PPARγ Agonist Rosiglitazone Improves Vascular Function and Lowers Blood Pressure in Hypertensive Transgenic Mice

Michael J. Ryan, Sean P. Didion, Satya Mathur, Frank M. Faraci, Curt D. Sigmund

Abstract—The peroxisome proliferator activated receptor (PPARγ) agonist rosiglitazone has been reported to yield cardiovascular benefits in patients by a mechanism that is not completely understood. We tested whether oral rosiglitazone (25 mg/kg per day, 21 days) treatment improves blood pressure and vascular function in a transgenic mouse expressing both human renin and human angiotensinogen transgenes (R+/A−). Rosiglitazone decreased systolic (138±5 versus 128±5 mm Hg) and mean blood pressure (145±5 versus 126±7 mm Hg) of R+/A− mice as measured by tail-cuff and indwelling carotid catheters, respectively. Relaxation of carotid arteries to acetylcholine and authentic nitric oxide, but not papaverine, was impaired in R+/A− mice when compared with littermate controls (RA−). There were no effects of rosiglitazone on RA− mice; however, relaxation to acetylcholine (49±10 versus 82±9% at 100 μmol/L) and nitric oxide (51±11 versus 72±6% at 10 μmol/L) was significantly improved in treated R+/A− mice. Rosiglitazone treatment of R+/A− mice did not alter the expression of genes, including endothelial nitric oxide synthase (eNOS), angiotensin 1 receptors, and preproendothelin-1, nor did it alter the levels of eNOS or soluble guanylyl cyclase protein. In separate studies, carotid arteries from R+/A− and RA− mice relaxed in a concentration-dependent manner to rosiglitazone, suggesting possible PPARγ-independent effects in the vasculature. This response was not inhibited with the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (200 μmol/L) or the PPARγ antagonist bisphenol A diglycidyl ether; 4,4′-isopropylidenediphenol diglycidyl ether (100 μmol/L). These data suggest that in addition to potential genomic regulation caused by PPARγ activation, the direct effect of rosiglitazone in blood vessels may contribute to the improved blood pressure and vessel function. (Hypertension. 2004;43:661-666.)

Key Words: nitric oxide ■ angiotensin ■ endothelin ■ carotid

Peroxisome proliferator activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and bind to DNA as a heterodimer with retinoid x receptor to regulate transcription. Three genes encode different PPARs (α, β/δ, and γ), and the gene products are differentially expressed in a variety of tissues.1 PPARs have been most widely characterized with respect to their role in adipocyte growth and differentiation.2

More recently, PPARγ has become the focus of attention for the treatment of noninsulin-dependent diabetes mellitus, due largely to successful treatment with the thiazolidinedione (TZD) class of drugs that act as specific PPARγ ligands. TZDs (ie, troglitazone, ciglitazone, pioglitazone, and rosiglitazone) activate PPARγ, leading to improved insulin sensitivity and reduced blood pressure in both humans and animals through a mechanism that has not been completely elucidated.3-6 These findings, coupled with the observation that PPARγ is expressed in both vascular muscle7 and endothelium,8 suggest that it may play an important role in the regulation of blood pressure and vascular tone. Indeed, patients with mutations in PPARγ imparting dominant negative activity on the receptor, exhibit early onset hypertension and insulin resistance.9 Moreover, TZDs have been shown to attenuate the development of atherosclerotic lesions,10 increase the release of vasodilators such as nitric oxide11 and c-natriuretic peptide,12 and decrease expression of angiotensin receptors13 and endothelin.14 These effects of TZDs have been predominantly elucidated either in cell culture, models of insulin-resistance, or models with only acute changes in blood pressure. However, there is scant evidence to date for the effect of TZD treatment on blood pressure and vascular function in chronic models of hypertension and vascular dysfunction. Therefore, the present study was designed to test whether treatment with the TZD rosiglitazone has beneficial effects on blood pressure and endothelial function in a mouse model of lifelong hypertension caused by overexpression of...
both human renin and human angiotensinogen transgenes (R\(^\text{A}'\)). The R\(^\text{A}'\) model has been previously established to have chronic hypertension,\(^{15}\) vascular remodeling and hypertrophy,\(^{16}\) impaired vessel responses to endothelium-dependent dilators and nitric oxide,\(^{17,18}\) and elevated levels of vascular superoxide.\(^{18}\)

### Methods

#### Drugs

Acetylcholine (ACh), papaverine, N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME), and bisphenol A diglycidyl ether; 4,4'-Isopropylidenediphenol diglycidyl ether (BADGE) were obtained from Sigma. Rosiglitazone maleate (Alexis Biochemical) was dissolved in ethanol at 10 mmol/L. Ethanol or ethanol plus maleate was used as vehicle. For oral gavage, rosiglitazone maleate (Avandia) was ground and used as a suspension in sterile water. The PPAR\(\gamma\) antagonist BADGE was dissolved in ethanol. Authentic nitric oxide was prepared as described previously.\(^{19}\)

#### Experimental Animals

Double-transgenic (R\(^\text{A}'\)) mice were genotyped as previously described.\(^{15-18}\) Because blood pressure is not different between non-transgenic (R\(^\text{A}^-\)) and single transgenic mice (R\(^\text{A}'\), R\(^\text{A}^-\)), responses from these groups were pooled and reported as RA\(^\text{A}'\) mice.\(^{15}\) Mice (R\(^\text{A}'\) and RA\(^\text{A}'\)) were randomly divided into 2 groups: those treated with vehicle and those treated with rosiglitazone maleate (25 mg/kg per day) via oral gavage for 21 days. Blood glucose was measured using Accu Chek advantage glucometer (Roche) on whole blood samples collected at death. Body weight was recorded daily. All experimental protocols were approved by the University of Iowa Animal Care and Use Committee.

#### Blood Pressure and Vascular Studies

Systolic blood pressure was measured with an automated tail-cuff device (Visitech Systems BP-2000).\(^{15-20}\) Mean arterial pressure was recorded in conscious unrestrained R\(^\text{A}'\) mice treated with either vehicle or rosiglitazone for 21 days using indwelling carotid artery catheters.\(^{20,21}\) In a subset of mice the thoracic aorta (protein and RNA extraction) and the common carotid arteries (vascular function) were isolated and prepared.\(^{17,19,20}\)

#### Molecular Methods

Aortic RNA was isolated using the RNeasy Protect Mini Kit (Qiagen) according to manufacturer’s instructions. Detailed instructions for tissue preparation can be found in an online supplement available at http://www.hypertensionaha.org. Real-time polymerase chain reaction (PCR) was used to quantify relative differences in endothelial nitric oxide synthase (eNOS), angiotensin receptor 1 (AT1), and preproendothelin-1 (PPET-1) gene expression using the BioRad iCycler (for more details see the online supplement). Probes and primers were designed using Beacon Designer 2.0 software (Premier Biosoft International) and generated at Integrated DNA Technologies (Corvalle) with the exception of eNOS, which has been published.\(^{22}\) Thoracic aorta from vehicle- and rosiglitazone-treated R\(^\text{A}'\) mice were used to isolate membrane and cytosolic protein fractions. Commercially available antibodies for \(\beta1\) subunit of soluble guanylyl cyclase (Cayman Chemical) and eNOS (Santa Cruz) were used to assess protein expression.

#### Statistical Analysis

All data are expressed as mean±SE. For concentration-response curves, comparisons were made with 1-way repeated measures ANOVA using a Student-Newman-Keuls post hoc test. All other comparisons were made using 1-way ANOVA or \(t\) test where appropriate. \(P<0.05\) was considered significant.

### Results

The present study tested whether treatment with rosiglitazone could reduce blood pressure and improve vascular function in R\(^\text{A}'\) mice. Body weight and nonfasted blood glucose were measured in vehicle- and rosiglitazone-treated animals. Rosiglitazone treatment did not affect body weight or blood glucose in either R\(^\text{A}'\) or RA\(^\text{A}'\) mice (Table).

Systolic pressure, measured with tail cuff, was significantly higher in R\(^\text{A}'\) mice compared with RA\(^\text{A}'\) mice \((P<0.01)\), and vehicle treatment did not affect these pressures. Whereas treatment with rosiglitazone did not affect systolic pressure in RA\(^\text{A}'\) mice, it decreased \((P<0.05)\) pressure in R\(^\text{A}'\) mice (Figure 1A). Mean arterial pressure was measured in a subset of R\(^\text{A}'\) mice using indwelling carotid artery catheters (Figure 1B). These data supported the tail cuff findings \((145±5\) mm Hg vehicle versus \(126±7\) mm Hg rosiglitazone, \(P<0.05)\). Heart weight to body weight ratio as a measure of cardiac hypertrophy was not affected (Table). The rosiglitazone product literature indicates that doses as low as 3 mg/kg per day increase heart weight in mice.

Rosciglitazone treatment did not affect ACh responses in RA\(^\text{A}'\) mice (Figure 2A), however it significantly improved the ACh response in R\(^\text{A}'\) mice at all doses (Figure 2B). Likewise, the NO response was unchanged by rosiglitazone in RA\(^\text{A}'\) mice but significantly improved at the highest dose in R\(^\text{A}'\) mice (Figure 3A). The concentration response to papaverine, a non-nitric oxide dependent dilator, was unaffected by rosiglitazone treatment in both RA\(^\text{A}'\) and R\(^\text{A}'\) mice and was not different between groups (Figure 3B).

### Table

<table>
<thead>
<tr>
<th>Physical Characteristics</th>
<th>Treatment</th>
<th>Body Wt (g)</th>
<th>Heart Wt/Body Wt (mg/g)</th>
<th>Blood Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>RA(^-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>28±2</td>
<td>10</td>
<td>4.2±0.1</td>
<td>113±7</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>27±2</td>
<td>10</td>
<td>4.8±0.3</td>
<td>110±9</td>
</tr>
<tr>
<td>RA(^\text{A}')</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>28±1</td>
<td>29</td>
<td>6.8±1.8*</td>
<td>133±16</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>28±1</td>
<td>29</td>
<td>6.3±0.4*</td>
<td>98±12</td>
</tr>
</tbody>
</table>

*\(P<0.05\) 1-way ANOVA compared to RA\(^-\) vehicle group.
We investigated whether rosiglitazone treatment altered mRNA and protein expression of several genes in the vascular wall. First, we measured the expression of a known PPARγ target gene, leptin, from subcutaneous white adipose using an RNAse protection assay. As expected, leptin expression was decreased (as a ratio of leptin mRNA/β-actin mRNA of 19.3±4 (n=4) versus 11.1±2 (n=5), t test P<0.04). Next, we measured mRNA levels using real time reverse transcription-PCR of eNOS, AT1, and PPET-1 from the aorta of vehicle- and rosiglitazone-treated RAA mice. PPARγ activation has been reported to regulate the expression of these genes either directly or indirectly in other model systems. In total, these experiments (Figure 4) suggest that rosiglitazone does not alter expression of these genes in RAA mice. Protein expression of soluble guanylyl cyclase β1 was not altered in rosiglitazone-treated RAA mice, nor was the ratio of membrane to cytosolic eNOS protein, which was used as an index of eNOS activity (Figure 5A and 5B).

Finally, we tested whether rosiglitazone had nongenomic effects on carotid arteries in nontreated RAA and RA mice. Concentration response curves to rosiglitazone were performed. Figure 6 demonstrates that rosiglitazone doses equal to or greater than 100 μmol/L were required before significant relaxation in isolated vessels was observed. That this is 1000 times greater than the dose of ACh required to cause similar relaxation suggests the nongenomic effects may only occur at pharmacological doses. Moreover, incubation with l-NAME (200 μmol/L) or BADGE (100 μmol/L) suggests that this relaxation is not caused by acute NO generation or PPARγ activation (Figure 7).

**Discussion**

The present study was designed to evaluate the effect of rosiglitazone, a specific PPARγ agonist, on blood pressure and vessel function in a mouse model of lifelong hypertension with vascular dysfunction (RAA). There are several important findings. First, rosiglitazone treatment reduces blood pressure and improves the carotid artery response to ACh and authentic NO. Second, the mechanism of this improvement does not appear to involve changes in NO.
mediated signaling. For example eNOS mRNA or protein nor soluble guanylate cyclase protein expression are not altered by rosiglitazone. Third, the expression of genes that mediate vascular contraction, AT1 or PPET-1, were not changed by rosiglitazone treatment. Finally, these data demonstrate that there may be PPARγ independent effects of rosiglitazone (at pharmacological concentrations) on vascular tone that may potentially contribute to the improved blood pressure.

We have previously examined the blood pressure and vascular phenotypes of R A mice. It has been demonstrated that the increase in blood pressure is the result of central and peripheral effects of angiotensin II. In the vasculature, we reported that the R A model exhibits impaired responses to NO-dependent dilators making the R A mouse an appropriate model of lifelong, renin-angiotensin induced hypertension and vascular dysfunction.

Blood pressure lowering effects of TZDs in humans and rodent models of insulin resistance have been previously reported. Similarly, others have demonstrated that the acute change in blood pressure and vascular function in rats caused by angiotensin II infusion (osmotic minipumps) or endothelin (deoxycorticosterone acetate [DOCA] salt) can be prevented by simultaneous treatment with TZDs. This is the first study, to our knowledge, demonstrating that treatment with rosiglitazone can ameliorate lifelong renin-angiotensin–induced hypertension and extends the beneficial blood pressure effects of TZDs to a murine model of hypertension.

In addition to blood pressure effects of TZDs, there is evidence that these drugs can also improve vascular function in diabetic rat models. Our data support and extend on these findings by showing the beneficial vascular effects of rosiglitazone in a lifelong model of hypertension with normal blood glucose. In contrast to Diep et al, who demonstrated that rosiglitazone treatment could prevent the development of impaired ACh responses after acute angiotensin II infusion, our findings show that rosiglitazone can improve both ACh and authentic NO responses. These data suggest that PPARγ activation leads to improved NO signaling in smooth muscle and endothelial cells, both of which are sites of PPARγ gene expression. However, the molecular mechanism of this improvement is not clear given that the expression of soluble guanylyl cyclase was not changed by rosiglitazone in either the membrane or cytosolic fraction of isolated aorta. It is important to note that the cellular localization of soluble guanylyl cyclase β1 is not restricted to the cytosol but also has high activity in the membrane fraction. In addition, the ratio of eNOS protein in the membrane and cytosolic fractions was not altered. Inactivated eNOS is bound in the membrane to caveolin-1 and dissociates to the soluble fraction on activation. Therefore we used this ratio as an index for estimating the amount of activated eNOS in the aorta following rosiglitazone treatment. Despite the reversal of impaired NO-dependent vessel responses after treatment with rosiglitazone demonstrates potentially important therapeutic benefits.

PPARγ is a nuclear transcription factor and therefore has the potential to regulate many genes. Because rosiglitazone is a specific high affinity ligand for PPARγ, we investigated the expression of various potential molecular targets for PPARγ in the vascular wall. In the present study we tested the effect of rosiglitazone on the expression of three genes (eNOS, AT1 receptor, and PPET-1) that are important for vascular function. In order to confirm the efficacy of rosiglitazone treatment at the genomic level, however, we first showed that adipocyte leptin gene expression was decreased following treatment. Based on the improved vessel response to ACh we hypothesized that expression of the eNOS gene may be increased in rosiglitazone-treated mice, however this was not the case. Furthermore, while this work was in progress Calnek et al reported that treatment of endothelial cells in culture with the PPARγ agonists ciglitazone or 15 deoxy PGJ2 did not effect the eNOS gene expression but increased its release through a post-transcriptional mechanism that remains undefined. Similarly, our finding that AT1 receptor expression is not changed by rosiglitazone treatment in the R A model differs from several reports that have demonstrated that AT1 receptor expression is attenuated by PPARγ activation in cultured vascular muscle. Similarly, expression of PPET-1, the precursor to the potent vasoconstrictor
endothelin, was unchanged by rosiglitazone treatment in the R'A' model. These findings also differ from what others have reported in cell culture models or in the prevention of hypertension and vascular dysfunction in rats. Interestingly, the 5' flanking regions of eNOS, PPET-1, and AT1 do not contain a PPARγ response element, suggesting that the effects of PPARγ activation on the expression of AT1 and PPET-1 in these other model systems may be due to indirect control of gene transcription by PPARγ.

In addition to potential genomic effects of rosiglitazone in the regulation of vascular tone and blood pressure there have been reports of PPARγ-independent effects of the TZD class of drugs that are thought to be due to activation or inhibition of ion channel activity. More specifically, Eto et al demonstrated in isolated vascular smooth muscle that rosiglitazone (100 μmol/L) attenuated inward calcium currents and enhanced calcium-activated potassium currents. The net effect would cause cellular hyperpolarization and, therefore, relaxation of the vessel. Our findings support this hypothesis as pure rosiglitazone maleate causes vessel relaxation in a concentration dependent manner beginning at 100 μmol/L. This relaxation is not altered in the presence of the eNOS inhibitor L-NAME or in the presence of the PPARγ antagonist BADGE, suggesting that this response was not dependent on activation of PPARγ or the release of NO by the endothelium. The relatively short time-course for the carotid relaxation response to rosiglitazone further supports the hypothesis that this occurs through a nontranscriptional mechanism but requires high doses. Importantly, BADGE has been shown to have a higher efficacy in inhibiting rosiglitazone-mediated responses than responses elicited by other TZDs. These data suggest that rosiglitazone may directly regulate vessel tone and thus contribute to the improved blood pressure in the R'A' model. However the mechanism by which this occurs is not clear.

Perspectives

In the present study, treatment with a specific PPARγ agonist, rosiglitazone, was able to lower blood pressure and improve vascular function in a normoglycemic mouse model with preexisting hypertension and vascular dysfunction. It is important to note the distinction between the present study demonstrating the potential utility of PPARγ agonists for the treatment of hypertension and vascular dysfunction versus the work of others that have demonstrated that angiotensin II– or endothelin-induced vascular dysfunction can be prevented by TZD treatment. Therefore, despite the absence of a definitive molecular mechanism for the blood pressure and vascular improvements in the rosiglitazone treated R'A' model, these studies may have important clinical implications for the treatment of patients with hypertension and vascular disease and underscore the necessity for further studies to elucidate these mechanisms.

Acknowledgments

We thank Deborah R. Davis for assistance with oral gavage of mice. This work was supported by National Institutes of Health grants HL-48058, HL-61446, HL-38901, NS-24621, and HL-62984 (to C.D.S. and F.M.F.). Dr Michael Ryan is a recipient of an Individual National Research Service Award, HL-10425. Dr Sean Didion is the recipient of an American Heart Association, National Scientist Development Award (0230327N). We gratefully acknowledge the generous research support of the Roy J. Carver Trust.

References


PPARγ Agonist Rosiglitazone Improves Vascular Function and Lowers Blood Pressure in Hypertensive Transgenic Mice

Michael J. Ryan, Sean P. Didion, Satya Mathur, Frank M. Faraci and Curt D. Sigmund

Hypertension. 2004;43:661-666; originally published online January 26, 2004;
doi: 10.1161/01.HYP.0000116303.71408.c2
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/43/3/661

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2004/03/01/43.3.661.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/