Shimane J. Med. Sci., Vol.17 pp.51-59, 1999

EFFECTIVE INHIBITION OF TISSUE ANGIOTENSIN-CONVERTING ENZYME ATTENUATES PRESSURE-OVERLOAD AORTIC HYPERTROPHY IN EARLY STAGE IN RATS

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(Accepted October 12, 1999)

Trandolapril, an angiotensin-converting enzyme (ACE) inhibitor, has been reported to have higher efficiency on tissue ACE than other ACE inhibitors. In this study, effect of trandolapril on pressure-overload cardiovascular hypertrophy in early stage was examined in rats by comparing with the effect of enalapril. Rats with abdominal aorta banded or sham-operated were orally treated with trandolapril (1 mg/kg/day), enalapril (20 mg/kg/day), or vehicle for 2 weeks after the surgical maneuvers. In vehicle-treated rats, the banding raised intra-aortic pressure, maximum velocity of pressure rise, left ventricular (LV) weight, LV ACE activity, and aortic mass. Although both ACE inhibitors equally reduced the aortic pressure, only trandolapril elicited a significant prevention of the aortic hypertrophy. In contrast, cardiac hypertrophy was insignificantly prevented by either inhibitor. The LV ACE activity was completely inhibited by trandolapril, whereas insignificantly suppressed by enalapril. These results suggest that effective inhibition of tissue ACE attenuates the 2-week pressure overload-induced hypertrophy of the aorta but not of the LV.

Key words: rat / aortic banding / cardiovascular hypertrophy / trandolapril / enalapril / organ difference

A number of clinical evidence has shown that cardiovascular hypertrophy is a substantial risk factor of morbidity and mortality from cardiovascular diseases including heart failure, myocardial infarction, and cardiac arrhythmia (1, 2). If cardiac hypertrophy is present, incidence of other cardiovascular diseases are increased by several times (3-5). In addition, cardiac hypertrophy is a morbid state resulting from over-adaptation of the heart to pressure load and is recognized as a direct cause of cardiac death (6). On the other hand, hypertrophy of large artery is also reported to be a risk of cardiovascular diseases such as arteriosclerosis, atherosclerosis, and aortic aneurysm (7-9). Consequently, it should be of great importance to prevent cardiovascular hypertrophy for improvement of the long-term prognosis of cardiovascular diseases.

As described above, pressure overload is one of the factors which develop cardiovascular hypertrophy. Therefore, antihypertensive agents of several different classes are capable of reducing the hypertrophy presumably in part by reducing pressure load. In addition, angiotensin-converting enzyme (ACE) inhibitors are suggested to be the most effective in regressing left ventricular (LV; 10-12) and vascular hypertrophy (13, 14) among antihypertensive agents of different classes when compared at equally antihypertensive doses. The reason for the differences is supposed that the tissue ACE which is upregulated by hypertensive stress in the heart (15) and large vessels (16-18) may aggravate cardiovascular hypertrophy by producing angiotensin II which has potent growth-promoting activity (19-21). Actually, we measured ACE activity quantitatively in hypertensive LV and aorta and demonstrated excess production of ACE in rat tissues (22). Furthermore, by administering trandolapril for 8 weeks and estimating the long-term effects, we suggested differential roles of the upregulated tissue ACE in the development of cardiovascular hypertrophy and in the cardiac collagen synthesis: the upregulated ACE is an important yet not an essential factor for cardiac hypertrophy, whereas it is the critical factor for LV collagen accumulation and aortic hypertrophy over a long-range period (22). However, there have been few studies that report the role of tissue ACE in cardiovascular hypertrophy in the early stage.

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To address this question, we established the rat models with their LV and upper aorta exposed to pressure overload by narrowing the abdominal aorta, and analyzed antihypertrophic effects of ACE inhibitors in cardiovasculature in the early stage (within 2 weeks). The present study employed trandolapril for its high efficiency in tissue ACE inhibition, while enalapril was used as a reference drug because it would be much less effective in inhibiting tissue ACE activity despite equal antihypertensive effect (23; Fig. 1). Taking advantage of the obvious difference in efficiency between these 2 agents and comparing the present data with those from our previous study in which these 2 ACE inhibitors were administered for 8 weeks (22), we intended to clarify the effects of ACE inhibitors in pressure overload cardiovascular hypertrophy as well as the role of tissue ACE therein in the early stage (2 weeks after aorta coarctation).

MATERIALS AND METHODS

Aortic banding

Male Wistar rats (8-week-old) weighing 210 to 250 g (SLC, Shizuoka) were randomly allocated into 6 groups. Groups 1, 2, and 3 underwent coarctation of the abdominal aorta (banded groups, n=5 each). Surgical maneuvers were performed as described previously (22). Briefly, under pentobarbital anesthesia (40 mg/kg b.w., i.p.), abdominal aorta was constricted at 3 sites in the area between the right renal artery and the superior mesenteric artery. Groups 4, 5, and 6 (n=5 each) were sham-operated groups in which the aorta was only exposed but no narrowing was made. The present study was conducted in line with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society and by the Committee of Shimane Medical University.

Administration of ACE inhibitors

Drug treatment by gavage via a stomach catheter (once daily) began on the day after the above mentioned surgery and lasted for 2 weeks. Groups 1 and 4 received the vehicle (0.25% carboxymethylcellulose-Na [CMC-Na] solution containing 1% dimethyl sulfoxide [DMSO]). Groups 2 and 5 received enalapril (20 mg/kg/day), while groups 3 and 6 were given trandolapril (1 mg/kg/day). Both of the ACE inhibitors were prepared as stock solutions in neat DMSO, the stock solutions which were diluted with CMC-Na solution to appropriate concentrations immediately before the daily dosing.

Hemodynamic measurements and tissue preparation

At 24 hrs after the final dosing of 2-week treatment, rats were anesthetized with pentobarbital (40 mg/kg, i.p.) and were inserted a polyethylene catheter into their right common carotid artery so that tip of the catheter was placed immediately above the aortic valve. Through this catheter which was connected to a pressure transducer (DX-312; Nihon Kohden, Tokyo), intra-aortic blood pressure (BP), maximum

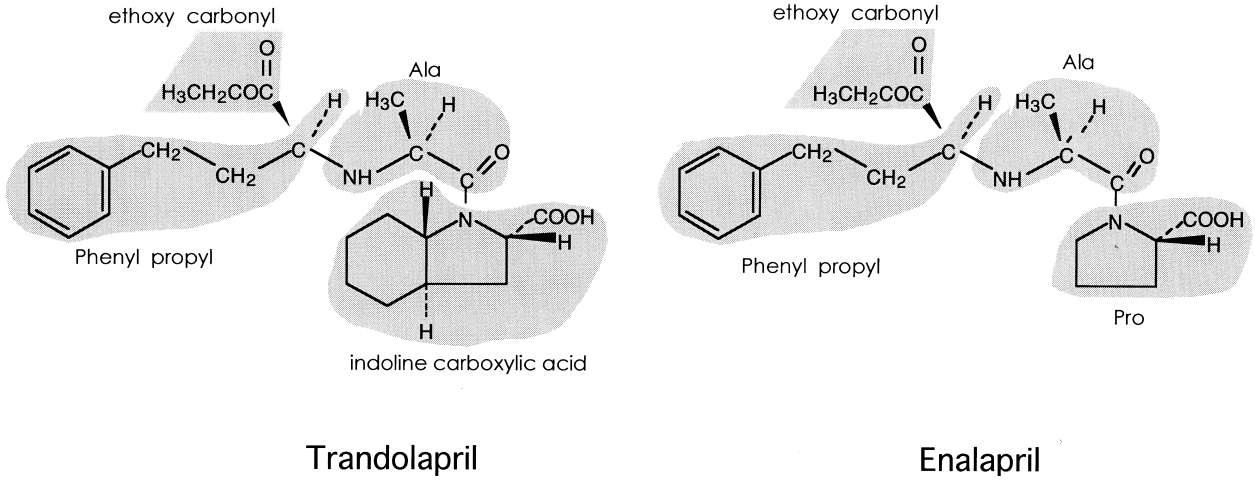

Fig. 1. Chemical structures of trandolapril and enalapril. The major and only one difference of trandolapril from enalapril is indolinecarboxylic acid substitution for proline. Due to this substitution, trandolapril is several times more liphophilic and tissue-penetrative than enalapril.

velocity of pressure rise (Ao dp/dt_{max}), and heart rate (HR) were measured by use of an pressure amplifier (AP-621G; Nihon Kohden), a differential operator unit (EQ-601G; Nihon Kohden), and a HR counter (AT-621G; Nihon Kohden).

After these measurements were accomplished, rats were sacrificed by an intra-arterial injection of 1 M KCl (1.5 ml) and then the heart and upper aorta including the aortic arch and thoracic aorta (down to the diaphragm) were excised. Wet weights were measured for the LV and upper aorta.

A cross-section of LV with 1 mm thickness was taken from the mid portion and the specimens of the LV and upper aorta were fixed in neutral buffered formalin. After fixation for 24 hrs, the slices of LV were photographed and the cross-sectional area of LV wall was measured by a 2-dimensional image analyzer (Nikon Cosmozone 1SA, version 2; Nikon, Tokyo). Then, the slices of LV and the aorta were embedded in paraffin to make 5 μm-thick sections, which were stained with hematoxylin-eosin and Azan for morphometric analysis. The areas of the collagen bundles in LV tissue were measured in Azan-stained sections using an image analysis processor (nexusQube; Nexus Co, Tokyo), and were expressed as a proportion to the unit LV area.

Ex vivo measurement of LV ACE activity

From the excised and weighed LV, tissue ACE was extracted together with the potentially remaining drugs within the tissue as noted previously (16). Tissues were minced and homogenized with 5-volume (v/w) of medium consisting of 20 mM Tris-HCl (pH 8.3), 5 mM Mg acetate, 30 mM KCl, 0.25 M sucrose, and 0.5% (v/v) Nonidet P-40. The homogenate was centrifuged (20,000×g for 20 min) at 4°C to obtain supernatant into which tissue ACE and residual drugs, if any, were extracted. A 50-μl aliquot of the supernatants was incubated with 5 mM hippuryl-His-Leu substrate (Peptide Institute, Minoh, Osaka) at 37°C for 30 min in the buffer containing 300 mM NaCl and 100 mM KH_2PO_4 (pH 8.3). These Cl^- concentration and pH were optimum for rat ACE activity toward the substrate (24). Reaction was stopped with 750 μl of 3% metapho-sphoric acid (Nacalai Tesque, Kyoto) and the denatured protein was removed by centrifugation (3000 rpm, 10 min). An aliquot of the supernatant was analyzed for its content of hippuric acid with a reverse-phase HPLC (LC-10AD, Shimadzu, Kyoto) equipped with a Puresil C18 column (4.6 mm i.d. × 150 mm; Waters, Milford, MA, USA) on the basis of absorbance at 228 nm. The column was eluted with a 1:1 mixture of 10 mM KH_2PO_4 (pH 3.0) and methanol at a flow rate of 0.7 ml/min with the column temperature maintained at 40°C. The blank for each sample was set by adding 5 mM EDTA-Na_2. The hippuric acid-liberating activity that was inhibitable with EDTA-Na_2 was defined as net ACE activity. One unit of ACE activity was the amount of the enzyme which produced 1 μmol/min of hippuric acid at the condition as above. Protein concentration of the tissue extracts was determined with BCA Protein Assay Reagent (Pierce, Rockford, IL, USA) according to the method by Smith *et al.* (25).

Chemicals

Enalapril maleate (Sigma Chemicals, St. Louis, MO, USA), CMC-Na (Maruishi Pharmaceuticals, Osaka), DMSO (Nacalai Tesque), and pentobarbital sodium (Dainabot, Osaka) were purchased from respective manufacturers. Trandolapril was provided from Roussel-UCLAF, Romainville, France.

Statistical analyses

The results are presented as the mean ±S.E.M. Statistical analysis was performed by Student's *t* test when a single treatment group was compared with a control group. Multiple comparison was made by ANOVA and *post-hoc* Bonferroni/Dunn's test. A difference was considered significant when $P<0.05$.

RESULTS

Systemic condition

After random allocation, there was no significant difference in body weight among the 6 groups. The body weight after 2-week treatment did not differ among 3 different treatment groups within the same subclass either banded or sham-operated. Neither symptoms nor organ abnormalities indicating congestive heart failure (dyspnea, edema, ascites) were evident in any of the animals at the end of experimental period.

Hemodynamics

In the [banded + vehicle] group, intra-aortic mean BP was increased by 43 mmHg compared with the [sham + vehicle] group and the increase in BP due to aortic banding was not suppressed significantly by the 2-week treatment with trandolapril or enalapril (Fig. 2A). Neither coarctation nor drug treatments resulted in significant alterations in the HR at the end of experimental period (Fig. 2B). The Ao dp/dt_{max}, an index of pressure overload onto the LV and upper aorta, was evidently increased by 1760 mmHg/s in the [banded + vehicle] group (Fig. 2C). Both enalapril and trandolapril lowered the increase in this index to the same extent, but failed to reverse the index completely to the normal level.

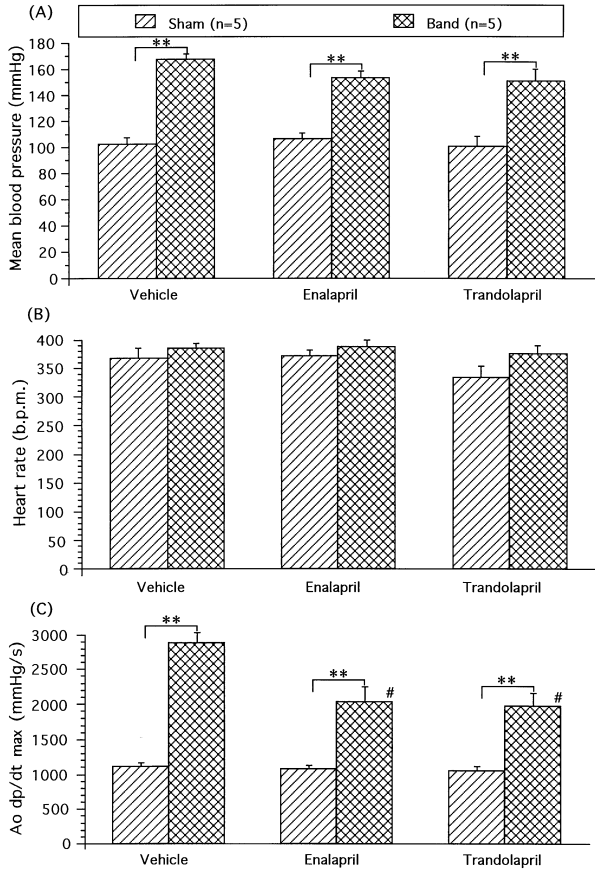

Fig. 2. Effects of abdominal aortic banding on aortic mean blood pressure (A), heart rate (B), and the maximum velocity of intra-aortic pressure rise (Ao dp/dt_{max}) (C), which were directly measured via intra-aortic catheter, and the influence thereon of enalapril and trandolapril treatments. Sham: 2 weeks after sham-operation (n=5 each). Band: 2 weeks after aortic banding (n=5 each). Data are shown as mean ±S.E.M. **P<0.01 vs. sham-operated group. #P<0.05 vs. [banded + vehicle] group.

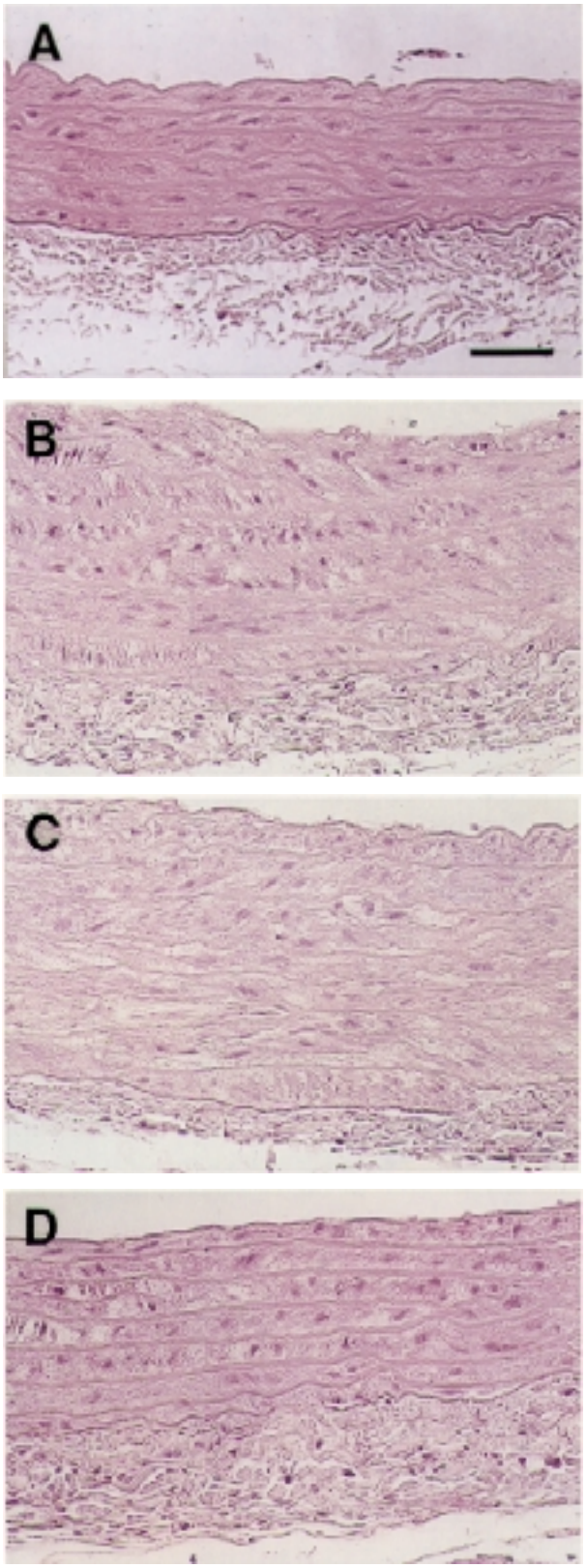

Fig. 3. Histological appearances of upper aorta (the section of 5 mm under the arch; hematoxylin eosin-stained) treated with vehicle, enalapril, or trandolapril for 2 weeks. A: Sham-operated group treated with vehicle. B: Aorta-banded group treated with vehicle. C: Aorta-banded group treated with enalapril. D: Aorta-banded group treated with trandolapril. Bars indicate 50 μm (original magnification=×100).

Morphological changes in the upper aorta

In comparison with the [sham + vehicle] group (Fig. 3A), the [banded + vehicle] group showed a remarkable thickening of aortic wall: increases in size and number of smooth muscle cells and disarrangement of smooth muscle cells were microscopically observed in tunica media of the cross-section (Fig. 3B). Enalapril showed little inhibitory effect on these morphological changes (Fig. 3C), while trandolapril inhibited the development of these alterations apparently (Fig. 3D). The weight of the upper aorta per unit length was increased significantly (73%) in the [banded + vehicle] group as compared with the [sham + vehicle] group (Fig. 4). Trandolapril suppressed the increase in aortic weight significantly, whereas enalapril did so insignificantly.

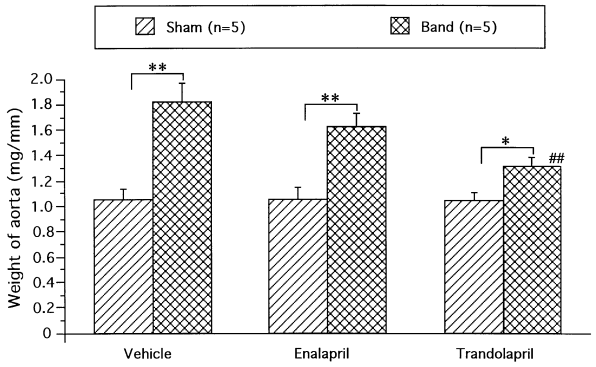

Fig. 4. Effects of abdominal aortic banding on the weight of upper aorta per mm length and the influence thereon of enalapril and trandolapril treatments. Sham: 2 weeks after sham-operation (n=5 each). Band: 2 weeks after aortic banding (n=5 each). Data are shown as mean±S.E.M. *P<0.05, **P<0.01 vs. sham-operated group. ##P<0.01 vs. [banded + vehicle] group.

Morphological changes and ACE activity in the LV

In comparison with the [sham + vehicle] group (Fig. 5A), the [banded + vehicle] group showed microscopic hypertrophy of cardiomyocytes (Fig. 5B). In the groups observed for 8 weeks (for reference), the [banded + vehicle] group showed not only cardiac hypertrophy but also marked accumulation of collagen bundles in the ventricular interstitium (Fig. 5D) as compared with other groups (Figs. 5A-C). In consistence with the morphological alterations, fractional area of collagen (percentage relative to the total LV area) was the highest in [banded + vehicle] group after 8 weeks among the four groups (Fig. 6). Other three groups showed very low collagen area.

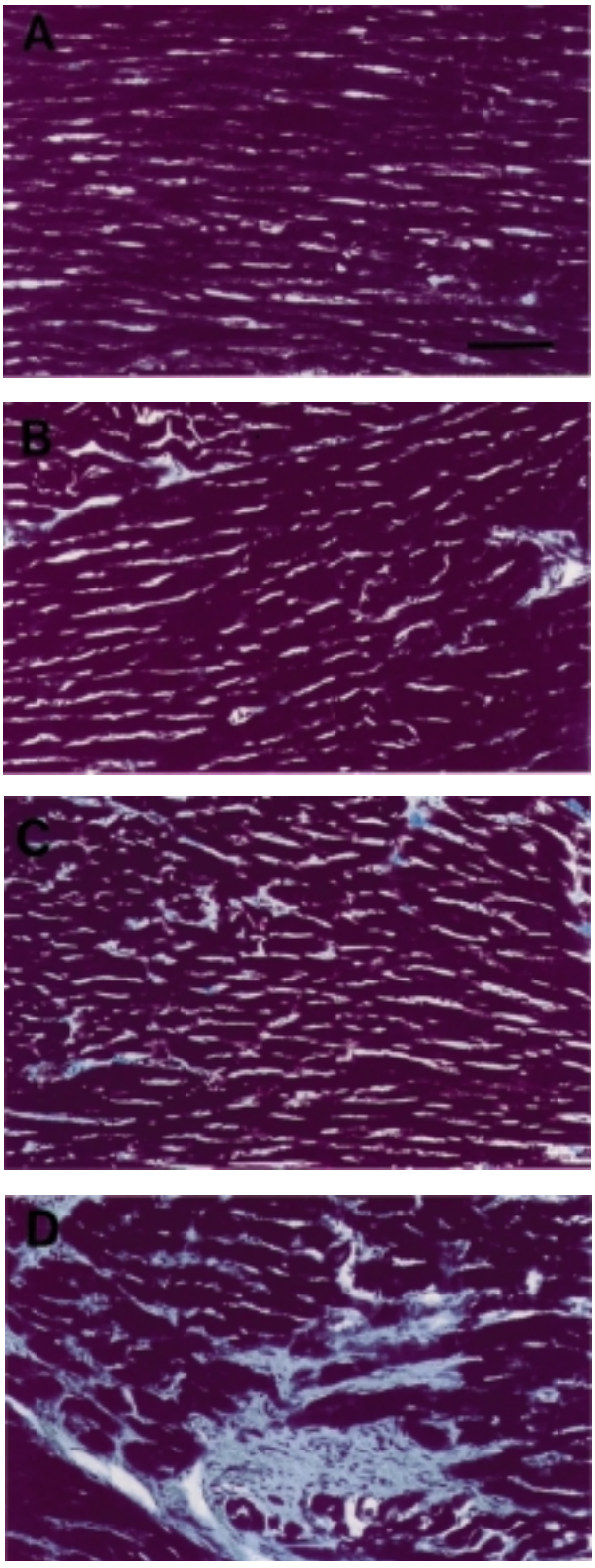

Fig. 5. Effects of abdominal aortic banding on histo-logical appearances of left ventricle (LV; coronal section of middle LV portion, Azan-stained). A: Sham-operated group tested for 2 weeks. B: Aorta-banded group tested for 2 weeks. C: Sham-operated group tested for 8 weeks. D: Aorta-banded group tested for 8 weeks. The data for C & D are adapted from our previous data (Wang *et al*., 1999). Bars indicate 100 μm (original magnification=×50).

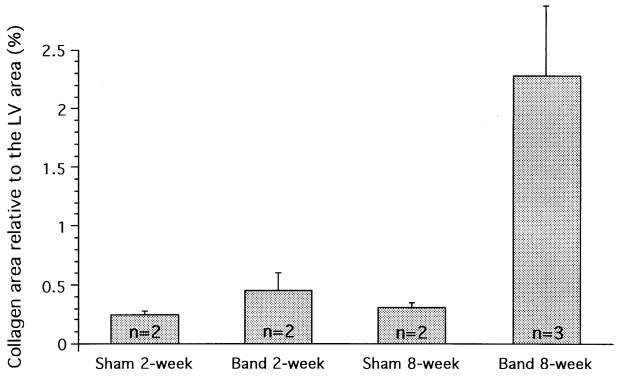

Fig. 6. Effects of abdominal aortic banding on collagen areas relative to the left ventricle (LV) area. Coronal sections of the middle LV portion which were Azan-stained were used. Sham 2-week: Sham-operated group tested for 2 weeks (n=2). Band 2-week: Aorta-banded group tested for 2 weeks (n=2). Sham 8-week: Sham-operated group tested for 8 weeks (n=2). Band 8-week: Aorta-banded group tested for 8 weeks (n=3). Data are shown as mean ±S.E.M. All the data as to 8-week were adapted from our previous study (Wang *et al.*, 1999).

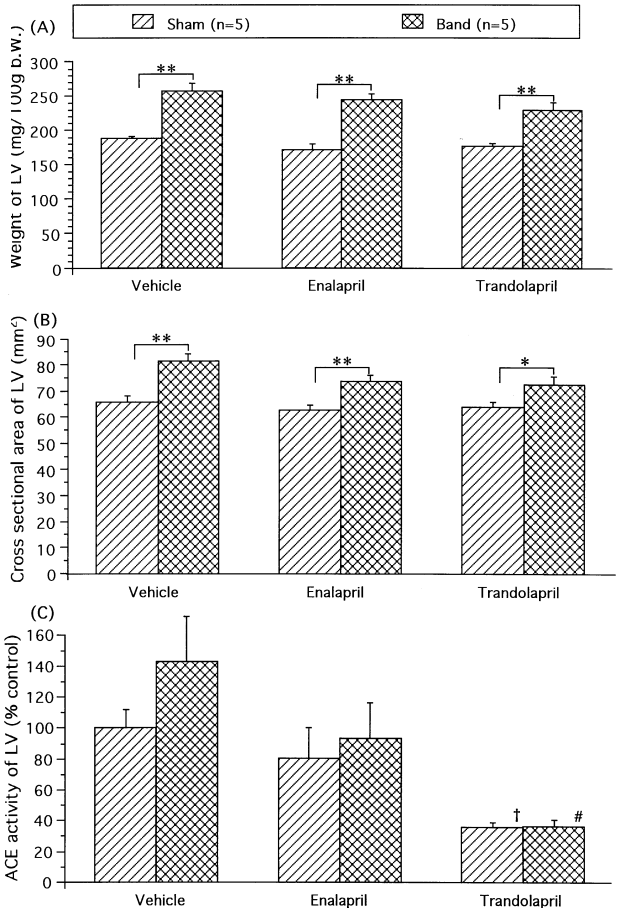

Fig. 7. Effects of abdominal aortic banding on left ventricular (LV) weight (A), LV cross sectional area (B), and LV ACE activity (C) and the influence thereon of enalapril and trandolapril treatments. Each LV ACE activity is expressed as % relative to the activity in the [sham + vehicle] group. Sham: 2 weeks after sham-operation (n=5 each). Band: 2 weeks after aortic banding (n=5 each). Data are shown as mean ±S.E.M. *P<0.05, **P<0.01 vs. sham-operated group. #P<0.05 vs. [banded + vehicle] group. †P<0.05 vs. [sham + vehicle] group.

In the [banded + vehicle] group, the LV weight was increased by 37% and cross-sectional area of LV wall was increased by 24% compared with the [sham + vehicle] group, respectively (Figs. 7A and B). Administration of either enalapril or trandolapril for 2 weeks insignificantly suppressed the cardiac hypertrophy. The LV ACE activity was increased by 43% in the [banded + vehicle] group, although it was not significantly different from that of the [sham + vehicle] group (Fig. 7C). The LV ACE activity that was measured 24 hrs after the final drug treatment revealed that enalapril did not significantly inhibit the activity of the banded group, whereas trandolapril completely suppressed the ACE activity to the level below that of the vehicle-treated heart.

DISCUSSION

In the present study, the pressure overload raised the intra-aortic BP, Ao dp/dt_{max} and ACE activity, and also induced the hypertrophy of the upper aorta and LV. Microscopically, obvious hypertrophy of vascular smooth muscle cells and cardiomyocytes was confirmed in the [banded + vehicle] group. The hypertrophy of cardiomyocytes was almost to the same extent that of our previously experiment, i.e. pressure overload for 8 weeks (22). On the other hand, accumulation of collagen in the LV was much less in the 2-week group than in the 8-week group. The aortic hypertrophy occurring within 2 weeks was prevented significantly by trandolapril, but not by enalapril, whereas the LV hypertrophy was not significantly prevented by either ACE inhibitor. These results suggest that trandolapril represents an effective antihypertrophic action in the early stage of aortic hypertrophy as well as in the later stage.

Quantitative alterations of aortic ACE activity were not measured in the present study, however, the activity was measured in our previous experiment for 8 weeks (22). Our previous measurement demonstrated that the % increase in aortic ACE

activity induced by aortic banding was as high as 265%. The results have led us to consider that the tissue ACE is an important factor so far as the development of aortic hypertrophy is concerned. Previous *in vitro* and *in vivo* studies (26, 27) have indicated that angiotensin II is capable of accelerating the proliferation of vascular smooth muscle cells and thereby support our contention as above. Furthermore, Clozel *et al*. (13) and Thybo *et al*. (14) compared the effects of ACE inhibitors on hypertensive vascular hypertrophy with those of other classes of antihypertensive agents. They reported that treatments with ACE inhibitors showed more pronounced normalization of vascular remodelling than did other classes of agents with equally hypotensive effects. These results are also consistent with our contention that the inhibition of aortic ACE activity is more directly related to the antihypertrophic effect than is the reduction of aortic blood pressure.

Antihypertrophic effects of enalapril and trandolapril on rat aorta were compared in the present study. Because both ACE inhibitors lowered the pressure overload to the same extent, the differential effects on aortic hypertrophy between the 2 agents may be attributed to the difference in their inhibitory efficiency on tissue ACE activity. The difference of trandolapril's chemical structure from that of enalapril is indolinecarboxylic acid moiety in place of proline moiety which is intrinsic to enalapril (Fig. 1). As predicted from this chemical substitution, trandolapril is several times more lipophilic, thereby can more readily distribute into the tissue than enalapril (23). In addition, as judged from the IC_{50} and Ki values of both drugs (23), trandolaprilat (active diacid form of trandolapril) is shown to have 3-fold higher affinity for the active site of the ACE molecule than enalaprilat (active diacid form of enalapril). Therefore, trandolapril's higher efficiency in tissue ACE inhibition can be explained by not only its high tissue-distributability but also by its high affinity to ACE molecules. Indeed, in this study, trandolapril, not enalapril, showed an antihypertrophic effect on pressure-overloaded aorta at 2 weeks after banding. In contrast to the present 2-week administration study, both enalapril and trandolapril prevented the aortic hypertrophy completely in the 8-week administration study (22). In that condition, both ACE inhibitors suppressed the aortic ACE activity significantly, although trandolapril showed a more marked ACE inhibition than did enalapril. These results indicate that trandolapril (high efficiency on tissue ACE) prevents the development of vascular hypertrophy in the early phase as well as in the late phase, while enalapril (low efficiency) exerts its antihypertrophic action only in the late phase.

In contrast to the case of aortic hypertrophy, even trandolapril failed to prevent the LV hypertrophy. The result may be ascribed in part to the fact that the drug could not totally remove the pressure load onto the LV and it is also likely that pressure stress may induce other cardiotrophic factors than ACE (21, 28, 29) since the LV ACE activity was inhibited completely by trandolapril. On the contrary, in our previous observation for 8 weeks, trandolapril revealed a significant suppression of LV hypertrophy with complete inhibition of ACE activity, although the suppression of hypertrophy was partial. In addition, it can be supposed that the LV hypertrophy that is a result of acute adaptation to maintain systemic circulation is more dominant in the early stage than in the later stage. Taken all together, the antihypertrophic actions of ACE inhibitors on LV might be submerged beneath a recognizably beneficial level in the early stage.

Our previous study also reported that the accumulation of collagen in LV was significantly suppressed by 8-week treatment with trandolapril or enalapril (22). Therefore, our previous study concluded that regulation of collagen synthesis depended on tissue ACE activity more than did the hypertrophy of cardiomyocytes. But the present study did not evaluate the effects of ACE inhibitors on collagen accumulation because pressure overload for 2 weeks induced little collagen accumulation.

In conclusion, our results suggest that effective inhibition of tissue ACE attenuates the aortic hypertrophy occurring in response to pressure overload within 2 weeks, whereas antihypertrophic effect was not evident on the LV in this early stage. These characteristics of ACE inhibitors should be considered to attain more benefit for the treatment of hypertensive cardiovascular diseases.

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