Effect of Rosiglitazone Treatment on Soluble CD40L in Patients With Type 2 Diabetes and Coronary Artery Disease

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Background—Interaction of CD40L with its receptor CD40 is critically involved in inflammatory cell activation in atherogenesis. In addition, serum levels of soluble CD40L are elevated in acute coronary syndromes and have been associated with increased cardiovascular risk in healthy subjects, thus making sCD40L an intriguing target to modulate the inflammatory response in the vasculature. PPARγ-activating thiazolidinediones, novel insulin-sensitizing antidiabetic agents, have recently been shown to exhibit antiinflammatory effects in the vessel wall. To examine whether thiazolidinedione treatment might modulate serum levels of sCD40L in high-risk patients, we performed a randomized, placebo-controlled, single-blinded trial to assess the effect of rosiglitazone on sCD40L levels in patients with type 2 diabetes and coronary artery disease (CAD).

Methods and Results—Thirty-nine patients with diabetes and angiographically proven CAD were randomized to receive rosiglitazone (4 mg BID) or placebo for 12 weeks. Baseline parameters did not significantly differ between groups. Rosiglitazone treatment, but not placebo, significantly reduced sCD40L serum levels within the first 2 weeks by 8.1% (17.1 to −32.7) (median percentage [interquartile range]; P<0.05 compared with baseline), further decreasing it by 18.4% (−5.0 to −33.1) after 6 weeks (P<0.05 compared with baseline), and by 27.5% (8.2 to −70.5) after 12 weeks (P<0.05 compared with baseline and with 2 weeks of treatment).

Conclusion—Treatment with the PPARγ-activating thiazolidinedione rosiglitazone reduces sCD40L serum levels in patients with type 2 diabetes and CAD. These data support an antiinflammatory and potentially antiatherogenic effect of thiazolidinediones. (Circulation. 2003;107:1954-1957.)

Key Words: diabetes mellitus ■ coronary disease ■ receptors

Patients with type 2 diabetes mellitus have an increased risk of developing extensive arteriosclerosis with its sequelae, unstable angina pectoris and acute myocardial infarction. During the past several years, experimental data have illuminated the role of inflammation in atherogenesis, and clinical studies have shown that this concept of inflammation in arteriosclerosis applies directly to human patients. Interaction of the multipotent immunomodulator CD40 ligand (CD40L or CD154) with its receptor CD40 has emerged as an important contributor to this inflammatory process in the vessel wall. CD40 and CD40L are expressed on endothelial cells, vascular smooth muscle cells, mononuclear cells, and platelets, and CD40–CD40L interaction has been shown to exhibit proinflammatory and proatherogenic effects in vitro and in vivo. In addition to the cell-associated form, CD40L also exists in a soluble, biologically active form (sCD40L), which has similar proinflammatory effects on vascular cells. Interestingly, sCD40L is associated with acute coronary syndromes as well as hypercholesterolemia, and elevated sCD40L levels predict an increased cardiovascular risk in healthy subjects. Therefore, CD40L has been suggested as a potential therapeutic target to modulate vascular inflammation and possibly influence cardiovascular risk. However, limited studies exist on such a counterbalancing mechanism, with conflicting data on the role of statins in sCD40L lowering.

Still, nothing is known about sCD40L levels in patients with diabetes or about mechanisms to modulate sCD40L levels in this high-risk group.

Recent experimental data suggest that a novel group of antidiabetic agents, thiazolidinediones (TZDs; glitazones), like rosiglitazone or pioglitazone, might—in addition to their metabolic effects—exhibit antiinflammatory properties in the vessel wall. These agents, used clinically to treat patients with type 2 diabetes mellitus, act via the nuclear transcription factor peroxisome proliferator–activated receptor gamma (PPARγ), thus regulating the expression of various target genes involved in the inflammatory response.
genes.\textsuperscript{10} In vascular cells, PPAR-\(\gamma\)-activating TZDs exhibit antiatherogenic effects in vitro and have been shown to decrease lesion size in animal models of arteriosclerosis (reviewed in Marx\textsuperscript{11}). In addition, a recent clinical study has demonstrated that rosiglitazone lowers serum levels of inflammatory biomarkers of arteriosclerosis such as C-reactive protein.\textsuperscript{12} However, nothing is known about the effect of TZDs on serum levels of sCD40L.

Therefore, we performed a randomized, placebo-controlled trial to examine the effect of rosiglitazone treatment on sCD40L serum levels in patients with type 2 diabetes and coronary artery disease (CAD).

**Methods**

**Patients and Study Design**

Patients were recruited from the Department of Internal Medicine II at the University of Ulm, Germany. Forty patients, aged 30 to 78 years, with established type 2 diabetes for at least 6 months (on the basis of 1997 American Diabetes Association criteria), who were on oral antidiabetic treatment only (eg, metformin, sulfonylurea) and had coronary heart disease (>50% stenosis by angiography) were included. Exclusion criteria were as follows: cardiogenic shock, unstable angina or myocardial infarction during the preceding 4 weeks, impaired liver function, insulin treatment, renal failure requiring hemodialysis, cancer, systemic inflammatory disease, previous TZD treatment, or recent major surgery or illness. The local ethics committee approved the study protocol, and all patients gave written, informed consent. Patients with diabetes and CAD were compared with 39 population-based, healthy control subjects and with 39 nondiabetic CAD patients, both frequency-matched for age, the latter recruited from the population-based MONICA/KORA myocardial infarction registry in Augsburg.\textsuperscript{13}

Patients with diabetes and CAD were randomized to receive either rosiglitazone (4 mg BID) or placebo on top of individual, conventional treatment for 12 weeks. One patient in the placebo group stopped treatment after 10 days because of nausea. Blood samples were taken before standard cardiac catheterization and after 2, 6, and 12 weeks for measurements of metabolic parameters and serum levels of sCD40L, as well as \(E\)-selectin, a marker of endothelial activation, and soluble interleukin-2 receptor (sIL2R), a marker for T-cell activation.

**ELISAs**

ELISAs were used to determine circulating levels of sCD40L (Bender MedSystem; detection limit 95 pg/mL), \(E\)-selectin, and sIL2R (both R&D systems).

**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 one-way repeated-measurement ANOVA followed by the appropriate post-hoc test. Skewed data were reported as median (interquartile range); all other data are reported as mean\(\pm SD\). A probability value \(p<0.05\) was regarded as significant.

**Results**

**Rosiglitazone Treatment Significantly Reduces sCD40L Serum Levels in Patients With Type 2 Diabetes and CAD**

sCD40L levels in diabetic CAD patients were significantly higher compared with age-matched healthy control subjects and with nondiabetic subjects with previous myocardial infarction (Figure, A). For the clinical trial, patients with diabetes and CAD were randomized to receive rosiglitazone treatment or placebo. Patients of the two groups did not significantly differ in any of the baseline characteristics other than use of metformin monotherapy (Table 1). Rosiglitazone treatment, but not placebo, significantly reduced HbA\textsubscript{1c} levels from 7.5\(\pm 1.5\)% to 7.0\(\pm 1.1\)% \((P<0.01)\), demonstrating that rosiglitazone exhibited its expected metabolic action. Blood glucose levels showed a trend to decreased values in the rosiglitazone group after 12 weeks from 132\(\pm 61\) mg/dL to 126\(\pm 30\) mg/dL, but the difference did not reach statistical significance. In addition, rosiglitazone did not significantly change total cholesterol (185.6\(\pm 26.9\) mg/dL before treatment; 202.7\(\pm 44.4\) mg/dL after 12 weeks; \(P=0.263\)) or LDL cholesterol (113.3\(\pm 24.8\) mg/dL before treatment; 118\(\pm 31\) mg/dL after 12 weeks; \(P=0.600\)) levels in treated patients.

sCD40L, although higher in the placebo group, was not significantly different between the two groups at baseline (placebo group, 6.3 ng/mL [3.6 to 7.9]; rosiglitazone group, 7.8 ng/mL [6.0 to 9.1]; \(P=0.12\)). Rosiglitazone significantly reduced sCD40L levels within the first 2 weeks by 8.1% (17.1 to \(-32.7\)) \((P<0.05, \text{compared with baseline})\), further decreasing it by 18.4% \((-5.0\text{ to }-33.1)\) after 6 weeks \((P<0.05, \text{compared with baseline})\), and by 27.5% (8.2 to \(-70.5)\) after 12 weeks \((P<0.05 \text{compared with baseline and with 2 weeks treatment})\). No such effect was observed in the placebo group. In addition, changes in sCD40L levels in the rosiglitazone group were significantly different from the placebo group after 12 weeks of treatment (Figure, B). The decrease in sCD40L levels produced by rosiglitazone did not correlate with body mass index \(r=0.154\); \(P=0.36\). Moreover, neither rosiglitazone nor placebo had an effect on levels of \(E\)-selectin, a marker for endothelial activation, or on sIL2R, an indicator for T-cell activation (data not shown). In
addition, sCD40L levels did not significantly correlate with E-selectin \( (r = 0.154; P = 0.36) \) or sIL2R \( (r = 0.154; P = 0.36) \).

**Discussion**

This randomized, placebo-controlled, single-blinded trial demonstrates significant reduction of sCD40L serum levels in patients with type 2 diabetes and stable CAD after treatment with the PPAR\(\gamma\)-activating TZD rosiglitazone. sCD40L levels in diabetic CAD patients were higher compared with age-matched, healthy control subjects and nondiabetic CAD patients. Although the study did not include diabetic patients without CAD, our data suggest that diabetes mellitus is an important contributor to elevated sCD40L levels, but further work is needed to address to what extent CAD itself increases sCD40L levels in patients with diabetes. Elevated sCD40L, comparable to the levels of the diabetic study population in this study \( (7.0 \text{ ng/mL at baseline}) \), have been reported in patients with hypercholesterolemia,\(^7\) a high-risk population for the development of acute coronary syndromes. In addition, sCD40L levels \( \geq 3.71 \text{ ng/mL} \) were associated with a 2.8-fold increase in cardiovascular risk in a nested case-control study in healthy, middle-aged women.\(^8\) Our data, showing elevated levels in patients with diabetes and CAD, thus potentially reflects the increased cardiovascular risk of this group. However, further studies should establish the prognostic value of sCD40L in subjects with diabetes.

Rosiglitazone significantly reduced sCD40L serum levels after 2 weeks of treatment, subsequently leading to a continuous decrease over the 12-week study period. Although the present study only examined the effect of rosiglitazone over this short period, the significant decrease of sCD40L from 2 to 12 weeks of treatment, combined with previous data showing a reduction of other inflammatory markers after 6 months of treatment with rosiglitazone, suggests that the effect on sCD40L might also last over a longer period. Interestingly, previous studies have shown that rosiglitazone exhibits its maximal glucose-lowering effects after 8 to 12 weeks.\(^14\) This difference in the early reduction of sCD40L strongly suggests that rosiglitazone might directly affect sCD40L levels independently of its metabolic action. Previous in vitro data have shown that TZDs modulate activation of endothelial cells and T cells, both potential sources of sCD40L, but the lack of an effect on E-selectin and sIL2R makes direct effects of rosiglitazone on these cells an unlikely explanation for our finding. In addition, given that rosiglitazone did not alter total cholesterol and LDL cholesterol levels, the decrease in sCD40L seems not to be caused by changes in the lipid profile. Interestingly, a recent clinical study demonstrated inhibitory effects of rosiglitazone treatment on serum levels of C-reactive protein, matrix-metalloproteinase-9, and white blood cell count.\(^12\) Our study extends the knowledge of these pleiotropic TZD effects by showing reduction of another cardiovascular risk marker, sCD40L, thus bolstering the hypothesis that TZDs might modulate inflammation in the vasculature. Still, further work is needed to establish the importance of sCD40L-lowering for the reduction of macrovascular events.

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References


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