An angiotensin-converting enzyme inhibitor, not an angiotensin II type-1 receptor blocker, prevents β-aminopropionitrile monofumarate–induced aortic dissection in rats

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Objective: Cystic medial degeneration (CMD) is a histologic abnormality that is common in aortic diseases such as aortic dilation, aneurysm, or dissection. Although little is known about the mechanism underlying CMD, we have previously demonstrated that angiotensin II signaling via angiotensin II type 2 receptor (AT2R) plays a central role in apoptosis of vascular smooth muscle cells (VSMCs) occurring in CMD associated with Marfan syndrome. The aim of this study is to elucidate the role of angiotensin II signaling in the pathogenesis of aortic diseases associated with CMD.

Method: We investigated the effects of angiotensin-converting enzyme inhibitor (ACEI), temocapril (n = 15), angiotensin II receptor type-1 (AT1R) blocker, CS-866 (n = 15), and vehicle control (n = 17) on 0.25% β-aminopropionitrile monofumarate (BAPN)-induced aortic dissection and histopathologic findings in a rat model.

Results: Temocapril significantly prevented aortic dissection (P < .05), CMD (P < .01), and VSMC apoptosis (P < .01) compared with vehicle control in BAPN-fed rats. However, CS-866 did not show any preventive effect. Reversed transcriptase-polymerase chain reaction demonstrated that expression of both AT1R and AT2R was detected in control rat aortas, and that AT2R expression was significantly upregulated in the aortas of BAPN-fed rats (P < .01). Blood pressure was significantly and equally lowered in both temocapril and CS-866 groups compared with control.

Conclusions: Differential expression of angiotensin II receptors and AT2R signaling are involved in the pathogenesis of CMD and aortic dissection in BAPN-fed rats. ACEIs might be of clinical value for the prevention and treatment of aortic diseases related to CMD. (J Vasc Surg 2002;36:818-23.)

Cystic medial degeneration (CMD) is a histologic abnormality of the aorta that is characterized by fragmentation of elastic fibers, fibrosis with collagen production, accumulation of amorphous matrix, and loss of cell nuclei. This histologic change is commonly seen in patients who have dilation, aneurysm, or dissection of the aorta associated with Marfan syndrome (MFS), but it is not specific to MFS and sometimes also occurs in atherosclerosis related to aging. Although little is known about the mechanism underlying CMD, we reported previously that apoptosis of vascular smooth muscle cells (VSMCs) is accelerated in MFS aortas, and also demonstrated that angiotensin II signaling via the angiotensin II type 2 receptor (AT2R) plays an important role in VSMC apoptosis occurring in CMD associated with MFS. On the basis of our previous findings, we hypothesized that angiotensin-converting enzyme inhibitors (ACEIs), but not angiotensin II type-1 receptor (AT1R) blockers, could prevent CMD and aortic dissection associated with VSMC apoptosis.

β-Aminopropionitrile monofumarate (BAPN) is known to induce aortic dissection in animals by inhibiting the cross-linking of collagen fibers. BAPN-induced aortic disease features vascular medial degeneration, fragmentation of elastic fibers, and apoptosis of VSMCs, findings that resemble the aortic changes in patients with CMD.

In the present study, to investigate our hypothesis, we examined the effects of an ACEI and an AT1R blocker on VSMC apoptosis and CMD in rats with BAPN-induced aortic dissection.

METHODS

Study protocol. Three-week-old Sprague-Dawley rats were divided into the following three groups: a temocapril (ACEI, a kind gift from Sankyo Pharmaceutical Co, Ltd.)-treated (5 mg/kg/day) group (n = 15), a CS-866 (AT1R blocker, a kind gift from Sankyo Pharmaceutical Co, Ltd.)-treated (3 mg/kg/day) group (n = 14), and a vehicle-treated control group (n = 17). These drugs were administered orally after 1 week of a diet containing 0.25% BAPN. Systolic blood pressure was measured by the indi-
rect tail cuff method at 2 weeks after the start of drug administration. Animals that died during the study were autopsied at that time, and animals that did not die were sacrificed by an overdose of sodium pentobarbital at 5 weeks after the start of drug administration. The ascending and descending aortas were harvested and immediately placed into 4% paraformaldehyde (PFA) for fixation or into normal saline for following experiments. The protocol of this study was approved by the ethics committee for animal experiments at our institution.

Histologic examination. The PFA-fixed aortas were embedded in paraffin, cut into 4 μm thick sections, and stained with hematoxylin and eosin, Masson trichrome, Alcian blue to detect mucopolysaccharides (MPS), and Victoria blue to detect fragmentation of elastic fibers. Medial degeneration was evaluated from the following histologic features: (1) destruction and fragmentation of elastic fibers demonstrated by Victoria blue staining and (2) deposition of an amorphous substance stained by Alcian blue representing MPS. The area of each of these features in cross-sections obtained from the distal part of descending aorta (undissected region) was measured and the Percent area was calculated using Density Slice of NIH image 1.61 analysis software (Dako, Kyoto, Japan). Immunohistochemical staining for smooth muscle α-actin was carried out with an anti-human smooth muscle α-actin antibody (1A4, Dako) using a LSAB kit (Dako). The investigators performing histologic evaluation were blinded to the grouping of the rats.

Evaluation of apoptosis. Terminal transferase-mediated dUTP nick end-labeling (TUNEL) was carried out to detect apoptotic VSMCs in deparaffinized 4-μm sections using an ApopTag in situ apoptosis detection kit (Oncor Inc) according to the supplier’s instructions. Sections were lightly counterstained with hematoxylin. Negative controls included omission of terminal deoxynucleotidyl transferase from the labeling mixture. Four sections were selected from the distal part of descending aorta (undissected region) and four fields per section were examined at a magnification of 400×. Two independent investigators counted TUNEL-positive VSMCs, and their observations were averaged. Then the apoptotic index was calculated using the following formula: 1000 × (number of TUNEL-positive nuclei per field/total number of nuclei per field).

Table I. PCR primers used in this study

<table>
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<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>PCR Products, bp</th>
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<tbody>
<tr>
<td>AT1R</td>
<td>Sense 5′-GCCAAAGTCACCTGCATCAT-3′</td>
<td>476</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′-AATTTTTTTCCAGAAAGCC-3′</td>
<td></td>
</tr>
<tr>
<td>AT2R</td>
<td>Sense 5′-TGAGTCGCCATTTAACGTG-3′</td>
<td>494</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′-ACCACTGAGCATTTTCGGA-3′</td>
<td></td>
</tr>
<tr>
<td>S16</td>
<td>Sense 5′-AGGAGCCATTTGCTTGTTGGA-3′</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′-GCTACCAGGCCCTTGGAGA-3′</td>
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Reverse transcription-polymerase chain reaction (RT-PCR). Total ribonucleic acid (RNA) was isolated from aortic specimens using TRIzol reagent (Gibco BRL) and reverse transcription of an aliquot (5 μg) of the total RNA sample was done using reverse transcriptase (Super-Script II, Gibco BRL). Then PCR of the complementary DNA to detect the rat AT1R and AT2R genes was performed by a programmed cycle of 94 for 30 seconds (denaturation), 58 for 30 seconds (annealing) and 72 for 45 seconds (polymerization). As an internal control, co-amplification of mouse S16 ribosomal protein was performed. The sequences of the oligonucleotide primers used for PCR and the sizes of the predicted PCR products are shown in Table I. The number of PCR cycles was 35, and products were analyzed on agarose gels. To determine whether the PCR was in the linear range, various amounts of total RNA were used to amplify each gene. Semiquantification of messenger ribonucleic acid (mRNA) expression was achieved by densitometric analysis using NIH image analysis software. This confirmed the linear amplification of RNA at amounts from 0.1 to 5.0 μg (data not shown), indicating that RT-PCR was performed within the linear range in the present study.

Table II. Effects of ACEI and AT1R blocker on blood pressure and aortic dissection

<table>
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<tr>
<th>Treatment</th>
<th>Systolic BP (mm Hg)</th>
<th>AD (%)</th>
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<tr>
<td>Vehicle (n = 17)</td>
<td>100 ± 2.2</td>
<td>10/17 (59%)</td>
</tr>
<tr>
<td>Temocapril (n = 15)</td>
<td>87.6 ± 4.3*</td>
<td>4/15 (27%)*</td>
</tr>
<tr>
<td>CS-866 (n = 14)</td>
<td>89.0 ± 4.7*</td>
<td>7/14 (50%)</td>
</tr>
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</table>

Data are mean ± SD. BP, Blood pressure; AD, aortic dissection.
* P < .05 vs vehicle.

Fig 2. Pathologic evaluation of cystic medial degeneration. A–F, Destruction and fragmentation were detected by use of Victoria blue staining (upper panels; magnification, 100×) and deposition of an amorphous protein was demonstrated by Alcian blue staining (lower panels; magnification, 100×). G, Percent area of elastic fibers is shown. Temocapril significantly maintained elastic fiber tissue. * P < .01. H, Percent area of amorphous protein deposition is shown. Temocapril significantly reduced Percent area of amorphous protein deposition. * P < .01.
Statistical analyses. Analysis was performed with SAS System 8e software (SAS Institute Inc, Cary, NC). Results are presented as the mean ± standard deviation (SD). χ2 Test was applied for the analysis of dichotomous data. Student t test was used for the analysis of continuous data. The normality of the distribution of data was evaluated by the Shapiro-Wilks one-sample test, and the F test was used to assess the homogeneity of variance testing. One-way analysis of variance (ANOVA) was used to test for statistically significant differences among the groups, and Dunnett multiple comparison method was applied when appropriate. Two-tailed probability (P) values of <.05 were considered to indicate statistical significance.

RESULTS

Effect of ACEI and AT1R blocker administration on aortic dissection. Spontaneous dissection was observed from the ascending aorta to the proximal portion of the descending aorta in BAPN-fed rats (Fig 1). The aortas were dilated and the findings resembled those in MFS patients. Temocapril significantly prevented aortic dissection when compared with that in control rats (26.7% vs 64.7%, P < .05). In contrast, CS-866 had no statistically significant effect on aortic dissection when compared with that in control rats, although temocapril and CS-866 lowered blood pressure to the same degree (Table II).

Effect of ACEI and AT1R blocker administration on CMD. Temocapril significantly maintained the area of elastic fibers (P < .01) and significantly suppressed the deposition of MFS when compared with vehicle-treated controls (P < .01). In contrast, CS-866 had no effect (Fig 2), suggesting that the ACEI, but not the AT1R blocker, could inhibit BAPN-induced CMD in rats.

Effect of ACEI and AT1R blocker administration on VSMC apoptosis. Temocapril significantly reduced apoptotic VSMCs, as evaluated by the TUNEL method, when compared with that in vehicle-treated controls (P < .01). In contrast, CS-866 did not alter apoptosis (Fig 3).

Angiotensin II receptor expression. RT-PCR demonstrated that both AT1R and AT2R were expressed in control rat aortas, and that AT2R mRNA expression was significantly upregulated in the aortas of BAPN-fed rats (P < .01, Fig 4).

DISCUSSION

Several studies have demonstrated that angiotensin II plays a pivotal role in the pathogenesis of aortic disease.3–7,10 Daugherty et al7 reported that aortic aneurysm with medial dissection could be induced by angiotensin II infusion in apo E-deficient mice, and Nishijo et al8 showed that salt-sensitive aortic dissection occurred in mice with overproduction of angiotensin II. We have previously shown by organ culture that AT2R expression is upregulated, and that ACEIs (but not AT1R blockers) can suppress VSMC apoptosis in aortas from patients with MFS.4 In addition, we have hypothesized that this inhibition of VSMC apoptosis by ACEI might be useful for the prevention and treatment of CMD or aortic dissection in patients.
with MFS. To test our hypothesis, we planned this in vivo study.

We demonstrated two major findings by using rats with BAPN-induced aortic dissection in the present study. First, an ACEI (but not AT1R blocker) could prevent CMD, VSMC apoptosis, and aortic dissection in BAPN-fed rats. This result is compatible with the hypothesis that was based on our previous in vitro studies.

Liao et al reported that the creation of experimental abdominal aortic aneurysms by elastase infusion was also inhibited by ACEIs (not by AT1R blockers) in rats. They also demonstrated the lack of association between blood pressure and occurrence of aortic aneurysm, but they did not reach the basic mechanism underlying the difference of inhibitory effect between ACEIs and AT1R blockers. 

Second, we showed that angiotensin II receptor expression was altered in diseased rat aortas, leading to a possible mechanism for the difference in the preventive effect on BAPN-induced aortic diseases between ACEI and AT1R blocker. Most of the known effects of angiotensin II in adults are attributable to AT1R, and signaling via AT2R has been shown to antagonize AT1R signaling. Recent studies have suggested that AT2R may play an important role in the regulation of apoptosis, AT2R expression level is generally high in fetal tissues but decreases rapidly after birth, although enhanced expression has been reported in adults with cardiovascular diseases such as cardiac hypertrophy, atherosclerosis, hypertension, myocardial infarction, and aortic disease associated with MFS. We demonstrated that both the AT1R and the AT2R were expressed in the rat aorta and that AT2R expression was upregulated in rats with BAPN-induced aortic dissection.

Taking our previous in vitro finding that AT2R mediates VSMC apoptosis in MFS aortas together with the finding of the present in vivo study that an ACEI (but not an AT1R blocker) can prevent aortic dissection in BAPN-fed rats, it appears that signaling via the AT2R to promote apoptosis may play a central role in pathogenesis of CMD and aortic dissection in this rat model.

Inhibition of the renin-angiotensin system (RAS) is believed to be important for the prevention of cardiovascular remodeling. In fact, many recent studies have proved that RAS-inhibition therapy can improve the prognosis and the quality of life in patients with heart failure. In the context of RAS inhibition, ACEIs and AT1R blockers might have the same effect when AT1R expression is abundant. However, in diseases associated with increased AT2R expression, the action of ACEIs and AT1R blockers should be different, as was shown in the present study.

On the other hand, ACEIs not only block the RAS pathway but also inhibit the breakdown of bradykinin and then activate nitric oxide synthase. Thus, it is possible that the beneficial effects of ACEIs in the present study may result from effects other than RAS inhibition. Further experi-

**Fig 4.** A, RT-PCR for AT1R, AT2R, and S16 as an internal control. B, Ratio of AT2R mRNA expression/S16 mRNA expression was significantly higher in BAPN-fed rat aortas than in aortas from rats without BAPN, whereas ratio of AT1R mRNA/S16 mRNA expression was not changed. *P < .01.
ments are necessary to elucidate the contribution of these alternative effects of ACEIs in the vasculature.

CONCLUSION

Our results suggest that differential expression of angiotensin II receptors and AT2R signaling are involved in the pathogenesis of CMD and aortic dissection in BAPN-fed rats. ACEIs might be of clinical value for the prevention and treatment of aortic diseases related to CMD.

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REFERENCES