Role of P-Glycoprotein-Mediated Secretion in Absorptive Drug Permeability: An Approach Using Passive Membrane Permeability and Affinity to P-Glycoprotein†

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Abstract □ It has been shown in vivo and in vitro that P-glycoprotein (P-gp) may be able to influence the permeability of its substrates across biological membranes. However, the quantitative contribution of the secretion process mediated by P-gp on the overall permeability of membranes has not been determined yet. In particular, observations need to be clarified in which substrates showing high affinity to P-glycoprotein, e.g., verapamil, apparently do not seem to be greatly influenced by P-gp in their permeability and consequently also with respect to their extent of GI-absorption after oral administration, whereas weaker substrates of P-gp, e.g., talinolol, have clearly shown P-gp-related absorption phenomena such as nonlinear intestinal permeability and bioavailability. Experiments with Caco-2 cell monolayers and mathematical simulations based on a mechanistic permeation model should aid in clarifying the underlying mechanism for these observations and quantifying the influence of passive membrane permeability and affinity to P-gp to the overall transmembrane drug flux. In addition, the concentration range of drug at which P-glycoprotein-mediated transport across the biological membrane is relevant should be examined. The permeability of various drugs in Caco-2 monolayers was determined experimentally and modeled using a combination of passive absorptive membrane permeability and a Michaelis–Menten-type transport process in the secretory direction. The passive permeabilities were experimentally obtained for the apical and basolateral membrane by efflux experiments using Caco-2 monolayers in the presence of a P-gp inhibitor. The Michaelis–Menten parameters were determined by a newly developed radioligand-binding assay for the quantification of drug affinity to P-gp. The model was able to accurately simulate the permeability of P-glycoprotein substrates, with differing passive membrane permeabilities and P-glycoprotein affinities. Using the outlined approach, permeability vs donor–concentration profiles were calculated, and the relative contribution of passive and active transport processes to the overall membrane permeability was evaluated. A model is presented to quantitatively describe and predict direction-dependent drug fluxes in Caco-2 monolayers by knowing the affinity of a compound to the exsorptive transporter P-gp and its passive membrane permeability. It was shown that a combination of high P-gp affinity with good passive membrane permeability, e.g., in the case of verapamil, will readily compensate for the P-gp-mediated reduction of intestinal permeability, resulting in a narrow range in which the permeability depends on the apical drug concentration. On the other hand, the permeability of compounds with low passive membrane permeability (e.g., talinolol) might be affected over a wide concentration range despite low affinity to P-gp.

Introduction

The presence of the exsorptive multidrug transporter P-glycoprotein (P-gp) in the apical membrane of absorptive cells throughout the gastrointestinal tract has been identified as a possible source of low or erratic absorption of drugs after peroral dosing. Furthermore, the possibility for drug–drug and drug–food interaction on this level has been pointed out.[1]

It is known that P-gp is abundant in several tissues under physiological conditions, especially in organs and tissues physiologically involved with excretion or distribution to specific organs, e.g., the liver, kidneys, and the blood–brain barrier.[2] In human tissues P-gp has been shown to be mainly expressed in two isotypes, MDR-1 and MDR-3, with distinct differences in substrate specificity.[3] MDR-3 is likely to be involved in the biliary excretion of phosphatidylcholine,[4] whereas the MDR-1 isoform is expressed in the human gastrointestinal tract and is responsible for interference with the intestinal absorption of a number of drugs.[5]

Recently, a model has been introduced to quantitate the affinity of compounds to human P-gp, based on the radioligand displacement principle.[6,7] Several drugs have been classified by this technique with respect to their affinity toward this secretory carrier. Nevertheless, until today, the relevance of the P-gp affinity for the overall transport rate of a P-gp substrate across a biological membrane remains unresolved.

For example, some authors have concluded from a small secretory P-gp-mediated drug efflux, that the respective compound may be a "noncompetitive" inhibitor or an "inhibitor but not a substrate" to P-gp, neglecting, however, the possibility that the P-gp-mediated, saturable secretory transport could be minimized due to high drug concentrations and/or high passive membrane permeability.[8]

Based on the information available today on P-gp-mediated drug secretion, a transport model can be constructed, which contains the relevant parameters controlling drug flux of P-gp substrates across biological membranes expressing the glycoprotein. The use of such a model should enable us to elucidate the quantitative contribution of the P-gp-mediated secretion to the overall membrane permeability of a P-gp substrate.

A scheme of the transport model is shown in Figure 1. As parameters, the model contains the affinity of a compound to P-gp and its passive permeability across the cell membrane. As a model membrane the human colonic carcinoma cell line (Caco-2) overexpressing P-glycoprotein was used. A general outline which describes the experimental approach applied for obtaining data for *passive drug permeability* (by efflux experiments for each compound) and for the *affinity to P-gp* (by a radioligand-binding assay (RBA)[6,7]) is outlined in Figure 2. In a first step, the passive permeation of the apical and basolateral membranes comprising the Caco-2 monolayer was determined, and the permeability was simulated for compounds exhibiting no affinity to P-gp and for P-gp substrates under permeation conditions, where the P-gp "pump" was inhibited by verapamil. In a second step, the model was extended to include

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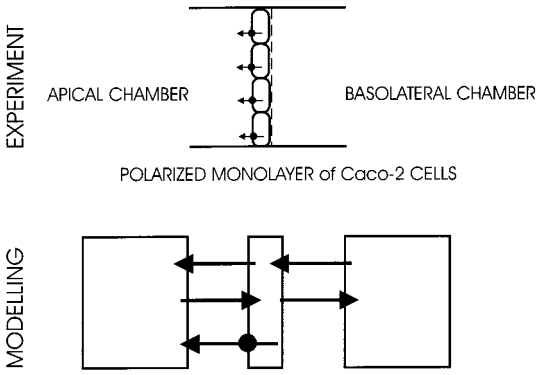

Figure 1—Outline of experimental and modeling approach depicting the compartments as well as passive fluxes and active, P-gp-mediated secretion between the three compartments.

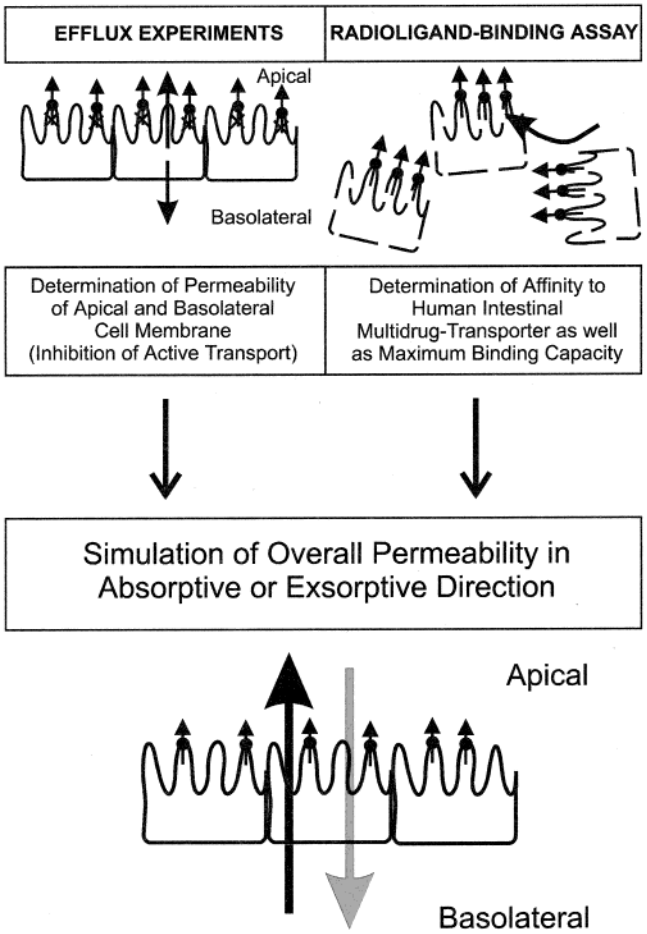

Figure 2—Scheme of experimental approaches used to obtain passive apparent permeablities of apical and basolateral membranes in Caco-2 monolayers by efflux studies as well as affinity to P-gp by radioligand-binding assay.

a saturable, carrier-mediated flux from the intracellular compartment to the apical compartment using affinity data obtained from RBA and estimates of V_{max} obtained from the carrier density and substrate turnover rates. These results were then correlated to data from transport experiments in order to investigate the predictive performance of the model.

Materials and Methods

Cell Culture—Dulbecco's Modified Eagle Medium, fetal calf serum (FCS), L-glutamine 200 mM, penicillin/streptomycin (10000 U/mL, 10 mg/mL), trypsin/EDTA, MEM nonessential amino acids (NEAA), Hanks's Balanced Salt Solution (HBSS), and phosphate-buffered saline (PBS) were from Life Technologies, Paisley, UK. Transwell cell culture inserts used for transport experiments (24 mm, 0.4 μm pore size, polycarbonate membrane) and all other cell culture materials were from Costar, Cambridge, MA. The Caco-2 cells obtained from the American Type Culture Collection (ATCC), Rockville, MD (starting with passage 74), were grown in the presence of 10 nM vinblastine sulfate for induction of P-gp expression. This vinblastine-adapted cell line has been characterized previously with respect to the extent and specificity of P-gp overexpression as well as to growth characteristics of monolayers.[6] For conducting the transport experiments, 90−95% confluent monolayers of vinblastine-induced Caco-2 cells were trypsinized and seeded into Transwell (24 mm, 0.4 μm pore size, polycarbonate membrane) (Costar, Cambridge, UK) cell culture inserts (300000 cells/well) and cultured for 14−17 days. Vinblastine-free medium was provided to the cells 8−16 h before performing binding or transport studies.

Compounds—[^{3}H]Atenolol, [^{3}H]metoprolol, pafenolol, and metoprolol were from Astra Hässle, Mölndal, Sweden. [^{3}H]Verapamil (25 μCi, 84 Ci/mmol) was purchased from New England Nuclear (Boston, MA), and [^{3}H]vinblastine (50 μCi, 16 Ci/mmol) and [^{3}H]-vincristine (50 μCi, 8 Ci/mmol) were from Amersham (Buckinghamshire, UK). Talinolol was a kind gift from Arzneimittelwerk Dresden (Radebeul, Germany). 2-(Morpholino)ethanesulfonic acid (MES) was from Fluka (Gothenburg, Sweden). All other compounds and reagents used were from Sigma (Malmö, Sweden) or BDH (Poole, UK).

Radioligand Binding Assay—The MultiScreen 96-well plate assay system was from Millipore (Eschborn, Germany), MultiScreen 96-well plates with Durapore membrane of 0.22 μm pore size were provided by Millipore (Malmö, Sweden). Liquid scintillation counting was performed by a WinSpectral 1414 counter from Wallac (Turku, Finland), using scintillation fluid Optiphase "Highsafe" 3 from Wallac (Loughborough, UK).

Transport and Efflux Experiments—An Electrical Voltage-Ohm-Meter (EVOM) equipped with "chopstick" electrodes (World Precision Instruments, Sarasota, FL) was used for monitoring transepithelial resistance of Caco-2 monolayers growing in Transwell inserts.

HPLC Analysis—The liquid chromatographic system consisted of a Pharmacia LKB 2150 pump (Uppsala, Sweden), a Perkin-Elmer ISS-100 autoinjector (Allerød, Denmark), a 206 PHD UV-absorbance detector from Linear (Reno, NV), or a Shimadzu RF 350 fluorescence monitor (Kyoto, Japan). Solvents used were of analytical grade and purchased from Skandinaviska Gentech (Kungsbacka, Sweden).

Determination of Affinity to P-gp by Radioligand-Binding Assay—The affinity of the test compounds to P-gp was investigated using a specific and sensitive radioligand binding assay described previously.[7] The displacement of the radioligand [^{3}H]verapamil from the P-gp-preparation by increasing concentrations of the nonlabeled compound of interest was determined. In short, porated cells of the P-gp-overexpressing Caco-2 cell line were incubated in duplicate with 16 different concentrations of each competitor at 37 °C in HBSS buffered with 10 mM MES at pH 7.0 for 30 min in the presence of ATP. Furthermore an ATP-regenerating system consisting of magnesium chloride (10 mM), creatine phosphate (10 mM), and creatine kinase (100 μg/mL) (19 mM) was present in the incubation mixture. The incubation was stopped by removing the liquid, and the filter membranes were washed twice with 100 μL of ice-cold HBSS buffered with 10 mM MES pH 7.0. The filter membranes were incubated with scintillation fluid for 12−16 h, and total radioactivity was determined by liquid scintillation counting.

Transport Experiments in Caco-2 Cell Monolayers—Transport experiments were performed with vinblastine at donor concentrations of 0.1, 1, 10, and 100 μM, with talinolol at 500 μM, and with quinidine at 0.25, 2.5, 25, and 250 μM. Furthermore transport experiments were carried out on atenolol (100 μM), metoprolol (100 μM), pafenolol (1000 μM), vincristine (0.1 μM), and verapamil (0.001 μM). Furthermore, for vinblastine (0.1 μM), vincristine (0.2 μM), talinolol (250 μM), and quinidine (25 μM), transport experiments in the presence of 0.5 mM verapamil were conducted for validation of the passive flux model under exclusion of P-gp-mediated transport. Transepithelial resistance of the vinblastine-induced Caco-2 monolayers (typically around 2500 Ω·

cm^2, for details see ref 6) was monitored prior to and after each transport experiment for verification of the integrity of the cell layer.

Transport experiments were conducted in HBSS buffered with 10 mM MES to pH 6.5 at 37 °C in correspondence to the experimental conditions of the radioligand-binding experiments. Each compound was added to the apical (A) or basolateral (B) compartment, and samples were taken from the corresponding acceptor compartment at predefined times. For vinblastine and vincristine, samples were taken every 30 min for 2.5 h from the acceptor compartments, for talinolol, metoprolol, atenolol, and pafenolol every 15 min for 1 h, and for verapamil and quinidine, samples were taken every 10 min for 1 h. To quantitate the flux across the monolayer, [^{3}H]vinblastine, [^{3}H]vincristine, [^{3}H]metoprolol, [^{3}H]verapamil, and [^{3}H]atenolol were determined by liquid scintillation counting, and talinolol, pafenolol, and quinidine were analyzed using HPLC methods.

Efflux Experiments—The apparent permeabilities of the apical and basolateral membranes of the vinblastine-induced Caco-2 cells were determined in monolayers grown on Transwell filters. The monolayers were loaded (30 min, 37 °C) with a solution of the drug at a concentration of interest in HBSS buffered with 10 mM MES, in the presence of 0.5 mM verapamil, for competitive inhibition of P-gp. When verapamil was studied, 0.5 mM of rhodamine 123 (Molecular Probes, Leiden, Netherlands) was used for competitive inhibition. The monolayers were washed gently with drug-free, ice-cold buffer of the same composition as used for loading of the cells. Efflux over the apical and basolateral membrane was started by placing the drug-loaded monolayer into a fresh six-well plate containing drug-free, prewarmed (37 °C) loading buffer (including verapamil or rhodamine 123) and adding the same buffer to the apical chamber of the Transwell. Samples were taken from the apical and basolateral compartments at 10, 20, and 30 min except for verapamil where samples were taken at 5.0, 7.5, 10.0, 12.5, and 15.0 min.

HPLC Methods—Validation and quality assurance was performed according to international recommendations.[9]

Talinolol and quinidine were determined as described previously.[6] Talinolol was analyzed using a chiral stationary phase (LiChrospher 100 Chiraspher NT, 250 × 4 mm i.d., Merck, Darmstadt, Germany) with ethanol:triethylamine (1000:0.5 [v:v]) and UV-absorbance detection at 245 nm after liquid−liquid extraction into dichlormethane:2-propanol (95:5 [v:v]) and reconstitution with methanol (internal standard pindolol). The method for the quantification of quinidine employed a LiChrospher RP-18 (Merck, Darmstadt, Germany) HPLC analytical column, 150 × 4.6 mm i.d., using methanol:acetonitrile:sulfuric acid (350:100:450 [v:v:v]), containing 10 mM octanesulfonic acid as the mobile phase and fluorescence detection at an excitation wavelength of 350 nm and an emission wavelength of 450 nm.

For the quantification of pafenolol, samples were analyzed by reversed phase HPLC using a LiChrospher RP-18 (Merck, Darmstadt, Germany) stationary phase (150 × 4.6 mm i.d.) and acetonitrile:0.02 M sodium dihydrogen phosphate pH 4.5:triethylamine (400:2000:1.5) as the mobile phase. UV-absorbance at 227 nm was monitored for detection. Pafenolol was determined with a precision and accuracy of 4.1 to 13.4% and −8.2 to 5.4% in the concentration range from 10 to 1000 ng/mL. For details on the analytical validation, see ref 6.

For talinolol, the precision and accuracy in the concentration range of 2.5 to 500 ng/mL per enantiomer was 3.5 to 12.7% and 1.2 to 15.4% ($n = 6$ for each concentration). The analytical method for the determination of quinidine exhibited in the concentration range from 1 to 1000 ng/mL a precision and accuracy of 1.9 to 9.0% and −4.9 to 5.6%.

Data Analysis—*A. Radioligand Binding Assay for P-gp*—A two-affinity model was fitted to the data obtained from competition experiments as described previosly.[6] SigmaPlot 2.01 (SPSS Science Software, Erkrath, Germany) was used for all nonlinear regression analysis.

B. Transport Experiments—The effective permeability (P_{eff}) was determined from the transport data according to

$$P_{eff} = \frac{dC/dt\,V}{AC_D} \tag{1}$$

where dC/dt is the flux across the monolayer, V is the volume of the acceptor chamber (3.0 mL for experiments in the apical to basolateral direction and 1.5 mL for experiments in the reverse direction), A is the apparent surface area of the monolayer used for the transport experiments (4.71 cm^2), and C_D represents the donor concentration of the respective drug. The flux across the monolayer was calculated as the slope of the amount transported vs time.

C. Efflux Experiments—The relationships between the drug efflux and time were adequately described by a monoexponential decay function.[10,11] The apparent permeability, P_{eff}, of the cell membrane was calculated from initial drug flux versus time into the respective compartment and the concentration used for loading of the cell monolayer.[10,11]

Modeling of Transport Experiments Using Efflux Permeabilities and Data from Radioligand Binding Assay—*A. Passive Permeability without P-gp Activity*—For modeling transport experiments of compounds lacking affinity to P-gp (e.g., atenolol), and transport with inhibition of P-gp, the passive drug fluxes for all three compartments depicted in Figure 1 were calculated according to

$$\frac{dM}{dt} = P_{eff,apical/basolateral} A C_{compartment} \tag{2}$$

where $P_{eff,apical/basolateral}$ was the apparent permeability of the apical or basolateral membrane, as determined from efflux experiments, A is the apparent surface area of the monolayer (4.71 cm^2), and $C_{compartment}$ is the concentration of the analyte in the respective compartment, calculated from the amout of drug in the compartment, the compartmental volume (1.5/3.0 cm^3 for the apical/basolateral compartment and 0.0247 cm^3 for the intracellular compartment of a Caco-2 cell monolayer with an area of 4.71 cm^2. The mean cell number of a monolayer amounted to 2.1×10^5 cells per cm^2 and was determined following trypsinization of monolayers grown in Transwells. The cell volume of 25 pL per Caco-2 cell was calculated on the geometrical dimensions of an ashlar with a basal square area of 4.8×10^{-6} cm^2 and a height of of 30 μm). The time interval used for numerical integration was 2−6 s. The concentration in the acceptor compartment was predicted for the various sampling times, and the apparent permeability of the substrate across the Caco-2 monolayer was calculated.

B. Transport Experiments Including Secretion by P-gp—For the transport experiments performed in the presence of functional P-gp, the carrier-mediated secretion of the drug from the cellular compartment was calculated according to

$$\text{carrier-mediated flux} = A_{max}\left(\frac{(1 - F_h)CC}{K_{i_1} + CC} + \frac{F_h CC}{K_{i_2} + CC}\right)t \tag{3}$$

where the maximum capacity of the P-gp pump, A_{max} [mol/s], is calculated from

$$A_{max} = N_{P-gp} A \tag{4}$$

N_{P-gp} is the number of P-gp molecules per cell, as determined by RBA-saturation experiments in P-gp-overexpressing Caco-2 cells as 800000 binding sites per cell,[6] and A is the average ATP-turn over per second (25 s^{-1}).[12] F_h is the fraction of high-affinity binding sites for the respective compound, K_{i_1} and K_{i_2} are the respective equilibrium dissociation constants ("affinity constants") to P-gp, both determined from competition experiments by radioligand-binding assay, CC is the intracellular concentration of the drug, and t is the interval of numerical integration (2−6 s). All simulations were performed using S-PLUS 3.3 (MathSoft, Seattle, WA).

Results

Permeability across Caco-2 Monolayer—*A. Passive Permeation without Involvement of P-gp*—In this first step, the diffusional transport, i.e., transport for nonsubstrates of P-gp or for P-gp substrates in the presence of 0.5 mM verapamil, was determined from the permeability of the apical and basolateral membranes in efflux experiments. The fluxes across the membranes were calculated from the passive permeabilities of the single membranes given in Table 1.

Table 1—Parameters Used for Predicting the Permeability in Caco-2 Monolayers of the Respective Compound. Passive Permeability (P_{apical} and $P_{basolateral}$) of the Apical and Basolateral Membrane of Caco-2 Monolayers as Determined by Efflux Experiments with Inhibition of P-gp, As Well As Affinity Constants (K_i) to P-gp and Fraction of High Affinity Binding Sites (f_H), As Determined by Radioligand Binding Assay

	passive permeability		affinity data		
compound	$P_{apical} \times 10^6$[cm/s]	$P_{basolateral} \times 10^6$ [cm/s]	K_{i_1} [μM]	K_{i_2} [μM]	f_H
atenolol	0.57	0.69	–	–	–
talinolol	1.3	3.6	72	1570	0.20
metoprolol	43	45	200	1750	0.46
pafenolol	0.70	1.3	5.5	3200	0.20
quinidine	75	96	2.6	225	0.21
verapamil	519	330	0.30	3.6	0.42
vinblastine	18	33	0.15	107	0.34
vincristine	0.33	0.29	0.50	150	0.20

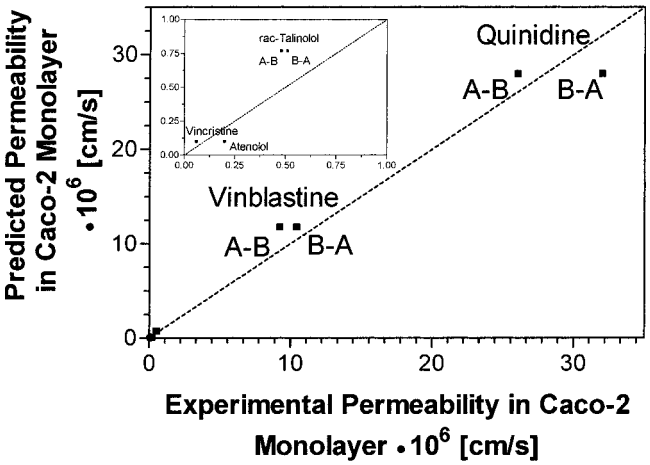

Figure 3—Correlation of experimentally obtained and predicted permeabilities in Caco-2 monolayers for nonsubstrate (atenolol, $C_D = 100$ μM) and different P-gp substrates under inhibition of P-gp (vinblastine, $C_D = 0.1$ μM, vincristine, $C_D = 0.2$ μM, talinolol, $C_D = 250$ μM, quinidine, $C_D = 25$ μM) to evaluate the validity of the passive-flux model used. The hatched line represents the line of identity.

The obtained apparent permeability coefficients were compared with those experimentally determined in transport experiments with Caco-2 monolayers. The results reveal a good correlation between experimental and predicted data, as shown in Figure 3. Thus the passive-flux model was considered an adequate basis for advancing to the next step, the carrier-mediated secretion.

B. Transport Experiments with Functional P-gp in Caco-2 Monolayers—Secretory flux mediated by P-gp was introduced into the permeability model by adding a two-affinity, Michaelis–Menten-type transport process. The values of the affinity constants and the relative proportions of high and low affinity binding sites determined from the radioligand-binding assay are listed in Table 1. The effect of the intracellular drug concentration on the flux mediated by the P-gp pump is shown for verapamil, vinblastine, quinidine, and talinolol in Figure 8. According to these results, the "working range" of P-gp, compared to a classical, single-affinity Michaelis–Menten process is greatly enhanced by the two-affinity mechanism.

The predictive model turned out to satisfactorily describe the permeability of the monolayer for a number of different solutes and permeation conditions, such as various concentrations and permeation directions.

Figures 4 and 5 show the correlations between the observed and predicted results in transport experiments on vinblastine and quinidine. The experiments were performed at four different concentrations covering a 1000-fold difference for both, apical to basolateral and the reverse direction across the P-gp overexpressing Caco-2 monolayers.

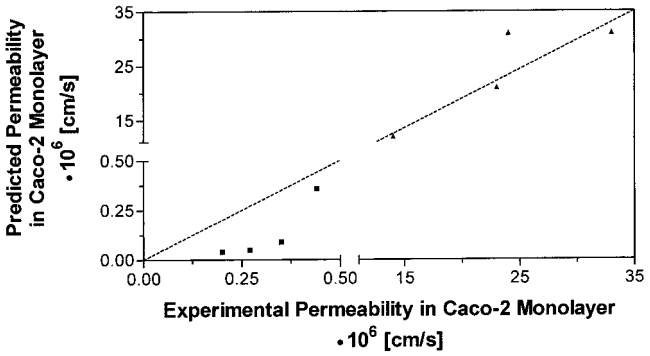

Figure 4—Correlation of experimentally obtained and predicted permeabilities in Caco-2 monolayers for vinblastine in A–B (■) and B–A (▲) direction.

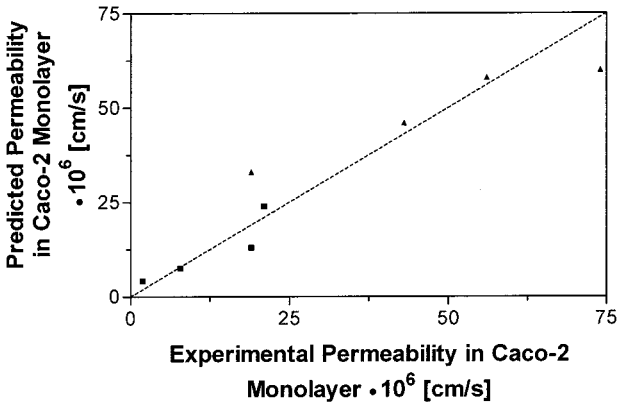

Figure 5—Correlation of experimentally obtained and predicted permeabilities in Caco-2 monolayers for quinidine in A–B (■) and B–A (▲) direction.

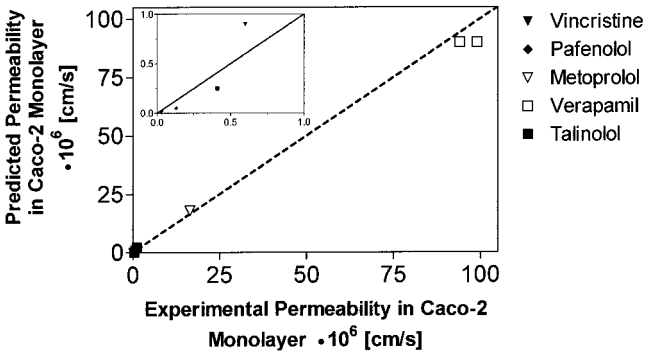

Figure 6—Correlation of experimentally obtained and predicted permeabilities in Caco-2 monolayers for verapamil, vincristine, metoprolol, talinolol, and pafenolol.

Similar results were obtained for various other lipophilic and hydrophilic compounds including examples with both high and low affinity to P-gp, Figure 6.

Dependence of Epithelial Permeability on Apical Drug Concentration—The results of simulations of the transport in the absorptive direction (A–B) in Caco-2 monolayers for four P-gp substrates with varying passive permeabilities and affinities to P-gp at different donor concentrations are given in Figure 7. The dashed line represents the permeability coefficient determined in the absence of P-gp-mediated secretion, in accordance with the fact, that for a diffusive transport process, the permeability is independent of the concentration of the permeating species.

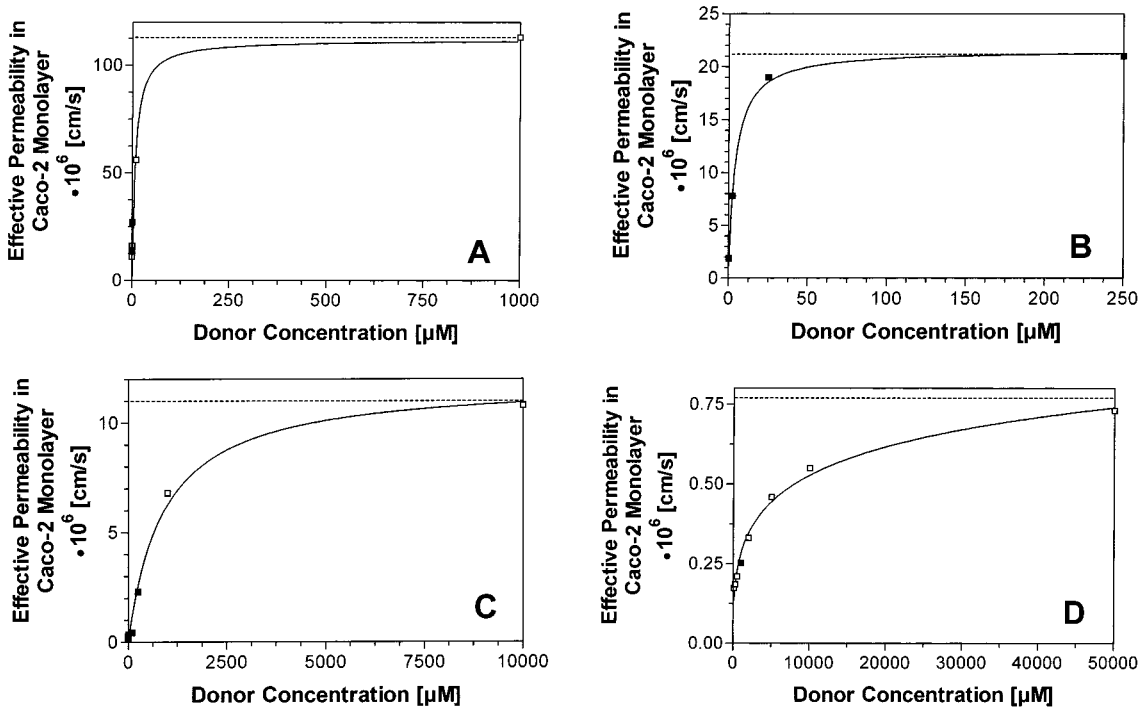

Figure 7—Simulated (□) and experimentally determined (■) effective permeabilities in Caco-2 monolayers (A–B direction) of verapamil (A), quinidine (B), vinblastine (C), and talinolol (D) at different donor concentrations, as well as passive permeability in absence of P-gp (----). The line is drawn according to the Michaelis–Menten equation.

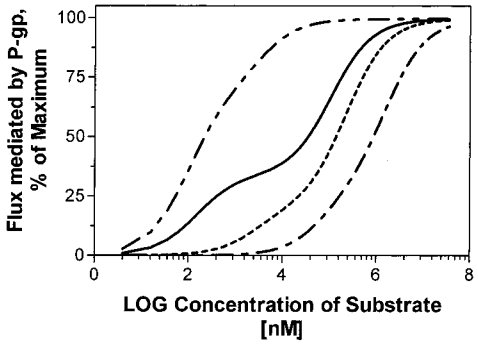

Figure 8—Secretory capacity of P-gp for verapamil (-•••-), vinblastine (—), quinidine (----) and talinolol (-•••-). Percentage of maximum secretion rate as a function of the respective substrate concentration.

Depending on the drug's affinity to P-gp and its passive membrane permeability, different permeability versus initial donor concentration profiles can be observed:

Verapamil—This drug exhibits very high passive permeability of the cell membrane and high affinity to P-gp (Table 1). This results in a pseudolinear concentration-independent permeability of the Caco-2 cell monolayer. Only at very low donor concentrations, the effect of P-gp is visible, resulting in a systematic trend toward lower permeability values.

Vinblastine and Quinidine—Both compounds are characterized by moderate-to-high passive permeability and moderate-to-high affinity to P-gp, resulting in a strong influence of P-gp on the permeability in the medium and low drug concentration range. For vinblastine this resulted in permeability values comparable to the paracellular flux of small molecules, e.g., mannitol, in Caco-2 monolayers.

Talinolol—This compound possesses moderate-to-low passive permeability and moderate-to-low affinity to P-gp. This results in a clearly visible dependence of P_{eff} on the apical drug concentration over a wide concentration range. Accordingly, the permeability approached that of paracellular flux in the lower concentration range.

Discussion

The results presented here were obtained using the same P-gp-overexpressing cell line for the transport experiments that has also been used for the binding studies. This cell line has been demonstrated to selectively overexpress P-gp and to exhibit expression of other cation transport systems only to a negligible extent. The permeabilites of various P-gp substrates determined in this system were accurately predicted by a model employing the passive drug flux across the apical and basolateral membranes and the P-gp-mediated flux. In the case of vinblastine an overestimation of the influence of P-gp by the model, especially in the low permeability range was obvious. The reason for this might be that only a part of the total P-gp identified in the binding assay is functional, and, e.g., intracellular P-gp may well contribute to substrate binding but not to its transport.[13] Another explanation could be that the model described here does not include paracellular permeation, which might become relevant at the very low permeability range.

The apparent permeability vs donor concentration profiles presented here allow an assessment of the possible compromising influence of P-glycoprotein on the membrane permeability of a drug. The four compounds for which the transport model was applied, verapamil, quinidine, vinblastine, and talinolol, show distinct differences in the quantitative contribution of P-gp-related secretion to the overall membrane permeability. For example, it can be stated that the membrane permeability of verapamil is hardly affected despite the high affinity of verapamil to P-gp. On the other hand, talinolol exhibits a strong influence of P-gp-mediated transport on the permeability over a wide concentration range, despite the fact that it has much lower affinity to P-gp than verapamil.

It can be concluded from the present findings that the passive permeability of a compound in the absence of P-gp-mediated transport equals the maximum possible permeability. Affinity to P-gp determines the shape and concentration range of the observed negative deviation from this

maximum permeability. In addition, this is governed by the intracellular concentration of the compound, being a function of its diffusive permeability of the apical and basolateral membrane.

Assuming 0.25 to 0.5 L of liquid available within the GI tract for dissolution of a perorally administered drug and the dose, an estimate of the local concentration range of a drug in the intestine can be guessed in order to apply the in vitro data to the in vivo situation. For talinolol, nonlinearity in absorption was detected following peroral dosing of four different doses spread over a 16-fold range (25−400 mg of the racemate) in humans.[14] Due to the absence of metabolic degradation, this effect was attributed to saturable intestinal secretion, most probably P-gp. These observations are well in accordance with the results presented here, thus demonstrating the in vivo relevance of this simulation approach. Furthermore, for verapamil, almost complete absorption has been found following p.o. administration of different therapeutic doses.[15,16] This is also in accordance with the predictions of the model presented here. For vinblastine, the bioavailability after peroral administration is negligible at therapeutic doses of 3.5 mg/m^2 body surface in adults, a fact that can also be derived from the P_{eff} vs donor concentration plots provided here.

The findings in this communication can thus be taken as an extension of the hypothesis by Hunter and Hirst[17] with respect to the influence of ATP-dependent efflux on the permeability of physiological barriers. The new findings reveal that relevant deviations from the maximum permeability by simple diffusion requires both affinity to P-glycoprotein as well as appropriate physicochemical properties of the permeating species to reach intracellular drug concentrations in the "working range" of the secretory carrier. Thus the approach chosen here reveals that affinity to P-gp may not necessarily compromise the absorption of a compound, even for a high affinity ligand, as, e.g., shown for verapamil.

In conclusion, high permeability drugs have a much decreased chance that their intestinal permeability is limited by P-glycoprotein activity, whereas low permeability drugs, irrespective of their affinity to P-gp, have a much greater chance of undergoing permability restriction mediated by P-gp at therapeutically applied peroral doses.

References and Notes

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