Effect of Rosiglitazone Treatment on Nontraditional Markers of Cardiovascular Disease in Patients With Type 2 Diabetes Mellitus

Steven M. Haffner, MD; Andrew S. Greenberg, MD; Wayde M. Weston, PhD; Hongzi Chen, PhD; Ken Williams, MS; Martin I. Freed, MD

Background—Markers of systemic inflammation (eg, C-reactive protein [CRP] and interleukin-6 [IL-6]) have been proposed to be “nontraditional” risk factors for cardiovascular disease in patients with type 2 diabetes mellitus. Matrix metalloproteinase-9 (MMP-9) has been implicated in the pathogenesis of atherosclerotic plaque rupture, which raises the possibility of the use of MMP-9 levels as a marker for future myocardial infarction or unstable angina. In vitro and animal studies suggest that thiazolidinediones can reduce the expression of these markers. The purpose of this analysis was to determine whether rosiglitazone alters serum concentrations of CRP, IL-6, MMP-9, and white blood cell count (WBC) and to examine the relationship of these effects with demographic and disease variables.

Methods and Results—CRP, IL-6, MMP-9, and WBC were analyzed from stored frozen serum samples obtained from patients with type 2 diabetes who completed a 26-week randomized, double-blind, placebo-controlled study. After 26 weeks of rosiglitazone treatment, the percentage reductions in mean CRP, MMP-9, and WBC levels were statistically significant compared with baseline and placebo (P<0.01). The percentage reduction in mean IL-6 was small and similar in the rosiglitazone and placebo groups. The change in each inflammatory marker from baseline to week 26 was significantly correlated (P<0.05) with each of the other markers, as well as with the homeostasis model assessment estimate of insulin resistance.

Conclusions—Rosiglitazone reduces serum levels of MMP-9 and the proinflammatory marker CRP in patients with type 2 diabetes, which indicates potentially beneficial effects on overall cardiovascular risk. (Circulation. 2002;106:679-684.)

Key Words: atherosclerosis ▪ cardiovascular diseases ▪ diabetes mellitus ▪ inflammation ▪ risk factors

Cardiovascular disease (CVD) accounts for ≈50% of all deaths worldwide. Type 2 diabetes mellitus is one of the most potent independent risk factors for the development of CVD and is seemingly related to accelerated atherosclerosis compared with the nondiabetic population.1,2 It is clear that alterations in traditional risk factors (eg, abnormal lipids and raised blood pressure) alone cannot explain the excess incidence of CVD in patients with type 2 diabetes.3

There is increasing recognition that chronic subclinical vascular inflammation plays a role in the pathogenesis of atherosclerosis, insulin resistance, and type 2 diabetes.4-6 Markers of subclinical inflammation, in particular C-reactive protein (CRP) and interleukin-6 (IL-6), have been shown to be powerful independent predictors of diabetes and CVD risk.5,7 In addition, elevated white blood cell count (WBC) may be a marker for inflammation and may predict future coronary heart disease and mortality.9

Preclinical studies demonstrate that peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists may affect inflammatory pathways through transcriptional mechanisms. These effects, seen in monocytes, macrophages, T-lymphocytes, and vascular smooth muscle cells, include decreases in cytokines, chemokines, and matrix metalloproteinases (MMPs).10 Treatment with troglitazone, a PPAR-γ agonist, is associated with declines in plasminogen activator inhibitor-1 levels.11 Taken together, these anti-inflammatory effects raise the prospect of reduced cardiovascular risk, either through improved metabolism or directly by activation of PPAR-γ in vascular or atherosclerosis-associated cells.12 To follow up these preclinical observations, we investigated the effects of rosiglitazone (RSG) on markers of inflammation (CRP and IL-6) and plaque stability (MMP-9) in patients with type 2 diabetes. Effects on WBC were analyzed as well. Potential relationships between effects on these markers and variables associated with type 2 diabetes were also examined.

Methods

Subject Material
Serum biomarkers were analyzed with samples obtained from 357 patients with type 2 diabetes mellitus who completed a 26-week...
randomized, double-blind, placebo-controlled study to assess the efficacy and safety of RSG (4 or 8 mg/d). Patients received instruction on a weight-maintenance diet throughout the study. Prior antidiabetic medications taken by patients were discontinued for a minimum of 4 weeks before randomization. Serum samples were obtained on the day of randomization (baseline) and at week 26 and stored at $-70^\circ$C until analyzed.

Analyses

Serum levels of IL-6 and MMP-9 were measured by ELISA (R&D Systems), and serum levels of CRP were assayed by another ELISA (Diagnostic Systems Laboratory Inc). Baseline and week 26 paired samples for any patient were assayed in the same batch to minimize interassay variability. The marker of insulin resistance, homeostasis model assessment estimate of insulin resistance (HOMA-IR), is defined as follows:

$$\text{HOMA-IR} = \frac{\text{Fasting plasma insulin (} \mu \text{U/mL) } \times \text{fasting glucose (mmol/L)}}{22.5}$$

Statistical Analyses

Within-treatment comparisons of mean change from baseline to week 26 of CRP, MMP-9, IL-6, and WBC levels; point estimates; and 95% CIs were presented for different treatment groups. For the assessment of differences between each RSG dosage group and placebo group with regard to continuous variables, an ANCOVA (using PROC MIXED in SAS software) with terms for treatment and baseline measurement was used that was based on log-transformed data. To determine whether analysis of only those who completed the study might bias our results, the same comparisons were also made for the study population as a whole. Patients missing values for an analyte were assumed to have had no change in that analyte.
and metabolic variables (Table 1). Correlations were determined for baseline variables (Table 2) and the relationship of percentage changes from baseline to week 26 with CRP, IL-6, MMP-9, and WBC with the prespecified changes in metabolic variables (Table 3). Baseline levels of analytes and the changes in analytes were nonnormally distributed; therefore, log-transformed data were used for determination of Pearson correlation coefficients.

Results

Each treatment group displayed similar demographic and baseline characteristics (Table 1), and there was no difference between this patient cohort and the original intent-to-treat study population (n=493) in terms of the magnitude of improvement in mean [SD] of glycosylated hemoglobin (HbA1c; RSG 4 mg/dL, -0.6% [1.2%]; RSG 8 mg/dL, -0.9% [1.2%]; placebo 0.6% [1.1%]) and fasting plasma glucose (RSG 4 mg/dL, -2.5 [2.6] mmol/L; RSG 8 mg/dL, -3.1 [2.9] mmol/L; placebo 0.2 [2.6] mmol/L). Median CRP levels were higher in women than in men (0.76 versus 0.39 mg/dL, P<0.001). There were no sex differences in IL-6, WBC, or MMP-9 levels.

Table 2. Pearson Correlations in the Entire Population (n=357)

<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>FPG</th>
<th>HbA1c</th>
<th>HOMA-IR</th>
<th>Ln HDLC</th>
<th>Ln LDLC</th>
<th>Ln TG</th>
<th>Ln WBC</th>
<th>Ln IL-6</th>
<th>Ln CRP</th>
<th>Ln MMP-9</th>
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<td>-0.15‡</td>
<td>-0.14‡</td>
<td>-0.18‡</td>
<td>0.20†</td>
<td>0.09*</td>
<td>-0.23‡</td>
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<tr>
<td>Ln LDLC</td>
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<td>0.06</td>
<td>0.07*</td>
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<tr>
<td>Ln TG</td>
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<td>Ln WBC</td>
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<tr>
<td>Ln CRP</td>
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<td>0.17‡</td>
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</tbody>
</table>

BMI indicates body mass index; FPG, fasting plasma glucose; Ln HDLC, log-transformed HDL cholesterol; Ln LDLC, log-transformed LDL cholesterol; and Ln TG, log-transformed triglycerides.

*P<0.05; all coefficients are treated as absolute values; †P<0.01; ‡P<0.001.

Table 3. Pearson Correlations in Changes Among All Individuals

<table>
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<tr>
<th></th>
<th>IL-6</th>
<th>MMP-9</th>
<th>WBC</th>
<th>BMI</th>
<th>HDLC</th>
<th>TG</th>
<th>LDLC</th>
<th>HbA1c</th>
<th>FPG</th>
<th>HOMA-IR</th>
<th>FFA</th>
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<td>0.07*</td>
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<td>0.04</td>
<td>0.14†</td>
<td>0.19†</td>
<td>0.07*</td>
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<tr>
<td>WBC</td>
<td>0.22‡</td>
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<td>0.04</td>
<td>0.04</td>
<td>0.14†</td>
<td>0.26‡</td>
<td>0.27‡</td>
<td>0.09*</td>
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<td>BMI</td>
<td>0.02</td>
<td>0.00</td>
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<td>TG</td>
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<td>LDLC</td>
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<tr>
<td>HbA1c</td>
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<td>0.31†</td>
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<td>HOMA-IR</td>
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</table>

BMI indicates body mass index; HDLC, HDL cholesterol; TG, triglycerides; LDLC, LDL cholesterol; FPG, fasting plasma glucose; and FFA, free fatty acids.

*P<0.05; all coefficients are treated as absolute values; †P<0.01; ‡P<0.001.

Relationship of Baseline Values With Metabolic and Disease Variables

As shown in Table 2, the natural logarithm (ln) of CRP was significantly (P<0.001) correlated with ln IL-6 (r=0.44) and ln MMP-9 (r=0.17) at baseline. Baseline ln CRP and ln IL-6 both correlated positively with body mass index (r=0.30 and r=0.11, respectively) and WBC (r=0.28 and r=0.27, respectively). Ln CRP was correlated with ln HOMA-IR (r=0.21), as was reported in other studies.6 Additionally, ln HOMA-IR was correlated with WBC (r=0.13) and ln IL-6 (r=0.13). Baseline ln MMP-9 showed a statistically significant positive correlation with baseline ln WBC (r=0.47) but was only weakly correlated with baseline triglycerides (r=0.07) and LDL cholesterol (r=0.07).

Effects of RSG Treatment on Weight, CRP, IL-6, MMP-9, and WBC

After 26 weeks of RSG treatment, patients in the placebo group lost 1.1 kg as opposed to a 1.8-kg increase in the RSG 4 mg/d group and a 3.5-kg increase in the RSG 8 mg/d group.
Both RSG treatment groups showed statistically significant ($P<0.05$) mean percentage reductions in CRP levels from baseline and placebo (Figure, A). The reductions in CRP did not appear to be dose related. There was no significant difference between the percentage reductions in CRP in the RSG 4- and 8-mg/d groups. After adjustment for the greater weight increases in the RSG groups, the decline in CRP was $-0.15$ mg/dL in the placebo group, $-0.52$ mg/dL in the RSG 4-mg/d group, and $-0.54$ mg/dL in the RSG 8-mg/d group. There was no significant percentage change in CRP from baseline in the placebo group (Figure, A). Mean percentage changes in IL-6 level were small and similar between the RSG and placebo groups (Figure, B). Statistically significant ($P<0.05$) and dose-ordered reductions from baseline to week 26 in CRP were observed for MMP-9 in the RSG treatment groups, whereas no change was observed in the placebo group (Figure, C). WBC also declined significantly with RSG (Figure, D).

Correlations Between Changes From Baseline to Week 26 in CRP, IL-6, and MMP-9 and Metabolic Variables

The change in ln CRP from baseline to week 26 was significantly ($P<0.05$) positively correlated with changes in IL-6 ($r=0.53$), MMP-9 ($r=0.19$), WBC ($r=0.19$), and HOMA-IR ($r=0.13$) and inversely correlated with changes in HDL cholesterol ($r=-0.17$; Table 3). The change in MMP-9 was significantly correlated with change in IL-6 ($r=0.22$), WBC ($r=0.40$), HbA1c ($r=0.14$), fasting plasma glucose ($r=0.19$), and free fatty acids ($r=0.11$). Change in IL-6 was correlated with change in WBC ($r=0.36$), HDL cholesterol ($r=-0.12$), and HOMA-IR ($r=0.09$). Multivariate analyses of the change from weeks 0 to 26 also illustrated that the strongest correlates of change were between CRP, MMP-9, WBC, and IL-6 (data not shown).

Discussion

These data show that RSG treatment significantly reduced serum CRP, MMP-9, and WBC compared with placebo. Elevation in CRP levels has been associated with both the development of type 2 diabetes and an increased risk of CVD.\textsuperscript{5-8,16} MMPs, in addition to being a known acute-phase reactant that increases inflammation, have also been implicated in plaque rupture.\textsuperscript{17} Interestingly, thiazolidinediones have been shown to decrease MMP-9 expression in vascular smooth muscle cells,\textsuperscript{10} with evidence for a transcriptional mechanism demonstrated by changes in protein and mRNA levels. Despite these intriguing data, there is no evidence to suggest elevated serum MMP-9 levels reflect MMP-9 levels in the arterial walls and only limited evidence to suggest an
increased propensity for plaque rupture. Increased blood levels of MMP-9 have been reported in premature atherosclerosis and in patients with acute coronary syndrome. After the initiation of cardiopulmonary bypass (a high-stress condition), circulating MMP-9 increased more than 6-fold. Reductions of MMP-9, CRP, and WBC could thus possibly be interpreted as reflecting a reduction in overall CVD risk, although this possibility should be tested in clinical controlled studies.

The percentage reductions in CRP with RSG in the present study were of a similar magnitude to those seen with the lipid-lowering statins. However, patients in the present study were diabetic and were more obese than in the typical CRP study with statins, and direct comparisons between the study were diabetic and were more obese than in the typical study should be done with a factorial study design. It is possible that the decrease of CRP in obese diabetic patients could reflect changes in insulin resistance rather than a vasculoprotective effect. Changes in CRP were independent of changes in LDL cholesterol, similar to the effect of statins on CRP. Additionally, RSG treatment effects on CRP and MMP-9 were still evident in a second, more conservative analysis of the study data in which patients with missing values were assumed to have no change in that parameter, which increases our confidence in these results.

CRP levels in the present report were higher than in some previous reports, which may reflect the present study population, who had diabetes and who were quite obese. In data from the Women’s Health Study report, which examined CRP in relation to the incidence of diabetes, median CRP was 0.67 mg/dL in patients who developed diabetes compared with 0.26 mg/dL in patients who did not develop diabetes. Median CRP in women in the present study was 0.76 mg/dL. Because glucose levels were also associated with higher CRP levels, it would be expected that the present population might have even higher levels than the obese prediabetic patients in the Women’s Health Study.

Although there was a positive correlation between changes in CRP and IL-6 in both the RSG and placebo treatment groups, there was no apparent effect of either treatment on serum IL-6 levels. Thiazolidinediones have been shown to reduce mRNA induction and expression of IL-6 in a mouse model of type 2 diabetes. Subcutaneous adipose tissue is a significant source of IL-6 expression, whereas thiazolidinedione treatment has been associated with weight gain and increases in subcutaneous fat. It is possible that our inability to detect differences between RSG and placebo with respect to this parameter may be related to the observed weight decrease in the placebo group (possibly leading to reductions in subcutaneous fat) and weight gain in the RSG group.

The correlations we observed between changes in CRP, IL-6, and MMP-9 were consistent with potential anti-inflammatory and antiatherogenic actions of various PPAR-γ agonists observed in preclinical and clinical studies. The correlation between HOMA-IR and the inflammatory markers IL-6 and MMP-9 at baseline in diabetic patients may suggest a relationship between insulin resistance and a chronic inflammatory state, as shown for CRP and insulin resistance with the frequently sampled intravenous glucose tolerance test in nondiabetic individuals. The decrease of WBC with RSG is consistent with the positive correlation of WBC with decreased insulin sensitivity in the Insulin Resistance Atherosclerosis Study (IRAS). In the West of Scotland Coronary Prevention Study (WOSCOPS), WBC was associated with the development of type 2 diabetes in univariate models. Additionally, WBC has been shown to predict CVD.

Relatively few data are available on correlations of circulating MMP-9 with demographic or metabolic variables. In the present report, MMP-9 was correlated with WBC, IL-6, and CRP at baseline. Baseline MMP-9 was positively but weakly correlated with baseline triglyceride and LDL cholesterol levels. However, changes in MMP-9 were correlated with changes in several other variables. Changes in MMP-9 were correlated with changes in CRP, IL-6, WBC, HbA1c, fasting plasma glucose, insulin resistance, and body mass index. The strongest correlations between 0 and 26 weeks for CRP were with other inflammatory factors. In one report, baseline MMP-9 was associated with lower HDL cholesterol, but no association of MMP-9 and HDL cholesterol was observed in another report. A positive association of MMP-9 and WBC was observed in one report. Additional studies need to be done on the epidemiological correlations of circulating MMP-9.

These data support the potential beneficial effects of insulin-sensitizing interventions such as use of thiazolidinediones on levels of markers for cardiovascular risk. Additional investigations of the effects of anti diabetic agents on cardiovascular outcomes are ongoing.

Acknowledgments
This study was funded by SmithKlineBeecham Pharmaceuticals. We thank Diane Fuell, GlaxoSmithKline, for her valuable contribution to this study.

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