Uptake of Enalapril by Rabbit Small Intestinal Brush-Border Membrane Vesicles

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Uptake of the angiotensin-converting enzyme (ACE) inhibitor enalapril by rabbit small intestinal brush border membrane vesicles was examined. In the presence of an inward H^+ gradient the uptake of this peptide mimetic drug was accelerated and an overshoot phenomenon was observed. The uptake was more stimulated by higher H^+ gradient. Initial uptake rate was saturable in the presence of an inward H^+ gradient, with apparent K_m value of 4.2 mM. These findings suggest the involvement of the H^+ -coupled carrier-mediated transport in the uptake of enalapril. The uptake was inhibited by ACE inhibitortrandolapril but hardly or only slightly inhibited by aminocephalosporins.

Key words ACE inhibitor; enalapril; small intestine; brush-border membrane vesicle; intestinal absorption

H^+ -coupled oligopeptide carrier plays an important role in the oral absorption of peptide-like drugs such as the β -lactam antibiotics cephadrine and cefixime.¹⁻³⁾ cDNA encoding peptide transporter (PepT1) has been cloned for human and rabbit,^{4,5)} and its involvement in the transport of the oral β -lactam antibiotics has been identified.^{6,7)} Like β -lactam antibiotics, angiotensin-converting enzyme (ACE) inhibitors such as enalapril maleate (hereafter referred to as enalapril), which are also peptide-mimetic drugs, have been suggested to be transported at least partly by the H^+ /oligopeptide carrier following experimental findings using perfusion,^{8,9)} Ussing chamber,¹⁰⁾ and everted intestinal rings.¹¹⁾ The concentration dependent permeability and the decrease by a dipeptide and cephadrine in a perfusion experiment has been reported.⁹⁾ A non-linear relationship between concentration and the transport was also observed by a Ussing chamber experiment.¹⁰⁾ Furthermore, H^+ -linked transport has been suggested in Caco-2 cells with observation of lowered intracellular pH when perfused across the apical surface of cell monolayers.¹²⁾

We recently reported the inhibition by ACE inhibitors enalapril, quinapril, benazepril, temocapril andtrandolapril on the uptake of an aminocephalosporin antibiotic, cefroxadine, by rabbit small intestinal brush-border membrane vesicles, and confirmed the affinity of these drugs to the H^+ /oligopeptide transporter.^{13,14)} Among these ACE inhibitors, enalapril is a relatively hydrophilic drug which is not likely to be beneficially absorbed through the lipid bilayer of intestinal epithelial membrane. Therefore, transport by carrier protein is probably involved in its absorption process. However, the transport of enalapril by a non-saturable, passive diffusion process has also been suggested by studies using everted rat intestinal rings and Caco-2 cells.¹⁵⁾

Therefore, to examine the involvement of the H^+ /oligopeptide carrier in the uptake process of enalapril, we investigated the characteristics of the uptake using brush-border membrane vesicles, a method appropriate to determine the transport mechanism of relatively hydrophilic drugs such as enalapril. We used rabbit vesicles because the major contribution of the dipeptide transporter to the uptake of aminocephalosporins has been reported in rabbit,³⁾ and because we have identified the nearly competitive inhibitory ef-

fect of enalapril on the uptake of the aminocephalosporin antibiotic, cefroxadine by rabbit vesicles.^{13,14)}

MATERIALS AND METHODS

Materials Enalapril maleate, cephadrine and cephalixin were obtained from Sigma (St. Louis, MO, U.S.A.). Trandolapril (Hoechst Marion Roussel, Cedex, France) was generously donated. All other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of Brush-Border Membrane Vesicle Brush-border membrane vesicles were isolated from rabbit small intestine as described previously^{13,14)} by the $CaCl_2$ precipitation method of Kessler *et al.*¹⁶⁾ and suspended in medium A (10 mM 4-(2-(hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-KOH (pH 7.5)), 100 mM KCl and 100 mM mannitol).

Measurement of Enalapril Uptake Enalapril uptake was measured by the rapid filtration technique of Okano *et al.*¹⁾ Membrane vesicles (10 μ l) were incubated at 37 °C with a nine-fold volume of medium B (100 mM KCl and 100 mM mannitol buffered with either 10 mM MES (2-(*N*-morpholino)ethanesulfonic acid)-KOH (pH 5.5—6.0) or 10 mM HEPES-KOH (pH 6.5—7.5)). From aliquots of the samples drawn, enalapril trapped on a nitrocellulose membrane filter was extracted with 300 μ l of distilled water and was used for determination by HPLC. Nonspecific absorption of enalapril to the membrane vesicles was estimated by incubating the vesicles with an ice-cold substrate mixture. Deviation of data at each extravesicular pH was within 21%.

Analytical Method The amount of enalapril absorbed by the brush-border membrane vesicles was determined by HPLC (L-6000, Hitachi, Ltd., Tokyo) using an L-4000 UV detector (Hitachi, Ltd.) after solid phase extraction of enalapril following Hammes *et al.*¹⁷⁾ with some modification. Separation was achieved on a reversed phase column (Mightysil, RP-18, 4.6 mm i.d., 250 mm) using a mobile phase consisting of methanol, water and phosphoric acid (50 : 50 : 0.05, v/v) at a flow rate of 1.0 ml/min. Absorbance was detected at 220 nm using temocapril as an internal standard. Protein was measured by the method of Lowry *et al.*¹⁸⁾ with bovine albumin as the standard.

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RESULTS AND DISCUSSION

We examined the enalapril uptake by rabbit small intestinal brush-border membrane vesicles in the presence or absence of an inward H^+ gradient. As shown in Fig. 1, in the presence of the gradient, the uptake occurred quickly, reached the maximum level in 1 min, and a slight overshoot phenomenon was observed. In contrast, permeation occurred slowly in the absence of H^+ gradient and reached the equilibrium concentration in 5 min. These findings are consistent with those on aminocephalosporins such as cephradine and dianionic cephalosporins such as cefixime.^{1,2)} The uptake was accelerated by higher pH gradient as shown in Fig. 2.

We next examined the concentration-dependence of the initial uptake rate of enalapril in the presence of an inward H^+ gradient. As shown in Fig. 3, the uptake rate was saturable. The concentration dependence of enalapril uptake rate was analyzed by assuming that the transport process

Fig. 1. Time-Course of Uptake of Enalapril (0.5 mM) at Extravesicular pH 6.0 and 7.5

○—○, at extravesicular pH 6.0; ●—●, at extravesicular pH 7.5. Intravesicular pH was 7.5. Data at pH 6.0 are means $\pm$ S.D. of 3 or 4 experiments and data at pH 7.5 are those of 5 to 7 experiments.

Fig. 2. Dependence of Uptake Rate of Enalapril (0.5 mM) on Extravesicular pH

Intravesicular pH was 7.5. Data are means $\pm$ S.D. of 4 (pH 5.5), 5 (pH 6.0), 6 (pH 6.5 and 7.5) or 8 (pH 7.0) experiments. Statistical significance of difference from the value at extravesicular pH 7.5 was assessed by Student's *t*-test after confirming the presence of significant difference among data at different extravesicular pH by analysis of difference: *p < 0.001.

consists of a carrier mediated process and simple diffusion using nonlinear least-squares regression analysis.¹⁹⁾ Kinetic parameters were calculated from the following equation:

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

where V is the initial uptake rate, $[S]$ is the extravesicular concentration, $V_{\max}$ is the maximum uptake rate by carrier-mediated process, K_m is the Michaelis constant, and K_d is the coefficient of simple diffusion. According to the analysis, K_m and $V_{\max}$ were 4.16 mM and 6.22 nmol/mg protein/30 s, respectively. K_d was 1.01 μ l/mg protein/30 s. The Michaelis constant resembles those of aminocephalosporins.²⁰⁾ Furthermore, it was similar to the inhibition constant, K_i , on cefroxadine uptake by rabbit small intestinal brush-border membrane vesicles (4.2 ± 1.6 mM),¹⁴⁾ suggesting the possibility

Fig. 3. Concentration Dependence of Enalapril Uptake Rate at Extravesicular pH 6.0

Solid lines indicate the total uptake (○) and carrier-mediated uptake (●), and broken line indicates the nonsaturable component. The inset shows a Lineweaver-Burk plot of enalapril uptake after correction for the nonsaturable component. V and $[S]$ represent the corrected uptake rate and extravesicular concentration of enalapril, respectively. Data for total uptake represent means $\pm$ S.D. of 3 (3 mM) or 4 experiments (other concentrations).

Fig. 4. Effects of Cephradine, Cephalixin and Trandolapril on Uptake of Enalapril (0.5 mM) at Extravesicular pH 6.0

a, control; b, with 10 mM cephradine; c, with 10 mM cephalixin; d, with 2 mM trandolapril. Data are means $\pm$ S.D. of 7 experiments. Statistical significance of differences from control value was assessed by Student's *t*-test after confirming the presence of significant difference among data at 4 different conditions by analysis of difference: *p < 0.01, **p < 0.001.

that enalapril is carried by the transporter which carries cefroxadine.

We also examined the effects of two aminocephalosporins (cephradine and cephalixin) and the ACE inhibitor trandolapril on the uptake of enalapril. As shown in Fig. 4, the uptake was inhibited by trandolapril. However, 20-fold concentration (10 mM) of cephalixin only slightly inhibited the uptake and that of cephradine had no effect. This is in contrast to the previous findings that uptake of cephalosporins such as cefroxadine was markedly inhibited by high concentrations of cephradine and cephalixin.²¹⁾

These findings suggest that H⁺-coupled transport at least partly contributes to the uptake of enalapril in small intestinal brush-border membrane. However, the involvement of the H⁺/oligopeptide transporter is still not clear because of no or only slight inhibition by aminocephalosporins. The kinetic analysis revealed above suggested that passive diffusion is also important for the uptake of enalapril in spite of its relatively hydrophilic nature. Furthermore, the possible involvement of some other transporter than H⁺/oligopeptide transporter in the uptake process of enalapril cannot be excluded.

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