A Functional Assay for Quantitation of the Apparent Affinities of Ligands of P-Glycoprotein in Caco-2 Cells

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Purpose. To develop a facile functional assay for quantitative determination of the apparent affinities of compounds that interact with the taxol binding site of P-glcoprotein (P-gp) in Caco-2 cell monolayers.

Methods. A transport inhibition approach was taken to determine the inhibitory effects of compounds on the active transport of $[^3H]$-taxol, a known substrate of P-gp. The apparent affinities (K_I values) of the compounds were quantitatively determined based on the inhibitory effects of the compounds on the active transport of $[^3H]$-taxol. Intact Caco-2 cell monolayers were utilized for transport inhibition studies. Samples were analyzed by liquid scintillation counting.

Results. $[^3H]$-Taxol (0.04 μM) showed polarized transport with the basolateral (BL) to apical (AP) flux rate being about 10–20 times faster than the flux rate in the AP-to-BL direction. This difference in $[^3H]$-taxol flux could be totally abolished by inclusion of (±)-verapamil (0.2 mM), a known inhibitor of P-gp, in the incubation medium. However, inclusion of probenecid (1.0 mM), a known inhibitor for the multidrug resistance associated protein (MRP), did not significantly affect the transport of $[^3H]$-taxol under the same conditions. These results suggest that P-gp, not MRP, was involved in taxol transport. Quinidine, daunorubicin, verapamil, taxol, doxorubicin, vinblastine, etoposide, and celiprolol were examined as inhibitors of the BL-to-AP transport of $[^3H]$-taxol with resulting K_I values of 1.5 ± 0.8, 2.5 ± 1.0, 3.0 ± 0.3, 7.3 ± 0.7, 8.5 ± 2.8, 36.5 ± 1.5, 276 ± 69, and 313 ± 112 μM, respectively. With the exception of that of quinidine, these K_I values were comparable with literature values.

Conclusions. This assay allows a facile quantitation of the apparent affinities of compounds to the taxol-binding site in P-gp; however, this assay does not permit the differentiation of substrates and inhibitors. The potential of drug-drug interactions involving the taxol binding site of P-gp can be conveniently estimated using the protocol described in this paper.

KEY WORDS: apparent affinity; Caco-2 cells; Michaelis-Menten constant; P-glycoprotein; transport.

INTRODUCTION

P-Glycoprotein (P-gp), the product of the multidrug resistance gene (MDR1), is an efflux transporter located on the plasma membranes of many cancer cells that exhibit the multidrug resistance phenomenon (1,2). P-gp can transport a variety of structurally unrelated lipophilic drugs and other xenobiotics from the interior of the plasma membranes out of the cells, thereby limiting the access of drugs and xenobiotics into cells (3,4). P-gp is also significantly expressed in many normal human tissues, e.g., epithelial cells in the intestinal mucosa, endothelial cells in the blood-brain barrier, hepatocytes of the liver, and proximal tubular epithelial cells of the kidney (2,5–7). The presence of P-gp in these normal tissues significantly affects the absorption through the intestinal mucosa, distribution into the brain, and elimination from the body via the liver and kidney for drugs that are substrates for P-gp. (7,8). Moreover, drugs that are substrates of P-gp also have the potential to exhibit drug-drug interactions during the processes of absorption, distribution and elimination (7,9,10). Therefore, knowledge about the substrate activity of a drug candidate for P-gp has become an integral part of drug discovery.

The objective of the present study was to develop a functional assay for quantitative determination of the apparent affinities (K_I values) of drugs or drug candidates for P-gp. Taxol, an anticancer drug, has been reported to be a substrate for P-gp (11). The transport of taxol across Caco-2 cell monolayers, a cell culture model of the intestinal mucosa (12), has been shown to be modulated by P-gp (13). The active efflux of taxol by P-gp exhibits Michaelis-Menten kinetics with a K_M value of 16.5 μM (13). In the present assay, a transport inhibition approach is used to determine the inhibitory effect of a test compound on the active efflux of $[^3H]$-taxol in Caco-2 cell monolayers. From the inhibitory effect, the apparent affinity (K_I value) of the test compound for the taxol binding site on P-gp can be quantitatively determined. A number of known P-gp ligands have been examined using this assay. The apparent affinities (K_I values) measured by this assay in general are comparable to the affinities reported in the literature. This facile transport functional assay is easy to conduct and has advantages over other assays used to assess P-gp substrate activity, e.g., full-range concentration-dependence transport assays, radioligand binding displacement assays, and P-gp containing immobilized artificial membrane chromatography assays (14–18).

MATERIALS AND METHODS

Materials

Vinblastine, daunorubicin, etoposide, quinidine, doxorubincin, and cyclosporin A (CsA) were purchased from Sigma Chemical Co. (St. Louis, MO). Taxol, probenecid, and (±)-verapamil were purchased from Aldrich Chemical Co. (Milwaukee, WI). $[^3H]$-Taxol was purchased from Moravek Biochemicals, Inc. (Brea, CA). Celiprolol was a gift from Rhone-Poulenc Rorer Pharmaceuticals Inc. (Collegeville, PA). Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD).

Caco-2 Cell Culture

Caco-2 cells (passage numbers 50–70) were grown on Transwell polycarbonate membranes (3.0 μm pore size, 4.71 cm^2 surface area) for 21–28 days as described in the literature (19,20). The integrity of Caco-2 cell monolayers was exam-

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ABBREVIATIONS: AP, apical; BL, basolateral; CsA, cyclosporin A; HBSS, Hank's balanced salt solution; MDR, multidrug resistance; MRP, multidrug resistance associated protein; P-gp, P-glycoprotein.

ined by measuring the flux of [14C]-mannitol, a paracellular transport marker, from the apical (AP) to the basolateral (BL) side of the cell monolayers. All cell monolayers used in the transport experiments had apparent permeability coefficients of [14C]-mannitol flux of less than 3.0×10^{-7} cm/sec.

Transport Across Caco-2 Cell Monolayers

Transport of [3H]-taxol from the BL to the AP side of Caco-2 cell monolayers was conducted at pH 7.4 and 37°C in a shaking water bath set at 55 rpm. The cell monolayers were rinsed three times with pH 7.4 HBSS buffer solution pre-warmed to 37°C (2.6 ml on the BL side and 1.5 ml on the AP side) before the transport experiments (20). In the transport experiment, the donor (BL side) solution contained [3H]-taxol (20–800 nM) in the absence or presence of the P-gp inhibitor (±)-verapamil (0.2 mM) (21) or a test compound at a known concentration in pH 7.4 HBSS buffer solution. It should be pointed out that any P-gp inhibitor that can completely inhibit the function of P-gp may be used instead of verapamil. The receiver (AP side) compartment was filled with pH 7.4 HBSS buffer solution in the absence or presence of a test compound at the same concentration as in the donor side solution. The purpose of adding a test compound also on the receiver (apical) side of the cells was to better maintain a constant concentration of the test compound on the donor (basolateral) side. Samples (100 μl) were taken from the receiver side at 20, 40, 60, 80, and 100 min and the volume of the receiver solution was replenished by adding fresh HBSS buffer solution (pH 7.4). A sample (50 μl) from the donor (BL) side solution was also taken at the beginning of the transport experiment. The samples were mixed with 3 ml of liquid scintillation cocktail (3a70B from Research Products International Corp., Mount Prospect, IL) and the radioactivity of the samples was determined using a liquid scintillation counter (Beckman LS 6000IC, Beckman Instruments, Inc., Schaumburg, IL). The apparent permeability coefficients (P_{app}) were calculated using the following equation:

$$P_{app} = (\Delta M / \Delta t) / (A \cdot C_O)$$

where $\Delta M / \Delta t$ is the appearance rate (in nmol/sec) of [3H]-taxol on the receiver (AP) side at the steady-state of the transport process, A is the surface area of Caco-2 cell monolayers (4.71 cm² for the Transwell membranes used in this study), and C_O is the initial concentration (in nmol/cm³) of [3H]-taxol that was determined from the sample taken at the beginning of the transport experiment from the donor (BL) side solution.

RESULTS AND DISCUSSION

Theory

Working Equations

Working equations 1–3 are used to calculate the apparent affinities (K_I values) of test compounds from the apparent permeability coefficient (P_{app}) values that were determined from the BL-to-AP transport experiments of [3H]-taxol in the absence and presence of P-gp inhibitors.

$$K_I = [(P_I/P_0)/(1 - P_I/P_0)] \cdot [I] \qquad (1)$$
$$P_0 = P_{app,\,1} - P_{app,\,3} \qquad (2)$$

$$P_I = P_{app,\,2} - P_{app,\,3} \qquad (3)$$

In working equations 1–3, P_I and P_0 are the permeabilities of [3H]-taxol for the active transport component mediated by P-gp in the presence and absence of a test compound, respectively. P_I/P_0 is a reflection of the inhibitory effect of the test compound on the active transport component of [3H]-taxol. [I] is the concentration of the test compound used in the transport experiment. $P_{app,\,1}$, $P_{app,\,2}$ and $P_{app,\,3}$ are the apparent permeability coefficients for the BL-to-AP transport of [3H]-taxol in the absence of any inhibitor, in the presence of the test compound, and in the presence of 0.2 mM (±)-verapamil, respectively. To avoid cell-cell variations, the transport experiments for the determination of $P_{app,\,1}$, $P_{app,\,2}$ and $P_{app,\,3}$ should always be conducted using the same batch of cells.

Equation Derivation

For an active transport process that exhibits Michaelis-Menten kinetics, the flux (J_I) of the substrate in the presence of a competitive inhibitor can be expressed as the following equation:

$$J_I = \{(J_{max}/K_S) \cdot [S]\} / (1 + [S]/K_S + [I]/K_I) \qquad (4)$$

where J_{max} is the maximum flux of the substrate; K_S is the Michaelis-Menten constant of the substrate ([3H]-taxol in this study); [S] is the concentration of the substrate; [I] is the concentration of the inhibitor (the test compound); K_I is the inhibition constant (the dissociation constant from the transporter) and the inverse of K_I is a reflection of the apparent affinity of the inhibitor. If the transport experiments are conducted at low substrate concentrations (assume [S] ≤ 1/20 K_S), Eq. 4 is reduced to the following:

$$J_I = \{(J_{max}/K_S) \cdot [S]\}/(1 + [I]/K_I) \qquad (5)$$

When the transport experiment is conducted at such a low substrate concentration and in the absence of the inhibitor (i.e., [I] = 0), Eq. 5 becomes

$$J_0 = (J_{max}/K_S) \cdot [S] \qquad (6)$$

where J_0 is the flux of the substrate for the active transport in the absence of the inhibitor. The fluxes in the presence and absence of the inhibitor are related to the permeabilities of the substrate for the active transport component in the following equations:

$$J_I = P_I \cdot A \cdot [S] \qquad (7)$$
$$J_0 = P_0 \cdot A \cdot [S] \qquad (8)$$

where P_I and P_0 are the permeabilities of the substrate in the presence and absence of the inhibitor and A is the surface area of the cell monolayers. Substitution of Eqs. 7 and 8 into Eqs. 5 and 6, respectively, gives the following equations:

$$P_I \cdot A \cdot [S] = \{(J_{max}/K_S) \cdot [S]\}/(1 + [I]/K_I) \qquad (9)$$

$$P_0 \cdot A \cdot [S] = (J_{max}/K_S) \cdot [S] \qquad (10)$$

Dividing Eq. 9 by Eq. 10 provides the following equation:

$$P_I/P_0 = 1/(1 + [I]/K_I) \qquad (11)$$

where P_I and P_0 are calculated by Eqs. 2 and 3 from the apparent permeability coefficients (P_{app} values) that can be

measured from the transport experiments in the presence and absence of the test compound and in the presence of 0.2 mM (±)-verapamil; [I] is the concentration of the test compound and K_I is the inhibition constant of the test compound to be evaluated by the assay. Rearranging Eq. 11 gives working equation 1.

Distinction of the Active Transport Component from Other Transport Components

The derivation of Eqs. 1–11 is based only on the active transport component (i.e., the component mediated by the transporter) for the flux of the substrate from the BL to the AP side of Caco-2 cell monolayers. P_I and P_0, as defined above, are direct measures of the active transport process and need to be extracted from the total transport, which is a combination of active transport and passive diffusion. The active-transport component of the total transport can be determined by subtracting the passive component from the observed total transport as depicted in Figure 1. The passive component ($P_{passive}$), a combination of paracellular and transcellular diffusion, is the observed transport when P-gp function is completely inhibited by 0.2 mM (±)-verapamil. Therefore, P_0 and P_I can be evaluated from working equations 2 and 3 (Figure 2), and the ratio of P_I/P_0, a reflection of the inhibitory effect of the test compound on the active transport of the reference substrate, can be used in working equation 1 to calculate the K_I value of the test compound for the transporter.

Accuracy and Simplicity Considerations

Experimentally, a 20–80% inhibitory effect in a transport study across Caco-2 cell monolayers can usually be determined with good accuracy. Rearranging Eq. 1 gives:

$$[I] = [1/P_I/P_0 - 1] \cdot K_I \qquad (12)$$

For a 20% inhibitory effect, which corresponds to a P_I/P_0 value of 80/100, the concentration of the inhibitor needs to be:
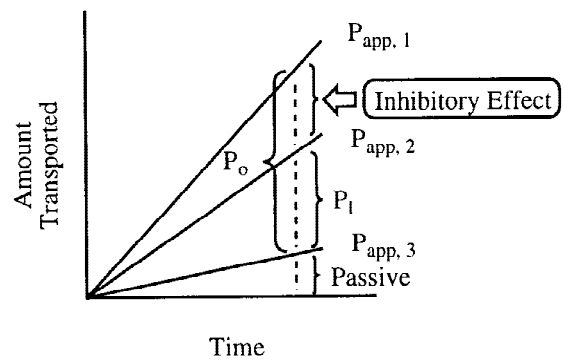
$$[I]_{20\%} = [1/(80/100) - 1] \cdot K_I = 0.25\,K_I \qquad (13)$$

Fig. 2. Determination of the inhibitory effect of a test compound on the active component of the substrate ([³H]-taxol) transport from the BL to the AP side of Caco-2 cell monolayers. $P_{app,\,1}$, $P_{app,\,2}$ and $P_{app,\,3}$ are determined from the BL-to-AP transport of the substrate ([³H]-taxol) in the absence of any inhibitor, in the presence of a test compound, and in the presence of an inhibitor of the transporter (P-gp), respectively. The permeabilities for the active component of the substrate ([³H]-taxol) transport in the absence (P_0) and presence (P_I) of a test compound, therefore, can be determined by subtracting $P_{app,\,3}$ (the passive component) from $P_{app,\,1}$ (total transport in the absence of any inhibitor), and $P_{app,\,2}$ (total transport in the presence of the test compound), respectively.

For an 80% inhibitory effect, which corresponds to a P_I/P_0 value of 20/100, the concentration of the inhibitor needs to be:

$$[I]_{80\%} = [1/(20/100) - 1] \cdot K_I = 4.0\,K_I \qquad (14)$$

For a test compound with totally unknown affinity for P-gp, a two-concentration experimental design can be used to determine quickly the apparent K_I value of the compound. Assume that two concentrations (16 μM and 250 μM) of the test compound are used; the K_I value of the test compound can be quantitatively determined with good accuracy if the K_I value of the test compound is in the range of 4–1000 μM or can be estimated to be either "less than 4 μM" or "greater than 1000 μM."
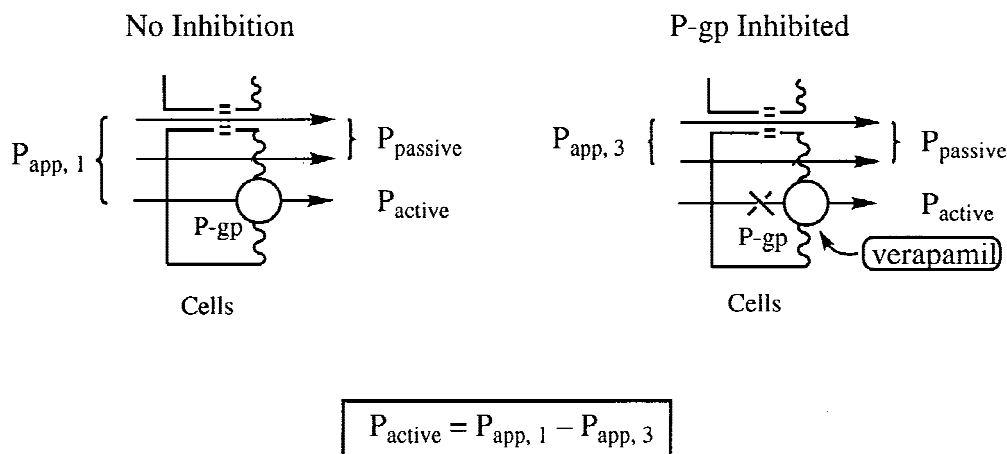
Fig. 1. Delineation of the active component from total transport. The active component of the transport can be determined by subtraction of the passive component from the total transport. The passive component in turn is determined by complete inhibition of the active transport process by an inhibitor (e.g., verapamil) of the transporter (e.g., P-glycoprotein, P-gp).

RESULTS

P-gp Activity

For the transport of $[^3H]$-taxol (40 nM) across Caco-2 cell monolayers in the absence of any inhibitor, fluxes of the substrate in the BL-to-AP direction were approximately 10 to 20 times greater than in the AP-to-BL direction (P_{app} values were $10 - 30 \times 10^{-6}$ cm/sec for BL-to-AP transport and $1 - 3 \times 10^{-6}$ cm/sec for AP-to-BL transport). The BL-to-AP fluxes could be decreased to approximately the same rate as in the AP-to-BL direction when 0.2 mM (±)-verapamil, a known inhibitor of P-gp and MRP (21), was included in the transport medium. However, the transport of $[^3H]$-taxol was not significantly affected when 1.0 mM probenecid, a known inhibitor of MRP (22), was included in the transport medium under the same conditions (Table I). These observations indicate that the transport of $[^3H]$-taxol across Caco-2 cell monolayers is mediated by P-gp but not by MRP. These observations also suggest that the levels of the functional expression of P-gp in these cells are satisfactory for the present assay.

Affinities of P-gp Ligands

Vinblastine, a ligand of P-gp, when included in the transport medium at 35 μM concentration, significantly inhibited the flux of $[^3H]$-taxol in the BL to AP direction (Figure 3). An approximately 50% inhibitory effect (corresponding to a P_I/P_0 value of 0.51) on the active component of the transport of $[^3H]$-taxol was observed, and the apparent K_I value for vinblastine was calculated to be 36.5 ± 1.4 μM, which is similar to the literature values of 34 μM and 19 μM (1,16). Similarly, for most of the other compounds tested, we observed approximately 30–80% inhibition (corresponding to P_I/P_0 values of
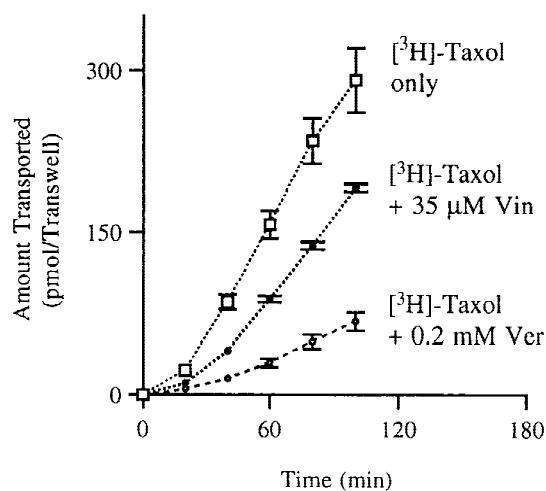

Fig. 3. Transport profiles of $[^3H]$-taxol (0.8 μM) from the BL to the AP side of Caco-2 cell monolayers in the absence of any inhibitor, in the presence of 35 μM vinblastine (Vin), and in the presence of 0.2 mM (±)-verapamil (Ver). The data points are the averages of triplicate determinations, and the bars represent the standard deviation. The inhibitory effect of Vin on the active component of $[^3H]$-taxol transport was determined by these three transport experiments. Based on the inhibitory effect, the apparent affinity (K_I value) of Vin for the taxol binding site in P-gp was quantitatively determined.

0.17–0.69) on the active component of the transport of $[^3H]$-taxol in the BL to AP direction. With the exception of quinidine, the K_I values of these P-gp ligands determined by this assay ranged from 1.5 to 313 μM, which are comparable in general to the literature values (Table I). For CsA (0.5 μM), also a P-gp ligand, a slight stimulatory effect rather than an

Table I. Inhibitory Effects (P_I/P_0) on the Active Component of $[^3H]$-Taxol Transport from the BL to the AP Side of Caco-2 Cell Monolayers and the Apparent Affinities (K_1 Values) of the Treated P-gp Ligands

Compounds	$P \times 10^6$, cm/sec			K_I, μM	
	P_I	P_0	P_I/P_0	Measured	Literature
(±)-Verapamil (5 μM)	10.1 (±0.61)	27.2 (±0.07)	0.371	3.0 (±0.3)	2.5[a]
Vinblastine (35 μM)	12.9 (±0.42)	25.5 (±3.95)	0.505	36.5 (±1.4)	34,[b] 19[c]
Taxol (15 μM)	1.84 (±0.41)	10.7 (±1.29)	0.172	~7.3 (±0.7)	16.5[d]
Daunorubicin (1.0 μM)	7.90 (±1.13)	11.4 (±0.70)	0.693	2.5 (±1.0)	1.5[e]
Etoposide (200 μM)	5.27 (±3.41)	9.25 (±0.89)	0.570	276 (±69)	213[f]
Celiprolol (1.0 mM)	2.25 (±0.61)	9.57 (±2.33)	0.235	313 (±112)	1000[g]
Quinidine (1.0 μM)	2.40 (±1.00)	10.7 (±1.29)	0.224	1.5 (±0.8)	303[h]
Doxorubincin (5 μM)	14.5 (±2.06)	23.5 (±1.10)	0.617	8.5 (±2.8)	
Probenecid (1.0 mM)	18.3 (±2.29)	16.4 (±1.95)	1.11	no inhibition	
CsA (0.5 μM)	35.1 (±1.61)	25.5 (±3.95)	1.37	stimulatory	

Note: Permeabilities for the active component of $[^3H]$-taxol transport in the absence of any inhibitor (P_0) and in the presence of a test compound (P_I) were calculated by equations $P_0 = P_{app,1} - P_{app,3}$ and $P_I = P_{app,2} - P_{app,3}$. $P_{app,1}$, $P_{app,2}$ and $P_{app,3}$ were the apparent permeability coefficients for the BL-to-AP transport of $[^3H]$-taxol in the absence of any inhibitor, in the presence of the test compound, and in the presence of 0.2 mM (±)-verapamil, respectively. $P_{app,3}$ values ranged from 1 to 3×10^{-6} cm/sec. K_I values were calculated from P_I/P_0 values by equation $K_I = [(P_I/P_0)/(1 - P_I/P_0)] \cdot [I]$ where [I] was the concentration of the test compound.
[a] Reference (23).
[b] Reference (16).
[c] Reference (1).
[d] Reference (13).
[e] Reference (24).
[f] Reference (25).
[g] Reference (15).
[h] Reference (16).

inhibitory effect was observed upon the active transport of [³H]-taxol in the BL to AP direction (Table I).

DISCUSSION

The functional assay described above is based on the inhibitory effects of test compounds on the active component of the transport of [³H]-taxol mediated by P-gp from the BL to the AP side of intact Caco-2 cell monolayers. The K_I values determined for the test compounds using this assay represent the extracellular concentrations of the test compounds at which the active transport component of [³H]-taxol is inhibited by 50%. Therefore, the apparent affinities of the test compounds for the taxol-binding site of P-gp can be quantitatively determined. However, mechanistic information about how the compounds interact with P-gp (i.e., substrates or inhibitors) cannot be determined using this assay. In addition, it should be pointed out that the K_I values determined from this assay are different from the IC_{50} values determined in many competition assays. K_I values are independent of the affinity and concentration of the reference substrate, while IC_{50} values are dependent on the affinity and concentration of the reference substrate. In addition, since P-gp is an ATP-driven efflux transporter, any compounds which can affect ATP supplies or which are ATPase inhibitors may also exhibit inhibitory effects on taxol transport.

Multiple substrate binding sites and a modulator binding site have been suggested for P-gp, and the interactions among these substrate/modulator binding sites have been observed (26,27). The interactions among these binding sites can be either stimulatory or inhibitory, depending on the specific ligands and, possibly, on the ligand concentrations used as well (26,27). The reason for the slight stimulatory effect of CsA on the active transport of [³H]-taxol is not clear. However, one possible explanation might be some positive interactions between the taxol and CsA binding sites on P-gp.

In the quinidine study, a 250 μM concentration of this compound was initially tested and its inhibitory effects on taxol transport were too large (~100%) to allow an accurate estimation of its K_I. Therefore, a lower concentration (50 μM) of quinidine was used and the K_I value was estimated to be 1.7 μM, which is approximately the same value obtained when a 1 μM concentration was used. The discrepancy between the K_I values of quinidine determined by this functional assay and the values reported in the literature could result from several differences in experimental conditions. The literature affinity value for quinidine was determined by a competition binding assay using permeabilized Caco-2 cells (16,28), while the present assay is a transport inhibition assay using intact Caco-2 cell monolayers. In addition, in the literature assay, radiolabeled verapamil was used as a reference ligand and verapamil binding was fit to a two-affinity binding model (16,28). In the present assay, [³H]-taxol was used as a reference P-gp substrate that exhibited simple Michaelis-Menten kinetics in the transport studies (13). Therefore, one possible reason for the discrepancy in the quinidine data between the present assay and the literature assay could be the use of different reference substrates that have different substrate activities or binding affinities for the different binding sites in P-gp.

There are a number of advantages of the present assay over existing methodology for quantitative determination of the affinities of compounds for P-gp. Intact Caco-2 cell monolayers are used in the present assay, in contrast to literature assays that use harvested and permeabilized cells or membrane vesicles (16,17,28). The intact Caco-2 cell monolayers better mimic the *in vivo* situation, and the K_I values determined by this assay are more relevant to the drug absorption process from the intestine. For compounds with totally unknown affinities for P-gp, the two-concentration experimental design can be applied to conveniently determine the apparent affinities of the compounds for P-gp with good accuracy. Compared to the traditional method in which a full range of concentrations are used to determine the Michaelis-Menten constant of a substrate for P-gp, this assay requires smaller amounts of material and less manpower and time. In addition, because a radiolabeled reference compound (e.g., taxol) is used, the test compounds themselves do not have to be detected. Moreover, because low concentrations (as low as 0.25 K_I without compromising the accuracy) of the test compound can be used, possible solubility problems for some compounds can be minimized. In the traditional methods where a full range of concentrations is studied, concentrations of the test compound higher than its K_M value are desirable to obtain an accurate estimate of the affinity of the compound for the transporter. The present assay can quantitatively determine the apparent affinities of both substrates and inhibitors for the taxol binding site on P-gp. In contrast, it could be difficult or impossible to do the same type of study using the traditional full range of concentration dependence transport assay for poor substrates and poor inhibitors. In the present assay, the K_I values of ligands are estimated and the K_I values of the ligands should not be significantly affected by the cell-cell variations in the level of P-gp expression. Data from different batches of cells or from different laboratories should be comparable. However, the present assay is limited to the determination of the apparent affinities of compounds for the taxol binding site in P-gp. To determine the ability of a compound to interact with the other binding sites of P-gp, assays using reference compounds specific for the other binding sites would have to be developed.

Drug-drug interactions mediated by transporters are becoming an important research subject with the accumulating knowledge that membrane transporters can significantly affect the absorption, distribution, and elimination of some drugs. The present assay should be useful in quantitatively determining drug-drug interaction potentials mediated by the efflux transporter P-gp. Assays for other membrane transporters located in biological barriers (e.g., the intestinal mucosa and the blood-brain barrier) may be developed based on the same methodology.

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