The Inhibitory Effects of Cephalosporin and Dipeptide on Ceftibuten Uptake by Human and Rat Intestinal Brush-border Membrane Vesicles

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Abstract—The types of inhibitory effects caused by compound V (an analogue of ceftibuten) and alanylproline (dipeptide) on the uptake of ceftibuten by brush-border membrane vesicles (BBMV) prepared from human and rat small intestine were analysed. In the presence of an inward H^+-gradient, the initial uptake rate of ceftibuten by both human and rat intestinal BBMV was concentration-dependent with apparent K_m and V_{max} values of 0·35 mM and 2·052 nmol (mg protein)$^{-1}$ min^{-1} for human BBMV, and 0·50 mM and 3·056 nmol (mg protein)$^{-1}$ min^{-1} for rat BBMV, respectively. For both human and rat BBMV, kinetic analysis by Dixon and Lineweaver–Burk plots demonstrated that the uptake of ceftibuten was competitively inhibited by compound V, whereas inhibition by alanylproline was noncompetitive or partially competitive. These results suggest that there is a stereospecific transport system which is common to ceftibuten and compound V, and that this system is not identical to the carrier system for the dipeptide, alanylproline.

There have been some reports with regard to the transport mechanism of ceftibuten, an orally active di-anionic cephalosporin antibiotic, across the intestinal brush-border membrane (Muranushi et al 1989; Yoshikawa et al 1989; Sugawara et al 1991a, b, 1992). Yoshikawa et al (1989) reported that the transport of ceftibuten is proton-coupled and stereoselective (*cis*-isomer specific). It was also pointed out that this route is a H^+-gradient-dependent oligopeptide transport system (Muranushi et al 1989). On the other hand, the same authors reported that the inhibitory effects of dipeptides on the H^+-gradient-dependent ceftibuten uptake differ from each dipeptide in both human and rat intestinal brush-border membrane vesicles, i.e. L-alanyl-L-alanine and L-phenylalanylglycine inhibited the process strongly, but glycylsarcosine and L-carnosine did not affect the uptake of ceftibuten in any way (Sugawara et al 1992). Moreover, there have been few studies concerning the analysis of the types of inhibition on ceftibuten transport by dipeptides or other cephalosporins, including analogues of ceftibuten. Studies on human intestinal brush-border membrane vesicles are valuable and important for evaluation of absorption mechanisms of these drugs. The main objective of this study was to compare the transport system of ceftibuten with that of dipeptides by determining the types of inhibition caused by compound V and L-alanyl-L-proline, which revealed the strongest inhibitory effect among tested dipeptides (data not shown), on the uptake of ceftibuten using human and rat intestinal brush-border membrane vesicles. The structures of ceftibuten, compound V and alanylproline are shown in Fig. 1.
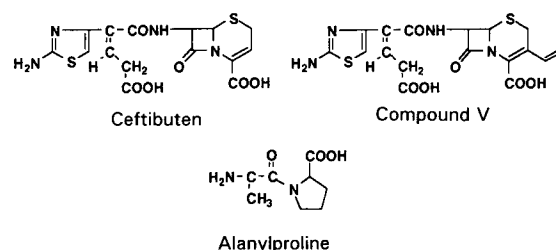
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Fig. 1. Structures of ceftibuten, compound V and alanylproline.

Materials and Methods

Chemicals
Ceftibuten, [^{14}C]ceftibuten (1·12 GBq mmol^{-1}) and compound V were kindly donated by Shionogi Co. (Osaka, Japan). L-Alanyl-L-proline was purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of the highest grade available.

Preparation of intestinal brush-border membrane vesicles
Brush-border membrane vesicles for the transport studies were isolated from human jejunum and rat whole intestine by CaCl$_2$ precipitation (Kessler et al 1978) as described previously (Iseki et al 1989; Sugawara et al 1991b). Brush-border membrane vesicles were suspended in a 20 mM HEPES/Tris, pH 7·5 buffer containing 100 mM D-mannitol and 100 mM KCl.

Uptake experiments
The uptake of ceftibuten was measured by a rapid filtration technique (Sugawara et al 1990). The reaction was initiated by the addition of 100 μL buffer (100 mM D-mannitol, 100 mM KCl, 20 mM Mes/Tris, pH 5·5) containing 61 KBq mL^{-1} [^{14}C]ceftibuten and various concentrations of non-labelled ceftibuten and inhibitor to 20 μL membrane vesicles
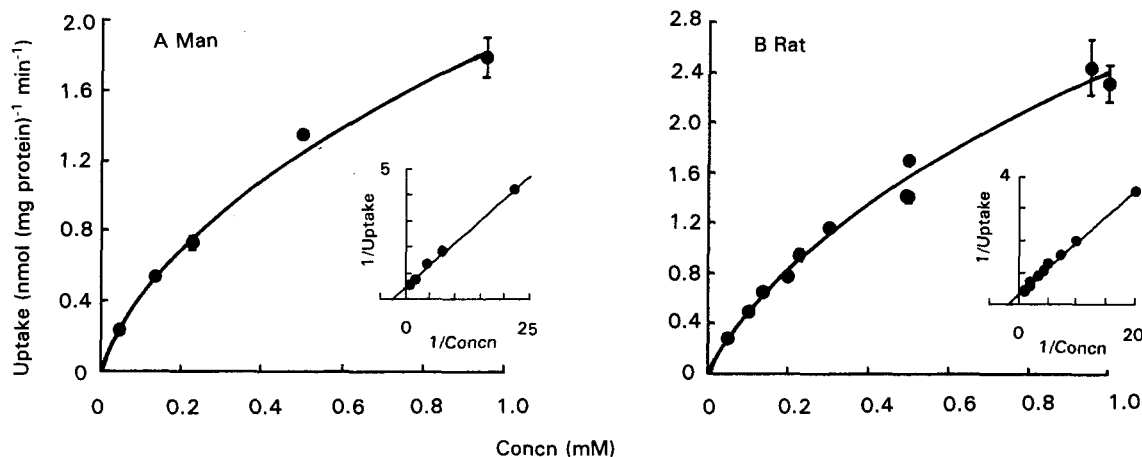
FIG. 2. Concentration-dependency of ceftibuten uptake by human (A) and rat (B) intestinal brush-border membrane vesicles. Each point represents the mean ± s.e.m. of three measurements. (Inset: Lineweaver-Burk plot of ceftibuten uptake.)

(10–15 mg protein mL^{-1}) at 25°C. After 30 s, the reaction was stopped by diluting the reaction mixture with 5 mL ice-cold buffer (150 mM NaCl, 20 mM Mes/Tris, pH 5·5). The contents of the tube were immediately filtered through a Millipore filter (HAWP, 0·45 μm, 2·5 cm diam.) which was washed once with 8 mL ice-cold buffer. Ceftibuten trapped on the filter was extracted with 300 μL distilled water.

Analytical methods

The concentration of [^{14}C]ceftibuten was determined by liquid scintillation counting. Protein was measured by the method of Lowry et al (1951) with bovine serum albumin as the standard.

Results

Kinetics of the uptake of ceftibuten by human and rat intestinal brush-border membrane vesicles

The concentration dependency of ceftibuten uptake in the

presence of an inward H$^+$-gradient was examined. The relationship between the uptake and the concentration of ceftibuten is shown in Fig. 2. The uptake rate of ceftibuten was concentration-dependent in both human and rat intestinal brush-border membrane vesicles. Apparent K$_m$ and V$_{max}$ values calculated from the Lineweaver-Burk plot (Fig. 2 inset) were 0·35 mM and 2·052 nmol (mg protein)$^{-1}$ min^{-1} for man, and 0·50 mM and 3·056 nmol (mg protein)$^{-1}$ min^{-1} for rat.

Kinetic analysis of the inhibitory effect of compound V on the uptake of ceftibuten

To clarify the type of inhibitory effect of compound V on the uptake of ceftibuten by human and rat intestinal brush-border membrane vesicles, the uptake of ceftibuten in the presence of various concentrations of compound V were studied. Fig. 3 shows the result using human intestinal brush-border membrane vesicles. As shown in Fig. 3A, Dixon plot analysis demonstrated that compound V inhib-
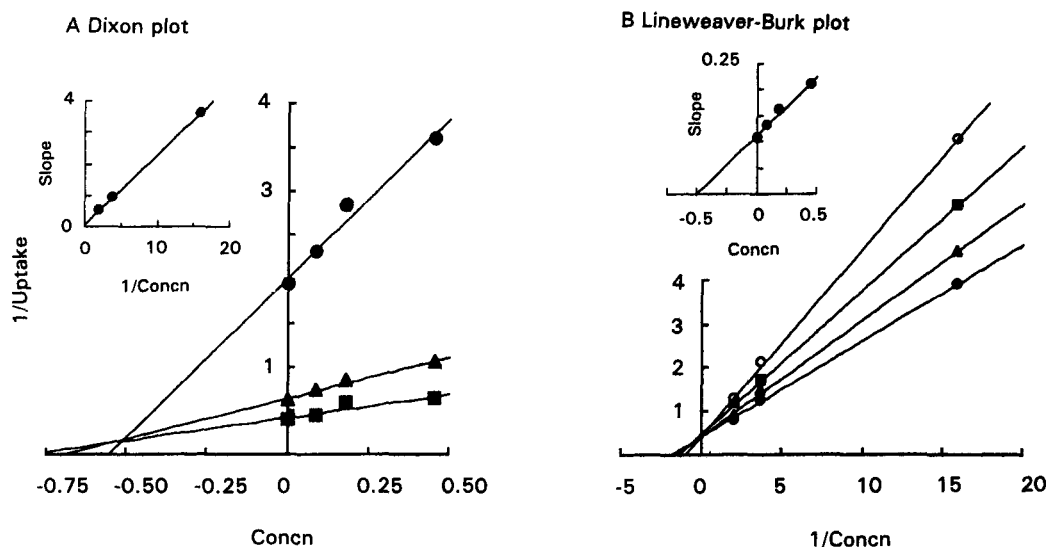

FIG. 3. Analysis of type of inhibition by compound V on ceftibuten uptake by human intestinal brush-border membrane vesicles. Each point represents the mean of 2–3 measurements. A. Dixon plot; final concentrations of ceftibuten were 0·063 (●), 0·273 (▲) or 0·500 mM (■). (Inset: replot of slopes of Dixon plot.) B. Lineweaver-Burk plot; final concentrations of alanylproline were 0 (●), 0·091 (▲), 0·182 (■) or 0·455 mM (○). (Inset: replot of slopes of Lineweaver-Burk plot.) Concentrations are mM and uptake values are nmol (mg protein)$^{-1}$ min^{-1}.
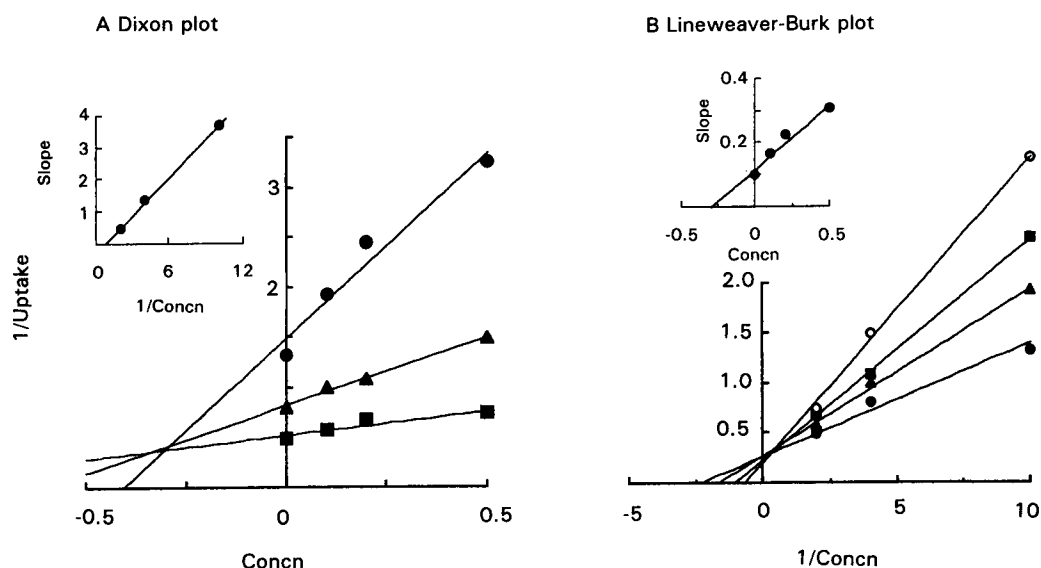
A Dixon plot

B Lineweaver-Burk plot

FIG. 4. Analysis of inhibition by compound V on ceftibuten uptake by rat intestinal brush-border membrane vesicles. Each point represents the mean of 2–3 measurements. A. Dixon plot; final concentrations of ceftibuten were 0·1 (●), 0·25 (▲) or 0·5mM (■). (Inset: replot of slopes of Dixon plot.) B. Lineweaver–Burk plot; final concentrations of alanylproline were 0 (●), 0·1 (▲), 0·2 (■) or 0·5mM (○). (Inset: replot of slopes of Lineweaver–Burk plot.) Concentrations are mM and uptake values are nmol (mg protein)$^{-1}$ min^{-1}.

ited the uptake of ceftibuten competitively or partially competitively. The regression line obtained from the replot of the slope of the Dixon plot almost coincided with the origin (Fig. 3A inset). Moreover, the lines of the Lineweaver–Burk plot intersect nearly at the same point on the vertical axis (Fig. 3B). These results suggest that the inhibition by compound V on the uptake of ceftibuten by human intestinal brush-border membrane vesicles is competitive in manner. Additionally, the apparent K_i value calculated from the replot of the slope of the Lineweaver–Burk plot (Fig. 3B inset) was 0·51 mM. The same analysis was applied to the

uptake by rat intestinal brush-border membrane vesicles (Fig. 4). As for human brush-border membrane vesicles, the inhibition by compound V on ceftibuten uptake was competitive and the apparent K_i was 0·30 mM.

Kinetic analysis of the inhibitory effects of alanylproline on the uptake of ceftibuten

Figs 5 and 6 show the relationship between the concentration of alanylproline in the medium and the uptake of ceftibuten by human (Fig. 5) and rat (Fig. 6) intestinal brush-border membrane vesicles. The intersection of the three lines on the

A Dixon plot
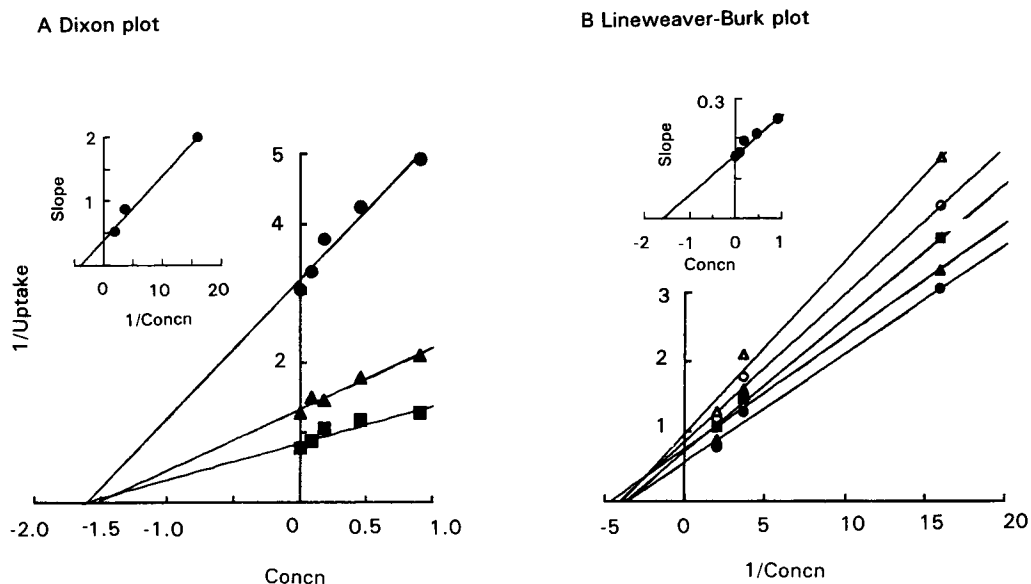
B Lineweaver-Burk plot

FIG. 5. Analysis of inhibition by alanylproline on ceftibuten uptake by human intestinal brush-border membrane vesicles. Each point represents the mean of six measurements. A. Dixon plot; final concentrations of ceftibuten were 0·063 (●), 0·273 (▲) or 0·500mM (■). (Inset: replot of slopes of Dixon plot.) B. Lineweaver–Burk plot; final concentrations of alanylproline were 0 (●), 0·091 (▲), 0·182 (■), 0·455 (○) or 0·909 mM (△). (Inset: replot of slopes of Lineweaver–Burk plot.) Concentrations are mM and uptake values are nmol (mg protein)$^{-1}$ min^{-1}.
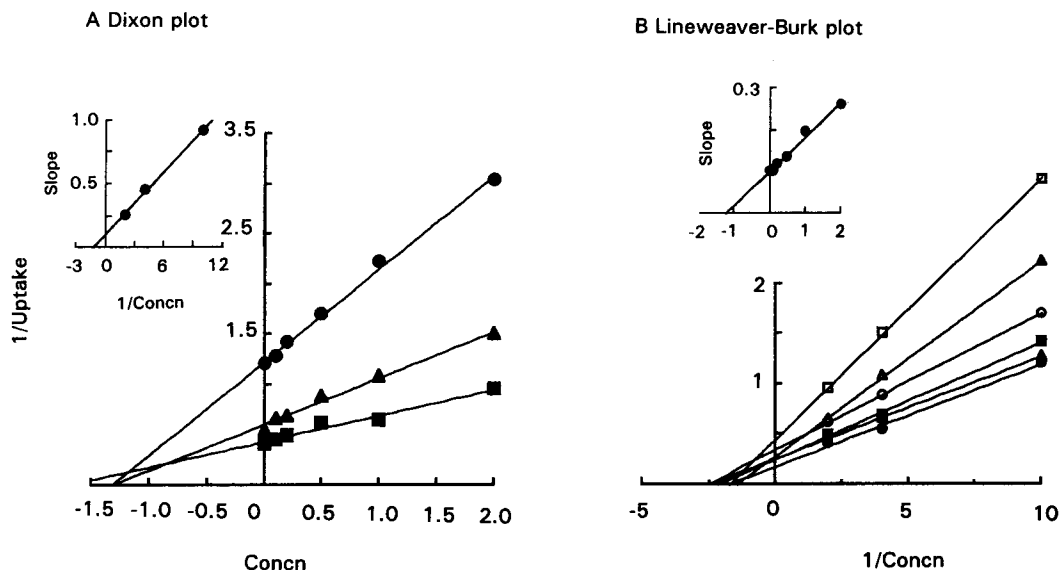
FIG. 6. Analysis of inhibition by alanylproline on ceftibuten uptake by rat intestinal brush-border membrane vesicles. Each point represents the mean of 2–3 measurements. A. Dixon plot; final concentrations of ceftibuten were 0·1 (●), 0·25 (▲) or 0·5 mM (■). (Inset: replot of slopes of Dixon plot.) B. Lineweaver–Burk plot; final concentrations of alanylproline were 0 (●), 0·1 (▲), 0·2 (■), 0·5 (○), 1 (△) or 2 mM (□). (Inset: replot of slopes of Lineweaver–Burk plot.) Concentrations are mM and uptake values are nmol $(mg\, protein)^{-1}\, min^{-1}$.

Dixon plot is located on the horizontal axis of the fourth quadrant, suggesting the inhibition is either noncompetitive, competitive or partially competitive (Figs 5A, 6A). Analysis of the Lineweaver–Burk plot (Figs 5B, 6B) and the replot of the slope of the Dixon plot (Figs 5A inset, 6A inset) demonstrated that alanylproline noncompetitively or partially competitively inhibited ceftibuten uptake, i.e. the lines of the Lineweaver–Burk plot did not intersect on the vertical axis and the regression curve obtained from the replot of the slope of the Dixon plot did not coincide with the origin. Moreover, K_m values of ceftibuten uptake obtained by extrapolation of this regression curve to the horizontal axis were 0·24 mM for man (Fig. 5A inset) and 0·76 mM for rat (Fig. 6A inset), respectively. These values were almost equal to the values of concentration dependency (Fig. 2). The K_i values calculated by the replot of the slope of the Lineweaver–Burk plot were 1·62 mM for man and 1·21 mM for rat (Figs 5B inset, 6B inset).

Discussion

There have been many reports concerning the transport mechanisms of zwitterionic (cephalexin, cephradine etc.) and di-anionic (cefixime, ceftibuten) orally active cephalosporin antibiotics (Okano et al 1986a, b; Tsuji et al 1987; Inui et al 1988; Iseki et al 1989; Muranushi et al 1989; Yoshikawa et al 1989; Sugawara et al 1990, 1991a, b, 1992; Wang et al 1992). Although there are structural differences, some of these reports suggested that these cephalosporins are transported via H^+-gradient-dependent dipeptide-carrier systems in the small intestine. However, studies concerning the oligopeptide transport in intestinal brush-border membrane are scarce compared with renal brush-border membrane studies (Miyamoto et al 1985, 1986; Takuwa et al 1985; Tiruppathi et al 1990, 1991; Daniel et al 1992).

H^+-Gradient-dependencies of the uptake of di- and tripeptides by intestinal brush-border membrane vesicles are different according to these reports. Some reports suggested that the uptake of dipeptides by small intestinal brush-border membrane vesicles was H^+-gradient-dependent (Ganapathy & Leibach 1983; Ganapathy et al 1984; Said et al 1988). In these reports, however, in contrast to the case of renal brush-border membrane vesicles, there was no overshoot phenomenon, with the exception of the study of Said et al (1988) who used suckling rat. On the contrary, Rajendran et al (1987) and Wilson et al (1989) reported that the transport of di- and tripeptides in the small intestinal brush-border membrane was not generated by an H^+-gradient.

It has already been suggested that ceftibuten uptake by human, rat and rabbit intestinal brush-border membrane vesicles results in an overshoot phenomenon in the presence of an inward H^+-gradient (Yoshikawa et al 1989; Sugawara et al 1991a, b, 1992). However, considering the conflicting evidence on the intestinal oligopeptide transport system mentioned above, further investigation is required to establish if ceftibuten is transported via the oligopeptide transport system. In this paper, accordingly, the inhibitory effects of compound V and alanylproline, which revealed the strongest inhibitory effect among tested dipeptides, on the uptake of ceftibuten were analysed. Previously, we observed that compound V was accumulated by rat intestinal brush-border membrane vesicles against the concentration gradient in the presence of an inward H^+-gradient and that the uptake of ceftibuten was inhibited by compound V (Sugawara et al 1991a, 1992). As shown in Fig. 2, ceftibuten uptake by human and rat intestinal brush-border membrane vesicles was concentration-dependent with apparent K_m values of 0·35 and 0·50 mM, respectively. The type of inhibitory effect of compound V on ceftibuten transport was competitive in both human and rat intestinal brush-

border membrane and the apparent K_i values were 0·51 and 0·30 mM, respectively (Figs 3, 4). Furthermore, the *trans*-stimulation effect of compound V was observed on the uptake of ceftibuten by human and rat intestinal brush-border membrane vesicles (not shown). Our previous and present results suggest that ceftibuten and compound V are transported through the common transport system, and its affinity is almost the same for human and rat intestinal brush-border membrane vesicles. In contrast with compound V, alanylproline-inhibited ceftibuten uptake is either non-competitive or partially competitive in manner with apparent K_i values of 1·62 and 1·21 mM for man and rat, respectively (Figs 5, 6). These data suggest that the ceftibuten and compound V transport system have a different structural specificity and an affinity with that of alanylproline.

In conclusion, we suggest that ceftibuten and compound V are transported through a common transport system which has at least a different structural specificity and an affinity with that of alanylproline (a neutral dipeptide).

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