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Transport characteristics of ceftibuten, cefixime and cephalexin across human jejunal brush-border membrane

MITSURU SUGAWARA, KEN ISEKI, KATSUMI MIYAZAKI, HIROSHI SHIROTO*, YUKIFUMI KONDO*, JUN-ICHI UCHINO*, *Department of Pharmacy and *First Department of Surgery, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan*

Abstract—The transport characteristics of orally active cephalosporins, ceftibuten, cefixime and cephalexin have been examined using brush border membrane vesicles isolated from human jejunum. In the initial uptake of ceftibuten, the stimulation and overshoot phenomena were observed in the presence of an inward H^+ gradient. Effects of H^+ gradient on the uptake of cefixime and cephalexin were low and no overshoot was observed. These transport characteristics, especially uphill transport phenomena, were in agreement with previous results obtained from rat intestinal brush-border membrane vesicles and suggest that these β-lactam antibiotics are absorbed by different transport systems, despite their similar molecular structures.

There have been many studies of transport characteristics of orally active cephalosporins across rat or rabbit intestinal brush-border membrane vesicles (Iseki et al 1985, 1988, 1989; Kimura et al 1985; Okano et al 1986a, b; Tsuji et al 1987; Inui et al 1988; Muranushi et al 1989; Yoshikawa et al 1989; Sugawara et al 1990, 1991). However, the extent to which passive or carrier-mediated transport systems participate in the absorption process has not been established. Moreover, the detailed permeation characteristics of β-lactam antibiotics across human intestinal brush-border membrane are unclear. Recently, Lowther et al (1990) examined the uptake of several cephalosporins using human intestinal brush-border membrane vesicles and suggested that aminocephalosporins utilized, at least in part, the H^+ activated transport system used by dipeptides. However, these authors used high drug concentrations (10 mM). In this paper, we have investigated the absorption mechanisms of orally active cephalosporins by studying the time course of uptake and by comparing the effect of an inward H^+ gradient on the uptake of ceftibuten, cefixime and cephalexin using human jejunal brush-border membrane vesicles.

Materials and methods

Chemicals. Ceftibuten, [^{14}C]ceftibuten (sp. act. 1.1 G Bq mmol^{-1}) and cephalexin were kindly donated by Shionogi Co. (Osaka, Japan). Radioactive ceftibuten was used for the uptake experiments at low drug concentration (0·1 mM). Cefixime was a gift from Fujisawa Pharmaceutical Co. (Osaka, Japan). D-[^{14}C(U)]Glucose (sp. act. 0·5 G Bq mmol^{-1}) was purchased from NEN Research Products (Boston, MA, USA). All other chemicals were of the highest grade available. The structures of β-lactam antibiotics tested are shown in Fig. 1.

Preparation of human intestinal brush-border membrane vesicles for transport studies. With informed consent of the subjects, segments (1–2 cm long) of histologically normal jejunum were obtained from the removed tissue of diseased segments when the lesioned region was excised from patients undergoing routine surgery. Usually, these tissues are sent for pathological examination. The tissue was rinsed with 0·9% NaCl, and mucosa was removed gently with a surgical knife. The mucosa obtained was

Correspondence: K. Miyazaki, Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan.

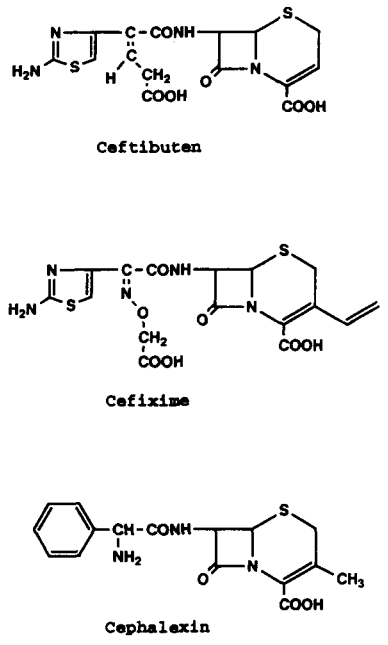

FIG. 1. Structures of β-lactam antibiotics tested.

wrapped in parafilm to exclude air and frozen at −70°C until use. Jejunal brush border membrane vesicles were isolated by $CaCl_2$ precipitation (Kessler et al 1978) as described previously for rat (Iseki et al 1989). Brush-border membranes were suspended in the buffer used for the transport studies. This experimental buffer contained (mM): mannitol 100, KCl 100 and either Hepes/Tris buffer (pH 7·5) 20, or Mes/Tris buffer (pH 5·5, pH 6·5) 20. The purity of the membrane was assessed by measuring the specific activity of alkaline phosphatase, which is increased 14·2(±4·9)-fold (mean±s.d., n=4) in the final membrane suspension compared with the concentration in the homogenate of intestinal scrapings. The enrichment factor of Na^+-K^+-ATPase, the marker enzyme for the basolateral membrane, was 0·4±0·6 (n=4). To examine the viability of isolated vesicles, the uphill transport of D-[^{14}C(U)]glucose (0·2 mM) was measured in the presence of a Na^+ or K^+ gradient. The initial rate of D-[^{14}C(U)]glucose uptake was markedly stimulated in the presence of an inward Na^+ gradient and offered an overshoot of 6·5 times the equilibrium value obtained at 10 min (Table 1).

Uptake experiments. The uptake of substrates was measured by a rapid filtration technique (Sugawara et al 1990). The reaction was initiated by the addition of 100 μL of a buffer containing the substrate to 20 μL of membrane vesicle suspension at 25°C. The cephalosporin trapped on the Millipore filter (HAWP, 0·45 μm, 25 mm) was extracted with 300 μL of distilled water and was measured by HPLC or scintillation counting. The composition of incubation media is given in the legends of figures.

Table 1. Uptake of D-[^{14}C(U)]glucose by human jejunal brush-border membrane vesicles.

Time (min)	Uptake (pmol (mg protein)$^{-1}$)	
	Na^+ (n = 11–12)	K^+ (n = 3)
0·5	387·3 ± 18·5	—
1	420·6 ± 28·2	36·0 ± 4·8
2	322·3 ± 33·6	—
10	64·5 ± 8·2	55·9 ± 27·8

Membrane vesicles were preincubated in 300 mM D-mannitol, 20 mM Hepes/Tris, pH 7·5 at 25°C for 2 min. The vesicles (20 μL) were incubated with 100 μL of 240 μM D-[^{14}C(U)]glucose, 60 mM D-mannitol, 20 mM Hepes/Tris, pH 7·5 buffer containing 120 mM NaCl or KCl at 25°C. Each value represents the mean ± s.e.m.

Analytical methods. Ceftibuten, cefixime and cephalexin were determined by HPLC (Hitachi L-6000, Hitachi Ltd, Tokyo, Japan) equipped with an L-4000 UV detector (Hitachi Ltd, Tokyo, Japan) at 262 nm for ceftibuten and cephalexin, or 280 nm for cefixime. For the determination of these compounds, the reversed phase column (ODS, Hitachi 3053, 5 μ, 4 mm i.d. × 250 mm) was used. The mobile phase was a mixture of methanol: 0·05 M citrate buffer (15:85, pH 2·5) for ceftibuten and cefixime, methanol: 0·1 M acetate (17:83, pH 6·0) for cephalexin at a flow rate of 0·7 mL min^{-1}. The limit of detection was 2 pmol for ceftibuten and cefixime, and 5 pmol for cephalexin. D-[^{14}C(U)]-Glucose and [^{14}C]ceftibuten were determined by liquid scintillation counting. Protein was measured by the method of Lowry et al (1951) with bovine serum albumin as the standard.

Results and discussion

Fig. 2 shows the time course of uptake of [^{14}C]ceftibuten by human jejunal brush-border membrane vesicles in the presence and absence of an inwardly directed H^+ gradient at the relatively low drug concentration of 0·1 mM. The initial rate of [^{14}C]ceftibuten uptake was markedly stimulated in the presence of an inward H^+ gradient, and a distinct overshoot phenomenon which peaked at 1 min was observed.

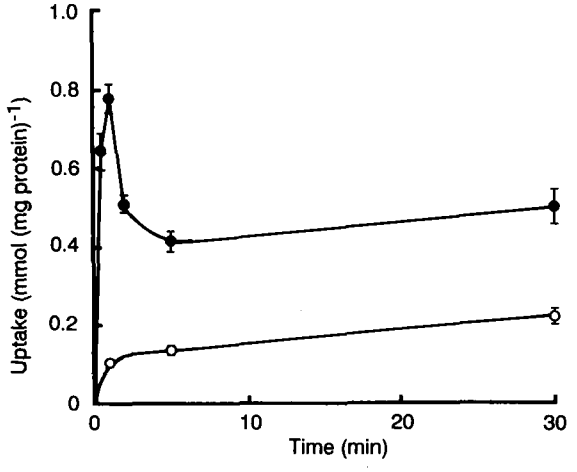

FIG. 2. Time course of uptake of [^{14}C]ceftibuten (0·1 mM) by human jejunal brush-border membrane vesicles. Membrane vesicles were preincubated in 100 mM D-mannitol, 100 mM KCl and 20 mM Hepes/Tris, pH 7·5 at 25°C for 2 min. The vesicles (20 μL) were incubated with 100 μL of 100 mM D-mannitol, 100 mM KCl, 120 μM ceftibuten and 20 mM Hepes/Tris, pH 7·5 (○) or Mes/Tris, pH 5·5 (●) at 25°C. Each point represents the mean ± s.e.m. of six measurements.

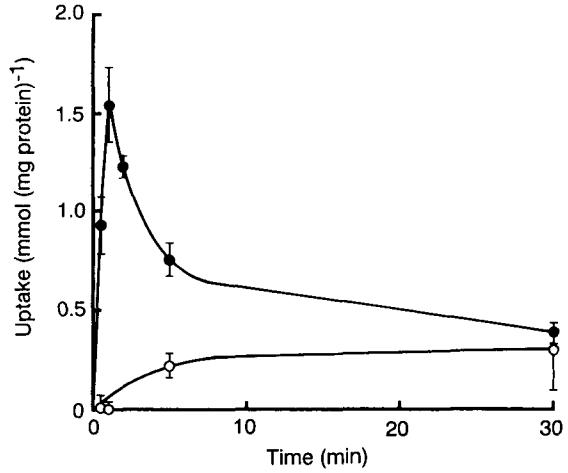

FIG. 3. Time course of uptake of ceftibuten (1 mM) by human jejunal brush-border membrane vesicles. Membrane vesicles were preincubated in 100 mM D-mannitol, 100 mM KCl and 20 mM Hepes/Tris, pH 7·5 at 25°C for 2 min. The vesicles (20 μL) were incubated with 100 μL of 100 mM D-mannitol, 100 mM KCl, 1·2 mM ceftibuten and 20 mM Hepes/Tris, pH 7·5 (○) or Mes/Tris, pH 5·5 (●) at 25°C. Each point represents the mean ± s.e.m. of three measurements.

As shown in Fig. 3, in the presence of an inward H^+ gradient, uphill transport was also observed in the ceftibuten uptake even at the higher drug concentration (1 mM). This overshoot phenomenon for ceftibuten was similar to our previous results obtained from rat small intestinal brush-border membrane vesicles (Sugawara et al 1991).

Yoshikawa et al (1989) reported that an inward H^+ gradient (pH 7·5 inside, pH 5·5 outside) stimulated the uptake of ceftibuten in rat small intestinal brush-border membrane vesicles. They also mentioned that ceftibuten is well absorbed in man, dog and rat and poorly absorbed in rabbit and monkey (Muranushi et al 1987). Our previous and present results for ceftibuten are consistent with those results (Yoshikawa et al 1989).

Fig. 4 shows the effect of an inward H^+ gradient on cefixime and cephalexin uptake by human jejunal brush-border membrane vesicles at drug concentrations of 1 mM. Even in the presence of an inward H^+ gradient, the initial uptake rates of these two drugs were significantly slower compared with that of ceftibuten and no overshoot was observed. These results for cefixime and cephalexin were in agreement with our previous results (Sugawara et al 1991) obtained from rat preparations except that the degree of stimulation by the H^+ gradient on the uptake in the rat brush-border membrane vesicles was relatively larger than that of man throughout the uptake periods.

Tsuji et al (1987) reported a carrier-mediated transport system for cefixime, cephalexin (Nakashima et al 1984) and cefadroxil (Nakashima & Tsuji 1985) in rats. Okano et al (1986b) found the overshoot phenomena in the rabbit small intestinal brush-border membrane vesicles. Muranushi et al (1989) indicated that the transport system of ceftibuten was different from that of aminocephalosporins (cephalexin and cefaclor) and that there was no overshoot in the cefaclor uptake even in the presence of an inward H^+ gradient in rat. On the other hand, in our previous studies using rat small intestinal brush-border membrane vesicles (Iseki et al 1985, 1988, 1989; Sugawara et al 1990, 1991), we observed that only ceftibuten and its analogues were concentrated and showed an overshoot in the presence of an inward H^+ gradient among those β-lactam antibiotics tested (ampicillin, amoxicillin, cephalexin, cephradine, cefadroxil, cefazolin, cefixime, ceftibuten and analogues of ceftibuten). Thus, there

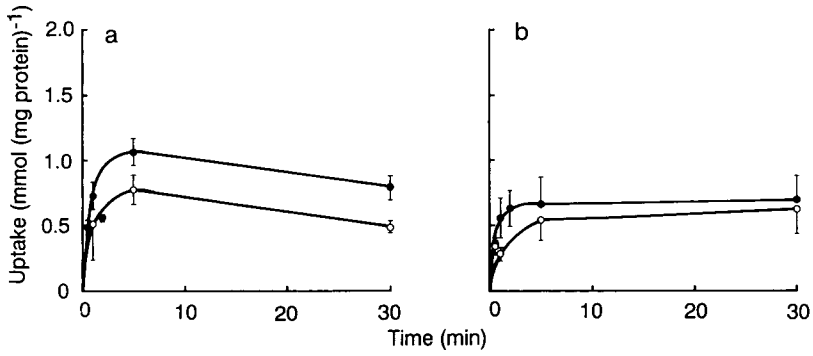

FIG. 4. Time course of uptake of cefixime (a) and cephalexin (b) by human jejunal brush-border membrane vesicles. Membrane vesicles were preincubated in 100 mM D-mannitol, 100 mM KCl and 20 mM Hepes/Tris, pH 7·5 at 25°C for 2 min. The vesicles (20 μL) were incubated with 100 μL of 100 mM D-mannitol, 100 mM KCl, 1·2 mM cephalosporin and 20 mM Hepes/Tris, pH 7·5 (○) or Mes/Tris (●) (pH 5·5 for cefixime, pH 6·5 for cephalexin) at 25°C. Each point represents the mean ± s.e.m. of three measurements.

remains uncertainty on the transport mechanisms of these cephalosporins. In the present study using human jejunal brush-border membrane vesicles, we observed that the transport characteristics of ceftibuten and the other cephalosporins (cefixime and cephalexin) were different, despite their similar molecular structure. Moreover, the present results suggest that brush-border membrane vesicle preparations from rat small intestine might be applied for the investigation of the transport characteristics of β-lactam antibiotics across human small intestinal brush-border membrane. It is unclear, however, whether the apparent H^+ stimulated uphill transport of ceftibuten is due to the utilization of a carrier-mediated transport system or the differences in physicochemical properties of ceftibuten compared with the other two cephalosporins.

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