ELSEVIER

Biochimica et Biophysica Acta 1191 (1994) 7–13

BBA Biochimica et Biophysica Acta

Transport mechanisms responsible for the absorption of loracarbef, cefixime, and cefuroxime axetil into human intestinal Caco-2 cells

Anne H. Dantzig *, Dale C. Duckworth, Linda B. Tabas

Lilly Research Laboratories, Lilly Corporate Center, Eli Lilly and Company, Indianapolis, IN 46285-0424, USA

(Received 5 August 1993)

Abstract

Loracarbef, cefixime and cefuroxime axetil are β-lactam antibiotics that are administered orally. Oral absorption of loracarbef is nearly complete, while that of cefixime and cefuroxime axetil is 30–50%. To investigate this we used the human intestinal cell line Caco-2 that possesses the proton-dependent peptide transporter that takes up cephalexin and cefaclor. Drug uptake was measured at pH 6 by high performance liquid chromatography or with radioactively labelled drug. The initial uptake rate of 1 mM cefixime was lower than that of 1 mM loracarbef. By 2 h both drugs were concentrated intracellularly against a gradient; however, the accumulation of cefixime was only 40% of that of loracarbef. The uptake rate of both drugs was sodium-independent, temperature- and energy-dependent, and was inhibited by dipeptides, cephalexin, cefaclor, but not by amino acids. Kinetic analysis of the concentration-dependence of the uptake rates for loracarbef and cefixime indicated that diffusion and a single transport system were responsible for uptake. The kinetic parameters for loracarbef and cefixime, respectively, were: K_m values of 8 and 17 mM and V_{max} values of 6.5 and 2 nmol/min per mg protein. Loracarbef and cefixime were competitive inhibitors of each other's uptake. By contrast, cefuroxime axetil was taken up and rapidly hydrolyzed to cefuroxime by Caco-2 cells. Cefuroxime axetil uptake was not dependent on energy and was not affected by dipeptides. Thus, cefuroxime axetil apparently enters Caco-2 cells by simple diffusion. By contrast, loracarbef and cefixime share a common transport mechanism, the proton-dependent dipeptide transporter. Cefixime was taken up less well than loracarbef due to a substantial reduction in the turnover rate and decreased affinity of the transporter for cefixime.

Key words: Carbacephem; β-Lactam; Loracarbef; Cefixime; Peptide transport; Caco-2; (Human intestine)

1. Introduction

Loracarbef is an oral β-lactam antibiotic used for the treatment of ear inflammation and infections of the kidney, skin, and respiratory tract. Loracarbef represents a new chemical structural class called carbacephem (Fig. 1). This β-lactam has enhanced chemical stability to hydrolysis in water or by enzymatic hydrolysis by β-lactamases when compared to other cephalosporin and penicillin antibiotics. Like two oral cephalosporin antibiotics, cephalexin and cefaclor, loracarbef is very well absorbed in man. The mechanism responsible for uptake has not been examined.

The human Caco-2 cell line has been used as an in vitro model to study the absorption of many antibiotics and drugs [1–9]. When cultured, these cells possess many biochemical and morphological properties of the intestinal enterocyte [10–12]. Confluent cells become polarized and possess microvilli on the apical surface (facing the growth medium) and a smooth surface on the basolateral (support) side. Transport carriers for nutrients and hydrolytic enzymes are distributed asymmetrically on the two cellular surfaces [8,10,13–16].

* Corresponding author. Fax: +1 (317) 2769086.

Abbreviations: cefaclor, 7-(D-α-amino-α-phenylacetamido)-3-chloro-3-cephem-4-carboxylicacid; cefixime, (6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)-acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylic acid; cefuroxime, (6*R*,7*R*)-3-carbamoyloxymethyl-7-[*Z*-2-methoxyimino-2-(fur-2-yl)acetamido]-ceph-3-em-4-carboxylate; cefuroxime axetil, 1-(acetyloxy) ethyl ester of cefuroxime; cephalexin, 7-(D-α-amino-α-phenyl-acetamido)-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; EBSS, Earle's balanced salt solution; Mes, 2-(*N*-morpholino)-ethanesulfonic acid; 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′-isothiocyanostilbene-2,2′-disulfonic acid; DIDS, 4,4′-diisothiocyano-2,2′-disulfonicstilbene.

SSDI 0005-2736(93)E0392-N

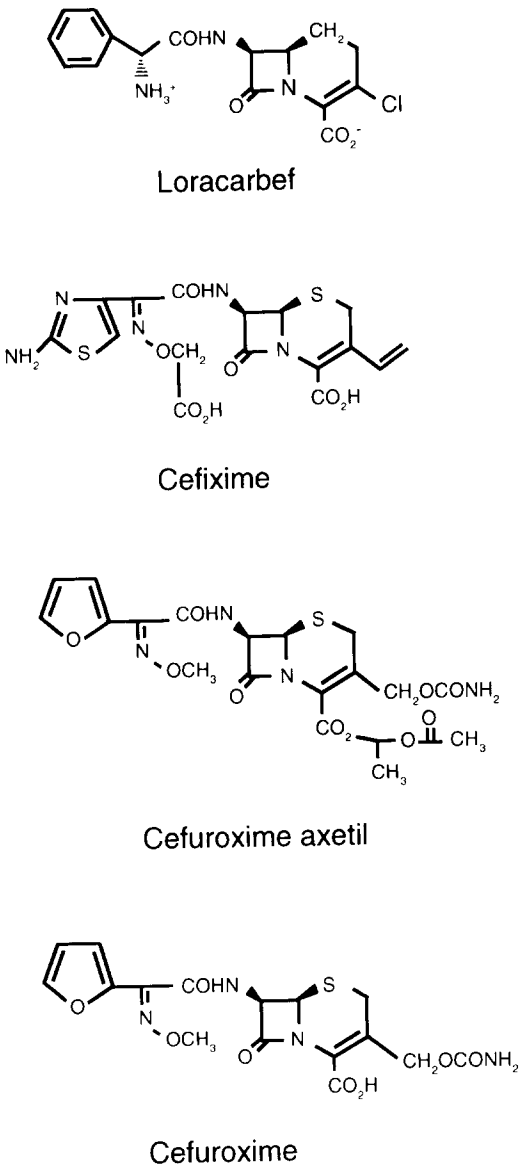

Fig. 1. Structures of loracarbef, a carbacephem antibiotic, and cephalosporin antibiotics used in study.

Located in the brush border membrane is the proton-dependent dipeptide transporter that takes up cephalexin and cefaclor [1,2] and located on the basolateral side is a second peptide transporter responsible for the translocation of cephalexin across the cell membrane [17].

The proton-dependent dipeptide transporter of Caco-2 cells has all of the salient features of the intestinal brush border proton-dependent peptide transporter as elucidated with the study of cephalexin and cefaclor uptake [1,2]. The transporter is proton- and energy-dependent. Uptake of these β-lactams is inhibited by the presence of dipeptides but not amino acids. The carrier has a higher affinity for the L-stereoisomer than the D-isomer of dipeptides and of cephalexin. The affinity of the transporter for cephalexin and cefaclor is within the range of reported values from studies with intestinal brush border membrane vesicles and in situ absorption studies.

The present study was undertaken to examine the mechanism by which loracarbef is orally absorbed into the human intestinal enterocyte and to compare the mechanism of uptake with that of cefixime and cefuroxime axetil. Cefixime is taken up by a proton-dependent peptide transporter in intestinal brush border membrane vesicles [18–21] and is 30–40% orally bioavailable in man. Cefuroxime axetil is 35–50% bioavailable in man and is a ester prodrug of the parenteral agent, cefuroxime [22,23]. The enhanced absorption of the prodrug compared with cefuroxime is thought to be due to the increased lipophilicity, resulting in a more rapid rate of diffusion. By contrast, loracarbef which is hydrophilic is nearly completely absorbed in man [24].

2. Materials and methods

Materials. Cephalexin, cefuroxime axetil, cefuroxime, and loracarbef were obtained from Eli Lilly, Indianapolis, IN. Cefixime was a kindly provided by Lederle. D-[9-^{14}C]Cephalexin (12.25 Ci/mol) and D-[^{3}H]loracarbef were prepared by Lilly/Amersham. Ethicon, Somerville, NJ generously supplied a collagen dispersion. 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 Caco-2 cell line was passaged as previously described [1]. 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 29 and 45.

Transport measurements and calculations. Drug uptake was measured using a cluster-tray technique [1,25]. 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 ± 5 mOsm/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 using bovine serum albumin as the standard [26].

For non-radiolabelled drugs, detection of the drug was measured employing high performance liquid chromatography. Cells were lysed with 250 μl of water.

A 20 μl sample was injected onto a Waters Nova C18 Radial Compression Module (8 × 100 mm with a guard insert) at 1200 p.s.i. with a flow rate of 2–2.5 ml/min. The solvent system was isocratic and differed for each drug. The solvent system for loracarbef was 7.5% acetonitrile/7.5% methanol/0.5% ammonium acetate/water and for cefixime 9% acetonitrile/9% methanol/0.5% ammonium acetate/water. For cefuroxime axetil and cefuroxime, the solvent system was 40% acetonitrile/0.1% trifluoroacetate/water. Drugs were detected at 260 nm and peaks were observed between 4 and 7 min depending on the drug. The peak area was used to calculate the amount of drug from a standard curve.

Calculations. The rate of loracarbef uptake was measured at 1, 2, 3 and 4 min; the rate for cefixime uptake was measured at 2, 3, 4, and 6 min. Initial uptake rates were calculated by linear regression from these points along with the estimated zero time as described above. In these experiments, the initial uptake rates of 1 mM loracarbef was 0.94 ± 0.14 and 1 mM cefixime was 0.55 ± 0.14 nmol/min per mg protein. Percent inhibition was calculated based on the uptake rate measured in the absence of inhibitor for each experiment. The distribution ratio is the intracellular drug concentration/extracellular drug concentration determined at 37°C [1]. Alternatively, uptake was measured at 4 min for loracarbef and 6 min for cefixime and cefuroxime axetil; values were corrected for trapped water. Kinetic parameters for the concentration-dependence of uptake were determined by a general nonlinear curve-fitting procedure, a Marquardt algorithm (IBM Share No. 3094; [27]). The non-saturable component (K_d) was estimated by measuring the uptake rate at 4°C.

3. Results

Time course for drug accumulation

As a first step in the characterization of the uptake of loracarbef, cefixime, and cefuroxime axetil, Caco-2 cells were incubated over a 2 h time course with drug in flux buffer at pH 6.0 which is within the reported pH range of the small intestine of 5.5–6.3 [28,29]. As shown in Fig. 2A, uptake of both 1 mM loracarbef and 1 mM cefixime was more rapid at 37°C than 4°C. At 37°C, loracarbef uptake was linear for the first four minutes and reached a plateau within 0.5 h. By contrast, cefixime uptake was linear for six minutes and reached a maximum plateau within 0.5 h. Uptake of both of these drugs was substantially reduced at 4°C and the intracellular concentration was not at equilibrium within the 2 h incubation period. A comparison of the the ability of Caco-2 cells to concentrate these drugs intracellularly is presented in Table 1. Cefixime

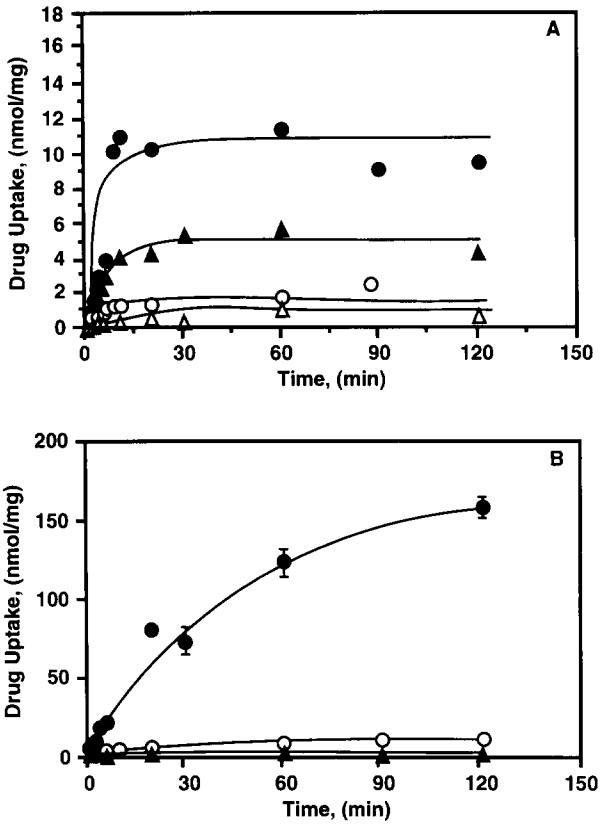

Fig. 2. Time-dependence of drug uptake into human intestinal Caco-2 cells. Cells were incubated with 1 mM drug for up to 2 h at 37°C (solid symbols) and 4°C (open symbols). (A) Uptake of loracarbef (circles) and cefixime (triangles). Curves are representative of eight or four independent experiments for loracarbef and cefixime, respectively. (B) Uptake of cefuroxime axetil (circles) and cefuroxime (triangles). Curves are representative of two independent experiments.

was accumulated less well than loracarbef, cephalexin or cefaclor.

Next, Caco-2 cells were incubated at 37°C with 1 mM cefuroxime axetil, the ester prodrug of cefuroxime, or with 1 mM cefuroxime, the parent drug. Initial experiments suggested that the prodrug was hydrolyzed

Table 1
The ability of human intestinal Caco-2 cells to concentrate β-lactam antibiotics intracellularly

β-Lactam	Distribution ratio
Loracarbef	3.9 ± 0.5
Cephalexin	3.5 ± 0.1 [a]
Cefaclor	3.2 ± 0.3 [b]
Cefixime	1.6 ± 0.7 [c]

Cells were incubated with 1 mM of each antibiotic at 37°C for up to 2 h in sodium-free flux buffer (pH 6.0). Values represent the mean ± S.E. of duplicates measured in eight or four independent experiments for loracarbef and cefixime, respectively.

[a] As previously reported [1].

[b] As previously reported [2].

[c] Significantly different ($P < 0.02$) than loracarbef by Student's t-test.

Table 2
Effect of energy poisons and protonophores on drug uptake

Incubation condition	% Inhibition		
	loracarbef	cefixime	cef. axetil
Oligomycin (25 μg/ml)	56±15 [a]	46±3 [a]	N.D. [b]
Sodium azide (10 mM)	76±15 [a]	81±2 [a]	20±2
2,4-Dinitrophenol (0.5 mM)	89±15 [a]	70±2 [a]	−7±8 [c]
Nigericin (10 μg/ml)	53±16 [a]	66±4 [a]	N.D. [b]
FCCP (10 μg/ml)	76±15 [a]	75±2 [a]	14±1

Drug uptake (1 mM) was measured in sodium-free buffer (pH 6.0) containing choline chloride. Cells were incubated for 15 min with the indicated compound prior to measuring uptake. Loracarbef uptake was measured for 4 min; cefixime and cefuroxime axetil uptake were each measured for 6 min. The uptake rate for untreated cells in sodium-free buffer was 1.03, 0.46, and 3.33 nmol/min per mg protein for loracarbef, cefixime, and cefuroxime axetil, respectively. Values represent the mean±S.E. of triplicate determinations measured in two or three independent experiments.

[a] Significantly different ($P < 0.05$) from untreated cells (Student's t-test).

[b] Not determined.

[c] Not significantly different ($P > 0.5$) from untreated cells (Student's t-test).

rapidly intracellularly to the parent drug, cefuroxime, since the presence of cefuroxime axetil was not detected even at the earliest time points in cell extracts. Consequently, the uptake of cefuroxime axetil was determined by following the appearance of cefuroxime intracellularly. When cells were incubated at 37°C with 1 mM cefuroxime axetil, the appearance of cefuroxime intracellularly was much more rapid than when incubated at 4°C or when cells were incubated with cefuroxime at 37°C (Fig. 2B).

Effect of energy poisons and protonophores

In order to determine whether drug uptake is energy-dependent or dependent on the inwardly directed pH-gradient that exists across the plasma membrane, the effect of metabolic inhibitors and protonophores was examined on the uptake of loracarbef, cefixime, and cefuroxime axetil. Table 2 summarizes the results of those studies. The uptake of 1 mM loracarbef and 1 mM cefixime was inhibited significantly by incubation of Caco-2 cells with energy poisons, oligomycin, sodium azide, and 2,4-dinitrophenol. Uptake of both drugs was also significantly inhibited by the presence of the protonophores, nigericin and FCCP. By contrast, incubation of Caco-2 cells with these inhibitors had little to no affect on the uptake of 1 mM cefuroxime axetil.

Effect of dipeptides, amino acids and other inhibitors on drug uptake

Next, drug uptake was examined in the presence of several dipeptides, amino acids, and other inhibitors. Table 3 summarizes the effect of these inhibitors on the uptake of 1 mM loracarbef, 1 mM cefixime, and 1 mM cefuroxime axetil. The uptake of loracarbef and cefixime was significantly inhibited by the presence of dipeptides. A ten-fold higher concentration of Gly-D-Phe than Gly-L-Phe was required to achieve the same level of inhibition. Uptake was not affected by the presence of either of the amino acids examined or by the presence of the agents, PAH, furosemide, SITS, or DIDS. By contrast, cefuroxime axetil uptake was reduced only slightly or not all by the presence of dipeptides and these other compounds. These results suggest that the uptake of loracarbef and cefixime are mediated by a transport carrier, whereas, the uptake of cefuroxime axetil is likely to be by passive diffusion.

Kinetic analysis of loracarbef and cefixime uptake

To further characterize the transport mechanisms responsible for the absorption of loracarbef and cefixime, initial uptake rates were measured over a wide concentration range at two temperatures 37°C and 4°C. Uptake rates measured at 4°C were used to estimate the non-saturable component due to simple diffusion. As shown in Fig. 3A, the uptake rate at 37°C of loracarbef increased with increasing concentrations and then appeared to saturate; whereas, the uptake rate was reduced substantially at 4°C and did not appear to

Table 3
Effect of dipeptides, amino acids, and organic anions and other inhibitors on the uptake of loracarbef, cefixime, and cefuroxime axetil

Compound	Conc. (mM)	% Inhibition		
		loracarbef	cefixime	cef. axetil
Dipeptides				
Gly-L-Pro	10	86±15 [a]	80±10 [a]	6±1
L-Phe-Gly	10	91±15 [a]	67± 4 [a]	−1±8 [b]
Gly-L-Phe	1	63±15 [a]	73± 7 [a]	N.D. [c]
Gly-L-Phe	10	92±15 [a]	83± 4 [a]	N.D.
Gly-D-Phe	1	22± 5 [a]	54±10 [a]	N.D.
Gly-D-Phe	10	74±16 [a]	75± 5 [a]	N.D.
Carnosine	10	69±15 [a]	70± 5 [a]	N.D.
Amino acids				
L-Proline	10	−16±10	20± 4 [a]	−4±2 [b]
L-Phenylalanine	10	13±13 [b]	23± 4 [a]	N.D.
Organic anions				
PAH	1	−33±16	25± 8 [a]	−11±9
Furosemide	1	35±15	5± 5 [b]	N.D.
Inhibitors				
SITS	1	32±16	7± 4 [b]	−5±1 [b]
DIDS	1	−4±16 [b]	15± 4 [a]	N.D.

Uptake of 1 mM drug was measured at 37°C in the absence and the presence of the indicated compound in sodium-free flux buffer (pH 6.0). Uptake was measured for 4 min for loracarbef and for 6 min for cefixime and cefuroxime axetil. Values represent the mean±S.E. of triplicate determinations measured in two or three independent experiments.

[a] Significantly different ($P < 0.05$) from the control (Student's t-test).

[b] Not significantly ($P > 0.5$) different from the control (Student's t-test).

[c] Not determined.

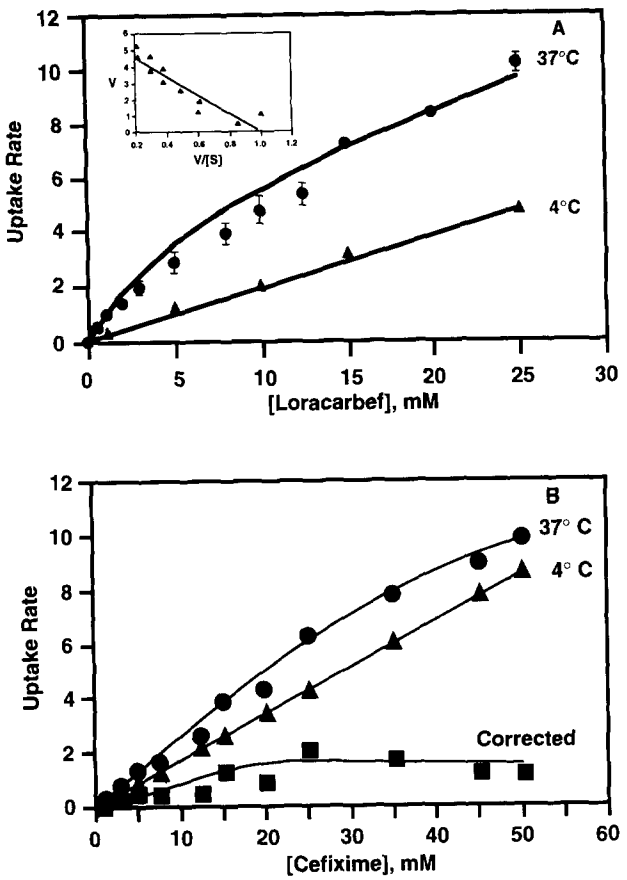

Fig. 3. Concentration-dependence of loracarbef and cefixime uptake. Initial uptake rates were measured at 37°C and 4°C. The units for the uptake rate are nmol/min per mg protein. The curve is the computer fit of the data to the sum of one Michaelis-Menten term plus a non-saturable term (Eq. (1)). Curves are representative of two independent experiments. (A) Concentration-dependence of loracarbef uptake. Initial rates of uptake were measured over the concentration range of 0.5–25 mM. An Eadie-Hofstee plot (inset) of the uptake rates after correction for the non-saturable portion of uptake. The line was drawn by the method of least squares. (B) Concentration-dependence of cefixime uptake. Initial uptake rates were measured over the concentration range of 1–50 mM. Uptake rates determined at 37°C were corrected for uptake rates measured at 4°C are shown (solid squares).

saturate over this concentration range. An Eadie-Hostee plot of these data after correcting for diffusion indicated the presence of a single transport system. When the uptake rates of cefixime were measured at the two temperatures, the uptake rate at 37°C appeared to saturate with increasing drug concentraions and the rates were greater than those measured at 4°C (Fig. 3B). Subsequently, the uptake rates were fitted to a Michaelis-Menten term and a non-saturable term:

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

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. Table 4 summarizes the kinetic parameters obtained for the uptake of loracarbef and cefixime

Table 4
Kinetic parameters for β-lactam uptake

Drug	V_{max} [a]	K_m [b]	K_d [c]
Loracarbef	6.5±0.3	8.1± 0.9	0.19
Cephalexin [d]	6.5±0.9	7.5± 2.8	0.18
Cefaclor [e]	7.6±0.9	7.6± 1.5	0.09
Cefixime	2.0±0.7	17.4±14.4	0.17

[a] nmol/min per mg protein.
[b] mM.
[c] nmol/min per mg protein per mM.
[d] Reported in Ref. [1].
[e] Reported in Ref. [2].

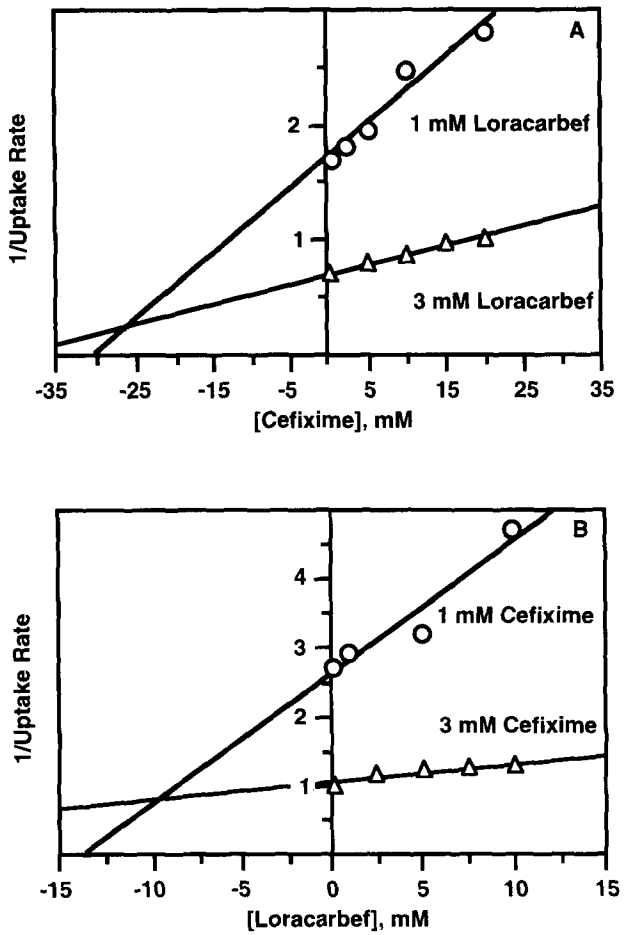

Fig. 4. Dixon-Webb plots. Initial rates of drug uptake were measured at 1 and 3 mM in the presence of increasing concentrations of the competing drug which is indicated on the abscissa. The units on the ordinate are (min per mg protein)/nmol. Each point represents the average of duplicate values. Values 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.

along with parameters for the uptake of cephalexin and cefaclor determined in earlier studies [1,2].

Next, to determine if loracarbef and cefixime share the same transport mechanism, competition studies were conducted. The uptake rate of each drug was measured at 1 mM and 3 mM in the presence of increasing concentrations of the second drug. Dixon-Webb plots of the data were prepared for both loracarbef and cefixime as shown in Fig. 4. Loracarbef uptake was competitively inhibited by the presence of cefixime with a K_i of 24.4 ± 1.7 mM; cefixime uptake was competitively inhibited by the presence of loracarbef with a K_i of 11.6 ± 2.1 mM.

4. Discussion

The present study examines the absorption of three oral β-lactam antibiotics, loracarbef, the new carbacephem antibiotic, cefixime and the prodrug, cefuroxime axetil into human intestinal Caco-2 cells. This is the first report of the mechanism responsible for the uptake of loracarbef into intestinal cells. In these studies, Caco-2 cells took up loracarbef much better than cefixime and also took up the ester prodrug cefuroxime axetil much better than the parent drug, cefuroxime. Both loracarbef and cefixime entered the intestinal enterocyte by simple diffusion and a carrier-mediated pathway. By contrast, the oral ester prodrug, cefuroxime axetil, entered the cell by simple diffusion and much more rapidly than the parenteral parent drug, cefuroxime. These findings are consistent with clinical studies that loracarbef is better absorbed orally than cefixime, and that cefuroxime axetil is absorbed orally while its parent drug, cefuroxime, is not.

The uptake of both loracarbef and cefixime was mediated by the proton-dependent peptide transporter that is responsible for the uptake of di- and tripeptides and many orally administered cephalosporin antibiotics. (a) Uptake of both drugs was sodium-independent and temperature-, energy-, and proton-dependent [30–34]. (b) Uptake was inhibited by peptides and not amino acids or inhibitors of the renal organic anion transporter [32,33,35,36]. (c) In addition, uptake was reduced more in the presence of the L-stereoisomer than the D-stereoisomer of Gly-Pro, indicating a preference for the L-isomer [32]. (d) The concentration dependence of uptake indicated that the maximal turnover rate (V_{max}) and the affinity (K_m) for loracarbef were, respectively, 6.5 nmol/min per mg protein. and 8.1 mM. These values are quite similar to the kinetic parameters for two other well absorbed β-lactam antibiotics, cephalexin and cefaclor (Table 4; Refs. [1,2]). The K_m for loracarbef is in excellent agreement with the K_i values that were determined in competition studies of cephalexin and cefaclor uptake, respectively, 7.7 and 6.4 mM [2]. By contrast, kinetic parameters for cefixime uptake were considerably different from those for loracarbef; the V_{max} was 2 nmol/min per mg protein. and the K_m was 17 mM. (e) Furthermore, competition studies indicate that loracarbef and cefixime are competitive inhibitors of each other's uptake with K_i values of 11.6 and 24 mM, respectively, also in close agreement with their K_m values. Taken together, these data indicate that loracarbef and cefixime share a common transport mechanism, the intestinal proton-dependent dipeptide transporter which is also responsible for the uptake of cephalexin and cefaclor into intestinal cells [1,2]. This intestinal transporter has also been shown to be responsible for the uptake of cefixime in studies employing rat brush border membrane vesicles [17,18,37–39].

Although the intestinal proton-dependent peptide transporter takes up both loracarbef and cefixime, cefixime uptake is considerably less than loracarbef. After a 2 h incubation, the cells concentrated cefixime to a level that was only 41% of loracarbef (1.6 versus 3.9-fold, respectively). This is in agreement with an earlier report that cefixime was accumulated to only 40% of the level of cephalexin in Caco-2 cells [17]. The reduced ability to accumulate cefixime is likely to be due to differences in the kinetic parameters of the transporter for these drugs. The maximal turnover rate for cefixime is ~30% of that of loracarbef and cephalexin, and the affinity for cefixime appears to be poorer 17–24 mM versus an affinity of 7–8 mM (Table 4). Thus, the difference in uptake is largely due to a reduced turnover rate of the transporter for cefixime as well as a decreased affinity for cefixime. Evidently an α-amino group to the peptide bond in the side chain of the β-lactam is not absolutely required for uptake by the transporter as previously proposed [40]. Its presence may, however, permit the carrier to take up the substrate more efficiently.

Next the uptake of two other β-lactams, the prodrug cefuroxime axetil and the parenteral drug cefuroxime, were examined (Fig. 1). Both β-lactams lack a free α-amino group in their side chain; neither enters the cell by a carrier-mediated pathway. Uptake of the prodrug was not sodium- or energy-dependent nor was uptake affected by the presence of dipeptides, amino acids, or other inhibitors. Even though uptake was not carrier-mediated, cefuroxime axetil entered the intestinal cell much more rapidly than the parent drug, cefuroxime, and higher intracellular concentrations were achieved than loracarbef or cefixime (Fig. 2). The enhanced diffusion of the ester prodrug relative to the parent drug may result from the increased lipophilicity due to the replacement of the 1-carboxyl group with the uncharged 1-acetoxyethyl ester side chain. In our studies, cefuroxime axetil apparently diffused readily into the cell where it was rapidly hydrolyzed presum-

ably by esterases to the less lipophilic parent drug whose exit from the cell was much slower than the entry of the prodrug. The apparent accumulation of cefuroxime (axetil) within the cell is due to differences in the lipophilicities of the parent drug and the ester prodrug. Thus, cefuroxime would be expected to be 'trapped' intracellularly. The unusually high drug concentration that can be trapped intracellularly might be expected to give better absorption than what is actually observed clinically. Hydrolysis of the ester prodrug in the intestinal lumen by esterases as previously reported [22,41] liberates cefuroxime in the gastrointestinal tract and thereby reduces the luminal cefuroxime axetil concentration resulting in reduced absorption of the prodrug. Hydrolysis in the intestinal lumen, no doubt, contributes to cefuroxime axetil's moderate oral bioavailability in man of 36–50%.

In summary, human intestinal Caco-2 cells took up loracarbef, cephalexin, cefaclor, much better than cefixime in excellent agreement with their known bioavailabilities in man. All four of these β-lactam antibiotics are taken up by a common transport mechanism, the intestinal proton-dependent peptide transporter. Decreased cefixime uptake is due to a reduced ability of the transporter to turn over when cefixime is bound as compared to the other antibiotics. This apparently reduces its bioavailability so that it is only moderately absorbed orally in man. In addition the transport of cefuroxime axetil was much greater than the parent parenteral agent, cefuroxime. Cefuroxime axetil was hydrolyzed rapidly intracellularly to cefuroxime. Together these data indicate that the human intestinal cell line is an excellent in vitro model for the study of absorption mechanisms for new peptidyl-drugs.

5. References

[1] Dantzig, A.H. and Bergin, L. (1990) Biochim. Biophys. Acta 1027, 211–217.
[2] Dantzig, A.H., Tabas, L.B. and Bergin, L. (1992) Biochim. Biophys. Acta 1112, 167–173.
[3] Ranaldi, G., Islam, K. and Sambuy, Y. (1992) Antimicrob. Agents Chemother. 36, 1374–1381.
[4] Wilson, G., Hassan, I.F., Dix, C.J., Williamson, I., Shah, R., Mackay, M. and Artursson, P. (1990) J. Control. Rel. 11, 25–40.
[5] Hilgers, A.R., Conradi, R.A. and Burton, P.S. (1990) Pharm. Res. 7, 902–910.
[6] Hu, M. and Borchardt, R.T. (1990) Pharm. Res. 7, 1313–1319.
[7] Artursson, P. (1991) Crit. Rev. Ther. Drug Carrier Syst. 8, 305–330.
[8] Burton, P.S., Conradi, R.A., Hilgers, A.R. and Ho, N.F.H. (1993) Biochem. Biophys. Res. Commun. 190, 760–766.
[9] Schasteen, C.S., Donovan, M.G. and Cogburn, J.N. (1992) J. Control Rel. 21, 49–62.
[10] Pinto, M., Appay, M.D., Simon-Assmann, P., Chevalier, G., Dracopoli, N., Fogh, J. and Zweibaum, A. (1982) Biol. Cell. 44, 193–196.
[11] Rousset, M. (1986) Biochemie 68, 1035–1040.
[12] Nath, S.K. and Desjeux, J.-F. (1990) J. Diarrhoeal Dis. Res. 8, 133–142.
[13] Hidalgo, I.J., Kato, A. and Borchardt, R.T. (1989) Biochem. Biophys. Res. Commun. 160, 317–324.
[14] Hidalgo, I.J., Raub, T.J. and Borchardt, R.T. (1989) Gastroenterology 96, 736–749.
[15] LeBivic, A., Quaroni, A., Nichols, B. and Rodriguez-Boulan, E. (1990) J. Cell Biol. 111, 1351–1361.
[16] Alvarez-Hernandez, S., Nichols, G.M. and Glass, J. (1991) Biochim. Biophys. Acta 1070, 205–208.
[17] Inui, K.-I., Yamamoto, M. and Saito, H. (1992) J. Pharmacol. Exp. Ther. 261, 195–201.
[18] Tsuji, A., Hirooka, H., Tamai, K. and Terasaki, T. (1986) J. Antiobiotics 39, 1592–1597.
[19] Tsuji, A., Terasaki, T., Tamai, I. and Hirooka, H. (1987) J. Pharmacol. Exp. Ther. 241, 594–601.
[20] Sugawara, M., Iseki, K., Miyazaki, K., Shiroto, H., Kondo, Y. and Uchino, J.-I. (1991) J. Pharm. Pharmacol. 43, 882–884.
[21] Lowther, J., Hammond, S.M., Russell, K. and Fairclough, P.D. (1990) J. Antimicrob. Chemother. 25, 183–185.
[22] Finn, A., Straughn, A., Meyer, M. and Chubb, J. (1987) Biopharm. Drug Dispos. 8, 519–526.
[23] Ginsburg, C.M., McCracken, G.H., Jr., Petruska, M. and Olson, K. (1985) Antimicrob. Agents Chemother. 28, 504–507.
[24] DeSante, K.A. and Zeckel, M.L. (1992) Am. J. Med. 92, 16S-19S.
[25] Gazzola, G.C., Dall'Asta, V., Franchi-Gazzola, R. and White, M.F. (1981) Anal. Biochem. 115, 368–374.
[26] Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Garter, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985) Anal. Biochem. 150, 76–85.
[27] Marquardt, D.W. (1963) J. Soc. Ind. Appl. Math. 11, 431–441.
[28] Lucas, M.L., Schneider, W., Haberich, F.J. and Blair, J.A. (1975) Proc. R. Soc. Lond. B 192, 39–48.
[29] Shimada, T. and Hoshi, T. (1988) Biochim. Biophys. Acta 937, 328–334.
[30] Berteloot, A., Khan, A.H. and Ramaswamy, K. (1981) Biochim. Biophys. Acta 649, 179–188.
[31] Ganapathy, V., Burckhardt, G. and Leibach, F.H. (1985) Biochim. Biophys. Acta 816, 234–240.
[32] Rajendran, V.M., Berteloot, A. and Ramaswamy, K. (1985) Am. J. Physiol. 248, G682–G686.
[33] Rajendran, V.M., Ansari, S.A., Harig, J.M., Adams, M.B., Khan, A.H. and Ramaswamy, K. (1985) Gastroenterology 89, 1298–1304.
[34] Calonge, M.L., Ilundain, A. and Bolufer, J. (1989) J. Cell. Physiol. 138, 579–585.
[35] Ganapathy, V., Mendicino, J.R. and Leibach, F.H. (1981) J. Biol. Chem. 256, 116–124.
[36] Rubino, A., Field, M. and Shwachman, H. (1971) J. Biol. Chem. 246, 3542–3548.
[37] Inui, K., Okano, T., Maegawa, H., Kato, M., Takano, M., and Hori, R. (1988) J. Pharmacol. Exp. Ther. 247, 235–241.
[38] Tsuji, A., Tamai, K., Hirooka, H. and Terasaki, T. (1987) Biochem. Pharmacol. 36, 565–567.
[39] Inui, K.-I. (1988) Yakugaku zasshi 108, 921–937.
[40] Bai, P.-F., Subramanian, P., Mosberg, H.I. and Amidon, G.L. (1991) Pharm. Res. 8, 593–599.
[41] Campbell, C.J., Chantrell, L.J. and Eastmond, R. (1987) Biochem. Pharmacol. 36, 2317–2324.