Effect of Rosiglitazone Treatment on Plaque Inflammation and Collagen Content in Nondiabetic Patients

Data From a Randomized Placebo-Controlled Trial

Franz Meisner, Daniel Walcher, Florence Gizard, Xaver Kapfer, Roman Huber, Anja Noak, Ludger Sunder-Plassmann, Helga Bach, Cornelia Haug, Max Bachem, Tatjana Stojakovic, Winfried März, Vinzenz Hombach, Wolfgang Koenig, Bart Staels, Nikolaus Marx

Abstract

Background—Therapeutic strategies to stabilize advanced arteriosclerotic lesions may prevent plaque rupture and reduce the incidence of acute coronary syndromes. Thiazolidinediones (TZDs), like rosiglitazone, are oral antidiabetic drugs with additional antiinflammatory and potential antiatherogenic properties. In a randomized, placebo-controlled, single-blind trial, we examined the effect of 4 weeks of rosiglitazone therapy on histomorphological characteristics of plaque stability in artery specimen of nondiabetic patients scheduled for elective carotid endarterectomy.

Methods and Results—A total of 24 nondiabetic patients with symptomatic carotid artery stenosis were randomly assigned to rosiglitazone (4 mg BID) or placebo in addition to standard therapy. In this population of nondiabetic patients, rosiglitazone treatment did not significantly change fasting blood glucose, fasting insulin, or lipid parameters. In contrast, rosiglitazone significantly reduced CD4-lymphocyte content as well as macrophage HLA-DR expression in the shoulder region, reflecting less inflammatory activation of these cells by lymphocyte interferon-γ. Moreover, rosiglitazone significantly increased plaque collagen content (7.7±1.6% versus 3.7±0.7% of plaque area; P=0.036) compared with placebo, suggesting that TZD treatment may stabilize arteriosclerotic lesions. In addition, rosiglitazone reduced serum levels of 2 inflammatory arteriosclerosis markers: C-reactive protein and serum amyloid A.

Conclusions—Four weeks of treatment with rosiglitazone significantly reduces vascular inflammation in nondiabetic patients, leading to a more stable type of arteriosclerotic lesion.

Key Words: plaque ▪ thiazolidinediones ▪ inflammation ▪ collagen ▪ diabetes

Uptake or fissuring of advanced arteriosclerotic lesions with subsequent formation of an occluding or nonoccluding thrombus are major causes of acute coronary syndromes (ACSs). These advanced plaques are characterized by the presence of a large necrotic lipid core and a covering fibrous cap, which separates the circulating blood from the plaque underneath. In addition, in such unstable lesions, numerous monocytes/macrophages as well as CD4-positive lymphocytes accumulate in the shoulder region of the plaque and through the expression of inflammatory mediators these cells largely contribute to plaque instability. CD4-positive lymphocytes, releasing proinflammatory cytokines like interferon-γ (IFN-γ), induce the expression of matrix-degrading enzymes (matrix metalloproteinases [MMPs]) in macrophages and foam cells. These MMPs, by degrading collagen and elastin, lead to a thinning of the fibrous cap and thus render the plaque more vulnerable. Therapeutic strategies to stabilize such advanced lesions may prevent plaque rupture and subsequently reduce the incidence of ACSs.

Thiazolidinediones (TZDs), like rosiglitazone or pioglitazone, are a novel class of oral antidiabetic agents currently used to treat patients with type 2 diabetes mellitus. These agents, activators of the nuclear transcription factor peroxisome-proliferator-activated receptor-γ (PPAR-γ) increase insulin sensitivity and, as such, have favorable effects on blood glucose levels and the lipid profile in treated patients. Beyond their metabolic action, TZDs have been shown to exhibit antiinflammatory and antiatherogenic effects in vascular cells in vitro and limit lesion development in various animal models of arteriosclerosis. In treated patients, TZDs reduce serum levels of inflammatory biomarkers of arteriosclerosis like C-reactive protein (CRP) as well as MMPs, considered to be surrogate parameters for plaque instability. Therefore, these agents may also directly modu

Original received November 2, 2005; final version accepted December 28, 2005.

From the Department of Thoracic and Vascular Surgery (F.M., X.K., L.S.-P.), University of Ulm, Germany; Department of Internal Medicine II—Cardiology (D.W., A.N., H.B., W.K., N.M.), University of Ulm, Germany; UR545 INSERM (F.G., B.S.), Département d’Athérosclérose, Université de Lille, France; Department of Neurology (R.H.), University of Ulm, Germany; Department of Clinical Chemistry (C.H., M.B.), University of Ulm, Germany; and Clinical Institute of Medical and Chemical Laboratory Diagnostics (T.S., W.M.I.), Medical University Graz, Austria.

F.M. and D.W. contributed equally to this study.

Correspondence to Nikolaus Marx, MD, Department of Internal Medicine II—Cardiology, University of Ulm, Robert-Koch-Str. 8, D-89081 Ulm, Germany. E-mail nikolaus.marx@medizin.uni-ulm.de

© 2006 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

DOI: 10.1161/01.ATV.0000203511.66681.7f

845
late the inflammatory process in the vessel wall and potentially influence lesion composition. Indeed, ultrasound data demonstrating a reduction of intima-media thickness (IMT) of the carotid artery13,14 as well as a reduction of neointima formation after coronary intervention on TZD treatment15,16 suggest that these drugs are capable of modulating plaque size and morphology in diabetic and nondiabetic patients. Still, nothing is known about the effects of TZDs on lesion composition and histomorphological characteristics of plaque stability.

Therefore, we performed a randomized, placebo-controlled, single-blind trial in nondiabetic patients scheduled to undergo elective carotid endarterectomy to evaluate the effect of 4 weeks rosiglitazone treatment on plaque composition and histomorphological parameters of plaque stability.

Methods

Study Design and Patient Selection
Twenty-four nondiabetic patients with symptomatic carotid artery stenosis (≥70%) were included in this randomized, placebo-controlled, single-blind trial. Nondiabetic state was assessed by a negative history for diabetes mellitus, no treatment with antidiabetic drugs, or assessment of fasting blood glucose. All patients were recruited at the Department of Thoracic and Vascular Surgery, University of Ulm, Germany. Exclusion criteria were as follows: diabetes mellitus, chronic heart failure (New York Heart Association class III/IV), impaired liver function (AST or ALT 2.5-fold above upper normal limits), renal insufficiency requiring hemodialysis, pregnancy, systemic inflammatory disease, and life expectancy of <6 months. Patients scheduled for carotid endarterectomy were randomized to placebo or 4 mg rosiglitazone BID after written informed consent was obtained. Study medication was given on top of regular treatment. Patients were seen after 2 weeks for clinical follow-up and were scheduled for surgery after 4 weeks. Blood was taken in a fasting state at baseline and the morning of the procedure. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the local ethics committee.

Immunohistochemical Staining
After surgery, plaque samples taken from the same part of the artery were immediately frozen and stored in liquid nitrogen. For immunohistochemistry, the following antibodies were used: mouse anti-human CD68 antibody (DAKO), anti-human CD4 antibody (DAKO), mouse anti-human CD34 class II antibody (DAKO), mouse anti-human α actin antibody (DAKO), mouse anti-human HLA-DR antibody (DAKO), rabbit anti-human MMP-3 (Abcam), rabbit anti-human MMP-8 antibody (Abcam), and rabbit anti-human MMP-9 antibody (Abcam).

Serial cryostat section of carotid specimens were cut, air dried onto microscope slides, and fixed in acetone at −20°C for 10 minutes. The sections were preincubated for 10 minutes with methanol containing 1% hydrogen peroxide and blocked for 30 minutes at 37°C with the appropriate serum. The primary antibody dilution in PBS was stained for 45 minutes, and for negative controls, type and isomatched IgG at similar concentration were used.

Finally, sections were incubated with the respective biotinylated secondary antibody (DAKO) followed by avidin-biotin-peroxidase complex (Vector Laboratories). Antibody binding was visualized with 3-amin-9-ethyl carbazole, and afterward, sections were counterstained with Gill’s Hematoxylin (Sigma).

Collagen content was assessed by Sirius red staining for interstitial collagen (types I and II). Frozen carotid plaque sections were fixed for 10 minutes in acetic, dehydrated with xylol and ethanol, and after rinsing with distilled water, the tissue sections were incubated with 0.1% Sirius red in saturated picric acid for 90 minutes. Sections were rinsed twice with 0.01N HCl and water. After dehydration with ethanol (70%, 90%, 100% Xylol), sections were observed under polarized light.

Computer-assisted image analysis was used to quantify staining on sections using Image-Pro Plus software (Media Cybernetics). In brief, the total plaque area of single microscopic sections of each patient was scanned and positive immunostaining was quantified using computer-assisted pixel detection. Positive pixels were expressed as percentage of total plaque area. Total plaque area was not significantly different between the 2 groups.

Measurement of Lipids and Inflammatory Biomarkers
Levels of total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol as well as triglycerides and LDL radius were assessed as described previously.17 Measurement of CRP and serum amyloid A (SAA) was performed as described previously.

Statistical Analysis
Differences between groups were analyzed by the Mann-Whitney U test. Differences between treatment time points were calculated using Friedman repeated measurement ANOVA or 1-way repeated measurement ANOVA followed by the appropriate post hoc test. Skewed data were reported as median (interquartile range); all other data were reported as mean±SD. Spearman rank correlation was used to analyze correlation between parameters. Sample size was calculated based on the results of a previous trial examining the effect of pravastatin treatment on plaque collagen content with 11 patients in the verum and 13 patients in the control group.19 A P value <0.05 was regarded as statistically significant.

Results

Clinical Data
Twenty-four nondiabetic patients with carotid artery stenosis requiring endarterectomy were enrolled in this study and randomized to receive placebo (12 patients) or rosiglitazone (12 patients) for 4 weeks on top of regular therapy. In the rosiglitazone group, body mass index was significantly higher compared with placebo, but there was no significant difference with respect to all other baseline characteristics, including cardiovascular risk factors, medication, glucose, and lipid parameters, as well as inflammatory biomarkers of arteriosclerosis (Table 1). No serious drug-related side effects were observed.

In this study population of nondiabetic patients, rosiglitazone treatment did not significantly change fasting blood glucose (5.3 [4.9 to 6.1] mmol/L versus 5.6 [4.7 to 6.4] mmol/L; P=0.56) or fasting insulin levels (14.0 [9.2 to 24.0] μU/mL versus 13.5 [7.7 to 38.3] μU/mL; P=0.95) compared with placebo at 4-week follow-up. In addition, rosiglitazone did not significantly change total cholesterol, HDL cholesterol, LDL cholesterol, or triglyceride levels (Table 2). Moreover, LDL particle size, shown previously to increase on TZD treatment,17 did not significantly change after rosiglitazone treatment for 4 weeks.

Inflammatory Cell Composition
To examine the effect of rosiglitazone treatment on inflammatory cell composition, we performed immunohistochemical staining of carotid artery specimens for CD4-positive lymphocytes, macrophages, as well as vascular smooth muscle cells (VSMCs). Rosiglitazone treatment for 4 weeks significantly reduced CD4-positive lymphocytes compared...
with placebo (0.14±0.08% of plaque area versus 0.76±0.29% of plaque area; P=0.048) but had no significant effect on macrophage (Figure 1) and VSMC (data not shown) content in the plaque.

Given the nodal role of CD4-positive lymphocytes in vessel wall inflammation, we next examined the expression of HLA-DR, a protein reflecting cell activation by IFN-γ released from CD4-positive cells.20 Compared with placebo, treatment with rosiglitazone significantly reduced immunoreactivity for HLA-DR (0.14±0.07% of plaque area versus 0.78±0.30% of plaque area; P=0.047), mainly in macrophages and to a lesser extent in VSMCs, suggesting limited IFN-γ–induced cell activation in the plaque (Figure 2). Moreover, HLA-DR content in the plaque significantly correlated with the presence of CD4-positive lymphocytes (r=0.677; P=0.016), underscoring the importance of lymphocyte-mediated inflammation in these lesions.

### Plaque Stability

Because inflammatory cell activation with subsequent release of matrix-degrading MMPs from macrophages is a critical determinant for lesion vulnerability, we next compared

---

**TABLE 1. Baseline Characteristics of Study Population**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Rosiglitazone</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>9/3</td>
<td>8/4</td>
<td>0.74</td>
</tr>
<tr>
<td>Age, y</td>
<td>62.4±9.8</td>
<td>66.4±9.7</td>
<td>0.30</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.0 (22.2 to 25.3)</td>
<td>27.8 (26.3 to 29.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Smoking</td>
<td>6</td>
<td>9</td>
<td>0.30</td>
</tr>
<tr>
<td>Hypertension</td>
<td>8</td>
<td>12</td>
<td>0.84</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.0 (4.8 to 5.9)</td>
<td>5.1 (4.9 to 5.9)</td>
<td>0.47</td>
</tr>
<tr>
<td>Fasting insulin, μU/mL</td>
<td>15.3 (9.2 to 25.3)</td>
<td>11.6 (6.7 to 20.0)</td>
<td>0.60</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.4 (4.7 to 6.2)</td>
<td>5.9 (4.7 to 6.3)</td>
<td>0.62</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4 (1.2 to 1.7)</td>
<td>1.2 (1.0 to 1.4)</td>
<td>0.09</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.6 (1.2 to 2.6)</td>
<td>1.8 (1.2 to 2.3)</td>
<td>0.89</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.2 (0.7 to 1.7)</td>
<td>2.0 (0.7 to 3.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>SAA, mg/dL</td>
<td>4.5 (3.0 to 7.9)</td>
<td>5.7 (3.6 to 7.8)</td>
<td>0.62</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>9</td>
<td>10</td>
<td>0.63</td>
</tr>
<tr>
<td>Clopidogrel/ticlopidine</td>
<td>0/1</td>
<td>3/0</td>
<td>0.49</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>6</td>
<td>4</td>
<td>0.42</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>4</td>
<td>3</td>
<td>0.67</td>
</tr>
<tr>
<td>AT1 receptor blocker</td>
<td>1</td>
<td>1</td>
<td>0.74</td>
</tr>
<tr>
<td>Statins</td>
<td>9</td>
<td>6</td>
<td>0.30</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>0</td>
<td>1</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Data are mean±SD, median (interquartile range), or n. ACE indicates angiotensin-converting enzyme; AT1, angiotensin II type 1.

---

**TABLE 2. Metabolic Parameters at Baseline and After 4 Weeks**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 Weeks</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.0 (4.8 to 5.9)</td>
<td>5.6 (4.7 to 6.4)</td>
<td>0.27</td>
</tr>
<tr>
<td>Fasting insulin, μU/mL</td>
<td>15.3 (9.2 to 25.3)</td>
<td>13.5 (7.7 to 38.3)</td>
<td>0.27</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.4 (4.7 to 6.2)</td>
<td>5.7 (4.3 to 6.2)</td>
<td>0.62</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.1 (2.6 to 4.0)</td>
<td>3.4 (2.4 to 4.3)</td>
<td>0.69</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4 (1.2 to 1.7)</td>
<td>1.2 (1.0 to 1.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.6 (1.2 to 2.6)</td>
<td>1.5 (1.2 to 2.2)</td>
<td>0.57</td>
</tr>
<tr>
<td>LDL radius, nm</td>
<td>8.9 (8.8 to 9.0)</td>
<td>8.7 (8.6 to 9.0)</td>
<td>0.13</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.1 (4.9 to 5.9)</td>
<td>5.3 (4.9 to 6.1)</td>
<td>0.56</td>
</tr>
<tr>
<td>Fasting insulin, μU/mL</td>
<td>11.6 (6.7 to 20.0)</td>
<td>14.0 (9.2 to 24.0)</td>
<td>0.51</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.9 (4.7 to 6.8)</td>
<td>5.9 (5.2 to 7.0)</td>
<td>0.68</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.5 (2.8 to 4.2)</td>
<td>3.4 (2.9 to 4.2)</td>
<td>0.80</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.2 (1.0 to 1.4)</td>
<td>1.2 (1.0 to 1.4)</td>
<td>0.79</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.8 (1.2 to 2.3)</td>
<td>1.2 (1.1 to 2.5)</td>
<td>0.79</td>
</tr>
<tr>
<td>LDL radius, nm</td>
<td>8.7 (8.2 to 8.9)</td>
<td>8.7 (8.5 to 8.9)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Data are mean±SD or median (interquartile range). P values for comparison of parameters between time points.
MMP-3, MMP-8, and MMP-9 content in the carotid artery specimens. Staining of parallel sections revealed that the MMPs examined mainly colocalize with plaque macrophages (Figure I, available online at http://atvb.ahajournals.org). In plaques of rosiglitazone-treated patients, we found a reduction of MMP-3, MMP-8, and MMP-9 expression compared with placebo, but the difference did not reach statistical significance (Table 3).

Next, we examined whether this reduction in macrophage MMP expression by rosiglitazone resulted in an increase in collagen content in the plaque. As shown in Figure 3, picrosirius red staining demonstrated significantly higher collagen content in the rosiglitazone group compared with placebo (7.7±1.6% of plaque area versus 3.7±0.7% of plaque area; \( P<0.05 \)) and lower microvessel density compared with placebo (Figure II, available online at http://atvb.ahajournals.org). Together, these immunohistochemical data suggest that plaques from rosiglitazone-treated patients may be more stable than those from patients in the placebo group.

Because recent data suggested that plaque microvessels are associated with plaque inflammation and may be indicators of lesion instability,21 we assessed CD34-positive tubuloluminal capillaries in plaques from both groups. Lesions from rosiglitazone-treated patients showed significantly lower CD34 immunoreactivity (0.04±0.02% of plaque area versus 0.18±0.04% of plaque area; \( P=0.004 \)), indicating lower microvessel density compared with placebo (Figure II, available online at http://atvb.ahajournals.org). Together, these immunohistochemical data suggest that plaques from rosiglitazone-treated patients may be more stable than those from patients in the placebo group.

**Markers of Vascular Inflammation and Plaque Stability**

To investigate whether the decrease in plaque inflammation in the rosiglitazone group is associated with a reduction of inflammatory biomarkers of arteriosclerosis, we measured serum levels of CRP and SAA. As shown previously in studies with diabetic as well as nondiabetic subjects, rosiglitazone treatment significantly reduced both CRP (−61.9% \([-69.4\% \text{ to } -31.8\%]\); \( P=0.016 \) compared with baseline) and SAA (−33.3% \([-39.6\% \text{ to } -6.6\%]\); \( P=0.005 \) compared with baseline) already after 4 weeks, whereas no such effect was observed in the placebo group (Figure 4).

**TABLE 3. MMP Content in Carotid Artery Specimens**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Rosiglitazone</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-3, % of plaque area</td>
<td>5.1±6.7</td>
<td>2.8±3.0</td>
<td>0.28</td>
</tr>
<tr>
<td>MMP-8, % of plaque area</td>
<td>4.7±7.2</td>
<td>1.0±1.0</td>
<td>0.09</td>
</tr>
<tr>
<td>MMP-9, % of plaque area</td>
<td>1.1±1.5</td>
<td>0.7±1.4</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Data are mean±SD.

\( P \) values for comparison of parameters between groups.
The present randomized, placebo-controlled, single-blind trial demonstrates that 4 weeks of treatment with rosiglitazone reduces inflammatory cell composition and cell activation in carotid artery specimen of nondiabetic patients. Moreover, rosiglitazone increased collagen content in the plaque, suggesting that TZD treatment renders these plaques more stable and less vulnerable.

Previous studies have shown that TZDs exhibit antiinflammatory and antiatherogenic properties in vascular cells in vitro, and data from animal models of arteriosclerosis demonstrated a reduction in lesion development on TZD treatment.8 Moreover, clinical studies have shown that TZDs reduce serum levels of inflammatory arteriosclerosis markers in treated patients11,12,22 and induce beneficial morphological changes in the vessel wall by reducing IMT of the carotid artery13 as well as neointima formation after coronary stent implantation.15,16 The present study extends our knowledge on the effect of TZDs in the vessel wall by showing that rosiglitazone treatment for 4 weeks directly affects plaque composition by reducing inflammatory cell activation in the lesion, thus promoting formation of a more stable type of plaque. The modulation of vascular inflammation is evident by a reduction in CD4-positive cell infiltration as well as limited HLA-DR expression. CD4-positive lymphocytes are major effectors of the inflammatory response in the vessel wall through their capacity to release proinflammatory Th-1 cytokines like IFN-γ and tumor necrosis factor-α. These cytokines are central stimulators of other cells like macrophages and VSMCs, thus orchestrating inflammatory cell activation within the plaque.1 CD4-DR is a marker of such cell activation by IFN-γ. Therefore, a reduction in macrophage and VSMC HLA-DR immunoreactivity, as demonstrated here, suggests limited cell activation by this proinflammatory cytokine. The reduction in macrophage activation is mirrored by a trend to decreased expression of matrix-degrading MMPs in plaques of rosiglitazone-treated patients. The lack of a reduction of macrophage content seen here is consistent with a recent study in apolipoprotein E2 transgenic mice for which rosiglitazone did not decrease the number of macrophages.23 Still, others reported a reduction of macrophages on TZD treatment in preclinical animal models of arteriosclerosis.24 In our study, a longer treatment period may have diminished plaque macrophage content, but our data suggest that short-term TZD therapy mainly affects inflammatory cell activation. Finally, we observed a significant increase in collagen content in the rosiglitazone group. This may be attributable to a reduction of IFN-γ release from CD4-positive lymphocytes by TZDs because IFN-γ, on the one hand, enhances matrix degradation by MMPs and, on the other hand, reduces VSMC collagen synthesis. TZDs may finally counterbalance both of these mechanisms. These data, together with our finding that microvessel density, a surrogate for plaque vulnerability, is reduced by rosiglitazone treatment, suggest that TZDs can directly stabilize arteriosclerotic lesions, thus rendering them less vulnerable and less susceptible to plaque rupture.

The effect of rosiglitazone on inflammatory plaque composition and plaque stability is likely to be independent of its metabolic action. First, our study was conducted in nondiabetic subjects, and we did not find any changes in blood glucose or insulin levels after 4 weeks of rosiglitazone treatment. In addition, we did not find changes in total cholesterol, HDL or LDL cholesterol, or triglyceride levels as well as LDL particle size, making it unlikely that the effects on plaque composition resulted from changes in the lipid profile or a shift from small-dense to large buoyant LDL particles. Moreover, the metabolic effects of TZDs usually occur after several weeks of treatment, whereas the histomorphological changes observed here are already present after 4 weeks. Still, we did not perform oral glucose tolerance testing in our patients and cannot exclude that some of the patients exhibit an impaired glucose tolerance, which may have been influenced by rosiglitazone treatment. However, the lack of an effect on blood glucose or insulin levels argues against a causal role of major metabolic effects for the histomorphological changes observed here. Patients in the rosiglitazone group had a significantly higher body mass index, potentially suggesting a more insulin-resistant state, which, on TZD treatment, may have contributed to the beneficial effects on plaque stability. On the other hand, glucose and insulin levels were not different before and after treatment, indicating that both groups are comparable with respect to insulin sensitivity.

The reduction of vascular inflammation is paralleled by a decrease in the inflammatory biomarkers CRP and SAA. These data are in line with previous studies, showing a reduction of these markers after short-time TZD treatment in nondiabetic subjects and may reflect the antiinflammatory and antiatherogenic properties in vascular cells in vitro, and data from animal models of arteriosclerosis demonstrated a reduction in lesion development on TZD treatment. These data are in line with previous studies, showing a reduction of these markers after short-time TZD treatment in nondiabetic subjects and may reflect the antiinflammatory action of these drugs in the vessel wall.26

The effects of rosiglitazone treatment on plaque inflammation and plaque vulnerability may have important therapeutic implications for the treatment of patients with vascular disease. Strategies to stabilize advanced arteriosclerotic lesions may prevent plaque rupture and reduce the incidence of macrovascular events. So far, only statins have been shown to have similar effects in patients with vascular disease.19,27 Our study now suggests an effect of TZD treatment on plaque stability, promoting the concept that PPARγ-activating...
TZDs, independent of their metabolic action, may exhibit direct protective action in the vessel wall. Such mechanisms may, in particular, be of importance with respect to the recently published PROactive trial. This study compared the effect of pioglitazone treatment versus placebo on macrovascular events in type 2 diabetic patients. Despite the fact that the combined primary end point (mortality, nonfatal myocardial infarction [MI], stroke, ACS, coronary intervention, leg amputation, and leg revascularization) was not significantly changed by pioglitazone, a significant reduction of 16% was observed for the combined secondary end point of mortality, nonfatal MI, and stroke. Because all of these events are closely related to lesion vulnerability and plaque rupture, the findings reported here may at least in part contribute to such beneficial cardiovascular TZD effects.

**Acknowledgments**

This work was supported by grants of the Deutsche Forschungsgemeinschaft (SFB 451, projects B9 and B11) and the Else-Kröner-Forschungsgemeinschaft (SFB 451, projects B9 and B11) to D.W. The Department of Internal Medicine II, University of Ulm, received an unrestricted grant from GSK. We would like to thank Miriam Grübl, Renate Durst, and Gerlinde Trischler for expert technical assistance.

**References**


Effect of Rosiglitazone Treatment on Plaque Inflammation and Collagen Content in Nondiabetic Patients: Data From a Randomized Placebo-Controlled Trial
Franz Meisner, Daniel Walcher, Florence Gizard, Xaver Kapfer, Roman Huber, Anja Noak, Ludger Sunder-Plassmann, Helga Bach, Cornelia Haug, Max Bachem, Tatjana Stojakovic, Winfried März, Vinzenz Hombach, Wolfgang Koenig, Bart Staels and Nikolaus Marx

Arterioscler Thromb Vasc Biol. 2006;26:845-850; originally published online January 12, 2006; doi: 10.1161/01.ATV.0000203511.66681.7f
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/4/845

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2006/01/12/01.ATV.0000203511.66681.7f.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology 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 Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Figure II
ONLINE FIGURE LEGENDS:

Figure I: MMP content is reduced in plaques from rosiglitazone-treated patients. Representative sections of shoulder regions stained for CD68-positive macrophages as well as MMP-3, -8, and -9 are shown from the placebo (A-H) and the rosiglitazone group (I-P). Panels B-H and J-P: adjacent sections stained with similar concentrations of the respective type and class matched IgG show no immunoreactivity. Observations from 12 patients in the placebo and 12 patients in rosiglitazone group yielded similar results.

Figure II: Microvessel density assessed by CD34 immunoreactivity is reduced in plaques from rosiglitazone-treated patients. Representative plaque areas from the placebo and rosiglitazone group areas show CD34 staining (A and D, stained in red, indicated by arrows) and parallel sections stained for control with type and class matched IgG to (C and E). B and E show high power views (x 200) of the areas indicated in panels A and D. G: Quantitative image analysis of CD34 plaque content in plaques from the placebo and the rosiglitazone group. Bars represent mean±SEM expressed as % of plaque area; * p< 0.05 compared with placebo, n=12 in each group.