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ORIGINAL ARTICLES

The Fixed Combination of Pioglitazone and Metformin Improves Biomarkers of Platelet Function and Chronic Inflammation in Type 2 Diabetes Patients: Results from the PIOfix Study

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Abstract

Background:

Type 2 diabetes mellitus (T2DM) is characterized by a proinflammatory and procoagulant condition. This study investigates the impact of a pioglitazone plus metformin therapy on biomarkers of inflammation and platelet activation in comparison to a treatment with glimepiride plus metformin.

Methods:

The study was designed as a multicenter, randomized, double-blinded two-arm trial. Patients with T2DM and dyslipidemia under metformin monotherapy with hemoglobin A1c value between 6.5% and 9.0% were eligible for trial participation. Blood was drawn at baseline and after 24 weeks of treatment from patients of five centers. Markers of inflammation and thrombocyte function (soluble CD40 ligand, thromboxane, vWillebrand factor, adhesion molecules, clotting reaction) were evaluated subsequently in a central laboratory.

Results:

A total of 46 patients were included in the final analyses. Mean (± standard deviation) age was 58.5 ± 9.0 years (13 women, 33 men; disease duration 6.3 ± 5.0 years; body mass index 32.0 ± 4.8 kg/m²). A total of 25 patients were treated with pioglitazone plus metformin, and 21 patients were in the glimepiride arm. There was a significant decline of E-selectin (-3.7 ± 4.8 ng/ml, $p < .001$ versus baseline), vWillebrand factor (-19.5 ± 32.0%, $p < .05$), and high-sensitivity C-reactive protein concentrations (-1.08 ± 0.91 mg/liter, $p < .05$) in the metformin + pioglitazone arm only (metformin + glimepiride, -0.5 ± 3.4 ng/ml, +1.4 ± 33.2%, + 0.08 ± 0.72 mg/liter, respectively, all not significant). Also, all other surrogate markers for platelet function and inflammation showed slight improvements in the metformin + pioglitazone arm but not in the metformin + glimepiride arm.

continued →

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Abbreviations: (HDL) high-density lipoprotein, (hsCRP) high-sensitivity C-reactive protein, (sCD40L) soluble CD40 ligand, (T2DM) type 2 diabetes mellitus

Keywords: inflammation, oral antidiabetics, pioglitazone plus metformin, platelet aggregation, type 2 diabetes, vascular function

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Abstract cont.

Conclusions:

The fixed metformin + pioglitazone combination treatment showed an overall improvement of laboratory surrogate markers, indicating improvement of platelet function and of chronic systemic inflammation, which was not seen with metformin + glimepiride.

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Introduction

The high prevalence of macrovascular morbidity and mortality is one of the major complications of type 2 diabetes mellitus (T2DM). Atherogenesis driven by diabetes contributes to ischemic macrovascular events such as coronary artery disease, stroke, transient ischemic attack, and peripheral artery disease. These conditions are well recognized as the basis for the increased rate of cardiovascular events in diabetes patients.[1] One of the important links between T2DM and atherogenesis is a procoagulant activity in the blood circulation of the patient, which is characterized by platelet dysfunction and elevated levels of inflammatory biomarkers.[2]

Vascular inflammation is associated with endothelial cell wall rupture, which is observed with an unusually high prevalence in diabetes patients. As a result, pro-inflammatory adhesion molecules such as E-selectin, vascular cell adhesion molecule, and intracellular adhesion molecule are released into circulation.[3] This secretion leads to activation of platelets, the pivotal cells in the clotting cascade. Soluble CD40 ligand (sCD40L), thromboxane, and vWillebrand factor are procoagulant mediators released by activated platelets.[3,4] Platelets aggregate via the glycoprotein IIb/IIIa receptor complex and, eventually, initiate thrombus formation. Platelets are essential to hemostasis, and their dysfunction leads to increased thrombus formation and may subsequently cause acute vascular events. Hyperglycemia and/or insulin resistance are contributors to hyperreactivity of platelets.[5] Platelet aggregation is counteracted by insulin. However, platelets of diabetes patients are considered resistant to insulin,[6,7] which, in turn, starts a prothrombotic vicious cycle.

Therefore, improvement of platelet function and reduction of chronic systemic inflammation should be taken into account as an important secondary objective of an anti-diabetes therapy beyond achievement of glycemic control, and biomarkers of coagulation and inflammation can be used as surrogate for the prothrombotic condition. This analysis of the multicenter PIOfix trial investigates the mentioned biomarkers in diabetes patients who received a fixed combination of metformin plus pioglitazone in comparison to a combination therapy of metformin and glimepiride.

Material and Methods

Study Design

The original study (Clinical Trials Registry No. NCT 00770653) was designed as a multicenter, randomized, double-blinded study investigating the effects of a pioglitazone plus metformin fix combination compared to a treatment with metformin plus glimepiride on diabetic dyslipidemia, with an observation period of 6 months.[8] The study showed statistically confirmed superiority of the pioglitazone + metformin treatment compared to glimepiride + metformin with respect to an increase of high-density lipoprotein (HDL) cholesterol after 24 weeks of therapy in metformin-pretreated T2DM patients with dyslipidemia. The study was conducted in accordance with the Declaration of Helsinki and was approved by the responsible ethical committees. All subjects gave written informed consent prior to any study-related procedure. In addition to the primary objective, surrogate markers of platelet activation were analyzed in a subset of patients participating at five selected clinical sites.

Main inclusion criteria were T2DM diagnosed according to American Diabetes Association criteria, treated with metformin monotherapy in a maximal individually tolerated daily dose of 850 to 2000 mg within the previous 12 weeks, hemoglobin A1c between 6.5% and 9.0%, and dyslipidemia defined as decreased HDL cholesterol

levels ≤1.03 mM and/or elevated triglycerides ≥1.7 mM. Main exclusion criteria were significant hepatic (glutamate-pyruvate-transaminase values >2.5 times normal value), renal (serum creatinine >1.2 mg/dl in women and >1.5 mg/dl in men) or cardiovascular disorders (New York Heart Association I–IV), or previous treatment with insulin or with other oral antidiabetes drugs in addition to metformin.

Patients eligible for the study were randomized to receive twice daily either a single tablet containing metformin (850 mg) and pioglitazone (15 mg) together with a placebo tablet or metformin (850 mg) plus glimepiride (1 mg). Concomitant statin therapy or anticoagulant treatment with clopidogrel or acetyl salicylic acid were to be kept constant during study participation. Blood was drawn for measurement of laboratory parameters at baseline (start of study treatment) and endpoint after 24 weeks of treatment.

Laboratory Analysis

For this analysis, the following parameters were determined according to each manufacturer's instructions in a central laboratory: sCD40L, E-selectin, and matrix metalloproteinase-9 (all by ELISA, R&D Systems, Minneapolis, MN). vWillebrand factor and high-sensitivity C-reactive protein (hsCRP) were measured by latex-assisted turbidimetry (Olympus, Hamburg, Germany). Patients with hsCRP values >10 mg/dl, representing a confounding general inflammation, were eliminated from the analysis.[9] Thromboxane B2 was assessed with an enzyme immune assay (Assay Designs, Ann Harbor, MI). Platelet function was additionally checked by investigation of clotting time with the PFA-100® technology (Siemens Healthcare Systems, Eschborn, Germany). The Verify Now™ System (Incomat Medizinische Geräte, Glashütten, Germany) was used to determine both platelet aggregation units (PAU) and inhibition of glycoprotein IIb/IIa complex.

Data Analysis

Calculations were made with the SAS® software package (version 9.2). In case of premature termination, the last available observation was used for analysis (last observation carried forward approach). Demographic and baseline characteristics were summarized descriptively. Standard descriptive summary statistics were presented for continuous variables (i.e., arithmetic mean ± standard deviation). The changes from baseline to endpoint were analyzed in an exploratory manner using a general model for analysis of covariance with fixed effect factor for treatment group and with the baseline value as covariate. In addition, two-sided 95% confidence intervals and two-sided p-values for within- and between-group treatment differences were calculated. Normality of residuals was checked with the Shapiro–Wilk test. Values of $p < .05$ were interpreted as significant.

Results

Forty-six of 288 subjects of the full-analysis set of the PIOfix study[8] were included into this subanalysis of platelet function. **Table 1** summarizes the demographic data of this cohort, which is representative for a typical German diabetes population. Concomitant medication was kept constant during trial participation.

As shown in **Table 2**, the majority of the laboratory markers of thrombocyte function and inflammation showed improvements with metformin + pioglitazone and minor

Table 1.
Summary of the Demographic and Clinical Data of the Analyzed Study Group

Parameter	Whole group	Pioglitazone + metformin	Glimepiride + metformin
N	46	25	21
Gender (male/female)	33/13	19/6	14/7
Age (years)	58.5 ± 9.0	59.4 ± 8.0	57.5 ± 10.1
Disease duration (years)	6.3 ± 5.0	6.7 ± 5.3	5.9 ± 4.7
Body mass index (kg/m²)	32.0 ± 4.8	32.0 ± 4.5	32.0 ± 5.2
Waist/hip ratio	0.99 ± 0.07	1.00 ± 0.06	0.97 ± 0.08
Hemoglobin A1c (%)	7.1 ± 0.7	7.4 ± 0.8	6.7 ± 0.5
Concomitant diseases (*n*, %)			
Hypertension	36 (78.3%)	17 (68.0%)	19 (90.5%)
Dyslipidemia	25 (54.3%)	11 (44.0%)	14 (66.7%)
Coronary artery disease	7 (15.2%)	1 (4.0%)	6 (28.6%)
Diabetic retinopathy	2 (4.3%)	2 (8.0%)	0
Diabetic neuropathy	2 (4.3%)	1 (4.0%)	1 (4.8%)
Erectile dysfunction	2 (4.3%)	1 (4.0%)	1 (4.8%)
Diabetic foot	1 (2.2%)	0	1 (4.8%)
Relevant concomitant medication (*n*, %)			
Antithrombotic agents	15 (32.6%)	6 (24.0%)	9 (42.9%)
Beta blockers	20 (43.5%)	7 (28.0%)	13 (61.9%)
Antihypertensive	26 (56.5%)	11 (44.0%)	15 (71.4%)
Lipidemics	19 (41.3%)	8 (32.0%)	11 (52.4%)

Table 2.
Changes of the Investigated Parameters by Treatment Arm from Baseline to Endpoint[a]

	Pioglitazone + metformin		Glimepiride + metformin	
	Baseline	Endpoint	Baseline	Endpoint
Hemoglobin A1c (%)	7.4 ± 0.8	6.6 ± 0.9[b]	6.7 ± 0.5	6.0 ± 0.5[b]
Biomarker of platelet function				
Thromboxane B2 (pg/ml)	1337 ± 1197	1121 ± 753	756 ± 433	1284 ± 1046
Glycoprotein II/III complex inhibition (PAU)	134 ± 67	143 ± 63	153 ± 41	144 ± 35
vWillebrand factor (%)	148 ± 62	128 ± 53[b]	129 ± 50	130 ± 31
Biomarker of chronic systemic inflammation				
hsCRP (≤10 mg/liter) (mg/liter)	2.32 ± 1.88	1.26 ± 1.12[b]	2.64 ± 2.58	2.71 ± 2.79[c]
sCD40L (pg/ml)	388 ± 379	348 ± 518	285 ± 158	387 ± 372
Matrix metallopeptidase 9 (ng/ml)	429 ± 197	461 ± 280	460 ± 203	511 ± 276
Soluble intracellular adhesion molecule (ng/ml)	274 ± 73	261 ± 78	276 ± 69	273 ± 84
Soluble vascular cell adhesion molecule (ng/ml)	752 ± 267	763 ± 269	630 ± 117	633 ± 190
E-selectin (ng/ml)	22 ± 9	18 ± 7[b]	20 ± 5	19 ± 4
Lipids				
Total cholesterol (mg/dl)	191 ± 33	194 ± 41	159 ± 48	163 ± 52
HDL cholesterol (mg/dl)	43 ± 7	45 ± 10	41 ± 7	40 ± 8
Low-density lipoprotein cholesterol (mg/dl)	105 ± 33	114 ± 34[b]	85 ± 30	100 ± 36[b]
Triglycerides (mg/dl)	263 ± 119	160 ± 52[b]	155 ± 52	149 ± 65[c]

[a] Data are given as mean values ± standard deviation.
[b] $p < .05$ versus baseline.
[c] $p < .05$ for changes between the groups from baseline.

changes in the metformin + glimepiride arm that did, however, not reach the level of statistical significance for changes between the groups from baseline except for E-selectin and hsCRP. In the metformin + pioglitazone arm, there was an improvement in triglyceride levels, and there were significant reductions from baseline in E-selectin, vWillebrand factor, and hsCRP concentrations. The other investigated parameters also showed tendencies indicating an improved platelet function in this group.

In contrast, metformin + glimepiride treatment resulted in a moderate decrease of glycoprotein IIb/IIIa complex and a constant level of the adhesion molecules and of vWillebrand factor and hsCRP. Also, the procoagulant factors thromboxane and sCD40L increased slightly but not significantly in the metformin + glimepiride therapy group. It is noteworthy that the body weight remained nearly constant in both treatment groups (change from baseline: metformin + pioglitazone, -0.24 ± 4.99 kg; metformin + glimepiride, + 0.10 ± 3.21 kg; not significant).

Figure 1 illustrates the relative changes of the biomarkers from baseline to endpoint by treatment arm. The asterisks mark the significant changes in the analysis in comparison to baseline. The courses of E-selectin ($p = .0138$) and hsCRP ($p = .0275$) differ significantly between the two investigated treatments.

Discussion

Detrimental alterations of the diabetic vascular state are characterized by a dysfunctional endothelium, an impaired coagulation cascade,[10,11] and activated and hyperreactive platelets.[10,12] This prothrombotic milieu is mainly triggered by a proinflammatory condition in diabetes patients.[1,13] Therefore, a successful diabetes therapy calls for not only a normalization of glycemic control, but also for additional antithrombotic and anti-inflammatory effects.

Sulfonylurea drugs are well established in reducing blood glucose levels to an acceptable range. However, this

type of drug is incapable of improving the vascular inflammation.[14–16] Our analysis confirms this observation. Glimepiride, even in combination with metformin, does not improve biomarkers of vascular inflammation or platelet function.

In contrast to glimepiride, pioglitazone has anti-inflammatory properties as demonstrated in several clinical studies.[14,17–20] These findings were confirmed in an observational trial investigating the efficacy and tolerability of a fixed pioglitazone plus metformin combination,[21] which was also applied in the pioglitazone arm of this actual study. Consistent with the previous results in diabetes patients and non-diabetic patients, platelet function was positively influenced by pioglitazone treatment in a study in non-diabetic patients with increased cardiovascular risk.[22] The biomarkers E-selectin and vWillebrand factor are essential parameters in the clotting reaction cascade. Both markers declined significantly with the fixed pioglitazone + metformin combination in comparison to the glimepiride + metformin treatment arm in this study. Also, hsCRP, an accepted biomarker for chronic systemic inflammation,[9] was improved with metformin + pioglitazone only, displaying the potential superiority of pioglitazone with respect to platelet function improvement and anti-inflammatory effects.

Platelet count may be reduced by pioglitazone[23] and might serve as a crude estimate for monitoring this pleiotropic therapeutic effect. In consequence, the platelet activation marker sCD40L release has been suggested as a potential target biomarker for successful treatment of cardiovascular disease.[24,25] Soluble CD40 ligand is considered to be dependent on thromboxane expression.[26] Accordingly, both biomarkers decreased in our study in response to pioglitazone. These data are in line with

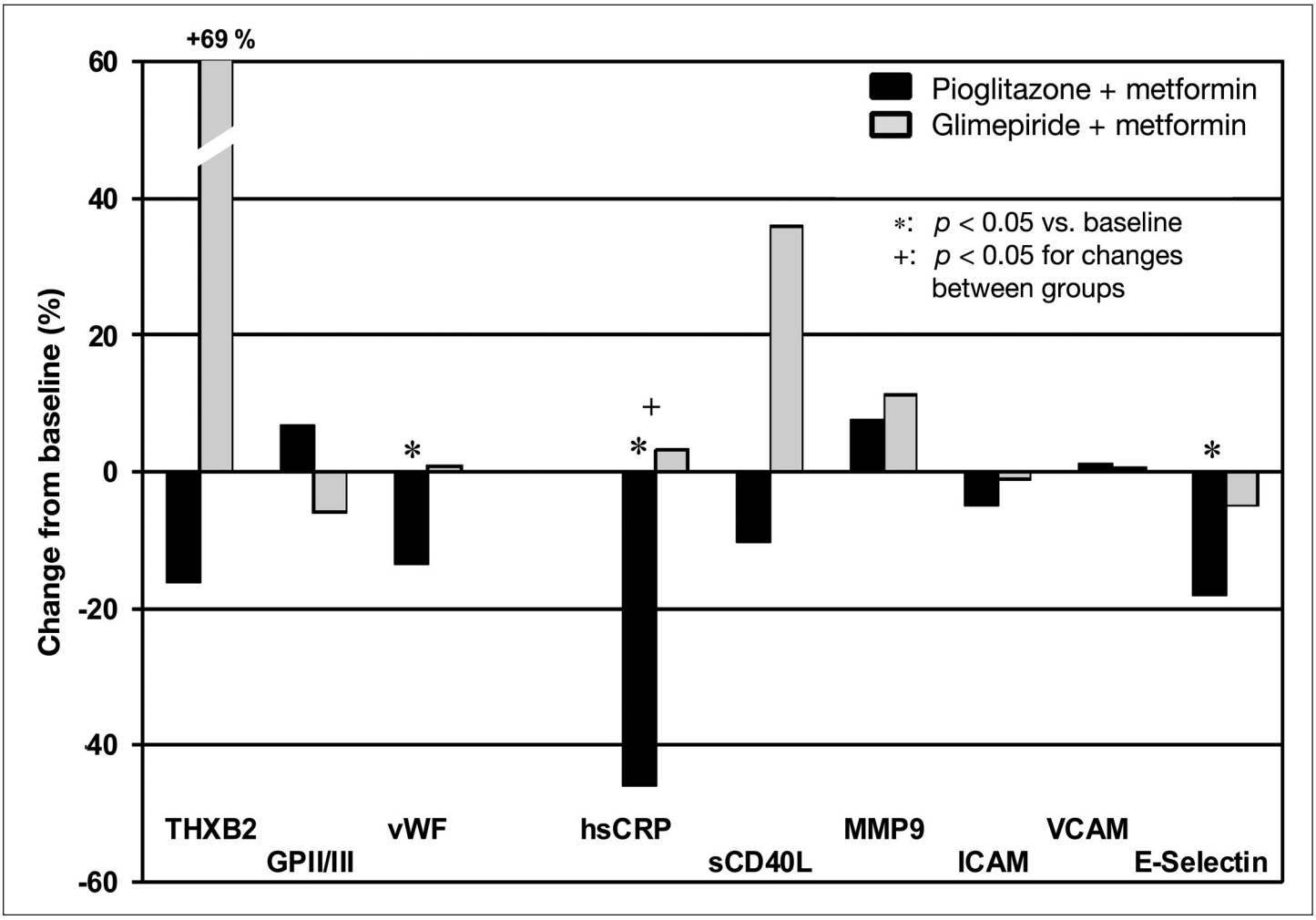

Figure 1. Individual relative changes of the investigated parameters by treatment arm from baseline to endpoint. The asterisks indicate statistically significant difference between the treatments. THXB2, thromboxane B2; GPII/III, glycoprotein complex inhibition; vWF, vWillebrand factor; MMP9, matrix metallopeptidase 9; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule.

the observation that improvement in insulin sensitivity significantly decreases thromboxane secretion in female patients with obesity.[27]

The positive effect of pioglitazone can be extended to the glitazone family. It was shown that rosiglitazone reduces E-selectin[28–30] and vWillebrand factor,[29] while similar effects could not be seen with glimepiride. Rosiglitazone treatment decreased sCD40L[30–32] and reduced platelet activity.[33] Additionally, leukocyte-platelet aggregates decreased significantly after therapy with rosiglitazone.[34]

A weakness of our actual analysis is its small population size. Many observations in this study failed to reach the level of statistical significance because of a large inter-individual variability. However, the direction of the observed changes with the fixed pioglitazone plus metformin combination, significant or not, are all in line with a coincident improvement of platelet function. This impact should be taken into account when considering an individual diabetes therapy with respect to cardiovascular risk, which has been shown for pioglitazone in several randomized clinical trials[35,36] as well as in the large outcome study PROactive.[37] In summary, pioglitazone plus metformin showed a beneficial impact on inflammation and platelet function in T2DM patients. It appears attractive to speculate that improved insulin sensitivity and improvement of endothelial function may be the basis for our findings.

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