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Characteristics of Cefdinir Uptake by Rabbit Small Intestinal Brush-border Membrane Vesicles

SHUJI KITAGAWA, YOSHIO SUGAYA, YOSHIHIKO KASEDA AND SHINJI SATO

Niigata College of Pharmacy, Kamishin'ei-cho 5-13-2, Niigata 950-21, Japan

Abstract

Aminocephalosporins with peptide-like structures have been shown to be absorbed by the intestinal peptide carrier. We investigated the transport mechanism of cefdinir, an oral monocarboxylic acid cephalosporin, using rabbit small intestinal brush-border membrane vesicles.

Transport of cefdinir showed a slow and almost linear uptake rate for concentrations up to 30 mM with and without an inward H^+ gradient. No overshoot phenomenon was observed in the presence of an inward H^+ gradient. The uptake rate increased only slightly with decreasing extravesicular pH, and a protonophore had little effect on the uptake. Aminocephalosporins such as cephalexin only slightly inhibited cefdinir uptake even in the presence of an inward H^+ gradient, and vice-versa. Monocarboxylic acids such as acetic acid and salicylic acid had little effect on cefdinir uptake.

These findings suggest that in contrast with other oral cephalosporins cefdinir uptake through the brush-border membrane is slow and involves a mechanism similar to passive diffusion.

Oral cephalosporins such as cephradine and cephalexin are effectively absorbed from the small intestine despite being present as ions at physiological pH and having very low lipid solubility. Studies with brush-border membrane vesicles of the rabbit small intestine have revealed that aminocephalosporin antibiotics such as cephradine are actively absorbed via an H^+-coupled dipeptide transport system (Okano et al 1986; Inui et al 1988; Yuasa et al 1993; Tomita et al 1995). Cefdinir, a monocarboxylic acid cephalosporin (Fig. 1), is also effective orally. Its transport mechanism is, however, unknown. Because cefdinir has pK_a values of 1·9 (–COOH), 3·3 ($-NH_2$) and 9·9 (–OH), it is present as an anion at physiological pH. Although its uptake by dipeptide and monocarboxylic acid transporters has been proposed (Tsuji et al 1993), the details are still unknown. We have compared the characteristics of uptake of cefdinir and aminocephalosporins into intestinal brush-border membrane vesicles. Rabbits were chosen because their small intestine takes up aminocephalosporins such as cephradine and cephalexin via an H^+-coupled transport system (Okano et al 1986; Inui et al 1988; Yuasa et al 1993; Tomita et al 1995) and because absorption of cefdinir in rabbits is reportedly much better than in rats and mice (Sakamoto et al 1988).

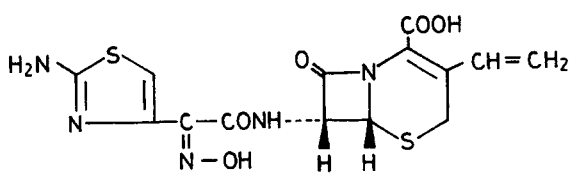

FIG. 1. The chemical structure of cefdinir.

Correspondence: S. Kitagawa, Niigata College of Pharmacy, Kamishin'ei-cho 5-13-2, Niigata 950-21, Japan.

Materials and Methods

Materials

Cefdinir (Fujisawa Pharmaceutical Co., Osaka, Japan) and cefroxadine (Ciba Geigy Japan Co., Tokyo, Japan) were kind donations. Carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone, glycylglycine, cephradine, cephalexin, cefadroxil and cefachlor were obtained from Sigma (St Louis, MO). All other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of brush-border membrane vesicles

Brush-border membrane vesicles were isolated from rabbit small intestine as described previously (Kitagawa et al 1995) by the $CaCl_2$ precipitation method of Kessler et al (1978) and suspended in buffer (10 mM Hepes-KOH pH 7·5, 100 mM mannitol and 100 mM KCl).

Measurement of cefdinir uptake

Cefdinir uptake was measured by the rapid filtration technique of Okano et al (1986). Membrane vesicles (10 μL) were incubated at 37°C, except where indicated otherwise, with nine volumes of the substrate mixture comprising 100 mM mannitol and 100 mM KCl buffered with either MES-KOH (pH 4.5-6.0) or Hepes-KOH (pH 6·5–7·5). Samples were taken and cefdinir trapped on the nitrocellulose membrane filter (0·45 μm, 2·5 cm diameter) was extracted with 300 μL of distilled water and the extract analysed by high performance liquid chromatography (HPLC). Deviation of data from different preparations of the vesicles was within 20·7% (n = 6).

Analytical method

The concentration of cefdinir was determined by HPLC (L-6000; Hitachi, Tokyo, Japan) with an L-4000 UV detector (Hitachi) at 290 nm. Separation was achieved on a reversed-

phase column (ODS, Shoudex C18-5A, 4·6 mm i.d. × 250 mm); the mobile phase was acetonitrile–50 mM citrate buffer (15:85, v/v), pH 3·0 at a flow rate of 0·6–0·7 mL min^{-1}. The aminocephalosporins were also separated using the same conditions, and their concentrations were determined at 262 nm (cephradine, cephalexin and cefaclor), 264 nm (cefadroxil) and 269·5 nm (cefroxadine). Protein was measured by the method of Lowry et al (1951) with bovine serum albumin as the standard.

Results

Dependence of cefdinir uptake on concentration

We examined cefdinir uptake by brush-border membrane vesicles at an intravesicular pH of 7·5, in incubation medium adjusted to pH 6·0. As shown in Fig. 2, in the presence of an inward H^+ gradient the initial uptake rate was almost linear with concentration up to 50 mM. Assuming that the transport process consists of a carrier-mediated process and a non-saturable process (possibly simple diffusion) non-linear least-squares regression analysis (Yamaoka et al 1981) was used to calculate the kinetic variables from the equation:

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

where V is the initial uptake rate, [S] is the initial concentration, V_{max} is the maximum uptake rate by saturable carrier-mediated process, K_m is the Michaelis constant and K_d is the permeability coefficient of non-saturable transport. According to the analysis, K_m and $V_{max} \pm$ s.d. were 0·22 ± 0·64 mM and 0·026 ± 0·020 nmol (mg protein)$^{-1}$ (15 s)$^{-1}$, respectively. K_d was 0·094 ± 0·004 nmol (mg protein)$^{-1}$ (15 s)$^{-1}$ mM^{-1}. The contribution of the saturable component was, therefore, very small. These findings differ from the reports of concentration-dependence of aminocephalosporins such as cephradine and cefroxadine which are transported mainly by a saturable component (Okano et al 1986; Kitagawa & Sugaya 1996).

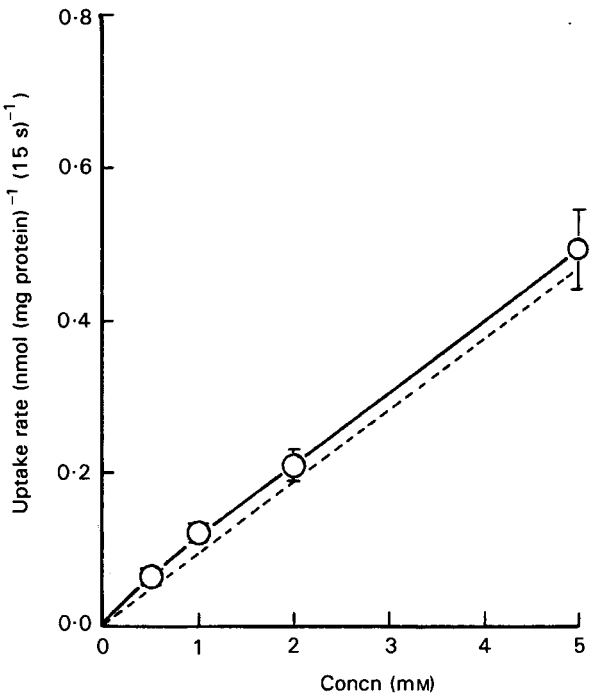

FIG. 2. The concentration-dependence of the rate of uptake of cefdinir by rabbit intestinal brush-border membrane vesicles at 37°C and extracellular pH 6·0. Membrane vesicles (10 μL) were incubated at 37°C for 15 s with nine volumes of substrate mixture comprising 100 mM mannitol and 100 mM KCl buffered with 10 mM MES-KOH (pH 6·0) or 10 mM Hepes-KOH. Cefdinir uptake was examined by a rapid filtration technique. Solid and dashed lines indicate the total uptake and the non-saturable component, respectively. Each value represents the mean ± s.d. of three determinations.

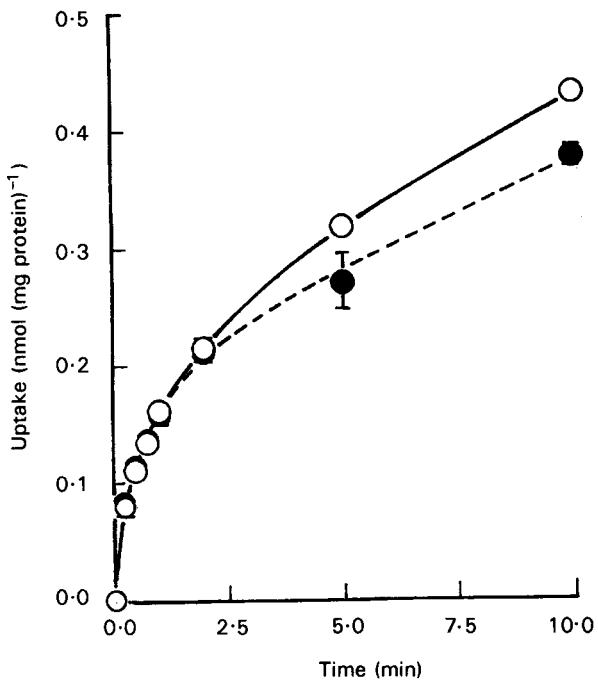

FIG. 3. Time-course of cefdinir uptake and effect of carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone at extravesicular pH 6·0. Membrane vesicles (10 μL) were incubated at 37°C with nine volumes of substrate mixture comprising 100 mM mannitol and 100 mM KCl buffered with 10 mM MES-KOH (pH 6·0) in the absence (○) or presence (●) of 36 M carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone. The final concentration of cefdinir was 0·5 mM. Cefdinir uptake was examined by a rapid filtration technique. Each value represents the mean ± s.d. of three determinations.

Temperature-dependence of cefdinir uptake in the presence of an H^+ gradient

We examined the time course of cefdinir uptake at a concentration of 0·5 mM at an extravesicular pH of 6·0. At 37°C cefdinir gradually reached the inside of the brush-border membrane vesicles (Fig. 3). In the presence of an inward H^+ gradient no overshoot phenomenon occurred even at 37°C. This is also in contrast with the behaviour of aminocephalosporins such as cephradine and cefroxadine (Okano et al 1986; Inui et al 1988; Kitagawa & Sugaya 1996). The protonophore carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone had little effect on cefdinir uptake. Between 15 and 37°C the uptake was slower with decreasing temperature. The Arrhenius plot of apparent cefdinir uptake rate constant k_p is shown in Fig. 4, assuming the pseudo-first-order transport equation because the initial uptake rate was almost proportional to the concentration of cefdinir (Fig. 2). The Arrhenius plot was linear between 15 and 37°C and activation energy was about

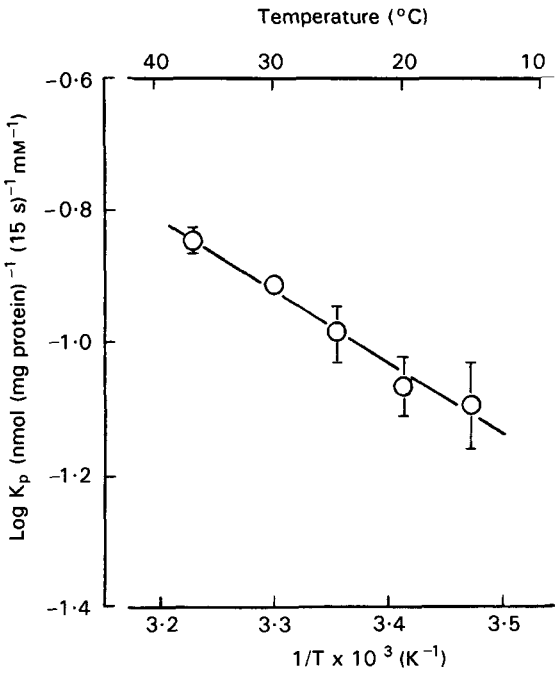

FIG. 4. Arrhenius plot of the apparent uptake rate constant, k_p, of cefdinir at extravesicular pH 6·0. Membrane vesicles (10 μL) were incubated at 15, 20, 25, 30 or 37°C for 15 s with nine volumes of substrate mixture comprising 100 mM mannitol and 100 mM KCl buffered with 10 mM MES-KOH (pH 6·0). The final concentration of cefdinir was 0·5 mM. Cefdinir uptake was examined by a rapid filtration technique and the apparent uptake rate constant was determined by assuming a pseudo-first-order transport equation. Each value represents the mean ± s.d. of three determinations.

22 kJ mol^{-1}. This value was much smaller than that for cefroxadine reported previously, which showed a break point at approximately 30°C and had an activation energy of 67 and 98 kJ mol^{-1} above and below the break point, respectively (Kitagawa & Sugaya 1996). The value was slightly smaller (31 kJ mol^{-1}) than the activation energy of cefixime transport at the physiological temperature, the Arrhenius plot of which also has a break point (22°C) (Tsuji et al 1987).

pH-Dependence of cefdinir uptake

Previous studies have shown that there is an optimum extravesicular pH of about 5·5–6·0 for uptake of aminocephalosporins such as cephradine and cefroxadine in brush-border membrane vesicles from rabbit small intestine (Inui et al 1988; Tomita et al 1990; Kitagawa & Sugaya 1996). The uptake rate of cefixime, dicarboxylic acid cephalosporin, also markedly depends on extravesicular pH with an apparent optimum pH at 4·5–5·0 (Inui et al 1988). We compared the pH-dependence of cefdinir with these other oral cephalosporins. As shown in Fig. 5, the uptake rate of cefdinir increased only slightly at a lower extravesicular pH, so that the pH-dependence differed from that of the other cephalosporins.

Effect of aminocephalosporins and monocarboxylic acids on cefdinir uptake

Aminocephalosporins at 20 mM and glycylglycine at 50 mM caused little or no inhibition of cefdinir uptake even in the presence of an inward H^+ gradient (Table 1). This is also in contrast with aminocephalosporins such as cephradine and cefroxadine (Inui et al 1988; Yuasa et al 1993; Kitagawa & Sugaya 1996). In the experiments of Tsuji et al (1993), cefdinir uptake was slightly inhibited by the monocarboxylic acids acetic acid and L-lactic acid, and the involvement of their transport system in cefdinir transport was suggested. We also investigated the effects of acetic acid and salicylic acid, therefore, but found they had little effect on cefdinir uptake (Table 1). This result suggests that the monocarboxylic acid transporter is probably not involved in cefdinir transport.

Effects of cefdinir on uptake of aminocephalosporins

As shown in Table 2, cefdinir only moderately (13·6–26.6%) inhibited the uptake of aminocephalosporins; this was true even at 20 mM, at which concentration aminocephalosporins inhibited the uptake of cefroxadine more markedly (79·2–88·2%; Kitagawa & Sugaya 1996). This tendency was consistent with the effects of cefdinir on the uptake of cephradine and cefixime reported by Tsuji et al (1993), although the inhibitory effect on cephradine uptake shown in Table 2 was slightly weaker.

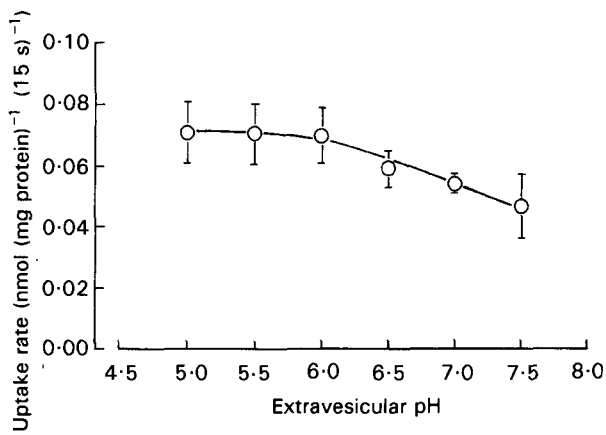

FIG. 5. Dependence of cefdinir uptake on extravesicular pH at 37°C. Membrane vesicles (10 μL) were incubated at 37°C for 15 s with nine volumes of substrate mixture comprising 100 mM mannitol and 100 mM KCl buffered with either 10 mM MES-KOH (pH 5·0–6·0) or 10 mM Hepes-KOH (pH 6·5–7·5). The uptake of cefdinir was examined by a rapid filtration technique. Each value represents the mean ± s.d. of three determinations.

Table 1. Effect of various compounds on uptake of cefdinir (0·5 mM) at 37°C and an extravesicular pH of 6·0.

Compound	Concn (mM)	Uptake (nmol (mg protein)$^{-1}$ (15 s)$^{-1}$)
None	–	0·0710 ± 0·0017
Glycylglycine	50	0·0704 ± 0·0062
Cephalexin	20	0·0592 ± 0·0043*
Cephradine	20	0·0631 ± 0·0050
Cefadroxil	20	0·0654 ± 0·0056
Salicylic acid	10	0·0699 ± 0·0114
Acetic acid	10	0·0649 ± 0·0063

Membrane vesicles (10 μL) were incubated at 37°C for 15 s with nine volumes of the substrate mixture at pH 6·0 in the presence or absence of test compounds. Cefdinir uptake was examined by a rapid filtration technique. Values represent means ± s.d. of three or four experiments. *$P < 0.05$, significantly different from the control value as assessed by Student's t-test for paired data.

Table 2. Effect of cefdinir on the uptake of 0·5 mM aminocephalosporins at 37°C and an extravesicular pH of 6·0.

Aminocephalosporin	Concn cefdinir (mM)	Uptake (nmol (mg protein)$^{-1}$ (15 s)$^{-1}$)
Cefroxadine	0	0·942 ± 0·007
	20	0·813 ± 0·016**
Cephalexin	0	1·018 ± 0·057
	20	0·747 ± 0·013*
Cefadroxil	0	1·075 ± 0·044
	20	0·884 ± 0·020*
Cefachlor	0	0·733 ± 0·062
	20	0·633 ± 0·069
Cephradine	0	0·957 ± 0·044
	20	0·797 ± 0·005*

Membrane vesicles (10 μL) were incubated at 37°C for 15 s with nine volumes of the substrate mixture at pH 6·0 in the presence or absence of 20 mM cefdinir. Uptake of aminocephalosporins was examined by a rapid filtration technique. The values represent means ± s.d. of three experiments. *$P < 0.01$, **$P < 0.001$, significantly different from the control value as assessed by Student's t-test for paired data.

Discussion

The absorption site of cefdinir in the gastrointestinal tract is mainly the middle intestine (Sakamoto et al 1988). Transfer through the epithelial membrane of the small intestine is probably via the transcellular pathway if the compound's transport mechanism is the same as those of the aminocephalosporins and with cefixime, dicarboxylic acid cephalosporin (Tsuji et al 1987; Inui et al 1988). The uptake rate of cefdinir in the rabbit brush-border membrane was, however, much lower than for other aminocephalosporins. For example, at an extravesicular concentration of 0·5 mM, the uptake rate of cefdinir was only about 10% of that of cefroxadine (Kitagawa & Sugaya 1996), although the difference was smaller at higher concentrations because of the non-saturable concentration-dependence of cefdinir uptake. The bioavailability of cefdinir in rabbit (32.3%) is much lower than those of the aminocephalosporins (Sakamoto et al 1988). Because cefdinir is barely metabolized after absorption and it is also stable in the stomach and small intestine (Sakamoto et al 1988), the relatively low bioavailability of this drug is possibly because it is transferred slowly through the intestinal epithelial-cell membranes. Low activation energy and the absence of saturation of concentration-dependence both contrast with the characteristics of aminocephalosporins such as cephradine, cefroxadine and dicarboxylic cefixime. This suggests that the uptake of cefdinir through the brush-border membrane is by passive diffusion. Sugawara et al (1990) indicated the involvement of simple diffusion in the transport of aminocephalosporins despite their presence as ions at physiological pH. Passive diffusion might also have an important role in the cefdinir transport.

Because cefdinir interacted weakly with aminocephalosporins such as cephradine, the dipeptide carrier transport system might also contribute slightly. Transport through the paracellular pathway, which has been found important for the uptake of ionic drugs (Barnett et al 1978), might also be involved in the uptake of cefdinir. The paracellular pathway participates in the intestinal transport of β-lactam antibiotics such as cephalexin and ampicillin (Yamashita et al 1984). Because transport of cefdinir through the brush-border membrane is slow, at least in rabbit intestine, contribution of the paracellular route might be more important for its intestinal uptake.

Acknowledgements

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