Intestinal Absorption Barriers and Transport Mechanisms, Including Secretory Transport, for a Cyclic Peptide, Fibrinogen Antagonist

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Purpose. The intestinal absorption of DMP 728, a cyclic peptide fibrinogen antagonist, was examined with the goals of identifying the cause(s) of its low oral bioavailability and understanding the mechanisms of its intestinal transport.

Methods. In vitro partitioning, metabolism, and permeation through rat intestinal segments were evaluated.

Results. DMP 728 had low lipophilicity and low intestinal permeation rates relative to model compounds. In addition, DMP 728 in vitro intestinal permeation in the secretory direction greatly exceeded transport in the absorptive direction. The secretory transport was saturable, glucose-dependent, and was inhibited by verapamil and by a monoclonal antibody to P-glycoprotein. DMP 728 likewise inhibited the secretory transport of verapamil. Mucosal-to-serosal permeation rates increased in going from the proximal to distal intestinal sites, but were lower than serosal-to-mucosal permeation rates for each site.

Conclusions. Net secretory transport and low lipophilicity are the major barriers to absorption of DMP 728.

KEY WORDS: DMP 728; fibrinogen antagonist; GPIIb/IIIa antagonist; intestinal transport; absorption; P-glycoprotein.

INTRODUCTION

Platelet aggregation is initiated by the binding of fibrinogen to an activated glycoprotein complex, GPIIb/IIIa, on the platelet membrane. Fibrinogen antagonists, or GPIIb/IIIa receptor antagonists, are potentially useful agents for preventing platelet aggregation and thrombosis (1). An Arg-Gly-Asp (RGD) sequence is the minimal structural component of fibrinogen required for binding to the platelet GPIIb/IIIa receptor. Inhibition of fibrinogen binding to GPIIb/IIIa can be accomplished using small peptides containing the RGD sequence, and these peptides furthermore inhibit platelet-dependent thrombosis (1). DMP 728 was recently identified as a cyclic RGD analog with high affinity and specificity for the platelet GPIIb/IIIa receptor, and is a potential new antithrombotic agent (2). DMP 728 is cyclo-[D-2-aminobutyryl-L-N^2-methyl-L-arginyl-glycyl-L-aspartyl-3-(aminomethyl)-benzoic acid].

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NOTATIONS: RGD, Arg-Gly-Asp; MDR, multidrug resistant; TFA, trifluoroacetic acid; M, mucosal; S, serosal.

Oral administration is most desirable for chronic drug therapy. However, like many peptide drugs, DMP 728 has relatively low oral bioavailability. Incomplete oral bioavailability of peptides can be caused by presystemic metabolism or poor intestinal permeation, or a combination of these problems (3,4). In order to gain the advantage of maximum oral bioavailability, it is necessary to first identify the barriers to oral absorption. One goal of this work was to identify the barriers to oral absorption of DMP 728. Both metabolism and permeation were considered. Permeation rates and partition coefficients were compared to model compounds with known in vivo oral absorption characteristics.

When intestinal permeability presents a major barrier to absorption, as in this case, it becomes very important to understand the mechanisms of intestinal permeation. There is little information in the literature on the mechanisms of intestinal permeation of linear or cyclic RGD peptides or analogs, or on the mechanisms of absorption of cyclic peptides. Many di- and tripeptides can permeate through the intestinal membrane via an active transport system coupled with proton movement. Some β-lactam antibiotics and angiotensin converting enzyme inhibitors are also absorbed by this peptide transporter, and this mechanism contributes to their having adequate oral bioavailabilities. Initially, a goal of this work was to examine whether DMP 728 is a substrate for the intestinal peptide transport system.

It has recently been shown that the intestinal absorption of some compounds is limited in part because they are transported preferentially in the secretory direction by an apically located transport system (5–7). One secretory intestinal transport system is P-glycoprotein. P-glycoprotein is the efflux transporter over-expressed in multidrug resistant (MDR) tumor cells. P-glycoprotein mediates drug efflux in Caco-2 cells (5–7) as well as normal rat intestine (8). Among the known substrates for intestinal efflux are some hydrophobic tripeptides (5) and cyclosporin (9). We report here that the intestinal permeation of DMP 728 is limited because it is subject to efflux by an intestinal epithelial transport system. DMP 728 secretory transport has some characteristics of P-glycoprotein-mediated transport.

MATERIALS AND METHODS

DMP 728 was prepared at The DuPont Merck Pharmaceutical Co. (Wilmington, Delaware). Theophylline, (±)-verapamil hydrochloride, D-tryptophan, ampicillin, sodium cefazolin, cephradine, Arg-Gly, Gly-Asp, L-carnosine, and porcine gastric mucin (crude, type II) were purchased from Sigma Chemical Company (St. Louis, Missouri). Anti-P-glycoprotein 170-180, a monoclonal antibody to the 170-180 kD multidrug resistance-related P-glycoprotein, was obtained from Boehringer Mannheim (Indianapolis, Indiana). Mouse IgG1 was obtained from Sigma.

Permeation experiments were performed using methods as described by Grass and Sweetana (10). The buffer used was Tyrode's buffer, containing 137 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.4 mM NaH_2PO_4 and 6 mM D-glucose. Male Sprague Dawley rats (Crl:CD(SD)BR, Charles River, Kingston, New York) weighing 300–350 g were used. Intestine from fasted rats

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was removed and rinsed in ice-cold saline. Intestinal segments, excluding Peyers patches, were placed in ice-cold Tyrode's buffer. Segments were pulled onto a glass rod, cut open with a scalpel, and spread onto a paper filter to expose the epithelial surface. Then the intestinal sheets were mounted onto the pins of the diffusion cell, and the half cells were clamped together. To the donor compartment was added 7 ml of drug solution and to the receiving compartment was added 7 ml of drug-free buffer. The temperature of the diffusion cells was maintained at 37°. The donor and receiving fluids were circulated by gas lift with O_2/CO_2. Receiving chamber samples (0.5 ml) were taken at various times and replaced with drug-free buffer. Samples were also taken from the donor chamber in some studies. TFA (0.5 ml of 0.1% solution) was immediately added to each sample. Permeation experiments lasted 2 hr. The exposed intestinal surface area was 1.78 cm^2. Most studies were done using jejunal segments, but site-dependence was studied using duodenum, ileum, and colon, as well.

The concentration in each receiving chamber sample was determined using HPLC. The cumulative amount of drug permeating the membrane was calculated and plotted vs. time for each experiment. Permeation rates were calculated from the linear portion of a plot of amount permeating vs. time, and were normalized for surface area to give the drug flux. Permeability coefficients were calculated by dividing flux by the donor concentration of the permeant. There were generally 4 or more experiments for each group.

Statistical comparisons between two groups were made using t-tests. For comparisons among more than two groups, analysis of variance was performed, followed by Duncan's multiple range tests.

This research was done in accordance with the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

RESULTS

Permeation and Partitioning Relative to Model Compounds

The intestinal absorption characteristics of DMP 728 and several model compounds were compared by measuring their permeation through excised rat jejunum in vitro. The model compounds used included a poorly-absorbed compound (cefazolin), a moderately-absorbed compound (ampicillin), and well-absorbed compounds (cephradine and theophylline). Cephradine is a known substrate of the absorptive peptide transporter. DMP 728 is zwitterionic at pH 7.4, as are D-tryptophan and ampicillin. These studies were run using pH 7.4 buffer in both donor and reservoir compartments, and 1 mM drug concentrations. Figure 1 shows the profile of the amount permeating vs. time for each of these compounds. The in vitro permeation rates of the model compounds correlated with their known extents of in vivo absorption, with the rank order cefazolin < ampicillin < cephradine and theophylline. No in vivo absorption data are available for D-tryptophan. DMP 728 had the lowest in vitro permeation. This is consistent with its low in vivo oral bioavailability in rats and dogs (unpublished). These results indicate that low oral bioavailability of DMP 728 in vivo is at least partly due to poor intestinal permeation.

The octanol/pH 7.4 buffer distribution coefficient of each of these compounds was measured. Excluding cephradine, there was a good correlation between these distribution coefficients and the intestinal permeation rates (Fig. 2). Poor membrane partitioning appears to be one factor contributing to poor intestinal permeation of DMP 728. Cephradine had high intestinal permeability, considering its distribution coefficient relative to the other compounds shown in figure 2. This deviation is expected though, because cephradine is absorbed by the peptide transport system, in addition to passive diffusion.

Metabolism as a Barrier

DMP 728 was not degraded within 6 hr in simulated gastric fluid containing pepsin, or in simulated intestinal fluid containing pancreatic proteases. This indicates that it is stable to the proteases found in the stomach and intestinal lumen. DMP 728 was also not degraded within 2 hr in a 10% homogenate of rat jejunal mucosa, and DMP 728 was stable

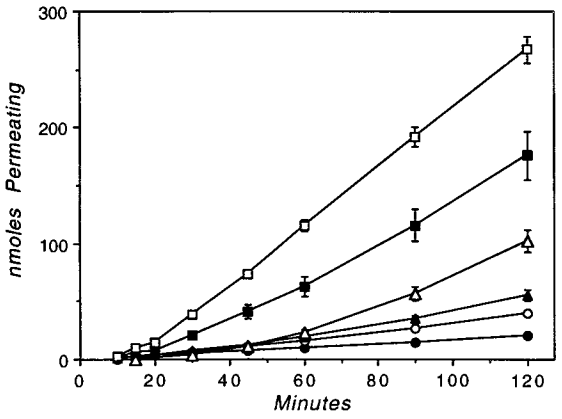

Fig. 1. Mucosal-to-serosal permeation profiles for DMP 728 (●), cefazolin (○), ampicillin (▲), cephradine (△), D-tryptophan (■), and theophylline (□), using rat jejunum and 1 mM drug solutions at pH 7.4. The data represent mean ± S.E.

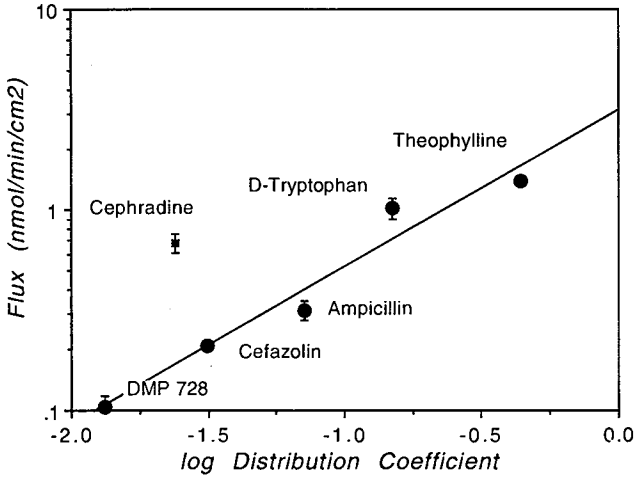

Fig. 2. Relationship between the octanol/pH 7.4 buffer distribution coefficient and mucosal-to-serosal drug flux through rat jejunum at pH 7.4. The line drawn is a regression of the mean values excluding cephradine.

Table I. Effects of Mucosal Solution pH and the Presence of a Proton Gradient on DMP 728 Intestinal Permeation in vitro

DMP 728 concentration	0.2 mM		1.0 mM	
Mucosal pH	7.4	5.0	7.4	5.0
N	9	4	9	10
Permeability coefficient (P)	1.25 ± 0.08	2.42 ± 0.25[a]	1.75 ± 0.22	2.72 ± 0.20[b]
P at pH 5/P at pH 7.4	1.87		1.55	

[a] $P < 0.01$ significantly different from pH 7.4.
[b] $P < 0.001$ significantly different from pH 7.4.

in the donor solution during the intestinal permeation studies. Thus, neither membrane-associated nor cytosolic peptidases degrade DMP 728, in contrast to many other peptides. Degradation within the intestinal lumen or by the intestinal cells does not appear to contribute to the poor oral bioavailability.

Effect of a Proton Gradient

One characteristic of many compounds absorbed by the peptide transport system is stimulation of permeation by the presence of a proton gradient (proton-coupled transport). The effect of a proton gradient on DMP 728 permeation was evaluated using mucosal solutions of pH 5 and serosal solutions of pH 7.4, and either 0.2 mM or 1 mM DMP 728 concentrations. The results in Table I show that DMP 728 permeation was stimulated in the presence of a proton gradient, since the permeability coefficients (P) were greater using a mucosal pH of 5. The effect of the proton gradient was greater at the lower DMP 728 concentration, as indicated by the greater ratio of P values (pH 5 to pH 7.4). In contrast, the permeation rates of cefazolin and theophylline, which are not substrates for the peptide transport system, were not increased in the presence of a proton gradient (data not shown). These results indicate a similarity between DMP 728 absorption and absorption by the peptide transport system. The pKa values of DMP 728 are 3.7 for the carboxylic acid and 13.6 for the guanidine (unpublished). DMP 728 is essentially completely in the zwitterionic form at pH 7.4 and at pH 5, and the lower pH did not significantly affect partitioning.

Concentration Dependence

Another characteristic of carrier-mediated absorption is saturability. Intestinal permeation of DMP 728 was measured using mucosal concentrations ranging from 0.2 to 5 mM. Permeability coefficients (P) represent flux divided by donor concentration. P values (cm/sec $\times 10^{-6}$) increased with increasing concentration, from 1.25 ± 0.08 at 0.2 mM DMP 728 to 1.75 ± 0.22 at 1 mM, 2.60 ± 0.38 at 2 mM, and 3.21 ± 0.39 at 5 mM. This is contrary to the hypothesis of saturation of carrier-mediated absorption, in which case P would decrease with increasing substrate concentrations. Increasing P with increasing concentrations suggests carrier-mediated efflux.

Directionality

To further examine the possibility of an efflux transport system, DMP 728 permeation was measured in the serosal-to-mucosal direction. The serosal-to-mucosal permeation rate was almost 4-fold greater than that in the mucosal-to-serosal direction. Theophylline, D-tryptophan, and cephradine were also evaluated for directionality of permeation. A comparison of these compounds is given in Table II. Theophylline and D-tryptophan, which are absorbed by passive diffusion, showed lower permeation in the serosal-to-mucosal direction than in the mucosal-to-serosal direction (the difference was significant for theophylline only). This is presumed to be due to a greater effective mucosal surface area. Cephradine, which is absorbed by the peptide transport system, also had a greater mucosal-to-serosal flux than in the reverse direction, and the mucosal-to-serosal transport by the peptide transport system could have also accounted for this difference.

Interactions with Verapamil

Verapamil is known to be an effective inhibitor of the P-glycoprotein-mediated efflux of drugs from both Caco-2 intestinal cells and MDR tumor cells. The inhibition of efflux by verapamil increases cellular uptake or increases the mucosal-to-serosal intestinal transport of P-glycoprotein substrates. The effects of verapamil on DMP 728 permeation, using 1 mM DMP 728 at pH 7.4, are summarized in Table III. Verapamil increased DMP 728 mucosal-to-serosal permeation, consistent with inhibition of efflux. Maximum inhibition (2.9-fold enhancement of flux) was observed at 0.2 mM verapamil, rather than at the maximum concentration tested. The reasons for this concentration dependence are not known.

We next examined the effects of verapamil in the presence of a proton gradient (mucosal pH 5, serosal pH 7.4)

Table II. Comparison of Mucosal-to-Serosal (Absorptive) and Serosal-to-Mucosal (Secretory) Permeation Rates of Various Compounds Through Rat Jejunum (1 mM Compound, pH 7.4 Mucosal and Serosal Buffers)

Compound	Mucosal-to-serosal flux (nmol/min/cm^2)[a]	Serosal-to-mucosal flux (nmol/min/cm^2)[a]
DMP 728	0.10 ± 0.01	0.40 ± 0.04[b]
Theophylline	1.40 ± 0.06	1.02 ± 0.06[c]
D-Tryptophan	1.02 ± 0.11	0.75 ± 0.06
Cephradine	0.68 ± 0.07	0.40 ± 0.03[d]

[a] Mean ± S.E. of 3 or more experiments.
[b] $P < 0.001$ Significantly different from mucosal-to-serosal.
[c] $P < 0.01$ Significantly different from mucosal-to-serosal.
[d] $P < 0.05$ Significantly different from mucosal-to-serosal.

Table III. Effects of Verapamil on DMP 728 Intestinal Permeation. Permeation of Rat Jejunum was Determined Using 1 mM DMP 728 in pH 7.4 Buffer

	DMP 728 Flux (nmol/min/cm^2)
Control	0.10 ± 0.01
+ 0.1 mM Verapamil	0.12 ± 0.02
+ 0.2 mM Verapamil	0.30 ± 0.03[a]
+ 0.5 mM Verapamil	0.20 ± 0.01
+ 1 mM Verapamil	0.22 ± 0.04

[a] Significantly ($P < 0.05$) different from control and 0.1 mM verapamil groups.

using 0.2 mM DMP 728. In the presence of 0.2 mM verapamil and the proton gradient, DMP 728 flux was 0.043 ± 0.005 nmol/min/cm^2. This was greater ($P < 0.1$) than the control flux in the presence of the proton gradient (0.029 ± 0.003 nmol/min/cm^2), which was in turn greater ($P < 0.001$) than flux in the absence of the proton gradient (0.015 ± 0.001 nmol/min/cm^2). Thus, the proton gradient and the presence of verapamil had additive effects in augmenting the permeation of DMP 728. This could be due to the combined effects of verapamil inhibiting secretory efflux and the proton gradient stimulating absorptive transport.

We have recently shown that verapamil is a substrate for preferential serosal-to-mucosal transport in rat intestine in vitro (11). Other workers have used verapamil as an inhibitor of P-glycoprotein-mediated basolateral-to-apical transport in Caco-2 intestinal epithelia (5,7) or serosal-to-mucosal transport in rat intestine (8). We investigated how DMP 728 affects verapamil transport, and whether these are mutual inhibitors. Verapamil flux in the secretory (serosal-to-mucosal) direction was greater ($P < 0.01$) than that in the absorptive direction (0.47 ± 0.02 nmol/min/cm^2 M-to-S vs. 1.26 ± 0.10 nmol/min/cm^2 S-to-M, using 1 mM verapamil at pH 7.4). DMP 728 (1 mM) increased mucosal-to-serosal verapamil permeation to 0.72 ± 0.02 nmol/min/cm^2 and reduced serosal-to-mucosal permeation to 0.83 ± 0.06 nmol/min/cm^2, eliminating the directional dependence of verapamil permeation. These results indicate that DMP 728 and verapamil are mutual substrates for a serosal-to-mucosal transport system.

Effect of Anti-P-glycoprotein 170-180

As another test for P-glycoprotein-mediated efflux, the effects of a monoclonal antibody to P-glycoprotein 170-180 were evaluated. Addition of the P-glycoprotein antibody to the mucosal solution resulted in a 2.7-fold increase ($P < 0.05$) in the mucosal-to-serosal transport of DMP 728 (from 0.029 ± 0.003 nmol/min/cm^2 to 0.078 ± 0.011 nmol/min/cm^2, using 0.2 mM DMP 728 at pH 5). In contrast, the intestinal permeation of D-tryptophan, a marker of passive diffusion permeation, was not affected by this antibody. As another control, the effects on DMP 728 permeation of a nonspecific antibody, mouse IgG, were examined. Mouse IgG (2 μg/ml) resulted in a 2.1-fold increase in DMP 728 permeation, which was also significantly different from control. Therefore, the P-glycoprotein antibody effect could have been nonspecific.

Interactions with Dipeptides and Other Peptide Transport System Substrates

To further investigate whether DMP 728 is absorbed by the peptide transport system, dipeptides and other substrates of the peptide transport system were evaluated as competitive inhibitors of DMP 728 mucosal-to-serosal permeation. Arg-Gly and Gly-Asp were selected because they are structural components of DMP 728. A proton gradient was present for these studies to stimulate the peptide absorptive transport system. Concentrations of DMP 728 and competing substrate were 0.2 mM and 10 or 20 mM, respectively. In the initial studies there was no inhibition of DMP 728 permeation with Arg-Gly, Gly-Asp, or ampicillin. We postulated that the lack of inhibition of net absorptive transport could be because these compounds also inhibited the secretory transport of DMP 728. Therefore, other inhibition studies were performed in the presence of verapamil (1 mM or 0.2 mM) or the P-glycoprotein antibody. Cephradine, L-carnosine, and ampicillin, which are known substrates of the peptide absorptive transport system, and Arg-Gly and Gly-Asp had no significant effects on DMP 728 permeation. Thus, these inhibition studies did not confirm that DMP 728 is absorbed by the peptide transport system.

Glucose Dependence

Active transport systems require metabolic energy. The energy dependence of DMP 728 permeation through jejunum in both the mucosal-to-serosal and serosal-to-mucosal directions was evaluated using 1 mM DMP 728 at pH 7.4. As one test for energy dependence, the buffer glucose was replaced with 3-O-methyl glucose (6 mM). This condition significantly ($P < 0.001$) raised the mucosal-to-serosal flux from 0.10 ± 0.01 nmol/min/cm^2 to 0.35 ± 0.04 nmol/min/cm^2, which was similar to serosal-to-mucosal flux. The absence of glucose did not affect the serosal-to-mucosal flux. The elimination of preferential transport in the serosal-to-mucosal direction is indicative of glucose dependence of secretory transport. But it should also be considered that energy depletion can also increase the permeability of tight junctions (12).

Site-Dependence

Site-dependence of mucosal-to-serosal DMP 728 permeation was studied by comparing duodenum, jejunum, ileum, and colon. As shown in Figure 3, mucosal-to-serosal DMP 728 permeation increased in going from proximal to distal intestinal sites. Colonic flux was significantly ($P < 0.05$) greater than duodenal and jejunal flux values. This is in contrast to the permeabilities of compounds absorbed by passive diffusion or paracellular permeation, which often decrease in progressing down the intestine from duodenum to colon (4,13,14). Serosal-to-mucosal DMP 728 flux was relatively constant along the intestinal tract, and was consistently greater than mucosal-to-serosal flux. The ratio of S-to-M/M-to-S was lowest for the colon.

DISCUSSION

The goals of this work were to identify the barriers limiting oral bioavailability of the cyclic peptide, fibrinogen antagonist, DMP 728, and to examine the nature of those bar-

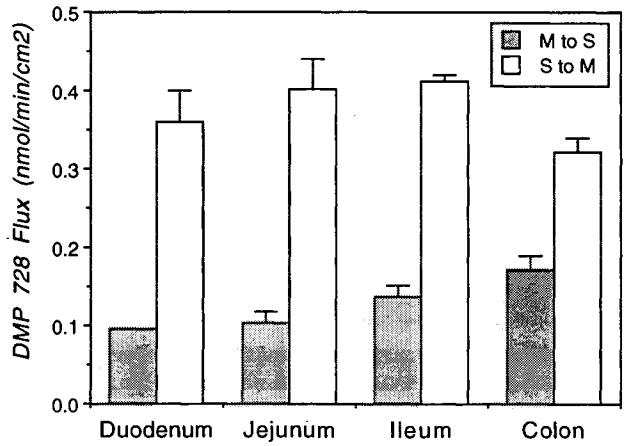

Fig. 3. Site-dependence of mucosal-to-serosal (M-to-S) and serosal-to-mucosal (S-to-M) DMP 728 flux values, using rat intestine and 1 mM DMP 728 at pH 7.4. Bars represent the mean + S.E. of at least 4 experiments.

riers and the mechanisms of transport. DMP 728 was known to be non-metabolized in vivo after i.v. dosing (unpublished), and we have shown that it was also metabolically stable when exposed to various gastrointestinal enzymes or intestinal tissues in vitro. DMP 728 had slow intestinal permeation and a low octanol/aqueous distribution coefficient, relative to several model compounds. Poor intestinal permeability is seen as the primary cause of low oral bioavailability. Poor lipophilicity contributes to the poor membrane permeation.

DMP 728 is zwitterionic at neutral pH, and thus is very hydrophilic (log octanol/aqueous distribution coefficient of -1.88), so permeation by transcellular passive diffusion is expected to be relatively slow. Based on its hydrophilicity, DMP 728 seems more likely to be absorbed paracellularly, as are other very hydrophilic solutes. But permeation by this mechanism could be limited by its molecular size (or molecular weight). The absorption of polyethylene glycols (PEGs) in man are highly dependent on the PEG molecular weight; jejunal absorption decreased from >50% to <10% as molecular weight decreased from 242 daltons to 594 daltons (14). The molecular weight of DMP 728 free zwitterion is 561 daltons. PEG absorption was also site-dependent, with the rank order jejunum > ileum > colon (14).

Secretory transport is a contributing cause of poor oral bioavailability of DMP 728. DMP 728 permeation in the serosal-to-mucosal direction was greater than absorptive permeation. The concentration dependence of DMP 728 intestinal permeation was consistent with saturability of secretory transport. Secretory transport exceeded absorptive permeation for all intestinal sites, but the difference was least for the colon. P-glycoprotein-mediated intestinal transport in the secretory direction has recently been demonstrated for several lipophilic peptides (5,9) and lipophilic, cationic, non-peptides (6–8). Verapamil was also transported in the net secretory direction by rat intestine, and this secretion was inhibited by a P-glycoprotein antibody (11). Secretory transport of DMP 728 was inhibited by verapamil. Furthermore, DMP 728 inhibited the secretory transport of verapamil, and increased its permeation in the absorptive direction, showing that these are mutual substrates for secretory transport. Mucosal-to-serosal permeation of DMP 728 was increased by a monoclonal antibody to P-glycoprotein, consistent with inhibition of secretory transport. However, mouse IgG also significantly increased mucosal-to-serosal DMP 728 permeation. Thus, the effect of this P-glycoprotein antibody could have been nonspecific. If DMP 728 is a substrate for P-glycoprotein, it is an unusual one. DMP 728 is very hydrophilic, whereas most known P-glycoprotein substrates are hydrophobic. Many P-glycoprotein substrates have a cationic functional group, as does DMP 728. Alternatively, DMP 728 may be secreted by other transport systems. Intestinal secretory transport of the organic cation guanidine has been demonstrated (15), and this system could contribute to DMP 728 transport. Guanidine did not significantly affect verapamil transport (11), indicating that verapamil and guanidine transporters are separate. We are continuing to evaluate substrate specificity of intestinal secretory transport.

Secretory transport can be envisaged as transport from the intracellular space into the intestinal lumen. Since DMP 728 has a very low partition coefficient, one might question how DMP 728 enters the cytoplasm to be a substrate for secretory transport. A specialized membrane uptake mechanism would seem the most likely explanation. We initially hypothesized that DMP 728 might be absorbed by the peptide transport system, since DMP 728 is a structural mimic of the tripeptide Arg-Gly-Asp and contains this structural motif. DMP 728 transport was stimulated by a proton gradient, one characteristic of the peptide transport system. But we did not demonstrate competitive inhibition. Other studies typically used to characterize specialized transport, such as concentration-dependence and the effects of inhibitors and other variables, were complicated by the possible involvement of these separate absorptive and secretory transport systems. Another possibility is that the secretory transporter can also perform membrane uptake in the absorptive direction, even though secretion is dominant. MDR tumor cell P-glycoprotein is reported to transport its substrates into the membrane in both the influx and efflux directions (16). Specialized transport might also operate at the basolateral membrane, as has been shown for the uptake of β-lactam antibiotics in Caco-2 cells (17). These possibilities remain to be investigated.

In summary, DMP 728 appears to be a structurally novel substrate for intestinal secretory transport. Its oral absorption is limited by its hydrophilicity and by the dominance of secretory transport. This secretory transport occurs throughout the rat intestinal tract.

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