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# Transepithelial transport of vinblastine by kidney-derived cell lines. Application of a new kinetic model to estimate in situ $K_m$ of the pump

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**We present a new transport model that may be useful for many kinds of transepithelial transport experiments. The model permits estimation of a pump $K_m$ and pump activity solely on the basis of transepithelial tracer fluxes. We apply the model to studies of a multidrug efflux pump, P-glycoprotein, which is normally located in the apical plasma membrane of certain transporting epithelia such as kidney proximal tubule cells. To determine the functional properties of this multidrug transporter in an epithelium, we studied the transepithelial transport of the chemotherapeutic drug, vinblastine, in epithelia formed by the kidney cell lines MDCK, LLC-PK1, and OK. We have previously shown that basal to apical flux of 100 nM vinblastine was about five times higher than apical to basal flux in MDCK epithelia, indicating that there is a net transepithelial transport of vinblastine across MDCK epithelia. Addition of unlabeled vinblastine reduced basal to apical flux of tracer and increased apical to basal flux of tracer in a concentration-dependent manner, a pattern expected if there is a saturable pump that extrudes vinblastine at the apical plasma membrane. The model permits estimation of a pump $K_m$ and pump activity solely on the basis of transepithelial tracer fluxes. According to the transport model the apical membrane pump has Michaelis-Menten kinetics with an apparent $K_m$ = 1.1 μM. Net basal to apical transport of vinblastine was also observed in LLC-PK1 cells and OK cells which are other kidney-derived cell lines. The order of potency of the transport is LLC-PK1 > MDCK > OK cells. The organic cation transporter is not involved in this vinblastine transport because vinblastine transport in MDCK cells was not affected by 3 mM tetramethyl- or tetraethylammonium. Inhibitors of vinblastine transport across MDCK epithelia, in order of potency, were verapamil > vincristine > actinomycin D > daunomycin. The transport pattern we observed is that predicted to result from the function of the multidrug transporter in the apical plasma membrane.**

## Introduction

Resistance of cultured cells to multiple chemotherapeutic agents may result from expression of the MDR1 gene [1]. This gene encodes a protein termed P-glycoprotein, which acts as an energy-dependent transport system for natural product cytotoxic drugs such as doxorubicin, vinblastine and actinomycin D. P-glycoprotein is found in several tissues, including the kidney [2–4]. In the kidney, it is located in the apical membrane of the proximal tubule [5]. The appearance of P-glycoprotein on the apical membrane of epithelia could result in basal to apical transepithelial transport, i.e., secretion of cytotoxic substrances.

To explore this hypothesis, we have studied epithelia formed by several cultured kidney cell lines which form epithelia able to transport electrolytes and non-electrolytes. We have recently reported that MDCK epithelia transport vinblastine, vincristine, daunomycin, actinomycin D and verapamil [6]. In the current work, we have developed a transport model that permits estimation of pump $K_m$ and pump activity solely on the basis of transepithelial tracer fluxes. We use the model to facilitate comparison of the $K_m$ for P-glycoprotein in situ in epithelia with the results of a study performed previously using membrane vesicles from multidrug-resistant KB cells [7]. We find that several different

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kidney cell lines transport vinblastine, and that the estimated $K_m$ of this transport based on our new model, in situ in epithelia, is similar to the $K_m$ for vinblastine transport by membrane vesicles from multidrug-resistant cells.

## Materials and Methods

*Cultured cells and epithelia.* MDCK and LLC-PK1 cells were obtained from the American Type Culture Collection, and OK cells were obtained from W.G. Strewler. Cells were grown in plastic tissue culture flasks in DMEM with 25 mM glucose, 5 mM glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin and 10% fetal bovine serum. Cells were passaged when confluent by trypsinization in $Ca^{2+}$- and $Mg^{2+}$-free PBS containing 2 mM EDTA. Confluent cells on Costar (Transwell™) filter bottom cups (Nuclepore™, 3.0 μm pores) were used for transport studies. Each 5 $cm^2$ Transwell™ cup was placed in the 35 mm well of a Costar Cluster Six tissue culture dish. There were 2 ml of medium outside (basolateral surface) and 2 ml inside (apical surface) the cup. Cells cultured on the filters were studied four days after seeding at confluent density.

*Transepithelial transport measurement.* To measure the transepithelial transport of $^3$H-labeled vinblastine from the apical side to the basal side of an epithelial monolayer, apical medium was replaced with 2 ml medium containing [$^3$H]vinblastine and basal medium was replaced with 2 ml medium containing unlabeled vinblastine at the same concentration as on the apical side. After 1 h, 2 h and 3 h of incubation at 37°C, duplicate 25 μl aliquots of medium from both the apical and basal sides were assayed for radioactivity to determine the flux of [$^3$H]vinblastine. Transepithelial transport of the $^3$H-drug from the basal side to the apical side was determined by reversing the side to which radioactivity was added. Experiments were performed with two to three independent filters for each direction of transport using epithelia seeded from the same pool of cells. Unless otherwise noted, the 1 h value was used to estimate transport velocity. Deviation in transport across epithelia formed from cells seeded at the same time is very small (Figs. 2–4), and there is no more than 2-fold variation in transport when experiments were repeated with cells seeded at another time. Transepithelial electrical resistance [8] was measured before and after the transport study to check for leaks in the epithelium.

*Calculation of transport by simple transport model.* If there is no pump which transports an agent from one side to the other side of the epithelium, apical → basal transport and basal → apical transport of the agent will be the same. Such transport depends on diffusion of the agent through apical and basal plasma membranes. If there is a pump in the apical plasma membrane which extrudes the agent to the outside of the cell, apical → basal transport of the agent will decrease and basal → apical transport will increase depending on the pump activity. Thus, the ratio of basal → apical and apical → basal transport can be a marker of the transport activity of the pump. This increase or decrease in the ratio, however, is not linear with transport activity of the pump since transepithelial flux will also be affected by diffusion across the apical and basal plasma membranes and by intercellular leaks. To characterize the properties of the pump and compare relative pump activity under different conditions, we used a simple transport model and calculated a component of the pump activity.

We assume the following: (1) Transepithelial transport to the recipient side depends on the intracellular substrate concentration. (2) Steady-state develops rapidly relative to the time scale of the measurements. (3) Concentration of label on the trans side of the epithelium is negligible, i.e., there is no back leak into cells. (4) The pump is the only mediated transport pathway. (5) There is no effect of membrane potential. We think these assumptions are valid for the following reasons: (1) Transport of substrate to the recipient side depends on the concentration of intracellular substrate concentration, because transepithelial transport is the same as transport through the plasma membrnae of the recipient side. (2) Transepithelial transport of vinblastine is linear within 2 h (Fig. 2), indicating that the intracellular substrate concentration is stable in this period and steady-state develops rapidly relative to the time scale of the measurement. (3) The concentration of label on the recipient side at 1 h is very low compared to the concentration of the label on the source side in these experiments, and transepithelial transport is linear for 3 h, indicating that the concentration of label on the trans side of the epithelium is negligible. (4) There is no evidence that vinblastine is transported by carriers other than P-glycoprotein. For example, it is not transported by the organic cation transport system in the kidney (Fig. 4). (5) Since vinblastine is a cationic agent, transport of vinblastine may be affected by membrane potential. We measured the potential difference between the apical side and basal side, and transepithelial resistance in MDCK epithelia. The potential difference between the apical side and the basal side is very small (< 2 mV), so that the effect of membrane potential on the transepithelial transport of vinblastine should be small.

The total substrate concentration on the source side and the recipient side is the same, and the source side has a low concentration of $^3$H-labeled substrate. $S_{total}$, total substrate concentration on source side and recipient side; $S$, $^3$H-labeled substrate on the source side; $M_{total}$, total intracellular substrate concentration ($M_{total}$ is the same in apical to basal transport studies and in

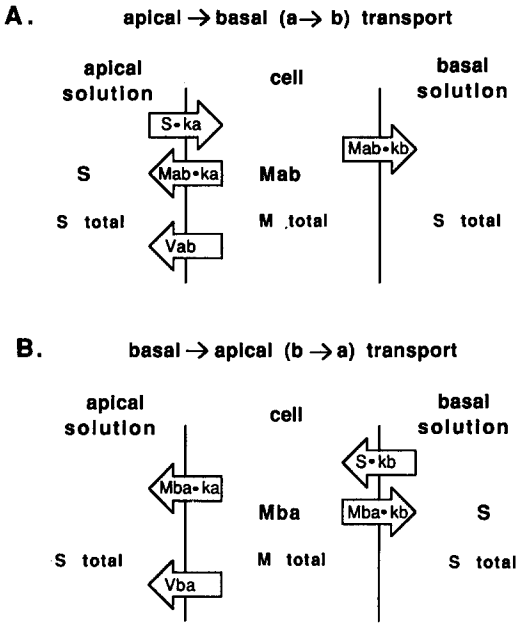


Fig. 1. Three compartment transport model. Symbols are defined in Materials and Methods.

basal to apical transport studies, because the total substrate concentration on the source side and the recipient side is the same); $M_{ab}$, intracellular $^3$H-labeled substrate concentration in apical → basal transport study; $M_{ba}$, intracellular $^3$H-labeled substrate concentration in basal → apical transport study; $k_a$, diffusion permeability constant (apical side); $k_b$, diffusion permeability constant (basal side); $V(M_{total})$, total substrate transport by the pump when the total intracellular substrate concentration is $M_{total}$; $V_{ba}$, $^3$H-labeled substrate transport by the pump in basal to apical transport study

$$V_{ba} = V(M_{total}) \cdot \frac{M_{ba}}{M_{total}} \tag{1}$$

$V_{ab}$, $^3$H-labeled substrate transport by the pump in apical to basal transport study

$$V_{ab} = V(M_{total}) \cdot \frac{M_{ab}}{M_{total}} \tag{2}$$

See Fig. 1 for the transport model.

Influx of total substrate $= S_{total} \cdot (k_a + k_b)$

Efflux of total substrate $= V(M_{total}) + M_{total} \cdot (k_a + k_b)$

Since

Influx = Efflux

$$S_{total} \cdot (k_a + k_b) = V(M_{total}) + M_{total} \cdot (k_a + k_b)$$

$$V(M_{total}) = (k_a + k_b) \cdot (S_{total} - M_{total}) \tag{3}$$

In the case of apical → basal (a → b) $^3$H-labeled substrate transport (see Fig. 1-A).

$$a \rightarrow b = M_{ab} \cdot k_b \tag{4}$$

$$\text{Influx} = S \cdot k_a$$

$$\text{Efflux} = M_{ab} \cdot k_a + M_{ab} \cdot k_b + V_{ab}$$

Since

Influx = Efflux

$$S \cdot k_a = M_{ab} \cdot k_a + M_{ab} \cdot k_b + V_{ab} \tag{5}$$

Substituting Eqn. 2 in Eqn. 5,

$$S \cdot k_a = M_{ab} \cdot k_a + M_{ab} \cdot k_b + V(M_{total}) \cdot \frac{M_{ab}}{M_{total}}$$

$$S \cdot k_a = M_{ab} \cdot \left( k_a + k_b + \frac{V(M_{total})}{M_{total}} \right) \tag{6}$$

In the case of basal → apical (b → a) $^3$H-labeled substrate transport (see Fig. 1-B).

$$b \rightarrow a = M_{ba} \cdot k_a + V_{ba} \tag{7}$$

$$b \rightarrow a = S \cdot k_b - M_{ba} \cdot k_b \tag{8}$$

$$\text{Influx} = S \cdot k_b$$

$$\text{Efflux} = M_{ba} \cdot k_a + M_{ba} \cdot k_b + V_{ba} \tag{9}$$

Substituting Eqn. 1 in Eqn. 9,

$$\text{Efflux} = M_{ba} \cdot k_a + M_{ba} \cdot k_b + V(M_{total}) \cdot \frac{M_{ba}}{M_{total}}$$

Since

Influx = Efflux

$$S \cdot k_b = M_{ba} \cdot \left( k_a + k_b + \frac{V(M_{total})}{M_{total}} \right) \tag{10}$$

Dividing Eqn. 6 by Eqn. 10,

$$\frac{k_a}{k_b} = \frac{M_{ab}}{M_{ba}} \tag{11}$$

Substituting Eqn. 3 in Eqn. 10,

$$S \cdot k_b = M_{ba} \cdot (k_a + k_b) + \frac{M_{ba} \cdot (k_a + k_b) \cdot (S_{total} - M_{total})}{M_{total}}$$

$$M_{total} = \frac{(k_a + k_b)}{k_b} \cdot \frac{S_{total}}{S} \cdot M_{ba} \tag{12}$$

Substituting Eqn. 11 in Eqn. 4,

$$a \rightarrow b = M_{ba} \cdot k_a \tag{13}$$

Adding Eqn. 8 to Eqn. 13,

$$(a \rightarrow b) + (b \rightarrow a) = M_{ba} \cdot k_a + S \cdot k_b - M_{ba} \cdot k_b \quad (14)$$

Dividing Eqn. 14 by Eqn. 13,

$$\frac{(a \rightarrow b) + (b \rightarrow a)}{(a \rightarrow b)} = \frac{S \cdot k_b - M_{ba} \cdot k_b + M_{ba} \cdot k_a}{M_{ba} \cdot k_a}$$

$$\frac{b \rightarrow a}{a \rightarrow b} = \frac{S \cdot k_b - M_{ba} \cdot k_b}{M_{ba} \cdot k_a}$$

$$M_{ba} = \frac{S \cdot k_b / k_a}{\frac{b \rightarrow a}{a \rightarrow b} + \frac{k_b}{k_a}} \quad (15)$$

Substituting Eqn. 15 for $M_{ba}$ in Eqn. 12,

Total intracellular substrate concentration ($M_{total}$)

$$= \frac{k_b / k_a}{\frac{b \rightarrow a}{a \rightarrow b} + \frac{k_b}{k_a}} \cdot S \cdot \frac{(k_a + k_b)}{k_b} \cdot \frac{S_{total}}{S}$$

$$= \frac{k_b / k_a}{\frac{b \rightarrow a}{a \rightarrow b} + \frac{k_b}{k_a}} \cdot \left( \frac{k_a}{k_b} + 1 \right) \cdot S_{total} \quad (16)$$

Substituting Eqn. 7 into Eqn. 13,

$$(b \rightarrow a) - (a \rightarrow b) = V_{ba} \quad (17)$$

Transport of $^3$H-labeled substrate by pump in basal → apical transport study

$$= (b \rightarrow a) - (a \rightarrow b)$$

From Eqn. 1,

$$V(M_{total}) = V_{ba} \cdot \frac{M_{total}}{M_{ba}} \quad (18)$$

Substituting Eqn. 12 in Eqn. 18,

$$V(M_{total}) = V_{ba} \cdot \frac{(k_a + k_b)}{k_b} \cdot \frac{S_{total}}{S} \quad (19)$$

Substituting Eqn. 17 into Eqn. 19,

Total transport by pump in basal to apical transport study

$$(V(M_{total})) = \{(b \rightarrow a) - (a \rightarrow b)\} \cdot \left(1 + \frac{k_a}{k_b}\right) \cdot \frac{S_{total}}{S} \quad (20)$$

We used $M_{total}$ as actual substrate concentration for the pump and $V(M_{total})$ as transport by the pump. We analyzed the kinetics of transport with an Eadie-Hofstee plot using these parameters (Fig. 1C).

In the inhibition experiment, we have to compare the pump activity with and without inhibitors. In this case, $S_{total}$ is the same. But $M_{total}$, the actual pump substrate concentration, is different in each condition, because $M_{total}$ depends on pump activity. Because $M_{total}$ is different in each condition, we cannot compare $V(M_{total})$ directly in an inhibition experiment. We assumed a reasonable intracellular total substrate concentration ($S_{in}$) and calculated the transport with or without inhibitors at the same intracellular total substrate concentration ($S_{in}$) to compare the transport activity of the pump ($V(S_{in})$). The value of $S_{in}$ is not important. It is just a tool to compare the transport activity of the pump at different substrate concentrations ($M_{total}$).

When the intracellular substrate is very low, transport by the pump will be almost linear to the intracellular substrate concentration.

When the intracellular concentration is $S_{in}$,

$$\text{Calculated transport by the pump} = V(M_{total}) \cdot \frac{S_{in}}{M_{total}} \quad (21)$$

Substituting Eqns. 20 and 16 in Eqn. 21:

Calculated transport by the pump

$$= \frac{\{(b \rightarrow a) - (a \rightarrow b)\} \cdot (1 + (k_a / k_b)) \cdot (S_{total} / S)}{\left( \frac{k_b / k_a}{\frac{b \rightarrow a}{a \rightarrow b} + \frac{k_b}{k_a}} \cdot ((k_a / k_b) + 1) \cdot S_{total} \right)} \cdot S_{in}$$

$$= \{(b \rightarrow a) - (a \rightarrow b)\} \cdot \frac{\frac{b \rightarrow a}{a \rightarrow b} + \frac{k_b}{k_a}}{\frac{k_b}{k_a} \cdot S} \cdot S_{in}$$

When the pump is inhibited by different concentrations of unlabeled substrate with the same concentration of $^3$H-labeled substrate:
From Eqn. 13,

$$(a \rightarrow b)_1 = M_{ba_1} \cdot k_a \quad (22)$$

$$(a \rightarrow b)_2 = M_{ba_2} \cdot k_a \quad (23)$$

where the subscripts 1 and 2 refer to different substrate concentrations from Eqn. 8,

$$(b \rightarrow a)_1 = S \cdot k_b - M_{ba_1} \cdot k_b \quad (24)$$

$$(b \rightarrow a)_2 = S \cdot k_b - M_{ba_2} \cdot k_b \quad (25)$$

Subtracting Eqn. 23 from Eqn. 22,

$$(a \rightarrow b)_1 - (a \rightarrow b)_2 = k_a \cdot (M_{ba_1} - M_{ba_2}) \quad (26)$$

Subtracting Eqn. 24 from Eqn. 25,

$$(b \rightarrow a)_2 - (b \rightarrow a)_1 = k_b \cdot (M_{ba_1} - M_{ba_2}) \quad (27)$$

Dividing Eqn. 27 by Eqn. 26,

$$\frac{k_b}{k_a} = \frac{(b \rightarrow a)_2 - (b \rightarrow a)_1}{(a \rightarrow b)_1 - (a \rightarrow b)_2} \quad (28)$$

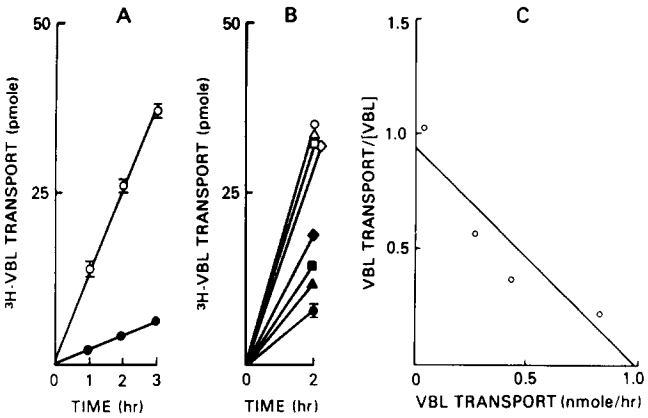


Fig. 2. Kinetics of transepithelial transport of $^3$H-labeled vinblastine in MDCK monolayers. (A) MDCK cells were cultured on filter bottom cups. Source side medium was replaced with medium that contained 100 nM [$^3$H]vinblastine (VBL) and recipient side medium was replaced with medium that contained 100 nM unlabeled vinblastine. Total substrate concentration on both sides of the epithelia is the same (100 nM). Transepithelial transport of [$^3$H]vinblastine from basal to apical surfaces (○) and from apical to basal surfaces (●) was measured at 1, 2 and 3 h. (B) Transepithelial transport of 100 nM [$^3$H]vinblastine from basal to apical (○, △, □, ◇) and apical to basal (●, ▲, ■, ◆) sides was measured at 2 h in MDCK epithelia. Unlabeled vinblastine was added to both sides of the epithelia so that the total concentration of vinblastine was 100 nM (○, ●), 1 μM (△, ▲), 2 μM (□, ■), and 5 μM (◇, ◆). When total vinblastine concentration is 1 μM, source side contains 100 nM labeled vinblastine and 900 nM unlabeled vinblastine. Recipient side contains 1 μM unlabeled vinblastine. (C) Eadie-Hofstee plot of the data shown in Fig. 1B. Estimated intracellular vinblastine concentration [VBL] was calculated by Eqn. 16 as $M_{total}$, and vinblastine transport by the pump was calculated by Eqn. 20 as $V(M_{total})$ in Materials and Methods. A least-squares fit of this plot yields an estimated $K_m = 1.1$ μM.

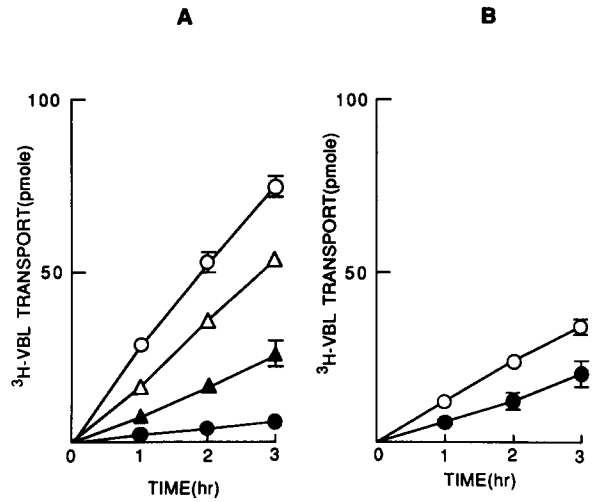


Fig. 3. Time course of transepithelial transport of vinblastine in LLC-PK1 and OK epithelia. (A) Transepithelial transport of 100 nM [$^3$H]vinblastine from basal to apical (○, △) and from apical to basal (●, ▲) sides was measured as a function of time in LLC-PK1 cells with 20 μM unlabeled vinblastine (△, ▲) or without (○, ●) unlabeled vinblastine. (B) Transepithelial transport of 100 nM [$^3$H]vinblastine from basal to apical (○) and from apical to basal (●) sides was measured as a function of time in OK cells.

Using Eqn. 28 to calculate $k_b/k_a$ from 100 nM [$^3$H]vinblastine transport across MDCK epithelia with or without 20 μM unlabeled vinblastine (data not shown), we obtained a $k_b/k_a$ value for vinblastine of 0.67.

## Results

*Transport of vinblastine by MDCK cells*

Transepithelial transport of $^3$H-labeled vinblastine across epithelia formed by MDCK cells was determined. As previously noted [6] basal → apical flux of 100 nM vinblastine at 1 h was about 5-times higher than apical → basal flux (Fig. 2A), indicating that there is basal → apical active transport of vinblastine in MDCK cells. Unlabeled vinblastine reduced basal → apical flux of tracer and increased apical basal flux of tracer at 2 h in a concentration-dependent manner (Fig. 2B), a pattern expected if there is a saturable vinblastine extruding pump at the apical plasma amembrane. Estimated intracellular substrate concentration and estimated transport by the pump were calculated from the data of Fig. 2B using equations in Materials and Methods. The results were plotted as transport by pump/[intracellular substrate] vs. transport by pump (Eadie-Hofstee plot, Fig. 2C). From this plot (correlation coefficient = −0.92) we estimate that vinblastine is

TABLE I

*Effect of verapamil and cytotoxic drugs on vinblastine transport by MDCK epithelia*

a → b, apical → basal transport; b → a, basal → apical transport; inhibitor concentration, 20 μM; vinblastine concentration, 100 nM; pump, estimated transport by pump when the intracellular substrate concentration is 100 nM.

| Inhibitor | Vinblastine transport (pmol/h) | | | | | | Inhibition (%) |
|---|---|---|---|---|---|---|---|
| | no inhibitor | | | with inhibitor | | | |
| | a → b | b → a | pump | a → b | b → a | pump | |
| Verapamil | 2.6 | 15.5 | 127.7 | 3.5 | 9.5 | 30.3 | 76 |
| Daunomycin | 1.9 | 13.1 | 126.5 | 2.2 | 9.6 | 55.6 | 56 |
| Vincristine | 1.7 | 8.3 | 54.7 | 3.8 | 7.6 | 15.1 | 72 |
| Actinomycin D | 1.7 | 8.3 | 54.7 | 2.0 | 5.7 | 19.4 | 64 |

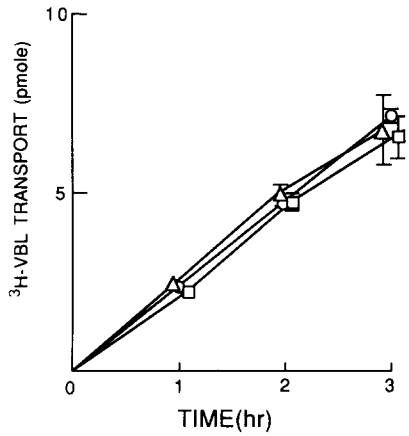


Fig. 4. Effect of tetramethyl- and tetraethyl-$NH_4$ on transepithelial transport of vinblastine in MDCK epithelia. Apical to basal transepithelial transport of 100 nM [$^3$H]vinblastine was measured in MDCK cells with 3 mM tetramethylammonium (△), 3 mM tetraethylammonium (□) or without these agents (○).

transported by the pump with Michaelis-Menten kinetics with an apparent $K_m = 1.1$ μM.

*Vinblastine transport in other kidney-derived cell lines*

Basal to apical transport of 100 nM [$^3$H]vinblastine after 1 h in LLC-PK1 cells is 13-times higher than apical to basal transport (Fig. 3A). This ratio is higher than that of MDCK cells (cf. Fig. 2). 20 μM unlabeled vinblastine inhibits this transport of tracer with the same inhibition pattern that was observed in MDCK cells. Basal to apical transport of 100 nM [$^3$H]vinblastine in 1 h in OK cells is 2-times higher than apical to basal transport (Fig. 3B). These data indicate that the order of potency for the transport of vinblastine is LLC-PK1 > MDCK > OK cells.

*Effect of tetramethyl or tetraethylammonium on vinblastine transport*

To test whether the organic cation transporter of the proximal tubule is involved in the transport of vinblastine, we added standard substrates for the organic cation transporter and studied the flux of [$^3$H]vinblastine. In contrast to the inhibition by unlabeled vinblastine at a concentration of 1 to 5 μM (Fig. 2B), the addition of 3 mM tetramethyl- or tetraethylammonium to both sides of the epithelium had no effect on apical to basal transport of [$^3$H]vinblastine (Fig. 4).

*Inhibition of vinblastine transport across MDCK epithelia*

Table I summarizes the effect of various drugs on vinblastine flux across MDCK epithelia. It is possible to use our transport model to calculate pump activity in the presence of inhibitors of transport. The inhibitors were present on both sides of the epithelia, and the effect of inhibition of the transporter is the same in apical to basal and basal to apical transport studies. 20 μM daunomycin, vincristine, actinomycin D and verapamil on both sides of the epithelium reduced net pumping of vinblastine. If the concentration of inhibitors at the pump is assumed to be same, then the order of potency of the inhibitors, based on estimated inhibition of the calculated pump activity is verapamil > vincristine > actinomycin D > daunomycin.

## Discussion

Previous studies have demonstrated that P-glycoprotein functions to maintain low intracellular concentrations of hydrophobic cytotoxic drugs and that P-glycoprotein is an ATP-driven pump [1,7]. On the basis of the cellular and tissue distribution of P-glycoprotein, we have suggested that its physiological function is the excretion of xenobiotics and unknown endogenous metabolites. Inhibition of the pump by unlabeled vinblastine reduced basal to apical transport and increased apical to basal transport of tracer in MDCK epithelia. The transport pattern we observed in this study and in our previous work [6] is that predicted to result from the function of P-glycoprotein in the apical plasma membrane. Although secretory transport could also result from an inward directed pump in the basolateral plasmaa membrane such as *p*-aminohippurate transport in the proximal tubule [9], such a transporter would increase the intracellular concentration of transported drugs and result in cytotoxicity.

Daunomycin, vincristine, actinomycin D and verapamil inhibit vinblastine transport in MDCK epithelia. This supports the idea that vinblastine transport results from the pump function of P-glycoprotein. The order of potency as inhibitors of vinblastine transport in MDCK epithelia is the same as that in plasma membrane vesicles prepared from drug-resistant KB cells, i.e., varapamil = vincristine > actinomycin D > daunomycin.

We used a new transport model for kinetic analysis using data from experiments with different concentrations of vinblastine. Based on the Eadie-Hofstee plot the estimated $K_m$ value for vinblastine transport is 1.1 μM. This value is similar to the value of 2.18 μM that was obtained in plasma membrane vesicles from drug-resistant KB cells [7].

Kinetic analysis of transport is needed for characterization of the transporter. Primarily, it is not possible to calculate the kinetic parameters of the pump in transepithelial transport studies using only steady-state flux studies such as these. In addition to estimating $K_m$ for the pump (Figs. 2C), we used Eqn. 28 to calculate $k_b/k_a$ from 100 nM [$^3$H]vinblastine transport across MDCK epithelia with or without 20 μM unlabeled vinblastine (data not shown). We obtained a $k_b/k_a$ value for vinblastine of 0.67. This ratio indicates that the diffusion of vinblastine through the apical side is greater than that through the basal side. The surface

area of the basolateral plasma membrane is larger than that of the apical plasma membrane and diffusion through the basal plasma membrane might therefore be expected to be faster than through the apical plasma membrane. However, basal membrane diffusion includes diffusion (in series) through the filter to which the basal plasma membrane is attached. These data indicate that diffusion of the substrate through the basal-lateral membrane and filter is lower than that through the apical plasma membrane.

The model that we present in this paper is generally applicable to other transepithelial transport studies. The model is useful not only for characterization of transport, but also allows calculation of the pump activity in order to compare function under different conditions.

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