Possible Involvement of Multiple P-Glycoprotein-Mediated Efflux Systems in the Transport of Verapamil and Other Organic Cations Across Rat Intestine

Hiroshi Saitoh[1,2] and Bruce J. Aungst[1,3]

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Purpose. We investigated the intestinal transport of verapamil, chlorpromazine, and propantheline, particularly their P-glycoprotein-mediated secretion. **Methods.** Permeation of rat intestinal segments *in vitro* was determined using diffusion cells. **Results.** Verapamil permeation in the serosal-to-mucosal direction was much greater than in the mucosal-to-serosal direction using duodenal, jejunal, and colonic membranes. The concentration dependence of jejunal permeation in the absorptive and secretory directions was consistent with saturability of a secretory transport system. Using a monoclonal antibody to inhibit P-glycoprotein-mediated secretion caused a significant enhancement of verapamil absorption through the jejunum. In contrast, the rat ileum did not preferentially transport verapamil in the secretory direction, and the P-glycoprotein antibody had no effect on ileal absorption. Chlorpromazine and propantheline enhanced the mucosal-to-serosal permeation of verapamil through the jejunum, most likely due to competitive inhibition of the P-glycoprotein-mediated secretory process. Vinblastine, tetraethylammonium, and guanidine did not affect verapamil permeation. Propantheline was also a substrate for P-glycoprotein-mediated secretory transport, but in contrast to verapamil, propantheline secretory transport was expressed in rat ileum. **Conclusions.** These results suggest that these cationic compounds are transported by plural P-glycoprotein-mediated efflux systems with different substrate specificities depending on the intestinal site.

KEY WORDS: verapamil; P-glycoprotein; absorption; intestine; propantheline; secretion; organic cation.

INTRODUCTION

Many types of tumor cells become resistant to cytotoxic drugs upon developing the ability to reduce the cellular accumulation of these agents (1). A common mechanism of reduced cellular drug accumulation is the increased expression of P-glycoprotein, a membrane transporter which efficiently removes drugs from these cells (2). Because P-glycoprotein has broad substrate specificity, tumor cells expressing P-glycoprotein are multidrug resistant (MDR). Immunocytochemistry, using a monoclonal antibody against P-glycoprotein from MDR tumor cell lines, showed that similar or identical P-glycoproteins were present in epithelia of normal human tissues, including the liver, brain, kidney, and gastrointestinal tract (3,4). The polarized expression of P-glycoprotein in excretory organs such as the kidney and liver, is likely to be related to a secretory detoxifying function (4). Although the physiological function of P-glycoprotein in the gastrointestinal tract is not known, one effect of this protein is to secrete or restrict the absorption of potentially toxic compounds (3,4). The intestinal epithelial P-glycoprotein could thus also influence the oral absorption of drugs. Several studies have recently shown that the P-glycoprotein-mediated efflux system contributes to the low oral bioavailability of some hydrophobic drugs and peptides (5–9). These studies utilized the human colorectal carcinoma-derived cell line, Caco-2, which is a useful *in vitro* model for the studying drug absorption (10,11). However, there is presently no information describing how P-glycoprotein-mediated efflux influences the absorption of drugs in commonly used animal models.

Recently, Hsing *et al.* (12) demonstrated the existence of P-glycoprotein on the brush-border membrane of rat intestinal mucosa. The molecular mass of the P-glycoprotein in brush-border membranes from rat jejunum was ~160 kDa, similar to that found in rat liver canalicular membrane vesicles (12). P-glycoproteins of lower molecular weights (143 kDa and 108 kDa) were also found in rat liver canalicular membranes by Müller *et al.* (13). Moreover, Croop *et al.* (14) indicated that three different types of mouse multidrug resistance genes are expressed in a tissue-specific manner in normal mouse tissues. These results suggest that there are several forms of P-glycoprotein expressed in normal rodent tissues, possibly including the intestine. It is not known whether Caco-2 cells express multiple forms of P-glycoprotein.

P-glycoprotein in MDR tumor cells appears to exhibit relatively broad substrate specificity (15). P-glycoprotein substrates are generally amphiphilic, have partition coefficients greater than 2, and are protonated at physiological pH(1). Among the compounds that have been reported as P-glycoprotein inhibitors or drug resistance modifiers are calcium channel blockers (15,16). Yusa and Tsuruo (17) demonstrated that verapamil, a potent inhibitor of P-glycoprotein, binds to the protein directly and is actively transported outward across the plasma membrane of MDR tumor cells. In studies using Caco-2 cells, verapamil has often been used as an inhibitor to prove the involvement of a P-glycoprotein-mediated efflux system (5,6), but it has not been shown that verapamil is transported by intestinal P-glycoprotein. Verapamil is well absorbed following oral administration to humans, dogs, and rats (18). If verapamil is a substrate of intestinal P-glycoprotein and is efficiently secreted by this efflux system, the *in vivo* absorption of verapamil might be expected to be low. However, it is possible that verapamil is not a direct substrate (competitive inhibitor), but is a non-transportable (noncompetitive) inhibitor of normal intestinal P-glycoprotein. Alternatively, the intestinal efflux system mediated by P-glycoprotein could be easily saturated after typical oral doses, and contribute little relative to passive diffusion. In this work we examined the involvement of P-glycoprotein in the transport of verapamil through rat small intestine *in vitro*. Because MDR tumor cell P-glycoprotein has broad specificity, other lipophilic cationic drugs were also evaluated as substrates for P-glycoprotein-mediated intestinal efflux, to begin to address the substrate

[1] Research and Development, The DuPont Merck Pharmaceutical Co., P.O. Box 80400, Wilmington, DE 19880-0400.
[2] Present address: Department of Pharmacy, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan.
[3] To whom correspondence should be addressed.

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specificity of rat intestinal P-glycoprotein. Known substrates of other secretory organic cation transport systems were examined as inhibitors of verapamil secretory transport, to determine whether these various secretory mechanisms transport verapamil. An important aspect affecting oral bioavailability is the site-dependence of permeation, and this was also evaluated in these *in vitro* intestinal permeation studies.

MATERIALS AND METHODS

Materials

Verapamil hydrochloride, chlorpromazine hydrochloride, vinblastine sulfate, propantheline bromide, tetraethylammonium chloride, sodium taurodeoxycholate, sodium deoxycholate, cefazolin sodium, and theophylline were purchased from Sigma Chemical Co. (St. Louis, MO). A monoclonal antibody to the 170–180 kD MDR-related P-glycoprotein (anit-P-glycoprotein 170–180) was obtained from Boehringer Mannheim (Indianapolis, IN). All other reagents were of the highest grade available.

Isolation of Intestinal Segments

All experiments used 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. The buffer was saturated with 95% O_2 : 5% CO_2 after preparation, and refrigerated until use. The pH was 7.4 in most experiments, unless stated otherwise.

The animal experiments were in compliance with the *Guide for the Care and Use of Laboratory Animals,* NIH publication 86–23. Male Sprague Dawley-derived rats (Crl: CD(SD)BR, Charles River, Kingston, NY) weighing 300–350 g were used. Rats were fasted at least 16 h before tissue procurement. Rats were anesthetized with ether and an abdominal incision was made. The entire small intestine was immediately removed and rinsed in ice-cold saline. Usually the 10 cm immediately distal to the pylorus was cut off, and jejunum was used. In studies to compare intestinal sites, segments of duodenum (immediately distal to pylorus), ileum (15 cm above the ileocecal junction), and colon were obtained. After flushing out the intestinal contents with ice-cold saline, 4 to 5 segments of approximately 3 cm length were isolated from each rat and placed in ice-cold Tyrode's buffer. Peyer's patches were identified visually, and sections containing them were not used in this study.

Intestinal Permeation Experiments

Permeation experiments were performed using methods similar to those described by Grass and Sweetana (19) and diffusion cells obtained from Precision Instrument Design (Los Altos, CA). The intestinal segment was pulled onto a glass rod and the fat adhering to the mesentery was removed. The segment was cut open with a scalpel and spread onto a paper filter to expose the epithelial surface. After washing the epithelial surface with ice-cold saline, the intestinal sheet was mounted onto the pins of the diffusion cells, 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, both of which had been pre-heated to 37°C. The temperature of the diffusion cells was maintained at 37°C using heating blocks. The donor and receiving fluids were circulated by gas lift with O_2/CO_2. Receiving chamber samples (0.5 ml) were removed at 15, 30, 45, 60, 90, and 120 min and replaced with drug-free buffer. Trifluoroacetic acid (0.1 ml of 0.3% solution) was immediately added to each sample and the samples were frozen until assayed. The surface area available for diffusion in this study was 1.78 cm^2.

The drug concentration in each receiving chamber sample was determined, and the cumulative amount of drug permeating the membrane was calculated based on the chamber volume and the volumes replaced with drug-free buffer. The cumulative amount that permeated the membrane was plotted *v.* time for each experiment. The permeation rate was calculated from the linear portion of the amount permeating *v.* time plot. Verapamil, theophylline, and cefazolin lag times were generally low ($\leq$ 15 min). Propantheline generally had slightly longer lag times ($\leq$ 30 min), and chlorpromazine had considerable lag times ($\approx$ 60 min). Permeability coefficients were calculated by dividing permeation rates by the initial drug concentration. There were 3 or more experiments for each group, using intestines from 3 or more separate rats. Statistical comparisons of permeation rates or permeability coefficients were made using unpaired *t*-tests.

Inhibition studies were performed with substrate and inhibitor added simultaneously. Various inhibitor concentrations were used, as reported in the results. The P-glycoprotein antibody was also added at the same time as the substrate, and was used at 2 μg/ml.

Partitioning

An aqueous drug solution (0.2 mM drug in Tyrode's buffer, pH 7.4) was prepared and 3 ml was shaken with an equal volume of n-octanol for 30 min, and then centrifuged to separate the phases. As a control, 3 ml of the same aqueous solution was shaken without n-octanol. The drug concentration in the n-octanol was obtained as the difference in the concentrations of aqueous test and control solutions.

Analyses

The concentrations of verapamil, propantheline, chlorpromazine, cefazolin, and theophylline were determined by HPLC using UV absorbance detection. Assay conditions for verapamil, propantheline, and chlorpromazine were as follows: column, Nova-Pak® C-18 (4μm, 3.9 × 150 mm, Waters, Milford, MA); mobile phase, acetonitrile/0.05 M phosphoric acid/triethylamine (40/60/0.1); flow rate, 0.6 ml/min; wavelength, 230 nm; and column temperature, 35°C. Cefazolin and theophylline were determined as follows: column, LiChrospher® 60 RP-select B (5 μm, 4 × 125 mm, E. Merck, Darmstadt, Germany); mobile phase, acetonitrile/0.01 M, pH 4 sodium acetate (12/88); flow rate, 0.6 ml/min; wavelength, 270 nm; and column temperature, ambient. The injection volume was 50-200 μl.

RESULTS

Characteristics of Intestinal Permeation of Verapamil

A comparison of the profiles of verapamil, cefazolin,

and theophylline permeation across rat jejunum in the mucosal-to-serosal direction is given in Figure 1. Theophylline, which is known to be well-absorbed orally (20), showed much higher intestinal permeability than cefazolin, which is a poorly membrane-permeable model compound (21). The difference in intestinal permeabilities of these two model compounds is consistent with their *in vivo* bioavailabilities. The permeability of verapamil was much lower than that of theophylline, and similar to that of cefazolin. Verapamil distributed almost completely into n-octanol from pH 7.4 buffer, confirming that this compound is highly lipophilic.

Theophylline had the same jejunal permeation rates in the mucosal-to-serosal and serosal-to-mucosal directions (Figure 2). Assuming that theophylline permeates the intestinal membrane by simple diffusion (20), this data suggests that passively absorbed compounds should show similar intestinal permeabilities in the absorptive and secretory directions. In contrast to theophylline, the serosal-to-mucosal permeation of verapamil was more than 4-fold greater than its mucosal-to-serosal permeation, suggesting that the net movement of verapamil across the rat jejunum is preferentially in the secretory direction.

Mucosal-to-serosal and serosal-to-mucosal permeability coefficients were determined at three different concentrations of verapamil. Permeability coefficients were obtained by dividing permeation rates by the donor verapamil concentration. As shown in Figure 3, permeability coefficients in the absorptive direction increased with increasing mucosal concentrations of verapamil. Permeability coefficients in the secretory direction decreased with increasing concentrations of verapamil in the donor compartment. The ratio of secretory/absorptive permeation rates therefore decreased with increasing verapamil concentrations. These data suggest that the rat jejunum has a potent efflux system, transporting verapamil effectively from the serosal side to the mucosal side, and that this secretory transport is concentration dependent. Secretory transport was greater than absorptive transport at all concentrations tested.

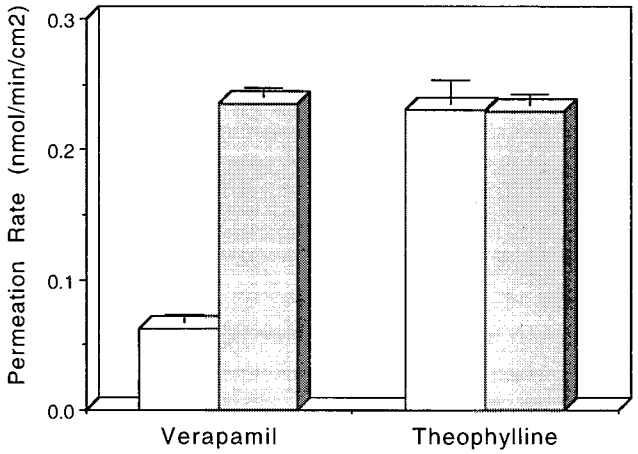

Fig. 2. Mucosal-to-serosal (open bars) and serosal-to-mucosal (shaded bars) permeation rates for verapamil and theophylline using rat jejunum. The concentration of each drug was 0.2 mM. Each bar represents the mean ± SE of 3-5 determinations.

Site-Dependence and Effects of P-Glycoprotein Monoclonal Antibody on Verapamil Permeation

The site-dependence of verapamil permeation was examined in additional studies using duodenum, ileum, and colon. As shown in Table I, duodenum and colon, like the jejunum, had lower mucosal-to-serosal permeation rates than in the serosal-to-mucosal direction. The mucosal-to-serosal permeation rates for these three sites were similar. However, the ileal mucosal-to-serosal permeation rate was about 4-fold greater than the permeation rates for duodenum, jejunum, or colon. Moreover, ileal permeation in the absorptive direction was the same as that in the secretory direction, suggesting the absence of any efflux system for verapamil in the rat ileum.

In order to clarify whether the efflux of verapamil is mediated by P-glycoprotein, the effect of a P-glycoprotein monoclonal antibody on mucosal-to-serosal permeation of

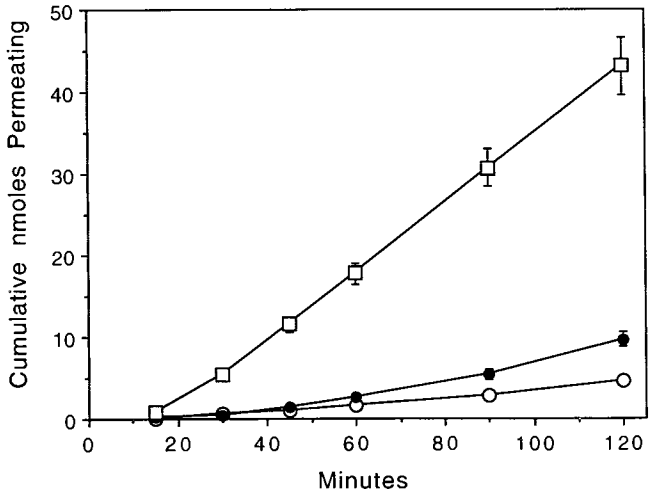

Fig. 1. Permeation profiles of verapamil (●), cefazolin (○), and theophylline (□) across rat jejunum. The concentration of each drug was 0.2 mM. Each point represents the mean ± SE of 3-5 determinations.

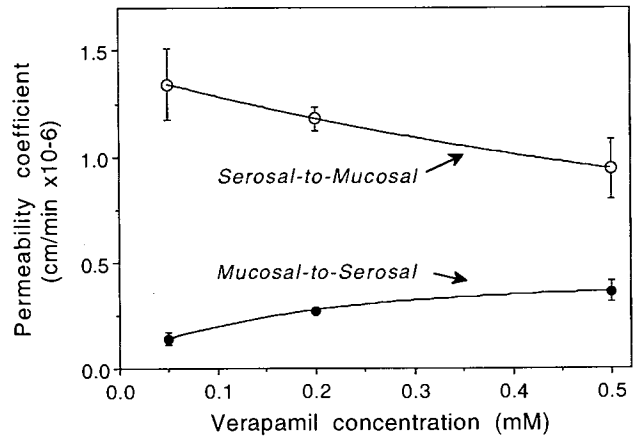

Fig. 3. Concentration dependence of verapamil permeability coefficients (P) for rat jejunum. Each point represents the mean ± SE of 4-5 determinations. The differences between 0.05 mM and 0.2 mM or 0.5 mM mucosal-to-serosal P values were significant, and the difference between 0.05 mM and 0.5 mM serosal-to-mucosal P values was significant.

Table I. Site-Dependence of Verapamil Permeation and the Effects of a Monoclonal Antibody to P-Glycoprotein (P-GP Antibody)[a]

Site	Permeation rate (nmol/min/cm^2)[b] Mucosal-to-serosal	Serosal-to-mucosal	S to M/ M to S
Duodenum	0.040 ± 0.005	0.184 ± 0.022	4.60
Jejunum	0.062 ± 0.002	0.235 ± 0.011	3.79
Ileum	0.201 ± 0.009	0.204 ± 0.025	1.01
Colon	0.051 ± 0.008	0.133 ± 0.025	2.61
Jejunum + P-GP antibody	0.110 ± 0.001[c]	ND[d]	
Ileum + P-GP antibody	0.232 ± 0.014[e]	ND[d]	

[a] The verapamil concentration was 0.2 mM, and the P-glycoprotein antibody was added to the mucosal solution at 2 μg/ml.
[b] Mean ± SE of 3–5 determinations.
[c] Significantly different ($p < 0.005$) from jejunum without P-GP antibody.
[d] Not determined.
[e] Not significantly different from ileum without P-GP antibody.

verapamil was examined using jejunum and ileum. In the presence of the antibody, mucosal-to-serosal verapamil permeation through the jejunum was markedly enhanced (Table I). In contrast, the permeation of verapamil through the ileum was not significantly affected. These data imply that jejunal mucosal-to-serosal permeation of verapamil is lower than ileal permeation because of P-glycoprotein-mediated transport out of the epithelial cells into the lumen. In experiments to be reported elsewhere, the effects of the P-glycoprotein antibody on D-tryptophan permeation were evaluated; D-tryptophan being used as a model polar compound not transported by P-glycoprotein. The antibody had no effect on D-tryptophan permeability.

Effects of Organic Cations on Mucosal-to-Serosal Verapamil Permeation

Competitive inhibitors of efflux are expected to increase mucosal-to-serosal verapamil permeation when added to the mucosal donor solutions. Table II shows the effects of various organic cations on verapamil permeation in the mucosal-to-serosal direction. Chlorpromazine (0.2 mM) and propantheline (1 mM) enhanced verapamil permeation by 155 and 195%, respectively. Vinblastine and tetraethylammonium (TEA) failed to significantly affect verapamil permeation in the absorptive direction. Chlorpromazine and vinblastine were only tested at 0.2 mM because of their limited aqueous solubilities. Verapamil permeation was not affected by the presence of a mucosal-to-serosal proton gradient. Guanidine did not significantly affect verapamil permeation in the presence of a proton gradient.

Chlorpromazine and Propantheline Permeation Across Jejunum and Ileum

Since chlorpromazine and propantheline affected verapamil permeation, these drugs could also be substrates for P-glycoprotein-mediated efflux. Therefore, the permeation characteristics of these two organic cations were examined. As shown in Figure 4, chlorpromazine permeation across rat jejunum was much greater in the secretory direction than in the absorptive direction. Propantheline permeation also strongly favored secretion, as summarized in Table III. Chlorpromazine and propantheline permeation through rat jejunum in the secretory direction may occur in part via the same intestinal efflux system responsible for verapamil secretion. As shown in Table III, the ileum also had net secretory permeation of propantheline, whereas this was not the case with verapamil. The ileal serosal-to-mucosal permeability coefficient of propantheline was 1.104 ± 0.113 ($\times 10^{-6}$ cm/min) at 0.2 mM, *v.* 0.481 ± 0.010 ($\times 10^{-6}$ cm/min) at 1 mM, showing concentration dependence. The mucosal addition of the P-glycoprotein antibody stimulated propantheline permeation in the absorptive direction by 155%. Therefore, it seems likely that P-glycoprotein contributes to the ileal efflux of propantheline, but not verapamil.

Bile Acid Effects

It was thought that one possible way for verapamil to escape the effects of P-glycoprotein could involve an elec-

Table II. Effects of Various Organic Cations on Mucosal-to-Serosal Verapamil Permeation Across the Rat Jejunum[a]

Organic cation	Concentration (mM)	Permeation rate (nmol/min/cm^2)[b]	Percentage of control
Control		0.062 ± 0.006	100
Chlorpromazine	0.2	0.096 ± 0.009[c]	155
Vinblastine	0.2	0.055 ± 0.007	89
Propantheline	1	0.121 ± 0.002[c]	195
Tetraethylammonium	0.2	0.059 ± 0.008	95
Tetraethylammonium	10	0.046 ± 0.008	74
Control, pH 5		0.058 ± 0.006	
Guanidine, pH 5	10	0.080 ± 0.013	138

[a] Verapamil permeation was determined from a 0.2 mM solution at pH 7.4, unless stated otherwise, in the absence (control) or presence of each organic cation listed.
[b] Mean ± SE of 3–5 determinations.
[c] Significantly ($p < 0.05$) different from control.

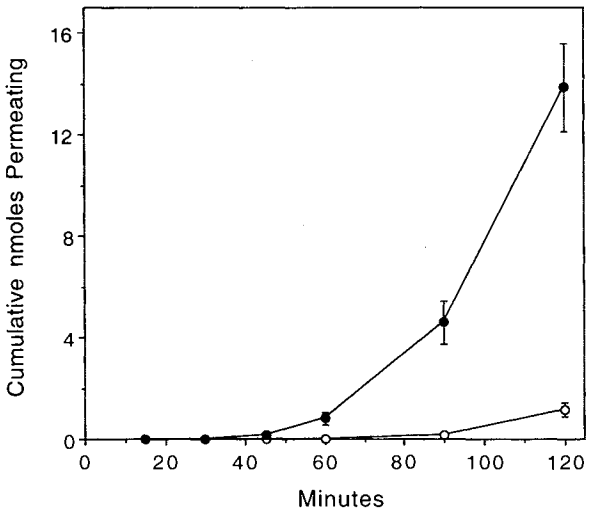

Fig. 4. Comparison of chlorpromazine permeation profiles in the mucosal-to-serosal (○) and serosal-to-mucosal (●) directions using rat jejunum. The chlorpromazine concentration was 0.2 mM. Each point represents the mean ± SE of 3 or 4 determinations.

trostatic interaction between verapamil and endogenous anions in the absorption process. To examine the effects of endogenous organic anions on verapamil permeation, taurodeoxycholic acid and deoxycholic acid were tested in separate permeation experiments. At 1 mM concentrations these two anions had no effect on verapamil permeation (data not shown).

DISCUSSION

Since Tsuruo *et al.* (22) demonstrated that verapamil reversed the resistance to vincristine and vinblastine displayed by MDR tumor cell lines, this compound has attracted considerable attention as a potent MDR modifier (23). According to Yusa and Tsuruo (17), verapamil directly binds to, and is transported by, MDR tumor cell P-glycoprotein.

P-glycoprotein was also identified in normal human tissues, including intestine (jejunum and colon), as indicated by the reaction to a mouse P-glycoprotein monoclonal antibody (3). Recent studies using Caco-2 cells have suggested the possibility that the P-glycoprotein efflux system is one of the major reasons for low oral bioavailability of some hydrophobic compounds (5–9). Since the localization of P-glycoprotein on the rat intestinal mucosa has also been described (12), and since verapamil has often been utilized as a potent inhibitor of MDR tumor cell and Caco-2 P-glycoprotein, we evaluated the mechanisms of verapamil permeation through rat intestine.

Table III. Permeation of Propantheline Across Rat Intestine[a]

Site	Permeation rate (nmol/min/cm²)[b] Mucosal-to-serosal	Serosal-to-mucosal	S to M/ M to S
Jejunum	0.090 ± 0.019	0.553 ± 0.068	6.14
Ileum	0.047 ± 0.006	0.481 ± 0.010	10.2
Ileum + P-GP antibody	0.073 ± 0.005[c]	Not determined	

[a] The propantheline concentration was 1.0 mM, and the P-glycoprotein antibody (P-GP antibody) was added to the mucosal solution at 2 μg/ml.
[b] Mean ± SE of 3 or 4 determinations.
[c] Significantly different ($p < 0.05$) from ileum without P-GP antibody).

The jejunal permeation rates of theophylline and cefazolin in the mucosal-to-serosal direction reflected their differences in oral bioavailability (Figure 1). Accordingly, it was expected that verapamil would exhibit relatively high jejunal permeability on the basis of its lipophilicity and its good absorption *in vivo*. However, this was only true for permeation in the serosal-to-mucosal direction. Verapamil permeation in the mucosal-to-serosal direction was much lower than that of theophylline, which was used as a model compound with good absorption. Verapamil permeation across the jejunum strongly favored the secretory direction (Figure 2). This is evidence of a potent efflux system. Decreased serosal-to-mucosal permeability coefficients and increased mucosal-to-serosal permeability coefficients with increasing verapamil concentrations (Figure 3), indicated that the efflux is concentration dependent. Moreover, a monoclonal antibody to MDR tumor cell P-glycoprotein enhanced the mucosal-to-serosal permeation of verapamil in the jejunum (Table I). The antibody apparently interacted with P-glycoprotein and inhibited its secretory function. The modification of drug efflux by a specific P-glycoprotein antibody has previously been shown using Caco-2 cells (8). In view of preferential transport in the secretory direction, concentration dependence, and the effects of the P-glycoprotein antibody, it can be concluded that verapamil is secreted by P-glycoprotein in the rat jejunum.

The duodenum and colon also showed greater serosal-to-mucosal permeation than mucosal-to-serosal permeation (Table I). Although the effect of the P-glycoprotein antibody was not examined in these segments, the P-glycoprotein-mediated efflux system appears to be expressed in these intestinal sites. Interestingly, verapamil permeation across the ileum was the same in both directions (Table I), suggesting the lack of an ileal efflux transport system. Absorption of verapamil from the ileum may be one reason why it is well absorbed *in vivo*. Sustained-release verapamil dosage forms, which deliver more of a dose to the lower intestine, do not result in a loss of bioavailability. Some studies have reported an increase in bioavailability with sustained release verapamil, relative to immediate-release dosage forms (24,25), even though verapamil is subject to extensive first-pass metabolism.

Hsing *et al.* (12) identified P-glycoprotein on the brush-border membrane of both rat jejunum and ileum. However, our results indicate that ileal P-glycoprotein is not involved in verapamil permeation. In the case of propantheline, in contrast, ileal permeation favored the secretory direction and permeation in the absorptive direction was enhanced in the presence of the P-glycoprotein antibody, consistent with a functioning ileal P-glycoprotein-mediated efflux system. Consequently, it appears that jejunal and ileal P-glycoprotein transport systems have different substrate specificities.

Chlorpromazine, which is very lipophilic and is known

as a potent multidrug resistance modifier (16), exhibited significant enhancement of verapamil mucosal-to-serosal permeation (Table II). In addition to competitive inhibition of P-glycoprotein, other mechanisms of permeation enhancement are possible. Chlorpromazine is highly bound to the brush-border membrane (26), and an interaction with verapamil binding could be related to enhanced permeation. Chlorpromazine also alters membrane fluidity (27), which could have enhanced passive membrane permeation. Based on the finding that chlorpromazine showed greater serosal-to-mucosal permeation than mucosal-to-serosal permeation in jejunum, similar to verapamil (Figure 4), it seems likely that chlorpromazine is a substrate of a jejunal efflux system.

It was previously shown by Turnheim and Lauterbach (28) that TEA and other quaternary ammonium compounds are transported by an intestinal secretory mechanism in guinea pig jejunum, and that secretory transport limits absorption. TEA is also a well known substrate for the organic cation secretory system located on the renal brush-border membrane, but is not a substrate for MDR tumor cell P-glycoprotein (29). In this study TEA did not alter verapamil permeation, even at a high concentration of 10 mM. It seems probable, therefore, that the mechanisms of TEA and verapamil transport across rat jejunum are independent.

A separate organic cation secretory transport system was also described based on studies with rabbit small intestine brush border membrane vesicles (30). Guanidine transport by this system was activated by a proton gradient (proton antiport), and was inhibited by imipramine but not by TEA. Verapamil permeation was not affected by the presence of a proton gradient and there was no interaction with guanidine (Table III). Thus, we found no evidence that the secretory transport of verapamil is mediated by this organic cation transport system.

The finding that vinblastine did not significantly alter verapamil permeation in the absorptive direction were against our expectations because this anticancer drug has been reported to be an efficient substrate of P-glycoprotein in Caco-2 (7,8) and MDR tumor cells (22). A possible explanation could be that vinblastine has lower affinity, relative to verapamil, for rat jejunal P-glycoprotein compared to its relative affinity for Caco-2 or MDR tumor cell P-glycoprotein. Vinblastine permeation in the presence and absence of verapamil and other organic cations is now under investigation.

In conclusion, verapamil is efficiently secreted as a substrate of intestinal P-glycoprotein in the upper intestine and colon. Rat ileal P-glycoprotein does not transport verapamil efficiently, but efficiently transports propantheline. The rat intestine thus appears to have multiple P-glycoprotein transport systems with distinct substrate specificities, depending on the intestinal site. These transport systems are expected to affect oral bioavailability. Because these transport systems are site-dependent, studies of the involvement of P-glycoprotein-mediated efflux in the intestinal absorption of drugs should be done using different intestinal sites. This information might not be obtained using epithelial cell cultures as an intestinal absorption model.

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