Hypertension Accelerated Experimental Abdominal Aortic Aneurysm Through Upregulation of Nuclear Factor κB and Ets

Suguru Shiraya, Ken Miwa, Motokuni Aoki, Takashi Miyake, Masako Oishi, Kazusaburo Kataoka, Shigetsugu Ohgi, Toshio Ogihara, Yasufumi Kaneda, Ryuichi Morishita

Abstract—In this study, we focused on the effect of hypertension on the transcription factors nuclear factor κB (NFκB) and ets in the mechanisms of abdominal aortic aneurysm (AAA), and we investigated how hypertension affects the progression of AAA. AAA was produced by elastase perfusion in hypertensive rats and normotensive rats. The size of AAA rapidly increased in hypertensive rats as compared with normotensive rats. Western blot analysis demonstrated that the expression of matrix metalloproteinase (MMP)-2, -3, -9, and -12, as well as intercellular adhesion molecule, was increased in hypertensive AAA rats, accompanied by upregulation of NFκB and ets. Moreover, in situ zymography showed that the activity of MMPs was increased in the aorta of a hypertensive AAA model as compared with that in a normotensive AAA model. Interestingly, transfection of chimeric decoy oligodeoxynucleotide (ODN) resulted in significant inhibition of aortic dilatation both in normotensive and hypertensive rats at 4 weeks after transfection. Destruction of elastic fibers was also significantly inhibited by transfection of chimeric decoy ODN in both hypertensive rats and normotensive rats. The expression of MMP-2, -3, -9, and -12, as well as intercellular adhesion molecule, was significantly attenuated by the chimeric decoy ODN, accompanied by inhibition of the migration of macrophages. Also, the effect of chimeric decoy ODN was confirmed in an organ culture. The present study demonstrated that hypertension accelerated the progression of experimental AAA through upregulation of NFκB and ets. Inhibition of NFκB and ets could be a novel therapeutic strategy to treat AAA in hypertensive patients. (Hypertension. 2006;48:628-636.)

Key Words: abdominal aortic aneurysm ♦ hypertension ♦ NFκB ♦ ets ♦ decoy ♦ MMP

Abdominal aortic aneurysm (AAA) is a common degenerative condition associated with aging and atherosclerosis.1 Because the main pathogenesis of AAA is considered to be based on atherosclerosis, patients with aneurysms and atherosclerotic disease share similar risk factors, including male sex,2 older age,3 and a lipid profile that includes lower high-density lipoprotein and higher triglyceride and low-density lipoprotein concentrations.4 However, the risk factors for atherosclerosis and AAA are not completely the same, because there are certain pathogenic, epidemiological, and genetic differences between these 2 diseases.5,6 Indeed, although hypertension induces sheer stress, oxidative stress, and vascular inflammation followed by atherosclerosis, a consistent relation between blood pressure and the prevalence of AAA has not been demonstrated.7,8 Hypertension has been reported to be both independently associated with AAA and not associated with AAA.8,9

Basic phenomena in the pathogenesis of AAA are degradation of extracellular matrix components and loss of structural integrity of the aortic wall.10 AAA disease typically involves tissue inflammation as seen by the presence of inflammatory cells, which are considered to participate in the immunopathogenesis of AAA leading to destruction of the aortic matrix.11,12 Recent investigations have emphasized disease mechanisms involving chronic aortic wall inflammation and the progressive degradation of fibrillar matrix proteins.13 From this viewpoint, we have reported previously that upregulation of the transcription factors nuclear factor κB (NFκB), which regulates inflammation, and ets, which regulates expressions of matrix metalloproteinases (MMPs), was closely related to the pathogenesis of AAA in an animal model. In this study, to clarify how high blood pressure is involved in the mechanisms of development of AAA, we examined the expression of NFκB and ets in a hypertensive rat elastase-induced AAA model. The elastase-induced AAA model is very popular and is considered to be suitable for analysis of the mechanisms of the pathogenesis of AAA, because several previous reports showed that elastase activity

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From the Division of Clinical Gene Therapy (S.S., K.M., T.M., R.M.), Department of Geriatric Medicine (M.A., T.O.), and Division of Gene Therapy Science (Y.K.), Graduate School of Medicine, Osaka University, Suita, Japan; Second Department of Surgery (S.S., K.M., S.O.), Faculty of Medicine, Tottori University, Yonago, Japan; and the Department of Pharmacy (M.O., K.K.), Osaka University Hospital, Osaka, Japan.
The first 2 authors contributed equally to this work.
Correspondence to Ryuichi Morishita, Division of Clinical Gene Therapy, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan. E-mail morishit@cgi.med.osaka-u.ac.jp © 2006 American Heart Association, Inc.
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plays an important role in the progression of AAA.14,15 In addition, using chimeric decoy oligodeoxynucleotide (ODN) against NFκB and ets, we demonstrated successful treatment of experimental AAA in a rat hypertension model, with the anticipation of developing future therapy.

**Methods**

### 5/6 Nephrectomy Model

Adult male Wistar rats (350 to 400 g; Charles River Breeding Laboratories), fed standard rat chow with free access to water, were subjected to subtotal renal ablation.16 Infarction of the left kidney was produced by ligation of 2 segmental renal arteries, and a week later, right nephrectomy was performed. After ablation, systolic blood pressure was measured by tail-cuff technique.

### Procedure of AAA Model and Transfection of Decoy ODN

Male Wistar rats (350 to 400 g; Charles River Breeding Laboratories) were anesthetized and underwent laparotomy. An elastase perfusion AAA model was prepared as reported previously, with modification.13,18 Briefly, the abdominal aorta was isolated from the level of the left renal vein to the bifurcation. After cannulation of the femoral artery, aortic perfusion with 2 mL of saline containing 25 U of elastase was performed for 30 minutes at 100 mm Hg. Transfection of decoy ODN was performed by wrapping a delivery sheet around the abdominal aorta at the same time as surgery. The delivery sheet consisted of 73 mg of hydroxypropyl cellulose (Wako) and 7.3 mg of polyethylene glycol 400 (Wako) and contained 400 nmol of decoy ODN. This delivery sheet is easily converted to a gel in wet conditions. This study was performed under the supervision of the Animal Research Committee in accordance with the Guidelines on conditions. This study was performed under the supervision of the Animal Research Committee in accordance with the Guidelines on conditions.

### Synthesis of ODN and Selection of Target Sequences

Sequences of the phosphorothioate ODN used were as follows: chimeric decoy ODN (consensus sequences are italicized), 5'-ACCGGAATGATTGGAGATTCTCCCC-3' and 3'-GGGCTTCTGATTAGGAAGGAGGAGG-5'; and scrambled chimeric decoy ODN, 5'-TGAGCAATACGTGACTGCGCTCAG-3' and 3'-AGCTGTATATGCGAGGCTGCTG-5'. Chimeric decoy cis- element double-stranded ODN binds to free NFκB and ets.19

### Electrophoretic Mobility Shift Assay

Rats were euthanized 1 week after the operation, and nuclear extracts were prepared from the aortic aneurysms of hypertensive and normotensive rats, as described previously.20 In brief, rat aortas were homogenized in ice-cold homogenization buffer (10 mmol/L of HEPES [pH 7.5], 0.5 mol/L of sucrose, 0.5 mmol/L of spermidine, 0.15 mmol/L of sperm, 5 mmol/L of EDTA, 0.25 mol/L of EGTA, 7 mmol/L of β-mercaptoethanol, and 1 mmol/L of phenylmethylsulfonyl fluoride). After centrifugation, each pellet was lysed in 1 volume of ice-cold homogenization buffer containing 0.1% Nonidet P-40. Then it was centrifuged, and the pellet nucleus was washed twice with ice-cold buffer containing 0.35 mol/L of sucrose. After washing, the nucleus was pre-extracted with 1 volume of ice-cold homogenization buffer containing 0.05 mol/L of NaCl and 10% glycerol for 15 minutes at 4°C. The nucleus was then extracted with homogenization buffer containing 0.3 mol/L of NaCl and 10% glycerol for 1 hour at 4°C. After pelleting the extracted nucleus at 12 000g for 30 minutes at 4°C, 45% (NH₄)₂SO₄ was added to the supernatant. Then, the mixture was stirred for 30 minutes at 4°C. The precipitated protein was collected at 17 000g for 30 minutes and resuspended in homogenization buffer containing 0.35 mol/L of sucrose.

ODN containing the NFκB binding site (5'-CCTTGAAGGGA-TTTCCCC-3'; only the sense strand is shown) and/or ets binding site (5' - GTGCCTGGGGTAGAATGGGGCTGGG-3'; only the sense strand is shown) were labeled as primers at the 3' end. Binding mixtures (10 μL) including 3P-labeled primers (0.5 to 1 ng, 10 000 to 15 000 counts per minute) and 1 μg of polydeoxyinosinonic-deoxyctydic acid (Sigma Chemicals) were incubated with 10 μg of nuclear extract for 30 minutes at room temperature and then loaded onto 6% polyacrylamide gel. As a control, samples were incubated with an excess (×100) of nonlabeled ODN.

### Ultrasoundography

Ultrasoundography was used to demonstrate dilatation of the AAA. A cardiovascular ultrasound system (Power Vision 6000, Toshiba) and a linear transducer (15 MHz) were used to image the abdominal aorta noninvasively in anesthetized rats. Rats were scanned transversely to obtain images for measurement of the luminal diameter and the area of the lumen of the aneurysm at the segment with maximum diameter. The aortic size was measured before and after laparotomy once a week and up to 4 weeks after operation.

### Quantification of Elastin

Rats were euthanized 4 week after the operation. The excised aorta was fixed in 10% neutral buffered formalin and processed for routine paraffin embedding. Aortic tissue cross-sections (6 μm) were stained with Miller’s elastin and van Gieson's stain (EVG) in a standard manner. The surface area occupied by elastic fibers stained with EVG was quantified using a computerized morphometry system (MacSCOPE version 2.2, Mitani Corporation), and expressed as a percentage of the surface area occupied by elastic fibers.21

### Western Blotting

Rats were euthanized 1 week after the operation and frozen. Aortic tissues were resuspended in 200 μL of lysis buffer (50 mmol/L of Tris-HCl [pH 8.0], 20 mmol/L of EDTA, 1% SDS, and 100 mmol/L of NaCl) containing a protease inhibitor mixture (Sigma), homogenized, and collected by centrifugation at 12 000g for 5 minutes. Samples (20 μg) were electrophoresed in SDS-PAGE acrylamide gels, transferred onto nitrocellulose membranes (Hybond ECL, Amersham), and incubated for 24 hours in PBS, 5% nonfat milk, and 0.2% Tween 20 at 4°C. Membranes were then incubated for 24 hours at 4°C with rabbit anti-rat MMP-9 (Torrey Pines Biolabs Inc, 1:2000 dilution), anti-rat intercellular adhesion molecule-1 (ICAM-1) (Seikagaku), anti-MMP-2, anti-MMP-3, or anti-MMP-12 goat polyclonal antibody (Santa Cruz Biotechnology, 1:200 dilution); washed in PBS and 0.1% Tween 20; incubated for 2 hours at room temperature with donkey anti-rabbit IgG secondary antibody for MMP-9 (Amersham Biosciences; 1:20000), sheep anti-mouse IgG for ICAM-1 (Amersham Biosciences; 1:10 000), or donkey anti-goat IgG and HRP for MMP-2, MMP-3, and MMP-12 (Promega, 1:10 000); and visualized using an ECLplus chemiluminescent kit (Amersham Biosciences) following the manufacturer’s instructions and exposed to XAR-5 x-ray film (Eastman Kodak Co).

### TABLE 1. Time Course of Systolic Blood Pressure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretigation</th>
<th>preNx</th>
<th>1 wk</th>
<th>2 wk</th>
<th>3 wk</th>
<th>4 wk</th>
<th>5 wk</th>
<th>6 wk</th>
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<tr>
<td>NT</td>
<td>137±5</td>
<td>128±3</td>
<td>129±1</td>
<td>129±2</td>
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<td>165±7</td>
<td>179±12</td>
<td>169±15</td>
<td>175±8</td>
<td>172±8</td>
<td>172±8</td>
</tr>
</tbody>
</table>

NT indicates normotensive AAA model; HT, hypertensive AAA model; PreNx, before right nephrectomy. AAA procedure was performed at 2 weeks after nephrectomy. N=15 per group.
Immunohistochemical Studies

Mouse anti-rat CD68 (Serotec, Ltd) and mouse anti-rat ICAM-1 (Seikagaku) were used for analysis. Immunohistochemical staining was performed using an immunoperoxidase avidin–biotin complex system with nickel chloride color modification. The abdominal aortas were immediately embedded in optimum cutting tissue compound (Miles) and frozen. Then, 5-μm-thick sections were deparaffinized, rehydrated before blocking endogenous peroxidase activity with 3% hydrogen peroxidase, and preincubated with 5% normal horse serum in sodium PBS for 30 minutes. Diluted primary antibodies (mouse anti-rat ICAM-1 monoclonal antibody [1:100] or mouse anti-rat CD68 [1:100]) were then applied to the sections, and these sections were incubated overnight at 4°C. For negative control experiments, the primary antibody was omitted. With intervening washing in PBS, sections were serially incubated with biotinylated anti-mouse IgG (Vector Laboratories) in PBS for 30 minutes and avidin-biotinylated horseradish peroxidase complex in PBS for 30 minutes, according to the manufacturer’s specifications (Vectastain Elite ABC kit, Vector Laboratories). Immune complexes were visualized using 0.05% 3,3’-diaminobenzidine (Vector Laboratories).

In Situ Zymography

Gelatinolytic activity in the rat aorta was analyzed with gelatin-coated film (Fuji Photo Film Co, Ltd) by the method reported previously.22 Aneurysms of rats were excised 1 week after the operation, and frozen sections of tissue samples were placed on this film. Films with specimens were incubated in a humidified chamber at 37°C overnight. Then, the film was stained with Biebrich Scarlet Stain Solution (WAKO Inc).

Organ Cultures of Human AAA Specimens

Aortic specimens were obtained from patients with AAA at the time of open surgical repair. The harvested aortic specimens were transported to a sterile tissue culture hood in cold DMEM supplemented with 1% BSA and antibiotics. Each specimen was immediately divided into 2-mm² segments of full-thickness aortic wall, followed by incubation in 20% collagen gel in DMEM containing each decoy ODN (100 μmol/L) for 1 hour at 4°C. After 48 hours, the conditioned medium was collected and used for ELISA of MMP-1 and MMP-9. Concentrations of MMP-1 and MMP-9 were measured by an ELISA from Amersham Biotech (Biotrak).

Statistical Analysis

All of the values are expressed as mean±SEM. ANOVA was used to determine the significance of differences in multiple comparisons. P<0.05 was considered significant.
Results

Acceleration of AAA in Rat Hypertensive Model

As shown in Table 1, the 5/6 nephrectomy model produced a significant increase in systolic blood pressure from the point of ligation, which was sustained for 4 weeks after the AAA operation ($P<0.01$). Consistent with the increase in blood pressure, as shown in Figure 1a and 1b, ultrasound analysis demonstrated that the size of AAA was significantly increased in hypertensive rats as compared with normotensive rats ($P<0.01$). Therefore, we further studied how hypertension affected the size of AAA. We measured MMPs in both the normotensive and hypertensive AAA models. Among various MMPs, MMP-2, -3, and -9 are considered to be especially important in the pathogenesis of the rat AAA model. Western blot analysis demonstrated that the expression of MMP-2, -3, -9, and -12, was increased in hypertensive AAA rats as compared with normotensive AAA rats (Figure 2a and 2b). Moreover, in situ zymography showed that the activity of MMPs was increased in the aorta of a hypertensive AAA model as compared with that in a normotensive AAA model (Figure 3), suggesting that hypertension accelerated inflammation and MMP activation. In addition, ICAM expression was also significantly increased in the hypertensive AAA model. Because this gene expression is regulated by NFκB and ets, we further examined the levels of NFκB and ets. Of importance, the binding activity of NFκB and ets, as assessed by gel mobility shift assay, was markedly increased in the aorta of the hypertensive rat model as compared with the normal rat model ($P<0.05$; Figure 4a and 4b).

Prevention of Development of AAA by Chimeric Decoy ODN in Hypertensive Rats

From these data, we speculated that hypertension accelerated the progression of AAA through an increase in MMP and ICAM expression induced by the upregulation of NFκB and ets. To confirm this hypothesis, we used chimeric decoy ODN, because our previous article demonstrated a suppressive effect of decoy ODN on activation of both NFκB and ets, and observed that in vivo transfection of chimeric decoy ODN using a delivery sheet significantly suppressed the upregulation of NFκB and ets induced by elastase. First, we used an ex vivo organ culture system of human vascular tissue from aneurysms and examined the effect of chimeric decoy ODN on the expression of MMP-1 and MMP-9, because MMP-1 and MMP-9 are considered to play a pivotal role in the pathogenesis in human AAA. MMP-1, as well as MMP-9, was readily detected in the conditioned medium from the organ culture system. Importantly, secretion of MMP-1 and MMP-9 from harvested human aortic aneurysms in organ culture was significantly decreased by transfection of chimeric decoy ODN as compared with untreated control or transfected scrambled decoy ODN, as shown in Table 2. This effect showed dose dependence.

Given the successful effect and dose dependence of chimeric decoy ODN, we applied chimeric decoy ODN to in vivo experiments. As shown in Figure 5a, ultrasound analysis demonstrated that treatment with chimeric decoy ODN significantly prevented the progression of aortic dilatation after elastase perfusion even in hypertensive rats, whereas scrambled decoy ODN did not affect the growth of AAA. Even 4
weeks after transfection, the progression of AAA was still completely inhibited by chimeric decoy ODN (Figure 5b; *P<0.01). Although low levels of MMPs were detected in the aorta of sham-operated (without elastase perfusion) animals as a baseline, no effect of decoy ODN on aortic size was observed (data not shown), because inflammation-induced activation of NFκB and ets does not occur or occurs to a very small degree in a hypertensive condition alone. Moreover, we evaluated the effect of hypertension on the destruction of elastic fibers. As shown in Figure 6a and 6b, significant degradation of elastic fibers was observed in the aneurysms of hypertensive rats as compared with those of normotensive rats (*P<0.01). Interestingly, treatment with chimeric decoy ODN markedly inhibited the proteolysis of elastin in both the hypertensive and normotensive rat model (Figure 6b; *P<0.01). In addition, transfection of chimeric decoy ODN significantly decreased MMP-2, -3, -9, and -12, as well as ICAM in the hypertensive AAA model (Figure 7; *P<0.01).

Consistently, immunohistochemical study demonstrated that the expression of ICAM, a well-known adhesion molecule regulated by NFκB, was markedly increased in the hypertensive rat model as compared with the normotensive rat model (Figure 8), whereas transfection of chimeric decoy ODN inhibited expression of ICAM in the intima and media. A reduction of ICAM was expected to inhibit macrophage recruitment. Indeed, infiltration of macrophages, which are the major cells secreting MMPs into the aortic wall, followed by vascular inflammation, was also significantly inhibited in the progression of AAA (Figure 9).

Discussion

Hypertension promotes arterial inflammation, which has emerged as central to the initiation and progression of atherosclerosis.25 Evidence from animal models, as well as patients, has suggested that hypertension exerts proinflammatory actions through increased expression of several mediators, including leukocyte adhesion molecules, chemokines, specific growth factors, heat shock proteins, endothelin-1, and angiotensin (Ang). Reports showing that lymphocytes and macrophages infiltrate the kidney of spontaneously hypertensive rats and that their reduction was associated with improvement of hypertension26 suggest that hypertension is an inflammatory disease. On the other hand, the mechanisms of AAA involve vascular inflammation related to several inflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor-α.11,12,27,28 Human aneurysm tissues are characterized by chronic aortic wall inflammation, and the progressive degradation of fibrillar matrix proteins13 and MMPs, including MMP-2 and MMP-9, are thought to contribute to aneurysm development.29 Also, the relation of inflammation to AAA is supported by a report demonstrating that monocyte chemoattractant protein-1 and its receptor, CCR2, play an important role in Ang II–induced acceleration of the atherosclerotic process and Ang II–induced AAA formation.30 Interestingly, a strong relationship between vascular inflammation and the progression of AAA was reported based on clinical findings.31,32 These reports showed that C-reactive protein levels were elevated in larger aneurysms, and serum high-sensitive C-reactive protein (hsCRP) was associated with aneurysm size, suggesting that in at least some patients, CRP may be produced by aneurysmal tissue.31,32 Moreover, these reports focused on the inflammatory

<table>
<thead>
<tr>
<th>Variable</th>
<th>Untreat</th>
<th>SD</th>
<th>Chimera 100</th>
<th>Chimera 600</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1 concentration, ng/mL</td>
<td>11.2±0.8</td>
<td>9.8±1.1</td>
<td>17.7±0.8*</td>
<td>5.9±0.3†</td>
</tr>
<tr>
<td>MMP-9 concentration, ng/mL</td>
<td>20.1±2.2</td>
<td>21.1±2.1</td>
<td>13.0±1.5*</td>
<td>9.8±1.8†</td>
</tr>
</tbody>
</table>

Untreat indicates untreated aortic specimens; SD, aortic specimens transfected with scrambled decoy ODN; Chimera 100, aortic specimens transfected with chimeric decoy ODN (100 μmol/L); Chimera 600, aortic specimens transfected with chimeric decoy ODN (600 μmol/L). N=8 per group.

*P<0.05 vs SD; †P<0.05 vs Chimera 100.

Figure 5. Effect of transfection of chimera decoy ODN on size of AAA as assessed by ultrasound. a, Representative ultrasound of aortic dilatation. b, Time course of aortic size after elastase perfusion assessed by ultrasound. HT-SD indicates hypertensive AAA model transfected with scrambled decoy ODN; HT-Chimera, hypertensive AAA model transfected with chimeric decoy ODN; NT-SD, normotensive AAA model transfected with scrambled decoy ODN; NT-Chimera N, normotensive AAA model transfected with chimeric decoy ODN. N=15 per group.
process in the pathogenesis of AAA and suggested that CRP produced in vascular tissue might contribute to aneurysm formation. This is also supported by evidence that treatment with anti-inflammatory agents, such as indomethacin, resulted in inhibition of enlargement of experimental AAA.\textsuperscript{33} Thus, we hypothesized that hypertension might accelerate the development of AAA through inflammatory changes, because a large number of articles reported a significant

\begin{figure}
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\includegraphics[width=\textwidth]{figure6.png}
\caption{Effect of transfection of chimera decoy ODN on elastin at 4 weeks after transfection. a, Histological sections of rat aorta stained with EVG at 4 weeks after transfection (\times 40). b, Quantification of elastin. The surface area occupied by elastic fibers stained with EVG was quantified using a computerized morphometry system, MacSCOPE Ver. 2.2 (Mitani Corporation), and expressed as percentage of the surface area occupied by elastic fibers. Sham indicates aorta from sham-operated rats without elastase perfusion; HT-SD, hypertensive AAA model transfected with scrambled decoy ODN; HT-Chimera, hypertensive AAA model transfected with chimeric decoy ODN; NT-SD, normotensive AAA model transfected with scrambled decoy ODN; NT-Chimera N, normotensive AAA model transfected with chimeric decoy ODN. N=6 per group.}
\end{figure}

\begin{figure}
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\includegraphics[width=\textwidth]{figure7.png}
\caption{Effect of transfection of chimeric decoy ODN on expression of MMPs and ICAM. Western blots of MMPs and ICAM in hypertensive AAA model at 1 week after transfection. a, Typical example of Western blots. b, Quantitative analysis. HT-SD indicates hypertensive AAA model transfected with scrambled decoy ODN; HT-Chimera, hypertensive AAA model transfected with chimeric decoy ODN. N=5 per group.}
\end{figure}
increase in the expression or activation of MMPs, ICAM, NFκB, and ets.34–37 Interestingly, this hypothesis was supported by the present study showing that the size of AAA rapidly increased in hypertensive rats as compared with normotensive rats, accompanied by a significant increase in the expression of MMPs, and well as ICAM.

In this study, we focused on 2 important transcription factors, NFκB and ets. NFκB is a well-known transcription factor that regulates various cytokines. In vascular cells, NFκB has been shown to regulate the expression of vascular adhesion molecule-1 and ICAM-1 as a part of the inflammatory response.38 Indeed, our previous study demonstrated that inhibition of NFκB activity by decoy ODN resulted in potent inhibition of the expression of adhesion molecules, such as ICAM and vascular adhesion molecule, after balloon injury, accompanied by significant inhibition of the migration and accumulation of macrophages in the neointima.39 Also, we have reported previously that inhibition of NFκB activity by decoy abolished the transactivation of IL-1 and IL-6 under tumor necrosis factor-α stimulation in human aortic endothelial cells.40 In addition to mediating inflammatory changes, NFκB regulates the transcription of MMP-1, -2, -3, and -9.41 Because the contribution of the inflammatory process is important in the pathogenesis of AAA, because macrophages from the intravascular or retroperitoneal space, induced by inflammation, are the main cells secreting MMP-9,42,43 NFκB is considered to be a key molecule in the pathogenesis of AAA. Interestingly, we demonstrated that expression of NFκB was significantly increased in a rat hypertensive AAA model. Other than inflammation, because NFκB directly regulates transcription of MMPs, its upregulation is considered to promote aortic dilatation in AAA.

The role of Ang II should be discussed. Because circulating Ang II is elevated in the 5/6 nephrectomy hypertensive model, a direct effect of Ang II on vascular injury might be involved in the progression of AAA in 5/6 nephrectomy

Figure 8. Effect of transfection of chimeric decoy ODN on ICAM protein. Immunohistochemical staining of ICAM. Sham indicates aorta from sham-operated rats without elastase perfusion; HT-SD, hypertensive AAA model transfected with scrambled decoy ODN; HT-Chimera, hypertensive AAA model transfected with chimeric decoy ODN; NT-SD, normotensive AAA model transfected with scrambled decoy ODN; NT-Chimera, normotensive AAA model transfected with chimeric decoy ODN.

Figure 9. Effect of transfection of chimeric decoy ODN on invasion of macrophages. Immunohistochemical staining of CD68 and counting of number of macrophages. HT-SD indicates hypertensive AAA model transfected with scrambled decoy ODN; HT-Chimera, hypertensive AAA model transfected with chimeric decoy ODN. Six sections from 6 animals (1 per each) were used for cell counting, and it was performed in a blinded manner.
model rats, in addition to high blood pressure. Moreover, because Ang II is well known to activate NfκB, it is possible that the potent activation of NfκB in the hypertensive AAA model might be mediated by not only high blood pressure but also activation of the circulating or tissue renin–Ang system. Thus, the mechanisms by which hypertension accelerated AAA are as follows: (1) activation of NfκB by high blood pressure, (2) activation of NfκB by circulating and/or tissue Ang II, and (3) a direct effect of Ang II on vascular injury.

In contrast, ets is also well known to regulate MMPs. The ets family activates the transcription of genes encoding MMP-1, stromelysin 1, MMP-9, and urokinase plasminogen activator, which are proteases involved in extracellular matrix degradation.\(^44,45\) Although the relationship between inflammation and ets upregulation is still unclear, some reports suggest that inflammation induces ets activation.\(^46,47\) Consistently, we reported previously that ets was induced in experimental AAA, followed by overproduction of MMPs.\(^19\) Interestingly, the present study indicated that high blood pressure significantly upregulated both NfκB and ets in a rat AAA model.

The hypothesis that hypertension accelerates the progression of AAA through an increase in matrix degradation and inflammation through the upregulation of NfκB and ets is supported by the observation that simultaneous inhibition of the upregulation of NfκB and ets by chimeric decoy ODN completely attenuated the progression of AAA. These data are consistent with a previous report that chimeric decoy ODN inhibited a broad spectrum of MMPs and the migration of macrophages, leading to the prevention of AAA in a normotensive AAA model.\(^19\) Suppression of AAA by chimeric decoy ODN could be mediated by 3 pathways: (1) direct inhibition of MMP gene expression driven by either the NfκB or ets binding site, (2) indirect inhibition of MMP secretion, and (3) inhibition of migration of macrophages, which secrete MMPs.

**Perspectives**

Because macrophages, which mainly produce MMPs, are recruited from the peritoneum to the vascular adventitia, our proposed strategy (delivery from outside vessels) would be ideal. With the further development of decoy delivery systems, such as sheet, gel type, and spray, low-invasive therapy using laparoscopy will be feasible. In considering the clinical application, questions such as how long the inhibitory effects of decoy ODN continue and whether decoy ODN would regress the size of established AAA should be addressed. Because NfκB negatively regulates the production of collagen and elastin,\(^48,49\) it might be possible that decoy ODN could regress the size of AAA.

Overall, the present study demonstrated that hypertension could be a risk factor for AAA development through upregulation of the transcription factors NfκB and ets. Because inhibition of expansion of experimental AAA was achieved using chimeric decoy ODN against both NfκB and ets, NfκB and ets might become important targets in developing a novel strategy to treat AAA in hypertensive patients.

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**Disclosures**

None.

**References**

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