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Short communication

Effect of metformin on selected parameters of hemostasis in fenofibrate-treated patients with impaired glucose tolerance

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Abstract:
Background: No previous study has assessed whether the addition of metformin potentiates fibrate action on hemostasis in prediabetic subjects.
Methods: Our study included 41 fenofibrate-treated patients with impaired glucose tolerance allocated to either metformin (3 g daily) or placebo.
Results: Twelve-week treatment with fenofibrate and metformin reduced plasma levels of fibrinogen and PAI-1 and tended to change the other hemostatic markers measured, as well as improved insulin sensitivity.
Conclusions: Our results show that high-dose metformin exhibits beneficial effects on coagulation and fibrinolysis in isolated IGT patients treated with a fibrate.

Key words:
metformin, fibrates, prediabetes, hemostasis, risk factors

Abbreviations: FFA – free fatty acids, HDL – high-density lipoprotein, IGT – impaired glucose tolerance, INR – international normalized ratio, LDL – low-density lipoprotein, PAI-1 – plasminogen activator inhibitor-1, vWF – von Willebrand factor

Introduction

In our previous studies, fenofibrate was found to produce multidirectional pleiotropic effects on coagulation and fibrinolysis in patients with mixed dyslipidemia [12], isolated hypertriglyceridemia [7], impaired glucose tolerance (IGT) [13] and type 2 diabetes [15]. In patients with type 2 diabetes, hemostatic effects of fenofibrate were particularly pronounced in the group simultaneously treated with metformin [15]. It is well documented that the latter agent administered to prediabetic subjects reduces the incidence of overt diabetes [6]. In type 2 diabetic patients, metformin treatment was associated with a reduction in cardiovascular risk [11], which is increased already in prediabetic patients (especially in subjects with IGT) [14]. Numerous studies have demonstrated that disturbances of coagulation and fibrinolysis contribute to the development and progression of atherosclerosis and that they affect the incidence of atherosclerosis-related clinical events [4, 17]. To the best of our knowledge no previous study has assessed whether the addition

of metformin to a fibrate increases the strength of hemostatic effects of the latter. Therefore, the aim of this single-blinded study was to investigate whether high-dose metformin (3 g daily) potentiates the anticoagulant effects of fenofibrate. Factor VII coagulant activity and plasma levels of fibrinogen, von Willebrand factor (vWF) and plasminogen activator inhibitor-1 (PAI-1), measured in our study, are considered cardiovascular risk factors and their increased levels/activities are associated with the earlier development and accelerated progression of atherosclerosis-related disorders [4, 5, 16].

Materials and Methods

To be included into the study, the participants had to meet the following inclusion criteria of isolated IGT: (1) fasting plasma glucose less than 100 mg/dl, and (2) plasma glucose concentration 2 h after a 75-g oral glucose load at least 140 mg/dl but less than 200 mg/dl. We included only patients with normal lipid profile, defined as plasma total cholesterol below 200 mg/dl, plasma LDL cholesterol below 130 mg/dl and plasma triglycerides below 150 mg/dl, complying with lifestyle interventions and treated with a constant dose of micronized fenofibrate (200 mg daily) for at least 6 months because of previously diagnosed mixed dyslipidemia or isolated hypertriglyceridemia. We excluded subjects with any acute and chronic inflammatory processes, grade 2 or 3 arterial hypertension, unstable coronary artery disease, myocardial infarction or stroke within 6 months preceding the study, symptomatic congestive heart failure, diabetes, impaired fasting glucose, autoimmune disorders, thyroid diseases, chronic pancreatitis, impaired renal or hepatic function, nephrotic syndrome, liver and biliary tract diseases and with body mass index above 35 kg/m^2, as well as treated with other hypolipidemic and/or oral antidiabetic drugs within 3 months before the study. The study complied with the principles of the Declaration of Helsinki and its protocol was approved by the Bioethical Committee of the Silesian University School of Medicine. All patients (n = 41) gave their written informed consent for the investigation. They were then randomized to receive metformin (3 g daily, n = 20) or placebo (n = 21). Both metformin and placebo were administered three times a day, whereas micronized fenofibrate once daily (at the same dose as previously) for 12 weeks without any changes in dosage during the entire study period. Patients who were already taking other drugs kept their pharmacologic schedule constant during the study. Compliance was determined twice monthly by the number of tablets returned.

Venous blood was collected at baseline and at the end of the treatment period. Samples were taken 12 h after the last meal, in constant daily hours (between 8.00 and 9.00 a.m.) to avoid circadian fluctuations of the parameters studied, and immediately coded so that the person performing laboratory assay was blinded to subject identity and study sequence. Plasma lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides), fasting and 2-h postchallenge plasma glucose, and plasma insulin were assayed by routine laboratory techniques (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 – insulin resistance (HOMA-IR) index [fasting serum glucose (mg/dl) × fasting insulin level (mU/ml)/405]. Total non-estrified free fatty acids (FFA) were determined by an enzymatic assay using reagents from Alpha Laboratories (Eastleigh, Hants, UK). The international normalized ratio (INR), the partial thromboplastin time, fibrinogen, factor VII coagulant activity, plasminogen activator inhibitor-1 (PAI-1) and von Willebrand factor (vWF) were assessed as previously described [7–10].

The obtained results were analyzed using the Kolmogorov-Smirnov test for data normality. Because of skewed distributions, values for HOMA-IR and the hemostatic variables were natural-log transformed to satisfy assumptions of normality and equal variance. Comparisons between the groups were performed using unpaired Student's *t*-test. The differences between the means of variables within the same treatment group were analyzed with Student's paired *t*-test. Kendall's tau test was used to evaluate the relationship between metabolic and hemostatic variables. Statistical significance was assumed at $p < 0.05$.

Results

Both groups were comparable in respect to demographics, medical background, clinical characteristics safety measurements and pharmacotherapy (Tab. 1).

Tab. 1. Baseline characteristics of patients[1]

	Fenofibrate + placebo	Fenofibrate + metformin
Number of patients	21	19
Age [years; mean (SD)]	52 (5)	50 (5)
Women [%]	43	47
Body mass index [kg/m^2; mean (SD)]	27.8 (2.4)	28.2 (2.8)
Waist circumference [cm; mean (SD)]	101 (6)	100 (7)
Smokers [%]	24	21
Coronary artery disease [%]	19	21
Mild hypertension [%]	24	26
Systolic blood pressure [mmHg; mean (SD)]	130 (7)	131 (8)
Diastolic blood pressure [mmHg; mean (SD)]	85 (4)	86 (4)
Plasma creatinine [mg/dl mean (SD)]	0.73 (0.08)	0.71 (0.07)
Creatinine clearance [ml/min mean (SD)]	114 (12)	120 (11)
Medications		
β_1-Adrenergic blockers	19	21
Angiotensin-converting enzyme inhibitors	10	11
Angiotensin II receptor blockers	5	10
Long-acting nitrates	10	5
Long-acting calcium channel blockers	10	11
Aspirin	14	16
Thienopyridines	55	5
Fasting glucose [mg/dl; mean (SD)]	92 (4)	91 (5)
2-h postchallenge plasma glucose [mg/dl; mean (SD)]	170 (13)	164 (16)
HOMA-IR [mean (SD)]	3.0 (0.5)	2.8 (0.5)
Free fatty acids [mmol/l; mean (SD)]	341 (43)	356 (49)
Total cholesterol [mg/dl; mean (SD)]	166 (14)	170 (15)
LDL cholesterol [mg/dl; mean (SD)]	101 (9)	104 (8)
HDL cholesterol [mg/dl; mean (SD)]	47 (5)	48 (4)
Triglycerides [mg/dl; mean (SD)]	121 (12)	123 (11)
INR	0.93 (0.06)	0.92 (0.07)
Partial thromboplastin time [s]	29.3 (1.8)	29.7 (1.9)
Fibrinogen [g/l]	3.7 (0.4)	3.6 (0.4)
Factor VII activity [%]	119.3 (14.2)	124.4 (16.0)
von Willebrand factor [IU/dl]	127.5 (15.3)	124.1 (11.4)
PAI-1 [ng/ml]	65.2 (8.9)	64.1 (10.5)

[1] Only data of 40 individuals who completed the study were included in the final analyses

One patient was withdrawn from the study because of metformin-induced nausea and diarrhea. Neither significant adverse effects nor any complications were reported throughout the entire study period in the remaining participants. Post-treatment blood pressure and renal function did not differ from pretreatment values (fenofibrate + placebo: systolic blood pressure: 132 ± 8 mmHg *vs.* 130 ± 7 mmHg, diastolic blood pressure: 86 ± 5 mmHg *vs.* 85 ± 4 mmHg, plasma creatinine: 0.75 ± 0.07 mg/dl *vs.* 0.73 ± 0.08 mg/dl, creati-

Tab. 2. The effect of metformin on glucose homeostasis markers, free fatty acids and hemostasis in fenofibrate-treated patients with isolated impaired glucose tolerance[1]

	Fenofibrate + placebo Mean (SD) [Δ%]	Fenofibrate + metformin Mean (SD) [Δ%]
Fasting glucose [mg/dl]		
Before randomization	92 (4)	91 (5)
At the end of the study	92 (5) [0]	87 (4) [–4]
2-h postchallenge plasma glucose [mg/dl]		
Before randomization	170 (13)	164 (16)
At the end of the study	173 (11) [2]	142 (12) [–13]** ###
HOMA-IR		
Before randomization	3.0 (0.5)	2.8 (0.5)
At the end of the study	3.2 (0.6) [7]	1.4 (0.4) [–50]*** ###
Free fatty acids [mmol/l]		
Before randomization	341 (43)	356 (49)
At the end of the study	328 (39) [–4]	251 (37) [–29]*** ##
INR		
Before randomization	0.93 (0.06)	0.92 (0.07)
At the end of the study	0.91 (0.08) [–2]	1.03 (0.08) [12]
Partial thromboplastin time [s]		
Before randomization	29.3 (1.8)	29.7 (1.9)
At the end of the study	29.8 (2.0) [2]	32.9 (1.9) [11]
Fibrinogen [g/l]		
Before randomization	3.7 (0.4)	3.6 (0.4)
At the end of the study	3.8 (0.4) [3]	2.9 (0.3) [–20]* ###
Factor VII activity [%]		
Before randomization	119.3 (14.2)	124.4 (16.0)
At the end of the study	125.0 (17.1) [5]	101.0 (10.7) [–19]
von Willebrand factor [IU/dl]		
Before randomization	127.5 (15.3)	124.1 (11.4)
At the end of the study	122.3 (14.4) [–4]	103.0 (13.1) [–17]
PAI-1 [ng/ml]		
Before randomization	65.2 (8.9)	64.1 (10.5)
At the end of the study	62.5 (9.2) [–4]	43.7 (7.8) [–32]*** ###

* $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$ *vs.* placebo-treated patients; ## $p < 0.01$, ### $p < 0.001$ *vs.* respective value before randomization. [1] Only data of 40 individuals who completed the study were included in the final analyses

nine clearance: 110 ± 12 ml/min *vs.* 114 ± 12 ml/min; fenofibrate + metformin: systolic blood pressure: 128 ± 7 mmHg *vs.* 131 ± 8 mmHg, diastolic blood pressure: 84 ± 5 mmHg *vs.* 86 ± 4 mmHg, plasma creatinine: 0.74 ± 0.08 mg/dl *vs.* 0.71 ± 0.07 mg/dl, creatinine clearance: 115 ± 10 ml/min *vs.* 120 ± 11 ml/min).

Placebo treatment administered for 12 weeks produced no effect on plasma lipids, glucose, HOMA-IR, FFA and the hemostatic variables. Metformin treatment of isolated IGT reduced 2-h postchallenge plasma glucose HOMA-IR and FFA. It also reduced plasma levels of fibrinogen and PAI-1 (Tab. 2). Moreover, metformin tended to increase INR ($p = 0.088$), to prolong the partial thromboplastin time ($p = 0.082$) and to reduce vWF ($p = 0.062$) and factor VII activity ($p = 0.058$). Fenofibrate + metformin were superior to metformin + placebo in reducing fibrinogen, PAI-1 and vWF.

At baseline, there were correlations between the assessed markers of hemostasis and HOMA-IR (r values between 0.48 and 0.60, $p < 0.001$) and FFA (r values between 0.40 and 0.53, $p < 0.001$). The effect of metformin on INR, the partial thromboplastin time, fibrinogen, PAI-1, factor VII and vWF correlated with its action on HOMA-IR (r values between 0.52 and 0.59, $p < 0.001$) and FFA (r values between 0.43 and 0.51, $p < 0.001$). No other correlations were found.

Discussion

Our study shows that metformin administered in high doses to fibrate-treated patients with IGT produce favorable effects on both glucose homeostasis and coagulation and fibrinolysis.

Epidemiological studies indicate that subjects with increased plasma levels of fibrinogen and PAI-1 are at a higher risk for coronary artery disease, acute coronary events, post-coronary angioplasty restenosis, pulmonary embolism, carotid stenosis and diseases of peripheral blood vessels [2, 3]. Because fibrinogen and PAI-1 exert multidirectional proatherogenic actions [4, 17], metformin-induced decrease in both these factors suggests that metformin may reduce the incidence of atherosclerosis and its complications in IGT patients.

Metformin treatment insignificantly affected the activated partial thromboplastin time, which quantifies the function of the intrinsic system, and INR, which in clinical practice measures the activity of the extrinsic pathway of coagulation [1]. This finding, indicating that metformin-fenofibrate combination treatment is superior to fenofibrate in the global effect on hemostasis, is in agreement with the results of our previous study, which included type 2 diabetic patients [15]. Considering that metformin strengthened the effect of fenofibrate on postchallenge plasma glucose, HOMA-IR, and FFA, fibrate-metformin combination seems to be an interesting treatment for patients with only slightly disturbed glucose homeostasis.

In our study, the effect on hemostasis was moderate and statistically significant changes were observed only for fibrinogen and PAI-1. It should, however, be underlined that the effect of the combination therapy was compared with the impact of long-term treatment with fenofibrate, which in IGT patients exerted a strong and multidirectional effect on coagulation and fibrinolysis [13]. Considering the role of all hemostatic variables measured as important risk factors for vascular diseases [4, 5, 16], the addition of high-dose metformin to fenofibrate may bring further clinical benefits for IGT subjects.

The occurrence of correlations between the hemostatic variables and glucose homeostasis markers indicates that abnormal hemostasis in IGT patients results in part from impaired insulin action and that hemostatic effects of metformin-fenofibrate combination treatment are a consequence of the improvement in insulin sensitivity.

Our study has some limitations. It did not investigate clinical outcomes, including morbidity or mortality, and the study population was relatively small. Therefore, our study can be considered only as a pilot study, the results of which should be confirmed in larger trials. Moreover, it should be underlined that metformin is not as yet registered for patients with pre-diabetes and treatment of IGT subjects with this agent is its off-label use. Finally, we included only patients with IGT and therefore, the study protocol does not allow us to conclude whether similar effects of metformin are observed in fibrate-treated patients with impaired fasting glucose.

In conclusion, our study shows that metformin partially normalizes IGT-induced disturbances in hemostasis in subjects treated with a fibrate. This suggests the existence of clinical benefits resulting from combination therapy with high-dose metformin and a fibrate in this group of patients.

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

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