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Effects of long-term Teatment of Captopril and Enalapril on Rat Intestinal Angiotenisin Converting Enzyme Specific Activities

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Objectives: *Angiotensin converting enzyme(ACE)has been shown to be an important peptidasse that play a role in digestion and assimilation of protein rich in proline such as casein, gliadin and collagen. Despite that ACE inhibitors have been popular for various types of hypertension and congestive heart failure, the effects of their long-term treatment on intestinal ACE activities are not known. Therefore, we measured intestinal specific activities in rats after four weeks' treatment of ACE inhibitors.*

Methods: *Thirty Wistar rats weighing about 200g in average were divided into three groups, and supplied with tap water, captopril solution and enalapril solution respectively for four weeks. After sacrificing, intestinal ACE specific activities were measured in homogenate and brush border membrane fraction respectively, which was prepared from three equally divided segments of removed small intestine.*

Results: *ACE specific activities of proximal, middle and distal segments of control group were 178.6±64.2, 180.3±60.2 and 48.6±13.1 in brush border membrane(mean±SD, nmol /min/mg protein) respectively. Those of captopril group were 314.2±72.5, 281.0±69.8 and 67.7±21.8 respectively, showing tendency of increase in proximal and middle segments ($p<0.01$ and 0.05 respectively). By contrast, those of enalapril group were 48.5±27.6, 70.7±15.6 and 11.6±4.4 respectively, which were significantly lower($p<0.01$) than those of control group.*

Conclusion: *Rat intestinal ACE specific activities were not inhibited by captopril treatment, but inhibited by enalapril treatment. This finding may explain why there has not been any case report of malabsorption in patients taking captopril. But the malabsorption of prolyl peptide could be possible in cases with long-term administration of enalapril*

Key Words : *Angiotensin converting enzyme(ACE), Brush border membrane, Captopril, Enalapril*

INTRODUCTION

Angiotensin converting enzyme(ACE) is a dipeptidylcarboxypeptidase which catalyzes cleaving dipeptides from C-terminal of various oligopeptides[1,2]. In vascular endothelium of various organs including lung, it catalyzes conversion of decapeptide angiotensin I to octapeptide angiotensin II and inactivation of bradykinin resulting in elevation of blood pressure[3,4]. ACE also exists in various organs such as small intestine, kidney, central nervous system and thyroid[5]. Although it has been suggested that it induces thirst or stimulates the secretion of ADH in central nervous system[6] and regulates the glomerular filtration or sodium, water absorption in renal tubular epithelium[7], its exact roles in small intes-

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tinal epithelium are not known. It has been reported that it participates in sodium and water absorption in small intestine via metabolism of kinin and angiotensin I[8,9] and that it also plays some role in digestion of proline-rich proteins[10-13]. Many kinds of ACE specific inhibitors are now used widely for the treatment of various types of hypertension and congestive heart failure[14,15]. Although it has been reported that intestinal ACE was inhibited site-specifically by various ACE inhibitors directly in vitro[16,17], it is not clear whether ACE specific activities are also inhibited by long-term ingestion of ACE inihbitors. The aim of this study is to observe the effects of long-term treatment of ACE inihbitors, captopril and enalapril on rat intestinal ACE specific activities.

MATERIALS AND METHODS

1. Subjects and Drug Administration

Thirty adult wistar rats, weighing 200g in average were divided into three treatment groups. Rats in the control group were supplied with tap water, captopril group with captopril-containing water(about 25mg/kg/day) and enalapril group with enalapril-containing water (about 12.5mg/kg/day). All the rats were fed with standard rat chows ad libitum for four weeks. Bovine serum albumin, Hippuryl-histidyl-leucine and o-phthalialdehyde were purchased from Sigma(St. Louis, USA) and p-nitrophenyl phosphate from Merk(New Jersey, USA). Captopril and enalapril were kindly provided by Squibb(Seoul, Korea) and Choong-oi Pharmaceuticals(Seoul, Korea) respectively.

2. Preparation of Tissue

After four weeks and midnight fasting, all the rats were sacrificed and their small intestines were removed, kept on ice and perfused with cold saline. Removed small intestine was divided equally into three segments. Mucosa was obtained from each segment with slide scraping method and immediately frozen to -20℃.

3. Preparation of Tissue Homogenate

Thirty ml of buffer solution (a mixture of 2 mM Tris chloride and 50 mM mannitol(pH 7.0) in 1 : 1 volume ratio) was added to each gram of stored mucosa which was homogenized with Waring blender for one minute. After adding 0.4M $CaCl_2$ to final concentration of 10 mM, the tissue solution was stirred to dissolve and let stand on ice for 20 minutes.

4. Preparation of Brush Border Membrane(BBM)

BBM fraction was extracted from tissue homogenate by minor modification of Kessler's methods[15]. Briefly, after centrifuging the previously prepared homogenate solution with 3,000 ×g in 4℃ for 15minutes, supernatant was taken and centrifuged again with 27,000×g for 30minutes. After discarding the supernatant, same volume of buffer solution as in preparation of homogenate was added to remnant pellet and homogenized 20 strokes with Potter-Elvehzen homogenizer. After adding 0.4M $MgCl_2$ to final concentration of 50 mM, it was centrifuged with 4,000×g in 4℃ for 15 minutes and supernatant was taken, centrifuged again with 27,000×g for 30 minutes. After discarding the supernatant, 5ml of phosphate buffered saline(pH 7.4) was added to remnant pellet and mixed well with glass rod and stored in -20℃.

5. Measurement of Protein Levels and Enzyme Specific Activities

Protein concentration was measured by Lowry method[16]. Alkaline phosphatase specific activity was estimated by Fujita's method[17] using 10mM p-nitrophenyl phosphate as substrate. ACE specific activity was measured by minor modification of Cushamn's method[18] using Hippuryl-Histidyl-Leucine as substrate. Buffer/substrate solution(pH 8.5) containing 0.0625 M HEPES and 0.375M NaCl. Twenty five μl of distilled water were added to 200μl of previously prepared buffer/substrate solution, mixed well and incubated in 37℃ water bath for 30minutes. After 30 minutes, 1.5ml of 0.3M NaOH and 100μl of o-phthalialdehyde solution(0.2% o-phthalaldehyde 2.5mg per 1 ml of methanol) were added to reaction mixture and incubated for 10minutes. After adding 200μl of 0.3M HCl to reaction mixture, it was incubated for 30 minutes until stabilized. Its relative fluorescence was measured using scanning spectrofluorometer (Farrand optical sys-3) with excitation at 365λ and emission at 500λ. Ten and twenty nmol Histidyl-Leucine was used as standard and the linearity of relative fluorescence between them was confirmed. Protein concentration was measured in $\mu g/10\mu l$ solution and enzyme specific activities were expressed in terms of substrate digested in 1 minute per 1 mg protein. ACE specific activity was calculated as follows.

F1/F2 × 1/30(min) × 10 × 1/2.5 × 1/protein(μg/10μl) × 1,000 × (dilution factor)(nmol/min/mg protein)

F1 : fluorescence of 25μl of diluted sample solution

F2 : fluorescence of 25μl of 10nmol standard solution

6. Statistical Analysis

All measured values were expressed in mean± standard deviation and statistical differences were tested among treatment groups on each segment using nonparametric Kruskal-Wallis test and Mann-Whitney U test.

RESULTS

1. Protein Concentration

As protein levels were used as denominators in calculating enzyme specific activities, the levels should be evenly distributed. There was no significant difference among treatment groups in homogenate solution. The result was similar in BBM as shown in Table 1.

2. Alkaline Phosphatase Specific Activities

Alkaline phosphatase was used as a marker enzyme because of its abundance in proximal small intestine and stability in intestinal brush border membrane. As expected, its specific activities were highest in the proximal segment and decreased abruptly along the distal segment, and there was no significant difference among treatment groups in each segment. There was also no significant difference among treatment groups in each segment. There was also no significant difference among treatment groups in BBM, but the enzyme specific activities were enriched about 10-foldl in them compared to those of homogenate, suggesting that BBM fraction was prepared well as shown in Table 2.

3. ACE Specific Activities in Homogenate

ACE specific activities of captopril group were increased in proximal and middle segments but it was not statistically significant. However, those of enalapril group were significantly decreased ($p < 0.01$) in middle and distal segments as shwon in Table 3.

4. ACE Specific Activities in BBM

ACE specific activities of BBM were about 10-fold higher than those of homogenate in the control group. Those of captopril group were significantly increased($p<0.01$ in proximal, $p<0.05$ in middle) compared to those of control group. By contrast, those of enalapril group were signifi

Table 1. Protein Contents of Rat Intestinal Mucosal Homogenate & Brush Border Membrane (μg/10μl)

	Proximal	Middle	Distal
Homogenate			
Control	18.5±2.9	18.8±4.1	17.0±3.8
Captopril	20.1±2.9	18.7±4.1	16.4±5.8
Enalapril	22.8±3.6	23.2±1.4	17.8±3.1
Brush Border Membrane			
Control	15.0±2.8	18.7±6.1	16.0±5.4
Captopril	10.7±2.0	13.0±3.2	11.3±5.0
Enalapril	22.5±15.7	22.1±10.2	16.1±5.5

Values are mean±S.D., n=10 per group. Control, Captopril & Enalapril groups were supplied with tap water, captopril(25mg/kg/day) & enalapril(12.5 mg/kg/day) respectively for four weeks. There was no statistically significant difference compared with control.

Table 2. Rat Intestinal Alkaline Phosphatase Activities in Mucosal Homogenate & Brush Border Membrane (nmole/min/mg protein).

	Proximal	Middle	Distal
Homogenate			
Control	134.8± 78.4	59.5± 10.9	54.1± 18.1
Captopril	138.1± 36.1	73.2± 8.6	69.1± 9.2
Enalapril	156.4± 57.7	55.3± 7.4	53.8± 9.9
Brush Border Membrane			
Control	1045.2±370.8	434.3±235.6	316.7±122.6
Captopril	1397.0±558.1	557.6±131.4	512.1±153.2*
Enalapril	1049.7±680.6	370.5± 81.4	314.7± 65.8

Values are mean±S.D., n=10 per group. Control, Captopril & Enalapril groups were supplied with tap water, captopril (25 mg/kg/day) & enalapril(12.5 mg/kg/day) respectively for four weeks. * $P<0.05$ compared with control.

Table 3. Rat Intestinal Angiotensin Converting Enzyme Activities in Mucosal Homogenate & Brush Border Membrane(nmole/min/mg protein)

	Proximal	Middle	Distal
Homogenate			
Control	15.3± 7.4	14.2± 3.1	5.0± 2.0
Captopril	22.4± 5.2	16.1± 5.8	5.0± 2.0
Enalapril	7.4± 3.6	5.7± 1.7**	2.4± 0.8*
Brush Border Membrane			
Control	178.6±64.2	180.3±60.2	48.6±13.1
Captopril	314.2±72.5**	281.0±69.8*	67.7±21.8
Enalapril	48.5±27.6**	70.7±15.6**	11.6± 4.4**

Values are mean±S.D., n=10 per group, Control, Captopril & Enalapril groups were supplied with tap water, captopril (25mg/kg/day) & enalapril (12.5mg/kg/day) respectively for four weeks. * $P<0.05$, ** $P<0.01$ compared with control.

cantly lower than those of control group in all three segments as shown in Table 3.

DISCUSSION

ACE has been shown to be an important peptidase that plays a role in digestion and assimilation of prolyl peptides[10-12]. ACE in brush border membrane prepared from human jejunum obtained during exploratory laparotomy showed its enzyme activity was comparable to those of other intestinal digestive enzymes and it was completely inhibited by ramipril, a potent ACE inhibitor[16]. Such findings suggest that long-term ingestion of ACE inhibitors as antihypertensive treatment mignt cause some impairments in the function of intestinal ACE in humans. But only few groups have reported the effects of longterm treatment of these inhibitors[22-24]. Forsuland et al. reported that serum ACE was increased after 4 weeks treatment with captopril 30mg/kg/day in rats and it reflected the increased activity of pulmonary vascular endothelial ACE, but those of other organs were the same as control group[25]. However, according to other study in which 2.5mg/kg and 12.5mg/kg body weight of captopril was given to rats for 4 weeks, there was no significant difference in specific activities of ACE between treatment and control group[26]. Actually, ACE specific activities were slightly higher in the treatment group. But the drawback of this study might be that the enzyme activities were measured only in the homogenate sample and the dose of ACE inhibitor was too small to assess its result. Therefore, the authors revised this study by adding another ACE inhibitor and increasing its dosages. The result of the present study is in accordance with others that intestinal ACE was inhibited by ACE inhibitors in vitro[16,17] but the long-term in vivo effects on intestinal ACE specific activities were not in consensus. Our finding that intestinal ACE activities were not decreased, but rather increased by in vitro captopril administration, suggests that there may be a compensatory mechanism for possible suppressed ACE by long-term ACE inhibitor administration in patients. However, discrepancy of this kind was not observed in enalapril administered rats. Several ACE inhibitors which are slightly different in their chemical structure are now in clinical use[14,15]. Little information is currently available whether there has been any difference in the frequency of the intestinal side effects according to their chemical structure in patients with chronic ACE inhibitor medication because of limited number of case reports[22-24]. But our data suggests that the digestion and assimilation of protein rich in prolime such as casein, gliadian and collagen could be inhibited in cases with long-term administration of enalapril. However, in the near future, if such information has accumulated enough for its statistical analysis to be possible, we colud correlate the significance of our finding-the difference of long-term effects of captopril and enalapril-to the real clinical setting.

REFERENCES

1. Yang HYT, Erdos EG, Levin Y: *Characterization of a dipeptide hydrolase(Kininase II: Angiotensin I-converting enzyme). J Pharmacol Exp Ther. 177 (1):291-1971*
2. Yoshioka M, Erickson RH, Woodley JF, Gulli R, Guan D, Kim YS: *Role of rat intestinal brush-border membrane angiotensin-converting enzyme in dietary protein digestion. Am J Physiol, 253:G781*

1987

3. Erdos EG. Bradykinin, Kallidin, and Kallikrein. In: Erdos EG, eds: *Handbook of Experimental Pharmacology. Berlin: Springer-Verlage, vol 25:427, 1970*
4. Cushman DW, Ondetti MA. Inhibitors of angiotensin converting enzyme. In: *Ellis GP, West GB, eds. Progress in Medical Chemistry. Amsterdam: North Holand, vol 17:41, 1980*
5. Cushman DW, Cheung HS: *Concentrations of angiotensin-converting enzyme in tissues of the rat. Biochim Biophys Acta 250:261, 1971*
6. Fitzsimon JT: *Angiotensin stimulation of central nervous system. Rev Physiol Biochem Pharmacol 87:117, 1980*
7. Wright FS, Briggs JP: *Feedback control of glomerular blood flow, pressure and filtration rate. Physiol Rev 59:958, 1979*
8. Levens NR: *Control of intestinal absorption by the renin angiotensin system. Am J Physiol 249 (Gastrointest Liver Physiol 12):G3, 1985*
9. Ward PE, Sheridan MA: *Converting enzyme, kininase and angiotensin of renal and intestinal brush border. Adv Exp Med Biol 156:835, 1983*
10. Yoshioka M, Erickson RH, Kim YS: *Digestion and assimilation of prolinecontaining peptides by rat intestinal brush-border membrane carboxy peptidases. J clin Invest, 81:1090, 1988*
11. Yoshioka M, Erickson RH, Kim YS : *Digestive and assimilation of proline-containing peptides by rat intesinal brush border membrane carboxypeptidase; Role of combined action angiotensin converting enzyme and carboxypeptidase P.J.Clin Invest. 81, 1090, 1988*
12. Yoshioka M, Erickson RH, Woodley JF, Gulli R, Guan D, Kim YS: *Role of rat intestinal brush border membrane angiotensin-convertong enzyme in dietary protein dietary protein. Am J Physiol. 253 : G781-786, 1987*
13. 윤병철, 김재준, 송인성, 김정룡 : 고 *proline*식에 의한 백서소장의 *angiotensin converting enzyme* 특이활동의 유도. 대한내과학회잡지 *44 : 795-802, 1993*
14. William WD: *Polypeptides-angiotensin, plasma kinins, and others In: Gilman AG, Goodman LS. Wall TW, Murad F, eds. 7th ed. The pharmacological basis of therapeutics. NY: MacMillans Publishing Company, 639, 1980*
15. The Acute Infarction Ramipril Efficacy(AIRE) Study Investigators: *Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. Lancet 1993*
16. Stevens BR, Fernandez A, Kneer C, Cerda JJ, Phillips MI, Woodward ER: *Human intestinal brush border angiotensin converting enzyme activity and its inhibition by anti-hypertensive ramipril. Gastroenterology 94:942, 1988*
17. Ward PE, Sheridon MA, Hammon KJ, Erdos EG: *Angiotensin I-convertingenzyme(kininase II) of brush border of human and swine intestine. Biochem Pharmacol 29:1525, 1980*
18. Kessler M, Acuto D, Storelli G, Murer H, Semenza GA: *A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport system. biochim Biophys Acta 506: 136, 1978*
19. Lowry OH, Rosebrouth NJ, Farr AL, Randal RJ: *Protein measurement with the Foin phenol reagent. J Biol Chem 193, 1951*
20. Fujita M, Ohta H, Matsui H, Nakao M: *Differential isolation of microvillous and basolateral plasma membranes from intestinal mucosa: Mutally exclusive distribution of digestive enzymes and ouabain sensitive ATPase. Biochim Biophys Acta 274:336, 1972*
21. Cheung HS, Cushman DW: *Inhibition of homogenous ACE of rabbit lung by synthetic venom peptides of Bothrops jararaca. Biochim Biophys Acta 293:451, 1973*
22. McMurray J, Mathews DM: *Consequences of fluid loss in patients treated with ACE inhibitors. Postgraduate Med J, 63:385, 1987*
23. Edwards IR, Coulter DM, Macintosh D: *Intestinal effects of captopril. Br Med J 304:359, 1992*
24. Gerckens U, Grube E, Mengden T, Siggel H, Wagner W-L, Lahn T, Irmisch R, Metzger H: *Pharmacokinetic and pharmacodynamic properties of ramipril in patients with congestive heart failure (NYHA III-IV). J Cardiovasc Phar-macol 13(Suppl. 3):S49, 1989*
25. Forsuland T, Kuovonen I, Fyhrquist F: *Tissue distribution of angiotensin converting enzyme in the rats: Effects of captopril treatment. Acta Pharmacol et Toxicol 54:124, 1984*
26. Kim NY, Jung HC, Song IS, Choi KW, Kim CY: *The effect of long-term captopril administration to mucosal angiotensin-converting enzyme activity in rat small intestine. Kor J Intern Med, 37(1):29, 1989*