Inhibitory Effects of Angiotensin-Converting Enzyme Inhibitors on Cefroxadine Uptake by Rabbit Small Intestinal Brush Border Membrane Vesicles

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Effects of angiotensin-converting enzyme (ACE) inhibitors, captopril, enalapril maleate and quinapril, on the uptake of aminoccephalosporin antibiotic, cefroxadine, by rabbit small intestinal brush border membrane vesicles were examined. These ACE inhibitors significantly inhibited the uptake of cefroxadine, which is transported by H^+ /dipeptide transporter in the membrane, in the order of captopril < enalapril < quinapril in the presence of an inward H^+ gradient. Inhibitory effect of quinapril was more marked than that of aminoccephalosporin cephradine, while in the absence of an inward H^+ gradient inhibition by these ACE inhibitors was much less. Dixon plot analysis showed that the inhibition by enalapril and quinapril in the presence of an inward H^+ gradient occurred in a competitive manner and estimated inhibition constants of these two drugs to be 5.3 mM and 0.46 mM, respectively. These results suggested the strong affinity of these ACE inhibitors, especially quinapril, on the H^+ /dipeptide transporter.

Key words ACE inhibitor; quinapril; enalapril; cefroxadine; small intestine; brush border membrane vesicle

It has been suggested that H^+ -coupled dipeptide carrier plays an important role in the oral absorption of peptide-like drugs such as β -lactam antibiotics like cephradine, cefixime and cefroxadine.^{1–4)} A complementary DNA clone encoding small intestinal H^+ /dipeptide transporter (Pep T1) has also been isolated and its involvement in the transport of the oral β -lactam antibiotics determined.^{5,6)} Like these antibiotics, the angiotensin-converting enzyme (ACE) inhibitors enalapril maleate (hereafter referred to as enalapril), captopril, lisinopril and quinapril have also been suggested to be transported at least partly by the dipeptide carrier, but details are still unclear.^{7–10)}

In this work we tried to clarify the affinities of ACE inhibitors, captopril, enalapril and quinapril, on the H^+ /dipeptide transporter by observing the inhibition of uptake of cefroxadine by small intestinal brush border membrane. We examined them for rabbit intestine because the major contribution of the dipeptide transport system to the uptake of aminoccephalosporins has been reported in rabbit,³⁾ and we earlier confirmed the major contribution of H^+ /dipeptide transporter to the uptake of cefroxadine in rabbit small intestinal brush border membrane.⁴⁾

MATERIALS AND METHODS

Chemicals Quinapril hydrochloride (Yoshitomi Pharmaceutical Industries Co., Osaka, Japan) and cefroxadine (Ciba Geigy Japan Co., Tokyo, Japan) were generously donated. Captopril, enalapril maleate and cephradine were obtained from Sigma (St. Louis, MO). All other reagents were purchased from Wako Pure Chemical Industries (Osaka).

Preparation of Brush Border Membrane Vesicles Brush border membrane vesicles were isolated from the rabbit small intestine as described previously⁴⁾ by the $CaCl_2$ precipitation of Kessler *et al.*¹¹⁾ The membrane vesicles

obtained were suspended in experimental buffer (100 mM mannitol, 100 mM KCl and 10 mM HEPES-KOH (pH 7.5)).

Measurement of Uptake of Cefroxadine The uptake of cefroxadine was measured by a rapid filtration technique as described.⁴⁾ Membrane vesicles (10 μ l) were incubated at 37 °C for 15 s with nine-fold volume of the substrate and inhibitor mixture comprising 100 mM mannitol and 100 mM KCl buffered with either MES-KOH (2-(*N*-morpholino)ethanesulfonic acid) (pH 6.0) or HEPES-KOH (pH 7.5). From aliquots of the samples drawn, cefroxadine trapped on a nitrocellulose membrane filter was extracted with 300 μ l of distilled water and was used for the determination by HPLC. Nonspecific absorption of cefroxadine to the membrane vesicles was estimated by incubating the vesicles with ice-cold substrate mixture. Deviation of data from preparation to preparation of the vesicles was within 25%.

Analytical Method The concentration of cefroxadine was determined by HPLC (L-6000, Hitachi Ltd., Tokyo) equipped with an L-4000 UV detector (Hitachi, Ltd.) at 269.5 nm. Separation was achieved on a reversed phase column (ODS, Shodex C18-5A, 4.6 mm i.d., 250 mm) using a mobile phase consisting of acetonitrile and 50 mM citrate buffer, pH 3.0 (15:85, v/v) at a flow rate of 0.6–0.7 ml/min). Protein was measured by the method of Lowry *et al.*¹²⁾ with bovine albumin as the standard.

RESULTS AND DISCUSSION

We examined the effects of three ACE inhibitors on the initial uptake of cefroxadine by brush border membrane vesicles, at intravesicular pH 7.5, in incubation medium adjusted to pH 6.0 or 7.5. The effects of 10 mM ACE inhibitors shown in Table 1 indicated that these inhibitors significantly inhibited the uptake of cefroxadine at extravesicular pH 6.0, at which an inward H^+ gradient was present, and H^+ -coupled rapid uptake of cefroxadine

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Table 1. Effects of 10 mM ACE Inhibitors and Cephadrine on Initial Uptake Rate of Cefroxadine (0.5 mM) at 37 °C at Extravesicular pH 6.0

Compound	Uptake rate (nmol/mg protein/15 s) ^{a)}
None	0.731 ± 0.058 (5)
Captopril	0.355 ± 0.023 (3) ^{b)}
Enalapril	0.226 ± 0.005 (3) ^{b)}
Quinapril	0.059 ± 0.002 (3) ^{b)}
Cephadrine	0.235 ± 0.012 (3) ^{b)}

a) Data represent means ± S.D. Numbers in parentheses indicate number of replicate experiments. b) $p < 0.001$.

Table 2. Effects of 10 mM ACE Inhibitors and Cephadrine on Initial Uptake Rate of Cefroxadine (0.5 mM) at 37 °C at Extravesicular pH 7.5

Compound	Uptake rate (nmol/mg protein/15 s) ^{a)}
None	0.156 ± 0.010 (5)
Captopril	0.141 ± 0.024 (3)
Enalapril	0.140 ± 0.016 (3)
Quinapril	0.096 ± 0.006 (3) ^{b)}
Cephadrine	0.125 ± 0.033 (3)

a) Data represent means ± S.D. Numbers in parentheses indicate number of replicate experiments. b) $p < 0.001$.

into brush border membrane vesicles was induced during the initial 30 s.⁴⁾ In this condition about 90% of the uptake was estimated to be due to specific carrier transport.⁴⁾ Inhibitory effects of these ACE inhibitors were in the order of captopril < enalapril < quinapril. Inhibition by quinapril was much more marked than that by the aminoccephalosporin cephadrine, whose inhibitory effect is also shown in Table 1 for comparison. As shown in Table 2, however, the inhibition was much less at extravesicular pH 7.5 at which the H^+ gradient was absent, although the inhibitory effect of quinapril was still marked compared with those of the other ACE inhibitors and cephadrine. These results suggest the high affinity of these ACE inhibitors, especially quinapril, on the H^+ /dipeptide transporter in the brush border membrane by which uptake of cefroxadine was conducted in the presence of an inward H^+ gradient.⁴⁾

We next examined the characteristics of inhibition of enalapril and quinapril, which showed stronger inhibition than captopril, on the uptake of cefroxadine by kinetic analysis in the presence of an inward H^+ gradient. The results shown in Figs. 1 and 2 suggest that inhibition by both enalapril and quinapril occurred in a competitive or a largely competitive manner. Estimated inhibition constants, K_i , were 5.3 and 0.46 mM, respectively. K_i value of quinapril obtained here in rabbit intestinal brush border membrane was similar to that of bestatin (0.47 mM) on the uptake of cephadrine,¹³⁾ whose K_m and V_{max} values resemble with those of cefroxadine.^{1,4)} These results suggested the strong affinity of these ACE inhibitors, especially quinapril, to the H^+ /dipeptide transporter. Relatively high hydrophobicity of quinapril may affect the affinity to the transporter, because it has larger n -octanol/water partition coefficient (26.7 at pH 4.3) than the other ACE inhibitors tested, cephadrine and cefroxadine. However, the result on enalapril was different from the previous finding by Yuasa *et al.*, which showed the non-

Fig. 1. Dixon Plot of the Initial Uptake Rate of Cefroxadine at Extravesicular pH 6.0 in the Presence of Various Concentrations of Enalapril

Concentrations of cefroxadine (mM) are: ○, 1; ●, 2; △, 4. Each value represents the mean ± S.D. of three experiments.

Fig. 2. Dixon Plot of the Initial Uptake Rate of Cefroxadine at Extravesicular pH 6.0 in the Presence of Various Concentrations of Quinapril

Concentrations of cefroxadine (mM) are: ○, 1; ●, 2; △, 4. Each value represents the mean ± S.D. of three experiments.

competitive inhibition of cephadrine uptake by this drug in rabbit intestinal brush border membrane.¹⁴⁾ The K_i value of enalapril was also much larger than the K_m value (0.07 mM) of enalapril absorption in rat intestine evaluated by a single-pass perfusion method, although species were different.⁷⁾ Therefore, its interaction with the H^+ /dipeptide transporter may be complicated.

Marked inhibition of H^+ -coupled uptake of cefroxadine by the ACE inhibitors tested, especially quinapril, suggests the contribution of H^+ /dipeptide transporter to their uptake in small intestinal epithelial cells. In line with this speculation, the possibility was recently suggested that the transport of quinapril is conducted *via* a combination of the carrier-mediated H^+ gradient-dependent peptide transporter and passive diffusion in Caco-2 cells.⁹⁾ Enalapril and captopril have also been suggested to share the H^+ -coupled mechanism involved in dipeptide transport.¹⁵⁾ As mentioned above, passive diffusion may also be important especially for the uptake of quinapril, because

the drug has a much larger *n*-octanol/water partition coefficient than the other ACE inhibitors and aminoccephalosporins. Another possibility also cannot be excluded: that these ACE inhibitors inhibited the cefroxadine uptake by modification of the binding of the drug to the H⁺/dipeptide transporter without being transported by that transporter.

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