Rapid Effects of Rosiglitazone Treatment on Endothelial Function and Inflammatory Biomarkers

Jürgen Hetzel, Bernd Balletshofer, Kilian Rittig, Daniel Walcher, Wolfgang Kratzer, Vinzenz Hombach, Hans-Ulrich Häring, Wolfgang Koenig, Nikolaus Marx

Background—Antidiabetic thiazolidinediones (TZDs), like rosiglitazone or pioglitazone, improve endothelial function in patients with type 2 diabetes or metabolic syndrome, but it is currently unknown, whether these beneficial effects of TZDs depend on their metabolic action or may be caused by direct effects on the endothelium. Therefore, the present study examined whether short-term rosiglitazone treatment influences endothelium-dependent vasodilation as well as serum levels of vascular disease biomarkers in healthy, non-diabetic subjects.

Methods and Results—Short-term treatment (21 days) of healthy subjects (n=10) did not significantly change blood glucose levels or lipid profile. In contrast, rosiglitazone significantly increased flow-mediated, endothelium-dependent vasodilation already within the first day from 5.3±2.7% at baseline to 7.8±2.6%, further increasing it to 9.4±3.0% at day 21. In addition, the early improvement of endothelium-dependent vasodilation was paralleled by a rapid reduction of serum levels of the biomarkers C-reactive protein (CRP), serum amyloid A (SAA), and sE-selectin. Moreover, after drug withdrawal all markers remained suppressed for the whole follow-up period of 7 days. In contrast, rosiglitazone treatment did not significantly affect tumor necrosis factor (TNF)-α, interleukin (IL)-6, sICAM-1, sVCAM-1, and sCD40L levels.

Conclusions—Our study suggests a direct effect of TZD treatment on endothelial function and inflammatory biomarkers of arteriosclerosis, promoting the concept that TZDs, independent of their metabolic action, may exhibit protective effects in the vessel wall. (Arterioscler Thromb Vasc Biol. 2005;25:0-0.)

Key Words:

The endothelium, initially seen as a “wall paper” covering the vessel wall, is critically involved in the maintenance of vascular homeostasis. Recent work has established various techniques for endothelial function testing and among them, assessment of flow-mediated endothelial-dependent vasodilation as well as measurement of circulating biomarkers like sE-selectin, are the most promising and have advanced our understanding of endothelial function and the pathophysiology of arteriosclerosis. Regular endothelial function mainly depends on the capacity of endothelial cells to produce nitric oxide (NO). NO, in addition to its vasodilatory function, exhibits vasoprotective effects in the vessel wall by limiting leukocyte adhesion, cytokine expression, smooth muscle cell migration, as well as by reducing platelet aggregation. Loss of endothelium-derived NO is accompanied by impaired endothelium-dependent vasodilation as well as by endothelial cell activation, thus increasing the propensity for inflammation and thrombosis. This state of endothelial dysfunction predisposes the vessel wall to vasoconstriction, leukocyte and platelet adhesion, as well as endothelial expression of pro-thrombotic and pro-inflammatory mediators, which in turn will induce hepatic biosynthesis of a variety of acute phase proteins like fibrinogen, C-reactive protein (CRP), and serum amyloid A (SAA). Moreover, recent experimental and clinical data have shown that endothelial dysfunction precedes the formation of arteriosclerotic lesions and is associated with an increased risk for future cardiovascular events, suggesting a crucial role of endothelial function for the clinical outcome of patients. Therefore, improving endothelial function in high risk patients, like those with hypercholesterolemia or diabetes mellitus, has become an emerging concept in vascular medicine over the past decade.

Among such therapeutic strategies, statins have been shown to improve endothelial function in hypercholesteremic patients, most likely through a direct effect on endothelial NO production. In addition, thiazolidinediones (TZDs), a novel class of antidiabetic drugs including rosiglitazone and pioglitazone, modulate endothelial dysfunction in patients with type 2 diabetes mellitus, as shown by increased flow-mediated, endothelium-dependent vasodilation. Interestingly, TZDs did not improve endothelium-dependent vasodilation in coronary artery disease patient without diabetes.
mellitus, suggesting that the favorable effects on endothelial function may depend on the antidiabetic action of TZDs. Still, we have recently shown that rosiglitazone reduces serum levels of inflammatory biomarkers of arteriosclerosis and endothelial function, like SAA or sCD40L as early as 2 weeks after the initiation of TZD treatment, whereas these agents exhibit their maximal glucose-lowering action after 8 to 12 weeks. These data favor an anti-inflammatory effect of TZDs in the vasculature independent of their metabolic action. Moreover, such results are in concert with experimental data, demonstrating that TZDs exhibit direct effects in vascular cells, eg, by modulating endothelial expression of proinflammatory and proatherogenic mediators in vitro. Thus, it remains unclear whether TZDs influence endothelial function in vivo independent of their metabolic action.

Therefore, the present study examined whether short-term rosiglitazone treatment affects flow-mediated, endothelium-dependent vasodilation in healthy, nondiabetic subjects and whether this treatment modulates serum levels of biomarkers of endothelial function and arteriosclerosis.

Methods

Study Population and Study Design
Healthy volunteers were recruited from the Department of Internal Medicine at the University of Ulm, Germany. Ten nondiabetic, nonobese, healthy men, aged 25 to 41 years, without any other cardiovascular risk factor (hypertension, smoking, hyperlipidemia) were included. Two subjects had a family history positive for coronary artery disease, but none had a family history for diabetes mellitus. Intake of any other medication served as an exclusion criteria. The study protocol was approved by the local ethics committee and all subjects gave written, informed consent.

Study participants were treated with rosiglitazone 4 mg twice daily for 21 days. Endothelial function was assessed at baseline, at days 1, 2, 3, 7, 14, and 21 after rosiglitazone treatment, as well as on days 1, 2, 3, and 7 after drug withdrawal. Blood samples were taken at each time point for measurements of metabolic parameters as well serum levels of inflammatory biomarkers.

Endothelial Function Testing

Standard flow-mediated, endothelium-dependent vasodilation was assessed as follows. All subjects were studied at rest in supine position. All measurements were performed in a quiet and temperature-controlled room (23°C) between 6 and 8 hours in the morning. Participants fasted for at least 8 hours before measurements. We used a high-resolution ultrasound system (Philips HDI 5000) with an internal electrocardiography package. The diameter of the brachial artery was measured from 2-dimensional ultrasound images using a 15-MHz linear array transducer (CL15–7; Phillips). Subjects had to rest for at least 10 minutes before the first scan was recorded. A pneumatic cuff was positioned just below the elbow. The brachial artery was scanned in longitudinal sections 2 to 10 cm above the elbow. Diameter measurements were performed according to the guidelines for measuring FMD. After measurement of baseline diameter and baseline flow, a pneumatic tourniquet was inflated on the forearm at least 50 mm Hg above systolic pressure for 5 minutes. Postischemic flow measurements were performed 15 seconds after cuff deflation; diameter measurements were performed 45 to 60 seconds after cuff deflation. After an additional 10 minutes (to allow vessel recovery), the endothelium-independent dilatation was assessed 4 minutes after sublingual administration of 0.4 mg glyceryl trinitrate.

ECG was monitored continuously. Vessel diameters were analyzed with the use of electronic calipers on frozen images over a length of the artery of 1 cm. Three measurements were taken at each scan for 3 cardiac cycles at the end of the diastole (incident with the R wave on the ECG), and the mean was then calculated. The difference in lumen diameter between rest and reactive hyperemia, expressed as percent change, was regarded as endothelium-dependent vasodilation, the difference at rest and after application of glyceryl trinitrate in percent as endothelium-independent vasodilation. Blood pressure was measured in supine position after 5 minutes of rest.

Laboratory Methods

Blood samples were taken after endothelial function testing. Tumor necrosis factor-α (TNF-α), IL-6, soluble-intercellular adhesion molecule-1 (sICAM-1), soluble-vascular adhesion molecule-1 (sVCAM-1), soluble-E-selectin (all R&D-Systems), and sCD40L (Bender Medsystems) were determined by enzyme-linked immunosorbent assay according to the manufacturers’ protocol. SAA and CRP were measured as previously described.

Statistical Analysis

Samples size was calculated based on the results of three previous trials examining the effect of TZDs on endothelium-dependent vasodilation in diabetic subjects (Dandona, Diabetologia 2001;44(Suppl 1):A36, Abs 136, Suzuki et al, Diabetes 2002 [ADA presentation], and). Based on these studies, we assumed a flow-mediated, endothelium-dependent vasodilation after arterial forearm compression of 5% at baseline with a 90% increase after treatment, resulting in 9 subjects to receive statistical significance with 80% power (α=0.05). 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 are reported as median (interquartile range); all other data were reported as mean±SD. P<0.05 was regarded as significant.

Results

Rosiglitazone Treatment Rapidly Increases Flow-Mediated, Endothelium-Dependent Vasodilation

At baseline, study participants exhibited normal metabolic parameters as well as normal serum levels of inflammatory biomarkers of arteriosclerosis (Table 1). Rosiglitazone treatment did not significantly affect blood glucose levels (5.3±0.1 mmol/L at baseline versus 5.1±0.4 mmol/L at day 21; P=NS) and did not induce significant changes in the lipid profile. Moreover, rosiglitazone did not change insulin sensitivity assessed by homeostasis model assessment of insulin resistance (Table 2).

Before treatment, flow-mediated, endothelium-dependent vasodilation after arterial forearm compression in healthy, nondiabetic subjects was within the normal range (5.3±2.7%). Treatment with rosiglitazone significantly increased flow-mediated, endothelium-dependent vasodilation already within the first day to 7.8±2.6%, further increasing it to 9.4±3.0% at day 21 (P<0.05 for both compared with baseline). Endothelium-dependent vasodilation remained significantly increased for 1 day after drug withdrawal (7.3±2.1%; P<0.05 compared with baseline) and reached baseline levels at day 7 after termination of rosiglitazone treatment (5.4±2.5%) (Figure 1A). Postischemic blood flow was not significantly different between days of measurement. Endothelium-dependent vasodilation, assessed after application of the direct NO donor glyceryl trinitrate, did not significantly change at any time point (Figure 1B).
Rosiglitazone Treatment Rapidly Reduces Serum Levels of Inflammatory Biomarkers of Arteriosclerosis

The increase in endothelium-dependent vasodilation as early as 1 day after initiation of rosiglitazone treatment was paralleled by a rapid reduction of serum levels of the vascular biomarkers CRP, SAA, and sE-selectin. Within 1 day of rosiglitazone treatment, CRP levels significantly decreased from 1.4 mg/L (0.3 to 2.0) to 1.0 mg/L (0.2 to 1.9) (*P*<0.05 compared with baseline), further decreasing to 0.3 mg/L (0.2 to 0.5) after 21 days (*P*<0.05, compared with day 1 of treatment). Interestingly, after drug withdrawal CRP levels remained significantly reduced for the whole follow-up period of 7 days (Figure 2A). Similar results were obtained for SAA with a significant decrease from 3.5 mg/L (2.2 to 5.1) at baseline to 2.4 mg/L (1.8 to 4.0) after 1 day of rosiglitazone treatment (*P*<0.05, compared with baseline) and a further...
syndrome have shown that TZDs improve endothelium-studies in diabetic subjects as well in those with metabolic already occur after 1 day of rosiglitazone treatment. Previous well as the reduction in CRP, SAA, and sE-selectin levels, sodilation and decreased serum levels of the biomarkers CRP, SAA, and sE-selectin. sE-selectin revealed a similar rapid reduction within 1 day of withdrawal for the whole follow-up-period of 7 days (Figure 2B). Measurement of the more endothelium-related biomarker SAA levels remained significantly reduced after drug withdrawal. Rosiglitazone treatment had no effect on serum levels of TNF-α, IL-6, sICAM-1, sVCAM-1, and sCD40L (Table 2).

Figure 2. Effect of rosiglitazone on serum levels of inflammatory biomarkers. Healthy subjects were treated with rosiglitazone 4 mg twice daily for 21 days and serum levels of CRP (A), SAA (B), and sE-selectin (C) were measured before (baseline, day 0), during and after treatment. Dots represent median; bars represent interquartile range. *P<0.05 compared with baseline, n=10.

Discussion
The present study demonstrates that short-term treatment of healthy subjects with rosiglitazone improves endothelial function as shown by increased endothelium-dependent vasodilation and decreased serum levels of the biomarkers CRP, SAA, and sE-selectin.

Both, the effect on endothelium-dependent vasodilation as well as the reduction in CRP, SAA, and sE-selectin levels, already occur after 1 day of rosiglitazone treatment. Previous studies in diabetic subjects as well in those with metabolic syndrome have shown that TZDs improve endothelium-dependent vasodilation after 8 to 16 weeks of treatment. These changes were paralleled by an improvement of insulin sensitivity and a decrease in blood glucose levels. In contrast, another study in nondiabetic coronary artery disease patients did not find a significant effect of rosiglitazone treatment on endothelium-dependent vasodilation after 24 weeks. Thus, it remained unclear whether the beneficial effects of TZDs on endothelial function depend on an improvement of glycemic control or other metabolic effects. The data presented here suggest that rosiglitazone directly influences endothelial function independent of its effects on glucose metabolism or lipid profile. First, our study was conducted in healthy, nonobese, nondiabetic subjects and we did not find any changes in blood glucose or insulin levels as well as homeostasis model assessment of insulin resistance after 21 days of rosiglitazone treatment. This is consistent with previous findings showing that short-term TZD treatment of healthy subjects does not have an effect on glucose metabolism. In addition, rosiglitazone treatment did not significantly alter total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglyceride levels, although there was a slight increase in total cholesterol and triglycerides. In a larger population, these changes may have reached statistical significance, but because these effects would not be vasculoprotective, it is unlikely that improved endothelial function results from changes in the lipid profile.

Second, the metabolic effects of TZDs usually occur after several weeks of treatment, whereas the improvement in vasodilatory function observed here is already present after 1 day of treatment. Previous studies reported higher baseline values in flow-mediated, endothelium-dependent vasodilation in healthy subjects than those measured in our population. Still, a recent study by Otto et al demonstrated low values (4.4±0.7%) of flow-mediated, endothelium-dependent vasodilation early in the morning suggesting decreased endothelial function in the early morning. Our data with a baseline value of 5.3±2.7% were assessed between 6:00 and 8:00 AM and are therefore in line with these results. Our data showing an increase in endothelium-dependent vasodilation in rosiglitazone-treated subjects are in contrast to a study by Sidhu et al, who did not find such an effect after rosiglitazone treatment in nondiabetic patients with coronary artery disease. This discrepancy might be caused by the fact that almost all coronary artery disease patients in this study received statin therapy, known to improve vascular function in treated patients and thus potentially blunting the beneficial effects of rosiglitazone.

The early increase in endothelium-dependent vasodilation goes in parallel with a rapid reduction of serum levels of CRP, SAA, and sE-selectin after 1 day of rosiglitazone treatment. sE-selectin is an established marker of endothelial activation and elevated serum levels have been shown to predict clinical outcome in patients with coronary artery disease. Furthermore, increased levels predict incident type 2 diabetes mellitus. The beneficial effect of rosiglitazone on both endothelium-dependent vasodilation and sE-selectin levels observed here suggest that TZDs may directly modulate endothelial activation. This is consistent with experimental
data showing that TZDs regulate the expression of inflammatory mediators in endothelial cells in vitro.\textsuperscript{29,30} Because endothelium-dependent vasodilatation\textsuperscript{1} and a reduction of endothelial E-selectin expression\textsuperscript{1} are mediated by an increased availability of NO, rosiglitazone treatment may directly enhance NO production in the endothelium. In vitro data have shown that TZDs augment the release of NO from endothelial cells\textsuperscript{31} and our study now suggests that similar mechanisms may hold true in humans in vivo. CRP and SAA are acute phase reactants induced by cytokines like TNF-\(\alpha\) and IL-6 and have been shown to predict cardiovascular events,\textsuperscript{32,33} most likely by reflecting the subclinical inflammatory state in arteriosclerosis.\textsuperscript{7} Moreover, recent work suggested a correlation between endothelium-dependent vasodilation and CRP levels.\textsuperscript{34} Previous studies have shown that TZDs reduce CRP and SAA levels in diabetic and nondiabetic subjects and we have reported a reduction of SAA levels as early as 2 weeks after initiation of rosiglitazone treatment in coronary artery disease patients with type 2 diabetes,\textsuperscript{13,14,35} The current study extents our knowledge on TZD-mediated modulation of these inflammatory biomarkers by showing a significant reduction already after 1 day of treatment in healthy nonobese subjects, thus bolstering the hypothesis that TZDs exhibit direct anti-inflammatory properties in the vessel wall, independent of their metabolic action. Interestingly, after drug withdrawal, CRP, SAA, and E-selectin levels remained significantly suppressed for the whole follow-up period of 7 days. Because rosiglitazone activates the nuclear transcription factor peroxisome proliferator-activated receptor gamma, thus regulating gene expression in various organs,\textsuperscript{36} the sustained reduction of CRP and SAA levels may stem from a prolonged inhibition of liver gene expression of these mediators. However, given that E-selectin levels, reflecting endothelial cell activation, also remain decreased for the 7 day follow-up period, prolonged inhibition of liver gene expression seems an unlikely explanation for our findings, with more favoring the hypothesis of a broad anti-inflammatory effect of these agents in the vasculature. Still, further studies should elucidate the underlying mechanisms for our observation. Interestingly, we did not observe a reduction in sICAM and sVCAM levels by rosiglitazone treatment in our study population. These data are in line with a previous study, reporting no effect of TZD treatment on sICAM and sVCAM levels in nondiabetic patients,\textsuperscript{13} potentially suggesting that E-selectin may be a better marker for endothelial function than sCAMs. In contrast to previous studies in diabetic patients,\textsuperscript{14,15,37} we did not see an effect of TZDs on TNF-\(\alpha\) and sCD40L. The lack of an effect of rosiglitazone treatment on these markers may be caused by lower levels in these healthy subjects compared with diabetic patients. Alternatively, given the small number of participants, we cannot exclude the possibility that our study might have been underpowered to correctly assess the effects on all markers measured. In addition, recent data suggest that sCD40L may mainly stem from activated platelets,\textsuperscript{38} potentially explaining the lack of an effect of rosiglitazone in our healthy subject population, because these subjects do usually not exhibit platelet activation.

Overall, our data are in line with previous studies suggesting vasculoprotective effects of TZDs in nondiabetic patients. As such, TZDs have been shown to reduce CRP, fibrinogen,\textsuperscript{39} and von Willebrand factor levels,\textsuperscript{13} limit circulating platelet activity,\textsuperscript{39} and reduce carotid intima media thickness progression in nondiabetic patients with coronary artery disease.\textsuperscript{40}

Limitations of the Study
A major limitation of the present study is the lack of a placebo-treated control group. Still, the increase in endothelium-dependent vasodilation in parallel with the significant reduction in serum levels of inflammatory biomarkers is a robust finding in our study, both being in accordance with previous findings in diabetic subjects. Moreover, so far, no data exist showing spontaneous changes in the parameters assessed in healthy subjects. Measurement of endothelium-dependent vasodilation as well as serum levels of inflammatory biomarkers have been shown to be highly reproducible parameters in untreated subjects, making it unlikely that chance accounts for the results observed. However, the present pilot study should initiate larger, randomized, placebo-controlled trials to assess the effect of TZD treatment on endothelial function and vascular inflammation in nondiabetic subjects. Furthermore, future studies should determine the effect of TZDs in NO bioactivity, eg, by urinary nitrates assessment.

Conclusion
Taken together, our study suggests a direct effect of TZD treatment on endothelial function and inflammatory biomarkers of arteriosclerosis, promoting the concept that peroxisome proliferator-activated receptor \(\gamma\) activating TZDs, independent of their metabolic action, may exhibit protective effects in the vessel wall. Still, ongoing trials assessing the effect of TZD treatment on cardiovascular mortality will reveal whether such protective effects may translate into the clinic and improve the prognosis of treated patients.

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