Lymphocyte-suppressing, endothelial-protective and systemic anti-inflammatory effects of metformin in fenofibrate-treated patients with impaired glucose tolerance

Robert Krysiak, Anna Gdula-Dymek, Bogusław Okopień

Department of Internal Medicine and Clinical Pharmacology, Medical University of Silesia, Medyków 18, PL 40-752 Katowice, Poland

Correspondence: Robert Krysiak, e-mail: r.krysiak@interia.pl

Abstract:
Background: No previous clinical study has been designed to assess the additive effect of metformin and a fibrate on lymphocyte secretory function. The aim of our study was to investigate whether metformin produces any effect on lymphocyte cytokine release in fibrate-treated patients with early glucose metabolism abnormalities.

Methods: The study included 80 patients with isolated impaired glucose tolerance and normal plasma lipids who complied with lifestyle modifications and received chronic fenofibrate treatment. These subjects were randomly assigned to 90 days’ treatment with either high-dose metformin (3 g daily in three divided doses) or placebo. Plasma lipids, glucose homeostasis markers, plasma C-reactive protein and intercellular adhesion molecule-1 levels, as well as lymphocyte release of proinflammatory cytokines were determined before randomization and at the end of the treatment.

Results: Beyond improving glucose homeostasis, metformin reduced plasma C-reactive protein levels and lymphocyte release of tumor necrosis factor-α and interferon-γ, as well as tended to reduce interleukin-2 release and plasma intercellular adhesion molecule-1.

Conclusions: Our study shows that metformin potentiates lymphocyte-suppressing, endothelial-protective and systemic anti-inflammatory effects of fenofibrate, and suggests that patients with impaired glucose tolerance may benefit the most from the combined treatment with a fibrate and high-dose metformin.

Key words: fibrates, inflammatory cells, low-grade inflammation, metformin, prediabetes

Abbreviations: CRP – C-reactive protein, FFA - free fatty acids, HDL – high-density lipoprotein, HOMA-IR – the homeostatic model assessment of insulin resistance ratio, hsCRP – high sensitivity C-reactive protein, ICAM-1 – Inter-Cellular Adhesion Molecule-1, IGT – impaired glucose tolerance, LDL – low-density lipoprotein, TNF-α – tumor necrosis factor-α

Introduction

Apart from lowering lipid levels, peroxisome proliferator-activated receptor α (PPARα) activators (fibrates) were found to produce anti-inflammatory,
antioxidant and antithrombotic properties, to regulate the growth and migration of smooth muscle cells and to improve endothelial function [1, 11, 12]. We have recently observed that the strength of systemic anti-inflammatory and monocyte-suppressing effects of fenofibrate was more pronounced in patients with impaired glucose tolerance (IGT) than in subjects with impaired fasting glucose [4]. Interestingly, in patients with type 2 diabetes, fibrate-metformin combination was superior to fenofibrate alone in reducing plasma levels of C-reactive protein (CRP) and monocyte release of proinflammatory cytokines [9]. To the best of our knowledge no previous clinical study has investigated whether the additive effect of metformin and a fibrate is superior to fenofibrate alone in affecting the secretory functions of inflammatory cells other than monocytes/macrophages. Therefore, this study was designed to investigate whether metformin, administered in high doses, has an impact on lymphocyte-suppressing, endothelial-protective and systemic anti-inflammatory effects of fenofibrate in patients with IGT. Previously, we observed that lymphocyte-suppressing effects of the latter were potentiated by the concomitant use of atorvastatin [5].

Materials and Methods

Subjects

The patients were eligible to participate in the study if they (1) were between the ages of 20 and 70 years, (2) met the criteria of isolated IGT (fasting plasma glucose below 100 mg/dl plasma glucose concentration 2 h after a 75-g oral glucose load at least 140 mg/dl but below 200 mg/dl), (3) complied with lifestyle modifications and received 200 mg fenofibrate daily for at least 6 months preceding the study, (4) did not have any lipid abnormalities on the day of allocation (total cholesterol below 200 mg/dl, LDL cholesterol below 130 mg/dl and triglycerides below 150 mg/dl), (5) were medically stable, and, in the judgment of the investigators, (6) otherwise acceptable for entry on the basis of the findings of medical history, physical examination, and routine laboratory tests. The exclusion criteria were as follows: unstable coronary artery disease, myocardial infarction or stroke within 6 months preceding the study, symptomatic congestive heart failure, diabetes, elevated cholesterol and/or triglyceride levels, moderate or severe arterial hypertension (ESC/ESH grade 2 or 3), any acute and chronic inflammatory processes, autoimmune disorders, thyroid diseases, chronic pancreatitis, impaired renal or hepatic function, nephrotic syndrome, liver and biliary tract diseases, body mass index above 35 kg/m², treatment with any hypolipemic and/or oral antidiabetic drugs agents within 3 months prior to the study, concomitant treatment with other drugs known either to affect plasma glucose and/or lipid levels or to interact with fibrates or metformin and poor patient compliance. The Bioethical Committee of the Medical University of Silesia approved the study protocol. All enrolled patients (n = 80) provided their written informed consent for the investigation and the study was performed according to the Declaration of Helsinki. At the beginning of the study, fenofibrate-treated patients were compared with the control group including 25 age-, sex- and weight-matched normolipidemic subjects with isolated IGT who had been receiving metformin (3 g daily) and following lifestyle modification for more than 6 months but had not been treated with any hypolipidemic agent.

Study design

Fenofibrate-treated IGT patients were randomly assigned to one of the two treatments: metformin (3 g daily; n = 41) or placebo (n = 39). Both metformin and placebo were administered in constant doses three times a day for 90 days. Throughout the study, all included patients continued to follow the Therapeutic Lifestyle Changes diet and received 200 mg fenofibrate daily. Compliance assessment was performed during each visit by tablet counts.

Laboratory assays

Blood samples for laboratory assays were obtained at approximately 8:00 a.m. following at least a 12-h overnight fasting before randomization and after 90 days of treatment. To minimize analytical errors, all assays were carried out in duplicate. Routine chemical methods were used to determine plasma concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, fasting and 2-h post-glucose load plasma glucose and insulin (bioMerieux France;
Beckman, Palo Alto, CA; Linco Research Inc., St. Charles, MO, USA). Fasting plasma glucose and insulin levels were used to calculate the homeostatic model assessment of insulin resistance ratio (HOMA-IR) \[\text{fasting serum glucose (mg/dL)} \times \text{fasting insulin level (µU/ml)}/405\]. Total non-estrified free fatty acids (FFA) were measured by an enzymatic assay using reagents from Alpha Laboratories (Eastleigh, Hants, UK). Plasma levels of CRP were measured using a high-sensitivity monoclonal antibody assay (hsCRP) (MP Biomedicals, Orangeburg, NY, USA). Phytohemagglutinin-stimulated T cells were cultured in triplicate as previously described [8]. Tumor necrosis factor-α (TNF-α), interferon-γ and interleukin-2 release, and plasma soluble Inter-Cellular Adhesion Molecule-1 (ICAM-1) levels were measured with commercial enzyme-linked immunosorbent assay kits obtained from R&D Systems (McKinley Place N.E. Minneapolis, MN, USA) [6, 7]. The intra- and interassay coefficients of variation for all the assessed markers were below 4.9% and 8.8%, respectively.

**Statistical analysis**

Because of the skewed distributions, results for HOMA and cytokine release were natural-log transformed to satisfy assumptions of normality and equal variance. Comparisons between the groups were made by the t test for independent samples. The differences between baseline and post-treatment values within the same treatment group were compared with Student’s paired t-test. Kendall’s τ test was used to evaluate the relationship between metabolic variables and inflammatory mediators. The level of significance was set at p < 0.05.

**Results**

Both treatment groups and the control group were similar with respect to age, sex, weight, medical background and baseline laboratory results (Tab. 1). Compared to lymphocytes from the control group, lymphocytes from fenofibrate-treated patients produced smaller amounts of TNF-α, interferon-γ and interleukin-2 and had lower plasma levels of hsCRP and ICAM-1 (Tab. 1). Two patients withdrew from the study due to metformin-induced diarrhea and lack of appetite. No serious adverse events were observed throughout the study in the remaining patients who completed the study protocol. No changes in plasma lipids, glucose homeostasis markers, hsCRP, ICAM-1 and cytokine release were observed during the entire study period in fenofibrate-treated patients receiving placebo (Tab. 2). Metformin administered to fenofibrate-treated patients decreased HOMA-IR, FFA and 2-h post-glucose load plasma glucose levels, as well as tended to reduce plasma triglycerides (p = 0.072) and fasting glucose (p = 0.069). Ninety days of metformin treatment decreased plasma hsCRP levels and lymphocyte release of TNF-α and interferon-γ, as well as tended to reduce plasma ICAM-1 levels (p = 0.086) and interleukin-2 release (p = 0.076). Metformin was superior to placebo in reducing HOMA-IR, FFA, post-challenge glucose levels, hsCRP, ICAM-1 and lymphocyte release of TNF-α and interferon-γ.

On the day of randomization, plasma hsCRP levels correlated with lymphocyte cytokine release (r values between 0.48 and 0.61, p < 0.001), as well as with plasma soluble ICAM-1 (r = 0.49, p < 0.001). Moreover, plasma hsCRP, ICAM-1 and cytokine release correlated with HOMA-IR (r values between 0.51 and 0.65, p < 0.001) and FFA (r values between 0.48 and 0.57, p < 0.001). A reduction in hsCRP correlated with the effect of metformin on ICAM-1 (r = 0.43, p < 0.001) and cytokine release (r values between 0.46 and 0.56, p < 0.001). The effect of metformin on hsCRP, ICAM-1 and cytokine release correlated with the reduction in HOMA-IR (r values between 0.52 and 0.64, p < 0.001) and in FFA (r values between 0.38 and 0.47, p < 0.001). No other correlations were found in both baseline conditions and after treatment.

**Discussion**

The major finding of our study is that high-dose metformin enhances lymphocyte-suppressing, endothelial-protective and systemic anti-inflammatory effects of fenofibrate in IGT patients. Lymphocytes are crucial cells involved in the development and progression of atheroclerosis, present in large numbers in the atherosclerotic plaque [2, 16], while TNF-α, interferon-γ and interleukin-2 produce a multi-directional proatherogenic effect [13, 14]. Considering the role of lymphocytes and all the cytokines assessed in athero-
genesis, as well as the association between low-grade inflammation [10] or impaired endothelial function [15] and the risk of myocardial infarction, stroke, peripheral arterial disease and sudden cardiac death, the effect of metformin seems to be clinically relevant. In our study, pleiotropic effects of the combination therapy were moderate but it should be remembered that metformin was administered to patients chronically treated with micronized fenofibrate and complying with lifestyle modifications. The same dose of fenofibrate administered to patients with type 2 diabetes reduced plasma hCRP as well as lymphocyte and monocyte cytokine release [5]. Both these findings suggest that lymphocyte-suppressing, endothelial-protective and systemic anti-inflammatory effects of the combination therapy would be even more evident if the control group did not receive any hypolipidemic treatment. Considering this suggestion as well as favorable effects of metformin on glucose homeostasis markers in our study and on the incidence of type 2 diabetes in IGT patients [3], it seems that high-dose metformin may bring further clinical benefits for IGT patients, treated with a fibrate. Interestingly, although metformin potentiated fenofibrate action, the effect of the isolated metformin treatment on cytokine release, ICAM-1 and hsCRP was weaker than that of fenofibrate.

The analysis of correlations shows that the impact of the combination therapy on lymphocyte secretory function, endothelial function and systemic inflammation is likely to be a consequence of the improvement in insulin action. Moreover, metformin-induced reduction in low-grade inflammation results from the additive effects of metformin on lymphocytes and endothelium. This may explain why plasma hsCRP does not seem to reflect accurately local effects of hypolipidemic and antidiabetic drugs.
In conclusion, our study shows that metformin enhances the effect of fenofibrate on lymphocyte cytokine release, endothelial function and systemic inflammation, which is accompanied by the improvement in insulin sensitivity and a reduction in FFA. It seems that patients with IGT, particularly those at high cardiovascular risk, may benefit the most from fibrate-high-dose metformin combination therapy.

**Conflict of interest:**
The authors declare no conflict of interest.

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**References:**


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