Differential Inhibition of Bradykinin Hydrolysis by Four ACE Inhibitors: a Possible Explanation for Differences in Induced Coughing

Manabu Sasaguri, Munehito Ideishi, Akio Kinoshita, and Kikuo Arakawa

Coughing is an adverse reaction to angiotensin-converting enzyme (ACE) inhibitors, but some ACE inhibitors cause more coughing than others. To test the hypothesis that this difference is related to a difference in their effects on bradykinin (BK), the potencies for inhibition of the hydrolysis of BK and angiotensin (Ang) I of four ACE inhibitors, captopril, enalaprilat, ramiprilat, and imidaprilat, were tested with purified canine lung ACE. The accumulation of BK relative to the inhibition of Ang II formation was significantly less with imidaprilat than with the three other ACE inhibitors. This may explain why imidapril was associated with less coughing than ramipril in clinical trials. ACE inhibitors may be classified by their relative potencies for the inhibition of the hydrolysis of BK and Ang I.

Key Words: angiotensin-converting enzyme, angiotensin-converting enzyme inhibitor, cough, bradykinin, angiotensin

Dipeptidyl carboxypeptidase I (angiotensin-converting enzyme (ACE), kininase II; EC 3.4.15.1) is a zinc metallopeptidase that plays a major role in blood pressure regulation (1). On the surface of endothelial cells, it converts the inactive decapeptide angiotensin (Ang) I to the vasopressor- and aldosterone-stimulating octapeptide Ang II. It also degrades bradykinin (BK), a vasodilatory nonapeptide that is involved in the regulation of vascular tone and has been implicated in inflammatory responses. ACE also cleaves substance P, LH-RH, and others (1).

ACE inhibitors are now widely used to treat hypertension and heart failure in the belief that the reduction of Ang II levels benefits patients with these diseases. Although ACE inhibitors are tolerated relatively well, one side effect that has been reported with increasing frequency is persistent dry coughing (2). Estimates of the incidence of this coughing have varied between 3 and 15 percent (3, 4). A recent cross-sectional epidemiological study found a cough prevalence of 19 percent among patients receiving ACE inhibitors (5). Although this coughing is usually not serious, about 5% of the patients treated with ACE inhibitors must discontinue treatment because of persistent coughing.

Although the mechanisms of this coughing remain unclear, various agents, such as BK and substance P, have been suggested to be involved. BK is primarily metabolized by ACE on lung endothelial cells. Inhibition of ACE would lead to an increase in the local BK concentration, which could explain the induction of coughing during treatment with ACE inhibitors.

In a double-blind study (6), the incidence of coughing caused by ramipril was 10.2%, but the incidence of coughing caused by captopril was only 5.3%. In other double-blind studies (7, 8), the incidence of coughing caused by imidapril was only 0.9%, but the incidence of coughing caused by enalapril was 7.0%, with equal antihypertensive effects in patients with essential hypertension (Table 1).

Results of a study in guinea pigs have also shown that the potentiating effect of imidapril on coughing induced by citric acid aerosol is less than those of other ACE inhibitors, such as enalapril, captopril, and delapril (12). In vitro studies with synthetic tripeptides as substrates have suggested that there is a significant difference among ACE inhibitors between the hydrolysis of Ang I and the hydrolysis of BK (13, 14).

These results prompted us to examine differences between ACE inhibitors (ramiprilat, enalaprilat, captopril, and imidaprilat) with regard to the ability to inhibit BK breakdown relative to the ability to inhibit Ang I conversion.

Materials and Methods

Ang I and BK were purchased from Peptide Institute (Osaka, Japan). Other chemicals, including captopril and substance P, were purchased from
Sigma (St. Louis, USA). The active metabolites of three other ACE inhibitors were used. Imidaprilat was a gift from Tanabe Seiyaku Co. (Osaka, Japan), enalaprilat from Merck Co. (New Jersey, USA), and ramiprilat from Hoechst Japan Co. (Tokyo, Japan). Canine lung ACE was purified as previously reported (15, 16). Briefly, the supernatant of a lung homogenate was subjected to affinity chromatography on a lisinopril-linked Sepharose column equilibrated with 20 mM phosphate buffer (pH 8.3) containing 0.3 M NaCl and 0.05% Triton X-100. The active fractions were eluted with 5 M urea, dialyzed against 20 mM phosphate buffer (pH 8.3), and subjected to a Sephadex G-200 column. Purified canine lung ACE was lyophilized and stored at −20°C until use.

Twenty microliters of ACE (55 μg, potency: approximately 20 μg of Ang II formed from 50 μg of Ang I in 30 min) was incubated with either 50 μg of Ang I or 10 μg of BK at 37°C for 30 min in 3 mM potassium phosphate buffer (pH 7.4) containing 0.1 M NaCl in a total volume of 300 μl with or without ACE inhibitors in various concentrations. The tubes containing incubation mixtures were heated in boiling water to stop the reaction, and then centrifuged at 1,000 g. Fifteen microliters of the supernatant of Ang I samples and 40 μl of the supernatant of BK samples were injected separately into a reverse phase HPLC (Nucleocil C18, 0.4 × 20 cm) equilibrated with 15% acetonitrile in 0.1% TFA. Ang II and BK were eluted at approximately 7.0 min and 9.2 min, respectively, with a linear gradient of 15% to 45% acetonitrile (5 to 24 min) and at a flow rate of 2 ml/min. The amounts of Ang II and BK were determined in duplicate according to the area under the peak of the HPLC curve, and averaged. The five concentrations of each ACE inhibitor used were chosen so that the percent inhibition of Ang II-formation ranged from about 10% to 90%, and was almost equal in the two groups. The concentrations of each ACE inhibitor in the three experiments were as follows:

i). enalaprilat; 7.5 × 10⁻⁹, 10⁻⁸, 2.5 × 10⁻⁸, 5 × 10⁻⁸, 7.5 × 10⁻⁹, 10⁻⁸, 2.5 × 10⁻⁸, 5 × 10⁻⁸ M, imidaprilat; 5 × 10⁻⁹, 7.5 × 10⁻⁹, 10⁻⁸, 2.5 × 10⁻⁸ M, ii) ramiprilat; 10⁻⁹, 5 × 10⁻⁹, 7.5 × 10⁻⁹, 10⁻⁸, 2.5 × 10⁻⁸ M, imidaprilat; 5 × 10⁻⁹, 7.5 × 10⁻⁹, 10⁻⁸, 2.5 × 10⁻⁸, 5 × 10⁻⁸ M, iii) captopril; 2 × 10⁻⁸, 3 × 10⁻⁸, 5 × 10⁻⁸, 3 × 10⁻⁷, 3 × 10⁻⁸, 7.5 × 10⁻⁷, 2 × 10⁻⁸, 3 × 10⁻⁸ M.

Three studies were done: imidaprilat was compared to each of the three other ACE inhibitors with regard to the ability to inhibit BK breakdown relative to the ability to inhibit Ang I conversion. Percent inhibition of Ang II formation was defined as [(amount of Ang II without ACEI)−(amount of Ang II with ACEI)] divided by (amount of Ang II

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<tr>
<th>Control drugs used</th>
<th>Drugs tested in double-blind trials</th>
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without ACEI). Percent inhibition of BK breakdown (percent remnant BK) was defined as [(amount of BK with ACEI)-(amount of BK without ACEI)] divided by (amount of BK added). The results of ten experiments done in each group were combined, with a total of 50 points in each group. The percent remnant BK and percent inhibition of Ang II formation of imidaprilat were compared to those of each of the three other ACE inhibitors with the paired t test. All data are expressed as mean ± S.E.

To compare the hydrolysis of substance P and BK by ACE, ACE (20, 40, and 80 μl) was incubated with substance P (12.2 μg) either with or without BK (10 μg) at 37°C for 30 min in 3 mM potassium phosphate buffer (pH 7.4) containing 0.1 M NaCl in a total volume of 200 μl. Thirty microliters of the supernatant of these samples was injected into a reverse-phase HPLC (Nucleocil C18) equilibrated with 10% acetonitrile in 0.1% TFA. Substance P and BK were eluted at approximately 24.3 min and 18.6 min, respectively, with a linear gradient of 10% to 40% acetonitrile in 30 min and at a flow rate of 1 ml/min.

Results
The relations between percent remnant BK and percent inhibition of Ang II-formation for imidaprilat vs. those of each of the three other ACE inhibitors are shown in Fig. 1. Percent remnant BK and percent inhibition of Ang II formation are shown in Fig. 2. Percent inhibition of Ang II formation was significantly lower with enalaprilat than with imidaprilat, with equal percent remnant BK (Fig. 2A). Percent remnant BK was higher with ramiprilat (B) and captopril (C) than with imidaprilat, although there were no differences in percent inhibition of Ang II formation. Each column shows a mean ± S.E., n = 50, * indicates p < 0.01, ** p < 0.001. NS = not significant.

Discussion
The present data show that the relative inhibitory potency of imidaprilat in Ang II formation and BK degradation differs from those of enalaprilat, ramiprilat, and captopril. The accumulation of BK was less with imidaprilat than with any of the other
ACE inhibitors. The fact that the accumulation of BK was less with imidaprilat than with enalaprilat may explain why imidapril was associated with less coughing than was enalapril in clinical trials (7, 8), and also why imidapril potentiates coughs in guinea pigs less than do other ACE inhibitors (12). Imidaprilat also potentiates the action of bradykinin less than does enalaprilat in isolated dog blood vessels (17).

Two studies examined the molecular mechanisms of ACE inhibitor interactions. Results of an in vitro study with synthetic tripeptides as a substrate suggest that ACE inhibitors can be classified into one of two types according to their preference for targeting Ang I or BK (13). In another study, the selectivity of ACE inhibitors for inhibition of kininase II was apparent when Ang I-like (Hip-His-Leu) and BK-like (Hip-Phe-Arg) peptides were used as substrates (14). To our knowledge, the present study is the first to show that different ACE inhibitors have different potencies in the hydrolysis of BK and Ang I. We used purified ACE from dog lungs. Based on physical properties, enzymatic characteristics, and immunological cross reactivity, ACE in different species and various tissues are quite similar, except for testicular ACE (I, 18). The interactions of canine ACE and ACE inhibitors shown in the present study are likely to be the same as those of human ACE and ACE inhibitors.

Recently, the primary structure of human endothelial ACE has been determined by cDNA cloning, and two homologous domains have been identified. The two domains (C and N) have an independently functional active site and zinc-dependent dipeptidyl carboxypeptidase activity, and are sensitive to competitive ACE inhibitors (19). The relative inhibitory potencies of captopril (C), enalaprilat (E), and lisinopril (L) for each domain are reportedly different: L > E > C for the C domain versus C > E > L for the N domain, with the synthetic tripeptide Hip-His-Leu (20). However, the relative potencies of different ACE inhibitors in the hydrolysis of Ang I and BK have not been previously studied.

The mechanism of the coughing associated with ACE inhibitors remains unknown. However, the accumulation of BK, prostaglandins, and other tachykinins caused by ACE inhibitors may be related to the occurrence of this coughing. Some evidence has been gathered regarding the role of BK. In animals, BK has been shown to activate both rapidly adapting receptors (21) and bronchial C fiber receptors (22). A controlled study in 10 hypertensive patients receiving ACE inhibitors showed that the incidence of coughing in response to BK was higher after administration of captopril and enalapril (23). Based on these studies, BK almost certainly has a role in this mechanism.

Results of several studies have suggested that the release of tachykinins from sensory nerves can mediate coughing. Tachykinin receptor antagonists prevent capsaicin-induced smooth muscle contraction and increases in vascular permeability. Substance P, one of these tachykinins, can be found in sensory nerves in the airways in several species, including humans (24). Capsaicin might induce coughing through the release of substance P. Substance P appears to be one of the most potent stimuli for coughing, and it can stimulate coughing at concentrations from $10^{-17}$ to $10^{-16}$ M, well below those of histamine ($10^{-8}$ to $10^{-7}$ M) (25).

Hydrolysis of substance P is catalyzed by ACE and neutral endopeptidase (24). Thus, ACE inhibitors can prevent the degradation of substance P. With the administration of ACE inhibitors, the local accumulation of substance P may induce coughing. However, in this study, we showed that ACE preferentially degrades BK (and not substance P), when the two are present simultaneously, since ACE binds more strongly to BK than to substance P (26). This in vitro finding suggests that ACE inhibitor-induced accumulation of BK in airways could mediate coughing. However, accumulated BK could, in turn, prevent the degradation of substance P by ACE, because accumulated BK inhibits Ang I.
hydrolysis (16). Thus, locally increased levels of substance P together with BK in airways may bring about coughing.

Based on the present data and the results of Japanese multi-center double-blind studies, ACE inhibitors may be classified into three types (A, B, and C): A-type ACE inhibitors such as imidapril may preferentially inhibit Ang I conversion, B-type drugs such as ramipril may preferentially inhibit BK breakdown, and C-type drugs are a combined or intermediate type, which may include most of the currently available ACE inhibitors such as captopril and enalapril. Type A ACE inhibitors may be useful for those patients who cannot tolerate coughing, and type B for those who are likely to benefit from BK (27, 28).

In summary, our study has shown that ACE inhibitors have different potencies for inhibiting the hydrolysis of BK and Ang I, and that there is less accumulation of BK with imidapril than with captopril, enalaprilat, and ramiprilat. This finding may explain, at least in part, why imidapril was associated with less coughing than ramipril in clinical trials. The finding that ACE inhibitors differ in their selectivity for inhibition of BK breakdown or Ang II formation may open a new direction for development of future ACE inhibitors.

Acknowledgement

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References

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