Comparison of Bidirectional Cephalexin Transport across MDCK and Caco-2 Cell Monolayers: Interactions with Peptide Transporters

Wendy S. Putnam,[1] Lin Pan,[2] Ken Tsutsui,[2] Lori Takahashi,[2] and Leslie Z. Benet[1,3]

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Purpose. Bidirectional transport studies were conducted to determine whether Madin-Darby canine kidney (MDCK) cell monolayers could be used as an alternative to the traditional Caco-2 assay as a fast-growing *in vitro* model of peptide transport.

Methods. Transport of cephalexin and glycylsarcosine across MDCK and Caco-2 cell monolayers was quantified using LC-LC/MS. Glycylsarcosine, p-aminohippuric acid (PAH), and tetraethylammonium chloride (TEA) were tested as inhibitors of cephalexin transport.

Results. The ratio of apparent cephalexin permeabilities (apical to basolateral/basolateral to apical) obtained from MDCK monolayers was almost 5-fold greater than that obtained from Caco-2 monolayers. The opposite trend was observed for glycylsarcosine. When MDCK monolayers were used, glycylsarcosine reduced the cephalexin/apparent permeability ratio almost 90%. PAH and TEA did not inhibit cephalexin transport across MDCK or Caco-2 cell monolayers.

Conclusion. MDCK cell monolayers may be a promising, fast-growing alternative to Caco-2 cells for identifying peptide transporter substrates. However, differences in the apical-to-basolateral transport of cephalexin and glycylsarcosine suggest that the basolateral transport mechanisms for these compounds are different in the two cell lines. Additionally, because the activity of the peptide transporter in MDCK cells was low, scaling factors may be required when using this cell line to predict *in vivo* drug absorption.

KEY WORDS: Caco-2; cephalexin; glycylsarcosine; MDCK; peptide transporter.

INTRODUCTION

Caco-2, a human adenocarcinoma cell line, has been widely used as an *in vitro* model of passive and carrier-mediated intestinal drug absorption. Although derived from the colon, Caco-2 cells differentiate under normal cell culture conditions into polarized monolayers having characteristics of the small intestine. Like enterocytes, Caco-2 cells develop microvilli on the apical surface and express brush-border enzymes (1) and intestinal transporters (2–5) for amino acids, peptides, bile acids, and sugars. Furthermore, because the permeability of drugs across Caco-2 cell monolayers has been correlated to human absorption (6), this system is routinely used in screening new drug candidates.

Caco-2, however, is not the ideal cell model for high-throughput screening because it is time-consuming to grow and maintain and its expression of transporters is variable (7). The standard assay requires 3 weeks for cells to differentiate into monolayers with intestinal characteristics. Although differentiated cells express many intestinal transporters, their expression levels may be higher or lower than those in the small intestine, producing deviations in absorption correlations (7). In particular, Caco-2 cells express a low level of the peptide transporter intestinal peptide transporter (PEPT1) (8). An H^+-coupled cotransporter, PEPT1 mediates the uphill transport of peptides with two and three amino acids, which are formed during protein hydrolysis in the gastrointestinal tract. Because of its low PEPT1 expression level, Caco-2 underpredicts the absorption of cephalexin and other peptide transporter substrates (8).

Alternative Caco-2 screening systems have been developed that have the potential to increase throughput by reducing the time required from seeding to experimentation. Increasing the seeding density produces a 4-day Caco-2 system that successfully predicts efflux by P-glycoprotein (9), but may be a less useful model for the peptide (10) and bile acid transporters (4), which require a longer growth period to become fully functional. An expensive 3-day system that requires proprietary media supplements and collagen-coated inserts to accelerate the differentiation process (11) is commercially available. However, this system has less discriminating power than the standard assay and also has variable transporter expression.

Madin-Darby canine kidney (MDCK) cells offer a promising alternative to Caco-2 cells for increasing the throughput of preclinical absorption studies. Because MDCK cells grow and differentiate quickly, they may be used for transport studies 3 days after seeding on filter inserts. MDCK cells have been previously used for predicting the passive permeability of drugs (12) and for demonstrating efflux by P-glycoprotein (13) and uptake by peptide transporters in the apical and basolateral membranes (14,15). Surprisingly, previous kinetic studies suggest that the apical peptide transporter in MDCK behaves like the intestinal transporter PEPT1, not the renal transporter PEPT2 (14).

Because Caco-2 cells seem to underexpress PEPT1, we investigated whether or not MDCK cells would be a more useful model for identifying substrates of the peptide transporter and predicting their absorption *in vivo*. We selected the antibiotic cephalexin as a model substrate because oral cephalosporins, unlike peptides, are not hydrolyzed by intestinal peptidases (see Dantzig and Bergin [3]). In the current study, we compared the bidirectional transport and accumulation of cephalexin across both MDCK and Caco-2 cell monolayers.

MATERIALS AND METHODS

Materials

Cephalexin, glycylsarcosine, tetraethylammonium chloride (TEA), and p-amino hippuric acid (PAH) were pur-

[1] Department of Biopharmaceutical Sciences, School of Pharmacy, University of California, 513 Parnassus Avenue, Room U-68 San Francisco, California 94143-0446.

[2] Affymax Research Institute, Santa Clara, California.

[3] To whom correspondence should be addressed. (e-mail: benet@itsa.ucsf.edu)

ABBREVIATIONS: A-to-B, apical-to-basolateral; B-to-A, basolateral-to-apical; LC-LC/MS, liquid-liquid chromatography mass spectrometry; MDCK, Madin-Darby canine kidney; PAH, p-amino hippuric acid; PEPT1, intestinal peptide transporter, PEPT2, renal peptide transporter; TEA, tetraethylammonium chloride.

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chased from Sigma Chemical Company (St. Louis, MO). Wild-type MDCK strain I cells were provided by Dr. Ira Pastan (National Institutes of Health, Bethesda, MD), and Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD). Cell culture media were purchased from the University of California, San Francisco, Cell Culture Facility (San Francisco, CA).

Cell Culture

To prepare monolayers for transport studies, MDCK (passages 59–65) and Caco-2 (passages 22–25) cells were cultured at 37°C in a humidified atmosphere with 5% CO_2. Cell confluence was assessed by light microscopy. Cells were passaged at 90–100% confluence using 0.05% trypsin EDTA and were seeded at an approximate density of 60,000 cells/cm^2 onto polyethylene terephthalate inserts in six-well plates (Becton Dickinson, Franklin Lakes, New Jersey).

MDCK cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Cells were fed 24 h postseeding and then every other day, including 24 h before use. Caco-2 cells were maintained in minimum essential Eagle's medium containing 2 mM L-glutamine, 5.5 mM glucose, and 2.2 g/l (26 mM) sodium bicarbonate, which was supplemented with 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 10% fetal bovine serum. Caco-2 cells were fed 24 h post-seeding and then twice weekly, including 24 h before use.

Bidirectional Transport Studies

Transport studies with cell monolayers were conducted 4–5 days after seeding MDCK cells and 20–26 days after seeding Caco-2 cells. On the day of each study, monolayer integrity was verified by measuring transepithelial electrical resistances using a Millipore Millicell-ERS system with "chopstick" electrodes (Millipore Corporation, Bedford, MA). MDCK cells had a lower average transepithelial electrical resistance value (207 ± 39 Ωcm^2) than Caco-2 cells (906 ± 197 Ωcm^2).

Transport media were prepared by adding 20 mM HEPES (pH 7.4) or 20 mM N-morpholino ethanesulfonic acid (pH 6.0) to Hank's balanced salt solution and adjusting the pH with NaOH. Cells were preincubated for 30 min at 37°C with 3 ml transport media (pH 7.4) in the basolateral compartment and 2 ml transport media (pH 6.0 or pH 7.4) in the apical compartment. The preincubation medium was then aspirated from both compartments and replaced with fresh medium, with 1.5 ml added to the apical side and 2.5 ml to the basolateral side. Transport medium containing the drug was added either to the apical or basolateral compartment. During inhibition studies, the inhibitor was added to both compartments.

At the specified time points, 150–200-μl samples were removed from the nondosing compartment and were replaced with fresh medium. Samples were analyzed with LC-LC/MS. Sink conditions were maintained throughout each transport study. Over the course of a 3-h experiment, <2% of the dose was transported to the opposite compartment.

Transport Calculations

Apparent permeability values were calculated using the following equation:

$$P_{aff} = dQ/dt \times 1/C_o \times 1/A$$

where dQ/dt is the permeability rate, C_o is the initial concentration in the donor compartment, and A is the surface area of the filter. Flux rates (dQ/dt × 1/A) were calculated by plotting the amount transported per unit area as a function of time and determining the slope of the line using linear regression.

Because the Caco-2 apical-to-basolateral (A-to-B) data (Fig. 1) deviated from linearity (r^2 = 0.89), apparent permeabilities were also calculated using average flux values for comparison. The permeability values obtained using linear regression were higher than those calculated using average fluxes; however, the trends in the kinetic results shown in Tables 1 and 2 were similar for both methods. The final results were calculated using linear regression because most of the data fit the equation well and because the nonlinearity in the Caco-2 A-to-B data did not affect the overall conclusions.

Unidirectional fluxes across apical and B membranes were calculated using the method of Naftalin and Curran (16). If A, B, and C refer to the apical, basolateral, and cell compartments; J is a steady-state flux; and R is the ratio of cellular accumulation of cephalexin across the apical membrane to cellular accumulation across the basolateral membrane, then

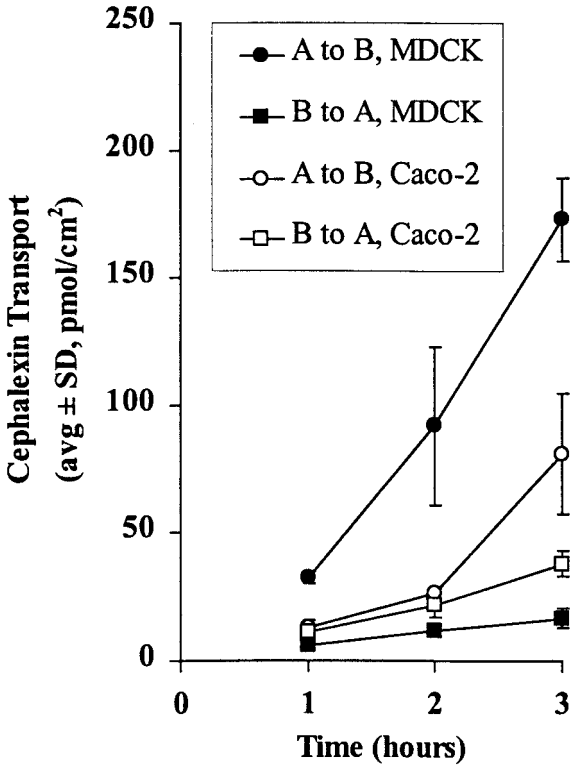

Fig. 1. Transepithelial transport of cephalexin (50 μM) across MDCK and Caco-2 cell monolayers. MDCK (closed symbols) or Caco-2 (open symbols) cell monolayers were incubated with drug in the apical (1.5 ml, pH 6.0) or basolateral (2.5 ml, pH 7.4) compartment. Transport to the opposite side was measured after 1, 2, and 3 h at 37°C. Each point represents the mean of 2–3 monolayers from a typical experiment.

Table I. Apparent Permeability Coefficients of 50 μM Cephalexin across MDCK and Caco-2 Cell Monolayers with pH Gradient[a]

Cell line	Apparent permeability (avg ± SE, nm/s) P_{A-B}	P_{B-A}	Ratio P_{A-B}/P_{B-A}
MDCK	3.9 ± 0.4	0.31 ± 0.01	12.6
Caco-2	1.9 ± 0.4	0.74 ± 0.07	2.6

[a] Apical pH, 6.0, Basolateral pH, 7.4.

$$J_{A-C} = J_{B-A} \cdot R + J_{A-B},$$

$$J_{C-A} = J_{B-A} \cdot (1 + R),$$

$$J_{C-B} = J_{A-B} \cdot (1 + 1/R) \text{ and}$$

$$J_{B-C} = J_{B-A} \cdot J_{A-B}/R.$$

Intracellular Accumulation Measurements

Intracellular accumulation was measured by adapting a method described by Sudoh *et al.* (17). After taking the last sample from a monolayer at the end of a 3-h transport study, the medium was aspired from the apical compartment and the filter with monolayer was rapidly washed three times with ice-cold phosphate-buffered saline solution. After drying, the filter was detached from the well. The cell monolayer was solubilized in 1 ml solvent, either a 70:30 methanol/water solution for cephalexin accumulation or nanopure water for glycylsarcosine accumulation. The solution was sonicated for 10 min and centrifuged for 10 min at 14,000 × *g* in a microcentrifuge (model 3531, Abbott, Abbott Park, IL). The supernatant was quantified with LC-LC/MS.

Analytical Methods

LC-LC/MS Instrumentation

Concentrations of cephalexin and glycylsarcosine were quantified using high-performance liquid chromatography (model 1100, Agilent Technologies, Palo Alto, CA), which consisted of a binary pump, vacuum degasser, autosampler, and column-switching valve. The mobile phase was composed of 0.2% formic acid in water (A) and 0.2% formic acid in acetonitrile (B). The LC separation was achieved on a 2 × 100 mm YMC AQ column with a particle size of 3 μm (Waters Corp., Milford, MA). The following gradient was used for both compounds: 0–0.2 min, 80% A; 0.2–0.3 min., 0% A; 0.3–0.8 min; 0% A; and 0.8–0.9 min, 80% A. Flow rates of 350 and 250 μl/min were utilized for cephalexin and glycylsarcosine, respectively. The injection-to-injection cycle time was 4 min with an injection volume of 5 μl for all samples. Both compounds were quantified using external calibration curves prepared according to the sample matrices.

Sample detection was performed on a PE Sciex (Concord, Ontario, Canada) API 365 with TurboIonspray using the following transitions in positive ion mode: cephalexin, m/z 348.1–158 and glycylsarcosine, m/z 146.9–90. Zero-grade air was used for the auxiliary and nebulizing gases, and ultra high pure nitrogen was used for the collision gas. The TurboIonspray interface was maintained at 400°C.

RESULTS

Bidirectional Transport of Cephalexin across MDCK and Caco-2 Cell Monolayers

Cephalexin transport was tested across MDCK and Caco-2 cell monolayers in the presence of a pH gradient: an initial pH of 6.0 in the apical compartment and a pH of 7.4 in the basolateral compartment. The time course of cephalexin transport is shown in Fig. 1. To determine whether cephalexin transport was polarized, transepithelial fluxes were measured in both directions. In both cell types, the flux of cephalexin was greater in the A-to-B direction than in the basolateral-to-apical (B-to-A) direction. The A-to-B apparent permeability in MDCK cells was twice that in Caco-2 cells, whereas the B-to-A permeability was almost 60% lower (Table 1). Overall, the net absorption of cephalexin, represented by the A-to-B/B-to-A permeability ratio, was almost 5-fold greater with the MDCK cells (12.6 vs. 2.6). The amount transported across the MDCK monolayers in 3 h was over 2-fold greater than that across Caco-2 cells.

Table 2 shows the bidirectional fluxes measured experimentally, along with the unidirectional fluxes calculated using the method of Naftalin and Curran (16). Without good estimates of intracellular concentrations, it was not possible to calculate unidirectional permeabilities for comparisons of permeability ratios across membranes. However, the data do allow for comparisons of unidirectional fluxes between MDCK and Caco-2 cells. Although the flux of cephalexin into the cell (J_{A-C}) was greater for Caco-2 than for MDCK cells, the flux out of the Caco-2 cells (J_{C-B}) was 2-fold lower than the exit flux from MDCK cells. The low transport of cephalexin across the basolateral membrane suggests that this was the rate-limiting step for transepithelial transport across Caco-2 cells.

Intracellular Accumulation of Cephalexin in MDCK and Caco-2 Cells

To further compare the characteristics of cephalexin transport in the two cell models, the intracellular accumulation of cephalexin in MDCK and Caco-2 cells was measured after 3 h of incubation in the presence of a pH gradient (Fig.

Table II. Transepithelial Bidirectional Fluxes of 50 μM Cephalexin and Calculated Unidirectional Fluxes[a]

Cell line	J_{A-B}	J_{B-A}	J_{NET}	R	J_{A-C}	J_{C-A}	J_{C-B}	J_{B-C}
MDCK	70.2 ± 7.6	5.5 ± 0.2	64.7 ± 7.6	3.7	90.6	25.9	89.2	24.5
Caco-2	34.1 ± 7.5	13.4 ± 1.2	20.7 ± 7.6	8.9	153.4	132.7	37.9	17.2

[a] Fluxes are expressed as pmol/cm^2/h (avg ± SE). R is the relative accumulation of cephalexin (accumulation from apical surface/accumulation from basolateral surface).

2). Overall, Caco-2 cells accumulated more cephalexin from both the apical and basolateral membranes than did MDCK cells. Accumulation from the apical membrane was 6-fold greater in the Caco-2 cells than in the MDCK cells, whereas accumulation from the basolateral membrane was 2.6-fold greater.

For both cell types, accumulation across the apical membrane was greater than that across the basolateral membrane. However, the accumulation from the apical side in the Caco-2 cells was almost 9-fold greater than that from the basolateral side, whereas a 3.7-fold difference was observed in the MDCK cells. Mass balance calculations following the transport and accumulation studies showed that good recovery of the drug was achieved, in a range from 95 to 104%.

pH Dependence of Cephalexin Transport across MDCK Monolayers

The effect of pH on cephalexin transport was examined by testing transport across MDCK monolayers at an apical pH of 6.0 and 7.4, while maintaining the basolateral pH at 7.4 (Fig. 3). Acidification of the apical compartment increased the cephalexin flux in the A-to-B direction and decreased the flux in the B-to-A direction. The ratio of apparent permeabilities in the two directions (A-to-B/B-to-A) increased almost 5-fold (2.7 vs. 12.6) in the presence of the pH gradient. The effect of pH on cephalexin transport across MDCK monolayers is consistent with a proton-coupled peptide transporter at the apical membrane.

Inhibition of Cephalexin Transport across MDCK Cell Monolayers

To determine whether cephalexin was transported by a peptide transporter in MDCK cells, the effect of glycylsarcosine, a substrate of the intestinal and renal peptide transporters, PEPT1 and PEPT2, was tested on cephalexin transport. The glycylsarcosine concentration selected for inhibition (20 mM) was 50-fold greater than the K_i (0.39 mM) previously reported for glycylsarcosine in Caco-2 cells (18). Glycylsarcosine (20 mM) placed in both the apical and basolateral compartments inhibited cephalexin transport across MDCK cell monolayers in the A-to-B direction and slightly increased transport in the B-to-A direction (Fig. 4). The apparent permeability in the A-to-B direction was reduced 84% (0.64 vs.

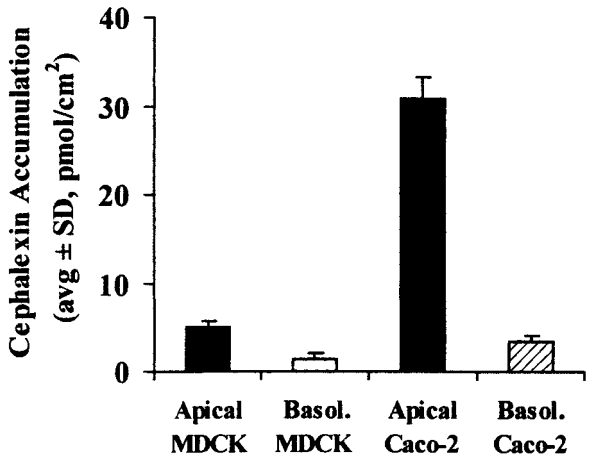

Fig. 2. Intracellular accumulation of cephalexin (50 μM) in MDCK and Caco-2 cell monolayers. Drug was added to the apical (1.5 ml, pH 6.0) or basolateral (2.5 ml, pH 7.4) compartment and accumulation within monolayers was measured after 3 h at 37°C. Each column represents the mean of 2–3 monolayers from a typical experiment.

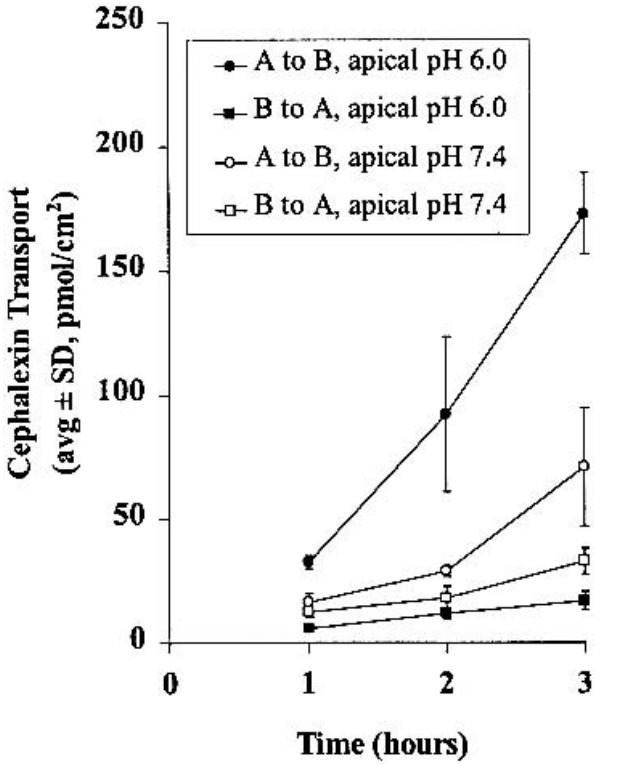

Fig. 3. Effect of apical pH on transepithelial transport of cephalexin (50 μM) across MDCK cell monolayers. Drug was added to the apical (1.5 ml, pH 6.0 or pH 7.4) or basolateral compartment (2.5 ml, pH 7.4) in the presence (closed symbols) or absence (open symbols) of a pH gradient. Transport to the opposite side was measured after 1, 2, and 3 h at 37°C. Each point represents the mean of 2–3 monolayers from a typical experiment.

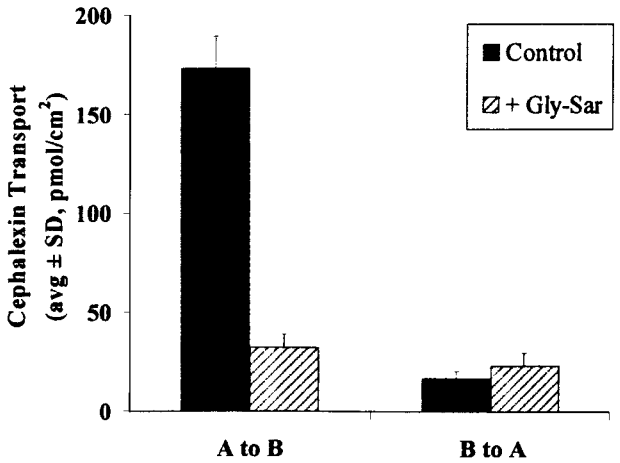

Fig. 4. Effect of glycylsarcosine on transepithelial transport of cephalexin (50 μM) across MDCK cell monolayers. Drug was added to the apical (1.5 ml, pH 6.0) or basolateral compartment (2.5 ml, pH 7.4) in the presence (hatched) or absence (solid) of 20 mM glycylsarcosine. Transport to the opposite side was measured after 1, 2, and 3 h at 37°C. Each point represents the mean of 2–3 monolayers from a typical experiment.

3.9 nm/s), whereas the permeability in the B-to-A direction was increased 45% (0.45 vs. 0.31 nm/s). The ratio of the apparent permeabilities (A-to-B/B-to-A) decreased almost 90% in the presence of glycylsarcosine (1.4 vs. 12.6).

As a control, glycylsarcosine was also tested as a substrate of the peptide transporters in MDCK and Caco-2 cell monolayers. Unlike cephalexin, A-to-B transport of glycylsarcosine was greater across Caco-2 cell monolayers than across MDCK cell monolayers (Fig. 5). The ratio of apparent permeabilities was almost 4-fold higher in Caco-2 than in MDCK cells (9.7 vs. 2.6 nm/s). Although transepithelial transport of glycylsarcosine was very low across MDCK cells, intracellular accumulation from both the apical and basolateral membranes was comparable to that observed with cephalexin (data not shown).

Effects of Organic Cation and Anion Inhibitors on Cephalexin Transport across MDCK and Caco-2 Cell Monolayers

To determine whether organic cation or anion transporters were also contributing to cephalexin transport, we tested whether TEA and PAH could competitively inhibit bidirectional cephalexin flux across MDCK and Caco-2 cell monolayers (Fig. 6). One-way analysis of variance showed that neither TEA or PAH had a significant effect on cephalexin flux in either the A-to-B or B-to-A direction for either cell line.

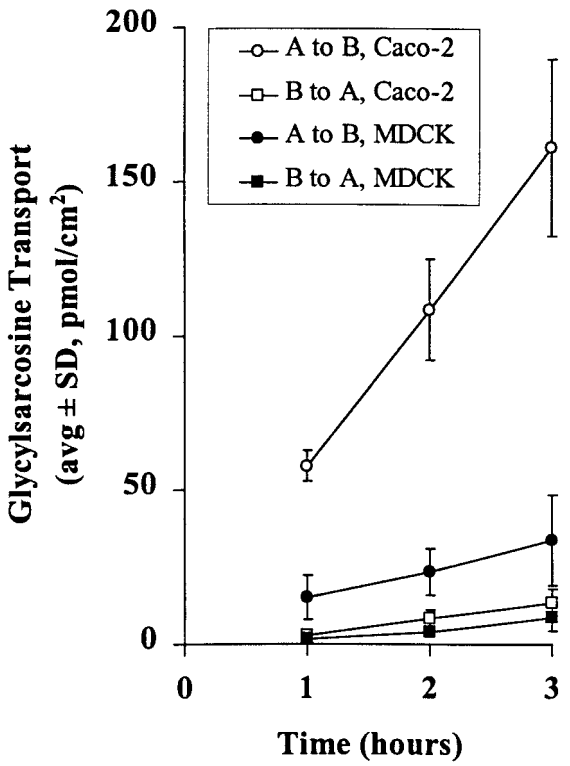

Fig. 5. Transepithelial transport of glycylsarcosine (75 μM) across MDCK and Caco-2 cell monolayers. MDCK (closed symbols) or Caco-2 (open symbols) cell monolayers were incubated with drug in the apical (1.5 ml, pH 6.0) or basolateral (2.5 ml, pH 7.4) compartment. Transport to the opposite side was measured after 1, 2, and 3 h at 37°C. Each point represents the mean of three monolayers from a typical experiment.

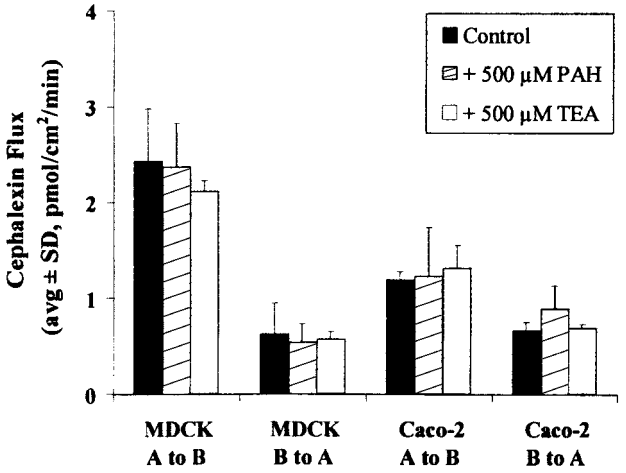

Fig. 6. Effect of organic cation and organic anion inhibitors on transepithelial transport of cephalexin (50 μM) across MDCK and Caco-2 monolayers. MDCK or Caco-2 cell monolayers were incubated with drug in the apical (1.5 ml, pH 6.0) or basolateral (2.5 ml, pH 7.4) compartment in the presence of 500 μM PAH (hatched) or TEA (clear) or in the absence of inhibitor (solid). Transport to the opposite compartment was measured after 3 h of incubation at 37°C. Each column represents the mean of 2–3 monolayers from a typical experiment.

DISCUSSION

Both MDCK and Caco-2 cells have been reported to express peptide transporters on the apical and basolateral membranes (14,15,19). The apical peptide transporter in both cell lines is pH dependent, similar to that in intestinal brush-border membrane vesicles. Western and Northern blots (20,21) have shown that Caco-2 cells contain the proton-coupled PEPT1, which is likely the peptide transporter on the apical membrane. Reverse transcription-polymerase chain reaction and Northern blot suggest that MDCK cells also express a PEPT1 homolog. A partial-length PEPT1 cDNA generated from MDCK shared 79 to 87% homology with other species (22). The identity of the basolateral transporter in the two cell lines has not been established. Functional data suggest that the transporter in Caco-2 may be a low-affinity, facilitative transporter (10,23), although Thwaites *et al.* (19) reported that it is pH dependent.

The differences between cephalexin transport and accumulation in MDCK and Caco-2 cells in this study demonstrate functional differences between the peptide transporters in the two cell models. The pH dependence of cephalexin transport in MDCK and its inhibition by glycylsarcosine suggest transport by a proton-coupled peptide transporter. However, cephalexin transport across MDCK cells was greater than that across Caco-2 cells, whereas cellular accumulation in MDCK was significantly less. The higher exit flux of cephalexin across the basolateral membrane may explain this difference, suggesting that the basolateral transporter in MDCK has a higher affinity for cephalexin than that in Caco-2 or that the transporters are distinct.

Furthermore, the transepithelial transport of glycylsarcosine was much lower across MDCK than across Caco-2 cell monolayers. The lower permeability across MDCK cells may be due to lower activity of the peptide transporter on the apical or basolateral membrane or both. However, because

MDCK cells accumulated comparable levels of glycylsarcosine from the apical membrane relative to cephalexin accumulation, lower activity of the apical transporter does not seem to account for this difference. Additionally, it is unlikely that the apical transporter was saturated because our substrate concentration (75 μM) was well below the reported K_M (1.3 mM) for glycylsarcosine uptake in MDCK cells (14). Differences in the affinity of the basolateral transporter for cephalexin and glycylsarcosine are a more likely explanation for the differences in transport.

The identity of the basolateral transporter in MDCK cells that is responsible for cephalexin transport is not known. Although PEPT2 is a high-affinity transporter for cephalosporins (24), this transporter has not been localized at the basolateral membrane of renal cells (25). Recently, a novel basolateral transporter was reported in MDCK cells, which has a high affinity for glycylsarcosine (K_M = 71 μM for uptake) (15). The basolateral transporter in MDCK cells that transports cephalexin seems to be distinct from this transporter and from the basolateral transporter in Caco-2 cells.

Multiple transporters in MDCK cells may be involved in transporting cephalexin across the basolateral membrane. For example, both an organic anion transporter (26) and an H^+-coupled organic cation antiporter (27) have been reported to transport cephalosporins in the kidneys. This is not surprising, considering that cephalexin is predominantly zwitterionic at pH 6.0 (28). In our studies, neither PAH or TEA significantly affected cephalexin transport in either the A-to-B or B-to-A directions, suggesting that organic anion and organic cation transporters are not contributing to cephalexin transport in these cell lines. However, these results do not preclude the possibility that another type of organic anion transporter may be involved in cephalexin transport across MDCK cells.

The first objective of this study was to determine whether MDCK cell monolayers were a fast-growing *in vitro* model system for identifying compounds that are substrates of peptide transporters. The success in using bidirectional transport across MDCK cells to identify peptide transporter substrates seems to be very compound dependent. For some compounds, like cephalexin and possibly other cephalosporins, this approach may be very useful for identifying peptide transporter substrates. However, for other compounds, such as glycylsarcosine, transepithelial transport across MDCK cells may be very low, particularly if carrier-mediated transport across the basolateral membrane is low.

Uptake systems may be the preferred approach for identifying drug candidates that are likely to be substrates of peptide transporters. Uptake measurements provide a good indication of interaction with a transporter and, unlike transepithelial transport studies, may be performed before cells have formed tight junctions (18). Uptake studies were previously used (14,15) to investigate peptide transporters on both the apical and basolateral membranes of MDCK cells. An uptake system with 7-day-old Caco-2 monolayers has also been developed to rank order the affinity of compounds for the peptide transporter based on their ability to inhibit uptake of a displaceable probe (18,29). Although compounds with a high affinity for a transporter are not necessarily substrates of that transporter, this system is useful for high-throughput screening.

The second objective of our study was to determine whether the permeability of a drug across MDCK cell monolayers correlated to *in vivo* absorption. The apparent permeability values that we obtained for cephalexin with a pH gradient were comparable to those previously reported for MDCK and Caco-2 cells without a gradient (12). In both cases, the permeabilities of cephalexin were about 2-fold greater in MDCK than those in Caco-2 cells. However, based on published apparent permeability correlations (12), our cephalexin permeabilities, even with an apical pH of 6.0, were an order of magnitude below the value required to accurately predict its human absorption value of 98–100%. Hence, scaling factors would be required to predict *in vivo* absorption from our *in vitro* data. In contrast, at this apical pH, Yamashita *et al.* (30) reported much higher cephalexin permeability across Caco-2 cells. This discrepancy suggests that differences in growth conditions between laboratories likely affected the expression of the peptide transporter.

Optimizing the growth media and experimental conditions will be an essential step in improving the *in vitro-in vivo* correlation obtained with MDCK cells. Brandsch *et al.* (14) showed that adding lactalbumin hydrolysate to the growth medium for MDCK cells increased the activity of the apical peptide transporter 4-fold. With our current experimental conditions, the primary advantage of MDCK over Caco-2 as an *in vitro* screening tool is its short growth period, not its ability to improve absorption predictions of peptide transporter substrates. Further studies using optimized experimental conditions and a variety of substrates are needed to substantiate the use of MDCK cell monolayers as an *in vitro* model of peptide transport.

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