Original Article

Different Effects of Imidapril and Enalapril on Aminopeptidase P Activity in the Mouse Trachea

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It has been reported that the incidence of angiotensin-converting enzyme (ACE) inhibitor-related dry cough is significantly less with the ACE inhibitor imidapril than with the ACE inhibitor enalapril in hypertensive patients. Bradykinin (BK) in the trachea is believed to play some role in this adverse effect. The present study was undertaken to evaluate the effects of imidapril and enalapril on the activity of aminopeptidase P (APP), one of the BK-metabolizing enzymes, in the mouse trachea. Imidapril (0.5 mg/kg) or enalapril (0.5 mg/kg) was given orally to mice once daily for 7 days. Drug concentrations and APP activity in the trachea were determined at the end of the experiment. Active metabolites (imidaprilat and enalaprilat), but not parent drugs (imidapril and enalapril) were detected in the trachea after a repeated dose for 7 days. Tissue concentrations of imidaprilat and enalaprilat did not significantly differ. The APP activity in the trachea did not significantly change after the 7th dose of imidapril. However, the enzyme activity was significantly inhibited after the final dose of enalapril. Thus, the present study showed that enalapril, but not imidapril inhibited the airway APP activity during repeated dosing. This finding is compatible with previous reports that the incidence of dry cough is lower with imidapril than with enalapril, and with the hypothesis that the dry cough induced by ACE inhibitors may be related to accumulation of BK in the trachea. (*Hypertens Res* 2005; 28: 243–247)

***Key Words*: imidapril, enalapril, dry cough, aminopeptidase P, bradykinin**

Introduction

Angiotensin-converting enzyme (ACE) inhibitors are widely used for the treatment of hypertension and related cardiovascular diseases. However, in 5 to 20% of patients, ACE inhibitors induce a bothersome dry cough, which leads to discontinuation of therapy in some cases (*1*). The mechanism of this adverse effect has not been fully evaluated, but the enhanced activity of airway C fibers by the elevated bradykinin (BK) is believed to be involved (*2*). Previous clinical studies have shown that the incidence of dry cough with the ACE inhibitor imidapril was significantly less than that with the ACE inhibitor enalapril (*3*, *4*). These data indicate that these two drugs may have different effects on the BK-mediated biological responses.

The main BK-metabolizing enzymes (*5*) are ACE, aminopeptidase P (APP), neutral endopeptidase and carboxypeptidase N. Inhibition of these enzymes may increase BK concentration and thereby enhance its effects. Recently, we observed that the potentiating effect of imidapril on the BK-induced writhing reaction was less than that of enalapril in mice (*6*). Since the inhibition of serum ACE activity was slightly greater with imidapril than with enalapril in that study (*6*), we speculated that the inhibitory effect of imidapril on other BK-metabolizing enzyme(s) was smaller than that of enalapril. To examine this hypothesis, we subsequently evaluated the effect of these ACE inhibitors on the activities of APP, neutral endopeptidase and carboxypeptidase N obtained from untreated mice (*7*). This study showed that APP activity

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Received May 11, 2004; Accepted in revised form November 29, 2004.

was inhibited by enalaprilat, an active metabolite of enalapril while imidaprilat, an active metabolite of imidapril, did not influence the enzyme activity (*7*). In contrast, these ACE inhibitors did not affect the activities of neutral endopeptidase and carboxypeptidase N.

The mechanism responsible for the difference in the incidence of dry cough between imidapril and enalapril (*3*, *4*) is unknown. To address this issue, we here evaluated the effects of imidapril and enalapril on airway APP activity.

Methods

Animals

Male ICR mice (Charles River Japan Inc., Atsugi, Japan), 4–5 weeks old and weighing 20–30 g, were kept under a 12-h light-dark cycle at 22±2°C. These animals had free access to food (MF containing Na^+ 2.6 mg and K^+ 7.5 mg per g food, Oriental Yeast Co., Ltd., Tokyo, Japan) and water.

All experiments were performed in accordance with the Jichi Medical School Guide for the Care and Use of Laboratory Animals.

Experiments

*Experiment 1 (*In Vitro *Study)*

APP activities in the lung and trachea were determined. To obtain a sufficient amount of microsome fraction to measure the APP activity, each organ (lung and trachea) was removed from 7 mice and homogenized together.

*Experiment 2 (*In Vivo *Study)*

Imidapril hydrochloride (imidapril) (Tanabe Seiyaku Co., Osaka, Japan) and enalapril maleate (enalapril) (Sigma, St. Louis, USA) were used in this study. A preliminary study showed that serum ACE activity (*8*) tended to be inhibited at 30 min and was significantly ($p<0.0001$ by analysis of variance [ANOVA]) inhibited at 60 min after oral dosing of imidapril 0.5 mg/kg or enalapril 0.5 mg/kg in mice ACE activity (IU/l) ($n=10$ in each) at 30 min: vehicle, 391±51; imidapril, 363±41; enalapril, 355±57; at 60 min: vehicle, 412±43; imidapril, 315±21; enalapril, 316±41. The effects on serum ACE activity did not differ significantly between the two drugs at either 30 or 60 min after administration. In this study, imidapril 0.5 mg/kg or enalapril 0.5 mg/kg was given orally, once daily for 7 days. Drug concentrations in the trachea were measured at 30 and 60 min after the final dose of each drug. These doses of 0.5 mg/kg/day (30 mg/60 kg/day) were higher than the clinically recommended dose for each drug (5–10 mg/day), but still were not considered extremely high.

*Experiment 3 (*In Vivo *Study)*

Imidapril 0.5 mg/kg, enalapril 0.5 mg/kg or vehicle alone was given orally, once daily for 7 days. APP activity in the trachea was determined just before and at 30 and 60 min after the final dose of each drug.

Preparation of Enzyme Fraction

Lungs and tracheas were removed and washed with ice-cold saline. The organs were weighed, minced, and homogenized at 4°C in 0.05 mol/l Tris-HCl buffer, pH 7.4, using a polytron homogenizer (Kinematica GmbH, Luzernerstrasse, Germany). Homogenates were fractionated at 4°C by sequential centrifugation at 1,000 × *g* for 10 min and 60,000 × *g* for 60 min. The pellets obtained from the final centrifugation were washed and resuspended in the above buffer. Microsome fractions were stored at −135°C.

Measurement of APP Activity

APP activity was measured using the intramolecularly quenched fluorogenic substrate Lys-(2,4-dinitrophenyl)-Pro-Pro-NH-CH_2-CH_2-NH-2-aminobenzoyl-2-tetrafluoroacetic acid (Peptide Institute, Inc., Osaka, Japan) (*9*). Ten microliters of the microsome fraction and 50 μl of the test solution were incubated at 40°C with 100 μl of 0.25 mmol/l substrate in 0.2 mol/l Tris-HCl, pH 8.0, containing 10 mmol/l trisodium citrate and 2.5 mmol/l $MnSO_4$. The reaction was stopped by the addition of 4 ml of 0.05 mol/l EDTA and 1 mmol/l dithiothreitol. The fluorescence produced by the enzymatic hydrolysis of the substrate (2-aminobenzoyl-Gly) was measured by a spectrofluorometer (RF-5000; Shimadzu Co., Kyoto, Japan) at an excitation wavelength of 320 nm and emission wavelength of 410 nm. The calibration curve for 2-aminobenzoyl-Gly was obtained by adding quinine sulfate (Sigma) to 0.1 mol/l sulfuric acid. A conversion factor ($K=1.40$) was applied, by which the fluorescence of the quinine sulfate solution was multiplied to obtain the fluorescence of an equimolar solution of 2-aminobenzoyl-Gly. The APP activity was measured as the initial velocity and expressed as the rate of formation of 2-aminobenzoyl-Gly during the incubation for 20 min. The initial velocity was normalized by protein concentration (mg). 1,10-Phenanthroline (Sigma) was used in control reactions to inhibit the APP activity.

Determination of Protein

Protein concentration was determined by using the bicinchoninic acid-detection reagent (Pierce Chemical Co., Rockford, USA). Bovine serum albumin was used as a standard.

Determination of Drug Concentrations in Trachea

Materials and Reagents

Imidapril, the methyl ester form of imidapril, imidaprilat, enalapril and enalaprilat were supplied by Tanabe Seiyaku Co. (Osaka, Japan). The methyl ester form of imidapril was used as an internal standard. All other reagents and solvents were obtained from Katayama Chemical Inc. (Osaka, Japan),

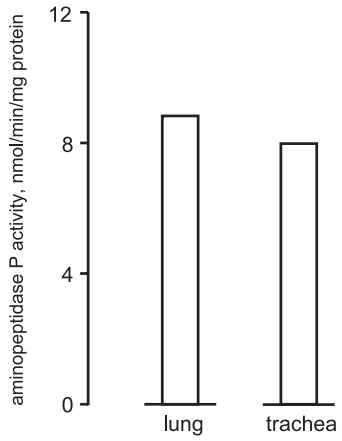

Fig. 1. *Aminopeptidase P activity in the mouse lung and trachea. Each organ was removed from 7 mice and homogenized together. The mean of 3 measurements is shown.*

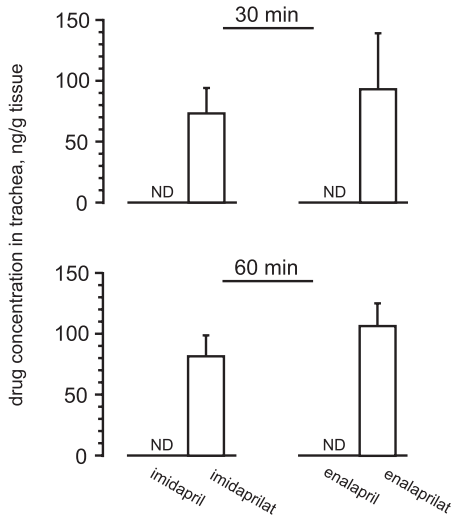

Fig. 2. *Drug concentrations in the mouse trachea at 30 and 60 min after the final dose of imidapril and enalapril. Means±SD,* n=*5 in each group. Imidapril 0.5 mg/kg or enalapril 0.5 mg/kg was given orally, once daily for 7 days. ND, not detected.*

and OASIS HLB 3 cc (60 mg) extraction cartridges were obtained from Waters (Milford, USA).

Instrumentation

A TSQ 7000 tandem mass spectrometer (Finnigan MAT; San Jose, USA) equipped with an ESI interface, a nitrogen generator (System Instruments, Tokyo, Japan) and a 2690 Separation Module (Waters) was used for all HPLC-ESI-MS-MS analyses.

Sample Preparation

Tracheas were removed under ether anesthesia, and washed with ice-cold saline. The organs were weighed, minced, and homogenized at 4°C with a volume of 20 times (w/v) 0.05 mol/l Tris-HCl buffer, pH 7.4. A 100-µl aliquot homogenate sample, 200 µl of distilled water and 200 µl of 6% (v/v) perchloric acid were mixed with vigorous stirring. The mixture was centrifuged at 1,500 × *g* for 3 min, and the supernatant applied to the OASIS HLB cartridge, which was previously conditioned with 3 ml of methanol, 3 ml of distilled water, and 1 ml of 2% (v/v) perchloric acid. The cartridge was washed with 2 ml of 0.1 mol/l hydrochloric acid and 1 ml of distilled water. Imidapril, imidaprilat, enalapril and enalaprilat, which were retained in the cartridge, were eluted with 1 ml of methanol into a disposable glass tube, and evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in 100 µl of internal standard solution (0.1 µg/ml mobile phase), and a 10-µl aliquot was analyzed by the HPLC-ESI-MS-MS system as described previously (*10*). The ion-transitions monitored were *m*/*z* 406 to *m*/*z* 234 for imidapril, *m*/*z* 378 to *m*/*z* 206 for imidaprilat, *m*/*z* 377 to *m*/*z* 234 for enalapril, *m*/*z* 349 to *m*/*z* 206 for enalaprilat and *m*/*z* 392 to *m*/*z* 220 for the internal standard. The detection threshold was 10 ng/g tissue.

Statistical Analysis

Data were expressed as the means or means±SD. Statistical analyses were performed using ANOVA. As a post-hoc test, Scheffe's test was used. Values of $p<0.05$ were considered to indicate statistical significance.

Results

The degree of APP activity detected in the lung was similar to that detected in the trachea (about 91% of the value for the lung) (Fig. 1). Active metabolites (imidaprilat and enalaprilat), but not parent drugs (imidapril and enalapril), were detected in tracheas after a repeated dose for 7 days (Fig. 2). The tissue concentrations of imidaprilat and enalaprilat did not differ significantly. The effects of repeated doses of ACE inhibitors on APP activity in the trachea are shown in Fig. 3. APP activity did not change significantly after the final dose of vehicle ($p=0.69$ by ANOVA) or imidapril ($p=0.19$ by ANOVA). However, the enzyme activity was significantly inhibited after the 7th dose of enalapril ($p=0.0004$ by ANOVA).

Discussion

BK, a potent vasodilator, is involved in the cardiovascular effects of ACE inhibitors (*5*, *11*). However, excess BK in the trachea can cause dry cough (*2*), especially in patients with bradykinin B2 receptor gene polymorphism (*12–14*). The persistent, bothersome cough, usually more severe at night, leads to discontinuation of ACE inhibitors in some patients (*1*). Saruta *et al*. (*3*) examined the incidence of dry cough

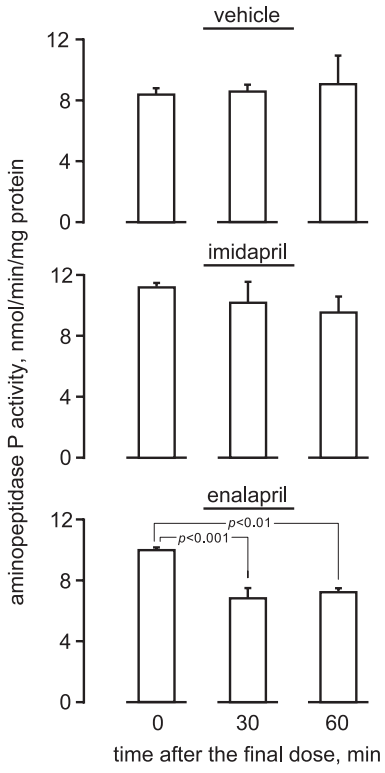

Fig. 3. *The effects of repeated dosing of imidapril or enalapril on aminopeptidase P activity in the mouse trachea. Means ±SD,* n = *5 in each group. Imidapril 0.5 mg/kg, enalapril 0.5 mg/kg or vehicle alone was given orally, once daily for 7 days. Significance was evaluated by Scheffe's test after ANOVA.*

induced by imidapril and enalapril in a double-blind, comparative study. Imidapril was given to 108 hypertensive patients and enalapril to 115 hypertensive patients for 12 weeks in their study. During the treatment period, cough was recorded in 0.9% (1/108) of the imidapril group and 7.0% (8/115) of the enalapril group. Based on these data, they concluded that the incidence of cough was significantly less with imidapril than with enalapril. Thereafter, Saruta *et al*. (*4*) confirmed their hypothesis in a comparative, crossover study. Moreover, an animal study using guinea pigs showed that the potentiating effect of imidapril on BK-induced airway microvascular leakage was significantly less than that of enalapril (*15*). These observations led us to speculate that the inhibitory effect on the metabolism of airway BK differs between imidapril and enalapril.

APP exists in various rat organs, with the highest activity occurring in the testis, lung, kidney and ovary (*9*). In the present study, similar levels of APP activity were detected in both the mouse lung and trachea. APP is one of the main BK-metabolizing enzymes (*5*) and largely contributes to the degradation of BK in several organs (*11*, *16*), especially under the inhibition of ACE activity (*17*). Since the increased BK exerts biological responses (microvascular leakage) in mouse tracheas after treatment with ACE inhibitors (*18*), we think that APP also contributes to the degradation of the increased airway BK under the conditions of ACE inhibition.

Recently, we showed that the potentiating effect of imidapril on the kaolin-induced writhing reaction, which is mediated by the increased BK, is less than that of enalapril (*6*). Serum ACE activity was slightly lower with imidapril than with enalapril in that study. Therefore, we speculated that the inhibition of BK-metabolizing enzymes other than ACE by imidapril would be weaker than that by enalapril. Actually, in a subsequent study we demonstrated that APP activity in the mouse lung was inhibited by enalaprilat, but not by imidaprilat (*7*). In addition, their inhibitory effects on other main BK-metabolizing enzymes (neutral endopeptidase and carboxypeptidase N) were negligible (*7*).

In the present study, the APP activity in the mouse trachea was not significantly decreased by repeated dosing of imidapril. On the other hand, the enzyme activity was significantly decreased after the 7th dose of enalapril. Since imidaprilat concentration in the trachea did not differ significantly from that of enalaprilat, we think that the difference between imidapril and enalapril was mainly due to the different pharmacological profiles of the two drugs on APP activity. Although the species difference remains to be determined, such a difference in the effects of imidaprilat and enalaprilat on airway APP activity may contribute to the different incidence of dry cough in hypertensive patients treated with these ACE inhibitors.

The mechanism of ACE inhibitor-induced dry cough is very complex, and several factors such as BK, substance P and prostaglandin E_2 are reported to be involved (*19*). Because ACE inhibitors cause enhanced prostaglandin E_2 production *via* BK (*20*), the fact that imidapril and enalapril have different effects on airway prostaglandin E_2 may also play some role in the different incidence of dry cough between the two.

In summary, the present study showed that enalapril, but not imidapril inhibited APP activity in the mouse trachea during repeated dosing. This finding lends further support to the idea that the incidence of dry cough is lower with imidapril than with enalapril, and further suggests that this difference is related to the lower accumulation of BK in the trachea by imidapril. Further studies will be needed to examine this hypothesis.

References

1. Jackson EK, Garrison JC: Renin and angiotensin, in Hardman JG, Limbird LE (eds): Goodman & Gilman's the Pharmacological Basis of Therapeutics. 9th ed. New York, McGraw-Hill, 1996, pp 733–758.
2. Fox AJ, Lalloo UG, Belvisi MG, Bernareggi M, Chung KF, Barnes PJ: Bradykinin-evoked sensitization of airway sensory nerves: a mechanism for ACE-inhibitor cough. *Nat Med* 1996; **2**: 814–817.

3. Saruta T, Omae T, Kuramochi M, *et al*: Imidapril hydrochloride in essential hypertension: a double-blind comparative study using enalapril maleate as a control. *J Hypertens* 1995; **13** (Suppl 3): S23–S30.
4. Saruta T, Arakawa K, Iimura O, *et al*: Difference in the incidence of cough induced by angiotensin converting enzyme inhibitors: a comparative study using imidapril hydrochloride and enalapril maleate. *Hypertens Res* 1999; **22**: 197–202.
5. Linz W, Wiemer G, Gohlke P, Unger T, Schölkens BA: Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacol Rev* 1995; **47**: 25–49.
6. Sakamoto K, Sugimoto K, Fujimura A: Different potentiating effects of imidapril and enalapril on kaolin-induced writhing reaction in mice. *Life Sci* 2001; **68**: 2415–2421.
7. Sakamoto K, Sugimoto K, Fujimura A: Different inhibition of enalaprilat and imidaprilat on bradykinin metabolizing enzymes. *Life Sci* 2000; **67**: 2159–2165.
8. Kasahara Y, Ashihara Y: Colorimetry of angiotensin-converting enzyme activity in serum. *Clin Chem* 1981; **27**: 1922–1925.
9. Holtzman EJ, Pillay G, Rosenthal T, Yaron A: Aminopeptidase P activity in rat organs and human serum. *Anal Biochem* 1987; **162**: 476–484.
10. Mabuchi M, Kano Y, Fukuyama T, Kondo T: Determination of imidapril and imidaprilat in human plasma by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *J Chromatogr* 1999; **734**: 145–153.
11. Squire IB, O'kane KPJ, Anderson N, Reid JL: Bradykinin B2 receptor antagonism attenuates blood pressure response to acute angiotensin-converting enzyme inhibition in normal men. *Hypertension* 2000; **36**: 132–136.
12. Zee RYL, Rao VS, Paster RZ, Sweet CS, Lindpaintner K: Three candidate genes and angiotensin-converting enzyme inhibitor-related cough. *Hypertension* 1998; **31**: 925–928.
13. Mukae S, Aoki S, Itoh S, Iwata T, Ueda H, Katagiri T: Bradykinin B2 receptor gene polymorphism is associated with angiotensin-converting enzyme inhibitor-related cough. *Hypertension* 2000; **36**: 127–131.
14. Sumino H, Ichikawa S, Ohyama Y, *et al*: Effect of hormone replacement therapy on serum angiotensin-converting enzyme activity and plasma bradykinin in postmenopausal women according to angiotensin-converting enzyme-genotype. *Hypertens Res* 2003; **26**: 53–58.
15. Murata T, Matsumoto Y, Kashida T, *et al*: Difference among angiotensin-converting enzyme inhibitors in potentiating effects on bradykinin-induced airway microvascular leakage in guinea pig airways. *Jpn J Pharmacol* 1995; **69**: 111–118.
16. Damas J, Liégeois J-F, Simmons WH: Potentiation of the pro-inflammatory effects of bradykinin by inhibition of angiotensin-converting enzyme and aminopeptidase P in rat paws. *Naunyn-Schmiederbergs Arch Pharmacol* 1996; **354**: 670–676.
17. Kim K-S, Kumar S, Simmons WH, Brown NJ: Inhibition of aminopeptidase P potentiates wheal response to bradykinin in angiotensin-converting enzyme inhibitor-treated humans. *J Pharmacol Exp Ther* 2000; **292**: 295–298.
18. Emanueli C, Grady EF, Madeddu P, *et al*: Acute ACE inhibition causes plasma extravasation in mice that is mediated by bradykinin and substance P. *Hypertension* 1998; **31**: 1299–1304.
19. Morice AH, Brown MJ, Higenbottam T: Cough associated with angiotensin converting enzyme inhibition. *J Cardiovasc Pharmacol* 1989; **13** (Suppl 3): S59–S62.
20. Regoli D, Barabe J: Pharmacology of bradykinin and related peptides. *Pharmacol Rev* 1980; **32**: 1–46.