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A comparison of the affinities of dipeptides and antibiotics for the di-/tripeptide transporter in Caco-2 cells

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Abstract

The intestinal peptide transporter(s) is involved in the absorption of natural di-/tripeptides and peptidomimetic drugs. Several key aspects of peptide transport such as number of peptide transporters and structural requirements for transport via this carrier(s) are not fully understood. In addition, recent studies showed that interaction with the di-/tripeptide transporter(s) does not necessarily lead to transcellular transport. The variety of structures which appear to interact with the transporter could be explained by the presence of several transporters or by multiple binding sites in a single transporter. The objective of this study was to determine whether there is a clear difference between dipeptides and amino-β-lactam antibiotics which may suggest the involvement of different transporters/binding sites. Experiments were carried out in Caco-2 cell monolayers grown on microporous membranes using cephalexin as the model compound. Inhibition constants (K_i) were calculated from Dixon plots assuming competitive inhibition. The strong correlation between K_i and IC_{50} (independently determined) indicates that the assumption of competitive inhibition was probably correct. Results show: (a) that dipeptides have greater affinity for the cephalexin transporter(s) than antibiotics; (b) among dipeptides, neutral dipeptides seem to have higher affinity for the carrier; and (c) a relationship between affinity for the transporter(s) and transepithelial transport could not be found.

Keywords: Cephalexin; Caco-2; Peptide transporter; Transcellular transport; Dipeptide transport

1. Introduction

The intestinal uptake and transepithelial transport of natural di-/tripeptides involves di-/tripeptide transporter(s) (Ganapathy and Leibach, 1985). There is also evidence that a large number of amino-β-lactam antibiotics undergo carrier-mediated intestinal transport (Inui et al., 1988; Iseki et al., 1989). The similarity in transport properties between di-/tripeptides and amino-β-lactam antibiotics indicates that the intestinal absorption of these two classes of compounds is mediated, at least in part, by the same peptide transporter(s). More recently, the transport of angiotensin converting enzyme inhibitors

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and renin inhibitors has also been shown to involve intestinal peptide transporter(s) (Hu and Amidon, 1988; Kramer et al., 1990). These observations suggest that the intestinal transport of these compounds is mediated by a transporter which has a broad specificity or involves multiple transporters. While the existence of multiple peptide transporters could explain the apparently broad substrate specificity of the intestinal di-/tripeptide transporter, the number of transporters involved in the intestinal transport of di-/tripeptides is not known. Existing evidence supports the existence of two transporters: (a) cefixime was found to be transported only in the presence of an inwardly directed pH gradient while cephradine was transported with and without a pH gradient (Inui et al., 1988); and (b) inhibition studies indicate that the transport process which mediates the uptake of amino-β-lactam antibiotics is different from that involved in the uptake of glycylglycine (Iseki et al, 1989).

The structural requirements for transcellular transport via this peptide carrier(s) are not fully understood. This information is particularly difficult to obtain without knowing the specific number of transporters involved. Most data on structural requirements for interaction with the transporter(s) have been provided by competition studies rather than by direct correlation between structure and transport. Mutual uptake inhibition between di-/tripeptides and/or amino-β-lactam antibiotics indicates that molecules from both classes of compounds can interact with the di-/tripeptide transporter(s) (Inui et al., 1988; Kramer et al., 1990, 1992). However, some molecules can bind to the peptide transporter(s) without necessarily undergoing transport themselves (Miyazaki et al., 1982; Ryan and Smith, 1989; Kramer et al., 1992). This lack of correlation between uptake and transcellular transport underscores the need to investigate these processes in greater detail.

Caco-2 cell monolayers have been characterized as a transport model system of the small intestinal epithelium (Hidalgo et al., 1989) and shown to express a functional peptide transporter(s) similar to that found in enterocytes (Dantzig and Bergin, 1990; Inui et al., 1992). In the present study, we have used Caco-2 cell monolayers grown on microporous membranes to investigate peptide transport. Caco-2 cells have the advantage that they can be used to determine both uptake and transcellular transport in the same system. Cephalexin was used as the model compound to assess the affinity of dipeptides and amino-β-lactam antibiotics for the peptide transporter(s). The affinity of these molecules was evaluated in terms of inhibition of cephalexin uptake. We also examined the effect of electrical charge of dipeptides on their affinity for the transporter(s). Finally, we compared the inhibitory effect of the antibiotics with known data on transepithelial transport to find out whether ability to inhibit the uptake of cephalexin (i.e., affinity for the transporter(s)) can be used to predict transepithelial transport.

2. Experimental

2.1. Materials

2.1.1. Cell culture

The Caco-2 cell line was obtained from The American Culture Collection (Rockville, MD) and used between passages 65 and 80. Dulbecco's modified Eagle's media (DMEM), Hank's balanced salts solution (HBSS), penicillin and streptomycin solution (10000 U/ml penicillin G and 10000 μg/ml streptomycin) and trypsin-EDTA solution (0.25% trypsin and 1 mM EDTA) were purchased from Gibco Laboratories (Grand Island, NY). Fetal bovine serum (FBS) and non-essential amino acids solution (NEAA) were obtained from Hazelton Research (Lenexa, KS). Transwell culture cluster plates (miniwells), 6.5 mm in diameter and 0.4 μm pore size, were purchased from Costar (Bedford, MA). Rat tail collagen, type I, was obtained from Collaborative Research (Bedford, MA). Hepes and 2-(*N*-morpholino)ethanesulfonic acid (Mes) were purchased from Sigma (St. Louis, MO).

2.1.2. Dipeptides and antibiotics

Dipeptides of arginine (Arg-Arg), lysine (Lys-Lys), aspartate (Asp-Asp), glutamate (Glu-Glu),

valine (Val-Val), and glycylproline (Gly-Pro) were obtained from Bachem Biosciences Inc. (Philadelphia, PA). Ampicillin, benzylpenicillin (BZP), cephalexin (CPX) and cephradine were purchased from Sigma. Amoxicillin was obtained from SmithKline Beecham. [^{3}H]Cephalexin ([^{3}H]CPX, 7.78 Ci/mmol) was synthesized in the Synthetic Chemistry Department, SmithKline Beecham Pharmaceuticals (King of Prussia, PA) and [*phenyl*-^{3}H]benzylpenicillin (26.5 Ci/mmol) was purchased from New England Nuclear-DuPont (Boston, MA).

2.2. Methods

2.2.1. Cell culture methods

Caco-2 cells were cultured in DMEM supplemented with 10% FBS, 1% NEAA and penicillin (100 U/ml) and streptomycin (100 μg/ml). Caco-2 cells were plated on Transwell plates previously coated with rat tail collagen (1.25 mg per ml of 70% ethanol) at a seeding density of 2×10^5 cell/miniwell. Medium was first changed in the apical chamber of the miniwell 24 h after seeding and then in both the apical and basolateral chambers every other day for 10 days and every day thereafter. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Stock cultures were routinely maintained by changing the media every 2–3 days.

2.2.2. Uptake studies

The uptake of [^{3}H]CPX was determined in Caco-2 cells grown at 37°C on 6.5 mm miniwells in the presence or absence (controls) of inhibitors such as dipeptides and antibiotics. On the day of the experiment, the apical side of the cells was rinsed with 10 mM Mes in HBSS, pH 6.0 (apical buffer) and the basolateral side was rinsed with 10 mM Hepes in HBSS, pH 7.4 (basolateral buffer). Plates were then pre-incubated at 37°C for 15 min with apical buffer bathing the apical membrane and basolateral buffer bathing the basolateral membrane. After the pre-incubation period, the control monolayers were incubated for 15 min at 37°C with apical buffer that contained [^{3}H]CPX on the apical side. The monolayers treated with inhibitors were incubated with [^{3}H]CPX plus inhibitor on the apical side. The basolateral sides of monolayers in both control and inhibition groups were maintained in basolateral buffer. Subsequently, the incubation medium was removed and the cells washed (4 ×) with ice-cold HBSS, pH 7.0 to stop further uptake and to remove unbound radioactivity. Filters containing the cells were then cut out, dissolved in Ready-Safe scintillation cocktail and the [^{3}H]CPX taken up counted in a Beckman Liquid Scintillation Counter, LS 6000 Series (Beckman Instruments Inc., Fullerton, CA). Percent inhibition of [^{3}H]CPX uptake (expressed as pmol/mg protein per min) due to the inhibitors was calculated against controls containing no inhibitors. The concentration of the inhibitor that produced 50% inhibition of control uptake (IC_{50}) was calculated from a plot of % inhibition of CPX uptake vs concentration of inhibitors. The type of inhibition and specific K_i values for the inhibitors were determined from Dixon plots (Segel, 1976).

2.2.3. Transepithelial transport studies

Transport studies were conducted in a side-by-side diffusion apparatus (Precision Instruments Design, Los Altos, CA) described previously (Hidalgo et al., 1991). The apical chamber received 5 ml HBSS containing 10 mM mannitol and the basolateral chamber received 5 ml of HBSS containing 8 mM glucose plus 2 mM mannitol. The pH of the apical chamber was adjusted to 6.0 or 7.4 as indicated in the appropriate figure legends. The concentrations of the model compounds ([^{3}H]cephalexin or [^{3}H]benzylpenicillin) were 100 μM. 1 ml samples were taken from the receiver sides at specified times and replaced with an identical volume of the same solution. Samples from the donor sides (100 μl) were taken only at zero time and at the end of the experiment and were not replaced.

3. Results and discussion

3.1. Comparison of affinities for the peptide transporter

Four aspects of peptide transport were investigated in this study: (a) the affinity of dipeptides

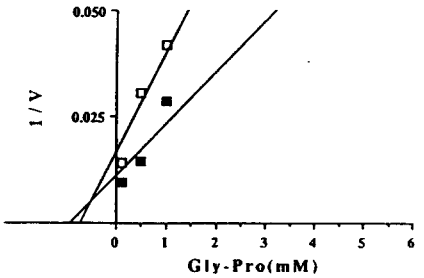


Fig. 1. Dixon plots of CPX uptake inhibition by Gly-Pro. The uptake of CPX, at two concentrations, was determined for 15 min at 37°C in the presence of increasing concentration of Gly-Pro. (□) 0.5 mM CPX; (■) 0.8 mM CPX); CPX uptake was expressed as pmol/mg protein per min. Values presented are the mean of at least two experiments. Each experiment included 3–4 monolayers. The coefficient of variation within experiment was usually about 15% and between experiments about 20% (see Table 1).

and amino-β-lactam antibiotics for the peptide transporter(s); (b) the possible existence of a threshold K_i value, below or above which, this parameter may be predictive of transepithelial transport; (c) comparison of IC_{50} and K_i values for the antibiotics and dipeptides; and (d) whether the electrical charge of dipeptides influences their affinity for the transporter(s). Results indicate the following: (a) the affinity of natural dipeptides for the di-/tripeptide transporter(s) was greater than that of the antibiotics; (b) K_i does not seem to be a good predictor of transepithelial transport at either low or high affinities; (c) the close agreement between K_i and IC_{50} indicates that the inhibition of cephalexin uptake was indeed competitive; and (d) di-cationic dipeptides had significantly lower affinity ($P < 0.05$) for the transporter(s).

Dixon plots of CPX uptake in the presence of several dipeptides are presented in Fig. 1. The point of intersection of the two lines is consistent with competitive inhibition (Segel, 1976). On the other hand, Dixon plots of CPX uptake in the presence of the antibiotics are more difficult to interpret because they intersect very close to the abscissa. The equation to calculate K_i values for competitve inhibitors is different from that for noncompetitive inhibitors (Segel, 1979). Although uncompetitive inhibition can be ruled out, these plots were compatible with either competitive or noncompetitive inhibition with the exception of cephradine (which showed competitive inhibition) (Fig. 2). However, results from previous studies suggest that inhibition by this type of molecule most likely is competitive (Nakashima et al., 1984). Thus, we assumed that CPX inhibition by the antibiotics examined was competitive and the K_i values were calculated as those for the dipeptides. A similar approach was necessary in a recent study (Daniel et al., 1992) with the renal peptide transporter(s). Whether the observed difference in mode of inhibition between dipeptides and antibiotics is due to interaction at different sites/transporters or simply to mixed (competitive and noncompetitive) inhibition by the antibiotics cannot be determined from results in this study. However, the close agreement between K_i and IC_{50} values indicates that the equation used

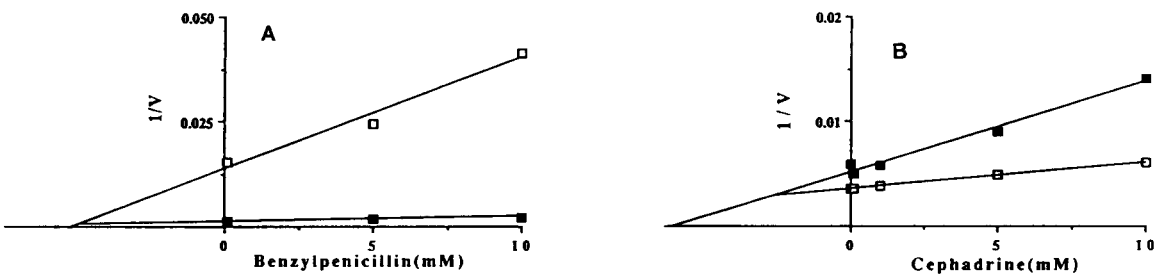


Fig. 2. Dixon plots of CPX uptake inhibition by antibiotics. The uptake of CPX, at two concentrations, was determined for 15 min at 37°C in the presence of the indicated concentration of inhibitors. (A) Benzylpenicillin [(□) 0.5 mM CPX; (■) 10 mM CPX]; (B) cephradine [(□) 0.5 mM CPX; (■) 1.0 mM CPX]. Uptake was expressed as pmol/mg protein per min. Each experiment included 3–4 monolayers. The coefficient of variation within experiment was usually about 15% and between experiments about 20% (see Table 1).

Table 1
Inhibition of cephalexin uptake by dipeptides and antibiotics in Caco-2 cells

Dipeptides	K_i (mM)	Antibiotics	K_i (mM)
Val-Val	0.41 (0.15)	amoxicillin	9.60 (0.63) [c]
Ala-Ala	0.55 (0.06)	cephradine	4.50 (1.53) [d]
Gly-Pro	0.22 (0.08)	benzylpenicillin	15.48 (3.93) [c]
Glu-Glu	0.54 (0.06)	ampicillin	5.79 (0.67) [c]
Asp-Asp	1.27 (0.44)		
Arg-Arg	2.23 (0.96) [a]		
Lys-Lys	3.43 (0.69) [a,b]		

K_i values are mean ± SD ($n = 3$).
[a] $P < 0.05$ compared to the di-neutral dipeptides.
[b] $P < 0.05$ compared to the di-anionic dipeptides.
[c] $P < 0.05$, compared to dipeptides.
[d] $P < 0.05$ compared to di-neutral and di-anionic dipeptides.

to calculate the K_i values (i.e., for competitive inhibition) was appropriate and that the inhibition of cephalexin uptake by both dipeptides and the antibiotics is mostly competitive.

The K_i values of antibiotics differed from those of dipeptides (Table 1). The dipeptides showed K_i values ranging from 0.22 to 3.43 mM and the K_i values for the antibiotics ranged from 4.5 to 15 mM (Table 1). These results indicate that, in general, dipeptides have greater affinity for the peptide transporter(s) than the antibiotics investigated. It is likely that the dipeptides used in the inhibition studies with cephalexin underwent some degree of metabolism during the experiments. Thus the true difference in affinity between dipeptides and antibiotics may be greater than that observed in this study.

The net charges carried by the dipeptides studied were: Gly-Pro and Val-Val, neutral; Asp-Asp and Glu-Glu, di-anionic; Arg-Arg and Lys-Lys, di-cationic. The K_i value for Lys-Lys was higher than that for di-anionic ($P < 0.02$) and di-neutral ($P < 0.01$). The K_i value for the other di-cationic dipeptide, Arg-Arg, showed a statistically significant difference from the di-neutral dipeptides ($P < 0.02$) and Glu-Glu ($P < 0.02$) but not from the di-anionic dipeptide, Asp-Asp. These results suggest that the presence of two positive charges decreases the interaction with the peptide transporter. However, the role of the negative charges is not clear. This observation does not agree with a previous study which examined the uptake of Gly-Pro by brush border membrane vesicles (Wootton and Hazelwood, 1989). The conclusion of that study was that negative charges were more detrimental to the affinity for the carrier than positive charges (Wootton and Hazelwood, 1989). This apparent discrepancy may be explained in several ways: (a) The model compound used in this study (cephalexin) and that used in the previous study may interact differently with the peptide transporter(s); (b) The current study was carried out in the presence of a pH gradient while the previous study with Gly-Pro were not. Thus, the two studies may have looked either at different carriers (e.g., pH-dependent vs pH-independent); and (c) interaction of the carrier with charged peptides at pH 7.4 may be different from that at pH 6.

3.2. Correlation between K_i and IC_{50}

K_i and IC_{50} have been used to quantitate the ability of inhibitors to interact with a specific carrier, receptor or enzyme. These parameters, IC_{50} and K_i, are not equivalent. IC_{50} is the concentration of inhibitor that causes 50% inhibition of uptake and K_i is the concentration of inhibitor that doubles the slope of the Lineweaver-Burke plot ($1/v$ vs $1/[S]$). In our calculation of K_i values for the antibiotics we assumed competitive inhibition and used the appropriate equation. Although this assumption was made based on previous studies by others who found competitive inhibition with related compounds (Nakashima and Tsuji, 1985; Dantzig and Bergin,

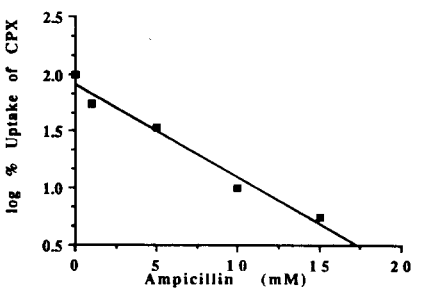


Fig. 3. Calculation of IC_{50} values. A plot of the log CPX uptake vs inhibitor concentration was a straight line. The parameters of the regression line were used to calculate the IC_{50} values.

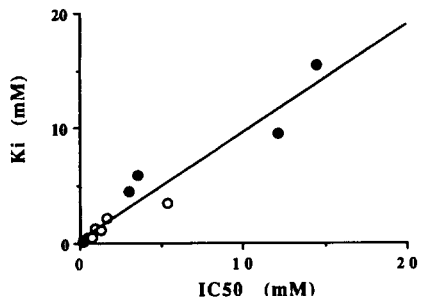
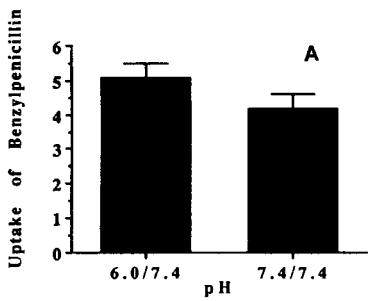


Fig. 4. Correlation between K_i and IC_{50} values for the dipeptides and antibiotics studied. (○) Dipeptides; (●) antibiotics ($R^2 = 0.981$). The strong correlation indicates that the underlying assumption (competitive inhibition) in choosing an equation to calculate K_i was probably correct.

1990), we decided to compared K_i and IC50 values. A close correlation between K_i and IC_{50} was interpreted as an indication that the K_i values were appropriately determined and that the type of inhibition was indeed competitive. IC_{50} values were calculated from linear transformations of % uptake vs concentration of the inhibitors as shown for ampicillin in Fig. 3. Results show a strong correlation between IC_{50} and K_i values ($R^2 = 0.981$) for dipeptides and antibiotics combined (Fig. 4). Both IC_{50} and K_i may be useful to compare affinity for the di-/tripeptide transporter(s). However, the Dixon plots used in determining K_i values can be used to distinguish among different modes of inhibition (i.e., competitive vs non-competitive).

3.3. Correlation between affinity for the peptide transporter and transepithelial transport for antibiotics

Benzylpenicillin inhibited the apical uptake and apical-basolateral transport of cephalexin but itself was not transported by the peptide transporter (Fig. 5) (Ryan and Smith, 1989; Inui et al., 1992). This observation led us to formulate the following hypotheses: (a) a weak binding to the transporter is required for transcellular transporter (and benzylpenicillin binds too strongly); and (b) transcellular transport requires a strong interaction with the transporter (and benzylpenicillin binds too weakly). Subsequently, we examined the transport and inhibitory properties of



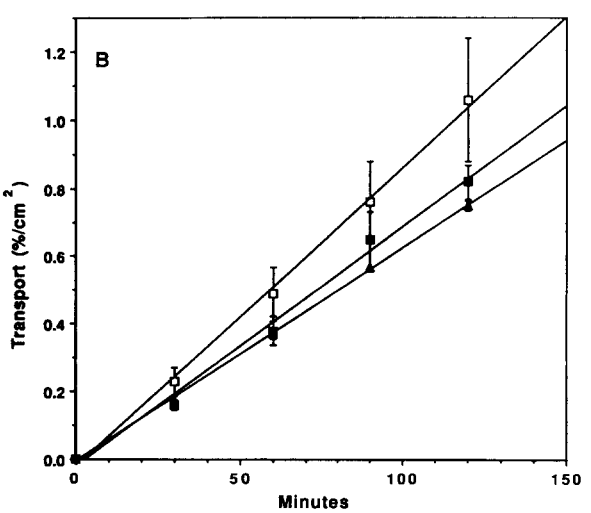


Fig. 5. Effect of pH gradient on the uptake and transepithelial transport of benzylpenicillin in Caco-2 cells. For BZP uptake (A) Caco-2 cell monolayers were incubated with 0.1 mM [^{3}H]BZP on the apical side for 15 min at 37°C. At the end cells were dissolved and radioactivity determined as described in section 2. (6.0/7.4) Apical uptake of BZP at apical pH 6.0 and basolateral pH 7.4; (7.4/7.4) apical uptake of BZP at apical pH 7.4 and basolateral pH 7.4. Values are mean ± SD ($n = 4$). For transepithelial transport (B), Caco-2 cell monolayers received 0.1 mM [^{3}H]BZP on the apical or basolateral bathing solutions, and the amount transported to the opposite chamber determined at specified times. (□) Apical-to-basolateral flux of BZP at apical pH 6.0 and basolateral pH 7.4; (■) apical-to-basolateral flux of BZP at apical pH 7.4 and basolateral pH 7.4; (▲) basolateral-to-apical flux of BZP at basolateral pH 6.0 and apical pH 7.4. Values are mean ± SD ($n = 4$).

other antibiotics to determine whether their behaviour revealed a correlation between affinity for the transporter and transcellular transport.

K_i values for benzylpenicillin and cephradine were 15.5 and 6 mM, respectively. We first examined whether this difference in K_i could be related to the type of interaction (e.g., competitive vs noncompetitive) with the peptide transporter. Benzylpenicillin, which shows low affinity for the peptide transporter(s) in our system, does not undergo carrier-mediated transport across the intestinal mucosa (Ryan and Smith, 1989).

Uptake and transepithelial transport of benzylpenicillin were also studied in Caco-2 cell monolayers. The uptake of [^{3}H]benzylpenicillin by Caco-2 cells was minimal and pH-independent, suggesting that it most likely is passive (Fig. 5A). Moreover, the apical-to-basolateral transport of [^{3}H]benzylpenicillin was pH-independent and comparable to that in the opposite direction (Fig. 5B). Both apical uptake and apical-to-basolateral transport of benzylpenicillin were comparable to those of mannitol, the internal marker of passive, paracellular diffusion (not shown). Together, these data suggest that the intestinal absorption of benzylpenicillin does not involve the di-/tripeptide transporter(s). In contrast, cephalexin, a compound whose transport involves the di-/tripeptide transporter(s), underwent pH-dependent uptake (Fig. 6) and transepithelial transport (not shown) under identical experimental conditions. Second, cephradine, which has a higher affinity for the transporter(s) in our system, was shown to be transported across Caco-2 cell monolayers, at least in part, via the di-/tripeptide transporter(s) (Inui et al., 1992).

In contrast to the apparent correlation between K_i and transepithelial transport for benzylpenicillin and cephradine, data with amoxycillin and ampicillin do not support a relationship between affinity for the carrier and transepithelial transport. The oral bioavailability of amoxycillin is higher than that of ampicillin (Benet and Sheiner, 1980). In addition, while some reports indicate that the intestinal absorption of amoxycillin involves the intestinal peptide transporter(s) (Paintaud et al., 1992), there is evidence that the transport of ampicillin is not carrier-mediated

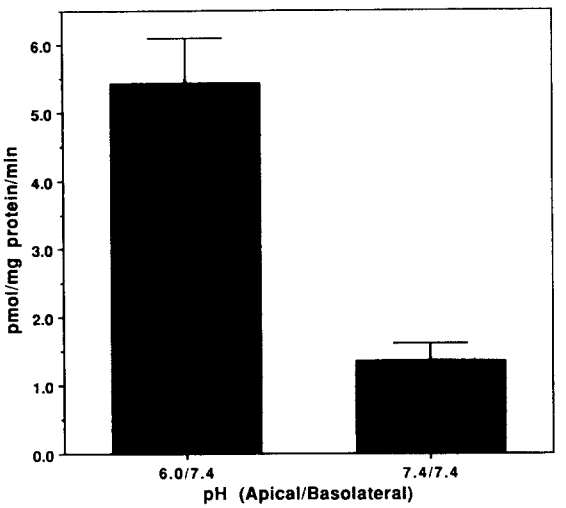


Fig. 6. pH-dependent uptake of cephalexin in Caco-2 cell monolayers. [^{3}H]CPX (0.1 mM) was applied to the apical bathing solution and incubated under identical conditions to those used for BZP. Values are mean ± SD ($n = 4$).

(Yamashita et al., 1984). Thus amoxycillin was expected to possess greater affinity for the carrier than ampicillin, in our uptake assay. However, this was not the case. Benzylpenicillin does not undergo carrier-mediated cellular uptake (Fig. 5A) or transepithelial transport (Fig. 5B) while cephradine has been reported to undergo carrier-mediated transport via the peptide transporter(s)) (Inui et al., 1992). Thus, the observation that K_i and IC_{50} values for amoxycillin are closer to those of benzylpenicillin than values for cephradine does not support a correlation between interaction with the transporter and transepithelial transport. Because of the small number of compounds evaluated in this study a definitive determination of a relationship between affinity (e.g., K_i or IC_{50}) and transepithelial transport cannot be made. A larger data base is required to establish this relationship.

In summary, these results show: (a) that dipeptides have greater affinity for the di-/tripeptide transporter(s) than antibiotics; (b) a trend in the relationship between electrical charge and affinity for the transporter(s) (neutral > di-cationic > di-anionic); (c) a strong correlation between K_i or IC_{50} indicates that both parameters can be used to evaluate the affinity for the transporter(s);

(d) with a limited number of compounds a from relationship between affinity for the transporter(s) and transepithelial transport could not be found. These results, however, indicate that the Caco-2 cell system may be utilized to predict absorption and transport of antibiotics in human intestinal mucosa for screening purposes.

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References

Benet, L.Z. and Sheiner, L.B., Design and optimization of dosage regimens; pharmacokinetic data. In Gilman, A.G., Goodman, L.S. and Gilman, A. (Eds), *The Pharmacological Basis of Therapeutics*, Macmillan, New York., 1980, pp. 1675–1737.

Daniel, H., Morse, E.L. and Adibi, S., Determinant of substrate affinity for the oligopeptide/H^+ symporter in the renal brush border membrane. *J. Biol. Chem.*, 267 (1992) 9565–9573.

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

Ganapathy, V. and Leibach, F.H., Is intestinal peptide transport energized by a proton gradient? *Am. J. Physiol.*, 249 (1985) G153–G160.

Hidalgo, I.J., Grass, G.M., Hillgren, K.M. and Borchardt, R.T., A new side-by-side diffusion cell for studying transport across epithelial cell monolayers. *In Vitro Cell. Dev. Biol.*, 28A (1992) 578–580.

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

Hu, M. and Amidon, G.L., Passive and carrier-mediated intestinal absorption components of captopril. *J. Pharm. Sci.*, 77 (1988) 1007–1011.

Inui, K.-I., Okano, T., Maegawa, H., Kato, M., Takano, M. and Hori, R., H^+ coupled transport of p.o. cephalosporins via dipeptide carriers in rabbit intestinal brush-border membranes: Difference of transport characteristics between cefixime and cephradine. *J. Pharmacol. Exp. Ther.*, 247 (1988) 235–241.

Inui, K.-I., Yamamoto, M. and Saito, H., Transepithelial transport of cephalosporins by monolayers of intestinal epithelial cell line Caco-2: Specific transport systems in apical and basolateral membranes. *J. Pharmacol. Exp. Ther.*, 261 (1992) 195–201.

Iseki, K., Sugawara, M., Saitoh, H., Miyazaki, K. and Arita, T., Comparison of transport characteristics of amino β-lactam antibiotics and dipeptides across rat intestinal brush border membrane. *J. Pharm. Pharmacol.*, 41 (1989) 628–632.

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

Kramer, W., Girbig, F., Gutjahr, U., Kowalewski, S., Adam, F. and Schiebler, W., Intestinal absorption of β-lactam antibiotics and oligopeptides. Functional and stereospecific reconstitution of the oligopeptide transport system from rabbit small intestine. *Eur. J. Biochem.*, 204 (1992) 923–930.

Miyazaki, K., Iseki, K. and Arita, T., Intestinal absorption of several β-lactam antibiotics: IV. Binding to the various components in the intestinal mucosa of rat and role in absorption process. *J. Pharmacobio-Dyn.*, 5 (1982) 593–602.

Nakashima, E. and Tsuji, A., Mutual effects of amino-β-lactam antibiotics and glycylglycine on the transmural potential difference in the small intestinal epithelium of rats. *J. Pharmacobio-Dyn.*, 8 (1985) 623–632.

Nakashima, E., Tsuji, A., Mizuo, H. and Yamana, T., Kinetics and mechanism of in vitro uptake of amino-β-lactam antibiotics by rat small intestine and relation to the intact-peptide transport system. *Biochem. Pharmacol.*, 33 (1984) 3345–3352.

Oh, D.-M., Amidon, G.L. and Sadee, W., Endogenous peptide transporter and exogenous proton/peptide cotransporter expressed in *Xenopus* oocytes. *Pharm. Res.*, 9 (1992) S-82.

Paintaud, G. Alvan, G., Dahl, M.L., Grahnen, A., Sjovall, J. and Svensson, J.O., Nonlinearity of amoxycillin absorption kinetics in human. *Eur. J. Clin. Pharmacol.*, 43 (1992) 283–288.

Ryan, F.M and Smith, P.L., Effects of altering fluid transport on the absorption of benzylpenicillin and mannitol across rat intestinal mucosa in vitro. *Pharm. Res.*, 6 (1989) S-88.

Segel, I.H., *Biochemical Calculations*, Wiley, New York, 1976, p. 252.

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

Wootton, R. and Hazelwood, R., Relative affinity of a series of charged dipeptides for the peptide carrier of rabbit intestinal brush border membranes. *Biochem. Soc. Trans.*, 17 (1989) 691–692.

Yamashita, S., Yamazaki, Y., Masada, M., Nadai, T., Kimura, T. and Sezaki, H., Investigations of influx and accumulation processes of β-lactam antibiotics and their role in the transmural transfer across rat jejunum. *J. Pharmacobio-Dyn.*, 9 (1986) 368–374.

Yamashita, S., Yamazaki, Y., Mizuno, M., Masada, M., Nadai, T., Kimura, T. and Sezaki, H. Further investigations on the transport mechanism of cephalexin and ampicillin across rat jejunum. *J. Pharmacobio-Dyn.*, 7 (1984) 227–233.