Function and Immunolocalization of Overexpressed Human Intestinal H+/Peptide Cotransporter in Adenovirus-Transduced Caco-2 Cells

Cheng-Pang Hsu, Elke Walter, Hans P. Merkle, Barbara Rothen-Rutishauser, Heidi Wunderli-Allenspach, John M. Hilfinger, and Gordon L. Amidon

ABSTRACT Purpose. To determine the localization of the human intestinal H^+/peptide cotransporter (hPepT1) and its function in intestinal epithelial cells after adenoviral transduction. Methods. Caco-2 cells grown on Transwell membrane filters were transduced with a recombinant replication-deficient adenovirus carrying the hPepT1 gene. The transport of Gly-Sar across both apical and basolateral membranes was measured after adenoviral transduction as a function of pH, temperature, inhibitors, and substrate concentration. The localization of hPepT1 was examined by immunocytochemistry using confocal laser scanning microscopy. Results. The apical-to-basolateral and basolateral-to-apical transport of Gly-Sar in Caco-2 cells after viral transduction was increased 3.3 and 3.5-fold, respectively. The similar magnitude of Gly-Sar permeability from either direction indicates involvement of identical transport pathways in both membranes. This was further confirmed by immunocytochemistry showing that hPepT1 was localized in the apical and basolateral membrane of Caco-2 cells after adenoviral transduction. In both directions, Gly-Sar transport was enhanced in the presence of a pH gradient. In addition, the basolateral-to-apical Gly-Sar transport was dependent on temperature, multiplicity of infection (MOI), and Gly-Sar concentration. It was inhibited in the presence of excess Gly-Pro and cephalexin. Conclusions. Caco-2 cell monolayers represent an appropriate model to study gene expression in intestinal epithelial cells. Transport characteristics of Gly-Sar from the basolateral to the apical side in adenovirus-transduced Caco-2 cells are in agreement with those from the apical to the basolateral side, indicating that hPepT1 is also expressed in the basolateral membrane and displays a similar level of transport enhancement after adenovirus mediated hPepT1 gene expression.

Keywords: hPepT1, Gene expression, Adenovirus, Caco-2 cells, Confocal microscopy.

Corresponding Author: Cheng-Pang Hsu, Johnson & Johnson, Drug Metabolism, Route 202, P.O. Box 300, Raritan, NJ 08869; telephone: (908)704-4816; facsimile: (908)704-8412; e-mail: chsu1@prius.jnj.com

INTRODUCTION

The intestinal uptake of di- and tripeptides is mediated by an H^+-coupled peptide transporter, PepT1, of which cDNA clones have been isolated from the intestine of rabbits, humans, and rats (1). PepT1 plays an important role in the absorption of dietary proteins and peptides; in addition, it mediates the intestinal absorption of b-lactam antibiotics, angiotensin-converting enzymeinhibitors, and other peptide-like drugs. It has been shown that PepT1 could be employed as a potential drug delivery system in combination with the design of peptidomimetic prodrugs. The uptake of acyclovir in Caco-2 cells was improved 10-fold when it was modified into an L-valyl ester, valacyclovir (2). Furthermore, the permeability of a-methyldopa-Phe across the rat intestine was increased more than 20 times compared to the parent drug a-methyldopa (3). These results demonstrate that targeting PepT1 by designing peptidomimetic prodrugs may be a powerful strategy for improving oral drug absorption.

The efficient transfer of DNA encoding hPepT1 to the intestinal epithelium presents a tremendous challenge to enhance the oral bioavailability of peptide and peptidomimetic drugs. Among various strategies, replication-deficient adenoviruses appear to be potent vectors that have been successful in transferring foreign DNA into a variety of cells and organs, including postmitotic cells, such as neural cells and hepatocytes (4). Previous studies have shown that recombinant replication-deficient adenoviruses were capable of transferring the hPepT1 gene into Caco-2 cells with high efficiency (5). The resulting overexpression of hPepT1 gave significant increase of dipeptide uptake in transduced Caco-2 cells. To understand where hPepT1 is located and how it functions after adenoviral transduction, we have used confocal microscopy to investigate the cellular distribution of hPepT1, focusing particularly on its localization in the brush border and basolateral membranes. Moreover, we have studied the function of hPepT1 on both apical and basolateral cell surfaces, particularly comparing their pH dependence and transport kinetics.

MATERIALS AND METHODS

Materials

[^{3}H]-Gly-Sar (400 mCi/mmol) was synthesized by Moravek Biochemicals (Brea, CA). All other chemicals were purchased from Sigma (St. Louis, MO), unless specified. Cell culture reagents were obtained from Gibco (Grand Island, NY) and culture supplies from Corning (Corning, NY) and Falcon (Lincoln Park, NJ).

Cell Culture

Caco-2 cells were grown as described previously (5). Cells were seeded on the membrane filter (0.4-µm pores, 4.71-cm^2 growth area) in the Transwell cell culture chamber system (Costar, Cambridge, MA) at a density of 600,000 cells/filter. They were transduced with Ad.RSVhPepT1 7 days after seeding and Gly-Sar transport was measured 2 days after transduction. To assess the integrity of the monolayer, transepithelial electrical resistance (TEER) was monitored by measuring the transmembrane resistance (EVOM, World Precision Instruments, Sarasota, FL). After subtracting intrinsic resistance (filter alone without cell monolayers) from the total resistance, TEER was corrected for surface area and expressed as W cm^2.

Adenoviral transduction of Caco-2 Cells

Ad.RSVhPepT1 is a replication-defective adenoviral vector based on a genomic backbone of adenovirus type 5 and contains the hPepT1 gene under the control of the Rous sarcoma virus (RSV) promoter. The vector was constructed by the University of Iowa Vector Core, Dr. Beverly L. Davidson, Director. High-titer stocks were made in 293 cells as described before (5). Viral titers were determined by plaque-forming assay in 293 cells and expressed as multiplicity of infection (MOI, plaque-forming unit per cell).

Transport Studies

For the transport experiments, the culture medium was removed from both sides of the monolayers and the cells were washed with PBS pH 7.4. The cell monolayers were preincubated for 20 minutes at 25°C with 1.5 and 2.5 ml of transport medium at the apical and the basolateral side, respectively. The transport medium contained 145 mM NaCl, 3 mM KCl, 1 mM NaH$_2$PO$_4$, 1 mM CaCl$_2$, 0.5 mM MgCl$_2$, 5 mM D-glucose, and 5 mM MES (pH 6.0) or 5 mM Hepes (pH 7.4). The osmolarity of the transport medium was 300 ± 5 mmol/kg as measured using a vapor pressure osmometer (Wescor, Logan, UT). After preincubation, the medium was removed and transport medium containing Gly-Sar (1 mM) was added to either the apical or basolateral side while plain transport medium (no drug) was added to the receiver compartment. In order to monitor the integrity of cell monolayers, PEG-4000 permeability was measured in all transport studies. For the kinetics study of Gly-Sar transport, Gly-Sar solution was prepared at concentrations ranging from 0.1 to 10 mM in transport medium at pH 6.0. The monolayers were incubated at 25°C and samples were withdrawn from the receiver compartment at different time intervals. Gly-Sar concentration was measured by liquid scintillation spectrometry (Model LS6000; Beckman, Fullerton, CA).

Immunocytochemistry

A rabbit anti-hPepT1 polyclonal antibody was provided by Dr. Wolfgang Sadée, University of California, San Francisco, CA. It was raised against the C-terminal 15 amino acids (SNPYFMSGANSQKQM) of hPepT1 using m-maleimidobenzoyl-N-hydroxysuccinimide as the linking agent and purified through affinity chromatography. Caco-2 cells were fixed with 3% paraformaldehyde for 15 minutes at 25°C, washed with PBS once, and incubated in 0.1 M Glycine for 5 minutes. The cells were permeabilized with incubation of 0.2% Triton X-100 for 15 minutes and incubated with anti-hPepT1 antibody at 1:250 dilution for 1 hour at 37°C. After washing with PBS, the cells were then incubated with a mixture of 2 µg/ml of Cy2-labeled goat anti-rabbit IgG (Amersham), 1.3 µg/ml of TRITC-labeled phalloidin, and 0.4 µg/ml 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Boehringer-Mannheim, Mannheim, Germany) for 90 minutes. Specimens were mounted in Lisbeth's embedding media (70% glycerol, 33 mM Tris-HCl and 5% n-propyl-Gallate) and examined by confocal laser scanning microscopy (Bio-Rad 600; Bio-Rad, Richmond, CA) based on the following emission peak wavelength: Cy2 (490 nm), TRITC (550 nm), and DAPI (260 nm). For the control study, Caco-2 cells were transduced with adenovirus carrying lacZ gene (Ad.RSVlacZ) at 75 pfu/cell and immunocytochemistry was performed under the

same experimental conditions as above. In another control study, Caco-2 cells were treated with plain medium instead of anti-hPepT1 antibody (followed by the same treatment as above) in order to evaluate the background of the secondary antibody (horseradish peroxidase conjugated anti-rabbit IgG).

Data Analysis

For the kinetics study of Gly-Sar transport, Km and Vmax were determined by non-linear regression using the modified Michaelis-Menten equation by including the term of passive diffusion:

$$V = \frac{V_{max} \times C}{K_m + C} + K_d \times C \quad (1)$$

where V is the initial transport rate, Vmax is the maximum transport rate, Km is the Michaelis-Menten constant, and Kd is the coefficient of simple diffusion. Each experimental point was determined in triplicate. Results are expressed as means ± SD of these replicates. Statistical significance was evaluated by Student's t-test.

RESULTS

Transepithelial Gly-Sar Transport in Adenovirus-

Transduced Caco-2 Cells

Caco-2 cells were seeded at a high density of 600,000 cells/filter and transduced with Ad.RSVhPepT1 at an MOI of 75 pfu/cell 7 days after seeding. Forty-eight hours after transduction, the transport of Gly-Sar across Caco-2 monolayers, apical-to-basolateral and basolateral-to-apical, was examined at pH 6.0 in the donor and pH 7.4 in the receiver compartment. Integrity of the monolayers after adenovirus treatment was monitored by TEER and the leakage of a nonpermeable marker, PEG-4000. The calculated transport parameters are summarized in Table 1. The apical-to-basolateral transport of Gly-Sar was increased 3.3-fold after transduction. Most interestingly, the basolateral-to-apical transport was also increased 3.5-fold. There was no significant difference among the permeability of PEG-4000 (all less than 0.006% hr^{-1} cm^{-2}) and the monolayer resistance (all remained ~1500 W cm^2) after transduction. The consistency of monolayer resistance and PEG-4000 permeability between treated and nontreated Caco-2 cells provided evidence that cell viability and monolayer integrity were maintained after adenoviral transduction.
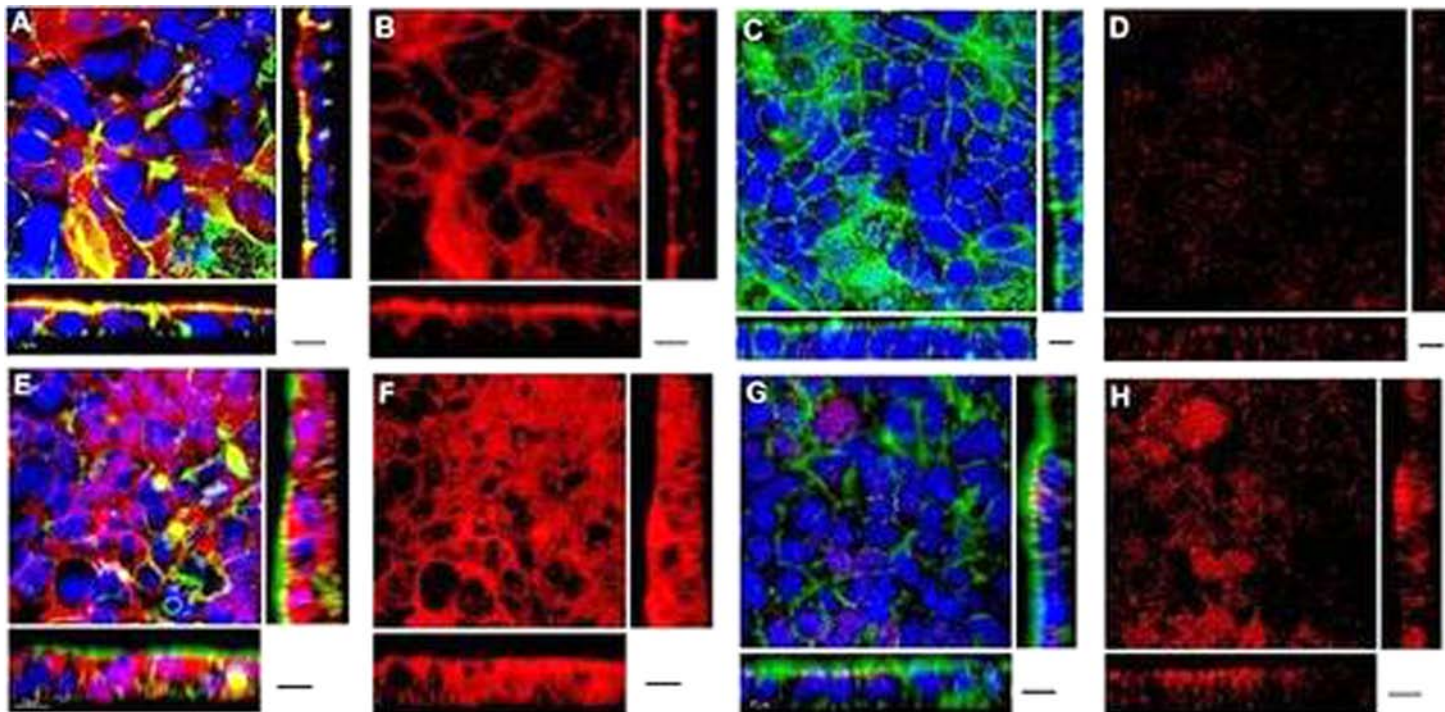

Figure 1. Immunocytochemical localization of hPepT1 in transduced Caco-2 cells. hPepT1 was stained with a rabbit anti-hPepT1 antibody and detected with Cy2-labeled anti-rabbit IgG (red) 2 days after viral transduction. In some figures additional staining of F-actin with TRITC-phalloidin (green) and of nuclei with DAPI (blue) is shown. Cells were transduced with Ad.RSVhPepT1 1 day post-seeding (A and B) or 7 days post-seeding (E and F). Untreated cells were used as controls for transduction experiments at day 1 (C and D) and day 7 (G and H). In B, D, F and H only hPepT1 staining is monitored. X-Z and Y-Z images are displayed at the side bars of each graph. Scale bar, 10 μm.

	Apical-to-basolateral		Basolateral-to-apical	
	Transduced	Control	Transduced	Control
Gly-Sar Permeability (cm/sec x 10⁷)	16.5 (0.9)*	5.0 (0.3)	16.2 (1.9)	4.6 (0.0)
PEG-4000 Permeability (cm/sec x 10⁷)	0.31 (0.10)	0.40 (0.13)	0.29 (0.04)	0.26 (0.01)
TEER (Ω cm²)	1460 (20)	1570 (7)	1530 (40)	1520 (80)

*Data are shown as means (SD) from three filters.

Table I. Transepithelial Gly-Sar transport and monolayer integrity in transduced and control Caco-2 cells.

Immunolocalization of hPepT1

Caco-2 cells develop functional and morphologic characteristics of enterocytes when grown on microporous membranes. Hidalgo et al. (6) reported that Caco-2 cells reached confluence by 6-8 days and developed constant resistance through day 17 when seeded at a density of 300,000 cells/filter. In our study, Caco-2 cells were seeded at double cell density (600,000 cells/filter) and transduced with Ad.RSVhPepT1 at day 1 or day 7. Cells were 90% confluent with no measurable TEER at day 1. In contrast, cells were fully confluent with TEER 1,500 W cm2 at day 7.

Figure 1 shows the cellular distribution of hPepT1 in transduced and nontransduced Caco-2 cells. X-Z and Y-Z images are displayed at the side bars of each graph. Cell structures were monitored by staining F-actin and nuclei. F-actin staining indicates the microvilli structure and is abundant at the inner side of the brush border membrane. By the outlines of the cellular structures, we can localize hPepT1 in the monolayers. The expression of hPepT1 in transduced Caco-2 cells Figure 1A and 1E was significant, whereas endogenous hPepT1 in nontransduced cells Figure 1C and 1G was hardly detectable. There was also a pronounced difference in hPepT1 expression between cells transduced at day 1 Figure 1B and those at day 7 Figure 1F. hPepT1 was enriched at the brush border membrane when cells were transduced at a premature stage Figure 1B. In contrast, an even distribution of transporter was found in cells transduced at a differentiated, mature stage Figure 1F. hPepT1 was distributed throughout the entire cell, including both apical and basolateral membranes. A low level of hPepT1 expression was detected in nontreated cells after 10 days in culture Figure 1H.

When cells were transduced with adenovirus carrying lacZ gene (Ad.RSVlacZ) at the same MOI (75 pfu/cell), we did not observe any difference in hPepT1 expression Figure 2A and 2B as compared with endogenous hPepT1 expression in Figure 1. Moreover, the secondary antibody (horseradish peroxidase conjugated anti-rabbit IgG) alone did not give any background in hPepT1 staining Figure 2C.
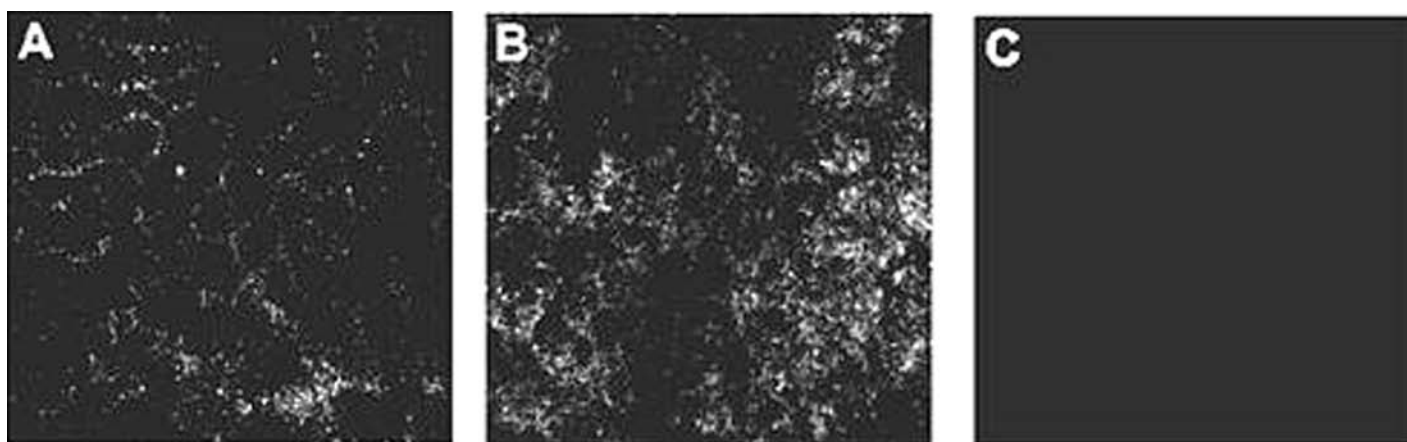
Figure 2. hPepT1 expression in Caco-2 cells infected with Ad.RSVlacZ at 1 day (A) or 7 days (B) after seeding. The effect of the secondary antibody (horse radish peroxidase conjugated anti-rabbit IgG) was evaluated in the absence of anti-hPepT1 during transduction (C).

Transduced cells

Control cells

n.d.   n.d.

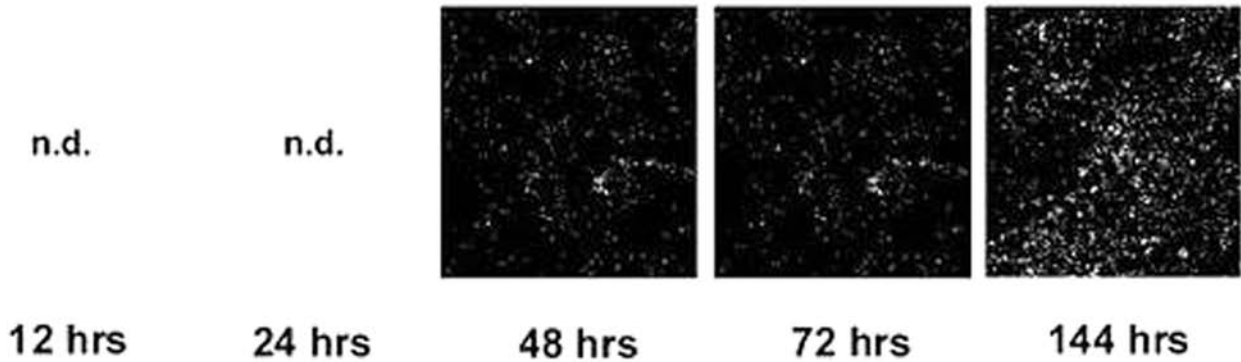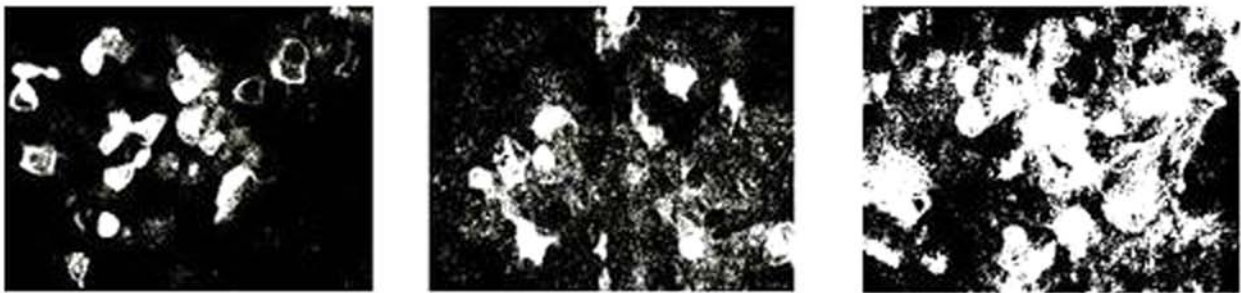12 hrs   24 hrs   48 hrs   72 hrs   144 hrs

Figure 3A. Time course of hPepT1 expression in Caco-2 cells after adenoviral transduction. Cells were transduced 1 day post-seeding and fixed at different times. n.d.= not determined.
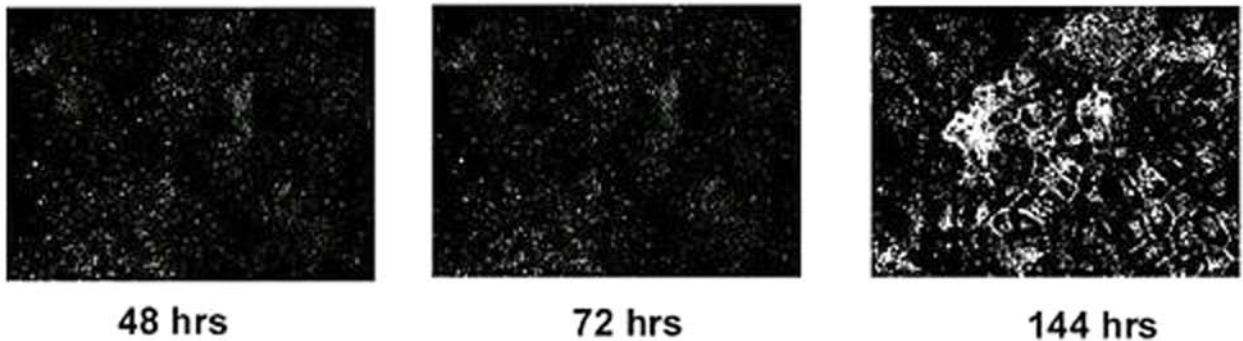
Transduced cells

Control cells

48 hrs   72 hrs   144 hrs

Figure 3B. Time course of hPepT1 expression in Caco-2 cells after adenoviral transduction. Cells were transduced 7 days post-seeding and fixed at different times.

Time Course of hPepT1 Expression in Caco-2 Cells After Adenoviral Transduction

Caco-2 cells were transduced at day 1 or 7 after seeding and fixed at different times after transduction.

As can be seen in Figure 3A, hPepT1 expression was not detected within 24 hours after transduction. After 48 hours, transporter expression was clearly more pronounced in transduced Caco-2 cells compared to nontransduced control cells and remained high up to 6 days. When cells were transduced at day 7 Figure 3B, hPepT1 could also be detected 48 hours posttransduction; however, the expression was increased over time throughout the study. At day 6 after transduction, strong staining of hPepT1 was seen in transduced Caco-2 cells, whereas little expression of endogenous hPepT1 was detected in control cells.
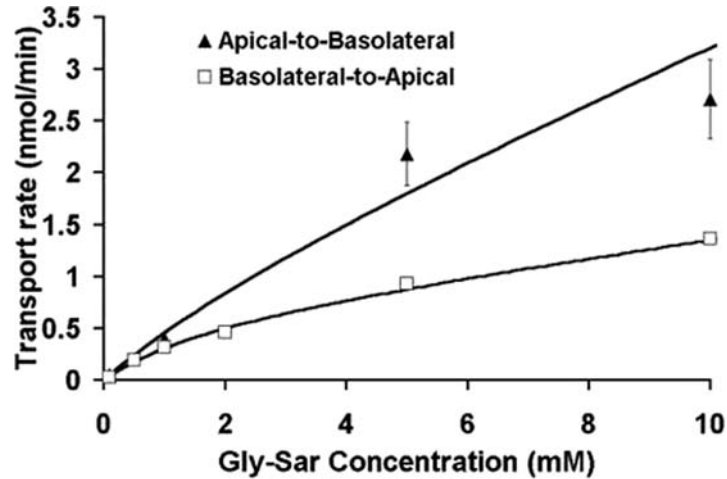
Figure 5B. Temperature dependence of Gly-Sar transport across basolateral membrane of adenovirus-transduced Caco-2 cells.

Figure 5C. Competition studies of basolateral-to-apical Gly-Sar transport in adenovirus-transduced Caco-2 cells. Gly-Sar (1 mM) transport was measured in the absence or presence of Gly-Pro (20 mM) or cephalexin (20 mM). Both Gly-Pro and cephalexin inhibited the Gly-Sar transport significantly ($p < 0.05$). Data are shown as means ± SD of 3 filters.

Figure 4. Kinetics of Gly-Sar transport across apical and basolateral membranes of adenovirus-transduced Caco-2 cells. Data are shown as means ± SD of 3 filters and fitted with equation 1 for determination of Km and Vmax.

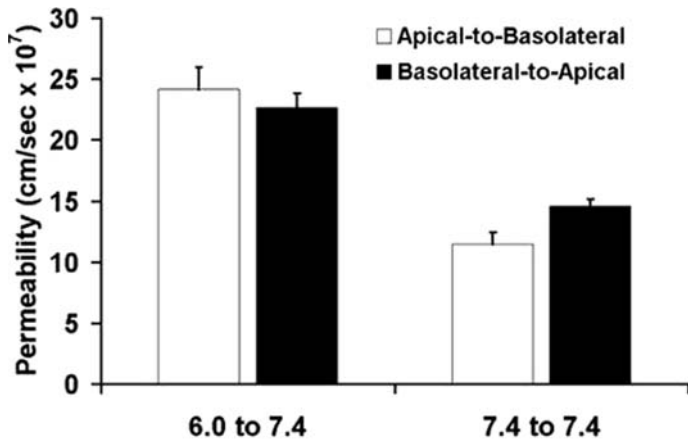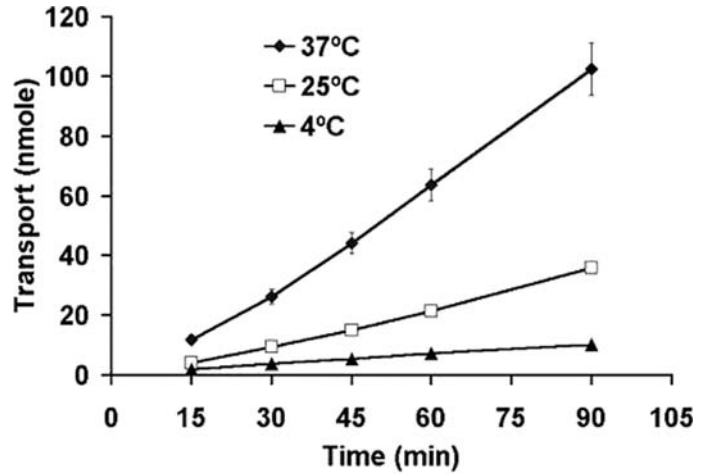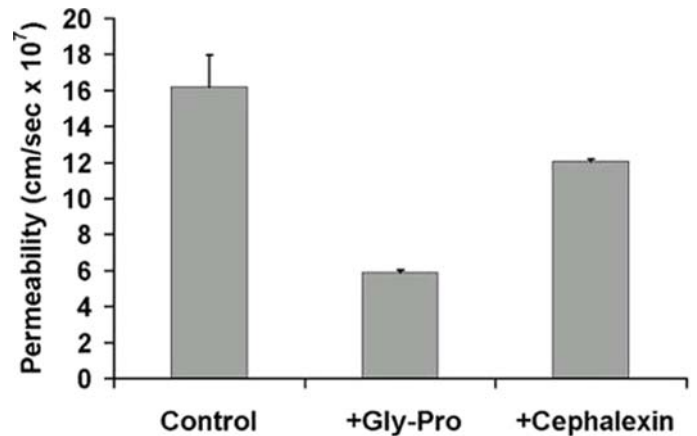**Figure 5A. pH dependence of Gly-Sar transport across apical and basolateral membranes of adenovirus-transduced Caco-2 cells.**
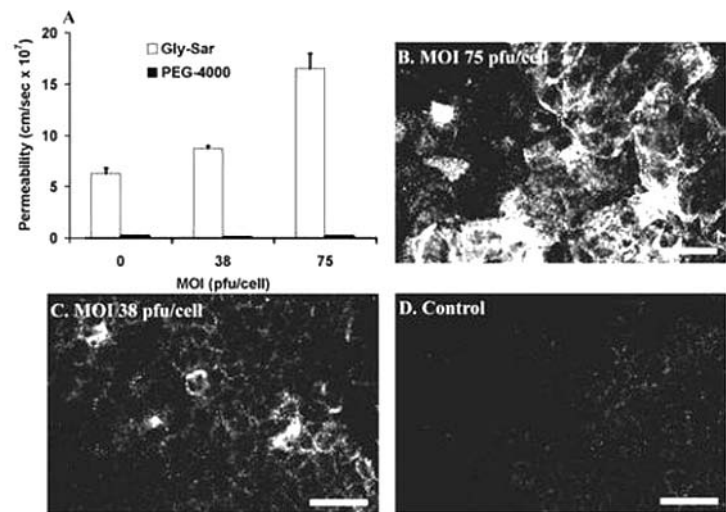
Figure 6. Effect of multiplicity of infection (MOI) on hPepT1 expression and Gly-Sar transport in adenovirus-transduced Caco-2 cells. (A) Basolateral-to-apical transport of Gly-Sar when cells were transduced at MOIs of 38 and 75 pfu/cell. (B, C, D) Corresponding immunocytochemical images from confocal laser scanning microscopy. Scale bar, 50 μm.

Concentration Dependence of Gly-Sar Transport

The existence of a carrier mediated transport mechanism triggering Gly-Sar flux across the basolateral membrane of Caco-2 monolayers was investigated by comparing the kinetic parameters of apical-to-basolateral and basolateral-to-apical Gly-Sar transport. Figure 4 shows the transport rate of Gly-Sar when it was added to either the apical or basolateral site of adenovirus-transduced Caco-2 cells. Gly-Sar transport at either direction was saturable. The apparent parameters for Km and Vmax for apical-to-basolateral transport are 3.46 mM, 0.9 nmol min^{-1} mg^{-1} and 1.89 mM, 0.65 nmol min^{-1} mg^{-1} for basolateral-to-apical transport.

Functional Analysis of hPepT1 in Caco-2 Cells After Adenovirus Transduction: Effect of Extracellular pH, Temperature, MOI, and Inhibitors

The effect of varying the pH of the donor side from 6.0 to 7.4 on Gly-Sar transport across both apical and basolateral membranes is shown in Figure 5A.

When the pH gradient was abolished, apical-to-basolateral and basolateral-to-apical transport of Gly-Sar was decreased by 52 and 36%, respectively. This pH dependence agrees with the function of hPepT1, which uses a proton gradient as driving force. The effect of temperature on the basolateral-to-apical transport of 1 mM Gly-Sar was determined by measuring the rate of transport at 4, 25, and 37°C Figure 5B.

The calculated permeabilities were 0.39 x 10^{-6}, 1.50 x 10^{-6}, and 4.33 x 10^{-6} cm/sec, respectively. At 37°C, the permeability of Gly-Sar was approximately 3 times higher compared to 25°C and 11 times higher compared to 4°C. Most important, the basolateral-to-apical transport of Gly-Sar was inhibited by addition of another dipeptide, Gly-Pro, and a b-lactam antibiotic, cephalexin Figure 5C.

Gly-Pro and cephalexin inhibited Gly-Sar transport by 63 and 25%, respectively. The effect of MOI on hPepT1 expression and Gly-Sar transport in adenoviral transduced Caco-2 cells is shown in Figure 6.

Gly-Sar permeability was measured Figure 6A after Caco-2 cells were transduced at MOIs of 38 and 75

pfu/cell. The corresponding immunocytochemical images of hPepT1 expression are given in Figure 6B-D. An increase of hPepT1 expression was observed as the dose of the virus was doubled. Compared to nontreated cells, the basolateral-to-apical Gly-Sar transport was enhanced 1.4- and 2.6-fold after cells were transduced at MOIs of 38 and 75 pfu/cell, respectively.

DISCUSSION

In the present study, we demonstrate that Caco-2 cells represent a useful model to study transporter expression in transduced intestinal epithelial cells. Based on immunocytochemistry, rat PepT1 is localized at the brush-border membranes of the absorptive epithelial cells along the small intestine but absent in crypt and goblet cells (7). This observation agrees with the PepT1 distribution in rabbit intestine studied by the same method (8). Interestingly, in our study we found hPepT1 expression to be located in both apical and basolateral membranes of adenoviral-transduced Caco-2 cells, which is consistent with results by Thwaites et al. (9) in nontransduced Caco-2 cells. They suggested that H$^+$-coupled dipeptide transporter was expressed at both membrane faces of Caco-2 cells according to similar pH dependence and substrate specificity in Gly-Sar transport across membranes. However, the distribution of hPepT1 in Caco-2 cells is controversial. Walker et al. (10) reported that hPepT1 was exclusively localized in the apical membrane of Caco-2 cells by a method of immunocytochemistry. The biological basis for the discrepancy of PepT1 distribution in Caco-2 cells versus rat or rabbit intestine is unknown. An intriguing possibility could be a difference in signaling for hPepT1 traffic and sorting in Caco-2 cells. In addition, the distribution of hPepT1 after viral transduction at distinct stages of Caco-2 differentiation revealed significant differences, which raises the question of a possible influence of cell growth on hPepT1 gene expression. Recently, Gonzalez et al. (11) found hPepT1 was not only present at the plasma membrane but also in intracellular vesicular structures of Caco-2 cells. Moreover, it was reported that hPepT1 was also localized in nuclei of vascular smooth muscle cells and in lysosomes of the exocrine pancreas (12). These results agree with our finding in Figure 1F, where the intracellular domains were stained by anti-

hPepT1 antibody. Physiologically, hPepT1 on the basolateral membrane may act as an exchanger with lower capacity for peptide exiting the cells due to the lack of an H+ gradient. The exact distribution and role of hPepT1 remain to be determined.

The proposed topological model of hPepT1 consists of 12 membrane-spanning domains, a large extracellular loop, and intracellular location of both the N- and the C-terminus (13). This model was further verified by using anti-hPepT1 polyclonal antibodies against peptide segments of the proposed extracellular loop (14). Concerning its broad substrate specificity, the H+-coupling and substrate binding sites are not known; however, our results indicate that the transport activity of hPepT1 on the basolateral membranes can be increased in a low-pH environment (pH 6). This implies that the orientation of hPepT1 in the basolateral membrane is the same as it is in the brush border membrane. It also raises the question whether hPepT1 is involved in the transport of dipeptides out of the cells. If this is the case, the substrate binding site and proton involvement have to be clarified.

Recently, the gastrointestinal tract has drawn increasing attention as a target site for gene transfer. It has been shown that intestinal epithelial cells are susceptible to gene transfer by lipofection, retrovirus, and adenovirus vectors (15). In our previous studies, the transduction of Caco-2 cells by adenoviral vector applied to the apical site resulted in serosal secretion of detectable amounts of IL-1 receptor antagonist (16). Furthermore, overexpression of hPepT1 in adenovirus-transduced Caco-2 cells could be achieved showing significant enhancement of dipeptide uptake (5). In this study, we demonstrated functional hPepT1 expression at the apical and basolateral membranes of transduced Caco-2 cells. These findings imply the interesting approach of targeting peptidomimetic prodrugs to both mucosal and serosal sites of transduced enterocytes. For site-directed gene therapy in the treatment of colonic cancer or other carcinoma occurring in the intestine, chemotherapeutic prodrugs that are substrates for hPepT1 could be administrated parenterally, reach the intestine through the circulation, and selectively gain access to transduced tumor cells via overexpressed hPepT1.

In conclusion, Caco-2 cells represent an attractive model to study gene transfer to intestinal epithelial cells, including polarity of transporter expression, apical and basolateral protein sorting, substrate binding and biological function of transgenic proteins, and regulation of gene expression. The current results, showing functional hPepT1 expression in both apical and basolateral membranes of Caco-2 cells after adenoviral transduction, make the prodrug approach of targeting hPepT1 more promising.

REFERENCES

1. Leibach FH, Ganapathy V. Peptide transporters in the intestine and the kidney. Ann Rev Nutr. 1996;16:99-119.

2. Han H-K, Vrueh RLA, Rhie JK, Covitz KY, Smith PL, Lee C-P, Oh D-M, Sadee W, Amidon GL. 5′-Amino acid esters of antiviral nucleosides, acyclovir and AZT, are absorbed by the intestinal PEPT1 peptide transporter. Pharm Res. 1998;15:1154-1159.

3. Hu M, Subramanian P, Mosberg HI, Amidon GL. Use of the peptide carrier system to improve the intestinal absorption of L-a-methyldopa: carrier kinetics, intestinal permeabilities, and in vitro hydrolysis of dipeptidyl derivatives of L-a-methyldopa. Pharm Res. 1989;6:66-70.

4. Brody SL, Crystal RG. Adenovirus-mediated in vivo gene transfer. Ann. N Y Acad. Sci. 1994;716:90-101.

5. Hsu C-P, Hilfinger JM, Walter E, Merkle HP, Roessler BJ, Amidon GL. Overexpression of human intestinal oligopeptide transporter in mammalian cells via adenoviral transduction. Pharm Res. 1998;15:1376-1381.

6. Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 1989;96:736-749.

7. Ogihara H, Saito H, Shin B-C, Terada T, Takenoshita S, Nagamachi Y, Inui K-I, Takata K. Immuno-localization of H+/peptide cotransporter in rat digestive tract. Biochem Biophy Res Comm. 1996;220:848-852.

8. Sai Y, Tamai I, Sumikawa H, Hayashi K, Nakanishi T, Amano O, Numata M, Iseki S, Tsuji A. Immunolocalization and pharmacological relevance of oligopeptide transporter PepT1 in intestinal absorption of b-lactam antibiotics. FEBS Letters 1996;392:25-29.

9. Thwaites DT, Brown CD, Hirst BH, Simmons NL. Transepithelial glycylsarcosine transport in intestinal Caco-2 cells mediated by expression of H+-coupled carriers at both apical and basal membranes. J Biol Chem. 1993;268:7640-7642.

10. Walker D, Thwaites DT, Simmons NL, Gilbert HJ, Hirst BH. Substrate upregulation of the human small intestinal peptide transporter, hPepT1. J Physiol. 1998;507:697-706.

11. Gonzalez DE, Covitz KY, Sadée W, Mrsny RJ. An oligopeptide transporter is expressed at high levels in the pancreatic carcinoma cell lines AsPc-1 and Capan-2. Cancer Res. 1998;58:519-525.

12. Bockman DE, Ganapathy V, Oblak TG, Leibach FH. Localization of peptide transporter in nuclei and lysosomes of the pancreas. Int J Pancreatology 1997;22:221-225.

13. Liang R, Fei YJ, Prasad PD, Ramamoorthy S, Han H, Yang-Feng TL, Hedinger MA, Ganapathy V, Leibach FH. Human intestinal H+/peptide cotransporter. Cloning, functional expression, and chromosomal localization. J Biol Chem. 1995;270:6456-6463.

14. Basu SK, Shen J, Elbert KJ, Okamoto CT, Lee VHL, Grafenstein HV. Development and utility of anti-PepT1 anti-peptide polyclonal antibodies. Pharm Res. 1998;15:338-342.

15. Lozier JN, Yankaskas JR, Ramsey WJ, Chen L, Berschneider H, Morgan RA. Gut epithelial cells as targets for gene therapy of hemophilia. Human Gene Ther. 1997;8:1481-1490.

16. Walter E, Croyle MA, Davidson BL, Roessler BJ, Hilfinger JM, Amidon GL. Adenovirus mediated gene transfer to intestinal epithelial cells as a potential approach for oral delivery of peptides and proteins. J Control Rel. 1997;46:75-87.