Cilostazol Attenuates Angiotensin II–Induced Abdominal Aortic Aneurysms but Not Atherosclerosis in Apolipoprotein E–Deficient Mice

Ryoko Umebayashi, Haruhito A. Uchida, Yuki Kakio, Venkateswaran Subramanian, Alan Daugherty, Jun Wada

Objective—Abdominal aortic aneurysm (AAA) is a permanent dilation of the abdominal aorta associated with rupture, which frequently results in fatal consequences. AAA tissue is commonly characterized by localized structural deterioration accompanied with inflammation and profound accumulation of leukocytes, although the specific function of these cells is unknown. Cilostazol, a phosphodiesterase III inhibitor, is commonly used for patients with peripheral vascular disease or stroke because of its anti-platelet aggregation effect and anti-inflammatory effect, which is vasoprotective effect. In this study, we evaluated the effects of cilostazol on angiotensin II–induced AAA formation.

Approach and Results—Male apolipoprotein E–deficient mice were fed either normal diet or a diet containing cilostazol (0.1% wt/wt). After 1 week of diet consumption, mice were infused with angiotensin II (1000 ng/kg per minute) for 4 weeks. Angiotensin II infusion increased maximal diameters of abdominal aortas, whereas cilostazol administration significantly attenuated dilation of abdominal aortas, thereby, reducing AAA incidence. Cilostazol also reduced macrophage accumulation, matrix metalloproteinases activation, and inflammatory gene expression in the aortic media. In cultured vascular endothelial cells, cilostazol reduced expression of inflammatory cytokines and adhesive molecules through activation of the cAMP–PKA (protein kinase A) pathway.

Conclusions—Cilostazol attenuated angiotensin II–induced AAA formation by its anti-inflammatory effect through phosphodiesterase III inhibition in the aortic wall. Cilostazol may be a promising new therapeutic option for AAAs.

Key Words: abdominal aortic aneurysm  angiotensin II  apolipoprotein E  atherosclerosis  cilostazol

Abdominal aortic aneurysm (AAA) is a permanent dilation of the abdominal aorta with an onset of symptoms that are often insidious until the overt manifestation of rupture. Associated with advanced age, male sex, cigarette smoking, and genetic predisposition, AAA is a common disease with a prevalence of 3% to 5% in males over 60 years of age.1 AAA and aortic dissection is the ninth leading cause of death in the United States. 2 The prevalence of AAA is increasing in the United States,2 and open and endovascular surgical repairs are the only available treatments for large AAAs (>5 cm). There is no proven medical therapy available, particularly for small AAAs. 2 The effects of antihypertensive and antidiyslipidemic therapies on AAA formation and growth, respectively, are controversial. 3–5

The pathogenesis of AAA is multifactorial, with complex interactions between MMP (matrix metalloproteinase)–driven enzymatic degradation of aortic elastin and damage to medial smooth muscle cells. Loss of smooth muscle cells, elastin degradation medial thinning, adventitial hypertrophy, accumulation of inflammatory cells, atherosclerosis, and thrombi are common pathological characteristics in human AAAs. 6,7 AAA is regarded as a chronic inflammatory disease as previous studies have demonstrated accumulation of macrophages and lymphocytes in the aortic wall8 and elevated plasma concentrations of IL (interleukin)–1β, IL-6, TNF-α (tumor necrosis factor-α), and interferon-γ in AAA patients. 9,10

Angiotensin II (AngII)–induced AAA is a commonly used model of aneurysm formation in mice. Chronic AngII infusion induces AAA in normo- and hyperlipidemic mice, with some succumbing to aneurysm rupture. 11,12 Some pathological features of this model resemble human AAAs, including medial elastin degradation and accumulation of inflammatory cells, including macrophages and T and B lymphocyte cells in the aortic wall. During the early phase of AngII-induced AAAs, macrophage accumulation occurs in the medial and adventitial layers of the suprarenal aorta. Macrophage accumulation into the medial layer is often accompanied by disruption of...
elastin fibers. Previous studies have demonstrated the roles of inflammation in aneurysmal formation in this model as deletion of cyclooxygenase-2, osteopontin, and microsomal prostaglandin E synthase-1–attenuated AngII-induced AAA formation. Bone marrow–derived MCP-1 (monocyte chemotactrant protein-1) receptor, caspase-1-dependent IL-1β production, IL-6, and TNF-α are also involved in the formation of AngII-induced AAA.

Cilostazol, a selective inhibitor of phosphodiesterase III (PDEIII), is commonly used in clinical practice as a anti-platelet drug for peripheral artery disease and stroke. PDEIII is expressed in platelets, heart, adipose tissue, liver, kidney, and arterial tissue. PDEs degrade cAMP synthesized by adenylate cyclase. By inhibiting PDEIII enzymatic activity, cilostazol increases intracellular cAMP and activates protein kinases, including PKA (protein kinase A) and exchange protein directly activated by cAMP. This leads to myriad effects, including vasodilation and inhibition of proliferation in the vasculature, inotropic and chronotropic effects in heart, anti-restenosis effects in coronary arteries, reduction of triglycerides, and increases of high-density cholesterol concentration in plasma, in addition to an antiplatelet effect.

Previous studies have shown that cilostazol has anti-inflammatory, antioxi-
dation of AngII-induced AAA. Despite these vasoprotective effects, the effect of cilostazol on AAA development remains unknown. In this study, we determined the effects of cilostazol on AngII-induced AAs in apolipoprotein E (apoE)–deficient (apoE−/−) mice. Our study demonstrated that cilostazol reduced inflammatory changes in the aortic wall and attenuated AAA development. These effects are associated with PDEIII inhibition, reducing inflammatory properties of endothelial cells.

Materials and Methods

Mice and Study Protocol

Male, 8- to 12-week-old, apoE−/− mice were purchased from The Jackson Laboratory (Bar Harbor, Cat. No. 2052). All mice were maintained in a barrier facility, and ambient temperature ranged from 20°C to 24°C. Mice were fed diet and water ad libitum. Only male mice were studied because female mice have low incidence of AngII-induced AAA as detailed in an ATPB Council statement. Cilostazol was a generous gift from Otsuka Pharmaceutical Company. Either vehicle or cilostazol-containing (0.1% wt/wt) diet were started 1 week prior to AngII infusion. For aneurysm quantification and histological analysis, saline or AngII (1,000 ng/kg per minute, Bachem, Cat. No. H-1705) was infused via Alzet mini-osmotic pumps (Model 2004; Durect Corp) for 28 days. Mini-osmic pumps were implanted subcutaneously on the right flank, as described previously. For mRNA and protein (Western blotting and gelatin zymography) analyses, AngII or saline was infused into mice for 7 days. The experimental protocol was approved by the Ethics Review Committees for Animal Experimentation of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical sciences.

Blood Pressure Measurement

Systolic blood pressure and pulse rate were measured by sphygmo-

anometer using a tail cuff system (BP-98A, Softron) following a published protocol. Conscious mice were introduced into a small holder mounted on a thermostatically controlled warming plate and maintained at 37°C during measurement.

Lipids Measurements

After an overnight fast, blood was obtained by cardiac puncture under anesthesia. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol concentrations were determined in individual plasma samples using commercially available enzymatic-based kits (Wako Chemicals Cat. No. 439–17501, No. 465–56701, and No. 432–40201).

Cilostazol Concentrations

Mice were fed either control or cilostazol-containing diet for 5 weeks. For systemic cilostazol concentration measurements, blood was obtained during the daytime without fasting, and plasma was separated. Cilostazol concentrations were measured using high-per-

Quantification of Aneurysms and Atherosclerosis

After 28 days of either saline or AngII infusion, mouse aortas were harvested for aneurysm and atherosclerosis quantification following the American Heart Association statement on atherosclerosis. Aortas were perfused with saline by left ventricular puncture and were fixed in 10% formalin overnight. Adventitial fat was removed, and the maximum external width of the suprarenal aorta was measured using computed morphometry (Lumina Vision software, Mitani Corp) as described previously. Aneurysm was defined as a 50% increase compared with saline-infused aorta. In saline-infused mice, the mean suprarenal width was 0.87 mm; consequently, we defined AAA as >1.30 mm. Atherosclerosis was quantified on intima on aortic arches as percent lesion area by en face method as described previously.

Histological Analysis

Mouse abdominal aortas were fixed in formalin, embedded in opti-

Gelatin Zymography

Either control or cilostazol-containing (0.1% wt/wt) diet were started 1 week prior to AngII infusion, and aortas were harvested 1 week after infusions. Proteins were extracted from aortas without any dis-

Isolation and Culture of Endothelial Cells

Aortic endothelial cells were isolated from apoE−/− mice by an

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA</td>
<td>abdominal aortic aneurysm</td>
</tr>
<tr>
<td>AngII</td>
<td>angiotensin II</td>
</tr>
<tr>
<td>ApoE</td>
<td>apolipoprotein E</td>
</tr>
<tr>
<td>MCP-1</td>
<td>monocyte chemotactrant protein-1</td>
</tr>
<tr>
<td>PDEIII</td>
<td>phosphodiesterase III</td>
</tr>
<tr>
<td>PKA</td>
<td>protein kinase A</td>
</tr>
</tbody>
</table>

Downloaded from http://ahajournals.org by on January 10, 2019
(BD, Cat. No. 354235) and then plated onto collagen-coated culture dishes. Subsequent passages were performed with 0.25% trypsin-EDTA, and cells were split in a 1:4 ratio. The identification of cells was confirmed by immunostaining for von Willebrand factor.

Cell Culture Conditions
Endothelial cells isolated from aorta of apoE−/− mice were used between passages 4 and 6. After overnight serum starvation, endothelial cells were incubated with selected concentrations of cilostazol (0, 1, and 10 μmol/L/H89 (Cayman chemical, Cat. No. 130964-39-5)/ forskolin (Cell Signaling, Cat. No. 9803) for 30 minutes and incubated in the presence of TNF-α (1 ng/mL; R&D, Cat. No. 410-MT) for 24 hours, and then media and cells were collected. Medium was used for the MCP-1 ELISA assay. Cell lysates were used for Western blotting and quantitative polymerase chain reaction (PCR).

Real-Time Polymerase Chain Reaction
mRNAs were extracted from aortas or cell lysates using RNeasy Fibrous Tissue Mini kits (Qiagen) or RNeasy Mini kits (Qiagen), respectively. Reverse transcription was performed using iScript cDNA synthesis kit (Bio Rad). PCR reactions were performed with an ABI Step One Real-Time PCR System (Applied Biosystems) using Fast SYBR Green Real-time PCR Mixture (Applied Biosystems). Primers for Ccl2 (chemokine [C-C motif] ligand 2/Mcp-1, II-1β), Cox2, iNos, Spp1, Icam-1, Mmp-2, Mmp-9, and 18s were available commercially (Takara Bio Inc.). Each sample was normalized to values for 18s mRNA expression (ΔΔCT method).

Western Blotting
Cultured endothelial cells were placed in lysis buffer (Cell Signaling, Cat. No. 9803). Samples were loaded onto 10% SDS-PAGE and transferred to polyvinylidene fluoride membrane, immunoblotted with primary antibodies (ICAM-1 [intercellular adhesion molecule 1]; R&D, Cat. No. AF796 and β-actin; Sigma, Cat. No. A1978), followed by secondary antibodies conjugated with horse radish peroxidase. Bands were detected by use of an enzyme-linked chemiluminescence detection kit (Merck Millipore). The density was quantified with luminiscence image analyzer (Fujifilm) and normalized to β-actin.

MCP-1 ELISA Assay
MCP-1 in culture media was quantified with a sandwich ELISA technique using the Quantikine Mouse/Rat CCL2/JE/MCP-1 immunoassay kit (R&D, Cat. No. EMJ 00) according to the manufacturer’s instructions.

Statistics
All plot and bar graphs were created with SigmaPlot v11.0 (Systat Software Inc.). All statistical analyses were performed using SigmaStat v3.5, incorporated into SigmaPlot v11.0. Data are presented as mean±SE. Statistical significance between multiple groups was assessed by 2-way analysis of variance with Holm–Šidák post hoc or 1-way analysis of variance with Student–Newman–Keuls post hoc, 1-way analysis of variance with Tukey’s post hoc, 1-way analysis of variance on Ranks with a Dunn’s post hoc, where appropriate. Percentage incidence and mortality of AAA were analyzed by Fisher Exact test. A P value <0.05 was considered statistically significant.

Results
Cilostazol Had No Effect on Body Weight, Pulse Rate, Blood Pressure, and Lipid Concentrations
Male, 8- to 12-week-old apoE−/− mice were fed either control or cilostazol-containing (0.1% wt/wt) diet. After 1 week of cilostazol administration, either saline or AngII (1000 ng/kg per minute) were infused for 28 days. Plasma cilostazol concentrations in mice fed cilostazol-containing diet were 198±10 ng/mL, while being undetectable in mice fed a control diet. AngII infusion had no effect on plasma cilostazol concentrations. The plasma cilostazol concentrations in mice were equivalent to the plasma concentrations 12 to 24 hours after oral administration of 100 mg in humans. There were no significant differences in body weight and pulse rate in saline or AngII infusions among the 4 groups. As expected, AngII infusion increased systolic blood pressure significantly, but there was no difference between the 2 groups (AngII+control diet and AngII+cilostazol-containing diet; Table I in the online-only Data Supplement). In addition, cilostazol had no effect on plasma concentrations of total cholesterol, triglyceride, and high-density lipoprotein cholesterol in apoE−/− mice during saline or AngII infusion (Table II in the online-only Data Supplement).

Cilostazol Attenuated Formation of AngII-Induced AAA but Did Not Influence AngII-Accelerated Atherosclerosis
To determine the effects of cilostazol on the development of AngII-induced AAs, male apoE−/− mice fed control or cilostazol-containing diet were infused with either saline or (saline: n=5, cilostazol: n=6) AngII for 28 days (AngII: n=20, AngII+cilostazol: n=17). No mice died of aneurysm rupture in saline-infused mice. In the AngII-control diet group, 1 mouse died of thoracic aortic aneurysm rupture and 3 died of AAA rupture. In the AngII+cilostazol group, 1 mouse died of thoracic aortic aneurysm rupture and 2 mice died of AAA rupture. At the end of study, ex vivo suprarenal aortic width was measured (Figure 1A). In saline-infused mice, cilostazol had no effect on ex vivo suprarenal aortic width (mean width of abdominal aorta: 0.87±0.04 mm in saline mice versus 0.88±0.03 mm in cilostazol mice). AngII infusion significantly increased aortic width (mean width of abdominal aorta: 1.94±0.16 mm; Figure 1A, representative photo in Figure 1B, left). Cilostazol administration attenuated dilatation of aortic width and decreased AAA formation (mean width of abdominal aorta: 1.53±0.17 mm, P=0.049; Figure 1A, representative photo in Figure 1B, right). Based on AAA being defined as a >50% increase in suprarenal aorta width, the incidence of AAs in AngII-infused mice not administered cilostazol was 79%. Cilostazol significantly decreased the incidence to 43% (P=0.029; Figure 1C). However, there was no significant difference in the aortic rupture rate between the AngII-infused group (mortality rate: 15% and 11%, respectively, n.s. Figure 1D). There were no differences in atherosclerotic lesion area as evaluated by en face method among the 4 groups (Figure 1 in the online-only Data Supplement).

Cilostazol Reduced Medial Disruption and Macrophage Infiltration
To evaluate the histological characteristics of abdominal aortas, we performed Verhoeff’s staining on tissue sections from suprarenal aortas. Pronounced disruptions of medial layers were observed in AngII-infused mice; however, elastin fragmentation induced by AngII infusion were markedly reduced in mice consuming cilostazol (Figure 2D through 2F). Because
it is known that macrophage-mediated inflammation plays an important role in AngII-induced AAA formation, we performed immunohistochemistry to determine the localization of macrophages in aortas. Immunohistochemistry of CD68 revealed increased macrophage accumulation in the region of medial disruption in aorta from AngII-infused mice, whereas less macrophage infiltration in medial layer was observed in mice consuming cilostazol (Figure 2G through 2I).

Figure 1. Cilostazol attenuated angiotensin II (AngII)–induced abdominal aortic aneurysm (AAA) formation. A, AngII infusion significantly increased ex vivo maximal diameters of abdominal aortas from AngII and AngII+cilostazol mice (P<0.001 and P=0.032, respectively, when comparing saline-infused group), while cilostazol attenuated enlargement of aortic diameter (P=0.049 when comparing the AngII-infused group). Aortic diameter measurements from each mouse are represented by a circle or an inverted triangle (● represent saline-infused mice without cilostazol administration, ○ represent saline-infused mice with cilostazol administration, ▼ represent AngII-infused mice without cilostazol administration, ◇ represent AngII-infused mice with cilostazol administration, ◇ represent means, and — represent SE. Statistical analysis was performed by 2-way analysis of variance (ANOVA). B, Representative images of abdominal aortas from experimental mice infused with AngII and fed control (left) or cilostazol-containing diets (right). C, The incidence of AAA (>50% increase in aortic width) was 79% in AngII mice (black bar) and was decreased significantly to 43% in AngII+cilostazol mice (white bar). Statistical analyses were performed by Fisher Exact test (P=0.029). D, The mortality ratio from AAA rupture in AngII-infused mice is shown in black bars, and the mortality ratio in AngII-infused mice administered cilostazol are shown in white bars. There was no significant difference in mortality from aneurysm rupture between these 2 groups by Fisher exact test.

Figure 2. Cilostazol reduced elastin degradation and macrophage accumulation in the aortic media. Elastic fibers were stained with Verhoeff's staining in tissues sections of suprarenal aortas (A–F). A, D, G, Samples from saline-infused mice. B, E, H, Samples from AngII-infused mice. C, F, I, Samples from AngII-infused+cilostazol mice. Disruption of elastic fibers and enlargement of aortic diameters were seen in aortas from AngII-infused mice (B and E). Degradation of elastic fibers and enlargement of aortic diameters were reduced in aorta from AngII-infused+cilostazol mice (G and F). Representative immunostaining images of macrophages with an anti-mouse CD68 antibody (G–I). Macrophages were localized predominantly in the media of suprarenal aortas from AngII-infused mice (H) but were only seen rarely in aortas from AngII-infused+cilostazol mice (I). * indicates the lumen of aorta, and arrow head indicates the perimeter of the media (bar = 200 μm).
Cilostazol Reduced MMP Activity in the Aorta

Gelatin zymography was performed to detect MMP-2 and MMP-9 activity in tissue extracts from aortas. After 7 days of AngII infusion, aortas without any discernable aneurysms were used for gelatin zymography. Pro-form MMP-2 (65 and 70 kDa), active-form MMP-2 (58 kDa), and MMP-9 (92 kDa) were detected in angiotensin II (AngII)-infused mice. Cilostazol diminished AngII-induced MMP-2 and MMP-9 activation.

Cilostazol Administration Reduced mRNA Abundance of Inflammatory Cytokines in Aortas

Gene expression of inflammatory cytokines, such as Ccl2/Mcp-1, Il-1β, Cox2, and Spp1, were increased significantly in aortas of AngII mice. Cilostazol administration reduced mRNA abundance of these molecules. These results suggested that cilostazol suppressed inflammation in aortic walls, leading to the reduction of dilatation of aortic width and incidence of AAAs.

Cilostazol Reduced MCP-1 and ICAM-1 Expression in Aortic Endothelial Cells

Because cilostazol decreased AAA formation in our model and is known to inhibit PDEIII function, we sought to investigate the site of PDEIII expression in developing AAAs. We performed immunohistochemistry for PDEIII in the aorta. PDEIII localized mainly to the intima and partly in media (Figure II in the online-only Data Supplement). Therefore, we investigated the effects of cilostazol on aortic endothelial cells.

Endothelial cells isolated from aortas of apoE−/− mice were incubated with selected concentrations of cilostazol (0, 1, and 10 μmol/L) for 30 minutes and then incubated with TNF-α (1 ng/mL) for 24 hours. mRNA was extracted for real-time PCR. TNF-α elevated Ccl2/Mcp-1 and Icam-1 mRNA expression, while cilostazol suppressed mRNA expression of them in a concentration-dependent manner (Figure 5A). Furthermore, cilostazol reduced TNF-α-stimulated Vcam-1 mRNA expression (Figure 5B). In agreement with this endothelial cell mRNA expression, concentrations of CCL2/MCP-1 in cultured media were reduced by cilostazol in a concentration-dependent manner (Figure 5C). By Western blotting, the abundance of ICAM-1 was increased during incubation with TNF-α but reduced by cilostazol, as expected (Figure 5D).

Cilostazol Exerted Protective Effects on Aortic Endothelial Cells Through PDEIII Inhibition

PDEIII inhibition increases intracellular cAMP, and cAMP exerts anti-inflammatory effects through the PKA pathway in vascular endothelial cells. Therefore, we hypothesized that activation of the cAMP–PKA pathway might be the target cilostazol during AAA development. To further elucidate the mechanism, we next performed an in vitro study using forskolin, an activator of adenylate cyclase, and H89, an inhibitor of PKA. Endothelial cells were incubated with cilostazol (10 μmol/L) or forskolin (10 μmol/L), cilostazol, and H89 (10 μmol/L) for 30 minutes, incubated with TNF-α (1 ng/mL) for 24 hours, and then mRNA was harvested from cell lysates and subjected to real-time PCR. TNF-α increased mRNA expression of Ccl2/Mcp-1 and Icam-1, but they were reduced by cilostazol and forskolin. Co-incubation of H89 blunted the effects of cilostazol (Figure 6). These results demonstrated that cilostazol reduced the expression of inflammatory cell adhesion molecules.
cytokines and adhesive molecules, such as CCL2/MCP-1 and ICAM-1, through the cAMP–PKA pathway.

**Discussion**

Chronic infusion of AngII into hyperlipidemic mice consistently induces AAA formation. The characteristics of this AAA model include activation of an inflammatory response and stimulation of a proteolytic cascade. In the present study, we demonstrated that cilostazol attenuated AAA development and reduced AAA incidence induced by chronic AngII infusion. Furthermore, this inhibitory effect of cilostazol on AngII-induced AAA formation was, at least in part, because of its anti-inflammatory effect to reduce activation of the cAMP–PKA pathway in endothelial cells.

Cilostazol is used widely as an antiplatelet drug. In addition, cilostazol has been reported to have many pleiotropic effects, including anti-inflammatory, antiproliferative, and vasodilatory. Previous studies have shown that cilostazol inhibited vascular inflammation by downregulation of VCAM-1 (vascular cell adhesion molecular 1) expression in a diabetic rat model and attenuated MCP-1 and MMP-9 expression in a balloon-injury model in vivo.

Cilostazol exerted anti-inflammatory effects by reducing MCP-1 production in vitro, TNF-α production induced by lipopolysaccharide stimulation, NF-κB (nuclear factor-κB) activation, and VCAM-1 expression. Genetic depletion of cyclooxygenase-2 and osteopontin (Spp1) attenuated AngII-induced AAA formation, indicating that these molecules play pivotal role in the development of AAA. Consistent with these previous studies, in our study, gene expression of Ccl2/Mcp-1, Il-1β, Cox2, and Spp1 were reduced in aortas from AngII-infused mice but were reduced by cilostazol administration. Statistical analyses were performed by 1-way analysis of variance (ANOVA).
During development of AngII-induced AAA, an initial event is medial accumulation of macrophages, which may lead to subsequent medial rupture. We investigated how cilostazol prevented vascular inflammation induced by AngII infusion. First, we investigated PDEIII localization in the murine aortic wall. PDEIII was reported previously to be localized in vascular smooth muscle cells, endothelium, and activated macrophages. In our study, PDEIII was localized mainly in endothelial cells. Accordingly, we investigated the effect of cilostazol on endothelial cells using murine primary cultured aortic endothelial cells. In vascular endothelium, cilostazol has been reported previously to increase NO synthesis to exert anti-inflammatory effects, including reduction of MCP-1 secretion and VCAM-1 expression. Furthermore, it is well established that increases in intracellular cAMP promote endothelial barrier integrity. We confirmed that cilostazol reduced expression of adhesion molecules and the production of inflammatory cytokines, thereby reducing the endothelial response to inflammation. Given these findings, we conclude that cilostazol, in part, reduced macrophage accumulation into the media through activation of the cAMP–PKA pathway in endothelial cells.

Figure 5. Cilostazol reduced mRNA abundance of inflammatory cytokines and adhesion molecules in cultured aortic endothelial cells isolated from apolipoprotein E (apoE)−/− mice. A, mRNA abundance of Ccl2/Mcp-1 and Icam-1 were increased by TNF-α (tumor necrosis factor-α) incubation (1 ng/mL, 24 hours) but suppressed by cilostazol (1 μmol/L and 10 μmol/L) in a concentration-dependent manner (n=3 in each group). Statistical analyses were performed by 1-way analysis of variance (ANOVA). B, Quantitative mRNA abundance of inflammatory cytokines and adhesion molecules in aortic endothelial cells (n=3 in each group). Expression of Ccl2/Mcp-1, Icam-1, and Vcam-1 were upregulated by TNF-α stimulation (black bar) and were significantly reduced by cilostazol incubation (white bar). Statistical analyses were performed by 1-way ANOVA. C, CCL2/MCP-1 concentrations in culture medium were measured by using a commercial ELISA kit. Aortic endothelial cells from apoE−/− mice were incubated with the indicated conditions for 24 hours. Cilostazol (10 μmol/L) administration reduced CCL2/MCP-1 in culture medium. Statistical analyses were performed by 1-way ANOVA. D, ICAM-1 expression in aortic endothelium was evaluated by Western blotting (n=3 in each group). The relative abundance of ICAM-1 protein was quantified by densitometry and normalized to the abundance under the control condition. Statistical analyses were performed by 1-way ANOVA. ICAM-1 indicates intercellular adhesion molecule 1; MCP-1, monocyte chemoattractant protein-1; and VCAM-1, vascular cell adhesion molecule 1.

Matrix metalloproteinases are increased in aortic tissue from AAA patients. Thus, these proteinases were considered to exert a pivotal role in aneurysm formation. In AngII-induced AAA, both MMP-2 and MMP-9 are also activated and are thought to be important for AAA development. Genetic disruption of MMP-9 has been reported to suppress elastase-induced AAA. Inhibition of MMP-2 also has been reported to attenuate AAA formation in CaCl2-induced AAs. A previous experimental study using elastase-induced AAA model revealed that cilostazol reduced MMP-2 and MMP-9 activities in the aortic wall. In our study, cilostazol administration suppressed AngII-induced MMP activity and, thereby, prevented matrix protein degradation and protected the structural integrity of the aorta.

Although AngII promotes atherosclerosis as well as atherosclerosis in hypercholesterolemic mice, the occurrence and degree of severity varies in previous reports. Indeed, atherosclerosis and AAA can respond differently to specific interventions. These findings imply that the mechanisms underlying atherosclerosis and aneurysms are different. In previous studies, cilostazol reduced atherosclerosis in low-density lipoprotein receptor−/− mice and apoE−/− mice fed a
high-fat diet. However, in our study, cilostazol had no effect on AngII-accelerated atherosclerosis in the aortic arch. AngII infusion also induces AAAs in normolipidemic mice, but the incidence is 3- to 4-fold lower than in hyperlipidemic mice.60 However, there is only weak evidence correlating total cholesterol concentrations and aneurysmal growth in humans.61,62 In the AngII model, concentrations of plasma apolipoprotein B–containing lipoproteins are more important to AAA formation than high-density lipoprotein cholesterol.63 Treatments with statin or Omega 3 polyunsaturated fatty acids have a certain effect on aneurysm development and growth.64,65 Both animal and clinical studies have shown that cilostazol decreases concentrations of serum triglyceride and increases high-density lipoprotein.24 However, in our study, cilostazol had no effect on serum lipid concentrations, implying that cilostazol attenuated AAA through other pharmacological effects. Additionally, this finding was consistent, with no improvement of atherosclerosis by cilostazol administration.

Several clinical studies reported that blood pressure is considered an important player in AAA formation and growth.66 Several animal studies have indicated that propranolol, a β-blocker, might attenuate aneurysmal growth on the bases of its hemodynamic properties and its biochemical effect on matrix cross-linkage.67,68 However, clinical studies failed to show the efficacy of propranolol on aneurysmal expansion. On the contrary, cilostazol sometimes causes tachycardia in clinical situations. In our animal study, cilostazol had no effect on blood pressure and pulse rate. It is likely that pulse rate may not influence AAA formation and growth.

There are several reports regarding usefulness of other antiplatelet therapy on AAA development and progression.69–71 Owens et al72 reported that platelet inhibitors, aspirin, and clopidogrel reduced death from AAA rupture but had no effect on dilatation of abdominal aorta in established AngII-induced AAAs. Furthermore, they also showed that administration of aspirin and P2Y12 inhibitors was associated with reduced death in AAA patients in a human retrospective study.72 Because cilostazol exerts its antiplatelet effect in a mechanism different from that of aspirin and clopidogrel, it may offer a beneficial effect on the reduction of aortic dilation in an established AAA model. However, further studies are warranted to test and clarify the effect of cilostazol regarding the aspect of antiplatelet effect on established AAA growth and rupture.

**Conclusion**

Cilostazol attenuated AngII-induced AAA formation by reducing inflammatory changes in the aortic wall. Cilostazol exerted this anti-inflammatory effect through PDEIII inhibition in aortic endothelial cells. Cilostazol could be a therapeutic agent for AAAs.

**Acknowledgments**

We thank Ms Yoshiko Hada for her technical assistance. We are also grateful to Ms Debra L. Rateri for carefully proofreading the manuscript.

**Disclosures**

None.

**References**


26. Umebayashi et al. Cilostazol Attenuates AngII-Induced AAA.


39. Umebayashi et al. Cilostazol Attenuates AngII-Induced AAA.


41. Umebayashi et al. Cilostazol Attenuates AngII-Induced AAA.
• Anti-inflammatory effects were partially because of reduction of inflammation in endothelial cells.

Highlights

- The phosphodiesterase III inhibitor, cilostazol, suppressed angiotensin II-induced abdominal aortic aneurysm.
- Cilostazol reduced the inflammation in aortic wall.
- Anti-inflammatory effects were partially because of reduction of inflammation in endothelial cells.