Drug Absorption Limited by P-Glycoprotein-Mediated Secretory Drug Transport in Human Intestinal Epithelial Caco-2 Cell Layers

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The hypothesis was tested that the operation of an ATP-dependent export pump localized at the apical (brush border) surface of the intestinal epithelium may limit substrate absorption kinetics. Human intestinal Caco-2 cell-layers display saturable secretion of vinblastine from basal to apical surfaces (K_m, 18.99 ± 5.55 μM; V_{max}, 1285.9 ± 281.2 pmol cm^{-2} hr^{-1}) that is inhibited by verapamil, consistent with the expression of the ATP-dependent P-glycoprotein drug efflux pump at the apical brush border membrane. Inhibition of P-glycoprotein by a variety of modulators (verapamil, 1,9-dideoxyforskolin, nifedipine, and taxotere) is associated with an increased vinblastine absorptive permeability. Vinblastine absorption displayed a nonlinear dependence upon luminal (apical) vinblastine concentration, and vinblastine absorption increased markedly at concentrations where vinblastine secretory flux was saturated (>20 μM). Upon inhibition of P-glycoprotein by verapamil and 1,9-dideoxyforskolin, vinblastine absorption increased and was linearly dependent on vinblastine concentration. The limitation of P-glycoprotein substrate absorption by active ATP-dependent export via P-glycoprotein is discussed, together with the possibility that other classes of substrate may be substrates for different ATP-dependent export pumps.

KEY WORDS: absorption limitation; Caco-2 cells; P-glycoprotein; multidrug resistance; intestine; oral drug delivery; vinblastine; verapamil.

INTRODUCTION

P-glycoprotein (*MDR*1 gene product) is a 170- to 180-kDa membrane glycoprotein associated with pleiotropic (multidrug) resistance (MDR) in tumor cells (1,2). P-glycoprotein functions as an ATP-dependent drug-efflux pump, thereby reducing the intracellular accumulation of a wide variety of chemotherapeutic agents (1,2). Immunohistochemical techniques have demonstrated the presence of P-glycoprotein in the apical region of several natural epithelia, especially in the gastrointestinal tract (small intestine, colon), liver (bile duct), and kidney (3,4). In the mouse small intestine, P-glycoprotein expression and vinblastine efflux are progressively increased from crypt to villus (5). Since expression of P-glycoprotein is at the apical epithelial cell border, transepithelial secretion of P-glycoprotein substrates is predicted. In human intestinal (colonic) T84 and HCT-8 epithelial layers, P-glycoprotein expression, confirmed by Western blotting of cell homogenates by a monoclonal antibody to P-glycoprotein, is associated with the transepithelial secretion of vinblastine (6). Such data suggested the hypothesis that P-glycoprotein may function in detoxification and to limit intestinal permeation of natural toxic compounds found as normal constituents of diet. It should be noted that many anticancer cytotoxic agents, e.g., vinblastine and vincristine, were originally isolated from plant and microbial natural sources [*vinca* alkaloids from *Catharanthus roseus* (7)].

Prediction of the likely oral bioavailability of drug candidates is often approximated by diffusion-solubility models utilizing correlations with octanol (or other hydrocarbon)/water partition coefficients (8). Extensions to such simple models have included corrections for unstirred layer phenomena, the pH-partition hypothesis for ionized molecules (9,10) and a parallel-pathway model of permeation comprising a lipophilic cellular path and a hydrophillic heteroporous barrier (cellular aqueous pores and paracellular pores) (11). Such models are often sufficient to encompass the behavior of many relevant chemical entities (11). For compounds such as the *vinca* alkaloids these models may fail to describe their transintestinal (epithelial) absorption since they are also substrates for ATP-dependent active transport. In this paper we tested the hypothesis that a high-capacity efflux pump located on the apical border of the intestinal epithelium may significantly alter drug absorption kinetics and explore the limits of such an effect.

Several epithelial cell lines are capable of forming intact epithelial layers when grown upon permeable matrices, including the renal MDCK cell line (12,13) and several cell lines derived from human intestinal adenocarcinomas, such as T_{84} and HCT-8 (6). The Caco-2 cell-line is increasingly used as an *in vitro* model for absorption studies (14–19). This human colonic adenocarcinoma cell line expresses brush border membrane hydrolases (17) and brush border Na^+-dependent membrane transporters (18) and the di-tripeptide transporter (19) consistent with a predominately small intestinal phenotype (17–19). Calcium antagonists (e.g., verapamil), calmodulin inhibitors (e.g., trifluoperazine), and other agents (e.g., reserpine) inhibit the active drug efflux and restore drug sensitivity in multidrug resistant cells (2,20) by a direct effect on P-glycoprotein (21). Such inhibition permits one to define P-glycoprotein-mediated fluxes (7,13). The Caco-2 cell line also displays verapamil-sensitive vinblastine secretion, with a maximal transport capacity for vinblastine an order of magnitude greater than that expressed in dog kidney renal cells (22).

MATERIALS AND METHODS

Materials

[^{3}H]Vinblastine sulfate and [^{3}H]mannitol were obtained from Amersham International (Little Chalfont, Bucks, UK). All tissue culture media and reagents (Gibco BRL), tissue culture plastics, and tissue culture inserts (Nunc) were supplied by Life Technologies Ltd. (Paisley, Scotland). 1,9-Dideoxyforskolin was obtained from Calbiochem (Novabiochem, Nottingham, UK). (R)- and (S)-isomers of verapamil were a gift from Dr. H. Tobias (Knoll AG, Ludwigshafen,

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Germany), and taxotere was a gift from J. L. Fabre (Rhône-Poulenc Rorer, Vitry sur Seine, France). All other chemicals were obtained from Sigma Chemical Co. (Poole, Dorset) or Merck (Poole, Dorset).

Cell Culture

Caco-2 cells (85–100 serial passages) were maintained in culture in DMEM (4500 mg L^{-1} glucose) supplemented with 10% fetal calf serum (FCS), nonessential amino acids (1%, v/v), 2 m*M* glutamine, and gentamycin (50 μg mL^{-1}). Confluent monolayers were subcultured every 7 days by treatment with 0.05% trypsin and 0.02% EDTA in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS). All cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air.

For flux experiments Caco-2 cells were grown as epithelial layers by high-density seeding (10^6 cells/mL, 3 mL/well) onto permeable filter matrices (Nunc 25-mm culture inserts, 4-cm^2 growth area). Culture inserts were incubated in six-well plates for 14–15 days. The formation of functional epithelial layers was monitored visually and by the development of a significant transepithelial resistance (R_T), as measured using a WPI Evometer fitted with "chopstick" electrodes to allow transepithelial current passage and potential sensing. Cell monolayers were used when the transepithelial resistance exceeded 300 Ω cm^2. Values of resistance for filter inserts alone, ~300 Ω cm^2, are subtracted from all data.

Measurement of Bidirectional Transepithelial [^{3}H]Vinblastine Sulfate Fluxes

Measurements of transepithelial solute flux were made essentially as described by Hunter *et al.* (6). Functional epithelial layers in filter cups were washed with 2 × 3 mL serum-free DMEM (including glutamine, gentamycin, and nonessential amino acids) and placed in fresh six-well plates containing 3 mL serum-free DMEM (basal solution); a further 3 mL serum-free DMEM was then pipetted into the upper chamber (apical solution) of the filter cup. Transepithelial resistance was measured following 30-min incubation of the cells at 37°C, as described above.

Under limited lighting conditions throughout, the medium on either the apical or the basal side of the monolayers was removed and replaced with 3 mL serum-free DMEM containing [^{3}H]vinblastine sulfate as tracer (or in some cases [^{3}H]mannitol), and that on the contralateral side replaced with 3 mL serum-free DMEM containing the same vinblastine sulfate concentration, in the presence or absence of modulators [dissolved in DMSO, so the total DMSO concentration of final solution did not exceed 1% (v/v)], followed by incubation in the dark at 37°C. In order to measure the bidirectional fluxes of vinblastine sulfate ($J_{a\text{-}b}$, flux from apical to basal solutions, and $J_{b\text{-}a}$, flux from basal to apical solutions), 100-μL samples of medium from either the tracer-containing compartment or the contralateral compartment were taken at regular intervals, and ^{3}H activities in these samples were determined by liquid scintillation counting. Each incubation was performed at least in triplicate. On completion of the flux experiments epithelial integrity was determined by measurement of transepithelial resistance. Net vinblastine flux was calculated as follows $J_{net} = J_{b\text{-}a} - J_{a\text{-}b}$ from paired filters where bidirectional fluxes were determined from the slope of the linear regression line of flux versus time (Fig. 1).

In order to pool data from separate experiments apparent permeabilities were calculated (the rate of vinblastine flux, in pmol cm^{-2} hr^{-1}, divided by the concentration of vinblastine added, i.e., $J_{a\text{-}b}/C$; units, cm hr^{-1}) to correct for differences in vinblastine concentrations used in the various experiments.

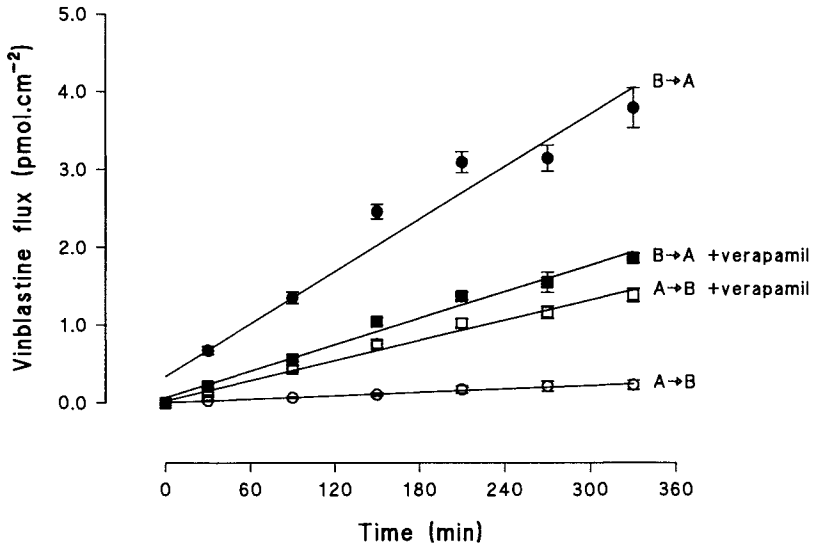

Fig. 1. Transepithelial flux of vinblastine across human intestinal Caco-2 cell layers. Vinblastine fluxes in the absorptive (A–B; ○) and secretory (B–A; ●) directions are plotted against time of incubation. Absorptive (□) and secretory fluxes (■) in the presence of 100 μ*M* verapamil are also illustrated. Concentration of vinblastine in all instances was 10 n*M*. Data are illustrated as mean ± SE (n = 6).

Statistics

Data are expressed as mean ± SE of n separate Caco-2 epithelial layers. Tests of significances of differences between mean values were made using a two-tailed Student's t test (for unpaired mean data) or Mann–Whitney U test (two-tailed, unpaired data), where appropriate. Kinetic constants for Michaelis–Menton kinetics were calculated by nonlinear regression with the method of least-squares fitting (Enzfitter, Elsevier, U.K.).

RESULTS

Transepithelial Vinblastine Flux

The fluxes of vinblastine sulfate across fully confluent monolayers of Caco-2 epithelial layers are linear with time up to 6 hr (Fig. 1) under all conditions. All subsequent flux rates were calculated by linear regression of such data. Transepithelial fluxes show a marked asymmetry (Fig. 1), with basal-to-apical flux ($J_{b\text{-}a}$) exceeding those from apical-to-basal surfaces ($J_{a\text{-}b}$). A net flux ($J_{net} = J_{b\text{-}a} - J_{a\text{-}b}$) of vinblastine sulfate was therefore observed in the secretory basal-to-apical direction for Caco-2 cells. The apparent permeability measured at 10 nM vinblastine (vinblastine flux/vinblastine concentration) in the absorptive direction (apical-to-basal) ($P_{a\text{-}b}$) was $0.37 \pm 0.04 \times 10^{-2}$ cm hr^{-1} (n = 58), an order of magnitude less than that observed in the basal-to-apical direction ($P_{b\text{-}a}$) $4.39 \pm 0.18 \times 10^{-2}$ cm hr^{-1} (n = 63; $P < 0.001$) (Fig. 2a). This difference is not observed with extracellular marker fluxes such as mannitol whose transepithelial fluxes are approximately equal [$J_{a\text{-}b} = 65.3 \pm 6.5$ (n = 6) fmol cm^{-2} hr^{-1}, $J_{b\text{-}a} = 60.0 \pm 5.8$ (n = 6) fmol cm^{-2} hr^{-1}; $P > 0.5$], consistent with transepithelial mannitol flux being passive. The data on vinblastine handling by epithelial layers of Caco-2 cells are, therefore, consistent with the apical expression of P-glycoprotein in these cells, as proposed for T_{84}, HCT-8, and MDCK cells (6,12,13).

The transepithelial resistance (R_T) is an index of the integrity of the epithelial layers and this measure has been shown to correlate with the restriction of inert hydrophillic molecules such as mannitol (see above) by each epithelial layer (16). Transepithelial resistance was not compromised by vinblastine (10 nM–50 μM) over the time course of the experiments; a slight increase in R_T was observed as Caco-2 layers were equilibrated to serum-free medium, from 571.4 ± 46.1 Ω cm^2 (n = 61) at time 0 to 658.0 ± 43.3 Ω cm^2 (n = 60) ($P < 0.1$) on completion of the experiment.

Inhibition of Vinblastine Sulfate Flux

Verapamil, at a concentration of 0.1 mM, reduced the net transport of vinblastine sulfate across the epithelial cell layers; net permeability was reduced from 4.02×10^{-2} to 0.88×10^{-2} cm hr^{-1} ($P < 0.001$) (Fig. 1), primarily by a reduction (approximately 50%) in basal-to-apical apparent permeability from $4.39 \pm 0.18 \times 10^{-2}$ to $2.19 \pm 0.08 \times 10^{-2}$ cm hr^{-1} (n = 23, $P < 0.001$). Further, the inhibition of net vinblastine secretion was accompanied by an approximately fourfold increase in absorptive apical-to-basal permeability

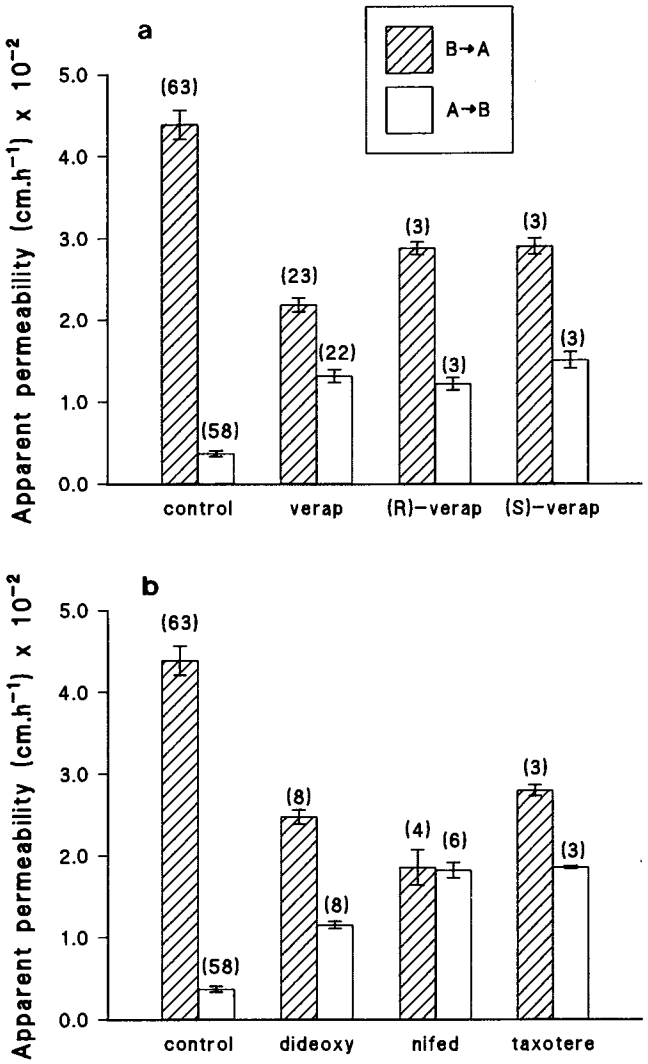

Fig. 2. Inhibition of secretory vinblastine flux is associated with increased absorptive vinblastine permeability. Inhibition of net vinblastine flux by (a) 100 μM verapamil (verap) and the stereoisomers (R)- and (S)-verapamil (100 μM) and (b) 100 μM dideoxyforskolin (dideoxy), 50 μM nifedipine (nifed), and 25 μM taxotere. Note the increased absorptive permeability (A–B) in all cases where secretory vinblastine flux (B–A) is reduced. Figures in parentheses are the number of separate epithelial layers used; data are mean ± SE.

to $1.32 \pm 0.08 \times 10^{-2}$ cm hr^{-1} (n = 22; $P < 0.001$, vs control absorptive permeability; Fig. 1).

No decrease in R_T was observed upon verapamil treatment; R_T prior to treatment with verapamil was 689.5 ± 71.6 Ω cm^2 (n = 36) and this increased to 923.1 ± 72.5 Ω cm^2, upon completion of the experiment. This indicates that the increase in apical-to-basal flux cannot be due to an increased paracellular leak as a result of disruption of the cell layer.

The action of verapamil as a modifier of the multidrug resistance phenotype *in vitro* is not stereospecific (23). This contrasts with the greater potency of the S-isomer of verapamil as a calcium channel antagonist (23). Apparent permeabilities for vinblastine flux in the presence of racemic verapamil and the two enantiomers of verapamil, the R- and S-forms are shown in Fig. 2a. No differences in the ability of the isomers or the racemic mixture of verapamil to inhibit

vinblastine secretory flux (basal to apical) were observed. With all three verapamil preparations, inhibition of net vinblastine secretion was associated with a similar increase in the vinblastine absorptive (apical-to-basal) flux across the monolayers. The transepithelial resistance of the cell monolayers was unaffected by either of the verapamil enantiomers (Table I). The similar results obtained with the two isomers of verapamil indicate that the inhibition of P-glycoprotein mediated vinblastine secretion is unrelated to calcium channel antagonism in these cells.

Several other modulators of P-glycoprotein activity were investigated, for their ability to inhibit vinblastine sulfate flux across the Caco-2 cell monolayers. A second calcium channel antagonist, nifedipine, at 50 μM, reduced secretory, basal-to-apical, permeability to $1.86 \pm 0.22 \times 10^{-2}$ cm hr^{-1} ($n = 4$; $P < 0.01$) while increasing the absorptive, apical-to-basal, permeability to $1.825 \pm 0.094 \times 10^{-2}$ cm hr^{-1} ($n = 6$; $P < 0.01$). Thus, with this inhibitor, net secretory flux was almost totally abolished. Transepithelial resistance measurements again indicated no nonspecific disruption of the cell layers during the experiment (Table I).

We have recently reported that an inactive analogue of the adenylate cyclase activator, forskolin, 1,9-dideoxyforskolin, inhibits P-glycoprotein mediated vinblastine secretion in renal MDCK cell layers (13). In human intestinal Caco-2 cells, a 45% inhibition in basal-to-apical permeability was measured in the presence of 100 μM 1,9-dideoxyforskolin, along with a threefold increase in absorption of vinblastine sulfate (apical-to-basal permeability). Again, no decrease in R_T was observed in the presence of 1,9-dideoxyforskolin (Table I).

Taxotere, an analogue of the anticancer agent taxol (24,25) at 25 μM, reduced secretory basal-to-apical permeability by 36% and increased absorptive apical-to-basal permeability by fivefold (Fig. 2b). Concentrations of taxotere of 50 μM and greater disrupted the cell monolayers, with a consequent reduction in R_T and increase in passive vinblastine leak.

Kinetics of [^{3}H]Vinblastine Sulfate Secretory Flux Across Caco-2 Cell Monolayers

Net basal-to-apical vinblastine sulfate flux (J_{net}) was found to be saturable across Caco-2 cell monolayers and followed Michaelis–Menten kinetics (Fig. 3). An apparent K_m of 18.99 ± 5.55 μM (df = 6) and V_{max} of 1285.9 ± 281.2 pmol cm^{-2} hr^{-1} were determined.

Absorptive Flux of Vinblastine Sulfate

As vinblastine concentrations were increased in the range 1–40 μM, the absorptive apical-to-basal flux displayed nonlinear characteristics: below 20 μM vinblastine absorptive flux remained low; above this limiting concentration of vinblastine, absorptive vinblastine flux increased markedly (Figs. 4A–D). This increase in vinblastine absorptive flux is correlated with saturation of the active transport system (Fig. 4). Thus it is likely that as increasing cytosolic vinblastine saturates the finite capacity of the active extrusion system, absorptive vinblastine flux increases.

An alternative way of testing this possibility is the use of inhibitors; as shown above a range of inhibitors of P-glycoprotein is associated with increases in vinblastine absorption. Both verapamil (50 and 100 μM) and dideoxyforskolin (50 and 100 μM) have a marked and similar action on the vinblastine absorption, increasing it at all concentration levels. Furthermore, this effect of P-glycoprotein inhibition is most marked at subsaturating (with respect to the secretory pump) vinblastine concentrations. In the presence of high concentrations of P-glycoprotein transport inhibitors, vinblastine absorption shows a simple linear dependence on vinblastine concentration, i.e., the simple rules of solubility/diffusion now govern transepithelial vinblastine absorption.

The slope (permeability) of the pooled linear absorptive flux curve in the presence of inhibitors was $2.83 \pm 5.3 \times 10^{-2}$ cm hr^{-1} ($n = 4$); this is an eightfold increase in vinblastine absorption permeability, compared to vinblastine

Table I. Modulation of Vinblastine Absorption by Inhibitors of P-Glycoprotein Action

Inhibitor	Concentration (μM)	Absorptive apical-to-basal permeability (cm h^{-1}) $\times 10^{-2}$	R_T (Ω cm^2)
Control	0	0.372 ± 0.035 ($n = 58$)	658 ± 43 ($n = 60$) [+15%][a]
Verapamil	100	1.316 ± 0.08 ($n = 23$)	923 ± 73 ($n = 36$) [+34%]
R-Verapamil	100	1.220 ± 0.08 ($n = 3$)	606 ± 18 ($n = 6$) [+57%]
S-Verapamil	100	1.510 ± 0.120 ($n = 3$)	508 ± 29 ($n = 6$) [+37%]
Dideoxyforskolin	100	1.160 ± 0.05 ($n = 8$)	791 ± 33 ($n = 15$) [+77%]
Nifedipine	50	1.825 ± 0.09 ($n = 6$)	334 ± 36 ($n = 12$) [+50%]
Taxotere	25	1.860 ± 0.02 ($n = 3$)	463 ± 48 ($n = 6$) [+64%]

[a] Figures in brackets give the percentage change from the R_T values measured prior to the experiments being performed.

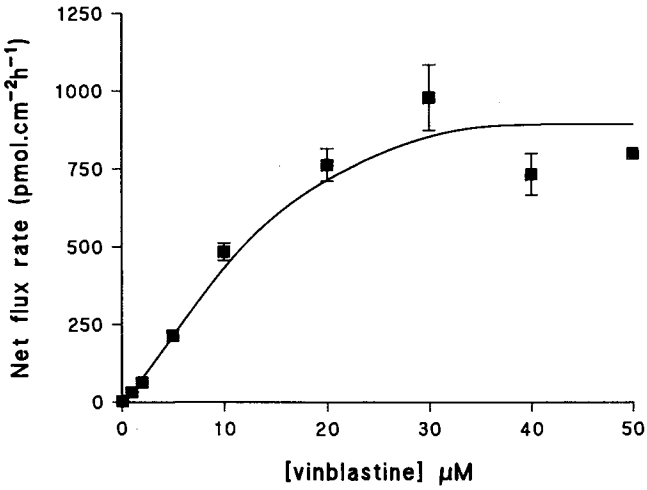

Fig. 3. Net vinblastine secretion displays saturation kinetics. Net vinblastine flux ($J_{b\text{-}a} - J_{a\text{-}b}$) was determined at concentrations up to 50 μ*M* vinblastine. The solid line is the least-squares fit for Michaelis–Menton kinetics (see Materials and Methods). Data are the mean ± SE of six determinations of kinetic parameters. At each concentration of vinblastine three pairs of cell monolayers were used for each determination, thus $n = 18$.

absorptive permeability values, when vinblastine concentrations are much less than the K_m for the secretory mechanism ($0.37 \pm 0.04 \times 10^{-2}$ cm hr^{-1} ($n = 58$)), and where the maximal effect of secretion in limiting absorption will be observed.

DISCUSSION

The demonstration of the functional expression of P-glycoprotein in the apical membrane of the human intestinal Caco-2 cell monolayers complements Western blotting and monoclonal antibody data (26). The calculated V_{max} for vinblastine secretion was 1285 pmol cm^{-2} hr^{-1}, at least one order of magnitude greater than that observed for dog renal epithelial cell layers (22).

The present results support the hypothesis that the drug-efflux mechanism at the apical surface of an intestinal epithelium renders that epithelium relatively impermeable to a substrate. The absorption kinetics of substrate (vinblastine) from the apical (lumen) compartment to the basal (blood) compartment show (a) a nonlinear dependence of vinblastine absorption upon vinblastine concentration; (b) an increase in absorption that is correlated with the saturation of the net secretory vinblastine flux; (c) a similar increase in absorption observed after inhibition of P-glycoprotein flux by a variety of inhibitors including verapamil, 1,9-dideoxyforskolin, nifedipine, and taxotere; and (d) a linear dependence of vinblastine absorption upon vinblastine concentration after P-glycoprotein inhibition.

Comparison of the absorptive permeability of vinblastine (measured at 10 n*M*) gives 0.37×10^{-2} cm hr^{-1}. This value is comparable to those permeability values obtained for acetylsalicylic acid (0.86×10^{-2} cm hr^{-1}) and practolol (0.32×10^{-2} cm hr^{-1}) in the identical Caco-2 cell system (16). The octanol/water partition coefficients [log*D* = −2.14 and −2.57, respectively (16)] emphasize the relative hydrophilicity of both acetylsalicylic acid and practolol. In the presence of P-glycoprotein inhibitors, Caco-2 cell monolayers have an increased permeability to vinblastine. Comparison of the passive permeability of vinblastine (in the absence of P-glycoprotein function, 2.83×10^{-2} cm hr^{-1}) to lipophilic drugs such as felodipine (8.17×10^{-2} cm hr^{-1}) and dexamethasone (7.8×10^{-2} cm hr^{-1}) [log*D* (octanol/water) values of 1.89 and 3.31, respectively (16)] emphasizes the native permeability of the apical cell border of the intestinal Caco-2 to vinblastine. This also reflects the relative lipophilicity of vinblastine as would be expected from a priori considerations of its molecular structure, indicated by its log*D* value of 2.9 (27).

The transepithelial permeability of a substrate for P-glycoprotein will be dependent not only on the passive permeability of the apical membrane to the substrate, but also on its affinity for the active transport site and the maximal capacity of P-glycoprotein contained in the apical membrane. A simple pump–leak balance will exist at the apical membrane (see Ref. 28). Limitation of absorptive uptake will be most pronounced with P-glycoprotein substrates when diffusional influx is low (at low luminal concentrations and/or with substrates with an intrinsically low passive permeability to the apical membrane lipids) and when ATP-dependent drug efflux is not limiting with respect to diffusional input. This condition is met if the drug has a high affinity with respect to ATP-dependent export and the cytosolic drug concentrations are nonsaturating. A minimal effect of P-glycoprotein in limiting absorption will be apparent with high diffusional fluxes (resulting from high intrinsic passive permeability or elevated external concentration) with respect to drug efflux (when the pump is saturated with respect to substrate or when substrates are of a low affinity with respect to export).

The current study was limited to vinblastine absorption. Multidrug resistance is associated with cross-resistance to a number of cytotoxic drugs, which include natural products such as anthracyclines, other *vinca* alkaloids, epipodophyllotoxins, colchicine, and actinomycin D, but not to drugs such as bleomycin, methotrexate, or alkylating agents (1,2). Thus, those natural products which demonstrate cross-resistance may also interact with intestinal P-glycoprotein with limited absorption at subsaturating concentrations. So verapamil is orally active and it has a low affinity with respect to inhibition of ATP-dependent drug export, epithelial vinblastine secretion, and potentiation of vinblastine mediated cytotoxicity (1,2,12,20). The inhibition of P-glycoprotein by forskolin and its analogue, 1,9-dideoxyforskolin, has been documented (13). These agents are highly lipophilic, and their relatively low affinity for P-glycoprotein suggests that cellular forskolin accumulation could not be limited by such a mechanism. Similar considerations apply to the absorption of the full range of P-glycoprotein substrates, e.g., nifedipine (2) and taxotere (24), a more potent analogue of the anticancer agent taxol (25). Noncompetitive mechanisms of P-glycoprotein inhibition would imply that such agents would not be subject to absorption restriction despite interacting with P-glycoprotein. Further, P-glycoprotein has been shown to transport a synthetic tripeptide (*n*-acetyl-leucyl-leucyl-norleucine) (29), raising the possibility that certain peptides may not permeate the apical border because of active export mediated by P-glycoprotein.

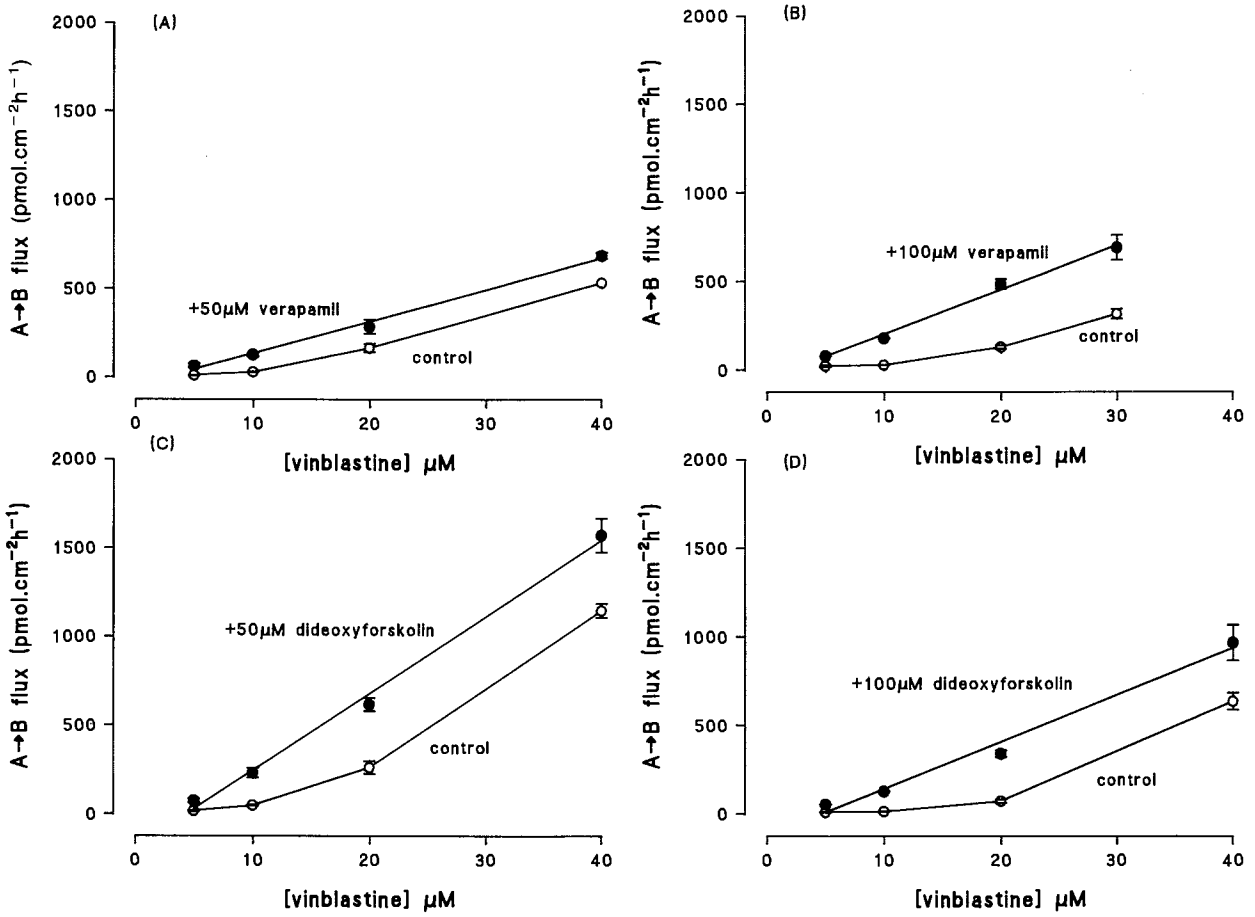

Fig. 4. Kinetics of vinblastine absorptive (A–B) flux in the presence and absence of inhibitors. (A) ○, Control data; ●, plus 50 μ*M* verapamil. (B) ○, Control data; ●, plus 100 μ*M* verapamil. (C) ○, Control data; ●, plus 50 μ*M* dideoxyforskolin. (D) ○, Control data; ●, plus 100 μ*M* dideoxyforskolin. Data are illustrated as mean ± SE (n = 3).

P-glycoprotein is expressed at the highest density at the villus enterocytes in close proximity to the luminal contents (5). The lower density of P-glycoprotein expression in the crypts implies that P-glycoprotein substrates may be preferentially absorbed into crypt cells. Data on the relative vinblastine transport secretory capacities in a segmental manner (between gastric, duodenal, ileal, and colonic sites) are currently unavailable but are of relevance to site-specific delivery of potential drug candidates whose absorption may be limited by a similar mechanism to that described for vinblastine.

The general principle illustrated by absorption limitation of a single substrate of one ATP-dependent export pump may be applicable to other systems. Thus, the ATP-dependent P-glycoprotein molecule is one example of a superfamily of such proteins [the ATP-binding cassette (ABC) superfamily (1,30)]. The cystic fibrosis transmembrane conductance regulator is a member of the ABC superfamily and is also expressed at the apical border of enterocytes (30). Though recognized as a Cl^- channel, this protein may also function to drive the ATP-dependent export of a different class of compounds (30). For drugs with unexpectedly poor oral absorption profiles, it should be tested whether ATP-dependent export functions render the intestinal membrane effectively impermeable.

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