J. Pharm. Pharmacol. 1999, 51: 35–40
Received June 29, 1998
Accepted September 1, 1998

The Role of an α-Amino Group on H^+-dependent Transepithelial Transport of Cephalosporins in Caco-2 Cells

SHAMSI D. RAEISSI*†, JIBIN LI* AND ISMAEL J. HIDALGO*

Drug Metabolism and Pharmacokinetics, Rhone-Poulenc Rorer Central Research, Collegeville, PA 19426-0107, USA and †Department of Pharmaceutics, Uppsala University, Sweden

Abstract

The role of an α-amino group on interaction with the intestinal and renal peptide carriers (PEPT 1 and PEPT 2, respectively) has been the subject of much investigation. Studies have differed in their conclusions about the role of an α-amino group on carrier-mediated absorption. Most studies have used brush-border membrane vesicles or perfused intestinal segments. These techniques enable the determination of membrane uptake and luminal disappearance, respectively, but not transepithelial transport. Transepithelial transport should be more predictive of absorption because it includes basolateral efflux, which could be the rate-limiting process in drug absorption. The objective of this study was to evaluate the influence of an α-amino group on PEPT 1-mediated transepithelial transport in Caco-2 cells.

The apical-to-basolateral permeability coefficients of cephalosporins with or without a free α-amino group were determined in the presence and absence of a pH gradient. Permeability coefficients obtained under these conditions were used to calculate a permeability ratio (i.e. P_{app} (pH 6·0)/P_{app} (pH 7·4)), which should indicate whether PEPT 1 is involved in transport. For cephalosporins with an α-amino group (cephalexin, cefaclor, cefadroxil, cephradine, cephaloglycin) the permeability ratios ranged between 1·77 and 2·77. In contrast, the permeability ratios for cephalosporins without an α-amino group were 1 (approx.; range = 0·74 – 1·26).

These data suggest that the presence of an α-amino group on cephalosporins increases their PEPT 1-mediated transepithelial transport in Caco-2 monolayers.

Peptide transporters are involved in the absorption or excretion of di- and tripeptides. The intestinal peptide transporter (PEPT 1) is located on the apical membrane of enterocytes and mediates the absorption of di- and tripeptides (Addison et al 1975). PEPT 1 plays a role in the intestinal absorption of β-lactam antibiotics (Okano et al 1986), angiotensin-converting enzyme inhibitors (Friedman & Amidon 1989), renin inhibitors (Kramer et al 1990) and the anti-cancer agent bestatine (Saito & Inui 1993). The broad substrate-specificity of this carrier makes it attractive as a potential means of enhancing the oral bioavailability of peptidomimetic drugs. The renal peptide transporter (PEPT 2) is located on the luminal surface of proximal tubule cells and mediates the re-absorption of di- and tripeptides from the glomerular filtrate. In man PEPT 1 and PEPT 2 differ in their affinity for cephalosporins and penicillins (Ganapathy et al 1995). This difference in substrate affinity is consistent with recent studies which indicate that the homology in amino acid sequence between PEPT 1 and PEPT 2 is only (approx.) 50% (Liu et al 1995).

Cephalosporins have low intrinsic membrane permeability, probably because of their low lipophilicity and zwitterionic character at physiological pH. Despite these unfavourable physicochemical characteristics the oral bioavailability of cephalexin, cefadroxil, cefaclor, cephradine and loracarbef is high (Smith et al 1992). This oral bioavailability is in part the result of PEPT 1-mediated transport across the intestinal brush-border membrane (Dantzig & Bergin 1990; Lowther et al 1990; Dantzig et al 1992). Cephalosporins have been used to probe the structural specificity of peptide transporters (Dantzig et al 1992; Wenzel

Correspondence: I. J. Hidalgo, Absorption Systems, Oakland's Corporate Center, 440 Creamery Way, Suite C, Exton, PA 19341, USA.

et al 1995). One structural feature that has been the focus of much attention is the free amino group. The role of this group in the interaction of peptidomimetic compounds with both PEPT 1 and PEPT 2 has been studied using cephalosporins, penicillins and model peptides (Tsuji et al 1987; Bai et al 1991; Sugawara et al 1991). Unrelated studies found that cephalosporins with a free α-amino group inhibited the uptake of [^{3}H]glycylglutamine in rabbit renal brush-border membrane vesicles (BBMV; Daniel & Adibi 1993) and the uptake of [^{14}C]cephalexin in Caco-2 cells (Dantzig et al 1992). In both instances, uptake was inhibited by cephalosporins containing an α-amino group, but not by cephalosporins lacking this group. On the basis of these studies it might be suggested that an α-amino group is needed for interaction with PEPT 1 and PEPT 2 (Dantzig et al 1992; Daniel & Adibi 1993), although other studies have found that an α-amino group is not needed for PEPT 1-mediated uptake. For example, two compounds lacking an α-amino group (cefixime and ceftibuten) underwent carrier-mediated uptake in intestinal BBMV (Dantzig et al 1994; Muranishi et al 1994) and the uptake characteristics of dipeptide analogs in perfused intestinal segments suggest that the presence of an α-amino group is not essential for PEPT 1-mediated transport (Bai et al 1991).

Experiments to determine structure – transport relationships are controversial partly because of the systems employed. Many studies have used brush-border membrane vesicles or perfused intestinal segments. These systems are useful for studying the intestinal absorption of drugs for which transepithelial transport is limited by uptake across the apical enterocyte membrane. However, when the basolateral efflux is the rate-limiting barrier for transepithelial transport (Gochoco et al 1994; Matsumoto et al 1994), use of brush-border membrane vesicles and perfused intestinal segments might lead to over-estimation of the true absorption potential of a compound. Observations made using perfused intestinal segments are based on the disappearance of the substrate from the perfusate. Equating the rate of disappearance from the perfusate with the rate of absorption might be misleading because sometimes the rate at which a compound disappears from the lumen is greater than the rate at which it crosses the intestinal mucosa (Sugawara et al 1990). Brush-border membrane vesicles and perfused intestinal segments are useful models for studying carrier-mediated apical transport but do not provide information about transepithelial transport. This limitation is important because there is evidence that binding of a drug to PEPT 1, or PEPT 1-mediated apical transport, might not be predictive of H^+-dependent transport (Ryan & Smith 1989; Gochoco et al 1994). The use of binding-uptake studies to predict transcellular transport assumes that the basolateral release of the compound is not the rate-limiting step in transcellular transport. This assumption is not supported by two studies, which suggested that the rate-limiting step in the transport of cephalexin across Caco-2 cells seems to be basolateral efflux and not apical uptake (Gochoco et al 1994; Matsumoto et al 1994). Few data are available on the structural requirements for interaction with the basolateral peptide transporter and so the possible importance of basolateral efflux on transepithelial transport cannot be ignored.

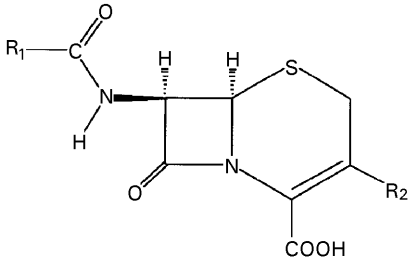
The objective of this study was to evaluate the influence of an α-amino group on the transport of cephalosporin across Caco-2 cell monolayers. The carrier-mediated transport (apical-to-basolateral) was determined for a series of structurally related cephalosporins (Figure 1) with or without an α-amino group. We conclude that the presence of an α-amino group is required for H^+-dependent transport of cephalosporin antibiotics across Caco-2 cell monolayers. Whether this observation is unique to cephalosporins or applicable to a wider variety of peptidomimetic compounds cannot be determined from these results.

Materials and Methods

The cephalosporins (Figure 1), HEPES (*N*-[2-hydroxyethyl]-piperazine-*N*′ and MES (2-[*N*-morpholino]ethanesulphonic acid) were purchased from Sigma (St Louis, MO). Dulbecco's modified Eagle's media (DMEM) and foetal bovine serum were obtained from Gibco BRL (Grand Island, NY). Penicillin G (10 000 units mL^{-1}), streptomycin solution (10 000 $\mu g\,mL^{-1}$), trypsin (0·25%)-EDTA (1 mM) solution and non-essential amino acids solution were obtained from JRH Biosciences (Kansas, MO). The Transwell culture inserts (4·71 cm^2 surface area and 0·4 μm pore size) were purchased from Corning Costar (Cambridge, MA). Caco-2 cells were obtained from The American Tissue Culture Collection (Rockville, MD) and used between passages 35 and 65. Caco-2 cells were cultured on polycarbonate Transwell inserts as described elsewhere (Hidalgo et al 1989) and used 15 – 20 days after seeding.

Apical-to-basolateral transport studies

Monolayer integrity was verified by measuring the flux of [^{14}C]mannitol. Apical-to-basolateral flux experiments were conducted in quadruplicate at 37°C. The pH of the basolateral side was 7·4 (10 mM HEPES in HBSS) and the pH of the apical



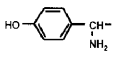
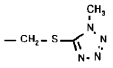
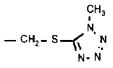
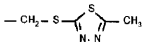
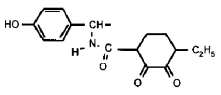
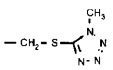
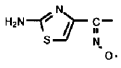
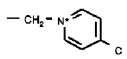
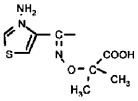
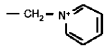
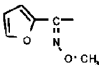
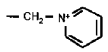
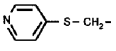
Cephalosporins	R^1	R^2
*Cefaclor		
*Cefadroxil		
Cefamandole		
Cafamandole Nafate		
Cefazolin		
Cefoperazone		
Cefotaxime		
Cefoxitin		
Cefsulodine		
Ceftazidime		
Cefuroxime		
*Cephalexin		
*Cephalogylycin		
Cephaloridine		
Cephalothin		
Cephapirin		
*Cephradine		

Figure 1 The structures of the cephalosporins tested in this study.

side was either 6 (10 mM MES in HBSS) or 7·4. Before being used to perform transport studies the cells were washed twice with HBSS. The cells were then pre-incubated with HBSS at pH 6·0 or 7·4 on the apical side for 30 min. The HBSS in the basolateral side remained at pH 7·4. The transport study was initiated by replacing the apical buffer with a solution containing 1 mM drug. Samples then were collected every 30 min up to 2 h. The apparent permeability coefficients (P_{app}) of cephalosporins were calculated according to equation 1. Previous studies showed pH-dependent transepithelial transport of cephalexin in Caco-2 cell monolayers (Gochoco et al 1994; Matsumoto et al 1994). Thus, in this study we assumed that transport at pH 7·4 is the result of passive diffusion and is pH-independent. For each cephalosporin we obtained both the P_{app} at pH 6·0 and the P_{app} at pH 7·4. The P_{app} at pH 6·0 was divided by the P_{app} at pH 7·4. This ratio (P_{app} (pH 6·0)/P_{app} (pH 7·4)) was assumed to indicate the H^+-dependent, or carrier-mediated, component of transepithelial transport, (equation 2).

$$P_{app} = (dQ/dt)/A.C_0 \quad (1)$$

$$P_{app}[H^+] = P_{app}\ (pH\ 6)/P_{app}\ (pH\ 7{\cdot}4) \quad (2)$$

where dQ/dt is the rate of transport (nmol s^{-1}), C_0 the initial concentration on the apical side (nmol mL^{-1}) and A is the diffusional area of the inserts (4·71 cm^2). P_{app} values (cm s^{-1}) are the means of results from 3 or 4 monolayers and $P_{app}[H^+]$ is the PEPT 1-mediated (H^+-dependent) component of transepithelial transport.

Sample analysis

HPLC was performed with a dual pump (model HPXL, Rainin Instrument, Woburn, MA), an automatic sampler (model AS-100T HRLC, Bio-Rad, Richmond, CA), and a UV detector (model 785A, Applied Biosystems). The HPLC system was operated by means of a Macintosh IIsi computer (Apple, Cupertino, CA). Data acquisition and integration were conducted using Rainin Dynamax software (Version 1.4). Samples were separated on a reversed-phase column (5 mm × 100 mm, 4 μm particle size, Waters C18 Radial-Pak cartridge) under isocratic conditions. The mobile phase, a mixture of acetonitrile and 10 mM ammonium acetate, was delivered at 0·8 mL min^{-1}. The acetonitrile content varied between 5 and 30%, depending on the drug being tested. Under these conditions the retention times of the compounds were between 2·3 and 12 min. UV absorbance was measured at 254 nm.

Results and discussion

Studies to determine the role of an α-amino group on the interaction of peptides and β-lactam antibiotics with PEPT 1 and PEPT 2 have led to different conclusions (Tsuji et al 1987; Bai et al 1991; Sugawara et al 1991; Daniel & Adibi 1993). This situation is partly because of the experimental systems used. In this study we evaluated the role of an α-amino group on the transepithelial transport of cephalosporins in Caco-2 cell monolayers, a model system of the small intestinal epithelium.

The intestinal absorption of cephalosporins generally involves two processes — passive diffusion and PEPT 1-mediated transport. Passive diffusion is pH-independent and PEPT 1-mediated transport is pH-dependent. In our study, the apical-to-basolateral transport of cephalexin increased threefold when the pH of the apical buffer was reduced from 7·4 to 6·0 (Figure 2). This observation is consistent with reports (Dantzig & Bergin 1990; Gochoco et al 1994) of PEPT 1-mediated, pH-dependent transport of cephalexin, a compound that is completely absorbed after oral administration (Began 1984). In contrast, the transport of cephalothin, a compound that is not absorbed after oral dosing (Klein et al 1964), was low and pH-independent (Figure 3). One structural difference between these compounds is that cephalexin contains an α-amino group and cephalothin does not (Table 1). Thus, these results support previous data which suggest that an α-amino group might be needed for interaction of cephalosporins with PEPT 1 (Dantzig et al 1992). An α-amino group also seems to influence the interaction of cephalosporins with PEPT 2. For example, the uptake of the model dipeptide glycylglutamine by rabbit renal brush-border membrane vesicles was inhibited by cephalosporins containing an α-amino group, but not by cephalosporins lacking this group (Daniel & Adibi 1993).

In the absence of PEPT 1 involvement, the permeability coefficient obtained with a pH gradient should be the same as that without. To assess the involvement of PEPT 1 on the transepithelial transport of cephalosporins, permeability coefficients obtained in the presence (P_{app} (6·0)) and absence (P_{app} (7·4)) of a pH gradient were used to calculate a permeability coefficient ratio (i.e. P_{app} (pH 6·0)/P_{app} (pH 7·4)) for each compound. For PEPT 1-mediated transepithelial transport this ratio should be greater than 1 and for passive transepithelial transport the ratio should be 1 (approx.) Our results show that for cephalosporins with an α-amino group P_{app} (pH 6·0)/P_{app} (pH 7·4) varied between 1·77 and 2·77, and that for cephalosporins without an α-amino group the ratio was 1 (approx.) (Table 1). This indicates that for cephalosporins containing an α-amino group a pH gradient increased transepithelial transport by at least 77%

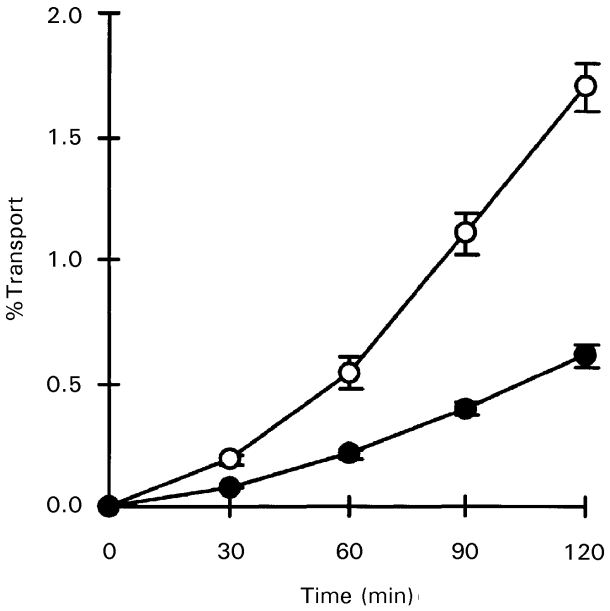


Figure 2. Apical-to-basolateral transport of cephalexin across Caco-2 cell monolayers: ○, Caco-2 cell monolayers incubated with pH 6·0 solution on the apical side and pH 7·4 on the basolateral side; ●, pH 7·4 on both the apical and basolateral sides. Results are the means ± standard deviations from four separate monolayers.

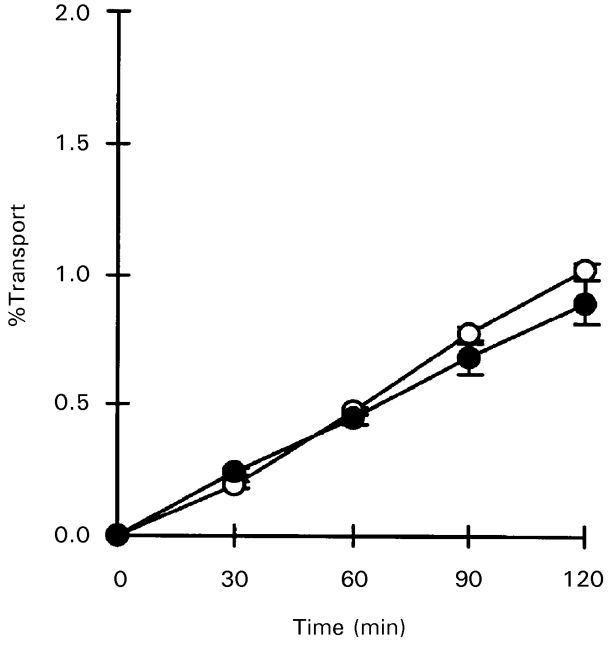


Figure 3. Apical-to-basolateral transport of cephalothin across Caco-2 cell monolayers: ○, Caco-2 cell monolayers incubated with pH 6 solution on the apical side and pH 7·4 on the basolateral side; ●, pH 7·4 on both the apical and basolateral sides. Results are the means ± standard deviations from four separate monolayers.

whereas a pH gradient had no effect on the transport of cephalosporins without this group. P_{app} (pH 6·0)/P_{app} (pH 7·4) found for cephalexin in this study (2·77) is comparable with that (2·3) found in a previous study of Caco-2 cells (Gochoco et al 1994).

Interestingly, the permeability coefficients for the cephalosporins used in this study seem to correlate well with their affinity for PEPT 1. In a recent study cephalosporins were assessed for their capacity to inhibit the uptake of [^{3}H]glycylglutamine in rabbit renal brush-border membrane vesicles (Daniel & Adibi 1993). The K_i values for the cephalosporins containing an α-amino group (63 – 1561 μM) were much lower than those for cephalosporins without an α-amino group (> 10 000 μM) (Daniel & Adibi 1993).

The structures of the aminocephalosporins tested in this study are similar (Figure 1). Physicochemical variables that could influence intestinal absorption were also considered. For example, the potential correlation with octanol – water partition coefficient (log P) and the number of hydrogen-bonds, parameters that have been reported to influence membrane permeability (Burton et al 1992) were evaluated. We found no correlation between either log P or the number of hydrogen-bonds with the permeability coefficients of the cephalosporins used in this study (data not shown).

In a recent study, we showed that these compounds have common conformations at their global energy minima (Li & Hidalgo 1996). The observation that an α-amino group had no effect on the energy-minimized conformation of cephalosporins led to the suggestion that the α-amino group might enhance the affinity of cephalosporins for PEPT 2 (Li & Hidalgo 1996). Although these results do not prove that an α-amino group is essential for PEPT 1-mediated transport, the data are supportive of an important role for this group on PEPT 1-mediated transepithelial transport. Unlike most previous studies which examined the role of an α-amino group on drug uptake or luminal disappearance, the current study assessed the impact of an α-amino group on transepithelial transport. This parameter is more representative of the steps involved in intestinal absorption. The importance of an α-amino group found in this study is probably a reasonable indication of the role of this group on PEPT 1-mediated intestinal peptidomimetic absorption.

In summary, we have examined the transepithelial transport of a series of cephalosporins across Caco-2 cell monolayers. The objective was to determine the role of an α-amino group on transepithelial transport instead of on uptake or luminal disappearance, as are measured by most studies. Results indicate that the H^+-dependent transepithelial transport of cephalosporins could be correlated with the presence of an α-amino group. This group seems to facilitate the interaction of

Table 1. Permeability coefficients for transport of cephalosporins across Caco-2 cell monolayers in the absence or presence of a pH gradient.

Compound	Permeability coefficient × 10^{-7} ($cm\,s^{-1}$)		P_{app} (pH 6)/P_{app} (pH 7·4)
	P_{app} (pH 6·0)*	P_{app} (pH 7·4)†	
Cephalexin‡	7·58 ± 0·85	2·73 ± 0·39	2·77
Cefaclor‡	9·69 ± 0·74	5·29 ± 0·47	1·83
Cefadroxil‡	8·80 ± 0·72	4·80 ± 0·84	1·83
Cephradine‡	8·17 ± 0·99	4·35 ± 0·41	1·88
Cephaloglycin‡	4·74 ± 0·44	2·67 ± 0·28	1·77
Cephapirin	3·71 ± 0·34	3·93 ± 0·46	0·94
Cephalothin	4·48 ± 0·25	3·95 ± 0·76	1·13
Cephaloridine	3·47 ± 0·05	3·81 ± 0·32	0·91
Cefoxitin	1·41 ± 0·09	1·56 ± 0·07	0·90
Cefsulodine	1·04 ± 0·13	1·20 ± 0·07	0·87
Cefazolin	3·11 ± 0·22	4·17 ± 0·17	0·74
Cefamandole	2·24 ± 0·04	2·69 ± 0·80	0·83
Cefamandole nafate	3·84 ± 0·65	3·62 ± 0·29	1·06
Cefuroxime	1·46 ± 0·28	1·52 ± 0·34	0·96
Ceftazidime	2·21 ± 0·31	2·46 ± 0·43	0·89
Cefoperazone	2·27 ± 0·13	2·92 ± 0·05	0·78
Cefotaxime	1·56 ± 0·21	1·24 ± 0·16	1·26

*P_{app} (pH 6·0) is the P_{app} value determined from incubations with pH 6 solution in apical chambers and pH 7·4 in basolateral chambers. †P_{app} (pH 7·4) is the P_{app} value determined from incubations with pH 7·4 solution in both apical and basolateral chambers. ‡Cephalosporins containing an α-amino group.

cephalosporins with PEPT 1. Additional studies will be required to determine whether this observation is unique to cephalosporins.

Acknowledgements
We thank Dr Michael P. Marietta for his support of this project and Dr Per Artursson for his valuable comments during the preparation of this manuscript.

References

Addison, J. M., Burston, D., Dalrymple, J. A., Matthews, D. M., Payne, J. W., Sleisenger, M. H., Wilkinsom, S. (1975) A common mechanism for transport of di- and tri-peptides by hamster jejunum in vitro. Clin. Sci. Mol. Med. 49: 313 – 322

Bai, P.-F., Subramanian, P., Mosberg, H. I., Amidon, G. L. (1991) Structural requirements for the intestinal mucosal-cell peptide transporter: the need for *N*-terminal α-amino group. Pharm. Res. 8: 593 – 599

Began, T. (1984) Pharmacokinetics of beta-lactam antibiotics. Scand. J. Infect. Dis. (Suppl.) 42: 83 – 98

Burton, P. S., Conradi, R. A., Hilgers, A. R., Ho, N. F. H., Maggiora, L. L. (1992) The relationship between peptide structure and transport across epithelial cell monolayers. J. Contr. Rel. 19: 87 – 98

Daniel, D., Adibi, S. A. (1993) Transport of β-lactam antibiotics in kidney brush-border membrane. Determinants of their affinity for the oligopeptide/H^+ symporter. J. Clin. Invest. 92: 2215 – 2223

Dantzig, A. H., Bergin, L. (1990) Uptake of the cephalosporin, cephalexin, by a dipeptide transport carrier in the human intestinal cell line, Caco-2. Biochim. Biophys. Acta 1027: 211 – 217

Dantzig, A. H., Tabas, B. L., Bergin, L. (1992) Cefaclor uptake by the proton-dependent dipeptide transport carrier of human intestinal Caco-2 cells and comparison to cephalexin uptake. Biochim. Biophys. Acta 1112: 167 – 173

Dantzig, A. H., Duckworth, D. C., Tabas, L. B. (1994) Transport mechanisms responsible for the absorption of loracarbef, cefixime, and cefuroxime axetil into human intestinal Caco-2 cells. Biochim. Biophys. Acta 1191: 7 – 13

Friedman, D. I., Amidon, G. L. (1989) Passive and carrier-mediated intestinal absorption components of two angiotensin converting enzyme (ACE) inhibitor prodrugs in rats: enalapril and fosinopril. Pharm. Res. 6: 1043 – 1047

Ganapathy, M. E., Brandsch, M., Prasad, P. D., Ganapathy, V., Leibach, F. H. (1995) Differential recognition of β-lactam antibiotics by intestinal and renal peptide transporters, PEPT 1 and PEPT 2. J. Biol. Chem. 43: 25672 – 25677

Gochoco, C. H., Ryan, F. M., Miller, J., Smith, P., Hidalgo, I. J. (1994) Uptake and transepithelial transport of the orally absorbed cephalosporin cephalexin, in the human intestinal cell line, Caco-2. Int. J. Pharm. 104: 187 – 202

Hidalgo, I. J., Raub, T. J., Borchardt, R. T. (1989) Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 96: 736 – 749

Klein, J. O., Eickhoff, T. C., Tilles, J. G., Finland, M. (1964) Cephalothin: activity in vitro, absorption and excretion in normal subjects and clinical observations on 40 patients. Am. J. Med. Sci. 248: 640 – 656

Kramer, W., Girbig, F., Gutjaher, U., Kleemann, H., Leipe, I., Urbach, H., Wagner, A. (1990) Interaction of renin inhibitors with the intestinal uptake system for oligopeptides and β-lactam antibiotics. Biochim. Biophys. Acta 1027: 25 – 30

Li, J., Hidalgo, I. J. (1996) Molecular modeling study of structural requirements for oligopeptide transporter. Drug Targeting 4: 9 – 17

Liu, W., Liang, R., Ramamoorthy, S., Fei, Y., Ganapathy, M. E., Hediger, M. A., Ganapathy, V., Leibach, F. (1995) Molecular cloning of PEPT 2, a new member of the H^+/peptide cotransporter family, from human kidney. Biochim. Biophys. Acta 1235: 461 – 466

Lowther, J., Hammond, S. M., Russel, K. (1990) Uptake of cephalosporins by human intestinal brush-border membrane vesicles. J. Antimicrob. Chemother. 25: 183 – 185

Matsumoto, S., Hideyuki, S., Inui, K. (1994) Transcellular transport of oral cephalosporins in human intestinal epithelial cells, Caco-2: interaction with dipeptide transport systems in apical and basolateral membranes. J. Pharmacol. Exp. Ther. 270: 498 – 504

Muranushi, N., Horie, K., Masuda, K., Hirano, K. (1994) Characteristics of ceftibuten uptake into Caco-2 cells. Pharm. Res. 11: 1761 – 1771

Okano, T., Inui, K., Takano, M., Hori, R. (1986) H^+-gradient-dependent transport of aminocephalosporins in rat intestinal brush-border membrane vesicles. Biochem. Pharmacol. 35: 1781 – 1786

Ryan, F. M., Smith, P. L. (1989) Effects of altering fluid transport on the absorption of benzylpenicillin and mannitol across rat intestinal mucosal in vitro. Pharm. Res. 6: S88

Saito, H., Inui, K. (1993) Dipeptide transporters in the apical and basolateral membranes of the human intestinal cell line, Caco-2. Am. J. Physiol. 265: G289 – G294

Smith, P. L., Wall, D. A., Gochoco, C. H., Wilson, G. (1992) Oral absorption of peptides and proteins. Adv. Drug Del. Rev. 8: 253 – 290

Sugawara, M., Saitoh, H., Iseki, K., Miyazaki, K., Arita, T. (1990) Contribution of passive transport mechanisms to the intestinal absorption of β-lactam antibiotics. J. Pharm. Pharmacol. 42: 314 – 318

Sugawara, M., Iseki, K., Miyazaki, K. (1991) H^+ coupled transport of orally active cephalosporins lacking an α-amino group across brush-border membrane vesicles from rat small intestine. J. Pharm. Pharmacol. 43: 433 – 435

Tsuji, A., Hirooka, H., Terasaki, T., Tamai, I., Nakashima, E. (1987) Saturable uptake of cefixime, a new oral cephalosporin without an α-amino group, by the rat intestine. J. Pharm. Pharmacol. 39: 272 – 277

Wenzel, U., Thwaites, D. T., Daniel, H. (1995) Stereoselective uptake of β-lactam antibiotics by the intestinal peptide transporter. Br. J. Pharmacol. 116: 3021 – 3027