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CLINICAL RESEARCH **Clinical Trial**

Improvement of Cardiovascular Risk Markers by Pioglitazone Is Independent From Glycemic Control

Results From the Pioneer Study

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OBJECTIVES This study was performed to assess whether the anti-inflammatory and antiatherogenic effects of pioglitazone suggested by animal experiments are reproducible in man and independent from improvements in metabolic control.

BACKGROUND Type 2 diabetes is associated with increased cardiovascular risk.

METHODS A total of 192 patients were enrolled into a six-month, prospective, open-label, controlled clinical study. They were randomized to receive either pioglitazone (45 mg) or glimepiride (1 to 6 mg, with the intent to optimize therapy). Biochemical and clinical markers to assess therapeutic effects included HbA1c, fasting glucose, insulin, adiponectin, lipids, high-sensitivity C-reactive protein (hsCRP), intracellular adhesion molecule, vascular cell adhesion molecule, vascular endothelial growth factor, fibrinogen, von Willebrand factor, matrix metalloproteinase (MMP)-9, monocyte chemoattractant protein (MCP)-1, soluble CD40 ligand, and carotid intima-media thickness (IMT).

RESULTS The study was completed by 173 patients (66 female, 107 male; age [± SD]: 63 ± 8 years; disease duration: 7.2 ± 7.2 years; HbA1c: 7.5 ± 0.9%; pioglitazone arm: 89 patients). A comparable reduction in HbA1c was seen in both groups ($p < 0.001$). In the pioglitazone group, reductions were observed for glucose ($p < 0.001$ vs. glimepiride group at end point), insulin ($p < 0.001$), low-density lipoprotein/high-density lipoprotein ratio ($p < 0.001$), hsCRP ($p < 0.05$), MMP-9 ($p < 0.05$), MCP-1 ($p < 0.05$), and carotid IMT ($p < 0.001$), and an increase was seen in high-density lipoprotein ($p < 0.001$) and adiponectin ($p < 0.001$). Spearman ranks analysis revealed only one correlation between the reduction in cardiovascular risk parameters and the improvement in the metabolic parameters (MMP-9 and fasting blood glucose, $p < 0.05$)

CONCLUSIONS This prospective study gives evidence of an anti-inflammatory and antiatherogenic effect of pioglitazone versus glimepiride. This effect is independent from blood glucose control and may be attributed to peroxisome proliferator-activated receptor gamma activation. (J Am Coll Cardiol 2005;45:1925–31) © 2005 by the American College of Cardiology Foundation

Patients with type 2 diabetes mellitus exhibit an increased propensity to develop extensive arteriosclerosis with its sequelae, unstable angina pectoris and acute myocardial infarction (1,2). Over the last years, experimental data have illuminated the role of inflammation in atherogenesis, while clinical studies have shown that this concept of inflammation in arteriosclerosis applies directly to human patients (3). As such, increased serum levels of inflammatory biomarkers of arteriosclerosis, like C-reactive protein, cytokines, like tumor necrosis factor-alpha or interleukin-6, as well as novel markers like monocyte chemoattractant protein (MCP)-1, soluble CD40 ligand (sCD40L), and matrix metalloproteinases (MMP) have been shown to predict cardiovascular risk and seem to reflect the overall burden of vascular disease in patients. Interestingly, some of these markers are elevated in patients with type 2 diabetes and insulin resistance, indicating a pivotal role of inflammation in this metabolic disorder (4–6). Recent data suggest that the release of inflammatory mediators like tumor necrosis factor-alpha and interleukin-6 from the visceral adipose tissue as well as an activation of vascular cells itself contribute to the inflammatory state in these patients with metabolic syndrome. Therefore, enhanced serum levels of sCD40L may reflect endothelial and platelet activation in diabetic subjects, while increased MMP-9 levels suggest the presence of unstable plaques with activated monocytes/macrophages (7–11). Moreover, elevated soluble adhesion molecules like soluble intracellular adhesion molecule (sICAM) and soluble vascular cell adhesion molecule (sVCAM) are markers of endothelial dysfunction in these patients. Given the increased risk of diabetic patients for macrovascular events, therapeutic strategies that limit inflammation in the vessel wall and reduce serum levels of inflammatory surrogate

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Abbreviations and Acronyms

HDL	= high-density lipoprotein
hsCRP	= high-sensitivity C-reactive protein
ICAM	= intracellular adhesion molecule
IMT	= intima-media thickness
LDL	= low-density lipoprotein
MCP	= monocyte chemoattractant protein
MMP	= matrix metalloproteinase
PPARγ	= peroxisome proliferator-activated receptor gamma
PROactive	= Prospective Pioglitazone Clinical Trial in Macrovascular Events
sCD40L	= soluble CD40 ligand
TZD	= thiazolidinedione
VCAM	= vascular cell adhesion molecule
VEGF	= vascular endothelial growth factor

parameters have been considered a promising tool to influence vascular disease in this high-risk population (12,13).

Recent experimental and clinical data suggest that a novel group of antidiabetic agents, thiazolidinediones (TZDs), like pioglitazone and rosiglitazone, may exhibit anti-inflammatory properties in the vessel wall (14,15). These agents act via the nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) and, in addition to their metabolic action, have been shown to regulate the expression of various target genes in vascular cells in vitro and in vivo, subsequently limiting inflammatory cell activation and lesion formation during atherogenesis. Furthermore, clinical data suggest that TZDs reduce inflammatory biomarkers of arteriosclerosis, like C-reactive protein or sCD40L, in treated patients, thus potentially modulating their cardiovascular risk (16–19). Still, most of these studies were placebo-controlled and, as such, did not allow the dissection of metabolic from nonmetabolic TZDs' effects, because TZD treatment, compared to placebo, significantly improved glucose metabolism in all of these studies. Therefore, we performed a six-month prospective, randomized, controlled trial to compare the effect of pioglitazone and sulfonylurea treatment on inflammatory biomarkers of arteriosclerosis, attempting to achieve comparable improvement in blood glucose control in both treatment groups.

METHODS

The prospective randomized monocentric study was approved by the responsible ethics committee and was performed in accordance with the Declaration of Helsinki and the guidelines for good clinical practice. All patient examinations were performed at the Clinical Department of the Institute for Clinical Research and Development (IKFE), Mainz, Germany. After written informed consent was obtained, 192 orally treated patients with type 2 diabetes without prior TZD treatment were enrolled into the trial. After randomization, they either received a fixed dose of pioglitazone (45 mg/day) in the morning or glimepiride (1 to 6 mg/day), titrated for optimal glycemic control. Inclusion criteria included an age of 40 to 75 years, HbA1c: 6.6% to 9.9%, absence of significant hepatic or renal disease, absence of congestive heart failure (New York Heart Association functional class II to IV), no cigarette smoking, and no known carotid artery disease. All study measurements were obtained at study entry and after 26 ± 2 weeks. In order to improve metabolic control, individual medical advice was given to every patient throughout the study. In the pioglitazone group, other additional oral antidiabetic therapy was permitted except for metformin, while only TZDs were excluded for additional treatment in the glimepiride group. All blood draws and measurements were performed in the morning after fasting of the patients from midnight onward.

Biochemical parameters. HbA1c was determined by means of high-pressure liquid chromatography (Adams TMA1c, Menarini Diagnostics, Florence, Italy). Therapy response was defined as an absolute decrease in HbA1c from baseline by at least 0.6% (normal reference range: 4.2% to 6.0%). Glucose was assessed using a standard glucose oxidase reference method (Ruhrtal Labortechnik, Mühnesee-Delecke, Germany), and lipids (total cholesterol, low-density lipoprotein [LDL], high-density lipoprotein [HDL], triglycerides) were measured by means of standard dry chemistry methods (OSR, Olympus Diagnostica, Hamburg, Germany). Insulin was measured using a chemiluminescence method (Sciema, Mainz, Germany), PAI-I by ELISA (American Diagnostica, Pfungstadt, Germany), endothelin by ELISA (Biomedica, Vienna, Austria), and adiponectin by radioimmunoassay (Linco, St. Charles, Missouri); ELISAs from R&D Systems (Wiesbaden, Germany) were used for the determination of the following parameters: ICAM, VCAM, vascular endothelial growth factor [VEGF], MMP-9, MCP-1. The sCD40L determinations were also performed by ELISA (Bender Medsystems, Vienna, Austria). Turbimetric methods were used for the following parameters: high-sensitivity C-reactive protein (hsCRP) (Olympus, Hamburg, Germany), fibrinogen (Dade Behring, Schwalbach, Germany), and von-Willebrand factor (Instrumentation Laboratory GmbH, Kirchheim, Germany).

High-sensitive C-reactive protein changes >15 mg/l during the observation period were attributed to other inflammatory processes (flu, cold, and so on) and were eliminated before analysis.

Carotid intima-media thickness (IMT). Carotid IMT was evaluated at all time points by a single operator with high-resolution B-mode ultrasound on a single machine (Caris Plus, Esaote SpA, Genoa, Italy) with a 10-MHz linear array transducer (LA 523). All recordings were performed in a standardized way, and readings were analyzed by a physician blinded to patient profile and treatment assignment as described previously (20,21).

Statistical analysis. The analysis of efficacy is based on the intention-to-treat population, which consists of all patients