The Effects of Rosiglitazone, a Peroxisome Proliferator-Activated Receptor-Gamma Agonist, on Markers of Endothelial Cell Activation, C-Reactive Protein, and Fibrinogen Levels in Non-Diabetic Coronary Artery Disease Patients

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OBJECTIVES

We sought to assess the effect of rosiglitazone on markers of endothelial cell activation and acute-phase reactants in non-diabetic patients with coronary artery disease (CAD).

BACKGROUND

Inflammation plays a key role in all stages of atherosclerosis and in the genesis of acute coronary syndromes. Rosiglitazone, a peroxisome proliferator-activated receptor gamma agonist, is used in the treatment of type 2 diabetes mellitus, and previous data suggest that it may have anti-inflammatory effects on atherosclerosis.

METHODS

Patients with stable, angiographically documented CAD without diabetes mellitus were investigated. Patients were randomized in a double-blind manner to receive treatment with placebo or rosiglitazone (4 mg/day for 8 weeks followed by 8 mg/day for 4 weeks) for 12 weeks. Eighty-four patients completed the study. Fasting glucose, insulin, lipid profile, markers of endothelial activation, and inflammatory markers were measured at baseline and after 12 weeks.

RESULTS

Rosiglitazone treatment resulted in a significant reduction in E-selectin (p < 0.03), von Willebrand factor (p < 0.007), C-reactive protein (p < 0.001), fibrinogen (p = 0.003) and the homeostasis model of insulin resistance index (p < 0.02), compared with placebo. Significant elevations in low-density lipoprotein and triglyceride levels were observed in the rosiglitazone group (p < 0.01). Within the rosiglitazone-treated group, reductions in C-reactive protein and von Willebrand factor were significantly correlated with a reduction in insulin resistance.

CONCLUSIONS

Rosiglitazone significantly reduces markers of endothelial cell activation and levels of acute-phase reactants in CAD patients without diabetes. Potential underlying mechanisms include insulin sensitization and direct modification of transcription within the vessel wall. (J Am Coll Cardiol 2003;42:1757–63) © 2003 by the American College of Cardiology Foundation

Inflammation is thought to play a key role in all stages of atherosclerosis and in the genesis of acute coronary syndromes (1). A key step in the formation and maturation of atherosclerotic plaques is endothelial cell activation (2). This is characterized by expression of cell adhesion molecules (CAMs; allowing adhesion and transmigration of leukocytes to the endothelium), a prothrombotic state, and impaired vascular reactivity (2). Elevated plasma levels of several markers of the inflammatory cascade have been shown to predict a future risk of cardiovascular events, including the acute-phase reactants, C-reactive protein (CRP), and fibrinogen (3). Peroxisome proliferator-activated receptor gamma (PPAR-gamma), a member of the nuclear receptor superfamily of ligand-activated transcription factors, is highly expressed in atherosclerotic plaques (4,5). The agonists of this receptor in clinical use are the thiazolidinediones rosiglitazone (Avandia) and pioglitazone (Actos). These agents have insulin-sensitizing actions and are used in the treatment of type 2 diabetes. Accumulating evidence suggests that PPAR-gamma agonists may have inhibitory effects on inflammatory processes in atherosclerotic plaques through indirect (insulin-sensitizing) and direct mechanisms.

Insulin resistance and hyperinsulinemia represent major risk factors for atherosclerotic disease (6). Thiazolidinediones both reduce insulin resistance and ameliorate the proatherogenic components of the insulin resistance syndrome, including dyslipidemia, the procoagulant state, and endothelial dysfunction (7–9). Insulin-resistant states are also associated with chronic, subclinical inflammation, and this may have a pathogenic role in atherogenesis and atherosclerotic disease complications (10). This association suggests that insulin sensitization may have an anti-inflammatory effect, and there are data to support this concept. Clinical studies, in obese or diabetic subjects without overt atherosclerosis, have shown that troglitazone and rosiglitazone can reduce levels of inflammatory markers (11–13).

Recent in vitro data suggest that PPAR-gamma agonists
may also act directly on the vessel wall and modify the transcription of pro-inflammatory genes within atherosclerotic plaques (14). One important molecular target of PPAR-gamma agonism is the transcription factor called nuclear factor-kappa-B (NF-kappa-B), which controls the synthesis of many of these pro-inflammatory genes (15). Although data from some animal studies suggest that PPAR-gamma agonists may reverse endothelial dysfunction and reduce markers of vascular inflammation (16–18), other in vitro studies failed to show any effect of PPAR-gamma agonists on adhesion molecule expression (19). Early studies showed that PPAR-gamma agonists may have a pro-atherogenic effect by upregulating the expression of the scavenger receptor CD36 in macrophages, which facilitates the uptake of oxidized low-density lipoprotein (LDL) (20). Recent data suggest that PPAR-gamma may actually have a dual action on cholesterol transport in macrophages as they also promote cholesterol efflux through induction of the ABCA1 transport protein, resulting in net lipid removal (21–23). Indeed, thiazolidinediones have consistently been shown to reduce atherosclerotic lesion formation in mouse models of atherosclerosis (24–27). To date, no clinical studies have examined the effects of PPAR-gamma agonists on inflammatory markers or endothelial cell activation in patients with atherosclerotic disease.

The aim of this study was to assess the effect of rosiglitazone on markers of endothelial cell activation (E-selectin and von Willebrand factor [vWF]) and acute-phase reactants (CRP and fibrinogen) in non-diabetic patients with coronary artery disease (CAD).

**METHODS**

**Study patients.** Consecutive patients with stable CAD were recruited from outpatient clinics to our institution. Inclusion criteria were angiographically documented CAD (≥50% lumen diameter reduction of at least one major coronary artery according to 2 independent observers) and age <75 years. Patients with any of the following were excluded: a previous clinical diagnosis of diabetes mellitus, history of acute coronary syndrome or revascularization in the previous three months, rest angina, cardiac failure, any change in cardiovascular medication during the preceding six weeks, malignant or hematologic disease, baseline alanine transaminase greater than two times the upper limit of normal, or women of child-bearing potential. The study was approved by the Local Research Ethics Committee, and all subjects gave written, informed consent before enrollment.

**Study design.** Subjects were randomized in a double-blind manner to receive placebo or rosiglitazone for 12 weeks. They received single-dose placebo or rosiglitazone 4 mg/day for the initial eight weeks, and the doses were doubled for the final four weeks. All other medications remained unchanged during the study. Rosiglitazone and matching placebo tablets were supplied by GlaxoSmithKline (U.K.).

**Study protocol.** Height, weight, and waist and hip circumferences were measured at baseline. Subjects had fasting blood samples collected in the morning for measurement of electrolytes, liver transaminases, lipid profile, insulin, glucose, markers of endothelial cell activation (E-selectin and vWF), and acute-phase reactants (CRP and fibrinogen). All measurements were repeated after 12 weeks of therapy.

**Biochemical parameters.** Electrolytes, glucose, total and high-density lipoprotein (HDL) cholesterol, and triglycerides were measured using a Beckman Coulter Synchron LX 20 analyzer (Beckman Coulter Inc., California). The LDL cholesterol was calculated according to the Friedewald equation (28). Serum insulin was measured by immunoassay (Elecsys, Roche Diagnostics, Mannheim, Germany). The homeostasis model of insulin resistance index (HOMA-R) was used as a measure of insulin resistance, where HOMA-R is calculated as fasting serum insulin (µU/ml) × fasting plasma glucose (mmol/l)/22.5 (29).

**E-selectin, vWF, fibrinogen, and CRP measurements.** E-selectin and vWF were measured by means of ELISA methods (R&D Systems [Abington, UK] and Dako Ltd. [Ely, UK], respectively). Fibrinogen was determined by the Clauss method. The CRP concentrations were measured by means of a high-sensitivity Immulite ELISA immunoassay (DPC Ltd., Gwynedd, UK).

**Statistical analysis.** Results are presented as the mean ± SD for continuous normally distributed variables, median (interquartile range) for continuous non-normally distributed data (CRP), and percentages for categorical data. The CRP levels were logarithmically (log10) transformed before being used in a comparative analysis. Comparisons between two mean values were performed by use of the unpaired, two-tailed t test. Differences between the repeated measurements of continuous variables were assessed by means of analysis of variance for repeated measurements. Categorical data were analyzed by means of the Fisher exact test. Correlations between continuous variables were assessed using the Pearson correlation coefficient. A p value <0.05 was considered to be statistically significant, and all reported p values are two-sided. Statistical analysis was performed with SPSS version 10.01 software.
Baseline characteristics and safety profile. Ninety-two patients were enrolled in the study, and 46 were assigned to each treatment. Two subjects were unwilling to attend for follow-up, and two subjects assigned to rosiglitazone withdrew due to side effects (dizziness and nausea, respectively). Four patients with baseline fasting plasma glucose >126 mg/dl (diagnostic criteria for diabetes mellitus) were excluded. Hence, the data on 84 subjects in total were analyzed. There were no other adverse events or biochemical side effects, in particular, any elevation in liver transaminases. Baseline clinical characteristics were similar in both groups (Table 1).

Comparison between treatment groups. METABOLIC PARAMETERS. Rosiglitazone treatment significantly reduced HOMA-R compared with placebo. In the rosiglitazone group, HOMA-R decreased from 2.45 ± 1.75 to 2.01 ± 1.78, whereas in the placebo group, there was a slight rise from 2.52 ± 1.43 to 2.84 ± 1.59 (p = 0.02) (Table 2). Compared with the placebo group, rosiglitazone treatment also significantly increased total and LDL cholesterol levels. In the rosiglitazone group, total cholesterol increased from 169 ± 29 mg/dl, with no significant change in the placebo group (p < 0.001) (Table 2). In the rosiglitazone group, LDL cholesterol increased from 102 ± 27 to 113 ± 27 mg/dl, with no significant change in the placebo group (p = 0.008) (Table 2). The HDL cholesterol levels showed no significant change in either group (Table 2).

RESULTS

Rosiglitazone treatment significantly increased triglyceride levels, compared with the placebo group. Triglyceride levels increased from 108 ± 63 mg/dl, whereas in the placebo group, there was no significant change (p = 0.005) (Table 2). There were no significant treatment effects on anthropometric measures (data not shown).

E-SELECTIN, vWF, FIBRINOGEN, AND CRP LEVELS. As shown in Table 3, baseline levels of E-selectin, vWF, fibrinogen, and CRP were similar in the rosiglitazone and placebo groups. After 12 weeks of treatment, patients who received rosiglitazone showed modest but significant decreases in E-selectin and vWF levels (p = 0.03 and p = 0.007, respectively) compared with the placebo group (Table 3, Fig. 1). In the rosiglitazone group, E-selectin decreased from 54 to 130 (p = 0.005), whereas in the placebo group, there was no significant change (p = 0.02) (Table 2).
creased from 48.9 \pm 16.4 to 43.4 \pm 16.0 \text{ng/ml}, whereas the placebo group showed no change. The vWF level decreased in the rosiglitazone group (138 \pm 46 to 131 \pm 43 \text{IU/dl}), whereas the vWF level increased by a similar degree in the placebo group (146 \pm 54 to 156 \pm 49 \text{IU/dl}).

The rosiglitazone group also showed significantly decreased CRP and fibrinogen levels (p < 0.001 and p = 0.003, respectively) compared with the placebo group (Table 3, Fig. 2). In the rosiglitazone group, the CRP level decreased from 0.56 (0.34 to 1.02) to 0.35 (0.26 to 0.50) mg/l, a relative reduction of 37\%, whereas the placebo group showed no change. The fibrinogen level decreased in the rosiglitazone group (3.81 \pm 1.12 to 3.38 \pm 0.65 g/l), whereas the fibrinogen level showed no change in the placebo group.

Within the rosiglitazone-treated group, reductions in CRP and vWF were significantly correlated with a reduction in HOMA-R (r = 0.37, p = 0.02 and r = 0.33, p = 0.04, respectively). No significant correlation was observed between a reduction in fibrinogen or E-selectin and a fall in HOMA-R (r = 0.18 and r = 0.12, respectively).

**DISCUSSION**

The most significant findings from this study are that rosiglitazone reduced circulating markers of endothelial cell activation (vWF and E-selectin) and acute-phase reactants (CRP and fibrinogen) in non-diabetic patients with CAD. Endothelial cell activation results in a release of the prothrombotic protein vWF from Weibel-Palade bodies and expression of CAMs, such as E-selectin, which facilitate entry of circulating monocytes to plaques. The plasma vWF level has been shown to be a predictor of cardiovascular...
events in patients with atherosclerotic disease (30). Although the pathogenic role of circulating CAMs that have been shed from the endothelium is unclear, plasma levels of soluble CAMs have been reported to be significant predictors of clinical outcome in patients with documented CAD (31,32). Elevated fibrinogen is a risk factor for death or recurrence of myocardial ischemia in patients with a previous coronary event, as well as a predictor of accelerated coronary atherosclerosis (33). An increase in fibrinogen levels may predispose to an atherothrombotic event through infiltration of the vessel wall by fibrinogen, rheologic effects due to increased blood viscosity, increased platelet aggregation and thrombus formation, and increased fibrin formation (33). C-reactive protein, produced in the liver in response to interleukin–6, has emerged as a marker of future cardiovascular risk among patients with stable and unstable angina (3). Recent evidence suggests that CRP may have direct pro-inflammatory effects (34). Data from statin trials suggest that reducing the inflammatory burden may improve the clinical outcome in atherosclerotic disease, even in patients with normal cholesterol levels (35). Notably, nearly all the patients in the present study were receiving long-term statin therapy, which has been shown to lower median CRP levels by 14% in CAD patients (36). Even so, we observed that rosiglitazone-treated patients showed a marked 37% reduction in median CRP levels after 12 weeks of therapy. The anti-inflammatory effects of rosiglitazone observed in our study may have therapeutic benefits, particularly in the high-risk group of patients with atherosclerotic disease and type 2 diabetes. It is likely that rosiglitazone modifies endothelial cell activation and expression of acute-phase reactants through indirect (metabolic) and direct actions on the vessel wall.

We studied non-diabetic patients with CAD to investigate the anti-inflammatory effects of rosiglitazone, independent of its hypoglycemic action. In our study cohort, the baseline insulin resistance (HOMA–R) in both the placebo and rosiglitazone groups was similar and mildly elevated compared with healthy non-diabetic control subjects (29). As expected, rosiglitazone treatment resulted in a significant reduction in insulin resistance compared with placebo. In the active treatment group, insulin resistance actually fell to within the normal range reported in epidemiologic studies (29). Our data also show that in the rosiglitazone-treated group, a reduction in CRP and vWF correlated closely with a reduction in insulin resistance. Previous studies have also shown that in insulin-resistant mice and humans, insulin-sensitizing agents decrease endothelial cell activation markers and acute-phase reactants (11–13,37). Therefore, it is likely that insulin sensitization is one mechanism by which rosiglitazone reduced inflammatory markers in this study. Because of logistical constraints related to the repeated investigation of a relatively large number of patients, insulin resistance was assessed using HOMA–R rather than more sensitive euglycemic insulin clamp techniques. Even so, we were able to demonstrate a significant insulin-sensitizing effect of rosiglitazone in our non-diabetic cohort of patients. Our results also showed that rosiglitazone treatment resulted in modest but significant rises in total and LDL cholesterol and triglyceride levels, with no effect on HDL cholesterol levels. These changes are in contrast to the lipid effects of rosiglitazone reported in most of the studies with type 2 diabetic patients. Randomized studies have shown that rosiglitazone treatment results in modest but significant increases in total, LDL, and HDL cholesterol, with triglyceride levels and the total/HDL cholesterol ratio remaining unchanged (38,39). However, one small observational study in type 2 diabetics also reported that rosiglitazone therapy caused modest increases in total and LDL cholesterol and triglycerides, with a trend toward decreased HDL cholesterol (40). Recent data also suggest that the thiazolidinediones may differ in their effects on lipid metabolism, with pioglitazone having less LDL cholesterol-raising effects than rosiglitazone (41). There is no clear mechanistic explanation for the effects of rosiglitazone on lipid metabolism in our non-diabetic patients. Rosiglitazone has been reported to increase the LDL particle size in diabetic patients (38). If the same effect was exerted in the non-diabetic patients investigated in our study, this may result in a rise in the absolute LDL concentration. Clearly, further studies are needed to determine the precise mechanisms by which PPAR-gamma agonists alter lipid metabolism. Regardless of the underlying mechanisms, the potentially deleterious effect of a rise in LDL cholesterol and triglyceride levels on endothelial cell activation and inflammatory burden deserves consideration. It is well established that LDL, after oxidative modification, activates endothelial cells to cause upregulation of endothelial adhesion molecules, selectins, and release of vWF (42). Hypertriglyceridemia has been shown to have a pro-inflammatory effect on atherosclerosis and enhances adhesion molecule expression (43). It is therefore possible that elevation of LDL cholesterol or triglyceride levels might increase endothelial cell activation and levels of acute-phase reactants. Whether the modest elevation in mean LDL cholesterol and triglyceride levels observed in the rosiglitazone group (11% and 20%, respectively) would have significant pro-inflammatory effects in vivo is open to debate. In summary, the effects of rosiglitazone on the lipid profile in our model do not explain the anti-inflammatory effect observed. Conversely, the elevation in triglyceride and LDL cholesterol levels may have a pro-inflammatory effect.

A potential mechanism by which rosiglitazone may exert an anti-inflammatory effect is through modifying transcription of pro-atherogenic genes in the vessel wall (14). This may explain why rosiglitazone reduced endothelial activation and inflammatory markers in our study population, despite elevating lipid levels. Previous in vitro studies have shown that PPAR-gamma agonists modulate transcription factors such as NF-kappa-B and thereby inhibit synthesis of pro-atherogenic gene products such as cytokines, chemo-
kines, matrix metalloproteinases, and adhesion molecules (44). In a study in LDL receptor-deficient mice, Li et al. (26) observed that rosiglitazone reduced aortic tissue expression of the cytokine tumor necrosis factor-alpha. This cytokine stimulates interleukin-6 production by smooth muscle cells, and interleukin-6 is the main hepatic stimulus for CRP production (3). In our study, we observed that in the rosiglitazone group, although a reduction in CRP and vWF correlated significantly with insulin sensitization, a reduction in E-selectin and fibrinogen levels did not show such a correlation. This suggests that insulin sensitization may not be the sole mechanism by which rosiglitazone exerted the anti-inflammatory effects observed. The mechanisms by which rosiglitazone reduces endothelial cell activation and levels of acute-phase reactants require further investigation. In order to assess the direct (vessel wall) effects of rosiglitazone, independent of metabolic effects, studies in patients with normal baseline insulin resistance are needed.

**Study limitations.** Our study has certain limitations that merit consideration. Although vWF and E-selectin levels fell significantly in the rosiglitazone compared with placebo group, the relative changes were modest (5% and 10%, respectively), and the biologic significance of these changes is questionable. Interestingly, statins have been shown to produce a similar 10% reduction in vWF levels in hypercholesterolemic patients (45). In the present study, additional indexes of endothelial activation were not measured, such as vascular cell adhesion molecule-1 or intercellular adhesion molecule-1 levels and endothelial vasomotor function.

**Conclusions.** We have demonstrated that the PPAR-gamma agonist rosiglitazone reduces markers of endothelial cell activation, CRP, and fibrinogen levels in non-diabetic patients with CAD. Potential mechanisms underlying this anti-inflammatory effect include insulin sensitization and direct modulation of transcriptional activity in the vessel wall. Further clinical studies, both in diabetics and non-diabetics, are warranted to determine whether this anti-inflammatory action translates into a therapeutic benefit in atherosclerotic coronary disease.

**REFERENCES**

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