Pharmacological Inhibition and Genetic Deficiency of Plasminogen Activator Inhibitor-1 Attenuates Angiotensin II/Salt-Induced Aortic Remodeling

Alec D. Weisberg, Francisco Albornoz, Jane P. Griffin, David L. Crandall, Hassan Elokdah, Agnes B. Fogo, Douglas E. Vaughan, Nancy J. Brown

Objective—To test the hypothesis that pharmacological plasminogen activator inhibitor (PAI)-1 inhibition protects against renin-angiotensin-aldosterone system-induced cardiovascular injury, the effect of a novel orally active small-molecule PAI-1 inhibitor, PAI-039, was examined in a mouse model of angiotensin (Ang) II-induced vascular remodeling and cardiac fibrosis.

Methods and Results—Uninephrectomized male C57BL/6J mice were randomized to vehicle subcutaneous, Ang II (1 μg/h) subcutaneous, vehicle + PAI-039 (1 mg/g chow), or Ang II + PAI-039 during high-salt intake for 8 weeks. Ang II caused significant medial, adventitial, and aortic wall thickening compared with vehicle. PAI-039 attenuated Ang II-induced aortic remodeling without altering the pressor response to Ang II. Ang II increased heart/body weight ratio and cardiac fibrosis. PAI-039 did not attenuate the effect of Ang II on cardiac hypertrophy and increased fibrosis. The effect of PAI-039 on Ang II/salt-induced aortic remodeling and cardiac fibrosis was comparable to the effect of genetic PAI-1 deficiency. Ang II increased aortic mRNA expression of PAI-1, collagen I, collagen III, fibronectin, osteopontin, monocyte chemoattractant protein-1, and F4/80; PAI-039 significantly decreased the Ang II-induced increase in aortic osteopontin expression at 8 weeks.

Conclusions—This study demonstrates that pharmacological inhibition of PAI-1 protects against Ang II-induced aortic remodeling. Future studies are needed to determine whether the interactive effect of Ang II/salt and reduced PAI-1 activity on cardiac fibrosis is species-specific. (Arterioscler Thromb Vasc Biol. 2005;25:1-7.)

Key Words: PAI-1 ■ angiotensin II ■ pharmacological inhibition ■ aortic remodeling ■ cardiac fibrosis

The renin-angiotensin-aldosterone system (RAAS) plays an integral role in cardiovascular homeostasis through its effects on vascular tone and volume. Activation of the RAAS has been associated with increased risk of ischemic cardiovascular events and renal injury, independent of effects on blood pressure. Interruption of the RAAS by angiotensin-converting enzyme inhibition or AT1 receptor antagonism reduces cardiovascular mortality and slows the progression of renal disease. There is substantial evidence that angiotensin (Ang) II contributes to cardiovascular and renal injury by inducing inflammation and oxidation, stimulating smooth muscle cell growth and proliferation, and inducing extracellular matrix formation.

One possible mechanism through which Ang II promotes the development of cardiac fibrosis and vascular remodeling is through induction of plasminogen activator inhibitor-1 (PAI-1) expression. Ang II induces PAI-1 expression in vascular smooth muscle cells, endothelial cells, and cardiomyocytes, whereas AT1 receptor antagonism decreases PAI-1 expression in several animal models. PAI-1, the principal inhibitor of plasminogen activation, can promote fibrosis by preventing the activation of matrix metalloproteinases (MMPs), enzymes that degrade collage- nous proteins, and the degradation of extracellular matrix by plasminogen activators and plasmin. Studies using PAI-1-deficient or overexpressing animals have implicated PAI-1 in the pathogenesis of fibrosis and/or sclerosis after chemical or immune-mediated injury.

Studies using PAI-1-deficient mice have also served to elucidate the role of PAI-1 in vascular remodeling. For example, inhibition of nitric oxide synthase using N\textsuperscript{\text{\textendash}}nitro\textsuperscript{\textendash}l-arginine-methyl-ester (l-NAME) induces cardiac remodeling and perivascular fibrosis in rodents, accompanied by increased PAI-1 expression and/or protein content in the endothelium and media of the aorta and coronary arteries. PAI-1 knockout mice are protected against l-NAME–induced...
hypertension and perivascular fibrosis. Furthermore, coadministration of an angiotensin-converting enzyme inhibitor abolishes L-NAME–induced PAI-1 expression and cardiovascular remodeling suggesting that, in this model, activation of the RAAS promotes cardiovascular injury through PAI-1.

Studies demonstrating that mice genetically deficient in PAI-1 are protected against perivascular fibrosis suggest that pharmacological inhibition of PAI-1 may provide similar protection. In the present study, we test this hypothesis by examining the effect of a novel oral small-molecule PAI-1 inhibitor, PAI-039, on Ang II–induced cardiovascular injury in uninephrectomized mice during high-salt intake for 8 weeks. The effect of pharmacological PAI-1 inhibition on Ang II/salt-induced aortic remodeling and cardiac fibrosis was compared with the effect of genetic PAI-1 deficiency.

Materials and Methods

Animals

Male C57BL/6J mice were purchased from Charles River Laboratory (Boston, Mass). Male PAI-1 knockout mice on a C57BL/6J background and C57BL/6J controls were obtained from Jackson Laboratory (Bar Harbor, Me). Mice were maintained in a temperature-controlled facility with a fixed light/dark cycle. All animal procedures were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Experimental Design

During the 2-month protocol, 32 8-week-old mice weighing <25 grams with a baseline systolic blood pressure <120 mm Hg were randomized into 4 experimental groups containing 8 mice each: vehicle for Ang II, Ang II, vehicle + PAI-039, or Ang II + PAI-039. Uninephrectomy was performed under anesthesia with pentobarbital (50 mg/kg intraperitoneal) on all mice at week 0, and each mouse was implanted with a subcutaneous mini-osmotic pump (Durect Corp., Cupertino, Calif) containing either saline or Ang II (50 mg/kg intraperitoneal) on all mice at week 0, and each mouse was implanted with a subcutaneous mini-osmotic pump (Durect Corporation, Cupertino, Calif) containing either saline or Ang II dissolved in saline (1 µg/mL). Mouse were fed either regular chow or chow supplemented with PAI-039 (1 mg/g chow) generously supplied by Wyeth Research. In pilot studies, this dose of PAI-039 yielded plasma concentrations of 5.5±0.6 µg/mL (n=17) in mice, whereas the in vitro IC₅₀ for PAI-1 inactivation of human PAI-1 is 2.7 µmol/L, or ~1.5 µg/mL. Previous drug safety and metabolism studies in rats indicated that PAI-039 exhibited a lack of significant toxicity such that the no toxic effect level was set at a dose of 2000 mg/kg per day, which resulted in a 24-hour exposure of 1508 µg/mL, equivalent to 1500 times the efficacious dose.17 Mini-osmotic pumps were replaced after 1 month. Mice were supplied 1% saline drinking water ad libitum. Systolic blood pressure was measured every 2 weeks and mice were euthanized at 12 weeks.

Histological Study

Investigators blinded to the treatment protocol assessed histopathology. Cardiac and renal interstitial fibrosis were scored semi-quantitatively on Masson trichrome from 0+ to 4+ for each high-power field, with 0+ indicating no fibrosis, 1+ indicating <25%, 2+ indicating 25% to 50%, 3+ indicating >50% to 75%, and 4+ indicating >75% of each high-power field. Glomerular sclerosis was scored as previously described.10 To evaluate aortic adventitial, medial, and left ventricular free wall thickness, Masson trichrome-stained aortic and cardiac cross-sections were photographed on a Zeiss AxioScop 40 using MRGgrab 1.0 and analyzed using ImageProPlus software (Media Cybernetics, Silver Spring, Md). Aortic medial thickness was measured from the inner border of the lumen to the outer border of the tunica media. Aortic adventitial thickness was also measured. Left ventricular free wall thickness was measured from the junction of the papillary muscles with the left ventricle to the outer border of the myocardium. The average of 2 cross-sectional measures was calculated for each animal.

Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction

Total cardiac RNA was extracted using RNAWiz (Ambion, Austin, Tex) and Rneasy Midi Kit (Qiagen, Valencia, Calif), and total aortic RNA was extracted using Rneasy Mini Kit (Qiagen). Reverse-transcription was performed using TaqMan Reverse Transcription Kit (Applied Biosystems, Branchburg, NJ). Quantitative real-time polymerase chain reaction was performed on the iCycler iQ Multi-Color, Real Time PCR Detection System (BioRad, Hercules, Calif) using iQ SYBR Green Supermix (Biorad). For information regarding primers, see http://atvb.ahajournals.org. Experimental cycle threshold (Ct) values were normalized to β-actin measured on the same plate, and fold differences in gene expression were determined using the 2⁻ΔΔCt method.

Biochemical Assays

Active murine PAI-1 was measured using a modification of a commercially available enzyme-linked immunosorbent assay kit (Molecular Innovations, Southfield, Mich). Briefly, the kit used a urokinase plasminogen activator-coated plate to capture active PAI-1, and a rabbit anti-mouse PAI-1 polyclonal primary antibody (ASMPAI-GF). Murine serum amyloid A was measured using an enzyme-linked immunosorbent assay kit (Immuno-Biological, Min-

To determine effect of genetic PAI-1 deficiency, 18 wild-type and 18 PAI-1−/− mice were each randomized after uninephrectomy into 4 experimental groups: vehicle, Ang II (1 µg/h), aldosterone (2.8 µg/d by sustained release subcutaneous pellet; Innovative Research of America, Fl), or Ang II + aldosterone. All mice were provided chow containing 3.15% sodium, 0.72% potassium (Purina Mills, Inc, St. Louis, Mo), and water ad libitum. Systolic blood pressure was measured every 2 weeks and mice were euthanized at 12 weeks.

Figure 1. Effect of treatment on systolic blood pressure (SBP) at weeks 0, 2, 4, 6, and 8. By ANOVA, Ang II increased SBP (P<0.001). PAI-039 decreased SBP compared with vehicle alone (P=0.028) but did not affect SBP during Ang II. For post-hoc comparisons, *P<0.05, **P<0.01 versus vehicle alone; †P<0.01 versus vehicle + PAI-039.
neapolis, Minn). Blood glucose was measured using a HemoCue (Angelholm, Sweden) B-Glucose Analyzer. Insulin was measured by radioimmunoassay (Linco Research Inc, St Louis, Mo). The homeostasis model assessment–insulin resistance index was calculated using the equation: [fasting glucose (mmol/L) × insulin (mU/L)]/22.5.

Statistics

Results are expressed as mean±SD in the text and tables, and as means±SEM in the figures. Repeated measures analysis of variance (ANOVA) was used to assess the effect of treatment on glucose, insulin, and systolic blood pressure. One-way ANOVA, followed by post-hoc testing, was used to compare continuous variables, such as PAI-1 activity, relative heart weight, medial thickness, and relative gene expression, among groups at a given time point. Results were confirmed using nonparametric tests. All tests were 2-tailed, and P<0.05 was considered statistically significant.

Results

Hemodynamic and Metabolic Parameters

There were no significant differences in baseline systolic blood pressure among the groups (Figure 1). Ang II infusion significantly increased systolic blood pressure (P<0.001). PAI-039 treatment significantly decreased systolic blood pressure (P=0.028) compared with vehicle but did not prevent the Ang II-induced increase in systolic blood pressure. Systolic blood pressure was similar in the Ang II-treated and Ang II+PAI-039–treated groups.

As illustrated in Table 2, body weight did not differ significantly among treatment groups. Ang II significantly increased heart weight (P=0.017 for the combined effect in the Ang II and Ang II+PAI-039 groups) and the heart/body weight ratio (P=0.004 for the combined effect). PAI-039 treatment alone had no effect on either parameter. Additionally, PAI-039 did not alter the effect of Ang II on these parameters. Despite the quantitative changes in heart weight and heart/body weight ratio, left ventricular free wall thickness was similar among treatment groups.

Cardiac Morphology and Histology

TABLE 1. Effect of Treatment on Metabolic and Inflammatory Parameters

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Ang II</th>
<th>Vehicle+PAI-039</th>
<th>Ang II+PAI-039</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active PAI-1, pg/mL</td>
<td>129.2±104.1</td>
<td>289.2±170.4*</td>
<td>33.5±4.1*§</td>
<td>298.0±263*</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>9.1±1.2</td>
<td>8.0±1.3</td>
<td>6.8±1.7§</td>
<td>8.3±1.2</td>
</tr>
<tr>
<td>Insulin, µU/L</td>
<td>0.4±0.2</td>
<td>0.5±0.4</td>
<td>0.5±0.3</td>
<td>0.7±0.4</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>3.4±1.8</td>
<td>3.8±3.3</td>
<td>4.4±2.8</td>
<td>6.4±4.5</td>
</tr>
<tr>
<td>SAA, µg/mL</td>
<td>987±1578</td>
<td>1227±1790</td>
<td>1004±1581</td>
<td>4076±4485†‡</td>
</tr>
</tbody>
</table>

P for effects of Ang II and PAI-039 by ANOVA are presented in the text. For posthoc analyses, *P<0.05 vs vehicle alone; †P<0.05 vs Ang II alone; ‡P<0.05 vs vehicle+PAI-039; §P<0.05 vs Ang II+PAI-039.

HOMA IR indicates homeostasis model assessment–insulin resistance index; SAA, serum amyloid A.

TABLE 2. Effect of Treatment on Cardiac and Aortic Morphology and Histology

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Ang II</th>
<th>Vehicle+PAI-039</th>
<th>Ang II+PAI-039</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>28.4±2.1</td>
<td>28.4±1.1</td>
<td>28.0±2.8</td>
<td>27.3±2.5</td>
</tr>
<tr>
<td>HW, mg</td>
<td>141±21</td>
<td>165±32</td>
<td>135±27</td>
<td>165±33</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>5.1±0.8</td>
<td>5.8±1.0†</td>
<td>4.9±0.7</td>
<td>6.1±1.1†</td>
</tr>
<tr>
<td>LVFW, mm</td>
<td>0.95±0.09</td>
<td>1.12±0.25</td>
<td>0.96±0.30</td>
<td>0.94±0.12</td>
</tr>
<tr>
<td>Cardiac</td>
<td>0.68±0.31</td>
<td>0.94±0.50</td>
<td>0.91±0.53</td>
<td>1.50±0.85*</td>
</tr>
<tr>
<td>Fibrosis index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic media, µm</td>
<td>63.1±9.6</td>
<td>86.2±32.0†¶</td>
<td>51.1±13.2</td>
<td>63.7±5.8</td>
</tr>
<tr>
<td>Aortic</td>
<td>22.4±4.7</td>
<td>55.3±25.1§</td>
<td>26.3±5.2</td>
<td>45.6±31.6*</td>
</tr>
<tr>
<td>Adventitia, µm</td>
<td>85.5±9.8</td>
<td>141.5±47.7†¶</td>
<td>77.3±10.5</td>
<td>109.3±30.8¶</td>
</tr>
</tbody>
</table>

BW indicates body weight; HW, heart weight; LVFW, left ventricular free wall thickness.

*P<0.05; †P<0.05; ‡P<0.05 vs Ang II+PAI-039; §P<0.01 vs vehicle alone; ¶P<0.001 vs vehicle+PAI-039.
Neither Ang II alone nor PAI-039 alone significantly increased the intracardiac fibrosis index compared with vehicle treatment (Table 2). However, the cardiac fibrosis index was significantly increased in the Ang II/PAI-039–treated group compared with the vehicle-treated group ($P<0.023$).

To determine the contribution of inflammation to the observed cardiac fibrosis, the effect of treatment on the systemic inflammatory marker serum amyloid A (SAA) was measured. There was no effect of Ang II alone on SAA. There was also no effect on SAA when PAI-039 was given alone, and that resulted in a reduced plasma PAI-1 activity. However, there was a significant interactive effect of Ang II and PAI-039 ($P=0.014$) on SAA concentrations such that SAA concentrations were higher in Ang II+PAI-039–treated mice than in the other groups (Table 1). No significant renal interstitial fibrosis or glomerular sclerosis was observed in any treatment group (data not shown).

### Aortic Morphology and Histology

As illustrated in Table 2 and Figure 2, Ang II caused significant medial, adventitial, and aortic wall thickening compared with vehicle alone. PAI-039 did not affect vascular structure in vehicle-infused mice. However, PAI-039 significantly attenuated Ang II-induced aortic medial and wall thickening, and tended to decrease adventitial thickening. Collagen volume correlated with adventitial thickness ($P=0.003$).

### Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction

Figure 3 shows the effect of treatment on aortic gene expression. By ANOVA, at 1 week Ang II significantly increased aortic expression of PAI-1, collagen I, collagen III, fibronectin, osteopontin, MCP-1, and F4/80, but not transforming growth factor (TGF)-β1. At 8 weeks, Ang II significantly increased aortic expression of PAI-1, collagen III, osteopontin, and TGF-β1, but not collagen I. PAI-039 did not significantly alter the effect of Ang II infusion on aortic PAI-1, collagen I, collagen III, fibronectin, MCP-1, F4/80, or TGF-β1 expression, but did significantly decrease the Ang II-induced increase in osteopontin expression at 8 weeks ($P=0.024$).

Figure 3 shows the effect of treatment on cardiac gene expression. At 1 week, Ang II significantly increased cardiac expression of PAI-1, collagen I, fibronectin, and osteopontin, but not collagen III, F4/80, TGF-β1, or MCP-1. At 8 weeks, Ang II significantly increased cardiac expression of PAI-1, collagen I, collagen III, F4/80, TGF-β1, and osteopontin, but not fibronectin ($P=0.152$) or MCP-1 ($P=0.983$). PAI-039 did not significantly alter the effect of Ang II infusion on cardiac PAI-1, collagen I, collagen III, fibronectin, MCP-1, osteopontin, or TGF-β1 expression at 1 week or 8 weeks. However, PAI-039 significantly decreased Ang II-induced
increase in F4/80 expression at 8 weeks ($P=0.018$) but not 1 week.

Effect of Genetic PAI-1 Deficiency on Aortic Remodeling and Cardiac Fibrosis

Ang II significantly increased systolic blood pressure from 101.2 ± 2.4 to 159.3 ± 12.9 mm Hg at 12 weeks ($P=0.0021$). There was no effect of aldosterone on systolic blood pressure ($P=0.862$) or on the pressor response to Ang II. The pressor response to Ang II was similar in wild-type and $\text{PAI-1}^{-/-}$ mice ($P=0.430$). Ang II, alone or in combination, increased heart/body weight ratio (Figure 4A). Aldosterone did not affect heart/body weight ratio. As in the case of pharmacological PAI-1 inhibition, genetic PAI-1 deficiency did not modulate Ang II-induced left ventricular hypertrophy. Ang II, aldosterone, and the combination induced cardiac interstitial fibrosis (Figure 4B). Significantly, cardiac fibrosis was increased in $\text{PAI-1}^{-/-}$ mice compared with wild-type mice. Treatment with Ang II alone or in combination, but not aldosterone alone, significantly increased aortic medial and adventitial thickness (Figure 4C and 4D). Ang II induced more adventitial thickening, left ventricular hypertrophy, and cardiac fibrosis after 12 weeks of 3.15% sodium chow than after 8 weeks of 1% saline drinking water. Genetic PAI-1 deficiency protected against Ang II-induced adventitial thickening.

Discussion

Activation of the RAAS plays a prominent role in the development and progression of cardiovascular disease. Previous studies in vitro and in animal models indicate that Ang II induces PAI-1 expression.6–11 The present study demonstrates that pharmacological inhibition of PAI-1 and genetic PAI-1 deficiency protect against Ang II-induced aortic remodeling through a blood pressure-independent mechanism, but not against ventricular hypertrophy or cardiac fibrosis.

The amount and composition of extracellular matrix depends on a balance between synthesis/deposition and degradation.19 In this study, the finding that pharmacological inhibition of PAI-1 protects against Ang II-induced aortic remodeling without altering Ang II-induced increases in collagen and fibronectin gene expression suggests that PAI-1 inhibition increased the availability of the plasmin/protease system for matrix degradation. In addition to fibrin, plasmin degrades the matrix proteins fibronectin and collagen and activates MMPs.20–22 As in the present study, previous investigations using plasminogen and PAI-1 knockout mice delineate an important role for the plasmin/protease system in modulating remodeling and fibrosis. Plasminogen knockout mice demonstrate impaired wound healing23 and increased fibrosis in response to chemical injury.24 Bleomycin-induced pulmonary fibrosis is decreased in PAI-1 knockout mice and increased in transgenic mice overexpressing the PAI-1 gene,25 and treatment of PAI-1 knockout mice with tranexamic acid, an inhibitor of plasmin formation, reverses the protective effect of PAI-1 deficiency.13 In an experimental model of glomerulonephritis, intravenous administration of a mutant PAI-1 that binds vitronectin normally but lacks protease inhibitory activity, restored plasmin generation, and reduced matrix accumulation of collagen I, collagen III, fibronectin, and laminin.26

Previous studies of insulin resistance and obesity in knockout animals suggest that genetic PAI-1 deficiency may protect against the development of insulin resistance.27 In the present study in a nonobese model, the protective effect of pharmacological PAI-1 inhibition could not be attributed either to metabolic or hemodynamic effects. A mechanism by which pharmacological inhibition of PAI-1 protected against Ang II-induced aortic remodeling may involve alterations in vascular osteopontin expression. As reported previously in vitro in vascular smooth muscle cells28 and in vivo,29 Ang II induced a dramatic increase in aortic and cardiac osteopontin.
expression. This is consistent with data indicating that transgenic mice overexpressing osteopontin have aortic medial thickening develop with aging and that osteopontin-deficient animals are protected against Ang II-induced cardiac fibrosis. Significantly, the protective effect of PAI-039 on aortic medial thickening was accompanied by attenuated aortic osteopontin expression at 8 weeks.

Osteopontin functions as a cell adhesion and migration molecule that can bind several ligands, including αvβ3 and CD44. Osteopontin may contribute to tissue fibrosis by promoting macrophage infiltration and associated TGF-β1 production, mediating migration, adhesion, and proliferation of fibroblasts, and modulating secretion of metalloproteinases. The mechanism whereby pharmacological PAI-1 inhibition decreased aortic osteopontin expression is not evident from the present study. However, we have similarly observed that genetic PAI-1 deficiency attenuates aldosterone-induced increases in renal osteopontin expression (unpublished data).

In contrast to the protective effect of PAI-1 inhibition on aortic remodeling, PAI-039 did not attenuate Ang II-induced cardiac hypertrophy, consistent with the lack of effect of drug on Ang II-induced hypertension. Moreover, although PAI-1 inhibition decreased Ang II-induced aortic remodeling, treatment with PAI-039 was associated with an enhanced cardiac profibrotic effect in the presence of Ang II. Several possible mechanisms could account for the divergent effects of PAI-1 inhibition on aortic remodeling and cardiac injury in the presence of Ang II. First, although the initial preclinical profile of PAI-039 indicated that the plasma drug concentration achieved during acute oral administration of 1 mg/kg of PAI-039 inhibited PAI-1 and was anthrombolic in a rat model of FeCl3-induced carotid artery injury, in the current study mice were used instead of rats. Whereas the dose of 1 mg PAI-039/g chow decreased PAI-1 activity in vehicle-treated animals, this dose was insufficient to attenuate the Ang II-induced increase in plasma PAI-1 activity after 1 week of therapy. Also, the amphipathic structure of PAI-039 could have a distinctly different binding profile in the presence of high concentrations of plasma Ang II.

To determine whether the profibrotic effect of PAI-039 + Ang II in the heart could have resulted from a proinflammatory effect of the compound that occurred with Ang II, independent of its mechanism of binding and inhibiting PAI-1 activity, we measured circulating concentrations of SAA. SAA concentrations provide evidence for a potential interactive pro-inflammatory effect of PAI-039 and Ang II in the present model. Importantly, however, whereas PAI-039 alone induced osteopontin expression, there was no effect of PAI-039 alone on SAA, and we have previously observed a reduction in SAA in a chronic model of atherosclerosis using apolipoprotein E knockout mice on a high-fat Western diet treated with the same 1 mg/g chow dosage of PAI-039.

The aforementioned not withstanding, the finding that both PAI-039 and genetic PAI-1 deficiency decreased Ang II/salt-induced aortic remodeling and increased cardiac fibrosis indicates that the effects of PAI-039 likely resulted directly from inhibition of PAI-1 rather than from a nonspecific effect of the drug. Furthermore, the opposing effects of either pharmacological PAI-1 inhibition or genetic deficiency on Ang II-induced aortic remodeling versus cardiac fibrosis point to different roles for PAI-1 in the pathogenesis of vascular remodeling and cardiac fibrosis. In the vasculature, PAI-1 may promote medial and adventitial thickening by inhibiting MMP activation and retarding extracellular matrix turnover, whereas in the heart PAI-1 may retard cellular infiltration and inflammation by impeding urokinase plasminogen activator-mediated or plasmin-mediated activation or release of latent growth factors. Consistent with this hypothesis, Moriwaki et al recently reported cardiac fibrosis in aged PAI-1-deficient mice and in mice overexpressing macrophage urokinase plasminogen activator. Similarly, the divergent effects of PAI-1 inhibition/deficiency on aortic remodeling and cardiac fibrosis in the present study parallel previously reported effects of genetic PAI-1 deficiency on perivascular fibrosis and myocardial inflammation and injury. For example, whereas PAI-1-deficient mice are protected from perivascular fibrosis induced by chronic administration of 1-NAME, PAI-1-deficient mice are not protected from cardiac inflammation and myocyte fibrosis after 2-week administration of Ang II/1-NAME.

In summary, this study used a novel orally active PAI-1 inhibitor to investigate the role of PAI-1 in tissue remodeling. Treatment with PAI-039 alone reduced plasma PAI-1 activity and lowered plasma glucose without affecting SAA. Treatment of Ang II-infused mice with PAI-039 provided protection against Ang II-induced aortic remodeling through mechanisms that may have involved alterations in osteopontin gene expression. This study provides proof of concept for the strategy of developing pharmacological inhibitors of PAI-1 to prevent vascular remodeling. However, like genetic PAI-1 deficiency, potent PAI-1 inhibition enhances the cardiac fibrotic effects of Ang II in this mouse model. Further studies are needed to determine whether the observed accentuation of Ang II-induced cardiac fibrosis is species-specific. Other chemical classes of in vitro PAI-1 inhibitors have been reported that include both direct-acting small-molecule inhibitors and antibodies, however, none has shown oral activity or has been profiled in chronic models of cardiovascular disease. Because PAI-039 is the first orally active PAI-1 inhibitor, it represents a unique pharmacological tool for determining the impact of inhibition of PAI-1 in the cause of cardiac and vascular remodeling.

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References


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