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ORIGINAL RESEARCH

Effect of Pioglitazone Versus Metformin on Cardiovascular Risk Markers in Type 2 Diabetes

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ABSTRACT

Introduction: Besides its critical role in metabolic homeostasis, peroxisome proliferator-activated receptor (PPAR)-γ modulates several cellular responses involved in atherothrombosis. This multicenter, double-blind, randomized study investigated the effects of two oral hypoglycemic agents on markers of inflammation, platelet activation, thrombogenesis, and oxidative stress in patients with type 2 diabetes.

Methods and Results: The primary objective of this study was to evaluate the effect on C-reactive protein (CRP) after a 16-week treatment period with either pioglitazone or metformin. Additionally, markers of vascular inflammatory response, platelet activation, thrombogenesis, oxidative stress, glucose, and lipid metabolism, as well as liver function, were measured. In total, 50 patients completed the study. Pioglitazone-treated patients were found to have statistically significantly larger decreases in mean CRP levels (–0.4 mg/dL) compared to those treated with metformin (–0.2 mg/dL) (P = 0.04), as well as greater reductions in levels of mean fasting plasma glucose (–27 vs. –9 mg/dL; P = 0.01), serum insulin (–2 vs. –1.9 mU/L; P = 0.014), homeostatic model assessment (HOMA) (–1.2 vs. –0.9; P = 0.015), and E-selectin (–12.4 vs. +3.4 µg/mL; P = 0.01). Mean glycated hemoglobin (HbA_{1c}) levels decreased in both treatment groups from baseline to week 16 (–0.4% in the pioglitazone group, –0.2% in the metformin

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group; $P = 0.36$). Pioglitazone treatment was also found to be associated with a statistically significant increase in total cholesterol levels (+10 mg/dL in the pioglitazone arm, –3 mg/dL in the metformin arm; $P = 0.05$) and a decrease in liver enzyme levels.

Conclusions: The favorable changes in markers of systemic and vascular inflammatory response with pioglitazone suggest that it may positively influence the atherothrombotic process in type 2 diabetes.

Keywords: Cardiovascular disease; Cardiovascular risk factors; Metformin; Oral pharmacologic agents; Pioglitazone; Type 2 diabetes

INTRODUCTION

Mechanisms underlying the association between diabetes and cardiovascular disease (CVD) are complex, and include hyperglycemia with increased levels of glycosylation products, enhanced oxidative stress, and inflammation, combined with insulin resistance and alterations of lipid metabolism [1]. Experimental and epidemiologic evidence has highlighted the role of the inflammatory cascade in the pathogenesis of atherosclerosis; in particular, C-reactive protein (CRP), a marker of inflammation, is also a predictor of cardiovascular events [2–4]. Considering that CRP has a direct proatherogenic effect through upregulation of angiotensin II type 1 receptors and through the stimulation of other proinflammatory factors, it is possible that a reduction in this parameter obtained either through lifestyle changes or drug therapy has clinical benefits [5]. Other nonconventional markers of cardiovascular risk include adhesion molecules of the immunoglobulin superfamily (intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1 [VCAM-1]) and the selectin family (P-selectin, E-selectin), which are upregulated during atherogenesis [6] and elevated in type 2 diabetes [7]. A growing body of evidence suggests that inflammatory pathways could also be involved in microvascular damage, participating in the pathogenesis of diabetes complications, such as retinopathy and nephropathy [8–9].

Thiazolidinediones (TZDs) can reduce CD40 ligand (CD40L) serum levels, suggesting an anti-inflammatory mechanism [10] and a possible modulation of platelet aggregation [11]. TZDs regulate not only the activity of genes involved in glucidic or lipidic metabolism, but also that of genes regulating the inflammatory response of endothelium, vascular smooth muscle cells, T-cells, and monocytes/macrophages [7]. Furthermore, peroxisome proliferator-activated receptor (PPAR) activation reduces the production of inflammatory cytokines and the expression and release of metallo-proteases from macrophages [12], while in animal models an effect of TZDs on oxidative stress production has also been reported [13, 14].

The anti-inflammatory effect of TZDs in patients with type 2 diabetes has been reported in several trials [14–20]. However, all these studies were designed with different endpoints (usually glycemic control), and the anti-inflammatory actions of TZD were observed only as a secondary outcome. To our knowledge, this is the first trial to date designed to assess the effect of repeated doses of the TZD, pioglitazone, on inflammation, having CRP as its principal endpoint. Pioglitazone was studied as monotherapy in order to avoid the possible interference of other drugs; metformin was chosen as a comparator because it is not a TZD agent but it does have a profile of action on blood glucose and insulin resistance that is as similar as possible to that of pioglitazone.

MATERIALS AND METHODS

Study Design

The study was designed as a 16-week, double-blind, randomized, comparative, multicenter, parallel-group trial. After a maximum 1-week run-in period, which was necessary to receive the results of screening laboratory tests for inclusion and exclusion criteria, eligible patients were assigned to pioglitazone or metformin. The overall treatment period was 16 weeks. In total, five clinic visits took place at the start and end of the run-in period, and after 4, 8, and 16 weeks following randomization. Evaluable patients were those who completed at least 8 weeks of treatment. The protocol was approved by the Ethics Committees of all the participating centers.

Study Participants

The study was conducted in 10 diabetes clinics. Patients of either sex were enrolled, provided they met all the following inclusion criteria: diagnosis of type 2 diabetes mellitus (defined using the American Diabetes Association criteria [21]); age 35 and 75 years; glycated hemoglobin (HbA_{1c}) levels ≤9.0%; no pharmacologic treatment for hyperglycemia in the previous 3 months; negative response to pregnancy test for female patients of childbearing potential; cooperative attitude and ability to be trained to use the investigational drugs correctly and to attain the study procedures; written informed consent provided.

Patients were considered not to be eligible if they met one or more of the following exclusion criteria: treatment with other oral antidiabetic drugs or insulin in the 3 months preceding study entry; pregnant or lactating females; presence of any disease with malabsorption; acute or chronic pancreatitis; familiar polyposis coli; past medical history of myocardial infarction, transient ischemic attacks, or stroke; congestive heart failure (New York Heart Association class I–IV); significant liver (alanine transaminase [ALT] >2.5 upper limit of normal range) or renal (serum creatinine >1.2 mg/dL) impairment; anemia of any etiology (defined as hemoglobin level <10.5 g/dL) or any other clinically relevant hematologic disease; diagnosis or suspicion of any neoplastic disease; history of chronic alcohol or drug/substance abuse, or presence of other conditions potentially able to affect study subjects' compliance; concomitant therapy with statins, antioxidant drugs (e.g., vitamins, Q10 coenzyme), beta-blockers, nonsteroidal anti-inflammatory drugs, aspirin, corticosteroids; known allergy, sensitivity, or intolerance to study drugs and/or study drugs' formulation ingredients; and participation in another trial in the 3 months preceding study entry.

Treatment Interventions

Patients were randomly assigned to receive either pioglitazone or metformin. Treatment was assigned centrally via telephone after verification of the inclusion criteria. The first patient was randomly allocated; for each subsequent patient, treatment allocation was identified through the minimization method, which minimizes the imbalance between groups at that time according to patient characteristics (center, use of angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists).

Pioglitazone 30 mg was taken once daily as a starting dose and up-titrated to 45 mg once daily in later visits in the case of poor response. The control group was treated with metformin, with a starting dose of 850 mg/day and up-titrated to 850 mg twice or three times a day in later visits depending on the glycemic response. Study drugs were titrated to higher doses when

fasting plasma glucose (FPG) was >140 mg/dL; in order to maintain the double blind design, in the case of up-titration, pioglitazone placebo was administered once or twice a day in addition to the active pioglitazone tablet. Tablets of each investigational study drug, as well as pioglitazone placebo, were encapsulated and packed in bottles and boxes by the manufacturing contractor (Farma Resa S.R.L., Cantù, Italy), according to Good Manufacturing Practices.

Efficacy Parameters

The primary efficacy variable was the change from baseline in CRP level measured after a 16-week treatment period. The secondary efficacy variables were the changes from baseline in levels of the following parameters: markers of inflammatory response (adhesion proteins [P-selectin, E-selectin, ICAM-1], interleukin [IL]-6, and CD40L); markers of platelet activation and thrombogenesis (urinary levels of 11-dehydro thromboxaneB2 [TXB2], circulating levels of tissue factor (TF), plasminogen activator inhibitor-1; markers of oxidative stress (nitrotyrosine); standard metabolic (blood glucose, HbA_{1c}, serum insulin) and lipidic (total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], very low-density lipoprotein cholesterol, triglycerides, nonesterified fatty acids) parameters.

All the parameters were centrally measured at baseline, and 8 and 16 weeks after randomization, with the exception of lipid profile, which was evaluated only at baseline and 16 weeks.

Analytical Methods

CRP was measured with a high sensitivity nefelometric assay (normal range 0–3 mg/L). The soluble forms of CD40L and the endothelium-derived adhesive molecules, P-selectin, E-selectin, ICAM-1, and VCAM-1 were measured in plasma using commercially available specific enzyme-linked immunoassay (ELISA) kits (RD Systems and Bender Med Systems, Prodotti Gianni, Milano, Italy). The circulating soluble form of TF was also measured using a commercial ELISA kit (IMUBIND® Tissue Factor, American Diagnostica, Intrumentation Laboratory, Milano, Italy).

TXB2 and 8-iso-prostaglandin F2α markers of platelet activation and oxidative stress, respectively, were measured in urine collected over 24 h. Urinary metabolites were purified through sequential extraction on Sep-Pak® C18 cartridges (Waters Associates and Sep-Pak® silica cartridges, Waters Associates Waters S.p.A., Milano, Italy). The eluates from the second extraction were dried, reconstituted in phosphate buffer, and frozen at –70°C until analysis by specific ELISA (Cayman Chemicals, Vinci Biochem, Florence, Italy).

Sample Size

The sample size calculation was based on the primary objective of demonstrating a reduction in CRP levels induced by pioglitazone as opposed to metformin. Based on previous evidence on troglitazone [22], the sample size was calculated to detect a reduction of 3 mg/L in CRP levels in patients treated with pioglitazone compared to those receiving metformin, with a statistical power of 90% ($\alpha = 0.05$), and assuming a standard deviation in CRP levels of 3 mg/L. Given these assumptions, a minimum of 20 subjects in each arm were needed. It was planned to enroll at least 50 individuals to allow for a 20% dropout rate.

Statistical Methods

Baseline demographic and background data were summarized as percentages for

qualitative variables and mean and standard deviations or median and interquartile ranges for quantitative variables. Categorical variables were compared using the Chi-square test. Student's *t*-test or Mann-Whitney *U*-test were used to compare continuous variables between treatment arms.

Within-group changes were tested using the paired *t*-test or the signed rank Wilcoxon test. For the assessment of differences between treatment groups with respect to primary and secondary endpoints, a repeated measures analysis of variance (ANOVA) of the change from baseline to the end of the study was applied using PROC MIXED software (SAS, release 9.1; SAS Institute, Cary, NC, USA). Continuous variables with substantial deviations from the normality assumption were mathematically transformed.

Statistical analyses were performed on the intention-to-treat population, i.e., all randomized patients who received at least one dose of study medication and who completed at least 8 weeks of treatment.

RESULTS

Overall, 67 patients were initially identified, of whom 9 were not randomized (Fig. 1). Of the 58 patients randomized (29 assigned to pioglitazone and 29 to metformin), 8 (5 assigned to pioglitazone and 3 to metformin) prematurely discontinued the study. Therefore, 50 patients completed the entire study period and were considered for all the efficacy analyses (24 in the pioglitazone group and 26 in the metformin group). Baseline patient characteristics according to allocated treatment are reported in Table 1 and show that the two groups were well matched for all the variables investigated. Results relative to all efficacy and safety laboratory parameters are reported in Table 2.

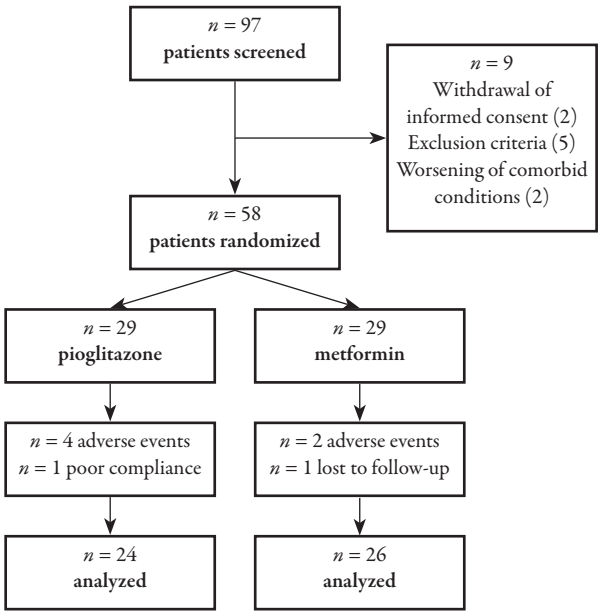

Fig. 1 Study flow diagram

Primary Efficacy Variable: CRP

A statistically significant decrease from baseline to week 16 was observed in the CRP levels of patients treated with pioglitazone ($P < 0.001$), compared to a smaller and nonsignificant decrease in those treated with metformin. The comparison between treatments showed a statistically significant difference ($P = 0.04$) in favor of pioglitazone.

Secondary Efficacy Variables

A statistically significant decrease from baseline to week 16 in E-selectin levels ($P < 0.05$) was observed in patients treated with pioglitazone compared to no changes in patients treated with metformin. The comparison between treatments showed a statistically significant difference in favor of pioglitazone for E-selectin levels ($P = 0.01$).

No statistically significant changes in markers of platelet activation and thrombogenesis, or nitrotyrosine levels, were observed with either treatment.

Table 1 Patient baseline characteristics according to study group

Characteristic	Pioglitazone	Metformin	*P* value
Number of patients	29	29	
Age (years)	59.1±6.8	56.4±7.9	0.17
Gender:			
Males	14 (48.3%)	19 (65.5%)	0.18
Females	15 (51.7%)	10 (34.5%)	–
BMI (kg/m^2)	31.1±3.2	31.7±3.6	0.52
Weight (kg)	84.1±12.5	87.8±11.5	0.24
Smoking (cigarettes/day):	24 (82.8%)	22 (75.9%)	0.70
0			
<10	1 (3.4%)	2 (6.9%)	–
10–20	2 (6.9%)	1 (3.4%)	–
>20	2 (6.9%)	4 (13.8%)	–
Diabetes duration (years)	4.4±3.2	3.9±2.2	0.50
CRP (mg/L)	1.9 (1.1–5.3)	2.2 (1.4–3.0)	0.86
FPG (mg/dL)	152±38	146±46	0.55
HbA_{1c} (%)	6.9±0.8	6.8±0.7	0.42
Insulin (mU/L)	9.1 (6.9–14.3)	10.4 (6.7–12.9)	0.96
HOMA index	3.3 (2.4–5.4)	3.5 (2.0–4.7)	0.65
Total cholesterol (mg/dL)	215±24	214±34	0.89
HDL-C (mg/dL)	40±10	39±9	0.66
LDL-C (mg/dL)	145±27	144±29	0.93
VLDL-C (mg/dL)	23 (18–35)	28 (21–36)	0.45
FFA (mmol/L)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.61
Triglycerides (mg/dL)	115 (90–167)	138 (104–182)	0.45
P-selectin (μg/mL)	38.9 (21.9–90.0)	41.8 (31.2–62.1)	0.96
E-selectin (μg/mL)	70.8 (53.9–79.6)	65.3 (59.1–82.7)	0.80
ICAM-1 (μg/mL)	290 (232–324)	253 (230–296)	0.42
CD40L (pg/mL)	1.5 (0.5–2.4)	1.2 (0.8–2.2)	0.70
TXB2 (pg/mg creatinine)	144 (75–207)	122 (85–284)	0.77
TF (pg/mL)	114 (102–149)	145 (108–180)	0.26
PAI-1 (ng/mL)	52.0 (24.5–78.6)	33.1 (24.7–81.7)	0.65
Nitrotyrosine (nM)	6.7±1.5	6.5±1.4	0.77

For continuous variables values are mean ± standard deviation or median and range
BMI body mass index, *CD40L* CD40 ligand, *CRP* C-reactive protein, *FFA* free fatty acid, *FPG* fasting plasma glucose, *HbA_{1c}* glycated hemoglobin, *HDL-C* high-density lipoprotein cholesterol, *HOMA* homeostatic model assessment, *ICAM-1* intercellular adhesion molecule-1, *LDL-C* low-density lipoprotein cholesterol, *PAI-1* plasminogen activator inhibitor-1, *TF* tissue factor, *TXB2* 11-dehydro thromboxaneB2, *VLDL-C* very low-density lipoprotein cholesterol

Table 2 Laboratory efficacy and safety variables with pioglitazone versus metformin

Parameter	Pioglitazone Baseline	Pioglitazone Week 16	Metformin Baseline	Metformin Week 16	*P* value
Number of patients	24	24	26	26	–
Markers of inflammatory response					
CRP (mg/L)	1.8 (1.1–4.7)	1.4 (0.5–2.5)*	2.0 (1.1–2.9)	1.8 (0.8–3.7)	0.04
P-selectin (μg/mL)	56.9 (26.7–140)	52.2 (29.3–126.8)	41.3 (31.2–68.1)	47.5 (29.2–74.1)	0.73
E-selectin (μg/mL)	70.2 (52.6–81.5)	57.8 (53.7–83.8)**	65.1 (59.1–79.9)	68.5 (62.9–78.3)	0.01
ICAM-1 (μg/mL)	292 (233–322)	269 (241–312)	251 (230–296)	252 (215–309)	0.87
CD40L (pg/mL)	1.6 (0.5–2.9)	2.0 (0.4–3.6)	1.3 (0.8–2.5)	1.4 (0.8–2.4)	0.98
Markers of platelet activation and thrombogenesis					
TXB2 (pg/mg creatinine)	146 (82–221)	121 (87–198)	123 (85–304)	159 (106–191)	0.61
TF (pg/mL)	113 (102–131)	139 (113–172)	141 (100–189)	145 (111–223)	0.23
PAI-1 (ng/mL)	55.1 (21.0–82.4)	35.8 (23.8–66.1)	32.7 (24.3–81.7)	39.5 (31.7–46.2)	0.69
Markers of oxidative stress					
Nitrotyrosine (nM)	6.7±1.5	6.6±1.6	6.5±1.4	6.3±1.0	0.82
Glucose parameters					
FPG (mg/dL)	153±40	126±25***	144±47	135±48*	0.01
HbA_{1c} (%)	6.9±0.9	6.5±0.8**	6.7±0.7	6.5±0.7*	0.36
Insulin (mU/L)	8.3 (6.7–14.7)	6.3 (4.7–9.2)***	10.0 (5.3–12.8)	8.1 (5.6–10.6)	0.014
HOMA index	3.2 (2.1–5.4)	2.0 (1.3–2.9)***	3.2 (2.0–4.1)	2.3 (2.1–3.3)	0.015
Lipid parameters					
Total cholesterol (mg/dL)	212±24	222±35**	215±35	212±35	0.05
HDL-C (mg/dL)	41±10	45±11*	40±9	42±9***	0.19
LDL-C (mg/dL)	141±26	148±34	147±29	142±27	0.07
VLDL-C (mg/dL)	22.8 (18.2–33.5)	23.8 (16.0–32.2)	24.3 (17.4–36.4)	26.4 (17.8–37.2)	0.94
FFA (mmol/L)	0.4 (0.3–0.5)	0.4 (0.2–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.6)	0.07
Triglycerides (mg/dL)	114 (91–168)	119 (80–161)	122 (87–182)	132 (89–186)	0.94
Safety parameters					
Hemoglobin (g/dL)	14.4±1.1	14.1±1.0	14.6±1.0	14.4±1.1	0.58
WBCs (10^9/L)	6.2±1.5	5.9±1.4**	6.5±1.9	6.3±1.7	0.60
Neutrophils (%)	51.4±8.0	50.2±7.2	53.5±7.8	53.7±9.3	0.72
ALT (U/L)	26.5 (20.5–33.0)	19.0 (17.0–23.5)***	28.0 (23.0–48.0)	27.5 (23.0–46.0)	<0.0001
AST (U/L)	20.0 (18.0–23.0)	18.5 (15.0–22.0)*	20.0 (17.0–24.0)	21.0 (16.0–26.0)	0.003
γGT (U/L)	28.0 (21.0–36.5)	19.5 (14.0–26.5)***	35.5 (24.0–40.0)	32.0 (23.0–40.0)	<0.0001

Values are mean ± standard deviation or median and range

ALT alanine transaminase, *AST* aspartate transaminase, *CD40L* CD40 ligand, *CRP* C-reactive protein, *FFA* free fatty acid, *FPG* fasting plasma glucose, *γGT* γ glutamyl transpeptidase, *HbA_{1c}* glycated hemoglobin, *HDL-C* high-density lipoprotein cholesterol, *HOMA* homeostatic model assessment, *ICAM-1* intercellular adhesion molecule-1, *LDL-C* low-density lipoprotein cholesterol, *PAI-1* plasminogen activator inhibitor-1, *TF* tissue factor, *TXB2* 11-dehydro thromboxaneB2, *VLDL-C* very low-density lipoprotein cholesterol, *WBCs* white blood cells

* $P < 0.01$ vs. baseline; ** $P < 0.05$ vs. baseline; *** $P < 0.001$ vs. baseline

A statistically significant decrease from baseline to week 16 in levels of FPG ($P < 0.001$), HbA_{1c} ($P < 0.05$), insulin ($P < 0.001$), and homeostatic model assessment (HOMA) index ($P < 0.001$) was observed in patients treated with pioglitazone, while patients on metformin had a significant decrease from baseline to week 16 in levels of FPG ($P < 0.01$) and HbA_{1c} ($P < 0.01$). The comparison between treatments showed a statistically significant difference in favor of pioglitazone for levels of FPG ($P = 0.01$), insulin ($P = 0.014$), and HOMA index ($P = 0.015$), but not for HbA_{1c}.

A statistically significant increase from baseline to week 16 in levels of total cholesterol ($P < 0.05$) and HDL-C ($P < 0.01$) was observed in patients treated with pioglitazone, whereas in those receiving metformin there was a statistically significant increase from baseline to week 16 in HDL-C levels ($P < 0.001$) without any modification in total cholesterol levels. The comparison between treatments showed a statistically significant difference for total cholesterol in favor of metformin ($P = 0.05$).

Safety

A total of 39 adverse events (AEs) were reported; 23 in patients treated with pioglitazone and 16 in patients treated with metformin. In total, 17 AEs (11 in patients treated with pioglitazone and 6 in patients treated with metformin) were considered to be adverse drug reactions, i.e., those with a certain, probable, or possible correlation with study drug. Four hypoglycemic episodes were reported in the pioglitazone group and none in the metformin group. No serious AEs were reported with either treatment. Only one AE (acute bronchitis) in a patient treated with metformin was of severe intensity, but was not considered to be related to study drug.

Six patients, four treated with pioglitazone and two with metformin, discontinued the study due to AEs. Causes of early discontinuations in patients treated with pioglitazone were hypoglycemia in two patients, general discomfort, vomiting, and diarrhea in one patient, and abdominal pain in another patient. In the metformin group, myocardial ischemia in one patient and flatulence in another were responsible for discontinuations.

Laboratory Safety Parameters

The results of hematology tests at baseline and week 16 showed a statistically significant decrease from baseline in white blood cell counts ($P < 0.05$) in patients treated with pioglitazone. No substantial changes from baseline in hemoglobin and neutrophil levels were observed with either treatment. The comparison between treatments in hematology parameters did not show statistically significant differences for any variable.

The results of liver function enzyme tests at baseline and week 16 showed a statistically significant decrease from baseline in levels of ALT ($P < 0.001$), aspartate transaminase (AST) ($P < 0.01$), and γ glutamyl transpeptidase (GT) levels ($P < 0.001$) in patients treated with pioglitazone, with no substantial changes in any variable in patients treated with metformin. The comparison between treatments showed a statistically significant difference in favor of pioglitazone for levels of ALT ($P < 0.0001$), AST ($P = 0.003$), and γGT ($P < 0.0001$).

DISCUSSION

It is increasingly recognized that markers of vascular inflammation play a role in the pathogenesis of type 2 diabetes, insulin resistance, and atherosclerosis [11, 23]; CRP in particular is an independent predictor of both type 2 diabetes and CVD [4, 24]. The clinical relevance

of lowering CRP values in terms of reducing major adverse cardiac events and mortality has been clearly demonstrated in clinical trials of statins [25, 26]. Previous studies have shown that PPAR-γ agonists may affect inflammatory pathways via transcriptional mechanisms, and decreases in cytokines, chemokines, and matrix metallo-proteinases have been demonstrated in monocytes/macrophages, T-cells, and vascular smooth muscle cells (VSMC) [27]. TZDs have been associated with an antiatherogenic effect, which cannot be completely accounted for by the observed improvement in glycemic control. In fact, in comparisons with sulfonylureas, the TZD, pioglitazone, has shown beneficial effects on intima-media thickness [28] and on the progression of coronary atherosclerosis [29], which are evident after a relatively short follow-up. Based on these observations, it can be speculated that the reduction in major cardiovascular events reported in the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) study [30] and confirmed by meta-analyses, including all available pioglitazone trials [31–32], is partly due to some extraglycemic action of the drug.

The anti-inflammatory effect of TZDs is a good candidate as a potential antiatherogenic mechanism independent of any glucose-lowering action. A reduction in levels of markers of inflammation induced by pioglitazone has been reported in previous experimental studies and clinical trials [10–20]. However, in previous clinical observations, its anti-inflammatory activity has been reported in comparisons with placebo or other glucose-lowering, noninsulin-sensitizing drugs. Therefore, on the basis of available data, it was not possible to distinguish between the specific effects of the drug and the effects induced by the improvement in glucose control and/or insulin sensitivity. The present study is the first to consider inflammation as the principal endpoint, excluding any concomitant treatments capable of interfering with the anti-inflammatory effects of the experimental drug. Interestingly, pioglitazone monotherapy produced a greater reduction in CRP levels than did metformin, which also has an insulin sensitizing effect despite sharing a similar overall glycemic control. It should be recognized that the effect of pioglitazone on insulin sensitivity was greater than that of metformin, meaning that theoretically, some of the differences between the two treatments could be related to the greater enhancement of insulin action. A beneficial effect of pioglitazone in reducing CRP in diabetic patients with high levels of this protein and high cardiovascular risk has been reported [33]. In the present study, the authors were able to show that in diabetic patients at low risk for cardiovascular disease, this beneficial (CRP-lowering) effect was superior to that achieved with metformin. The lack of any significant effect of treatment on platelet activity and on markers of platelet activation and thrombogenesis is inconsistent with previous studies [11, 34]. This result could be explained by the fact that in the present trial, the patients enrolled were drug-naïve and they had relatively moderate hyperglycemia. It is possible that some of the previously described benefits of TZD treatment on these parameters were related to the improvement in blood glucose in patients with a greater degree of impairment in metabolic control.

In this study, pioglitazone treatment was found to be associated with a statistically significant increase in total cholesterol and HDL-C levels. It is well recognized that pioglitazone positively affects the lipid profile by increasing HDL-C and reducing trigycerides. The effect on total cholesterol is less clear but a recent meta-analysis reported that treatment with this drug was associated with a significant

reduction in total cholesterol [35]. Furthermore, it is known that pioglitazone modifies LDL particle size, reducing its atherogenic effects [36]. Recently, a post-hoc analysis of the PROactive study showed that the beneficial effect of pioglitazone on cardiovascular outcome was mainly due to an increase in HDL-C, rather than an amelioration of HbA_{1c} [37].

In our study, pioglitazone treatment significantly decreased levels of liver enzymes. The positive effect of pioglitazone on liver enzymes and nonalcoholic fatty liver disease (NAFLD) is well known and our results confirm this effect. A recent meta-analysis showed that in patients with NAFLD, pioglitazone improved histologic disease activity, slowed fibrosis progression, and extensively ameliorated cardiometabolic endpoints [38].

Metformin is recognized by almost all available guidelines and treatment recommendations as the drug of choice for patients newly diagnosed with type 2 diabetes [39–42]. Obviously, such a position, which is based on an overall assessment of short- and long-term efficacy, tolerability, safety, and cost data cannot be modified by any single study showing the superiority of another drug with respect to a parameter other than a hard endpoint. Furthermore, it is not possible to anticipate the long-term effects associated with a reduction in CRP levels of the magnitude detected in our study, although the association between the levels of this marker and cardiovascular risk appears to be linear [43]. Only long-term, large-scale trials specifically designed for cardiovascular outcomes can provide reliable information on the effects of drugs on cardiovascular risk. However, such trials are unavailable at the present time for most of the agents currently used in the treatment of type 2 diabetes. This study suggests that alternative parameters (other than glucose control), that are diversely affected by glucose-lowering drugs, should be considered in the overall assessment of treatments. Moreover, the positive effects of the two drugs investigated in the present trial on inflammatory and metabolic parameters could be regarded as useful consequences in the treatment of type 2 diabetic patients. Recently, a retrospective cohort study using data from the UK-based General Practice Research Database showed that a combination of metformin plus pioglitazone appeared to provide superior clinical outcomes compared with the most commonly used regimen, represented by the association of metformin with a sulfonylurea [44].

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APPENDIX

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