Pharmacological treatment of abdominal aortic aneurysm

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Abdominal aortic aneurysm (AAA) is a common degenerative condition with high mortality in older men. Elective surgical or endovascular repair is performed to prevent rupture of large AAAs. In contrast, despite gradual expansion, small AAAs have a low risk of rupture, and there is currently no well-defined treatment strategy for them. Therefore, a pharmacological approach for AAA is expected in the clinical setting. Indeed, several therapeutic effects of pharmacological agents have been reported in experimental models, and some agents have undergone clinical trials. Treatment with statins, angiotensin-converting enzyme-inhibitors, antibiotics, and anti-inflammatory agents appears to inhibit the growth rate of AAA in humans. However, as the sample size and follow-up period were limited in these studies, a large randomized study with long-term follow-up of small AAA should be performed to clarify the effect of these agents. Recently, the regression of AAA using molecular pharmacological approaches was reported in experimental studies. The characteristics of these strategies are the regulation of multiple molecular mediators and the signalling networks associated with AAA formation. On the basis of the results of these investigations, it may be possible to repair the injured aortic wall and obtain the remission of AAA using pharmacological therapy.

KEYWORDS
Abdominal aortic aneurysm; Pharmacology; Decoy; NFκB; ets

1. Introduction
Abdominal aortic aneurysm (AAA) is defined as a permanent segmental dilatation of the abdominal aorta. Diagnosis is typically performed by non-invasive imaging methods, and an abdominal aorta of 3 cm or larger in maximal diameter is generally considered to indicate aneurysm formation.1 Ultrasound screening studies suggest that the prevalence of AAA is ~5% in the adult population over 60 years of age, and an increase in the detection of AAA will be observed in the next decade with the advent of screening for AAA.1,2 Importantly, despite improvement in the surgical treatment and peri-operative care, AAA is among the 15 leading causes of death in the USA.3 The high mortality of AAA is mainly due to aneurysm rupture. Maximum diameter of AAA is thought to be the most important predictor of AAA rupture.4,5 As patients with a large AAA, at least 5.5 cm in diameter, have an increased risk of rupture, elective surgical or endovascular repair is performed in these patients to prevent rupture. In contrast, despite gradual expansion, AAAs with a diameter <5.5 cm, small AAAs, have a low risk of rupture, and there is currently no well-defined treatment strategy for them. Although a large number of asymptomatic patients with AAA have been detected during routine abdominal screening, ~90% of these patients have an aneurysm diameter of <5.5 cm.6,7 Interestingly, two large randomized trials reported that survival was not improved by elective surgical repair of small AAA.8,9 Therefore, these patients do not receive effective treatment for the early stage of AAA.

For that reason, the development of a non-invasive therapeutic approach for treating AAA is awaited. With advances in vascular biology, the mechanism of AAA formation has been elucidated at the cellular and molecular levels, but it is still not fully understood. However, a pharmacological approach is expected to offer effective treatment for small AAA. Indeed, several therapeutic effects of pharmacological agents have been reported in experimental models, and some agents have undergone clinical trials. To develop a novel therapeutic approach, this review describes molecular targets based on current evidence on the pathogenesis of AAA and pharmacological strategies for treating AAA.

2. Pathophysiology of AAA formation
The pathological features of AAA are characterized by chronic aortic wall inflammation, destruction of the elastic media, neovascularization, and depletion of vascular smooth muscle cells (VSMC). Recent studies have revealed...
that a number of molecular mediators and extracellular matrix-degrading proteases contribute to the pathological process of aortic wall degradation, and the histological changes in the aneurysm wall are thought to result from complex interactions among these factors.

2.1 Inflammation in aortic wall

Chronic inflammation of the aortic wall plays an important role in the pathogenesis of AAA. Studies of human AAA tissue have shown extensive inflammatory infiltrates containing macrophages and lymphocytes in both the media and adventitia, and increasing aneurysm diameter was associated with a higher density of inflammatory cells in the adventitia. Activated macrophages are the main cells secreting various proteases, leading to the disruption of the orderly lamellar structure of the aortic media. The trigger of inflammation is not clear, but angiotensin (Ang) II is considered to be one of the factors inducing aortic inflammation. Ang II is the main effector peptide in the renin–angiotensin system (RAS) and exerts pro-inflammatory actions through an increase in the expression of several mediators including leucocyte adhesion molecules and chemokines. Sustained infusion of Ang II leads to aneurysmal lesions in the atherosclerosis-prone ApoE−/− mouse, without the presence of systemic hypertension, and the initial identified event in AAA formation is the medial accumulation of macrophages. In addition, increasing evidence suggests the importance of tissue RAS in the vasculature. Therefore, Ang II has emerged as a central factor in the initiation and progression of AAA.

Infiltration of lymphocytes is also associated with AAA formation. The dominant lymphocytes are Th2-restricted CD3+ lymphocytes expressing IL-4, -5, -8, and -10 and tumour necrosis factor (TNF)-α for the regulation of the local immune response. Infiltrated immune cells, especially Th2-type lymphocytes, release Fas ligand and FAP-1 as well as cytokines, leading to the apoptosis of VSMC.

Recently, the accumulation of mast cells has been identified in the outer media and adventitia in the human aneurysm wall. Moreover, degranulated mast cells were increased in the aneurysm wall compared with atherosclerotic aorta. Mast cells synthesize and release several proteases, pro-inflammatory cytokines, growth factors, and chemokines, such as chymase and cathepsin G. Numerous studies suggest that these mediators induce adventitial inflammation, apoptosis of VSMC, activation of matrix metalloproteinases (MMPs), and neovascularization in the arterial wall. In addition, mast cells promote the activation of T lymphocytes and macrophages by releasing pro-inflammatory cytokines. Indeed, mast cell-deficient mice showed resistance to aneurysm formation. Therefore, the accumulation of mast cells is thought to be an important factor in AAA formation.

2.2 Proteolysis of extracellular matrix proteins

Aneurysm development involves a complex remodelling process with an imbalance between the synthesis and degradation of connective tissue proteins. Various extracellular matrix proteins participate in the process of the destruction of the human aortic wall; in particular, MMPs are considered to be the predominant proteases. Several MMPs have been focused on in AAA, including four that degrade elastic fibres (MMP-2, -7, -9, and -12), several that degrade interstitial collagen (MMP-1, -2, -8, -13, and -14), and others that degrade denatured collagen (MMP-2 and -9). Particularly, MMP-2 and MMP-9 have attracted interest in the process of AAA development. Patients with AAA have elevated MMP-2 and MMP-9 protein levels in the vasculature remote from the aorta, and the increase in these proteins was correlated with aneurysm diameter.

The activation of MMPs is tightly regulated by tissue inhibitors of metalloproteinases (TIMPs), and mRNA levels of TIMPs were decreased in AAA tissue. Other proteases are also reported to contribute to the initiation and progression of AAA. Uptregulation of cysteine proteases is detected in the aneurysm wall as well as in atherosclerosis. Cathepsins are members of cysteine proteases and are regulated by the inhibitor cystatin C. Abisi et al. reported that the activities of cathepsin B, H, L, and S were significantly higher, and the level of cystatin C was lower in the aneurysm wall than in the aortic wall of occlusive aortic disease. In addition, a clinical study of AAA demonstrated that increased AAA diameter correlated negatively with serum cystatin C level.

2.3 Production of extracellular matrix proteins

Elastin and collagens are the major structural components of the aortic wall. Collagens are responsible for tensile strength and prevent aneurysm rupture. The major fibrillar collagens in the aortic wall are type I and III. The turnover of type III collagen was enhanced in the serum and aneurysm wall tissue of patients with aneurysms. However, the newly synthesized collagen was mainly present in the media and resulted in impaired fibril formation of the aneurysm wall.

In contrast, the production of pro-collagen type I was maintained at a low rate in serum and in the aneurysm wall. Elastic fibres maintain the structure of the vascular wall against haemodynamic stress, resulting in the prevention of aortic dilatation. In adults, elastin turnover is slow and its production is almost absent. To maintain a steady state of synthesis, various factors participate in the downregulation of elastin synthesis, but a recent study revealed that macrophages and VSMC begin to synthesize elastin in the human aneurysm wall.

2.4 Oxidative stress

Recent studies suggest an association of oxidative stress with the formation of AAA. Several stimuli enhance reactive oxygen species (ROS) and reactive nitrogen species (RNS) production, leading to cell and tissue damage in many physiological conditions. In human studies, ROS and RNS were increased in the aneurysm wall compared with the normal aorta and adjacent non-aneurysmal aortic wall. Infiltrated inflammatory cells are the main source of ROS production such as O2· and H2O2 through the upregulated activity of NADPH oxidase. In addition, pro-inflammatory cytokines, mechanical stretch, growth factors, and lipid mediators might upregulate NADPH oxidase in resident vascular cells, resulting in an increase in the production of ROS and lipid peroxidation products. Overexpressed ROS and NO increased the expression of MMPs through the activation of nuclear factor-kappaB (NFkB) and induced apoptosis of VSMC in the aneurysm wall.
3. Pharmacological therapy for AAA

On the basis of increasing evidence of the molecular mechanisms in the process of AAA formation, numerous strategies have been proposed to prevent AAA development. Pharmacological therapy has also been regarded as an effective approach for treating AAA, and some agents have undergone clinical trials. Potential targets for AAA treatment are as follows: (i) inhibition of proteolytic activity, (ii) inhibition of inflammatory response, (iii) suppression of oxidative stress, and (iv) upregulation of synthesis of extracellular matrix proteins (Figure 1).

3.1 Statins

Hydroxymethylglutaryl-coenzyme A reductase inhibitors, also known as statins, are widely prescribed for their lipid-lowering effects. Although the reduction of lipid levels prevents the progression of atherosclerosis, additional effects of statins, so-called pleiotrophic effects, have been increasingly recognized in recent years. Despite the absence of a clear relationship between serum cholesterol level and AAA growth rate, statin therapy is expected to prevent AAA development, because the pleiotrophic effects of statins include an anti-inflammatory effect, anti-oxidative effect, and the reduction of MMP secretion.

In experimental studies, simvastatin suppressed AAA progression in a mouse model, accompanied by a reduction of MMP-9 and an increase of TIMP-1, whereas inflammatory cell infiltration was not inhibited.41,42 We have also demonstrated an inhibitory effect of atorvastatin in an elastase-induced rat AAA model. However, atorvastatin suppressed macrophage recruitment into the vascular wall through the inhibition of ICAM-1 and MCP-1 expression, leading to the inhibition of MMP-12, but not MMP-9 expression. Therefore, it is suggested that the suppression of AAA development by atorvastatin was mainly dependent on its anti-inflammatory effect. In an ex vivo human organ culture system, the application of cerivastatin reduced the tissue level of MMP-9 in a concentration-dependent manner, accompanied by the inhibition of the activation of infiltrated inflammatory cells.43 These results suggest that, at least in part, distinct statins affect different signal transduction pathways to prevent AAA progression. In addition, several observational studies have shown beneficial effects of statins in patients with AAA.44,45

3.2 Angiotensin-converting enzyme-inhibitors and Ang II receptor blockers

Both angiotensin-converting enzyme (ACE)-inhibitors and Ang II receptor blockers (ARBs) are currently widely used in the treatment of cardiovascular disease, such as hypertension and chronic heart failure. In accordance with increasing evidence of an association between Ang II and AAA formation, several studies have been performed to examine the effect of blockade of RAS at several points on the formation of AAA. Interestingly, these agents showed different actions depending on the animal model. Liao et al.46 reported that three different ACE-inhibitors (captopril, lisinopril, and enalapril), but not an ARB (losartan), suppressed the development of elastase-induced AAA in rats. These therapeutic effects of ACE-inhibitors were observed independent of their lowering of arterial blood pressure and inflammatory response in the arterial wall. Consistent with these experimental data, patients taking ACE-inhibitors before admission were significantly less

Figure 1  Pathological mechanisms of AAA formation and therapeutic targets for pharmacological agents. Potential targets for AAA treatment are as follows: (A) inhibition of inflammatory response (antibiotics, ACE-inhibitors, NFκB-ets decoy, etc.); (B) inhibition of proteolytic activity (MMP inhibitor, statins, JNK inhibitor, etc.); (C) suppression of oxidative stress (vitamins, statins), and (D) upregulation of synthesis of extracellular matrix proteins (statins, JNK inhibitor, NFκB–ets decoy).
likely to present with a ruptured aneurysm than those not taking ACE-inhibitors in a population-based case–control study. Conversely, this protective effect was not observed for β-blockers, calcium channel blockers, α-blockers, ARBs, or thiazide diuretics. In contrast, Daugherty et al. demonstrated that the formation of Ang II–induced AAA was totally inhibited by losartan, and the administration of an AT2 receptor antagonist increased the incidence and severity of aneurysms in ApoE−/− mice. The discrepant effect of losartan on AAA formation might be explained by the different disease processes in each model. The initial event in Ang II–induced AAA is a focal dissection in the suprarenal region, leading to aortic dilatation. This process could resemble Marfan syndrome. Treatment with losartan prevented an increase in TGF-β signalling, resulting in the suppression of aneurysm formation in a mouse model of Marfan syndrome. Moreover, recent clinical evidence from the Nikei Heart Study clearly demonstrated a significant decrease in the incidence of aortic dissection by additve treatment with an ARB, valsartan. In addition, we have previously demonstrated that treatment with valsartan significantly prevented the progression of experimental AAA through the inhibition of NFκB activation, MMP expression, and infiltration of macrophages. These results suggest that ACE-inhibitors and ARBs might be useful for treating AAA and Marfan syndrome. Further investigations should be performed to demonstrate more clear evidence.

### 3.3 Antibiotic agents

The presence of microbacterial organisms has been found in the aneurysm wall as well as in atherosclerotic plaque. It has been reported that aneurysm progression correlated with evidence of chronic Chlamydia pneumonia infection. A randomized clinical trial using roxithromycin, an antibiotic against C. pneumonia, demonstrated that once-daily administration of 300 mg roxithromycin reduced the growth rate of small AAAs compared with placebo. However, the preventive effect of roxithromycin appeared in the first year, but not in the second year of follow-up. Tetracyclines are also used as antibiotics to treat C. pneumonia infection and are well known to be non-specific inhibitors of MMPs. A prospective, double-blind, randomized, placebo-controlled study was performed to evaluate the effect of doxycycline on AAA formation. Thirty-two patients with AAA received either doxycycline (150 mg daily) or placebo for a 3 month period. The aneurysm expansion rate in the doxycycline group was significantly lower than that in the placebo group. Recently, the effect of rapamycin on aneurysm formation was examined in a rat AAA model. Rapamycin is an immunosuppressive agent that is commonly used to control transplant rejection by modulating the inflammatory cascade. Rapamycin significantly reduced the rate of aneurysm expansion by 40%, accompanied by a reduction of MMP-9 expression and NFκB activation.

### 3.4 Anti-inflammatory agents

Prostaglandins act as a class of biological mediators in the.process of inflammation, including MMP production, and are converted from arachidonic acid by cyclooxygenase (COX) and terminal prostaglandin synthases. It has been reported that migrating macrophages upregulated the expression of COX-2 with increased production of PGE2 in human AAA tissue, whereas COX-1 expression was not altered. Walton et al. demonstrated that VSMC from human atherosclerotal aortic explants are more sensitive to PGE2-induced cell death compared with VSMC derived from normal aortas. In addition, the authors performed a small case–control study, in which the administration of non-steroidal anti-inflammatory drugs was associated with slower aneurysm growth rates. Recently, the effect of selective inhibition of COX-1 or COX-2 in attenuating aneurysm formation was evaluated in an Ang II–induced ApoE−/− mouse AAA model. A selective COX-2 inhibitor, celecoxib, decreased the incidence and severity of AAA formation, whereas selective COX-1 inhibition had no effect on AAA formation. In addition, after 28 day infusion of Ang II, AAA incidence in wild-type mice was 54%, whereas AAAs were not detected in COX-2-deficient mice. Therefore, selective inhibition of COX-2 by pharmacological agents is expected to be useful for treating AAA.

### 4. Novel therapeutic strategies for treating AAA

On the basis of basic investigations, many researchers have searched for new molecular targets, and novel therapeutic strategies have been proposed to treat AAA. Recently, the regression of AAA by pharmacological treatment has been reported in an experimental study.

#### 4.1 Inhibitors of mast cell degranulation

The effects of mast cell stabilization on AAA formation were examined in an experimental study. Application of disodium cromoglycate, an inhibitor of mast cell degranulation, reduced aortic expansion by 40% in an elastase-induced mouse AAA model, accompanied by the inhibition of recruitment of mast cells and macrophages. Similarly, Tsuruda et al. demonstrated that treatment with tranilast attenuated AAA progression in a CaCl2–induced rat AAA model. These mast cell stabilizers have been used clinically to control allergic disorders, such as bronchial asthma. Therefore, the impact of mast cell stabilizers on AAA formation needs to be clarified in a clinical study.

#### 4.2 c-Jun N terminal kinase inhibitor

Recently, pharmacological inhibition of c-Jun N terminal kinase (JNK) has been reported to regress AAA. The authors demonstrated that phosphorylated JNK was elevated in human AAA tissue, leading to the activation of MMP-9 and pro-inflammatory signalling in VSMC. In addition, selective inhibition of JNK using a specific inhibitor not only prevented AAA formation, but also caused the regression of established AAA in CaCl2-induced mouse AAA and Ang II–induced ApoE−/− mouse AAA models. Importantly, in addition to the suppression of MMP activation and migration of inflammatory cells, the inhibition of JNK restored the architecture of aortic tissue. They demonstrated that the activation of JNK suppressed genes encoding extracellular matrix biosynthetic enzymes in cultured rat VSMC. This study showed an important role of the upregulation of extracellular matrix protein synthesis in the treatment of AAA. Thus, a clinical trial using a JNK inhibitor is eagerly anticipated, because a JNK inhibitor might regress AAA in humans as well as in an animal model.
4.3 Oligodeoxynucleotide-based therapy

Oligodeoxynucleotide (ODN)-based therapy is a new technique for inhibiting target gene expression, and the transfection of cis-element double-stranded ODNs, referred to as ‘decoy’ ODNs, has been utilized for treating AAA in experimental models.61–63

4.3.1 Decoy strategy

The regulation of gene expression is usually achieved at the level of DNA transcription, a process that controls which genes are transcribed into RNA by the enzyme RNA polymerase, although post-transcriptional regulation is also important. The transcription of specific genes is controlled by regulatory proteins, known as transcription factors, which have been grouped in families on the basis of shared DNA-binding motifs.64 The decoy approach is a new class of anti-gene strategy for gene therapy by the modulation of endogenous transcriptional regulation. Synthetic decoy ODNs containing the consensus sequences of the binding site of the target transcription factor block the binding of the transcription factor at the promoter region in the target cells, preventing the transactivation of various genes associated with physiological processes. In contrast, decoy ODNs against a negative transcription factor enhance the expression of otherwise suppressed genes.65 In addition, we have developed a chimeric decoy strategy to regulate multiple transcription factors, because several transcription factors participate in the process of disease progression (Figure 2).

As inflammation and matrix degradation contribute to the progression of AAA, we focused on the transcription factors NFκB and ets. Cigarette smoking, hypercholesterolaemia, and oxidative stress promote NFκB activation. In addition, our previous study demonstrated that hypertension accelerated the progression of experimental AAA through the upregulation of NFκB and ets.66 NFκB is well known to regulate transcription of many genes involved in immune and inflammatory responses. Numerous cytokines and chemokines, including IL-1, IL-2, IL-6, IL-8, and TNF-α, are regulated by NFκB.65,67,68 As NFκB also regulates ICAM-1 and VCAM-1 expression, the activation of NFκB could result in the migration of inflammatory cells.69,70 Importantly, NFκB directly controls the expression of MMP-1, MMP-2, MMP-3, and MMP-9.71–73 Furthermore, recent studies demonstrated that NFκB inhibited the transcription of the elastin and collagen genes, leading to the suppression of their synthesis.74,75 In contrast, members of the ets family play important roles in regulating gene expression in response to multiple developmental and mitogenic signals, including cell growth, differentiation, and apoptosis. The expression of the Fas ligand gene in VSMC is controlled by ets-1, whereas ets-2 rescues macrophages from apoptosis through the upregulation of anti-apoptotic Bcl-XL.76,77 ets is also known to be a regulator of transcription of MMP-1, MMP-2, and MMP-9.76–80 In addition, ets suppresses the induction of the collagen type I gene in human fibroblasts (Figure 3).81 Importantly, we have confirmed marked activation of both NFκB and ets in human AAA tissue.61 These findings suggest that the activation of NFκB and/or ets might be one of the major factors in the process of aortic dilatation in humans.

4.3.2 Regression of AAA by chimeric decoy ODN

To inhibit both NFκB- and ets-binding activity, we employed chimeric decoy ODNs containing consensus sequences of these transcription factors. Importantly, inhibitory effects of chimeric decoy ODNs on MMP-1 and MMP-9 expression were confirmed by ex vivo experiments using human aorta organ culture.61 These findings strongly support the feasibility of the chimeric decoy strategy to treat human AAA. The effect of chimeric decoy ODNs has also been evaluated in animal models (Figure 4). Transfection of decoy ODNs was performed by wrapping a delivery sheet containing decoy ODNs around the aorta. Treatment with chimeric decoy ODNs successfully inhibited the progression of AAA in elastase-induced rat and rabbit models.62,63 Moreover, our current study clearly demonstrated the regression of AAA using chimeric decoy ODN in a rabbit already-formed AAA model, through the re-balance of matrix synthesis and degradation.
degradation. Chimeric decoy ODNs reduced MMP-2 and MMP-9 activities and induced macrophage apoptosis. The regression of AAA was also associated with an increase in elastin and collagen synthesis in the aneurysm wall.61

4.3.3 Unresolved issues in decoy strategy
Given the successful regression of AAA using chimeric decoy ODNs in an animal model, pharmacological therapy targeted to inhibit NFκB and ets simultaneously might provide a new therapeutic strategy for treating AAA. However, ODN-based therapy still has many unsolved problems such as the short half-life and rapid degradation of ODN. Especially, treatment with decoy ODN in vivo requires local application of ODN, because of the degradation of ODN by nucleases. Therefore, we used a cellulose-based delivery sheet containing decoy ODNs. Although this approach is useful to introduce decoy ODN into the outer aneurysm wall, low-invasive procedures, such as laparoscopy, might be necessary. However, considering the clinical usage of this strategy, the development of a non-invasive approach, such as intravenous application, is also necessary. Additional structural modification of ODNs to increase their stability against nucleases will facilitate the potential clinical utility of the decoy strategy. Also, it is necessary to perform further studies to develop effective and safe delivery systems.

5. Conclusion and future directions
A number of pharmacological therapies have the potential to limit AAA progression. Among them, statins, ACE-inhibitors, antibiotics, and anti-inflammatory agents appear to inhibit the AAA growth rate in humans. However, the sample size and follow-up period of studies were limited. Therefore, a large randomized study with long-term follow-up of small AAA should be performed to clarify the effect of these agents.

On the other hand, considering the future of AAA therapy, a pharmacological approach for treating small AAA would not only suppress the development of AAA but also induce regression. As AAA results from multiple interactions of intracellular signalling pathways, an effective approach for AAA could reverse several pathogenic phenomena associated with AAA formation. Recently, molecular pharmacological treatment using a JNK inhibitor and decoy ODNs against NFκB and ets has been reported to regress AAA in animal models—these strategies regulate numerous molecular mediators and signalling networks. From these investigations, it could be possible to repair the injured aortic wall and obtain remission of AAA using pharmacological therapy.

In addition, although the mechanisms of AAA formation have been elucidated at the cellular and molecular levels,
the factors that initiate and maintain the abnormal intracellular signalling pathways, such as inflammation, are not yet clear. Also, the interaction of distinct mediators and signal pathways is not understood. If these points could be clarified, the most effective molecular target would be identified, leading to important new discoveries and therapies for small AAA.

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