absorption results in vivo. The experimental data correlated well with the extent of didanosine absorption reported in dogs and humans after the administration of immediate- and delayed-release didanosine dosage forms. The results of the correlation further support the experimental results and suggest that the oral absorption of didanosine is permeability-limited and intestinal site-dependent with absorption decreasing down the small intestine.

Key words: Didanosine; Regional oral absorption; Site-specific oral absorption; Permeability; Human; Dog; Rat; Intestinal perfusion

1. Introduction

Didanosine (2′,3′-dideoxyinosine, ddI) is a purine nucleoside analogue approved for the treatment of human immunodeficiency virus (HIV) infection. Incomplete and erratic oral absorption of ddI, manifest as low to moderate and variable oral bioavailability, is observed in humans (Knupp et al., 1991). Bioavailability variability may result from a variety of factors such as variable gastric emptying and intestinal transit,

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solubility limitations, intestinal and hepatic metabolism, chemical instability, and intestinal permeability limitations. Erratic systemic availability results are meaningful in two ways. From identical oral doses of drugs with narrow therapeutic windows, a significant underdosage can occur in some patients, leading to therapeutic failure, while other patients may receive too much drug, resulting in therapy limiting toxicities. For example, since the bioavailability of ddI after a 10.2 mg/kg oral dose ($\approx$ 600 mg) in humans is 54 $\pm$ 25% (Knupp et al., 1991), then 179–481 mg of the dose is systemically available (based on area under the curve calculations). Although the therapeutic impact of the incomplete and variable oral absorption of ddI has not been assessed clinically, patients at the lower and upper absorption limits may experience therapeutic failure.

The mean oral bioavailability of didanosine in humans is 38–43% decreasing to 24% at doses greater than 15.2 mg/kg (Knupp et al., 1991). Similar results are seen in dogs with an average bioavailability of 48%, decreasing to 12% at the 500 mg/kg dose (Stolz et al., 1989; Sinko et al., unpublished data). It is unlikely that the incomplete oral absorption and dose dependency of didanosine are attributable to solubility limitations, since the solubility of ddI at pH 6.5 and 7.3 (25°C), corresponding to the pH of human and dog small intestine, is 27 and 30 mg/ml, respectively (Anderson et al., 1988). While ddI is unstable at acidic pH, with a half-life of 24 min at pH 1.9 (Anderson et al., 1988), it is also unlikely that stability is limiting since the maximal oral bioavailability was only 54% when ddI was dosed with buffers (Knupp et al., 1991). The potential for the intestinal and hepatic metabolism of didanosine was recently demonstrated in rats (Bramer et al., 1993); however, intestinal metabolism appeared to play a minor role and the degree of metabolism in humans is unknown.

When didanosine was administered to dogs and humans in two delayed-release, enteric-coated granule formulations to protect it from gastric acidity, the bioavailability of didanosine was reduced from 43% to 35 and 25% in humans (Hartman et al., 1991) and from 48% to 22 and 10% in dogs (Stolz et al., 1989), suggesting that its absorption is intestinal site-dependent in vivo. Recent reports from other investigators also suggest that nucleoside drug analogues may have regional oral absorption differences. For example, Bramer et al. (1993) reported that the time to peak didanosine plasma levels was greater after ileal infusion than after duodenal infusion in rats. These results suggest that the rate of didanosine absorption was reduced in the lower small intestine consistent with a decrease in distal small intestinal permeability and surface area. The regional differences in didanosine intestinal permeability, however, were not investigated in those studies. In this report, the regional dependence of didanosine intestinal permeability was investigated. Results from these investigations indicate that the low and regionally dependent intestinal permeability of didanosine may be a significant factor in the incomplete and variable oral absorption of didanosine.

2. Materials and methods

2.1. Materials

Ketaset and Rompun were obtained from A.J. Buck, Owings Mills, MD, ddI (didanosine) was supplied by Bristol-Myers Squibb Co. and the National Institute of Allergy and Infectious Diseases of the NIH. Hepes, Mes (2-[*N*-morpholino]ethanesulfonic acid) and PEG 3350 were obtained from Sigma Chemical Co., St. Louis, MO. [^{14}C]PEG 3350 was purchased from Du Pont NEN, Boston, MA. Sodium chloride, potassium chloride, HPLC-grade methanol, monobasic potassium phosphate and ScintiVerse BD were obtained from Fisher Scientific, Fair Lawn, NJ. All materials were used as received.

2.2. Acute single pass intestinal perfusion method

The acute single pass perfusion procedure (Johnson and Amidon, 1988) was used to characterize the site-specific oral absorption of didanosine in rats. Briefly, viral-free male Sprague-Dawley rats weighing 250–350 g were used. Following an overnight fast (12–18 h) anesthesia was induced by an i.m. injection of ketamine-xylazine

(60 mg/kg ketamine, 8 mg/kg xylazine). After the rats were immobilized, an s.c. injection (60 mg/kg ketamine, 8 mg/kg xylazine) was used to complete the induction of anesthesia. The rats were then placed on a warming pad (Vetko Thermal Barrier, Harvard Apparatus, South Natick, MA) under a surgical lamp to maintain body temperature. Upon verification of the loss of pain reflex, a midline abdominal incision of 3–4 cm was made, the intestinal segment was located, and a cannula was inserted. For the jejunal segment, the first cannula was inserted 2–4 cm from the ligament of Treitz and the second was placed 6–15 cm aboral to the first incision. The mid-small intestine was located (approx. 50 cm from the pylorus). A cannula was inserted 5–7 cm proximal and 5–7 cm distal to the mid-gut location. The terminal ileal segment was isolated by locating the caecum and inserting the cannula 2–4 cm proximal to the ileal – caecal junction. A second cannula was then inserted 10–15 cm proximal to the first. The exposed intestinal segments were covered to decrease moisture loss. The intestinal segment was perfused with didanosine (0.01 mM) in an iso-osmotic Mes buffer containing 15 mM Mes, 100 mM NaCl, 5 mM KCl, 0.01% (w/v) PEG 3350 and a tracer amount of its ^{14}C isotope (Du Pont NEN, MA). PEG 3350 was used as a nonabsorbable marker for the purpose of assessing net intestinal water transport. Typical perfusion flow rates varied from 0.1 to 0.3 ml/min. After about 30 min, when steady state was achieved, six outlet samples were collected at 15 min intervals. Outlet samples were diluted 1:1 with 15 mM Hepes buffer (pH 8) and immediately put on ice. The outlet didanosine concentrations were corrected for net water flux based on the changes in [^{14}C]PEG concentrations between the inlet and outlet cannulae. At the conclusion of the experiment, the intestinal segment was measured without stretching and the animals were killed with an overdose of pentobarbital administered intravenously.

2.3. Analytical methods

Didanosine concentrations were quantitated using an HPLC method. HPLC analyses were performed using a Waters solvent delivery module (Model 600E), a multiwavelength UV detector (Model 590E) and a WISP (Model 717) (Waters, Milford, MA). Separations were performed on a Supelcosil LC-18S column (25 cm × 4.6 mm) protected by a guard column Supelguard LC-18S (2 cm) (Supelco, Inc. Bellefonte, PA). The flow rate was 1.0 ml/min and the detection wavelength was 254 nm. The mobile phase consisted of 0.1 M potassium phosphate containing 20% (v/v) methanol. The mobile phase was filtered through 0.45 μm HPLC certified membrane filters (Gelman Sciences, Ann Arbor, MI). Standard curves were prepared for didanosine and were linear ($R^2 = 0.9996$) over the concentration range of 0.5–5 μg/ml. [^{14}C]PEG 3350 was measured using liquid scintillation counting (Beckman Instruments, LS 5000TD, Fullerton, CA) by adding 0.5 ml of the sample to a liquid cocktail ScintiVerse BD.

2.4. Data analysis

The perfusion data were analyzed using the modified boundary layer (MBL) analysis. A detailed presentation of the MBL analysis was presented elsewhere (Johnson and Amidon, 1988). Briefly, the experimentally measured permeability is known as the apparent or effective permeability (P_e) and is calculated from the following equation:

$$P_e^* = \frac{1 - (C_m/C_o)'}{4\ Gz} \quad (1)$$

where the asterisk (*) indicates a dimensionless quantity ($P^* = P \cdot R/D$, where R is the radius of the intestine and D denotes the aqueous diffusivity of didanosine), the prime (') represents a volume corrected quantity, C_o and C_m are the inlet and outlet didanosine concentrations, respectively and Gz is the Graetz number. The Graetz number is a dimensionless flow parameter that is defined as:

$$Gz = \frac{\pi DL}{2Q} \quad (2)$$

where L is the length of the perfused intestinal segment and Q denotes the volummetric flow

rate. From Eq. 1, P_e^* is clearly dependent upon the flow conditions of the experiment (i.e., hydrodynamics). Therefore, P_e^* is considered to be 'biased' by the experimental flow conditions. P_e^* can be defined in terms of two components: the aqueous permeability, P_{aq}^*, and the wall permeability, P_w^*,

$$\frac{1}{P_e^*} = \frac{1}{P_{aq}^*} + \frac{1}{P_w^*} \tag{3}$$

P_{aq}^* can be estimated from the MBL analysis where,

$$\left(P_{aq}^*\right)^{-1} = A\ \mathrm{Gz}^{1/3} \tag{4}$$

$0.004 \le \mathrm{Gz} \le 0.01 \quad A = 10.0\ \mathrm{Gz} + 1.01$
$0.01 \le \mathrm{Gz} \le 0.03 \quad A = 4.5\ \mathrm{Gz} + 1.065$
$0.03 \le \mathrm{Gz} \quad A = 2.5\ \mathrm{Gz} + 1.125$

Since P_e^* is experimentally measured and P_{aq}^* is estimated, P_w^* can be calculated by rearranging Eq. 3:

$$P_w^* = \frac{P_e^* P_{aq}^*}{P_{aq}^* - P_e^*} \tag{5}$$

Since P_w^* is considered 'unbiased', it can be used to compare perfusion results from experiment to experiment and laboratory to laboratory.

2.5. Statistical analysis

Results are expressed as mean ± SE (standard error of the mean). All statistical calculations were performed using BMDP Dynamic statistical software (BMDP Statistical Software, Inc., Los Angeles, CA). A p value of 0.05 was used as the significance level for all tests. One-way ANOVA analyses were performed to assess the significance of group differences. If significant differences were detected, Levene's F-test was performed to test for equality of variances. Based on the results of Levene's F-test, pooled or separate variance t-tests were performed to test for differences in group means.

3. Results and discussion

The intestinal permeability of didanosine was calculated using a modified boundary layer analysis (MBL; Johnson and Amidon, 1988). The MBL is a 'loss' method, i.e., permeability is calculated based on the loss of didanosine from the perfusing solution. In order to accurately calculate intestinal permeabilities, the loss of didanosine can only be due to absorption into intestinal tissue, otherwise, artificially high permeabilities will result. Some of the potential causes of drug loss are binding to intestinal mucin or tissue and chemical or enzymatic degradation. Although didanosine is known to be unstable in acidic solutions, it has been reported to be significantly more stable at pH 6.5, the pH of the intestinal perfusions (Anderson et al., 1988). In order to verify the stability of didanosine under typical perfusion conditions, two stability studies were performed. The first was conducted with didanosine (0.01 mM) in unperfused Mes buffer at pH 6.5 and 290 mOsm/kg at room temperature for 2 h, the length of the perfusion study. Didanosine stability was also determined at 37°C in Mes buffer that was previously perfused through the upper small intestine. The conditions of the two studies were typical of pre- and post-perfusion conditions, respectively. As seen in Fig. 1, less than 2% of didanosine was degraded in either study over the 2 h period, indicating adequate stability for this investigation. A second potential source for the loss of didanosine from the perfusing solution is through intestinal metabolism; however, in a recently published study (Bramer et al., 1993), it was reported that didanosine intestinal metabolism in rats accounted for 1% or less of the loss of didanosine in their experiments. Finally, since the binding of didanosine to intestinal mucin or tissue may decrease the concentration of didanosine in the perfusing solution, the binding of didanosine to intestinal tissue was investigated using an in vitro membrane vesicle technique. Brush-border intestinal membrane vesicles were prepared using the method of Kessler et al. (1978) with some minor modifications. The uptake of didanosine into membrane vesicles was investigated at four osmolarities; 310, 400, 620 and 1000 mOsm/kg. Drug-tissue binding is assessed by evaluating the uptake of drug at infinite osmolarity (Meier et al., 1984), i.e., when the vesicles are completely collapsed. As seen in Fig.

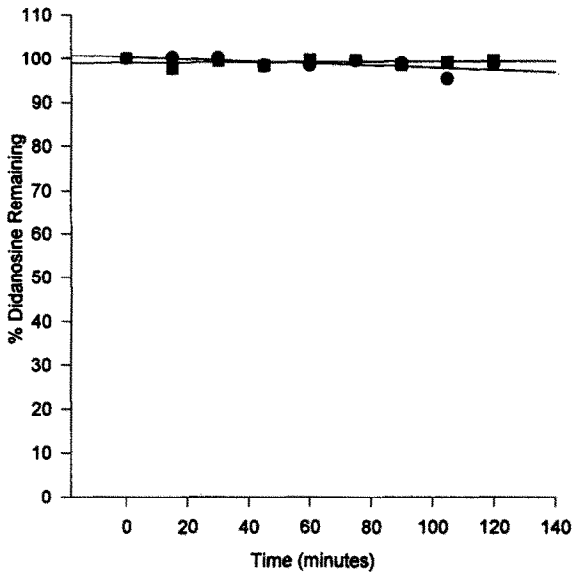

Fig. 1. Stability of didanosine in perfused and unperfused Mes, iso-osmotic buffer. The % of didanosine remaining in the perfusion solution is plotted as a function of time. The lines represent the best-fit lines as determined by linear regression analysis. ddI is stable in both perfused (37°C) (■) and unperfused (room temperature) (●) buffers at pH 6.5 (290 mOsm/kg).

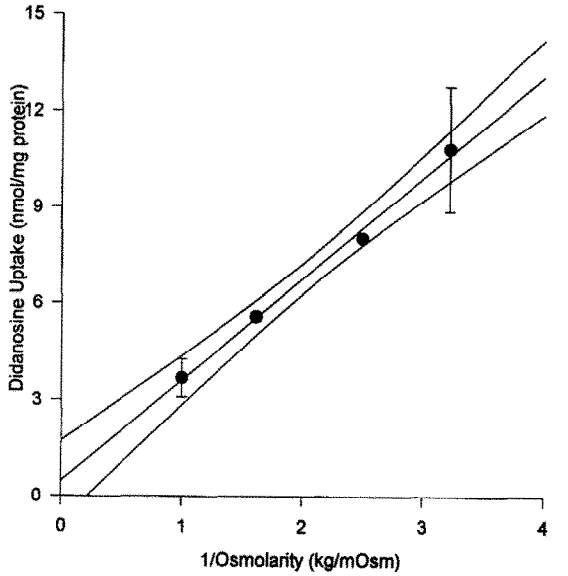

Fig. 2. Determination of the vesicle-associated didanosine. The uptake of didanosine into rat brush border membrane tissue is plotted as a function of inverse osmolarity. At infinite osmolarity, the vesicles are collapsed and didanosine cannot be taken up into the vesicle; therefore, uptake at infinite osmolarity is related to the amount of didanosine associated with (or bound to) the intestinal tissue. From these results, the binding of didanosine to intestinal tissue is minimal.

2, the uptake of didanosine at infinite osmolarity is low and not different from zero based on the calculated 95% confidence interval, indicating that didanosine binding to intestinal tissue is not significant. Since the SPIP is a steady-state method, the binding sites of tissue or mucin will be at a local equilibrium with the perfusing solution and will not affect didanosine concentrations in the perfusate. The existence of steady-state conditions was confirmed experimentally by the constancy of the corrected C_m/C_o values which was achieved within 30 min. Based on the results of the validation studies, it appeared that the loss of didanosine from the perfusing solution was only due to transport into intestinal tissue.

Given the results of the validation studies, didanosine intestinal perfusions were performed in the upper, middle and lower small intestinal segments of rats. The mean ($\pm$SD) data gathered from the perfusion experiments are given in Table 1. Table 1 lists the mean values of C_m/C_o, H_o/H_m, the length (L) of the perfused intestine, and the flow rate (Q) used in the experiment for all perfusions. Two different flow rates were used to measure P_w^* in the lower small intestine. Using the mean values for all rats reported in Table 1, the wall permeabilities calculated for the upper, middle and lower small intestine (0.25, 0.16, and 0.07, respectively) agree with the values calculated from individual rats (0.26 ± 0.09, 0.17 ± 0.03, and 0.09 ± 0.04, respectively). The dimensionless intestinal wall permeabilities (P_w^*) of didanosine

Table 1
Summary of the experimental results of didanosine intestinal perfusions in rats

Segment	n	C_m/C_o	SD	H_o/H_m	SD	L	SD	Q (ml/min)
Upper	10	0.99	0.03	0.95	0.02	13.4	1.4	0.191
Middle	5	1.02	0.03	0.93	0.04	17.3	3.0	0.191
Lower	5	1.00	0.02	0.92	0.04	18.8	1.1	0.076
Lower	2	1.01	0.01	0.95	0.00	16.4	0.4	0.191

and the associated net water flux were determined using the modified boundary layer analysis (Eq. 1, 3 and 4) and are shown in Table 2. The rank order of didanosine P_w^* (at pH 6.5, 290 mOsm/kg) was proximal jejunum > mid-small intestine > distal ileum. The intestinal permeability of didanosine was 1.5-fold lower in the mid-small intestine (SI) and 2.9-fold lower in the lower SI when compared to the permeability in the upper SI. A one-way ANOVA was performed on the permeability and net water flux measured in the upper, middle, and lower small intestine and the results are shown in Table 3a and b, respectively. Results of the ANOVA indicate that the regional differences in intestinal permeability were significant whereas net water flux differences were not. Results of *t*-tests indicate that all permeability differences were significant between intestinal regions. Ongoing intestinal transport studies in our laboratory suggest that the intestinal absorption pathway of didanosine may include a significant paracellular component. It is possible, therefore, that the permeability difference between segments was an artifact due to differences in water flux. Therefore, net water flux was monitored during all studies and, although water absorption (negative water flux) occurred in all three intestinal segments (Table 2), it was not significantly different between segments and was not likely to bias the permeability results. These results are consistent with data reported by Park and Mitra (1992) for another nucleoside analog, zidovudine (AZT, Burroughs-Wellcome). In their studies, the permeability of AZT was 2.5-fold lower in the lower jejunal-ileal segment than in the upper jejunum. Since the mid and lower small intestinal segments were grouped together in the aforementioned study, it is difficult to compare their results with the present studies; however, preliminary data with AZT in our laboratories demonstrate a 2.5-fold decrease in AZT permeability between the upper and lower intestinal segments consistent with the present didanosine studies. The colonic permeability of didanosine could not be estimated as a result of limitations of the single pass perfusion model. In all of the colon perfusion studies, the flow rates and lengths of the intestinal segments available for perfusion were limiting.

The low intestinal permeability calculated from the present results suggests that didanosine oral absorption may be permeability-limited in vivo. In order to estimate the effect of low didanosine intestinal permeability on its extent of absorption (F), a theoretical model correlating intestinal permeability and F was used. It was previously demonstrated that the absorption number (An), a dimensionless parameter comprised of the intestinal wall permeability determined in rats and flow scaling factors, correlated well with extent of

Table 2
Summary of intestinal permeability and net water flux results for didanosine in rats

Site	Mean	SE	n
Intestinal wall permeability (P_w^*)			
Proximal jejunum	0.26	0.03	10
Mid-small intestine	0.17 [a]	0.01	5
Distal ileum	0.09 [a,b]	0.01	7
Water transport (%/cm)			
Proximal jejunum	−0.38	0.03	10
Mid-small intestine	−0.38	0.07	5
Distal ileum	−0.38	0.08	7

[a] Difference with respect to upper small intestinal permeability is significant ($p < 0.05$).
[b] Difference with respect to middle small intestinal permeability is significant ($p < 0.05$).

Table 3
Summary of analysis of variance results on the differences in didanosine intestinal permeability (a) and net water flux (b) as a function of intestinal region

Sources of variance	D.F.	Sum of squares	Mean squares	F Ratio	Significance of F
(a) Analysis of variance for permeability in three intestinal sites					
Between sites	2	0.136	0.068	13.895	0.0001 [a]
Within sites	21	0.103	0.005		
Total	23	0.239			
(b) Analysis of variance for water flux in three intestinal sites					
Between sites	2	0.015	0.007	0.22	0.81 [b]
Within sites	21	0.704	0.034		
Total	23	0.719			

[a] Indicates significantly different P_w^* at the three sites.
[b] No two sites have significantly different water flux at the 0.05 level.

absorption results in humans (Sinko et al., 1991). Using this correlation it can be demonstrated that the wall permeability of didanosine is consistent with extent of absorption results reported in dogs and humans. As seen below, An is defined as:

$$An = P_w^* \, Gz \tag{6}$$

and the extent of absorption, F, based on the plug flow model, where Gz = 1.27, is:

$$F = 1 - e^{-2An} \tag{7}$$

Using the above equations and the experimental results, an in situ-in vivo correlation establishing the permeability-limited and site-specific oral absorption of ddI is developed below.

3.1. Permeability-limited absorption of ddI

Using the P_w^* determined in the proximal jejunum in rats and Eq. 7, F in dogs and humans is estimated to be 48%. The predicted value compares favorably with the bioavailability of didanosine reported in humans and dogs, 38–43 and 48%, respectively (Stolz et al., 1989; Hartman et al., 1991; Sinko et al., unpublished data). Whether the overestimate in F for humans is due to an overestimate in P_w^*, since the correlative model does not account for the distal differential in P_w^*, or if gastrointestinal and hepatic metabolism may occur in humans cannot be resolved from the present studies or literature; however, this correlation establishes that the intestinal permeability of didanosine is potentially a major factor in its limited oral absorption. The apparent dose dependence of didanosine absorption was not investigated in this study; however, preliminary mechanistic studies performed in our laboratory suggest that didanosine absorption is not mediated by the intestinal nucleoside carrier systems.

3.2. Intestinal site (regional) dependence

The correlation can also be extended to determine regional absorption differences. For the purposes of correlating the in situ and in vivo results, the intestine was arbitrarily divided into three regions: the upper, middle and lower small intestine. The location dependence of didanosine intestinal permeability is plotted in Fig. 3 along with the best-fit line. The use of normalized intestinal length facilitates the comparison between the dog, human and rat, since the absolute intestinal length of each of the species is significantly different. The intestinal permeability for the upper and lower regions used in the calculation of the absorption number was estimated from the midpoint of each region from the best-fit line. The other correlating parameter, the extent of absorption in dogs and humans, was taken from the literature (Stolz et al., 1989; Hartman et al., 1991; Sinko et al., unpublished data). To complete the correlation, the in vivo data were classified according to the site where the majority of didanosine absorption occurred. The intestinal segment where the majority of didanosine absorption occurred in vivo was estimated from the absorption time (time to peak plasma levels minus 15–30 min) and the small intestinal transit time in dogs (90 min) and humans (180 min). Gastric emptying may also affect total transit time but, in the cases cited, the effects were expected to be negligible since the dosage forms

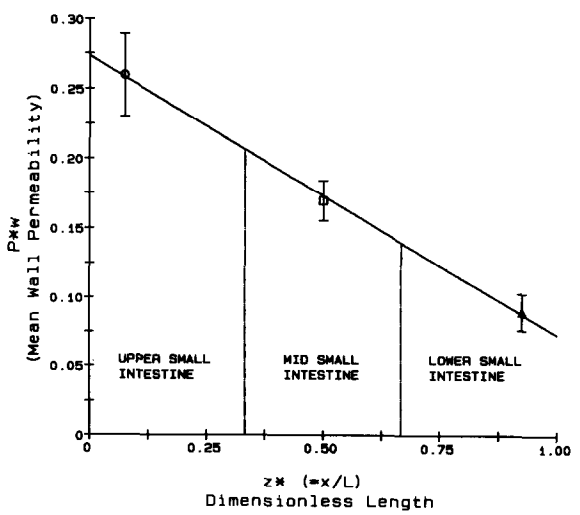

Fig. 3. Dependence of the intestinal permeability of didanosine on dimensionless intestinal length. The mean wall permeability (±SE) of didanosine was determined in rats using the single pass intestinal perfusion procedure. The best-fit line was determined by linear regression analysis ($R^2 > 0.99$). (○) Proximal jejunum, (□) mid SI, (△) distal ileum, (———) best-fit line.

are solutions or granules which readily pass through the stomach (Davis et al., 1986). Since the actual site of ddI release could not be verified, the midpoint of the appropriate intestinal region was used. Studies are being performed in intestinal-vascular access port (IVAP) dogs to confirm the regional dependence of didanosine absorption. The IVAP dog model allows regional oral absorption to be studied through the precise placement of surgically implanted intestinal drug-infusion ports. Six sets of in vivo data were used in the correlation, three sets of human data (Hartman et al., 1991) and three sets of dog data (Stolz et al., 1989; Sinko et al., unpublished data). The bioavailability of ddI, when dosed as enteric coated granules, was 35 and 26% in humans and 22 and 10% in dogs. Using the previously described method, the 35% absorption result in humans was determined to occur in the middle third of the small intestine whereas the 26% in humans and 22% absorption results in dogs were estimated to occur in the lower third of the small intestine. The results reflecting 10% absorption in dogs corresponded to colonic release of didanosine. Given that the permeability of ddI could not be calculated in the colon, this point was not considered in the correlation. The correlation between An and the bioavailability of didanosine reported in the literature is plotted in Fig. 4. The theoretical prediction based on Eq. 7 is depicted by the solid line. As seen in Fig. 4, the correlation between An (permeability in rats and flow) and extent of absorption in dogs and humans is in excellent agreement with the theoretical prediction. The results of the correlation between the regional permeability in rats and the regional extent of absorption of didanosine in humans and dogs indicate that permeability limitations may be responsible for the regional decrease in didanosine bioavailability. The distally occurring differential changes in intestinal permeability, interindividual differences in small intestinal residence time and changes due to the progression of HIV disease will all significantly affect An and extent of absorption and may account for the high degree of bioavailability variability observed in humans.

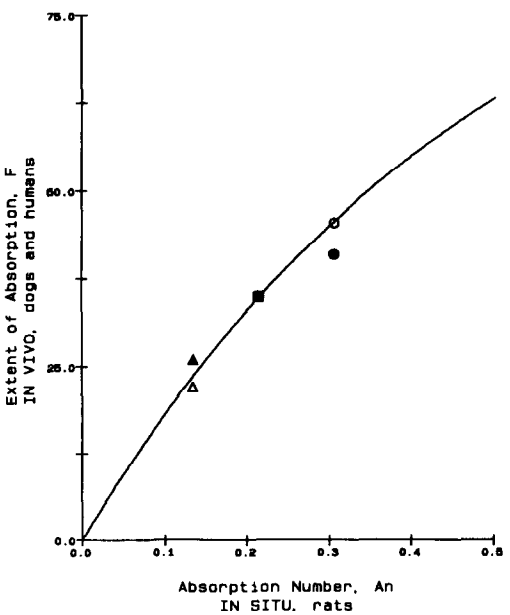

Fig. 4. Correlation of the extent of didanosine oral absorption in dogs and humans and the absorption number, An. An is calculated from the permeability in rats and flow parameters. The theoretical line is generated using Eq. 7. The correlation suggests that the decreased bioavailability observed with enteric coated granules may be due a decreased intestinal permeability of didanosine in the middle and lower small intestine. (———) Theoretical prediction ($F = 1 - \exp(-2An)$); (●) upper SI, humans, solution; (■) mid SI, humans, enteric granules; (▲) lower SI, humans, enteric granules; (○) upper SI, dogs, solution; (△) lower SI, dogs, enteric granules.

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