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H^+ coupled transport of orally active cephalosporins lacking an α-amino group across brush-border membrane vesicles from rat small intestine

MITSURU SUGAWARA, KEN ISEKI, KATSUMI MIYAZAKI, *Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan*

Abstract—The effect of an inwardly directed H^+ gradient on the transport characteristics of ceftibuten, cefixime and analogues of ceftibuten in rat intestinal brush-border membrane vesicles have been investigated. In the presence of a transmembrane H^+ gradient, ceftibuten and its analogues exhibited a peak to equilibrium overshoot and an accumulation in the vesicles against the concentration gradient. However, the uptake of cefixime and S-1006 [(6*R*, 7*R*)-(7-[(Z)-2-(2-aminothiazol-4-yl)-2-pentenoylamino]-8-oxo-3-carbamoyloxy-methyl-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid), which lacks a carboxyl group at position 4 of carboxyethylidene structure, exhibited no overshoot, although the equilibrium uptake was increased by a H^+ gradient. The equilibrium uptake was dependent on the pH of the final incubation medium and the H^+ gradient. These data suggested that the orally active cephalosporins were transported into rat intestinal brush-border membrane by the transmembrane H^+ gradient and the pH of the medium.

There have been many studies of intestinal transport of β-lactam antibiotics (Okano et al 1986; Tsuji et al 1987a; Inui et al 1988; Iseki et al 1989; Sugawara et al 1990) because their uptake by the small intestine is dependent upon their structure. Recently, it was reported that ceftibuten (Yoshikawa et al 1989) and cefixime (Tsuji et al 1987b) were transported into the rat intestinal brush-border membrane by a proton gradient. Neither ceftibuten nor cefixime has an α-amino group at the 7 position of the cephalosporin skeleton. Tsuji et al (1987a, b) have reported that cefixime used the common carrier-mediated transport system of amino β-lactam antibiotics via the dipeptide carrier system. However, Muranushi et al (1989) indicated that the transport of ceftibuten was different from the transport system of cephalexin and cefaclor. Additionally, they reported that there was no overshoot phenomenon for cefaclor uptake in the presence of an H^+ gradient. In our previous studies, we reported that there was lower dependence on an inward H^+ gradient transport system for the absorption of amino β-lactam antibiotics than that of glycylglycine from the rat small intestine (Iseki et al 1989; Sugawara et al 1990). Subsequently, we found that the uptake of cefixime by rat intestinal brush border membrane vesicles did not show any overshoot phenomenon under an inward H^+ gradient. Therefore, to clarify the extent to which the H^+ gradient-dependent transport system participates in the absorption process of a series of β-lactam antibiotics, we have compared the transport characteristics of cefixime, ceftibuten and the analogues of ceftibuten.

Materials and methods

Chemicals. Ceftibuten, compound V [(6*R*,7*R*)-7-[(Z)-2-(2-aminothiazol-4-yl)-4-carboxy-2-butenoylamino]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid], compound C [(6*R*,7*R*)-7-[(Z)-2-(2-aminothiazol-4-yl)-4-carboxy-2-butenoylamino]-8-oxo-3-carbamoxymethyl-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid], S-1006 [(6*R*,7*R*)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-pentenoylamino]-8-oxo-3-carbamoyloxymethyl-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid], were kindly donated by Shionogi Co. (Osaka, Japan). Cefixime was a gift from Fujisawa Pharmaceutical Co. Ltd, Osaka. All other chemicals were of the highest grade available. The structures of these compounds are shown in Fig. 1.

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.

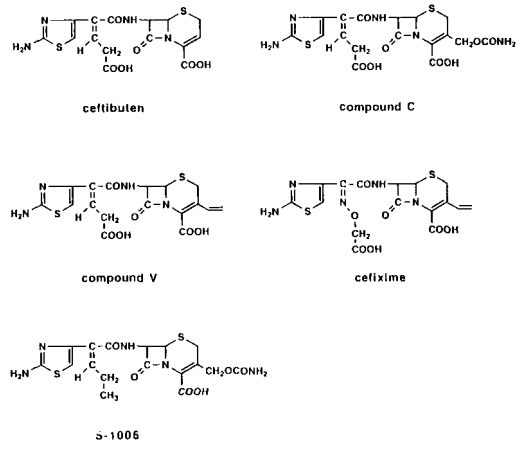

FIG. 1. Structures of β-lactam antibiotics and their analogues.

Prepararation of brush-border membrane vesicles and uptake experiments. Adult male Wistar rats, 180–230 g, were used. The entire small intestine was excised under ether anaesthesia and brush-border membrane vesicles isolated according to Kessler et al (1978) as described by Iseki et al (1989). The uptake of substrates (1·0 mM) by the freshly isolated membrane vesicles was measured at 25°C by a rapid filtration technique using a Millipore filter (HAWP, 0·45 μm, 2·5 cm diameter) (Iseki et al 1989). In a separate experiment, non-specific adsorption onto a Millipore filter was determined using the test buffer in place of the brush border membrane suspension. This value was subtracted from the uptake data.

All other procedures were as described previously (Sugawara et al 1990).

Analytical method. The concentrations of ceftibuten, cefixime, compound V, compound C and S-1006 were determined by HPLC (Hitachi L-6000) equipped with an L-4000 UV detector (Hitachi Ltd, Tokyo, Japan) with detection at 262 nm for ceftibuten, compound C and S-1006, or 280 nm for cefixime and compound V. Separation was achieved on a reversed phase column (ODS, Hitachi 3053, 5 μm, 4 mm i.d. × 250 mm) using a mobile phase consisting of methanol:0·05 M citrate buffer, pH 2·5 (1:9; ceftibuten, 15:85; compound V and S-1006, 7:93; compound C) at a flow rate of 0·7 mL min^{-1}. For cefixime, methanol: 0·1 M acetate (13:87, pH 6·0) was used as a mobile phase at a flow rate of 0·6 mL min^{-1}. The limit of detection was 2 pmol for ceftibuten, cefixime, compound C and V, and 4 pmol for S-1006. Protein concentrations were determined by the method of Lowry et al (1951) with bovine serum albumin as standard.

Results and discussion

The uptake of cefixime, S-1006, ceftibuten, compound V and compound C (each 1 mM) by intestinal brush-border membrane

vesicles, measured in the presence or absence of an inward H^+ gradient is shown in Fig. 2. The initial uptake of ceftibuten, compound V and compound C exhibited an overshoot phenomenon and accumulation against the concentration gradient in the presence of an H^+ gradient.

Yoshikawa et al (1989) found that ceftibuten uptake was stimulated by an inward H^+ gradient (pH 5·5 outside/pH 7·5 inside), which was confirmed in these studies for ceftibuten and its analogues which have a carboxyethylidene group at the 7 position of the cephalosporin nucleus (Fig. 2A). On the other hand, although cefixime uptake equilibrium was increased in the presence of an inward H^+ gradient, there was no overshoot of its uptake by brush border membrane vesicles (Fig. 2B) despite its similarity in structure to ceftibuten and compound V. For S-1006, similarly, no overshoot phenomenon was observed in the presence of an H^+ gradient, and H^+-stimulated uptake was relatively small compared with cefixime. This suggests that a carboxyethylidene group at the 7 position might be related to the transport of these compounds against the H^+ gradient into the intestinal brush border membrane.

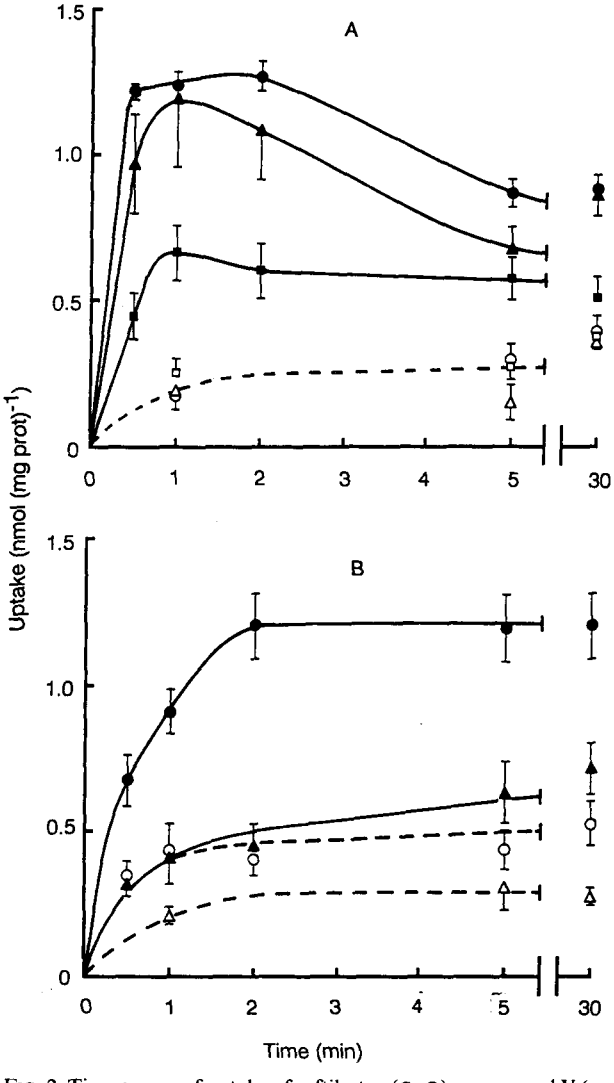

FIG. 2. Time course of uptake of ceftibuten (●, ○), compound V (▲, △), compound C (■, □) (A), and cefixime (●, ○) S-1006 (▲, △) (B) by rat intestinal brush border membrane vesicles in the presence (closed symbol) or absence (open symbol) of an H^+ gradient. Membrane vesicles were preincubated in 100 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 20 mM Hepes/Tris buffer, pH 7·5 or 20 mM Mes/Tris buffer, pH 5·5 (for ceftibuten, compound V, compound C and S-1006), 20 mM citrate/Tris buffer, pH 5·0 (for cefixime), each containing 100 mM D-mannitol, 100 mM KCl and 1·2 mM cephalosporin at 25°C. Each point represents the mean ± s.e.m. of six measurements with different preparations of vesicles.

We examined the effect of pH on the uptake of ceftibuten because there was a marked difference between the equilibrium uptake in the presence and absence of an H^+ gradient in the present study. As shown in Fig. 3, the equilibrium value depended upon the pH of the final incubation medium; at the lower pH (pH 5·5, closed symbol in Fig. 3), the uptake at equilibrium was approximately twice that at pH 7·5 (open symbol in Fig. 3). This pH-dependence was also observed in the uptake of cefixime, S-1006, compound V and compound C. We have already reported (Iseki et al 1989; Sugawara et al 1990) that similar equilibrium uptake was observed regardless of medium pH for amino β-lactam antibiotics such as cephalexin, cephradine and ampicillin, and that there was a good correlation of uptake by the membrane vesicles and the concentration of the substrate in the medium. Consequently, it is reasonable to suggest that the intravesicular space, calculated to be 0·8–1·0 μL (mg protein)$^{-1}$ on the basis of the equilibrium uptake of D-glucose (Kessler et al 1978) and cephradine, cephalexin and ampicillin (Iseki et al 1989; Sugawara et al 1990), can accomodate about 0·8–1·0 nmol (mg protein)$^{-1}$ at equilibrium in the presence of 1 mM of the substrate. However, only low uptake of those cephalosporins which lack the α-amino group was found following 30 min incubation, medium pH 7·5. (ceftibuten; 393·5 ± 51·5, compound V; 358·8 ± 27·1, compound C; 274·3 ± 22·8, cefixime; 527·5 ± 75·5, S-1006; 279·1 ± 32·2 pmol (mg protein)$^{-1}$, mean ± s.e.m., n = 5–6).

There may be a species difference in the transport of peptide-like molecules in an inwardly directed H^+ gradient as shown by the results of Ganapathy & Leibach (1983) in the rabbit and Rajendran et al (1987) in the mouse. Wilson et al (1989) reported that the uptake of the tripeptide, Gly-Gly-L-Pro, in human jejunal brush-border membrane vesicles was not increased by a Na^+ or H^+ gradient.

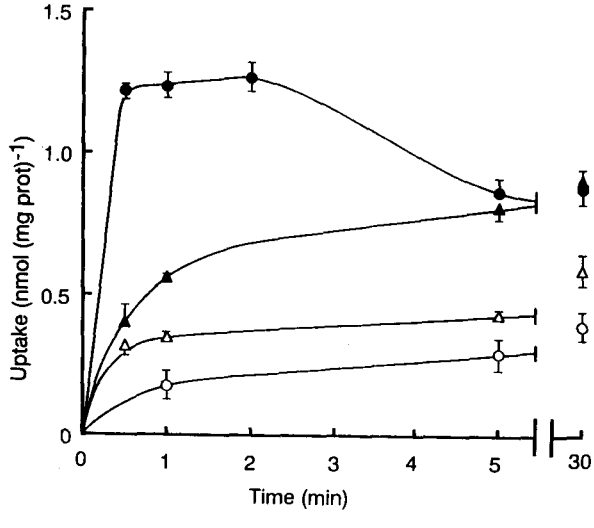

FIG. 3. Effect of pH on ceftibuten uptake by rat intestinal brush border membrane vesicles. Membrane vesicles were preincubated in 20 mM Hepes/Tris buffer, pH 7·5 (●, ○) or 20 mM Mes/Tris buffer, pH 5·5 (▲, △), containing 100 mM D-mannitol, 100 mM KCl at 25°C for 2 min. The vesicles (20 μL) were incubated with 100 μL of 20 mM Hepes/Tris buffer, pH 7·5 (○, △) or 20 mM Mes/Tris buffer, pH 5·5 (●, ▲), containing 100 mM D-mannitol, 100 mM KCl and 1·2 mM ceftibuten at 25°C. Each point represents the mean ± s.e.m. of six measurements with different preparations of vesicles.

On the other hand, Tsuji et al (1987b) reported an overshoot phenomenon in the uptake of cefixime by rat intestinal brush-border membrane vesicles in the presence of an H^+ gradient and although it was also reported that ceftibuten uptake was remarkably stimulated by an inward H^+ gradient in the rat intestinal brush-border membrane (Yoshikawa et al 1989), there was a discordance in the optimal effect of extravesicular pH between these two reports. Although some investigators (Tsuji et al 1987a, b; Inui et al 1988; Kramer et al 1988) reported that uptake of orally active cephalosporins at 1–2 mM was inhibited by the presence of dipeptides using intestinal brush-border membrane vesicles, the specific transport mechanism of many nutrients, (i.e. permeation characteristics driven by ion gradient or H^+ gradient) has been revealed at lower substrate concentrations (50–100 μM). In a previous study (Iseki et al 1989), we reported that there was no overshoot of cephradine and ampicillin uptake even at 50 μM by rat intestinal brush-border membrane in the presence of an inward directed H^+ gradient despite the finding that glycylglycine uptake was significantly stimulated by an H^+ gradient under the same conditions. Recently Muranushi et al (1989) reported that no overshoot phenomenon was observed for the uptake of cefaclor even in the presence of an H^+ gradient, and that H^+-stimulated uptake was extremely small compared with that for ceftibuten.

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