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

**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. (Arterioscler Thromb Vasc Biol. 2006;26:000-000.)

**Key Words:** plaque ■ thiazolidinediones ■ inflammation ■ collagen ■ diabetes

Rupture or fissioning 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.

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-
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 artery\textsuperscript{13,14} as well as a reduction of neointima formation after coronary intervention on TZD treatment\textsuperscript{15,16} suggest that these drugs are capable of modulating plaque size and morphology in diabetic and non-diabetic 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 non-diabetic 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 non-diabetic patients with symptomatic carotid artery stenosis (>70%) were included in this randomized, placebo-controlled, single-blind trial. Non-diabetic 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 (NYHA 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 or actin antibody (DAKO), mouse anti-human HLA-DR antibody (DAKO), rabbit anti-human antibody MMP-3 (Abcam), rabbit anti-human MMP-8 antibody (Abcam), and rabbit anti-human MPP-9 antibody (Abcam).

Serial cryostat section of carotid specimens were cut, air dried onto microscope slides, and fixed in acetone at $-20^\circ\text{C}$ for 10 minutes. The sections were preincubated for 10 minutes with methanol containing 1% hydrogen peroxide and blocked for 30 minutes at $37^\circ\text{C}$ with the appropriate serum. The primary antibody diluted in PBS was stained for 45 minutes, and for negative controls, 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 acetone, 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% Xyol), 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.\textsuperscript{17} Measurement for CRP and serum amyloid A (SAA) was performed as described previously.\textsuperscript{18}

Statistical Analysis

Differences between groups were analyzed by the Mann-Whitney U test. Differences between treatment time points were calculated using Friedman RM ANOVA or 1-way repeated measurement ANOVA followed by the appropriate post hoc test. Skewed 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.\textsuperscript{19} A $P$ value < 0.05 was regarded as statistically significant.

Results

Clinical Data

Twenty-four non-diabetic 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 atherosclerosis (Table 1). No serious drug-related side effects were observed.

In this study population of non-diabetic 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] mmol/L versus 13.5 (7.7 to 38.3) mmol/L; $P$=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,\textsuperscript{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-\( \gamma \) 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-\( \gamma \)-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.

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>0.74</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>9/3</td>
<td>8/4</td>
<td></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, ( \text{kg/m}^2 )</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, ( \mu \text{U/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>
</tbody>
</table>

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, ( \mu \text{U/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>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 Weeks</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosiglitazone</td>
<td></td>
<td></td>
<td></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>( \beta )-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.

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...
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 = 0.036).

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.

Because recent data suggested that plaque microvessels are associated with plaque inflammation and may be indicators of lesion instability, 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% to −53.8%]; P = 0.016 compared with baseline) and SAA (−33.3% [−39.6% to −26.6%]; P = 0.005 compared with baseline) already after 4 weeks, whereas no such effect was observed in the placebo group (Figure 4).
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 atherosclerosis demonstrated a reduction in lesion development on TZD treatment.8 Moreover, clinical studies have shown that TZDs reduce serum levels of inflammatory atherosclerosis 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 lesion, thus promoting formation of a more stable type of plaque.1 HLA-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 APOE2 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 atherosclerosis.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 atherosclerotic 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,25 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 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 atherosclerotic 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 ACS. 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 Fresenius-Stiftung to N.M., as well as by a grant of the Deutsche 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 Grüb, 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. published online January 12, 2006;
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/early/2006/01/12/01.ATV.0000203511.66681.7f.citation

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 I
**Figure II**

**A.** CD34 in Placebo

**B.** CD34 in Placebo

**C.** IgG in Placebo

**D.** CD34 in Rosiglitazone

**E.** CD34 in Rosiglitazone

**F.** IgG in Rosiglitazone

**G.**

<table>
<thead>
<tr>
<th>% of plaque area</th>
<th>Placebo</th>
<th>Rosiglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*Significant difference.*
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.