Trandolapril Reduces Infarction Area after Middle Cerebral Artery Occlusion in Rats

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In this study, we investigated whether angiotensin-converting enzyme (ACE) is involved in the progression of cerebral infarct lesions after middle cerebral artery (MCA) occlusion in rats. After placebo or trandolapril was administered orally for 7 days, we infarcted in the territory of the right MCA by extracranial vascular occlusion and studied the effect of trandolapril on brain ACE activity and infarct size 7 days after MCA occlusion. In placebo-treated rats, brain ACE activity in the infarct side was increased by a significant 1.34-fold compared with that in the non-infarct side 7 days after MCA occlusion. Brain ACE activities in the infarct sides were suppressed to 39.8% by trandolapril treatment. The ratios of unilateral infarcts to the total coronal sectional areas in placebo- and trandolapril-treated rats were 48.1 ± 3.3% and 37.4 ± 2.3%, respectively, and the difference between these values was significant. These results demonstrate that inhibition of the increased brain ACE activity in infarct lesions can reduce the infarction area after MCA occlusion. *(Hypertens Res 2002; 25: 583–588)*

**Key Words:** angiotensin converting enzyme, cerebral infarction, macrophage, inhibitor

**Introduction**

Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxylase which converts angiotensin I to angiotensin II, and this enzyme is known to be expressed in endothelial cells, macrophages and smooth muscle cells (1, 2). Angiotensin II plays a crucial role in tissue remodeling via increasing growth factors, cytokines, chemokines and extracellular matrix, in addition to regulation of blood pressure (2–4). In particular, after myocardial infarction or in atherosclerotic lesions, macrophages that express ACE are activated when angiotensin II stimulates the angiotensin II type 1 (AT 1) receptors on their surfaces, suggesting that ACE in macrophages promotes the conversion of angiotensin I to angiotensin II and that the generated angiotensin II accelerates the activation of macrophages (5–8). Therefore, the inhibition of ACE may result in the inactivation of macrophages. Macrophages contain inflammatory-induced substances such as superoxide, cytokines and chemokines, all of which play important roles in tissue damage and the accumulation of macrophages when macrophages are activated (6, 9). The tissue protection afforded by ACE inhibition may be dependent on the inactivation of macrophages and a reduction of the accumulation of macrophages in lesions with inflammation. In fact, after myocardial infarction, ACE inhibition has been shown to suppress the accumulation of macrophages and to inhibit cardiac remodeling (10–12). On the other hand, in cerebral infarction after middle cerebral artery (MCA) occlusion, an accumulation of macrophages was observed at the border between the infarct and non-infarct areas, and such an accumulation may induce the expansion of cerebral infarction (13–15). However, it remains unclear whether ACE is expressed in macrophages after cerebral infarction and whether it promotes tissue damage.

The renin-angiotensin system plays an important role in...
several functions, such as in drinking behavior and anti-opioid action in the central nervous system (15–17). Previously, we demonstrated that treatment with trandolapril (5 mg/kg, p.o.) for 7 days induced an antiinflammatory effect along with a suppression of brain ACE activity (17), suggesting that trandolapril can penetrate the blood-brain barrier. However, the role of ACE on cerebral damage after cerebral infarction is unclear. In this study, we investigated whether brain ACE inhibition by trandolapril treatment influences infarct size after MCA occlusion in rats.

Materials and Methods

Animals
Male Wistar Kyoto (WKY) rats weighing 280–310 g were purchased from Japan SLC (Shizuoka, Japan). All rats were housed individually in metabolic cages for measurement of urinary albumin 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).

MCA Occlusion Model
Focal cerebral ischemia was accomplished by using the intraluminal filament model (4–0 nylon monofilament suture) of proximal MCA occlusion as described previously (18, 19). The right common carotid artery was exposed through a lateral incision, separated from the vagus nerve, and ligated. For the permanent MCA occlusion, a 4–0 nylon monofilament whose tip was rounded by heating was introduced from the bifurcation of the internal carotid artery and advanced until resistance was felt. By means of this procedure, each rat had an infarct area of similar size after the MCA occlusion was completed. Rectal temperature was monitored routinely to maintain body temperature between 36.75 °C and 37.25 °C during the surgical procedure.

Effect of Trandolapril in Normal Rats
To determine the inhibitory effect of trandolapril on brain ACE activity in normal rats, WKY rats were divided into 2 groups, a placebo-treated group and a trandolapril (5 mg/kg per day)-treated group. After placebo or trandolapril was administered for 7 days, the body weights of the rats were measured, and then a PE-10 catheter (Clay Adams, Parsippany, USA) was inserted into the left femoral artery, connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo, Japan), and used to measure systolic blood pressure. After this measurement, the animals were anesthetized with 35 mg/kg of sodium pentobarbital intraperitoneally (i.p.). A blood sample was obtained through a catheter from the aorta, and the brain was harvested for the analysis of ACE activity.

Effect of Trandolapril after MCA Occlusion
To study the effect of trandolapril on cerebral infarction after MCA occlusion, 7 days after treatment with placebo or trandolapril, the territory of the right MCA was infarcted by extracranial vascular occlusion, and then the rats were kept in cages for 7 days without placebo or trandolapril treatment. After anesthesia (35 mg/kg of sodium pentobarbital, i.p.), a blood sample was collected and the brain was removed for analysis of ACE activity and for the histological study.

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. Brain extracts for measurement of ACE activity were prepared as described previously (20). First, the forebrain, separated from the cerebellum, was cut in half into left and right hemispheres sections, and each section was minced and homogenized in 5 volumes (w/v) of 20 mmol/l Tris–HCl buffer, pH 8.3, containing 5 mmol/l Mg(CH3COO)2, 30 mmol/l KCl, 250 mmol/l sucrose and 0.5% NP-40. The homogenate was centrifuged at 20,000 × g 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 was incubated for 30 min at 37 °C with 5 mmol/l HHL in 250 μl of 10 mmol/l phosphate buffer, pH 8.3, containing 0.3 mol/l NaCl. The reaction was terminated by addition of 750 μl of 3% metaphosphoric acid, and then the mixture was centrifuged at 20,000 × g for 5 min at 4°C. The supernatant was applied to a reversed-phase column (4 mm i.d. × 250 mm; IRICA Instrument, Kyoto, Japan) that had been equilibrated with 10 mmol/l KH2PO4 and CH3OH (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 μmol hippuric acid/min.

Histological Study
For histological study, other groups of animals were used. The brain segments were fixed in 10% neutral buffered formalin overnight and embedded in paraffin. Two coronal blocks were taken from the forebrain region at 6 mm and 8 mm from the tail of a pineal body, and sections of 5 μm thickness were cut from each block. To determine the infarct and non-infarct areas, the sections were stained with hematoxylin-eosin. The morphometric measurement of the manually outlined surface area was performed using an image an-
alyzing system (VM-30; Olympus Optical Co., Tokyo, Japan).

Statistical Analysis

All data are expressed as the means ± SE. Statistical significance was determined with Student’s t-test. Values of \( p < 0.05 \) were considered to indicate statistical significance.

Results

Effects of Trandolapril on Body Weight, Systolic Blood Pressure and ACE Activity in Normal Rats

Body weights of 292 ± 3 and 297 ± 5 g in normal rats before treatment with placebo and trandolapril were not changed by these treatments for 7 days (Table 1). The systolic blood pressure of 112 ± 4 mmHg in the pretreated rats tended to decrease by treatment with trandolapril, but the difference was not significant. The diastolic blood pressure in the pretreated rats was 79 ± 3 mmHg, and that in the rats treated with trandolapril for 7 days was 77 ± 3 mmHg; the difference was not significant.

Plasma ACE activities in the placebo- and trandolapril-treated rats were 2.19 ± 0.04 and 0.67 ± 0.05 mU/mg protein, respectively, and this difference was significant (Fig. 1). In the placebo-treated rats, the ACE activities of the left and right brain segments were 3.21 ± 0.14 and 3.20 ± 0.15 mU/mg protein, respectively (Fig. 1). On the other hand, in trandolapril-treated rats, the ACE activities of left and right brain segments were 0.63 ± 0.04 and 0.72 ± 0.03 mU/mg protein, respectively (Fig. 1). The ACE activities in both the left and right segments in the trandolapril-treated rats were significantly decreased compared with these activities in the placebo-treated rats.

Effects of Trandolapril on ACE Activities after MCA Occlusion

In placebo-treated rats, the ACE activities of the left (non-infarct side) and right (infarct side) brain segments were 3.51 ± 0.06 and 4.69 ± 0.11 mU/mg protein, respectively, and this difference was significant (Fig. 2). The brain ACE activity in the placebo-treated rats before MCA occlusion was regarded as 100%, and 7 days after MCA occlusion, this brain ACE activity was 134%.

Table 1. Effects of Trandolapril on Body Weight, Systolic Blood Pressure and Diastolic Blood Pressure in Normal Rats

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<tr>
<td>Body weight (g)</td>
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<td>Systolic blood pressure (mmHg)</td>
<td>113 ± 3</td>
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<td>Diastolic blood pressure (mmHg)</td>
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the left and right brain segments were 35.0% and 39.8%, respectively, in comparison to the 100% values of the placebo-treated animals (Fig. 3).

**Effects of Trandolapril on Infarct Area after MCA Occlusion**

The ratios of the infarct areas to the total right-side areas of the brain in the placebo-treated rats 7 days after MCA occlusion were 48.1 ± 3.3% and 68.1 ± 3.2% at 6 mm and 8 mm, respectively, whereas each of these ratios was significantly reduced to 37.4 ± 2.3% and 51.0 ± 0.6%, respectively, by treatment with trandolapril (Fig. 4).

**Discussion**

In this study, we demonstrated for the first time that the brain ACE activity in the infarct side was significantly increased compared with that in the non-infarcted side 7 days after MCA occlusion. However, the brain ACE activity was significantly suppressed by treatment with trandolapril (5 mg/kg per day, p.o.) for 7 days, and this significant suppression remained in both sides 7 days after withdrawal of trandolapril. In agreement with the present results, repeated treatments with high lipophilic ACE inhibitors such as trandolapril have been reported to significantly suppress brain ACE activity and to continue to suppress brain ACE activity at 7 days after withdrawal of trandolapril, despite the recovery of plasma ACE activity (21). On the other hand, in our previous study (17) and in those of other investigators (22, 23), treatment with hydrophilic ACE inhibitors such as enalapril did not inhibit brain ACE activity, despite their inhibition of ACE in plasma and peripheral tissues. Previously, we reported that repeated treatment with trandolapril has an antinociceptive effect in addition to suppressing brain ACE activity, but that treatment with enalapril neither exerts an antinociceptive effect nor inhibits brain ACE activity, despite its suppression...
of ACE activities in plasma and peripheral tissues (17). Therefore, lipophilic ACE inhibitors such as trandolapril, ramipril and perindopril should be used for studying the role of ACE in cerebral tissues (17, 23). In this study, trandolapril, an ACE inhibitor that can penetrate the blood-brain barrier, reduced the brain ACE activity that had increased after MCA occlusion, and also decreased the size of the infarct area.

Macrophages have been reported to increase at the border between infarct and non-infarct areas after MCA occlusion (13, 14). The increase of ACE expression in macrophages is thought to activate macrophages when angiotensin II formed by ACE stimulates AT 1 receptors on the surface of macrophages (7). The activated macrophages induce nuclear factor-κB, and this in turn induces an inflammatory cytokine, interleukin-1, and a chemokine, monocyte chemoattractant protein (MCP)-1 (24, 25). It is well known that accumulated macrophages at the border of infarct lesions promote an increase of superoxide anions, which also induce tissue damage (26, 27). AT1 receptor antagonists and ACE inhibitors have been shown to reduce the accumulation of macrophages (28, 29). The accumulation of macrophages at the border of infarct lesions may contribute to the expansion of infarct lesions, especially in the late ischemic period after MCA occlusion. In the present study, the reduced accumulation of macrophages via trandolapril-induced inhibition of brain ACE activity may have contributed significantly to the suppression of the infarct area after MCA occlusion.

On the other hand, an ACE (kininase II) has also been shown to degrade bradykinin to inactive forms, and bradykinin levels are known to increase by treatment with ACE inhibitor—an increase that induces tissue protection via an increase of nitric oxide (30). In hypertensive patients, the lower limit of cerebral blood flow autoregulation is shifted toward higher blood pressure levels (31). ACE inhibitors are reported to shift the lower limit of the cerebral autoregulation curve to lower pressure levels and to be useful for the treatment of hypertensive patients with cerebrovascular disorders (32, 33). Recently, Takada et al. (34) reported that the protective effect of an ACE inhibitor on cerebrovascular disorders in hypertensive patients was dependent on the increase of bradykinin. However, in this study we used normotensive animals, and the trandolapril dose did not significantly decrease either systolic or diastolic blood pressures. Therefore, nitric oxide, and not blood pressure change, induced by the bradykinin increase may be involved in the cerebral protection afforded by treatment with trandolapril after MCA occlusion.

Angiotensin II is well known to induce a contractile response in various isolated arteries, but not in isolated cerebral arteries (35). For example, perindopril does not reduce cerebral blood flow in spite of its penetration of the blood-brain barrier, and the Perindopril Protection Against Recurrent Stroke Study (PROGRESS) and animal studies suggest that perindopril may have beneficial effects on cerebral arter}

References


