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# Cefaclor uptake by the proton-dependent dipeptide transport carrier of human intestinal Caco-2 cells and comparison to cephalexin uptake

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The human Caco-2 cell line spontaneously differentiates in culture to epithelial cells possessing intestinal enterocytic-like properties. These cells possess a proton-dependent dipeptide transport carrier that mediates the uptake of the cephalosporin antibiotic cephalexin (Dantzig, A H. and Bergin, L. (1990) Biochim. Biophys. Acta 1027, 211–217). In the present study, the uptake of cefaclor was examined and found to be sodium-independent, proton-dependent, and energy-dependent. The initial rate of D-[3-*phenyl*-$^3$H]cefaclor uptake was measured over a wide concentration range; uptake was mediated by a single saturable transport carrier with a $K_m$ of 7.6 mM and a $V_{max}$ of 7.6 nmol/min per mg protein and by a non-saturable component. Uptake was inhibited by dipeptides but not amino acids. The carrier showed a preference for the L-isomer. The effect of the presence of a 5-fold excess of other β-lactam antibiotics was examined on the initial rates of 1 mM cefaclor and 1 mM cephalexin uptake. Uptake rates were inhibited by the orally absorbed antibiotics, cefadroxil, cefaclor, loracarbef, and cephradine and less so by the parenteral agents tested. The initial uptake rates of both D-[9-$^{14}$C]cephalexin and D-[3-*phenyl*-$^3$H]cefaclor were competitively inhibited by cephalexin, cefaclor, and loracarbef with $K_i$ values of 9.2–13.2, 10.7–6.2, and 7.7–6.4 mM, respectively. Taken together, these data suggest that a single proton-dependent dipeptide transport carrier mediates the uptake of these orally absorbed antibiotics into Caco-2 cells, and provide further support for the use of Caco-2 cells as a cellular model for the study of the intestinal proton-dependent dipeptide transporter.

## Introduction

A proton-dependent dipeptide transport carrier, located in the brush-border membrane of the small intestinal enterocyte [1], is believed to be a major route for the intestinal assimilation of dipeptides [2–9], tripeptides [3,10,11], and certain hydrophilic drug peptide mimetics. Drugs taken up by the transporter include orally absorbed β-lactam antibiotics and angiotensin converting enzyme (ACE) inhibitors [12–19]. The properties of the peptide transporter have been extensively studied in everted sacs, enterocytes, and intestinal brush-border membrane vesicles from chicken, rabbit, rats, hamsters, and humans [2,4–10,20–23]. Studies have also demonstrated the presence of a closely related proton-dependent dipeptide transport carrier in the human intestinal Caco-2 cell line [12]. Caco-2 cells spontaneously differentiate in culture to polar cells possessing microvilli and many enterocytic-like properties [24–26]. These cells grow as a monolayer forming a tight epithelium and expressing several of the intestinal transport carriers and pepti-

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Abbreviations: amiloride, 3,5-diamino-*N*-(aminoiminomethyl)-6-chloropyrazinecarboxamide; cefaclor, 7-(D-α-amino-α-phenylacetamido)-3-chloro-3-cephem-4-carboxylic acid; cephalexin, 7-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid; loracarbef, 7-(D-α-amino-α-phenylacetamido)-1-carba-1-dethia-3-chloro-3-cephem-4-carboxylic acid; cefadroxil, 7-(D-α-amino-α-(*p*-hydroxyphenyl)acetamido)-3-methyl-3-cephem-4-carboxylic acid; cephradine, 7-(D-α-amino-α-(1,4-cyclohexadiene-1-yl)acetamido)-3-methyl-3-cephem-4-carboxylic acid; cephalothin, 7-(D-2-thienylacetamido)-3-(acetyloxymethyl)-3-cephem-4-carboxylic acid; cefamandole, 7-(D-mandelamido)-3-[(1-methyl-H-tetrazol-5-yl)-thio]methyl]-3-cephem-4-carboxylic acid; cefoperazone, 7-[D(−)-α-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-α-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid; BBMV, brush-border membrane vesicles; EBSS, Earle's balanced salt solution; Mes, 2-(*N*-morpholino)ethanesulfonic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; ouabain, 3-[(6-deoxy-α-L-mannopyranosyl)oxy]-1,5,11α,14,19-pentahydroxycard-20(22)-enolide; L-Phe-Gly, L-phenylalanylglycine; Gly-D-Phe, glycyl-D-phenylalanine; Gly-L-Phe, glycyl-L-phenylalanine; Gly-L-Pro, glycyl-L-proline; L-Pro-Gly, L-prolylglycine; SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid.

dases present in the small intestine [26–30]. When differentiated, Caco-2 cells have a proton-dependent dipeptide transport carrier [12]. β-Lactam antibiotics, which possess certain structural features of peptides including a peptide bond with an α-amino group and a free carboxylic acid group (Fig. 1), are ideal substrates with which to characterize peptide transport in intact tissue without complications due to hydrolysis by peptidases or metabolism. Using the β-lactam antibiotic cephalexin as the substrate (Fig. 1), the properties of the dipeptide transport carrier of Caco-2 cells were found to closely resemble the properties of the intestinal proton-dependent dipeptide transport carrier of other species. The cephalexin transport carrier exhibits sodium-independence, proton-dependence, energy-dependence, and a preference for the L-stereoisomer. Cephalexin uptake into Caco-2 cells is competitively inhibited by dipeptides and is unaffected by the presence of amino acids. Thus, Caco-2 cells may provide a convenient in vitro model for the study of absorption via the proton-dependent dipeptide transport carrier in the small intestine.

The present study was undertaken to evaluate the specificity of the proton-dependent dipeptide transport carrier of Caco-2 cells for other cephalosporin antibiotics. Studies were conducted with cefaclor, a close structural analog of cephalexin (Fig. 1). The uptake of cefaclor was found to be mediated by the proton-dependent dipeptide transporter. Kinetic properties of the transporter for cephalexin and cefaclor are compared.

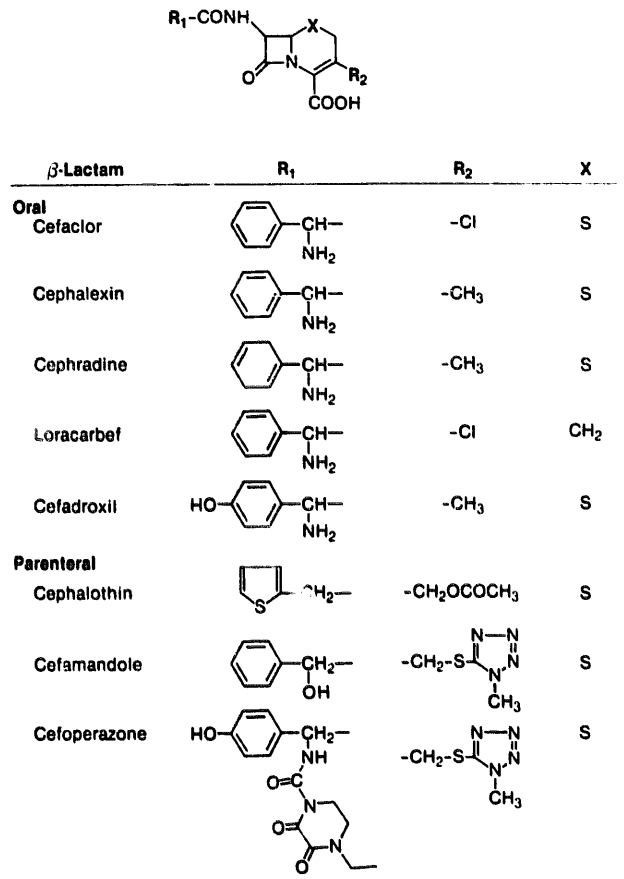


Fig. 1. Structures of β-lactam antibiotics used in this study.

## Methods and Materials

*Materials.* Cephalexin, cefaclor, and loracarbef were obtained from Eli Lilly, Indianapolis, IN. D-[9-$^{14}$C]Cephalexin (12.25 Ci/mol) and D-[3-*phenyl*-$^{3}$H]-cefaclor (1.0 Ci/mmol) was prepared by Lilly/Amersham. A dispersion of collagen was generous gift of Ethicon, Somerville, NJ. Growth media and EBSS was purchased from Gibco, Grand Island, NY. The other reagents were purchased from Sigma, St. Louis, MO.

*Cell culture.* The human adenocarcinoma cell line Caco-2 was obtained from Dr. J. Fogh at the Research Unit of Memorial Sloan-Kettering Cancer Center in Rye, NY. The cells were passaged as previously described [12]. For flux measurements, $(0.5–1.0) \cdot 10^5$ cells were grown in collagen-coated multiwell dishes (24 well) for 13–18 days and the medium was replaced every two to three days. The cells were mycoplasma-free and were used between passage numbers 28 and 44.

*Transport measurements and calculations.* Drug uptake was measured at 37°C using D-[9-$^{14}$C]cephalexin or D-[3-*phenyl*-$^{3}$H]cefaclor employing a cluster-tray technique [12,31]. The flux buffer was bicarbonate-free EBSS containing 25 mM Mes titrated to pH 6.0 with KOH. The sodium-free flux buffer contained choline chloride in place of sodium chloride. The osmolality of the flux buffer was adjusted to $300 \pm 5$ mosmol/kg with choline chloride. The extracellular fluid that adhered to the cells during the washing procedure was estimated using [$^{3}$H]inulin as the marker and used to estimate the zero time for the determination of the rate of uptake. Fresh solutions of the cephalosporins, dipeptides, SITS, and DIDS were prepared daily. Protein was measured by the method of Lowry et al. [32].

*Calculations.* Uptake was measured at 1, 2, 3 and 4 min. Initial uptake rates were calculated by linear regression from these points along with the estimated zero time as described above. Initial rates of 1 mM drug uptake ranged from 0.7 to 2.2 nmol/min per mg protein for cephalexin and from 0.3 to 1.7 nmol/min per mg protein for 1 mM cefaclor depending on the lot of serum and collagen. Percent inhibition was calculated based on the control uptake rate measured in each experiment. Alternatively, uptake was measured at 4 min and was corrected for trapped water. A general nonlinear curve-fitting procedure, a Marquardt algorithm (IBM Share No. 3094), was used to deter-

mine the kinetic parameters for the computer fitting of the data [33]. The uptake rates measured at 4°C were used in the computer fitting to estimate the $K_d$ for the non-saturable component.

## Results

### *Time course for cefaclor uptake into Caco-2 cells and effect of extracellular pH*

The accumulation of 1 mM D-[3-*phenyl*-$^3$H]cefaclor into Caco-2 cells was examined over a 2-h time course at pH 6.0 which is within the reported pH range of 5.5 to 6.3 present in the lumen of the small intestine [34,35]. As illustrated in Fig. 2, cefaclor uptake was much more rapid at 37°C than at 4°C. At 37°C, uptake of 1 mM cefaclor was linear for the first six min (data not shown) and had reached a plateau by 2 h with an intracellular drug concentration of 3.2 ± 0.3 mM. By contrast the drug intracellular concentration was only 0.5 ± 0.2 mM when cells were incubated at 4°C with 1 mM cefaclor for 2 h. When cells were incubated for 2 h at 37°C in buffer at pH 7.3 (close to the reported intracellular pH of 7.35 [36]), an intracellular concentration of only 0.5 ± 0.1 was achieved. Unless indicated otherwise in the experiments described below, the initial rates of cefaclor uptake were measured over a 4 min time course in sodium-free buffer pH 6.0.

### *Effect of energy poisons, protonophores, and the presence of sodium gradient*

To ascertain whether uptake was energy-dependent, the effect of metabolic inhibitors and protonophores was examined on the initial uptake rate of 1 mM D-[3-*phenyl*-$^3$H]cefaclor. As illustrated in Table I, uptake was inhibited significantly by 68 to 81% by oligomycin, 2,4-dinitrophenol, sodium azide, and the two protonophores, nigericin and FCCP. To further examine the role of the pH gradient across the plasma membrane, the intracellular pH of the cells was clamped to pH 6.0 by incubation of the cells with nigericin in pH 6.0 buffer containing 90 mM KCl; the rate of cefaclor uptake was reduced significantly by 41% (data not shown). To determine the effect of sodium, the uptake of 1 mM D-[3-*phenyl*-$^3$H]cefaclor was measured in the presence of sodium; no effect was observed on the rate of drug uptake. Furthermore, when the cells were incubated in sodium-containing buffer containing 1 mM ouabain, an inhibitor of $Na^+,K^+$-ATPase, no significant effect was measured on the rate of 1 mM cefaclor uptake (Table I).

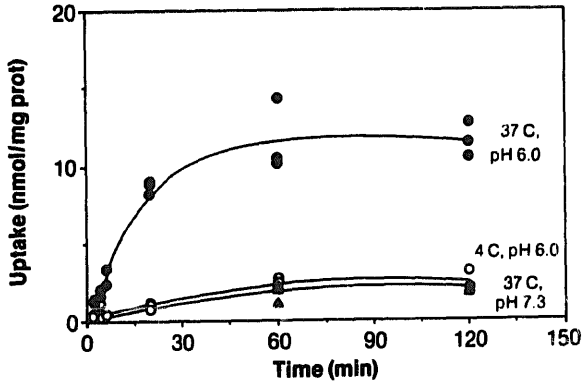


Fig. 2. Time dependence of D-[3-*phenyl*-$^3$H]cefaclor uptake into Caco-2 cells. Cells were incubated with 1 mM cefaclor for up to 2 h at 37°C (●) and 4°C (○) at pH 6.0 or at 37°C at pH 7.3 (▲). The curves are representative of two independent experiments.

TABLE I

*Effect of energy poisons, protonophores, and sodium on the uptake rate of 1 mM cefaclor*

The rate of 1 mM D-[3-*phenyl*-$^3$H]cefaclor uptake was measured in sodium-free buffer pH 6.0 containing choline chloride or in buffer pH 6.0 containing sodium chloride in place of choline chloride. Cells were incubated for 15 min with the indicated compound prior to measuring uptake. Initial uptake rates were determined in duplicate over a 4 min time course. The uptake rate for untreated cells in sodium-free buffer was 0.34 ± 0.04 nmol/min per mg protein (mean ± S.E.). The results are representative of two independent experiments.

| Incubation condition | % Inhibition |
|---|---|
| Oligomycin (25 μg/ml) | 68 ± 11 [a] |
| Sodium azide (10 mM) | 69 ± 11 [a] |
| 2,4-Dinitrophenol (0.5 mM) | 81 ± 11 [a] |
| Nigericin (10 μg/ml) | 64 ± 11 [a] |
| FCCP (10 μg/ml) | 77 ± 11 [a] |
| NaCl [b] | 5 ± 11 [c] |
| NaCl [b] plus ouabain (1 mM) | −5 ± 11 [c] |

[a] The uptake rates were analyzed by covariance and were found to be significantly different from untreated cells ($P < 0.005$).
[b] Sodium chloride containing buffer, pH 6.0.
[c] The uptake rates were analyzed by covariance and were found not to be significantly ($P = 0.5$) different from untreated cells.

### *Kinetic analysis of cefaclor uptake*

The initial rate of cefaclor uptake was determined over the concentration range of 1 to 25 mM at 37°C and at 1, 5, and 10 mM at 4°C (Fig. 3). An Eadie-Hofstee plot of the data indicated the presence of a single transport system (inset, Fig. 3). The uptake rates were subsequently fitted to a single Michaelis-Menten term and a non-saturable term:

$$v = \frac{V_{max}[S]}{K_m + [S]} + K_d[S] \qquad (1)$$

where $v$ is the velocity of uptake, [S] is the substrate concentration, $V_{max}$ is the maximum turnover rate, $K_m$ is the substrate concentration at which the velocity is half-maximal, $K_d$ is a constant for a non-saturable component. The kinetic parameters for the drug transport carrier were determined to be: $V_{max}$ of 7.6 ± 0.9 nmol/min per mg protein, $K_m$ of 7.6 ± 1.5 mM, and

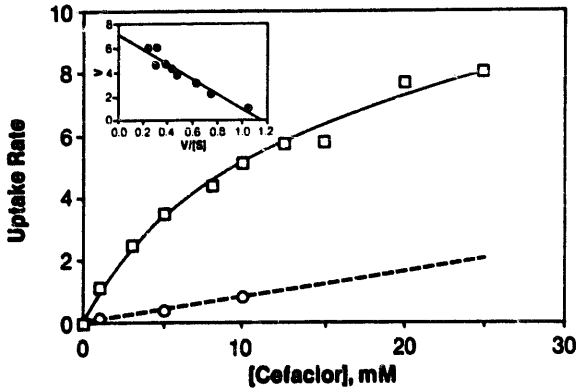


Fig. 3. Concentration dependence of the rate of D-[3-*phenyl*-$^3$H]-cefaclor uptake. The initial uptake rate was measured over the concentration range of 1 to 25 mM at 37°C. The initial rate of drug uptake was also measured at 1, 5, and 10 mM at 4°C. Each point is the mean of 4–6 points determined from two independent experiments. The curve is the computer fit of the data to the sum of one Michaelis-Menten term plus a non-saturable term (Eqn. 1). An Eadie-Hofstee plot of the uptake rates after correction for the non-saturable portion of uptake is shown in the inset. The line was drawn by the method of least squares ($r^2 = 0.92$). The plot indicates the presence of one transport system with an estimated $V_{max}$ of 7.1 nmol/min per mg protein and $K_m$ of 6.2 mM.

for the non-saturable component, the $K_d$ was estimated to be $0.09 \pm 0.02$ nmol/min per mg protein per mM.

*Effect of amino acids and dipeptides on cefaclor uptake*

To examine the properties of the transport carrier for cefaclor, the effect of addition of dipeptides, amino acids, organic anions and SITS and DIDS was examined on the uptake of 1 mM D-[3-*phenyl*-$^3$H]cefaclor. Table II summarizes the results. Uptake was inhibited significantly by the presence of 1 or 10 mM concentrations of neutral dipeptides but not by the presence of 10 mM amino acids, 1 mM SITS, or 1 mM DIDS. Cefaclor uptake was inhibited to a greater extent by the presence of the L-isomer than the D-isomer of the dipeptide, Gly-Phe; a 10-fold higher concentration of Gly-D-Phe than Gly-L-Phe was required to achieve roughly 50% inhibition. The renal organic anion transporter has also been reported to take up dipeptides and cephalosporins [37]. Two substrates, *p*-aminohippuric acid and furosemide, and two inhibitors, SITS and DIDS, of the renal organic anion transporter were without affect on the uptake of 1 mM cefaclor (Table II).

*Effect of cephalosporins on cefaclor and cephalexin uptake*

Next, the effect of the presence of other β-lactam antibiotics on drug uptake via the dipeptide transport carrier was examined. The initial rate of 1 mM D-[3-*phenyl*-$^3$H]cefaclor or 1 mM D-[9-$^{14}$C]cephalexin up-

TABLE II

*Effect of dipeptides, amino acids, organic anions, SITS and DIDS on the uptake of 1 mM cefaclor*

Uptake of 1 mM D-[3-*phenyl*-$^3$H]cefaclor was measured in the presence of the indicated compound for 4 min in triplicate. The control uptake rate was $1.47 \pm 0.10$ nmol/min per mg protein. The results are representative of two independent experiments.

| Compound | Concn. (mM) | % Inhibition |
|---|---|---|
| Dipeptides | | |
| Gly-L-Pro | 10 | 71 ± 1 [a] |
| Gly-L-Phe | 10 | 72 ± 1 [a] |
| | 1 | 54 ± 1 [a] |
| Gly-D-Phe | 10 | 53 ± 3 [a] |
| | 1 | 16 ± 1 [a] |
| L-Phe-Gly | 10 | 68 ± 4 [a] |
| Carnosine | 10 | 46 ± 3 [a] |
| Amino acids | | |
| Glycine | 10 | −8 ± 3 |
| L-Proline | 10 | −5 ± 6 |
| L-Phenylalanine | 10 | 11 ± 7 |
| Organic anions | | |
| *p*-Aminohippuric acid | 1 | −2 ± 4 |
| Furosemide | 1 | 15 ± 3 [b] |
| Inhibitors | | |
| SITS | 1 | −5 ± 5 |
| DIDS | 1 | 1 ± 3 |

[a] Significantly different from the control ($P < 0.005$) by Student's *t*-test.

[b] Significantly different from the control ($P = 0.02$) by Student's *t*-test.

TABLE III

*Effect of β-lactam antibiotics on the uptake rate of 1 mM cefaclor*

The uptake rate of 1 mM D-[3-*phenyl*-$^3$H]cefaclor was measured in duplicate in the absence and presence of 5 mM of each compound in sodium-free buffer. The control uptake rate was 0.7 nmol/min per mg protein. The percent inhibition by each compound was normalized to that of 5 mM Gly-L-Pro which was 52 ± 11% (mean ± S.E.). Inhibition by the dipeptide, Gly-L-Pro, represents the contribution due to the carrier-mediated pathway under these assay conditions. The results are representative of two independent experiments.

| Compound (5 mM) | % Inhibition |
|---|---|
| Orally absorbed | |
| Cephalexin | 33 ± 11 [a] |
| Cefaclor | 47 ± 11 [a] |
| Cephradine | 66 ± 11 [a] |
| Loracarbef | 50 ± 11 [a] |
| Cefadroxil | 82 ± 11 [a] |
| Parenteral | |
| Cephalothin | 17 ± 11 [b] |
| Cefamandole | 30 ± 11 [a] |

[a] The uptake rate was significantly different ($P < 0.05$) than the control when analyzed by covariance.

[b] The uptake rate was not significantly different than the control ($P > 0.5$).

TABLE IV

*Effect of β-lactam antibiotics on the uptake rate of 1 mM cephalexin*

The uptake rate of 1 mM D-[9-$^{14}$C]cephalexin was measured in the absence and presence of 5 mM of each compound in sodium-free buffer. The control uptake rate varied between 0.9–1.7 nmol/min per mg protein. The percent inhibition by each compound was normalized to that of 5 mM Gly-L-Pro which was 62 ± 4% (mean ± S.D.). Inhibition by the dipeptide, Gly-L-Pro, represents the contribution due to the carrier-mediated pathway under these assay conditions.

| Compound (5 mM) | % Inhibition |
|---|---|
| Orally absorbed | |
| Cephalexin | 48 ± 3 [a] |
| Cefaclor | 58 ± 3 [a] |
| Cephradine | 51 ± 3 [a] |
| Loracarbef | 53 ± 3 [a] |
| Cefadroxil | 70 ± 6 [a] |
| Parenteral | |
| Cephalothin | 8 ± 8 [b] |
| Cefamandole | 17 ± 3 [b] |
| Cefoperazone | 9 ± 4 [b] |

[a] Values represent the means ± S.D. of 3–5 independent determinations. Data are significantly different ($P < 0.05$) than the control when analyzed by Student's *t*-test.

[b] Values represent the means ± range of two independent determinations.

take was measured in the presence and absence of 5 mM of several β-lactam antibiotics. The results are summarized in Tables III and IV; the structures of these antibiotics are shown in Fig. 1. The uptake rates of both cefaclor and cephalexin were significantly inhibited by 33 to 70% in the presence of the orally absorbed β-lactam antibiotics, cephalexin, cefaclor, cephradine, loracarbef, and cefadroxil. The uptake rate

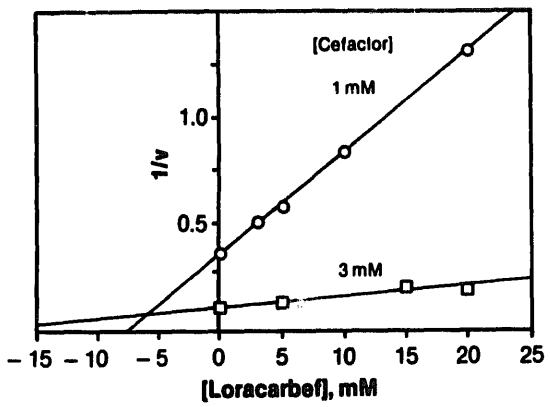


Fig. 4. Dixon-Webb plot of the rate of D-[3-*phenyl*-$^{3}$H]cefaclor uptake in the presence of loracarbef. Initial rates of drug uptake were measured at 1 and 3 mM cefaclor in the presence of increasing concentrations of loracarbef. The units on the ordinate are nmol/min per mg protein. Each point represents the average of duplicate values. Values were not corrected for diffusion. The plot is representative of two independent experiments. Lines were drawn by linear regression of the points for each drug concentration. The $K_i$ was determined from the intersection of the two lines.

TABLE V

*Affinity of the transport carrier for selected β-lactam antibiotics, cephalexin, cefaclor, and loracarbef*

Initial uptake rates of D-[9-$^{14}$C]cephalexin or D-[3-*phenyl*-$^{3}$H]cefaclor were measured at 1 and 3 mM in the presence of increasing concentrations of cefaclor, cephalexin, or loracarbef. The $K_i$ was determined from a Dixon-Webb plot as described in Fig 4.

| β-Lactam competitor | $K_i$ (mM) | |
|---|---|---|
| | cephalexin | cefaclor |
| Cephalexin | 9.2 | 13.2 |
| Cefaclor | 10.7 | 6.2 |
| Loracarbef | 7.7 | 6.4 |

of cefaclor was inhibited 30% by the presence of cefamandole and not significantly by the presence of another parenteral agent, cephalothin (Table III); the uptake rate of cephalexin was not affected by the presence of three parenteral agents examined, cephalothin, cefamandole, and cefoperazone (Table IV).

*Competition by β-lactams for cefaclor and cephalexin uptake*

The affinity of the transport carrier for cephalexin, cefaclor, and loracarbef was determined. The initial uptake rates of D-[3-*phenyl*-$^{3}$H]cefaclor and D-[9-$^{14}$C]cephalexin were measured at 1 mM and 3 mM in the presence of increasing concentrations of the indicated β-lactam antibiotic. The data were analyzed by a Dixon-Webb plot as illustrated in Fig. 4 for the inhibition of D-[3-*phenyl*-$^{3}$H]cefaclor uptake by loracarbef. All three β-lactams showed competitive inhibition of both substrates. The $K_i$ values are summarized in Table V.

## Discussion

The present study demonstrates that the uptake of the cephalosporin antibiotic, cefaclor, is mediated by a proton-dependent dipeptide transport carrier in Caco-2 cells. The properties of the transport carrier are consistent with that of the intestinal transporter responsible for the uptake of dipeptides, tripeptides, and orally absorbed cephalosporin antibiotics [1]: (a) Cefaclor uptake is sodium-independent, energy- and proton-dependent [2,4–9,13,14,16,21–23,38,39]. Previous studies conducted with rabbit intestinal brush-border membrane vesicles indicate that the rate of 1 mM cefaclor uptake is stimulated markedly by an inward directed proton-gradient which results in drug accumulation against a concentration gradient [14]. (b) Drug uptake can be inhibited by dipeptides and not by amino acids or inhibitors of renal organic anion transporter [3,22,40]. This is consistent with a $H^+$-cefaclor cotrans-

porter that also transports dipeptides and not amino acids. (c) Greater inhibition of cefaclor uptake was observed in the presence of the dipeptide, Gly-L-Phe than Gly-D-Phe, indicating a preference for the L-stereoisomer [6]. (d) The kinetic parameters for cefaclor uptake via the transporter are: $K_m$ of 7.6 mM and $V_{max}$ of 7.6 nmol/min per mg protein. The affinity of the transporter compares favorably with the reported $K_m$ of 3.0 and 16.1 mM for the rat cefaclor transporter examined, respectively, in intestinal brush-border membrane vesicles and in situ [41,42]. Thus, these properties of the cefaclor transport carrier in Caco-2 cells agree favorably with the properties described for the intestinal proton-dependent dipeptide transport carrier of other species.

The absorption of peptides and certain oral cephalosporin antibiotics including cefaclor has been proposed to be mediated by multiple transport carriers located in the intestine and the kidney [2,11,12,15,37, 43–45]. In studies conducted with rat intestinal brush-border membrane vesicles, the uptake of 0.5 mM cefaclor was estimated to be mediated by two distinct transporters, a proton-dependent transport mechanism (responsible for only 34% of the drug's uptake) and a proton-independent transport mechanism (accounting for 54%); the remainder was due to diffusion [11]. By contrast, studies conducted with rabbit intestinal brush-border membrane vesicles indicate that approximately 70–75% of 1 mM cefaclor uptake was mediated by a proton-dependent transport mechanism [14]. Previous studies from our laboratory indicate that different dipeptide transport carriers are responsible for the uptake of cephalexin in the two intestinal cell lines, HT-29-A1 and Caco-2. The HT-29-A1 cell line appears to possess an energy-independent dipeptide transporter that takes up cephalexin [46]; whereas, the Caco-2 cell line possesses a single proton- and energy-dependent dipeptide transport carrier for cephalexin uptake [12]. Moreover, the transport of dipeptides is mediated by two independent proton-dependent dipeptide transport systems with different affinities in rat renal brush-border membrane vesicles [45].

Although there have been no reports of multiple proton-dependent dipeptide transport carriers in the intestine, the present study investigates the possibility that the uptake of cefaclor and cephalexin may be mediated by distinct transport carriers in Caco-2 cells. The results indicate that the uptake of both cephalexin and cefaclor is mediated by a single, shared proton-dependent dipeptide transport system. (a) Both drugs are taken up by sodium-independent, proton- and energy-dependent transport carrier [12]. (b) The uptake of both antibiotics was significantly inhibited by the presence of a 5-fold excess of orally absorbed β-lactam antibiotics including cephalexin, cefaclor, cephradine, loracarbef, and cefadroxil and inhibited less well or not all by the parenteral β-lactam antibiotics tested. (c) Kinetic analysis of the rate of cefaclor uptake over the concentration range of 1 to 25 mM indicated the presence of a single transport carrier (Fig. 3) and that this transport system is responsible for approx. 90% of the uptake rate of 1 mM cefaclor. Previous studies examining the uptake of cephalexin over the same concentration range also indicated that a single transport system was responsible for cephalexin uptake [12]. (d) Both cefaclor and cephalexin competitively inhibited the uptake of the other, indicating a common transport mechanism. (e) The $K_i$ values for cefaclor and cephalexin were determined to be very close to the calculated $K_m$ values from the kinetic analysis of their respective drug uptake. If one transport system is responsible for drug uptake, the affinity of the transporter for the drug should be the same whether uptake was measured directly or indirectly as a competitor for the uptake of the another substrate of the transport carrier [47]. Thus, the affinity of the transporter for cefaclor was determined to be 10.7 mM when measured as a $K_i$ and was determined to be 7.6 mM when determined as the $K_m$ value from the kinetic analysis of cefaclor uptake. Similarly, the affinity of the transporter for cephalexin was determined to be 13.2 mM as the $K_i$, which agrees favorably with the previously reported $K_m$ of 7.5 mM [12]. If more than one transport system exists in these cells and if the putative transporters have different affinities for these two drugs (as has been demonstrated for the uptake of dipeptides in the brush-border of the kidney [45]), then the $K_i$ values and the $K_m$ values would not be expected to be in such good agreement. (f) When drug uptake was measured in the presence of a third β-lactam antibiotic, loracarbef, the uptake rates of both cefaclor and cephalexin were competitively inhibited. In addition, the affinity of the cefaclor and cephalexin transporter for loracarbef (measured as a $K_i$) were in close agreement, respectively, 6.4 and 7.7 mM. Taken together, these data indicate that cefaclor and cephalexin share a single proton-dependent transport system for uptake into Caco-2 cells [12]. No kinetic evidence could be found to support the presence of a second saturable transport system contributing significantly to the uptake of either of these two drugs at pH 6.0 in Caco-2 cells. Cephalexin and cefaclor have been proposed to be taken up by a common proton-dependent dipeptide transport carrier in the intestine of rabbits [14].

The Caco-2 cell line can be grown as a tight epithelium on permeable membrane supports to examine the vectorial flux of substrates across the intact epithelium [30,48–52]. Recently, the permeability of the Caco-2 monolayer for a series of small peptides and the tripeptide, thyrotropin-releasing hormone (a substrate of the a rat intestinal peptide transporter [19]) and analogs was reported. In these studies, no detectable flux of

any of these compounds across the monolayer was mediated by the dipeptide transporter when the cell monolayers were incubated in pH 7.4 buffer [53,54]. These findings are consistent with the peptide transport carrier requiring a proton-gradient across the lumenal cellular membrane of the Caco-2 cell as demonstrated for the uptake of both cefaclor and cephalexin [12]. When evaluating potential substrates of proton-dependent transport mechanisms, incubation conditions should be used that fall within the (acidic) pH range of the small intestine. One advantage of the intestinal Caco-2 cells is that the cells remain viable under these acidic conditions and incubation conditions may be easily manipulated. Thus, Caco-2 cells provide a convenient in vitro intestinal epithelial model for examination of the absorption of solutes.

## References


1 Ganapathy, V. and Leibach, F.H. (1991) Curr. Opinion Cell Biol. 3, 695–701.
2 Matthews, D.M. and Payne, J.W. (1980) Curr. Top. Membr. Transp. 14, 331–425.
3 Rajendran, V.M., Ansari, S.A., Harig, J.M., Adams, M.B., Khan, A.H. and Ramaswamy, K. (1985) Gastroenterology 89, 1298–1304.
4 Berteloot, A., Khan, A.H. and Ramaswamy, K. (1981) Biochim. Biophys. Acta 649, 179–188.
5 Ganapathy, V., Burckhardt, G. and Leibach, F.H. (1985) Biochim. Biophys. Acta 816, 234–240.
6 Rajendran, V.M., Berteloot, A. and Ramaswamy, K. (1985) Am. J. Physiol. 248, G682–G686.
7 Rajendran, V., Harig, J.M. and Ramaswamy, K. (1987) Am. J. Physiol. 252, G281–G286.
8 Ganapathy, V. and Leibach, F.H. (1983) J. Biol. Chem. 258, 14189–14192.
9 Said, H.M., Ghishan, F.K. and Redha, R. (1988) Biochim. Biophys. Acta 941, 232–240.
10 Wilson, D., Barry, J.A. and Ramaswamy, K. (1989) Biochim. Biophys. Acta 986, 123–129.
11 Muranushi, N., Yoshikawa, T., Yoshida, M., Oguma, T., Hirano, K. and Yamada, H. (1989) Pharm. Res. 6, 308–312.
12 Dantzig, A.H. and Bergin, L. (1990) Biochim. Biophys. Acta 1027, 211–217.
13 Okano, T., Inui, K.-I., Takano, M. and Hori, R. (1986) Biochem. Pharmacol. 35, 1781–1786.
14 Okano, T., Inui, K.-I., Maegawa, H., Takano, M. and Hori, R. (1986) J. Biol. Chem. 261, 14130–14134.
15 Inui, K.-I. (1988) Yakugaku Zasshi 108, 921–937.
16 Nakashima, E., Tsuji, A., Mizuo, H. and Yamana, T. (1984) Biochem. Pharmacol. 33, 3345–3352.
17 Tsuji, A., Hirooka, H., Tamai, K. and Terasaki, T. (1986) J. Antiobiotics. 39, 1592–1597.
18 Lowther, J., Hammond, S.M., Russell, K. and Fairclough, P.D. (1990) J. Antimicrobial Chemother. 25, 183–185.
19 Friedman, D.I. and Amidon, G.L. (1990) J. Control. Rel. 13, 141–146.
20 Calonge, M.L., Ilundain, A. and Bolufer, J. (1989) Rev. Esp. Fisiol. 45, 373–376.
21 Calonge, M.L., Ilundain, A. and Bolufer, J. (1989) J. Cell. Physiol. 138, 579–585.
22 Ganapathy, V., Mendicino, J.R. and Leibach, F.H. (1981) J. Biol. Chem. 256, 116–124.
23 Matthews, D.M. and Burston, D. (1984) Clin. Sci. 67, 541–549.
24 Nath, S.K. and Desjeux, J.-F. (1990) J. Diarrhoeal Dis. Res. 8, 133–142.
25 Rousset, M. (1986) Biochemie 68, 1035–1040.
26 Pinto, M., Appay, M.D., Simon-Assmann, P., Chevalier, G., Dracopoli, N., Fogh, J. and Zweibaum, A. (1982) Biol. Cell. 44, 193–196.
27 Vincent, M.L., Russell, R.M. and Sasak, V. (1985) Human Nutr.-Clinical Nutr. 39C, 355–360.
28 Mohrmann, I., Mohrmann, M., Biber, J. and Murer, H. (1986) Am. J. Physiol. 250, G323–G330.
29 Blais, A., Bissonnette, P. and Berteloot, A. (1987) J. Membr. Biol. 99, 113–125.
30 Dix, C.J., Hassan, I.F., Obray, H.Y., Shah, R. and Wilson, G. (1990) Gastroenterology 98, 1272–1279.
31 Gazzola, G.C., Dall'Asta, V., Franchi-Gazzola, R. and White, M.F. (1981) Anal. Biochem. 115, 368–374.
32 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
33 Marquardt, D.W. (1963) J. Soc. Ind. Appl. Math. 11, 431–441.
34 Lucas, M.L., Schneider, W., Haberich, F.J. and Blair, J.A. (1975) Proc. R. Soc. Lond. B. 192, 39–48.
35 Shimada, T. and Hoshi, T. (1988) Biochim. Biophys. Acta 937, 328–334.
36 Watson, A.J., Levine, S., Donowitz, M. and Montrose, M.H. (1991) Am. J. Physiol. 261, G229–G238.
37 Moller, J. and Sheikh, M.I. (1983) Pharmacol. Rev. 34, 315–358.
38 Ganapathy, V. and Leibach, F.H. (1985) Am. J. Physiol. 249, G153–G160.
39 Sleisenger, M.H., Burston, D., Dalrymple, J.A., Wilkinson, S. and Matthews, D.M. (1976) Gastroenterology 71, 76–81.
40 Rubino, A., Field, M. and Shwachman, H. (1971) J. Biol. Chem. 246, 3542–3548.
41 Yoshikawa, T., Muranushi, N., Yoshida, M., Oguma, T., Hirano, K. and Yamada, H. (1989) Pharm. Res. 6, 302–307.
42 Sinko, P.J. and Amidon, G.L. (1988) Pharm. Res. 5, 645–650.
43 Skopicki, H.A., Fisher, K., Zikos, D., Flouret, G., Bloch, R., Kubillus, S. and Peterson, D.R. (1988) Am. J. Physiol. 255, C822–C827.
44 Skopicki, H.A., Fisher, K., Zikos, D., Bloch, R., Flouret, G. and Peterson, D.R. (1991) Am. J. Physiol. 261, F670–678.
45 Daniel, H., Morse, E.L. and Adibi, S.A. (1991) J. Biol. Chem. 266, 19917–19924.
46 Dantzig, A.H. and Bergin, L. (1988) Biochem. Biophys. Res. Commun. 155, 1082–1087.
47 Christensen, H.N. (1975) Biological Transport, pp. 369–404, W.A. Benjamin, Reading, MA.
48 Wilson, G., Hassan, I.F., Dix, C.J., Williamson, I., Shah, R., Mackay, M. and Artursson, P. (1990) J. Control. Rel. 11, 25–40.
49 Hilgers, A.R., Conradi, R.A. and Burton, P.S. (1990) Pharm. Res. 7, 902–910.
50 Riley, S.A., Warhurst, G., Crowe, P.T. and Turnberg, L.A. (1991) Biochim. Biophys. Acta 1066, 175–182.
51 Artursson, P. and Karlsson, J. (1991) Biochem. Biophys. Res. Commun. 175, 880–885.
52 Artursson, P. (1991) Crit. Rev. Ther. Drug Carrier Syst. 8, 305–330.
53 Lundin, S., Moss, J., Bundgaard, H. and Artursson, P. (1991) Int. J. Pharm. 76, R1–R4.
54 Conradi, R.A., Hilgers, A.R., Ho, N.F.H. and Burton, P.S. (1991) Pharm. Res. 8, 1453–1460.