Intestinal Transport of β-Lactam Antibiotics: Analysis of the Affinity at the H^+/Peptide Symporter (PEPT1), the Uptake into Caco-2 Cell Monolayers and the Transepithelial Flux

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Purpose. This study on the intestinal transport of β-lactam antibiotics was undertaken to investigate the correlation between cellular transport parameters and the bioavailability.

Methods. Transport of 23 β-lactam antibiotics was characterized by measuring their ability to inhibit the uptake of glycylsarcosine into Caco-2 cells, their uptake into the cells and their total flux across the cell monolayers.

Results. Ceftibuten and cyclacillin were recognized by PEPT1 with affinity constants comparable to those of natural dipeptides (K_i = 0.3 and 0.5 mM, respectively). Cefadroxil, cefamandole, cephradine, cefaclor, cefuroxime-axetil, cefixime, cephalotin, cephalexin and ampicillin also interacted with PEPT1 (K_i = 7–14 mM). In contrast, cefapirin, cefodizime, cefuroxime, cefmetazole, ceftazidime, benzylpenicillin, ceftriaxone, cefpirome, cefotaxime, cefepime, cephaloridine and cefsulodin displayed no affinity to the transport system (K_i > 20 mM). The uptake into the cells and the transepithelial flux was highest for those β-lactam antibiotics, which showed the strongest inhibition of [^{14}C]Gly-Sar transport ($p < 0.0001$). Exceptions were cefuroxim-axetil and cephalotin.

Conclusions. The probability of oral bioavailability for β-lactam antibiotics is mainly determined by their affinity to PEPT1. A threshold K_i value of 14 mM with respect to Gly-Sar uptake is required.

KEY WORDS: PEPT1; peptide transport; β-lactam antibiotics; Caco-2 cells; cephalosporins; penicillins.

INTRODUCTION

Many β-lactam antibiotics are transported by the peptide transporter PEPT1 expressed in the luminal membrane of enterocytes (1–11). This transporter is driven by a transmembrane H^+ gradient and catalyzes the cotransport of its substrates with H^+ (12–15). PEPT1s natural occurring substrates are di- and tripeptides. The recognition of exogenous compounds such as β-lactam antibiotics, angiotensin converting enzyme inhibitors, renin and thrombin inhibitors and the antineoplastic agent bestatin by PEPT1 is based on the phenomenon that these compounds possess peptide-like chemical structures (1,2,12–16). Most of the chemically diverse substrates share structural features such as a peptide bond with an α-amino group and a C-terminal carboxylic acid group. β-lactam antibiotics bear sterical resemblance to the backbone of physiologically occurring tripeptides (1,2,16).

Pharmacologically active peptidomimetics that are accepted by PEPT1 can very often be administered orally. Hence, the intestinal peptide transporter is an important determinant of the bioavailability of these drugs (1,2). Likewise, the fact that β-lactam antibiotics are substrates for transport system PEPT2, which is present in the renal brush-border membrane (16) and catalyzes active transport from tubular lumen back to blood, determines—among other factors such as stability in plasma—the circulatory half-life of penicillins and cephalosporins.

The current investigation was undertaken to study in a systematic approach the structure-transport relationship of 23 β-lactam antibiotics by measuring both their recognition by PEPT1 and the cellular uptake as well as total transepithelial flux across Caco-2 cell monolayers. Comparing these data with the bioavailability reveals threshold values for the oral delivery route.

MATERIALS AND METHODS

Materials

The human colon carcinoma cell line Caco-2 was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cell culture media and supplements, fetal bovine serum and trypsin solution were purchased from Life Technologies, Inc. (Germany). [1-^{14}C]Mannitol (specific radioactivity 57 mCi/mmol) and [glycine-1-^{14}C]glycylsarcosine (specific radioactivity 53 mCi/mmol) were obtained from Amersham International (UK). Cefadroxil, cefamandole, cephradine, cefaclor, cephalotin, cephalexin, ampicillin, cefapirin, cefmetazole, ceftazidime, benzylpenicillin, ceftriaxone, cephaloridine and cefsulodin were purchased from Sigma (Germany). Cefotaxime, cefodizime, cefuroxime and cefpirome were from Hoechst AG (Germany). Cyclacillin, cefixime and ceftibuten were generous gifts from Dr. F.H. Leibach, Medical College of Georgia, USA. Cefuroxim-axetil was a gift from Glaxo Welcome (Germany). Cefepime was from Bristol-Myers Squibb (Germany). All other chemicals were of analytical grade.

Methods

Cell Culture

Caco-2 cells were routinely cultured in 75-cm^2 culture flasks with Minimum Essential Medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and 1% nonessential amino acid solution (8,17,18). Subconfluent cultures were treated 5 min with Dulbeccos phosphate-buffered saline followed by a 2-min incubation with 0.25% trypsin solution. For most experiments, the cells (passages #9–55) were seeded in 35-mm disposable petri dishes (Becton Dickinson, UK) at a density of 0.75×10^6 cells per

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dish. The uptake measurements were performed on the fourth to sixth day after confluence.

Caco-2 cells were also cultured on permeable polycarbonate Transwell® cell culture inserts (diameter 24.5 mm, pore size 3 μm, Costar GmbH, Bodenheim, Germany) (18). Subcultures (passages #49–81) were started at a cell density of 43.000 cells/cm^2 and cultured for 17 to 23 days. The lower (receiver) compartment contained 2.6 ml medium and the upper (donor) compartment 1.5 ml medium.

Transport Studies

Uptake of [^{14}C]Gly-Sar in cells cultured on plastic dishes was measured at room temperature as described earlier (8,17). The uptake buffer contained 25 mM Mes/Tris (pH 6.0), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, 10 μM radiolabeled Gly-Sar and unlabeled β-lactam antibiotics (0–31.6 mM or concentration of maximal solubility, respectively). After incubation for 10 min, the cells were quickly washed four times with ice-cold buffer, solubilized with 1-ml of 0.2 M NaOH containing 1% sodium dodecyl sulfate and prepared for liquid scintillation spectrometry.

The increase of extracellular osmolality alone by up to 10% at high substrate concentrations had no effect on Gly-Sar uptake. In presence of excess amounts of unlabeled mannitol (31.6 mM) in the uptake medium, [^{14}C]Gly-Sar transport was 102 ± 7% of control (n = 4). The non-saturable component of Gly-Sar uptake (diffusion, adherent radioactivity) was determined by measuring the uptake of radiolabeled Gly-Sar in the presence of increasing concentrations of unlabeled Gly-Sar (0–31.6 mM). At the [^{14}C]Gly-Sar concentration of 10 μM used throughout this study, the nonspecific uptake represented 10.7% of the total uptake. This value was taken into account during non-linear regression analysis of inhibition constants.

Transepithelial flux of β-lactam antibiotics across Caco-2 cell monolayers cultured on permeable filters was measured as follows (18). All experiments were performed at 37°C in a shaking water bath. After washing the inserts with buffer (25 mM Hepes/Tris (pH 7.5), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose) for 10 min, uptake was started by adding uptake buffer (pH 6.0, 1.5 ml) containing β-lactam antibiotics (1 mM) to the donor side. At time intervals of 10, 30, 60 and 120 min, 200 μl samples were taken from the receiver compartment and replaced with fresh buffer (pH 7.5). Samples were stored until HPLC analysis. After 2 h, the filters were quickly washed four times with ice-cold uptake buffer, cut out of the plastic insert, transferred to vials containing 0.5 ml deionized water and prepared for HPLC. The mannitol flux through Caco-2 cell monolayer was estimated by adding [^{14}C]mannitol (5 μM) to the donor compartment. Samples for radioactivity measurements were taken from the receiver compartment (10–120 min).

HPLC Analysis

HPLC analysis was done according to a laboratory standard procedure using an HPLC-system with diode array detector (Kontron, Germany) and a reversed phase column RP-18 (5 μm, 12.5 cm). The eluent was 15 to 40% acetonitril/0.5% phosphoric acid in water. UV-detection was done at 210 to 270 nm. Injection volume was 20 μl, flow rate one ml/min and the retention times were usually 2.5 to 5 min.

Data Analysis

Experiments were done in duplicate or triplicate and each experiment was repeated two to three times. Results are given as means ± S.E. IC_{50} values (i.e. concentration of the unlabeled β-lactam antibiotic necessary to inhibit 50% of radiolabeled Gly-Sar carrier-mediated uptake) were determined by non-linear regression with all single data points in a multiple fitting procedure using the four parameter logistic equation (linear dose vs. effect) $y = \min + (\max\text{-}\min)/(1 + (X/IC_{50})^{-P})$ where max is the initial y value (=100%), min the final y value (=10.7%) and the power P represents Hill coefficient (=1). For several compounds the IC_{50} values were near the end or out of the measurement range because of low inhibition at the highest concentration studied (maximal solubility or 31.6 mM, respectively). To quantify at least the rank order of inhibition, approximated IC_{50} values were mathematically extrapolated by non-linear regression of uptake data. Inhibition constants (K_i) were calculated from IC_{50} values (8). Flux data were calculated after correction for the amount taken out by linear regression of appearance in the receiver well vs. time.

RESULTS AND DISCUSSION

Inhibition of Gly-Sar Uptake

Uptake of Gly-Sar into confluent monolayers of Caco-2 cells is driven by an inwardly directed H^+ gradient and mediated by a single transport system (K_t = 1.1 ± 0.1 mM), which has been identified as the low affinity, high capacity system PEPT1 present in human small intestine (8,9,17). In the presence of an inwardly directed H^+ gradient, the concentration-dependent inhibition of [^{14}C]Gly-Sar uptake by 20 cephalosporins and 3 penicillins was measured. The chemical structures of the compounds used are given in Table I. The inhibition curves for all compounds are shown in Fig. 1A and B. From these curves, the K_i values were calculated (Table I). The cephalosporin ceftibuten and the penicillin cyclacillin were recognized by PEPT1 with affinity constants comparable to those of natural dipeptides (K_i = 0.3 and 0.5 mM, respectively). This is in agreement to previous studies (4,6,8,10). Cefadroxil, cefamandole, cephradine, cefaclor, cefuroxime-axetil, cefixime, cephalotin, cephalexin and ampicillin also interacted with PEPT1 (K_i between 7 and 14 mM). In contrast, cefapirin, cefodizime, cefuroxime, cefmetazole, ceftazidime, benzylpenicillin, ceftriaxone, cefpirome, cefotaxime, cefepime, cephaloridine and cefsulodin displayed no affinity to the transport system ($K_i > 20$ mM). Even if the affinity constants reported in the literature differ considerably depending on culture conditions and methods employed for transport assay (2,19), e.g., cells on plastic supports (5,8,17,24) versus cells grown on filters (6,9), extracellular pH (e.g., for the anionic cefixime 9,24), temperature and stirring, many values and especially the general rank order of affinities at PEPT1 in this study correspond well with those of previous studies (2,4,8,10,19). Our data support the concept that the following structural features are of relevance for the recognition of β-lactam antibiotics by the intestinal peptide carrier: (a) sterical resemblance to the tripeptide backbone, (b) N-terminal peptide bond with an α-amino group (e.g., cefadroxil, cephradine, cefaclor, cephalexin, ampicillin, cyclacillin) or hydroxyl group (cefamandole), (c) C-terminal peptide bond

Table I. Affinity Constants, Uptake, and Transepithelial Flux of β-Lactam Antibiotics

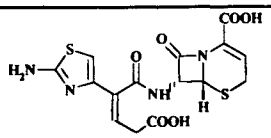
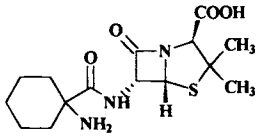
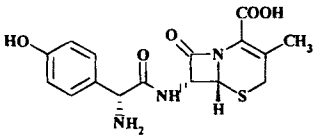
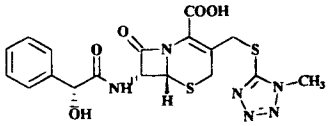
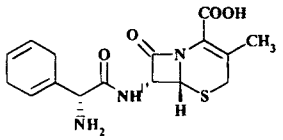
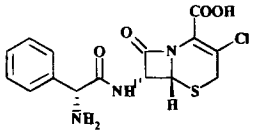
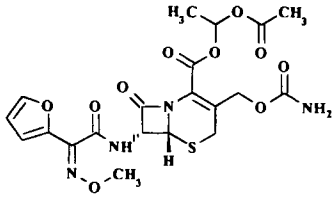
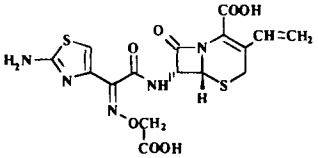
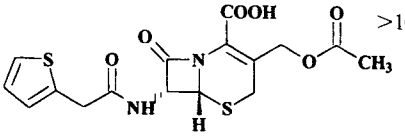
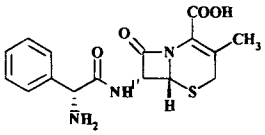
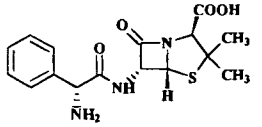
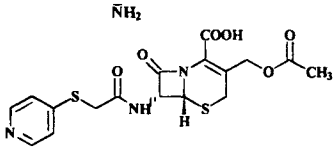
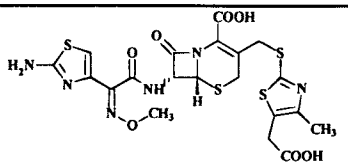
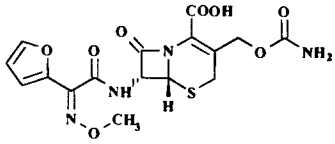
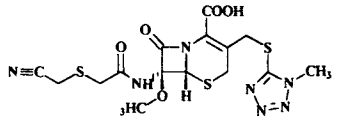
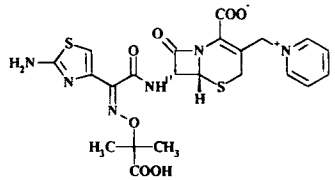
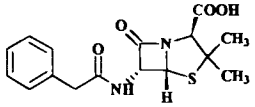
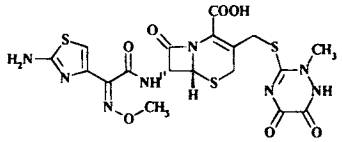
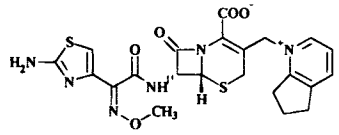
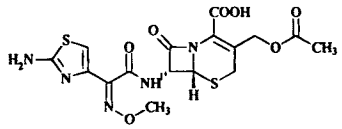
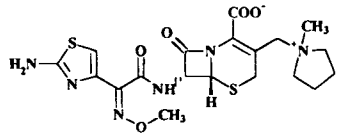
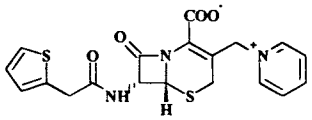
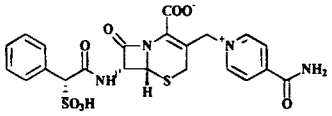
Compound	Structure	K_i(mM)	Uptake (nmol/(cm²·2 h))	Flux (nmol/(cm²·h))	Administration bioavailability p.o.
1. Ceftibuten		0.34 ± 0.03	10.7 ± 1.3	3.8 ± 0.4	Oral 84%
2. Cyclacillin		0.50 ± 0.05	1.0 ± 0.1	5.2 ± 0.2	Oral 70%
3. Cefadroxil		7.2 ± 0.8	12.3 ± 0.7	7.0 ± 0.6	Oral ≈100%
4. Cefamandole		8.1 ± 0.8	0.2 ± 0.1	3.2 ± 0.2	Parenteral <1%
5. Cephradine		9.8 ± 1.2	7.2 ± 0.4	2.8 ± 0.4	Oral ≈100%
6. Cefaclor		>10 (≈ 11)[b]	5.9 ± 0.3	2.5 ± 0.2	Oral ≈ 100%
7. Cefuroxime-axetil		>5[a] (≈ 12)[b]	6.9 ± 0.8[a]	5.9 ± 0.3[a]	Oral 40–50%
8. Cefixime		12 ± 2	2.4 ± 0.2	1.2 ± 0.2	Oral 40–50%
9. Cephalothin		>10 (≈ 14)[b]	0.07 ± 0.03	0.24 ± 0.01	Parenteral <5%
10. Cephalexin		14.4 ± 2.4	5.3 ± 0.7	3.3 ± 0.4	Oral ≥95%
11. Ampicillin		14.5 ± 1.7	0.32 ± 0.08	2.1 ± 0.4	Oral 30–40%
12. Cefapirin		>10 (≈ 20)[b]	0.19 ± 0.01	0.90 ± 0.04	Parenteral

Table I. Continued.

Compound	Structure	K_i(mM)	Uptake (nmol/(cm^2·2 h))	Flux (nmol/(cm^2·h))	Administration bioavailability p.o.
13. Cefodizime		22 ± 3	0.28 ± 0.07	0.30 ± 0.02	Parenteral
14. Cefuroxime		26 ± 4	0.12 ± 0.01	0.31 ± 0.01	Parenteral <1%
15. Cefmetazole		28 ± 3	0.12 ± 0.02	0.75 ± 0.12	Parenteral
16. Ceftazidime		>10 (≈ 40)[b]	0.17 ± 0.02	0.25 ± 0.07	Parenteral <1%
17. Benzylpenicillin		>30 (≈ 40)[b]	not measurable	1.3 ± 0.05	Parenteral
18. Ceftriaxone		>30 (≈ 40)[b]	0.17 ± 0.03	0.26 ± 0.03	Parenteral <5%
19. Cefpirome		>30 (≈ 45)[b]	0.17 ± 0.02	0.10 ± 0.02	Parenteral
20. Cefotaxime		>30 (≈ 50)[b]	0.10 ± 0.01	0.24 ± 0.02	Parenteral <2%
21. Cefepime		>30 (≈ 70)[b]	not measurable	0.16 ± 0.03	Parenteral
22. Cephaloridine		>30 (≈ 100)[b]	0.12 ± 0.02	0.29 ± 0.08	Parenteral <5%
23. Cefsulodin		>30 (≈ 150)[b]	0.16 ± 0.05	0.38 ± 0.06	Parenteral

Note: K_i-values of Gly-Sar uptake inhibition were calculated from data given in Figure 1A and B. Transepithelial flux was calculated by linear regression of data shown in Figure 2A and B. n = 4.

[a] Cefuroxime plus cefuroxime-axetil.

[b] K_i-values extrapolated beyond measurement range because of limited solubility of compounds or low inhibition. See Fig. 1 for maximal substrate concentrations used.

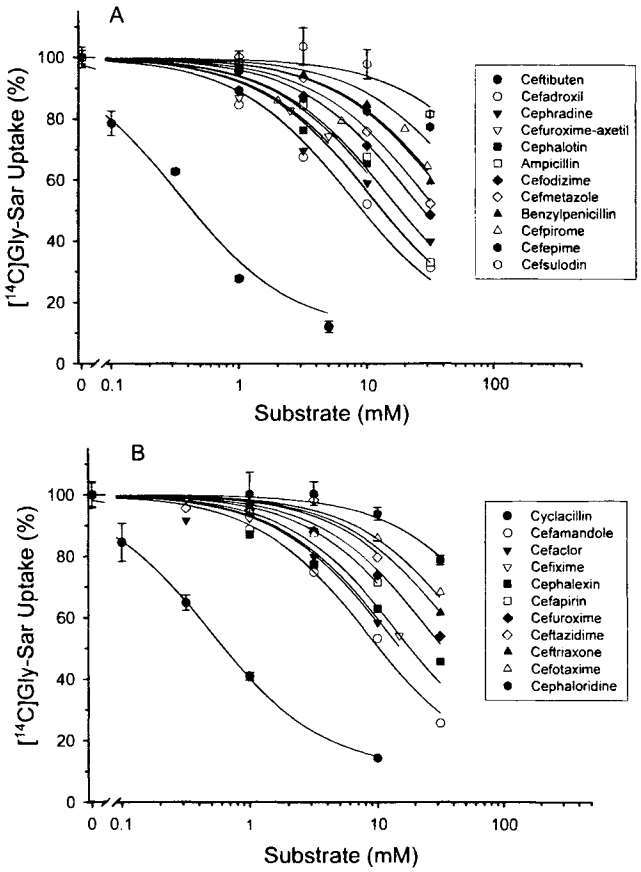


Fig. 1. Inhibition of [^{14}C]Gly-Sar uptake by β-lactam antibiotics in Caco-2 cells (A and B). Uptake of 10 μM [^{14}C]Gly-Sar was measured for 10 min in monolayer cultures of Caco-2 cells at pH 6.0 in the absence and presence of increasing concentrations of the substrates (0–31.6 mM or concentration of maximal solubility, respectively). Uptake of Gly-Sar measured in the absence of the inhibitors was taken as 100%. Data are shown as means. Representative S.E. are shown for 4 compounds. n = 4.

in the lactam ring of the cephem (cephalosporins) or penam (penicillins: cyclacillin, ampicillin, benzylpenicillin), respectively, (d) carboxy group at the dihydrothiazine ring of the cephalosporins or the thiazolidine of the penicillins and (e) substituents and saturation of the N-terminal ring systems (e.g., cyclacillin vs. ampicillin, cefadroxil vs. cephalexin vs. cephradine). New insight into the structural requirements of PEPT1 and PEPT2 is gained from computer-based analyses. For the renal peptide transporter, the structural determinants of the affinity at peptide transporters have been studied in detail (16) and subsequently used for molecular modeling studies (20). To characterize the pharmacophore of the intestinal peptide carrier, Swaan and Tukker (21) recently performed computer-aided conformational analysis with data collected from the literature. The data of the present study should be useful for such modeling studies because (a) the K_i values were measured under identical experimental conditions and (b) they reflect the direct interaction of β-lactam antibiotics with the binding site of PEPT1.

The affinity constants measured in this study are very well correlated with the bioavailability data of the compounds (Table I, 22,23). With the exception of cefamandole and cephalotin, only those β-lactam antibiotics with $K_i < 14$ mM are orally available. Therefore, when testing newly developed β-lactam antibiotics using the Caco-2 system, under the conditions described an affinity constant at PEPT1 of less than 14 mM is needed for oral availability via peptide carrier-mediated transport.

Transepithelial Flux

Measurement of Gly-Sar uptake inhibition only allows estimation of the affinity of β-lactam antibiotics at the binding site of PEPT1. To correlate the affinities of β-lactam antibiotics at PEPT1 with total net transport and the bioavailability, experiments using permeable filters were performed. Advantages and limitations of the Caco-2 model for oral absorption were reviewed in detail recently (19). In our study, the integrity of the Caco-2 cell monolayers grown on permeable filters was verified using [^{14}C]mannitol as a space marker. Under the conditions described, the transepithelial flux of [^{14}C]mannitol through the Caco-2 cell monolayers was 0.07 ± 0.002%/(cm^2·h). For comparison, the flux of [^{14}C]Gly-Sar was found to be 20-fold higher: At a substrate concentration of 30 μM, 616.9 ± 39.6 pmol/(cm^2·h) were measured at the receiver side of the monolayers (= 1.4%/(cm^2·h), data not shown). The permeability coefficients (P_{app}) of mannitol and Gly-Sar were $0.03 \cdot 10^{-5}$ cm/s and $0.57 \cdot 10^{-5}$ cm/s, respectively. Under these conditions, the total transepithelial flux of the β-lactam antibiotics through the Caco-2 cell monolayers differed by a factor of 70 and was highest for cefadroxil (Fig. 2A and B, Table I). All β-lactam antibiotics were apparently chemically stable during uptake and sample preparation with the exception of cefuroxim-axetil, which was completely hydrolyzed inside the cell within 2 h to cefuroxime. Table I also shows the intracellular accumulation of β-lactam antibiotics measured after the 2-h incubation. The uptake into the cells differed by a factor of 176 and was also highest for cefadroxil (12.3 nmol/(cm^2 · 2 h)). Peptides and β-lactam antibiotics that are recognized by PEPT1 are cotransported with H^+ uphill against a concentration gradient into the cell (6,9,17,24). Assuming an intracellular volume of 3.6 μl/mg of protein (6,17,25) and a protein content of 0.4 mg/cm^2 (this study), cefadroxil is enriched inside Caco-2 cells against the concentration gradient ≈9-fold. Likewise, ceftibuten, cephradine, cephalexin and cefaclor are accumulated in the cells at an intra- to extracellular concentration ratio of 4–7.

The transepithelial flux and the intracellular uptake are significantly correlated (linear correlation coefficient $r = 0.774$, $p < 0.0001$). More importantly, the transepithelial flux of the β-lactam antibiotics is well correlated with the K_i values for Gly-Sar uptake inhibition (Fig. 3, $r = 0.776$, $p < 0.0001$ for the exponential regression shown). Figure 3 (inset) also shows the ranks of total transport (i.e., the sum of intracellular uptake in 2 h plus the transepithelial flux per 2 h) plotted versus the ranks of affinity constants. This more robust procedure reveals a Spearmen's rank correlation coefficient r_s of 0.795 ($p < 0.0001$). Because of the strong correlation between transepithelial flux and intracellular uptake, rank correlation analyses of flux versus K_i values and uptake versus K_i values give similar results ($r_s = 0.790$, $p < 0.0001$ and $r_s = 0.791$, $p < 0.0001$, respectively). We conclude that the overall variation of transepithelial flux is mainly determined by the variation of the affinity at PEPT1 ($r^2 = 0.61$). However, as shown in Fig. 3, transport of some β-lactam antibiotics is not so well correlated with the

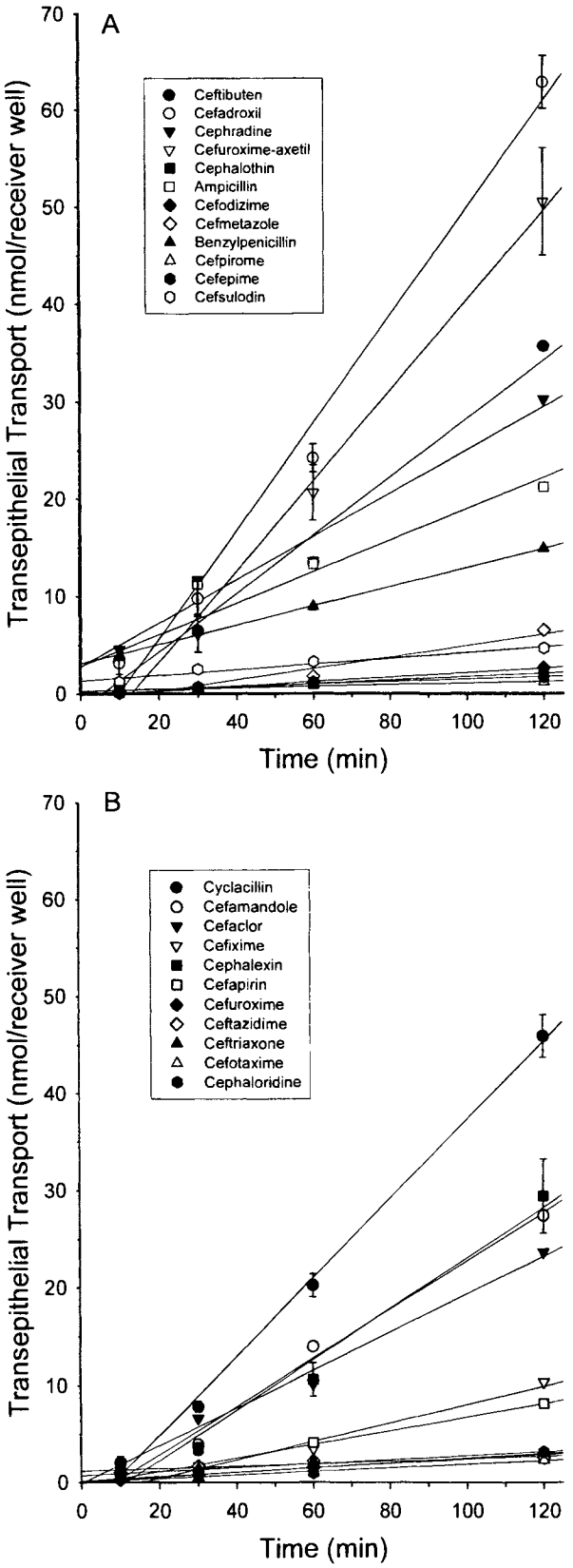


Fig. 2. Transepithelial flux of β-lactam antibiotics across Caco-2 cell monolayers (A and B). Substrates (1 mM) were added to the donor compartment (1.5 ml) of Transwell systems in uptake buffer (pH 6.0). After the time intervals indicated, samples (200 μl) were taken from the receiver compartment (pH 7.5) and replaced with buffer. Samples were analyzed by HPLC as described in Methods. Data are shown as means. Representative S.E. are shown for 4 compounds. n = 4.

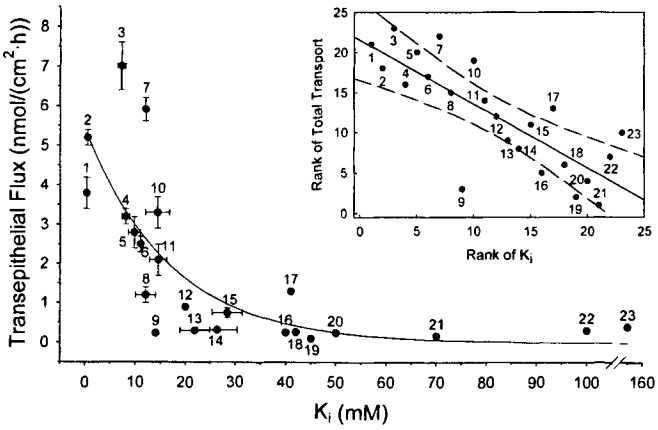


Fig. 3. Correlation between affinity constants (K_i-values of [^{14}C]Gly-Sar inhibition by β-lactam antibiotics) and flux data. Numbers of compounds and data correspond to Table I. K_i values of compounds 6,7,9,12, and 16–23 are extrapolated non-linear regression estimates beyond measurement range and shown without S.E. Inset: Ranks of total transport (= sum of uptake into the cells in 2 h plus transepithelial flux across the cell monolayer per 2 h) versus ranks of K_i values. Dashed line: 99% confidence interval of linear regression.

respective affinity constant at PEPT1, in particular the intracellular uptake of cyclacillin, cefamandole and cephalothin and the flux of cefadroxil, cefuroxime-axetil and cephalothin. Our experiments so far only concern the (a) direct interaction of β-lactam antibiotics with PEPT1 and (b) the net transport. Net transepithelial flux, however, is composed of: (a) transcellular and paracellular simple diffusion in both directions; (b) activity of one or more transport systems; (c) intracellular accumulation and degradation; (d) efflux across the luminal cell membrane back into the apical compartment; and (e) transport across the basolateral membrane. In the case of cefuroxime-axetil, the transepithelial flux is much higher than can be expected from the affinity constant. This can easily be explained with the very high lipophilicity of this compound (5). Several studies suggested the existence of multiple carrier systems for certain β-lactam antibiotics such as a second peptide carrier (3,4), the monocarboxylic acid carrier (26) and organic cation and organic anion transport systems for secretory transport (27). The contribution of each process to the total absorption of a particular compound varies, e.g., for the cephalosporins, ceftibuten and cefaclor (4). Hypothetically, efflux into the apical compartment could explain, for example, the low net flux of cephalotin in our study. Consequently, cephalotin has to be administered parenterally (Table I). It has to be noted, however, that the contribution of additional transport systems for absorption of β-lactam antibiotics is still in discussion. Gochoco *et al.* (7) studied in detail the uptake and transepithelial transport of cephalexin in Caco-2 cells and concluded that cephalexin uptake involves mainly the H^+/peptide transporter. The rate-limiting step in transepithelial transport of cephalexin appears to be the basolateral efflux and not the apical uptake (7). Using the Xenopus laevis expression system, Tamai *et al.* (28) also demonstrated the predominant role of PEPT1 in the carrier-mediated intestinal absorption of β-lactam antibiotics. Wenzel *et al.* (9) conclude from their study that PEPT1 is capable of transport of neutral as well as anionic/dianionic β-lactams in their zwitterionic form.

The correlation between transepithelial flux and the route of administration (Table I, 22,23) is unequivocal, cefamandole being the only exception. In approximation, the data in Table I show that oral availability >50% is based upon a minimal transepithelial flux of ≈2 nmol/(cm^2·h).

In summary, both the transepithelial flux of β-lactam antibiotics in Caco-2 cell cultures and the bioavailability correlate very well with the affinity to the peptide transporter. Therefore, PEPT1 is the major absorption route for oral antibiotics. The oral bioavailability of newly synthesized compounds such as peptide prodrugs can be predicted within certain limits by measuring affinity, uptake and flux in Caco-2 cell monolayers. Differences between laboratories, culture conditions and transport assays can be taken into account by comparing all three parameters with the respective transport data of both mannitol and a well described cephalosporin such as ceftibuten or cephalexin. To elucidate the structural requirements for transport of β-lactam antibiotics by the peptide carrier, computer-based molecular modeling analyses is required because of the complexity and diversity of the substrates.

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