Original Article

Significant Target Organs for Hypertension and Cardiac Hypertrophy by Angiotensin-Converting Enzyme Inhibitors

Shinji TAKAI, Denan JIN, Masato SAKAGUCHI, and Mizuo MIYAZAKI

To clarify the mechanisms by which angiotensin-converting enzyme (ACE) inhibitors lower blood pressure or inhibit cardiac hypertrophy, we analyzed the correlations among tissue ACE activities, blood pressure and cardiac hypertrophy. In spontaneously hypertensive rats (SHR), blood pressure, heart weight and ACE activities in plasma and various tissues were measured 3, 24 and 48 h after repeated daily treatment for 2 weeks with the ACE inhibitors trandolapril, perindopril, temocapril and enalapril. For all four ACE inhibitors, blood pressure and ACE activities in the plasma, aorta and kidney were significantly reduced 3 h after the last treatment. Although hypotensive effects were maintained at 24 h, ACE activities in plasma were not suppressed by temocapril and enalapril. Even at 3 h, enalapril could not suppress ACE activity in the brain, and temocapril and enalapril could not inhibit ACE activities in the heart. Significant correlations between ACE activity in the aorta and blood pressure were observed for all four ACE inhibitors, while the ACE activities in the heart and brain were not correlated with changes in blood pressure. Significant decreases in the ratio of heart weight to body weight were observed in SHR treated with trandolapril and perindopril, whereas they were not observed with temocapril and enalapril. The ratio of heart weight to body weight was significantly correlated with ACE activity in the heart. ACE activities in vascular tissues and the heart may be important targets in terms of the ability of ACE inhibitors to lower blood pressure or inhibit cardiac hypertrophy, respectively. (*Hypertens Res* 2004; 27: 213–219)

Key Words: angiotensin II, angiotensin-converting enzyme, blood pressure, cardiac hypertrophy

Introduction

Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxylase which converts an inactive peptide, angiotensin I, to an active peptide, angiotensin II. Angiotensin II generated by ACE plays a crucial role in regulation of blood pressure. In general, the regulation of blood pressure is known to be dependent on vascular tonus, cardiac function, renal function and the central nervous system. ACE expression has been detected in various tissues, such as brain, kidney, heart and vascular tissues, and it remains unclear where ACE activities play an important role in the regulation of blood pressure. In spontaneously hypertensive rats (SHR) and two-kidney, one

clip (2K1C) renal hypertensive rats, the renin and ACE activities in plasma are apparently normal or low in the chronic stage of hypertension (1–3). On the other hand, the ACE activity in vascular tissues, but not in the brain, kidney and heart, is increased, and there is an increase in the local production of vascular angiotensin II in the chronic stage of these hypertensive models (1–3). These findings suggest that an increase in ACE activity in vascular tissues may play a crucial role in the pathogenesis of hypertension.

ACE inhibitors are known to inhibit the conversion of angiotensin I to angiotensin II and to reduce blood pressure both clinically and in animal models of hypertension. ACE activity is easily detected in plasma, and the inhibition of ACE activity in plasma has been used as an indicator of

From the Department of Pharmacology, Osaka Medical College, Takatsuki 569–8686, Japan.

Address for Reprints: Shinji Takai, Ph.D., Department of Pharmacology, Osaka Medical College, Takatsuki 569–8686, Japan. E-mail: Pha010@art.osaka-med.ac.jp

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ACE inhibition after treatment with ACE inhibitors. In fact, immediately after an ACE inhibitor is administered, ACE activity in plasma is reduced along with blood pressure. However, in a previous study in which SHR were treated with a single dose of a water-soluble ACE inhibitor, enalapril, the ACE activity in plasma recovered to the control level within 24 h, although blood pressure continued to be suppressed (4). This finding suggests that inhibition of plasma ACE activity is not necessarily a blood pressure-lowering mechanism for ACE inhibitors. In another study in which enalapril was administered centrally to stroke-prone SHR, the blood pressure was reduced, suggesting that inhibition of ACE in the brain may play an important role in the antihypertensive mechanism of this drug (5). However, after oral administration of enalapril, it has been reported that ACE activity in the brain was not suppressed because enalapril could not penetrate the blood-brain barrier (6), although the blood pressure was reduced. Although oral administration of ACE inhibitors has been widely used for hypertensive patients, it remains unclear which organs these drugs target in order to achieve their antihypertensive effects. ACE inhibitors also reduce cardiac hypertrophy. However, the mechanism of this reduction is also unclear, and it has not been established whether the hypotensive effect or the inhibition of ACE activity in tissues is more important.

In this study, to clarify the mechanisms by which ACE inhibitors reduce blood pressure or inhibit cardiac hypertrophy, we analyzed the correlations among tissue ACE activities, blood pressure and cardiac hypertrophy.

Methods

Animals

Eighteen-week-old male SHR weighing 280–310 g were purchased from Japan SLC Inc. (Shizuoka, Japan). All rats were housed at room temperature (23–26°C) with a 12-h light-dark cycle and had free access to standard food (F-2; Funahashi Co., Tokyo, Japan) and water. The experimental procedures for animals were in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

SHR were daily treated with placebo, 1 mg/kg trandolapril, 3 mg/kg temocapril, 3 mg/kg perindopril or 10 mg/kg enalapril for 2 weeks ($n = 6$, each group). After placebo or ACE inhibitors were administered for 2 weeks, the body weights of the rats were measured. After this measurement, the animals were anesthetized with 35 mg/kg of sodium pentobarbital intraperitoneally. Then a PE-10 catheter (Clay Adams, Parsippany, USA) was inserted into the left carotid artery and connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo, Japan), and the systolic blood pressure was measured. A blood sample was obtained through a catheter from the carotid artery, and the weights of the brain, heart and kidney were measured. The tissues were

harvested for the analysis of ACE activity. The data on blood pressure, tissue weight and ACE activities 3 h after the last treatment with placebo were used as a control.

Plasma and Tissue ACE Activities

The plasma was separated from the blood samples by centrifugation at 3,000 rpm for 15 min at 4°C. Tissue extracts for measurement of ACE activity were prepared according to a previously described method with modifications (7). First, tissue was minced and homogenized in 10 volumes (w/v) of 20 mmol/l Tris-HCl buffer, pH 8.3, and the homogenate was centrifuged at 20,000 $\times g$ for 30 min at 4°C. The supernatant was discarded and the pellet was re-homogenized in 5 volumes (w/v) of 20 mmol/l Tris-HCl buffer containing 5 mmol/l $Mg(CH_3COO)_2$, 30 mmol/l KCl, 250 mmol/l sucrose and 0.5% NP-40. The homogenate was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was used for the measurement of ACE activity and protein concentration.

The ACE activity in plasma or tissue extract was measured using a synthetic substrate, hippuryl-His-Leu (HHL), specifically designed for ACE (Peptide Institute Inc., Osaka, Japan). Fifty microliters of tissue extract or plasma were incubated for 30 min at 37°C with 5 mmol/l HHL in 250 μ l of 10 mmol/l phosphate buffer, pH 8.3, containing 300 mmol/l NaCl (7). The reaction was terminated by addition of 750 μ l of 3% metaphosphoric acid, and then the mixture was centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant was applied to a reversed-phase column (4 mm i.d. $\times$ 250 mm; IRICA Instrument, Kyoto, Japan), which had been equilibrated with 10 mmol/l KH_2PO_4 and CH_3OH (1:1, pH 3.0), and eluted with the same solution at a rate of 0.3 ml/min. Hippuric acid was detected by ultraviolet absorbance at 228 nm. One unit of ACE activity was defined as the amount of enzyme that cleaved 1 μ mol hippuric acid/min.

Statistical Analysis

Significant differences among the mean values of multiple groups were evaluated by one-way ANOVA followed by a *post-hoc* analysis (Fisher's test). $P < 0.05$ was used as the threshold for statistically significant differences.

Results

Blood Pressure

Effects of ACE inhibitors on blood pressure are shown in Fig. 1. All four ACE inhibitor treatments resulted in a significant decrease in blood pressure between 3 to 48 h after the last treatment compared with the control values. The blood pressure recovered gradually and was completely restored by 48 h. When the change in blood pressure 3 h after the last treatment of each ACE inhibitor in comparison with the control was regarded as -100%, trandolapril showed the most

Fig. 1. (A) Effects of trandolapril, temocapril, perindopril and enalapril on systolic blood pressure (SBP) at 3, 24 and 48 h after the last treatment. (B) Changes in SBP by trandolapril, temocapril, perindopril and enalapril at 24 and 48 h after the last treatment; each ACE inhibitor induced a change in blood pressure 3 h after the last treatment in comparison with the control group.

continuous hypotension of the four ACE inhibitors, maintaining a reduction of -79% at 48 h. Temocapril showed the least continuous hypotensive effect, -37%, at 48 h, although animals treated with this inhibitor still showed significant hypotension.

Tissue ACE Activities

The effects of ACE inhibitors on tissue ACE activities are shown in Fig. 2. Although all four ACE inhibitors caused significant reduction of ACE activities in plasma 3 h after the last treatment, ACE activities in the animals treated with temocapril and enalapril had recovered to the control levels by 24 h. ACE activity in the heart was significantly suppressed by trandolapril and perindopril at both 3 and 48 h, but not by temocapril and enalapril even at 3 h. In the brain, ACE activity was significantly suppressed by trandolapril, temocapril and perindopril, but not by enalapril, at 3 h. In the kidney, as in plasma, ACE activities were significantly reduced at 3 h by all four ACE inhibitors. ACE inhibition in the kidney continued in the groups receiving trandolapril and perindopril even at 48 h, but had recovered to the control levels in the temocapril and enalapril groups.

Correlation between Blood Pressure and Tissue ACE Activities

In analysis of the correlations between blood pressure and tissue ACE activities for all ACE inhibitors, we only observed a significant correlation between blood pressure and ACE activities in vascular tissues by using data at 3, 24 and 48 h after the last treatment (Fig. 3).

Correlation between Heart Weight and Tissue ACE Activities

Body weight, brain weight, and kidney weight were not affected by treatments with any of the four ACE inhibitors (Table 1). Heart weight was significantly reduced by treatments with trandolapril and perindopril, but not by temocapril and enalapril. Furthermore, the ratios of heart weight to body weight in trandolapril- and perindopril-treated SHR were significantly lower than that in the controls, while those in temocapril- and enalapril-treated SHR were not (Fig. 4). A significant correlation between ACE activity in the heart and the ratio of heart weight to body weight was observed (Fig. 5).

Discussion

In the present study, a significant correlation between blood pressure and ACE activity in vascular tissues was observed in treatments with each of the four ACE inhibitors. Up to 48 h after the last treatment, inhibition of ACE activity in vascular tissues and reduction of blood pressure was observed for all four ACE inhibitors; this association did not hold for ACE activity in other tissues. These findings suggest that inhibition of ACE activity in vascular tissues may be most important for the hypotensive mechanism of ACE inhibitors. In 2K1C renal hypertensive rats, plasma renin activity was increased along with blood pressure during the acute phase after the clipping, while renin activity was recovered to normal levels in spite of continuing hypertension during the chronic phase (3). In this model, ACE activity in vascular tissues, but not in other tissues, was significantly increased. As in the present study using a rat model, studies using dog and hamster models of 2K1C renal hypertension have reported that ACE activity in vascular tissues was increased during the chronic phase after the clipping, but neither ACE activities in other tissues nor plasma renin activity were changed in comparison with the control levels (8, 9). Similarly in SHR, it has been shown that only ACE activity in vascular tissues increases along with blood pressure during the chronic phase of hypertension (1, 2). Taken together, these findings and the results of the present study indicate that ACE levels in vascular tissues may play a more important role in the regulation of blood pressure than ACE levels in other tissues or plasma.

Vicaut and Hou (10) treated SHR with trandolapril, enalapril or perindopril for 2 weeks, and found that trandolapril and perindopril resulted in a significant inhibition of microcirculation that persisted 48 h after the last dose in comparison with untreated SHR, whereas enalapril did not significantly inhibit the microcirculation. Moursi *et al.* (11) compared oral 2-week-treatment of enalapril, ramipril and perindopril in stroke-prone spontaneously hypertensive rats (SHR-SP), and found that ramipril and perindopril lowered tissue ACE activity in the kidney, heart, vascular tissues and

Fig. 2. Effects of trandolapril, temocapril, perindopril and enalapril on ACE activities in the aorta, kidney, heart, brain and plasma at 3, 24 and 48 h after the last treatment. * $p < 0.05$ and ** $p < 0.01$ vs. the control group.

Fig. 3. The correlations between blood pressure and aorta ACE activities in the trandolapril-, temocapril-, perindopril- and enalapril-treated groups.

brain more potently than enalapril did. In the present study, ACE activity in the vascular tissues in trandolapril-, temocapril- and perindopril-treated SHR were suppressed 48 h after the last treatment, while that in enalapril-treated SHR was recovered completely. In the kidney, 24 h after the last treatment, trandolapril and perindopril continued to significantly suppress ACE activity, while temocapril and enalapril did not. In the heart, even 3 h after the last treatment, trandolapril and perindopril continued to suppress ACE activity, but temocapril and enalapril did not. These different effects on tissue ACE inhibition by each ACE inhibitor may be due to their different lipophilicities (12). The most lipophilic compound is trandolapril, while the lipophilicities of the others are lower and similar (13). Temocapril and trandolapril are known to be excreted in bile, and temocapril, like trandolapril, is believed to be a lipophilic compound (14). However, it appears that the high excretion in bile is dependent on the structural formula of temocapril and not on its lipophilicity (14, 15). Temocapril is excreted into bile via an ATP-dependent primary active transporter, but trandolapril and the other ACE inhibitors are not (14, 15). In fact, inhibition of ACE activities in all tissues might be weaker by temocapril than trandolapril, although temocapril is more rapidly excreted in bile than trandolapril (14). On the other hand, perindopril, which has the same levels of lipophilicity, caused a stronger inhibition of ACE activities in all tissues than did temocapril or enalapril. In plasma, perindopril continued to inhibit ACE activity in plasma even 48 h after the last treatment, while in treatments with temo-

Table 1. Effects of ACE Inhibitors in Body Weight, Brain Weight, Heart Weight and 2 Kidneys Weight

	Control	Trandolapril	Temocapril	Perindopril	Enalapril
Body weight (g)	328 ± 4	325 ± 2	328 ± 6	323 ± 2	329 ± 2
Brain weight (g)	1.91 ± 0.02	1.88 ± 0.02	1.90 ± 0.01	1.90 ± 0.01	1.89 ± 0.01
Heart weight (g)	1.23 ± 0.05	1.11 ± 0.02*	1.20 ± 0.02	1.08 ± 0.02**	1.19 ± 0.03
Kidneys weight (g) (2 kidneys)	2.28 ± 0.05	2.26 ± 0.02	2.31 ± 0.03	2.30 ± 0.04	2.27 ± 0.03

ACE, angiotensin converting enzyme. * $p < 0.05$ and ** $p < 0.01$ vs. Control.

Fig. 4. The ratios of heart weight to body weight in the control group (C), and trandolapril (Tr)-, temocapril (Te)-, perindopril (Pe)- and enalapril (En)-treated groups. * $p < 0.05$ and ** $p < 0.01$ vs. the control group.

may play an important role in suppressing the progression of cardiac hypertrophy. In contrast, 2-week-treatments with trandolapril and perindopril significantly reduced cardiac hypertrophy. However, the blood pressure after treatments with perindopril and trandolapril tended to be lower than that after treatments with enalapril and temocapril, although this difference was not significant. Therefore, the suppression of cardiac weight by treatments with perindopril and trandolapril may be responsible for the hypotensive action of these agents. Kim *et al.* (23) reported that administration of high doses of amlodipine for 4 weeks suppressed cardiac hypertrophy in SHR-SP, but this suppression by amlodipine was significantly weaker than that by ACE inhibitors which exhibit the same degree of hypotensive action. This report suggests that the inhibition of cardiac ACE may be more important than the hypotensive action for the suppression of cardiac hypertrophy. We also found that ACE inhibitors that inhibited cardiac ACE activity significantly decreased cardiac weight, while ACE inhibitors that did not inhibit cardiac ACE activity did not. This result suggests that the suppression of cardiac ACE activity, in addition to the hypotensive action, may be important for the suppression of cardiac weight.

Kinoshita *et al.* (24) reported that when tissues are extracted, ACE inhibitors with strong affinity to tissue ACE retain their inhibitory effect, while ACE inhibitors with weak affinity to tissue ACE lose their inhibitory effect. This finding suggests that in agents with weak tissue affinity, such as enalapril, the binding between ACE and the inhibitor is disrupted when ACE is extracted from tissues. In the present study, enalapril suppressed approximately 95% of renal ACE activity 3 h after the final administration, indicating that the extraction alone, or at least the extraction method that was employed in the experiment, did not eliminate ACE inhibitors, even those with weak affinity such as enalapril. However, it is possible that the inhibitory effect may be more potently estimated in ACE inhibitors with strong tissue affinity than in inhibitors with weak affinity. It is therefore necessary to take into consideration the effect of the method of ACE extraction from tissues when studying the tissue ACE inhibition by ACE inhibitors.

In the present study, we studied each of the four ACE inhibitors at only one dose. In our previous study on the hypotensive effects of a single administration of trandolapril and enalapril to SHR, we reported that not only the hypotensive effects but also the inhibition of tissue ACE activity occurred dose-dependently (1). It is possible that enalapril and temocapril, which were found to exert weak tissue ACE inhibition in the present study, may show stronger tissue ACE inhibition when used in higher concentrations. Moreover, it is possible that the effects of these inhibitors on tissue ACE inhibition differ between rats and humans. Further studies using different doses and species will be needed.

In conclusion, inhibition of ACE activity in vascular tissues may be the most important mechanism for the antihy-

pertensive effects of ACE inhibitors, and inhibition of ACE activity in the heart may contribute to the suppression of cardiac hypertrophy.

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