Rosiglitazone Reduces the Development and Rupture of Experimental Aortic Aneurysms

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Background—Development and rupture of aortic aneurysms involve a combination of complex biological processes. Rosiglitazone, a peroxisome proliferator–activated receptor-γ agonist, has been shown to have a broad spectrum of effects in vivo. The hypothesis that rosiglitazone would reduce aneurysm expansion or rupture was tested in the angiotensin II (Ang II)–induced hypercholesterolemic mouse model.

Methods and Results—Apolipoprotein E–deficient mice, 12 months of age, were allocated to 4 groups. Three groups were infused with Ang II (1 μg · min⁻¹ · kg⁻¹), and the fourth was infused with saline. Rosiglitazone was given 1 week before infusion and 1 week after infusion. Before 30 days, aortic size was measured, and tissues were collected for analyses. Both pretreatment and posttreatment with rosiglitazone inhibited the occurrence of fatal rupture (11 of 30 versus 0 of 30 versus 0 of 15; P=0.0013) and reduced maximal dilatation of the aorta (4.6±0.13 versus 2.4±0.48 versus 2.15±0.46 mm²; P<0.0001). Blood glucose, total cholesterol, body weight, and atherosclerosis did not differ between groups. Pretreatment with rosiglitazone inhibited the Ang II–induced expression of angiotensin type 1a Ang II receptor while having no effect on the angiotensin type 2 Ang II receptor, in addition to reducing Ang II–induced expression of E-selectin, tumor necrosis factor-α, and interleukin-6.

Conclusions—Pretreatment or posttreatment with RGZ reduced aortic expansion and rupture in this mouse model. Reduction of lesions in animals pretreated with rosiglitazone is concomitant with decreased expression of inflammatory mediators. Further studies are needed to elucidate the precise mechanism. (Circulation. 2009;119:3125-3132.)

Key Words: aneurysm ■ aneurysm, ruptured ■ angiotensin ■ inflammation ■ polymerase chain reaction ■ PPAR gamma

Rosiglitazone reduces the development and rupture of experimental aortic aneurysms. 

Uptake of an aortic aneurysm is the third-commonest cause of sudden death after myocardial infarction and stroke. Approximately 5% of men >60 years of age will develop an abdominal aortic aneurysm. Currently, the only treatment option for patients with aneurysms is surgical repair when the aneurysm expands past a critical point (usually a diameter threshold of 5.5 cm). Screening programs have begun to identify a large number of patients with small aortic aneurysms who would benefit from targeted pharmacotherapy to reduce aneurysm expansion and rupture.

Clinical Perspective on p 3132

Any potential pharmacological strategy to modulate the natural history of the aneurysmal process must be targeted to the biological process that mediates aneurysm expansion and rupture. Much of our understanding of the human pathogenesis of abdominal aortic aneurysm, obtained from analysis of aneurysmal biopsies during open surgery, is limited to analysis of the end-stage disease. Histological examination has identified that degeneration of the medial elastic fibers and compensatory deposition of collagens are accompanied by adventitial hypertrophy and infiltration of macrophages and T and B lymphocytes. Atherosclerosis and thrombus formation also are features of abdominal aortic aneurysm.1–4 It is most likely that the dynamic remodeling process mediating the vascular changes observed in aneurysm development is the result of an initial inflammatory response. Involvement of inflammation as an instigating mechanism has been confirmed in 3 widely used experimental models of aneurysm, which reiterate to varying degrees elements of the human pathology.5–7

Because the effects of biomechanical wall stress, proteolytic degradation of connective tissue, and inflammation/immune responses contribute to aortic expansion, we suggest...
that a pleiotropic drug capable of redressing a number of these biological events may be effective in reducing abdominal aortic aneurysm growth rates. One such class of drugs, the thiazolidinediones, comprises high-affinity ligands for the nuclear hormone receptor family transcription factor, peroxisome proliferator–activated receptor-γ (PPARγ). These drugs are able to increase insulin sensitivity and have favorable effects on blood glucose and lipid profiles in diabetic patients.8 There are 2 isoforms of PPARγ derived from alternative promoters, PPARγ1 and PPARγ2. PPARγ2 is expressed mainly in adipocytes, whereas PPARγ1 is ubiquitous, found in many cell types located in blood vessels such as the endothelium, vascular smooth muscle cells, and monocyte/macrophages.9–11 The general mechanism of gene transcription modulation by PPARs is well described; once bound to a specific ligand, the factors dimerize with retinoid X receptor and bind to specific binecramic DNA sequences known as PPAR-responsive elements. This binding, activation, and heterodimerization recruit various coactivators and corepressor proteins that modulate gene expression.12 There are now >70 PPAR target genes with functional PPAR-responsive elements.13 PPARγ activation is known to blunt macrophage activation, as demonstrated by inhibition of gelatinase B (matrix metalloprotein [MMP]-9), induction of nitric oxide synthase, and tumor necrosis factor-α release.14,15 PPARγ agonists such as rosiglitazone (RGZ) are now known to modulate vascular structure and function independently of their role in lipid metabolism and to protect against ischemia/reperfusion injury in vivo through mechanisms related to the antiinflammatory effects of the drug.16 In addition, the thiazolidinediones have been reported to have blood pressure–lowering effects.17–19

In this study, we have used the angiotensin (Ang) II–induced murine model of aortic aneurysm to investigate the hypothesis that treatment with RGZ will have ameliorative effects on aneurysm development and rupture. This study has demonstrated that RGZ has a marked effect on both aneurysm rupture and development, consistent with the view that application of drugs with a broad spectrum of effects is a suitable approach to treat pathologies with complex origins.

Methods

Animal Model
Abdominal aneurysms were induced in 12-month-old ApoE−/−/C57Bl6 mice by a 28-day continuous infusion of Ang II (1 μg · min−1 · kg−1) (Sigma Aldrich, Gillingham, Kent, UK) according to established methodology.20 To ascertain the clinical effect of RGZ, a total of 87 mice were allocated to 4 groups (Figure 1). All animals had an osmotic pump inserted subcutaneously in the interscapular region that released Ang II (1 μg · min−1 · kg−1) in the positive control group (Ang II; n = 30), pretreatment group (Ang II/RGZ5; n = 30), and postinduction treatment group (Ang II/RGZ3; n = 15). The sham-operated group received saline (n = 12). The pretreatment group received RGZ (10 mg · kg−1 · d−1) in their drinking water 1 week before pump insertion and continuously during the following 28-day release of Ang II. The postinduction treatment group was given RGZ (10 mg · kg−1 · d−1) 1 week after the implantation of the osmotic pump delivering Ang II, and this regimen was continued for 3 weeks. Mechanistic and histological experiments were performed on subsets of the total animal group as defined in each of the results sections. Any animal that died before termination of the experimen-

![Figure 1. Schematic illustrating the experimental design. Animals were allocated to 1 of 4 groups. Osmotic pumps (Alzet 2400) were inserted subcutaneously in the interscapular region at time 0. The negative control group (sham) had pumps delivering PBS; the 3 treatment groups had pumps delivering Ang II at 1 μg · kg−1 · min−1. The pretreatment group (Ang II/RGZ5) had access to RGZ (10 mg · kg−1 · d−1) from 1 week before pump insertion. The posttreatment group (Ang II/RGZ3) had access to RGZ (10 mg · kg−1 · d−1) from 1 week after pump insertion. All animals were fasted for 6 hours before termination of the experiment 28 days after pump insertion, and the abdominal aortas were scanned by MRI during the fasting period. At termination, blood was collected by cardiac puncture, and animals were perfused at physiological pressures with an RNAlater before the harvesting of tissues for further analysis.](http://circ.ahajournals.org/)

Plasma RGZ Analyses
Two blood samples were collected at the termination of the experiment: 1 in heparin for measurement of MMPs and 1 in EDTA for measurement of lipids. Plasma MMP concentration was determined with commercially available ELISA kits (R&D Systems, Abingdon, Oxfordshire, UK) according to the manufacturer’s recommendations. Lipid concentrations were measured enzymatically with commercially available kits (Roche, Mannheim, Germany).

Magnetic Resonance Imaging
For details on magnetic resonance imaging (MRI), see the online-only Data Supplement.

Tissue Collection and Processing
Planned termination of the experiments occurred 28 days after insertion of the osmotic pumps. At termination, animals were fasted for 6 hours, during which time MRI scanning was performed. Whole blood was collected for plasma purification of plasma by cardiac puncture. Animals were then perfused with RNAlater (Ambion,
Figure 2. Ang II–induced suprarenal aortic aneurysms in Apo E–deficient mice. a, Photograph showing a representative suprarenal aneurysm generated by constant infusion of Ang II (1 μg · kg⁻¹ · min⁻¹) into hypercholesterolemic mice for 28 days. Lesions were evaluated with 4.7-T MRI-scanned images taken as 2-mm slices as indicated. b, Arrows indicate historically stained maximally dilated section showing a dissection of the elastic lamellae (low-magnification image stained with hematoxylin and eosin; high-power image stained with elastic van Gieson).

Huntingdon, UK) via a cannula inserted into the left ventricle of the heart. Infusion was maintained with a Harvard pump at 100 mm Hg. Perfusion was continued for 15 minutes, after which the aortic tree was dissected. The suprarenal aorta was excised between the last pair of intercostals and the right renal artery. A 2-mm section of this tissue block was embedded in optimal cutting temperature (Raymond A Lamb, UK), frozen slowly in hexane vapor above liquid nitrogen, and stored at −80°C until processed. The remaining tissue was stored at −20°C in RNAlater until RNA extraction.

Measuring the Extent of Atherosclerosis

Isolated hearts were fixed, mounted in optical coherence tomography, and cryostat sectioned (10 μm) as described previously. Sections were discarded until the 3-valve cusps at the junction of the aorta and heart were clearly observed. Three more sections were collected and stained with Oil Red O. The extent of staining evaluated the extent of atherosclerosis, which was quantified from low-power bright-field images by 2 independent observers blinded to treatment groups using the Axiomat image analysis system (Zeiss UK, Hertfordshire, UK), version 4.2.3.

Staining for Collagens I and II

Optical coherence tomography–mounted tissue was cryosectioned (10 μm) onto Superfrost–charged (Menzel-Glaser, Braunschweig, Germany) slides. Suprarenal aortic sections (5 per sample) were fixed in acetone for 10 minutes, dehydrated in xylol and ethanol, and after being rinsed in distilled water, were incubated with 0.1% Sirius Red in saturated picric acid for 90 minutes. Sections were then rinsed twice with 0.01 mol/L HCl and water and cleared with ethanol (70%, 90%, 100% xylol). Five random images per section were collected and stained with Oil Red O. The extent of staining was evaluated with 0.01% Sirius Red with the Axiomat image analysis system (Zeiss UK), version 4.2.3.

Total RNA Extraction and cDNA Synthesis

Tissue samples were ground to a fine powder in liquid nitrogen, and total RNA was extracted following the manufacturer’s protocol (RNeasy Fibrous Tissue RNA extraction kit, Qiagen, UK, West Sussex, UK). The integrity of the extracted total RNA was confirmed with Agilent PicoChip technology according to manufacturer’s recommendations. These samples were reverse transcribed with a high-capacity cDNA archive kit (Applied Biosystems, UK, Warrington, Cheshire, UK) according to the recommended protocol. The resulting cDNA mixture was stored at −80°C until further use.

Table 1. Incidence of AA Formation After RGZ Treatment

<table>
<thead>
<tr>
<th>Pump Content</th>
<th>Water Addition</th>
<th>n</th>
<th>Early Rupture*</th>
<th>AA Formation*</th>
<th>Percentage Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Nil</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ang II</td>
<td>Nil</td>
<td>30</td>
<td>11</td>
<td>16</td>
<td>81</td>
</tr>
<tr>
<td>Ang II</td>
<td>RGZ5</td>
<td>30</td>
<td>0</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>Ang II</td>
<td>RGZ3</td>
<td>15</td>
<td>0</td>
<td>5</td>
<td>30</td>
</tr>
</tbody>
</table>

The table summarizes the clinical data showing the percentage of animals with early rupture and aortic aneurysm (AA) lesions at the termination of the experiment. Thirty-six percent of animals given Ang II infusion died of early rupture before the end of the experiment. In the groups of animals given rosiglitazone (Ang/II/RGZ5, Ang/II/RGZ3) (10 mg · kg⁻¹ · d⁻¹), none were lost to aortic rupture.

*P<0.001.

Real-Time Quantitative Analysis

The online-only Data Supplement provides details of the real-time quantitative analysis.

Statistics

Data are reported as mean±SD and analyzed with 1-way ANOVA followed by the Bonferroni posttest for multiple comparisons using GraphPad Prism version 4.0. Percentage incidence of aortic aneurysms and early rupture was analyzed with χ² analysis. A value of P<0.05 was considered significant.

Results

Preaneurysm or Postaneurysm Treatment With RGZ Reduced the Incidence of Early Aortic Rupture and Development of Aneurysms

Ang II infusion produced suprarenal aneurysms, which were quantified by MRI scanning. Representative photomicrographs of histological analysis, demonstrating luminal expansion (Figure 2a) and disruption of medial elastin (Figure 2c), confirmed the pathological manifestation of this aneurysm model as previously described (Figure 2). Ten percent of animals in all groups developed paraplegia (no significant differences between groups), which may have been related to the physical effects of pump insertion.

The most important finding of the present study was the clinical effect of RGZ on aneurysm development and rupture (Table 1). In the positive control group, 37% of the animals died of aortic rupture, and 53% of the group developed aneurysms. This gave a combined end point of aneurysm development or aortic rupture in 90%. Pretreatment with RGZ (Ang II/RGZ5; n=30) abolished death caused by rupture and reduced aneurysm formation to 20% (P<0.0001). Similarly, posttreatment with RGZ (Ang II/RGZ3; n=15) abolished death caused by rupture and reduced aneurysm formation to 20% (P<0.0001).

The whole aneurysmal process is retarded by RGZ in that the maximum aortic diameters of aneurysms in the RGZ pretreatment and posttreatment groups were significantly smaller than those in the positive control group (4.6±0.13 mm versus 2.4±0.48 mm before treatment versus 2.4±0.46 mm after treatment; P<0.0001), although the animals with early aortic rupture were excluded from this analysis (Figure 3).

All animals with free access to RGZ had significant plasma levels of the drug, although there was a wide range (172.9 to...
RGZ reduces aortic dilatation in the Ang II–induced model. Graph summarizing the data showing the relative maximal aortic area of those animals that survived to 28 days after infusion (termination of the experiment). Aortic aneurysm was defined as a 50% increase in aortic area (dashed line). Only 20% of the animals given RGZ maleate (10 mg · kg⁻¹ · d⁻¹) developed aneurysms, which were significantly smaller compared with the positive control group of animals. Data represent mean±SD (n as indicated). **P<0.001; *P<0.05.

664.5 ng/mL). Because RGZ maleate, not its primary metabolites, was measured and in view of the fact that this drug has a short elimination half-life (0.5 to 1 hour), the observed range of concentration may reflect variations in the drinking patterns of individual animals. All animals with free access to RGZ, given as either pretreatment or 1 week after the induction of the aneurysm, had significant levels of RGZ in their plasma at termination. There was no correlation between the actual plasma level and the degree of inhibition of aneurysm formation.

The hypercholesterolemic 12-month-old apolipoprotein E–deficient mice used in these experiments had a mean cholesterol of 655.2±54.74 mg/dL and atherosclerotic plaques throughout the aorta (data not shown). There was no significant difference between the 4 experimental groups in terms of body weight, glucose, and lipid profile (Table 2), except for the plasma concentration of high-density lipoprotein cholesterol in the posttreatment group (Ang II/RGZ3), which was elevated compared with control (14.69±1.51 versus 38.15±2.84 mg/dL; P=0.032). The extent of atheroma, as determined by measurement of the extent of lipid lesions in the aortic root, did not change in response to RGZ (P=0.873; Figure 4).

Figure 3. RGZ reduces aortic dilatation in the Ang II–induced model. Graph summarizing the data showing the relative maximal aortic area of those animals that survived to 28 days after infusion (termination of the experiment). Aortic aneurysm was defined as a 50% increase in aortic area (dashed line). Only 20% of the animals given RGZ maleate (10 mg · kg⁻¹ · d⁻¹) developed aneurysms, which were significantly smaller compared with the positive control group of animals. Data represent mean±SD (n as indicated). **P<0.001; *P<0.05.

Table 2. Lipid Profile, Body Weight, and Plasma Glucose in Response to Ang II and RGZ Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sham (n=12)</th>
<th>Ang II (n=8)</th>
<th>Ang II/RGZ5 (n=16)</th>
<th>Ang II/RGZ3 (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>655.2±54.7</td>
<td>683.7±72.37</td>
<td>822.9±114.7</td>
<td>796.6±159.2</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>605.9±55.4</td>
<td>628.7±63.64</td>
<td>793.3±87.0</td>
<td>745.0±153.9</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>13.88±1.21</td>
<td>14.69±1.51</td>
<td>12.89±3.37</td>
<td>38.15±2.84*</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>199.1±35.35</td>
<td>204.4±42.54</td>
<td>256.4±146.5</td>
<td>194.0±38.15</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>30.2±3.4</td>
<td>29.3±2.9</td>
<td>31.2±3.0</td>
<td>30.9±19.4</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>145.0±16.2</td>
<td>139.7±18.3</td>
<td>144.9±17.7</td>
<td>138.3±19.4</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD.

*P<0.032.
vater inhibitor-1, but there was no significant reduction in the level after pretreatment with RGZ (Table 3). There was no induction of tissue plasminogen activator expression with Ang II or any effect of pretreatment with RGZ.

Pretreatment With RGZ Restores the Ang II–Induced Reduction of Collagen I and II Deposition

The collagen content of these aortas was estimated semiquantitatively with picrosirius red, which identifies collagen types I and II. The photomicrographs shown in Figure 5 are representative images. Collagen is shown as bright orange, mainly in the adventitial region of the vessel. Ang II infusion (Figure 5b and 5e) reduces the amount of collagen seen in the sham-operated animals (2.21±0.37 versus 0.37±0.13; \( P<0.01 \); Figure 5a and 5d). Animals with access to RGZ (Figure 5c and 5d) show a significant elevation in the extent of collagen deposition (0.37±0.13 versus 1.48±0.49; \( P<0.05 \)). Using polarized light microscopy and Axiomat version 4.2.3 to quantitate the image, we found a 2-fold increase in collagen deposition after RGZ pretreatment (Figure 5).

Pretreatment With RGZ Inhibits Ang II–Induced Expression of Inflammatory Mediators

Angiotensin II infusion resulted in an increased expression of E-selectin, tumor necrosis factor-\( \alpha \), and interleukin-6. Pretreatment with RGZ inhibited the Ang II–mediated increase (Table 4). Despite a trend toward a reduction in interleukin-10 induction in the RGZ-pretreated group, the data did not reach significance.

### Table 3. Relative Expression of Genes in Suprarenal Aortas in Response to RGZ Treatment

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sham vs Ang II</th>
<th>Ang II vs Ang II/RGZ</th>
<th>( P )</th>
<th>Sham vs Ang II/RGZ</th>
<th>Ang II vs Ang II/RGZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2</td>
<td>0.71±0.20</td>
<td>1.43±0.20</td>
<td>1.08±0.24</td>
<td>0.0193</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MMP-9</td>
<td>0.89±0.21</td>
<td>1.66±0.33</td>
<td>1.69±0.19</td>
<td>0.0123</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>tPA</td>
<td>1.10±0.06</td>
<td>1.35±0.06</td>
<td>1.01±0.20</td>
<td>0.0347</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.08±0.07</td>
<td>0.86±0.06</td>
<td>0.70±0.22</td>
<td>0.0305</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>uPA</td>
<td>0.71±0.08</td>
<td>1.10±0.07</td>
<td>0.80±0.14</td>
<td>0.0069</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

\( uPA \) indicates urokinase-type plasminogen activator; \( tPA \), tissue plasminogen activator; and \( PAI-1 \), plasminogen activator inhibitor-1. Data showing relative expression are mean±SD. \( n=10 \).

### Discussion

This is the first study to demonstrate that RGZ, a member of the thiazolidinedione family, exerted a protective effect on aneurysm development and rupture in an established experimental model of aortic aneurysm. RGZ pretreatment resulted in a clinically significant decrease in the combined endpoint of fatal aortic rupture plus nonfatal aneurysm formation and significantly reduced the size of those aneurysms that formed. Administration of RGZ 1 week after induction of aortic dilatation also resulted in a significant reduction in aneurysm growth and development. Because we are currently unable to identify patients who will form aneurysms before the phenotype is manifest, these observations may be more relevant to clinical practice.

There are a number of potential mechanisms through which RGZ might mediate its effect to modulate the aneurysmal process in this model, including modulation of blood pressure, lipid concentrations, matrix remodeling by activation of matrix proteases via the AT1a Ang II receptor, and finally induction of inflammation. In the present study, the effect of RGZ on each of these mechanisms has been considered.

Although Ang II clearly elevates systolic blood pressure in this model, hypertension per se is unlikely to have a causal effect on aneurysm formation. Indirect evidence in support of this idea comes from a number of studies in which agents such as doxycycline, vitamin E, or 17beta-estradiol have had an inhibitory effect of aneurysm formation but have failed to show a concomitant reduction in blood pressure.23–25 In a more recent study, direct evidence in support of the idea that...
blood pressure does not play a role in this model comes from an elegant study by Cassis and coworkers. This study showed that elevating blood pressure to a similar degree with norepinephrine does not result in aneurysm formation, that the use of subpressor concentrations of Ang II results in a reduction in the number of aneurysms formed, and finally that a reduction in Ang II–induced hypertension with hydralazine does not affect the generation of aneurysm formation.

The extent of atherosclerosis, as defined by the extent of lipid-stained lesion in the root of the ascending aorta, was unaffected by the infusion of Ang II or pretreatment or posttreatment with RGZ, suggesting that RGZ did not exert its effect by modulating global atheroma. Similarly, the lipid profile of the animals was not significantly altered by Ang II treatment or RGZ therapy. In a recent study, Zhou and coworkers reported that RGZ treatment of fat-fed apolipoprotein E–deficient mice had no effect on total cholesterol, but their findings agree with ours in showing a significant elevation in plasma high-density lipoprotein cholesterol levels. Direct comparison is complicated by the aggressive development of atherosclerotic lesions in these animals, which do not compare to the lesions developed after 12 months on normal chow, and by the fact this group used significantly lower concentrations of RGZ (0.6 mg · kg$^{-1}$ · d$^{-1}$). The resting lipid profiles (quantified in sham-operated animals) were comparable to those reported by Tham and colleagues.

Human aneurysms are characterized by excessive degradation and remodeling of the extracellular matrix. The earliest pathological feature of abdominal aneurysms is loss and fragmentation of the medial elastic lamellae, mediated through increased matrix metalloproteinase activity. In the present study, it was observed that pretreatment with RGZ did not influence the Ang II–induced expression of MMP-2 and MMP-9 in aortic tissue. Similarly, plasma concentrations of the active enzymes showed no significant differences when measured 28 days after Ang II infusion, although the serum levels of these enzymes do not reflect tissue activity. Examination of the effects of pretreatment with RGZ on the expression of genes known to influence MMP activity and expression (urokinase-like plasminogen activator, tissue plasminogen activator, and plasminogen activator inhibitor-1) revealed that there was a trend for RGZ to reduce the Ang II–induced increase in urokinase-like plasminogen activator and plasminogen activator inhibitor-1, although this was not statistically significant.

The concentrations of MMP-2 and MMP-9 are increased in human abdominal aortic aneurysm and have been shown to be necessary for the generation of experimental aneurysm. The ameliorative mechanism of RGZ cannot be explained in terms of the effects on these proteins because neither MMP-2 nor MMP-9 expression was influenced by pretreatment with RGZ. However, because the analysis was restricted to gene expression, the data may not reflect the bioavailability of these enzymes, which could not be quantified in the volume of tissue available for analysis. The observation that RGZ was unable to reduce the Ang II–induced expression of MMP-9 is consistent with observations of carotid atherosclerotic plaques. In the study from Meisner and coworkers, who randomized patients to RGZ or placebo, plaques from patients on RGZ had protein concentrations of MMP-9 similar to those in subjects receiving placebo.

In established aneurysms, loss of medial elastin is compensated for by accumulation of a disorganized collagen network in the adventitial layer. The present experiment demonstrated that RGZ pretreatment resulted in an increased deposition of collagen in the aortic adventitia. This may be mediated through a reduction in the Ang II induction of AT1a receptor observed in this study. Although these observations are supported by data derived from a study of effects of thiazolidinedione on human carotid plaque matrix, they are contrary to a previously reported rodent study. Clearly, further work is needed to examine collagen with a fully quantitative analysis.

Inflammation has been documented as an early event in aneurysm formation in clinical practice, and inhibition of this process may stimulate a reduced expansion rate. The current investigation revealed that pretreatment with RGZ reduced the Ang II–induced increased expression of tumor necrosis factor-α, E-selectin, and interleukin-6, although no change was found in the antiinflammatory cytokine interleukin-10. Further studies are needed to confirm that the clinical benefit we observed in animals that received RGZ 1 week after the initiation of aneurysm is related to the ability of the thiazolidinedione to modulate the inflammatory process. It is also possible that RGZ may have the potential to work further down the inflammatory signaling cascade. The ability of RGZ to influence mitogen-activated protein kinase signal transduction pathways has been well documented, with reference to the inhibition of the stress-activated protein kinase Jun N-terminal kinase/activating protein-1 cascade.

The recent study by Yoshimura and colleagues showing that N$_2$-Jun N-terminal kinase inhibition could mediate aneurysmal regression suggested another putative mechanism of action of the thiazolidinediones that warrants further investigation.

The present study has demonstrated that RGZ, administered as a pretreatment or given after induction of aneurysm formation, can inhibit aneurysm formation and growth in this
model. Equally significantly, RGZ therapy had a significant effect in reducing aneurysm rupture, which is the most serious clinical event. Clinical translation of these data raises concern about the suitability of RGZ for long-term clinical administration. A recent meta-analysis37 raised some doubt about the long-term safety of these agents, particularly in terms of cardiovascular death. In this analysis, the overall number of events was small, resulting in little or no power to detect differences between trials. In a subsequent interim report of the Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycaemia in Diabetes (RECORD) study,38 which was specifically designed to assess cardiac outcomes in respect to RGZ treatment, there was no evidence of any increase in death resulting from cardiovascular causes.

Conclusions
This is the first study to demonstrate that treatment with RGZ, a PPARγ agonist, is able to reduce aneurysm development and rupture in vivo. The mechanism of action appears unlikely to involve the ability of RGZ to modulate lipids and hence atherosclerosis. The drug is more likely to influence the availability of early inflammatory mediators involved in aneurysm formation, to alter the expression of the AT1a Ang II receptor, and to influence downstream targets of activation. The precise molecular mechanism that mediates these observations will be defined by analyses of more detailed longitudinal studies. However, these findings are consistent with the concept that drugs with a broad spectrum of effects will be successful in treating complex pathologies. Further studies are required to investigate the mechanism of action of this drug and the efficacy of other PPARγ agonists and to assess whether they can be used to stabilize existing aneurysms.

Acknowledgments
We wish to thank Professor Alan Daugherty for advice in establishing the aneurysm model and the Biological Research Facility staff for their animal care. We also thank the Analytical Unit for measuring plasma RGZ.

Sources of Funding
This work was supported by the Garfield Weston Foundation and a European Union Grant FP7-HEALTH-2006-47-A-EC, Fighting Aneurysm Diseases.

Disclosures
None.

References
Abdominal aneurysms pose a considerable health burden and are the third-commonest cause of sudden death in the elderly. Many countries have now instituted health screening programs that incorporate an abdominal ultrasound. Screening for aneurysms reveals a prevalence of 5% in men >65 years of age. The vast majority of these aneurysms are below the threshold for surgical repair. At present, there is no proven medical therapy that will retard the expansion of abdominal aneurysms or reduce the incidence of fatal rupture. Targeted pharmacotherapy is clearly needed to offer effective management of patients with aneurysms. The pathological processes underpinning aneurysm expansion include inflammation, proteolysis, apoptosis, and angiogenesis, which must be targeted in potential medical strategies to retard the aneurysmal process. Previous studies have suggested that agents with pleiotropic actions may be the most effective, especially if directed toward early targets in the signaling cascade. The present study has suggested that activation of peroxisome proliferator-activated receptor-γ through the thiazolidinedione rosiglitazone, is able to reduce aneurysm expansion and rupture in a well-validated experimental model. Although the exact mechanism of action requires further investigation, the findings are encouraging and stimulate consideration for a translational study. A proof-of-concept study in a small number of patients appears justified to establish compliance and safety, with a larger study required to prove efficacy in reducing the expansion of small abdominal aortic aneurysms.
Rosiglitazone Reduces the Development and Rupture of Experimental Aortic Aneurysms
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Circulation. 2009;119:3125-3132; originally published online June 8, 2009;
doi: 10.1161/CIRCULATIONAHA.109.852467

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SUPPLEMENTAL MATERIAL

Methods

Plasma RGZ analysis  The plasma concentration of RGZ was measured by LCMS analysis. Following acetonitrile extraction of sample or standard (20 µL diluted 1:2 with control plasma or de-ionised water mixed for 5 mins with 25 µL internal standard containing 1 µL glibenclamide and 500 µL acetonitrile), extracts were evaporated to dryness and reconstituted in 200 µL 50% methanol: 50% de-ionised water containing ammonium acetate (final concentration 2mMol/L), 10 µL was injected onto the LCMS column and quantified in comparison to a known standard.

Magnetic Resonance Imaging  MRI images of the murine aorta were obtained using a Varian Unity INOVA imaging system fitted with a 4.7 T horizontal bore magnet. Anaesthetised mice were positioned supine within a 63 mm diameter quadrature imaging coil, into which heated air was blown to maintain normal body temperature. Coronal scout images were acquired to allow reproducible positioning of axial images aligned with the central slice at the superior end of the left kidney. Because of the ferromagnetic core of the infusion pump spin echo (rather than gradient echo) images were acquired with strong slice selection and readout gradients to minimise image distortions. Nine axial $T_1$ weighted spin echo images were acquired with 2 mm thick contiguous slices with TR 400 ms, TE 8.5 ms and 16 averages. Outer slice 12 mm thick saturation bands were used to suppress the high-intensity blood signal of the lumen. In-plane image resolution was 512 by 256 over a 4 cm field of view and zero-filled to 1024 by 1024 for region-of-interest (ROI) analysis of abdominal aorta. Varian ImageBrowser software was used to delineate a ROI to determine the abdominal aorta cross-sectional area.
**Real time quantitative RT-PCR** The mRNA levels of AT1a, AT2 receptor, MMP-9, MMP-2, uPA, tPA, PAI-1, E-selectin, IL6, IL10 and TNFα, were determined by QRT-PCR using TaqMan® Gene Expression Assays (Assays-on-Demand™ Gene Expression Products, Applied Biosystems, UK), which have a FAM™ reporter dye at the 5' end of the Taqman® MGB probe and a nonfluorescent quencher at the 3'-end of the probe (Table 1) QRT-PCR reaction mixtures were established according to manufacturer’s recommendations. Each sample was run in triplicate and for all reactions, negative controls were run with no template present. In addition, total RNA from tissue samples was used for sham reverse transcription reactions with no reverse transcriptase present and then subjected to standard PCR using 18S ribosomal RNA (rRNA) primers to verify that no amplification was produced.

QRT-PCR was carried out using an Mx4000 Multiplex Quantitative PCR System (Stratagene, UK), The PCR cycle started with an initial 10 min denaturation step at 95°C, followed by 40 cycles of shuttle heating at 95°C for 15 s and 60°C for 1 min. The associated Mx4000 software was used to analyze the data and determine the threshold count (Ct). The specimens were normalized to the endogeneous reference control 18S rRNA. For each sample, Ct\textsuperscript{target gene} and Ct\textsuperscript{18S rRNA} were determined, and

\[
\Delta C_t = C_t\textsuperscript{target gene} - C_t\textsuperscript{18S rRNA}
\]

The relative quantification of target gene normalised to 18S rRNA was determined by calculating 2-\(\Delta\Delta C_t\).

For the 2-\(\Delta\Delta C_t\) calculation to be valid, preliminary experiments were performed to verify that the efficiencies of target gene amplification and the efficiency of 18S rRNA amplification to be approximately equal. To achieve this, standard curves of template dilution and Ct were obtained for each target gene and 18S rRNA. In this study, the
values of the slope of log amount total RNA vs C_t was sufficiently small (< 0.1 or > - 0.1) for efficiencies of amplification of target genes and 18S rRNA to be considered equal (Table 1, Figure 1).
Table 1 Quantitative RT-PCR probe sets used in this study:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ref Seq</th>
<th>AOD Code</th>
<th>Slope Log total RNA vs ΔCt</th>
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<tbody>
<tr>
<td>uPA</td>
<td>NM-008873.3</td>
<td>Mm00447054_m1</td>
<td>-0.980</td>
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<td>tPA</td>
<td>NM-008872.1</td>
<td>Mm00476931_m1</td>
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<tr>
<td>PAI-1</td>
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<td>Mm00435860_m1</td>
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<td>IL10</td>
<td>NM-010548.1</td>
<td>Mm01288386_m1</td>
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<td>IL6</td>
<td>NM-031168.1</td>
<td>Mm00446191_m1</td>
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<tr>
<td>TNFα</td>
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<td>Mm00443258_m1</td>
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<tr>
<td>E-selectin</td>
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<td>MMP-9</td>
<td>NM-013599.2</td>
<td>Mm00600163_m1</td>
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</table>

Gene accession number and Assay-on-demand™ code is provided for each probe set. Amplification efficiency, calculated from the standard curve for each probe set is given as the slope of log RNA vs ΔCt.
Figure 1 Example of standard curve using probe sets for uPA and TNFα as indicated.
Reference