



Extralipid effects of micronized fenofibrate in dyslipidemic patients

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Abstract:

The aim of this study was to evaluate the levels of lipid and extralipid parameters in patients with atherogenic dyslipidemia. We investigated the lipid-lowering therapeutic efficacy of fenofibrate and its extralipid influence on oxidized low-density lipoprotein (oxLDL), C-reactive protein (CRP), Fibrinogen, factor VII and plasminogen activator type 1 (PAI-1) during 1-month treatment. Fourteen individuals with HLPiIb were treated with micronized fenofibrate (267 mg/d) for 1 month. The control group included twelve volunteers. Lipidograms were determined with enzymatic kits. ELISA method was used to measure oxLDL and PAI-1. Plasma CRP levels were measured spectrophotometrically. Fibrinogen and factor VII serum levels were evaluated with automatic coagulometer.

After 1-month therapy with micronized fenofibrate, we observed a significant reduction of total cholesterol (TC) (277.2 to 217.8 mg/dl, $p < 0.05$), LDL (183.6 to 129.4 mg/dl, $p < 0.05$), triglyceride (TG) (316.7 to 220.6 mg/dl, $p < 0.05$), oxLDL (68.7 ± 5.5 to 39.7 ± 3.7 U/l, $p < 0.001$) and increase in high-density lipoprotein (HDL) (35.1 to 41.9 mg/dl, $p < 0.05$). Fibrate treatment also decreased CRP (5.81 ± 0.26 to 5.08 ± 0.06 mg/l, $p < 0.001$), PAI-1 (120.4 ± 9.7 to 84.7 ± 5.9 ng/ml; $p < 0.05$), fibrinogen (3.65 ± 0.17 to 3.44 ± 0.16 g/l, ns) and factor VII ($159.7\% \pm 56.7$ to $141\% \pm 42.4$, ns).

The micronized fenofibrate at a daily dose of 267 mg demonstrated a highly beneficial effect on all lipid parameters and advantageous influence on inflammatory and thrombogenic plasma risk factors in patients with dyslipidemia HLPiIb.

Key words:

hyperlipoproteinemia, fenofibrate, oxLDL, CRP, PAI-1

Abbreviations: PAI-1 – plasminogen activator inhibitor type 1, CRP – C-reactive protein, oxLDL – oxidized low density lipoprotein

Introduction

Coronary heart disease (CHD) continues to be the predominant cause of mortality among adults in de-

veloped countries. Although an increased low-density lipoprotein (LDL) serum level remains the primary target of CHD therapy, decreased high-density lipoprotein (HDL) and an elevated triglycerides (TG) are now recognized to be independent risk factors [1]. Atherogenic dyslipidemia is also characterized by a different LDL modifications, mainly oxidation. Recent data attribute also a fundamental role to a local inflammatory processes in the initiation, development and evolution of atherosclerotic lesions [15].

Atherothrombosis often occurs in subjects without overt hyperlipidemia. Among the biological markers, growing evidence suggests the leading role for C-reactive protein (CRP). CRP is a non-specific but sensitive acute-phase reactant that has been shown in multiple prospective epidemiological studies to be associated with an increased risk of myocardial infarction, stroke, sudden cardiac death and peripheral arterial disease [16]. Elevated CRP serum level seems to be at least equally strong acute coronary syndrome (ACS) predictor as LDL concentration and, furthermore, it tends to identify different high-risk groups [17].

In the natural history of atherosclerosis, the physiologically anticoagulant endothelium is induced by different forms of injury to have procoagulant properties [18]. In addition, an impaired balance of blood coagulation and fibrinolysis may predispose susceptible individuals to thrombosis and the development of ACS [24]. Major serum markers of coagulation include plasma fibrinogen levels, identified as an important risk factor for cardiovascular diseases [8] and factor VII. On the other hand, the reduced endogenous fibrinolysis, secondary to an increased plasminogen activator inhibitor type 1 (PAI-1) activity, may play a role in the early progression of atherosclerotic lesions [19]. The finding of immunocytochemical localization of PAI-1 in human endothelial cells (EC) and vascular smooth muscle cells (VSMC) suggests an action of the peptide not only in the vessel lumen, but also on the vascular wall [4].

The above-mentioned disorders, leading to premature atherosclerosis and heart disease, coexist and characterize individuals with type IIb mixed hyperlipoproteinemia (HLP IIb according to the Fredrickson classification). Fenofibrate, as a fibric acid derivative, exerts its hypolipidemic effect *via* the activation of the peroxisome proliferator-activated receptor (PPARs), which acts as a transcription factor and regulates a number of genes involved in metabolism [3]. This study aimed to evaluate serum levels of the following risk factors: oxLDL, CRP, fibrinogen, factor VII and PAI-1, and to investigate the ability of micronized fenofibrate (LIPANTHYL) to affect the above-mentioned atherogenic and thrombogenic plasma risk factors.

Materials and Methods

The study was carried out on 26 subjects (15 women and 11 men, aged from 42 to 64 years) subdivided

into two groups. The study group included 14 patients with the biochemical diagnosis of combined hyperlipoproteinemia-HLP IIb who did not respond to a 3 month-low-fat diet and antioxidant (vitamins C and E) treatment alone. Additionally, their anamnesis revealed familial history of hyperlipidemia among parents or siblings. The control group included 12 individuals matched for age and sex with biochemical confirmation of normolipemia.

The inclusion criteria for HLP IIb were as follows: total cholesterol (TC) concentration higher than 200 mg/dl, LDL level higher than 135 mg/dl and the level of TG higher than 200 mg/dl.

The main exclusion criterion in the treated group was the secondary type of HLP IIb (adiposity or overweight regarded as body mass index (BMI) > 27, hypothyreosis, nephrotic syndrome, diabetes mellitus, liver diseases, alcoholism) or other type of dyslipidemia (chylomicronemia or dysbetalipoproteinemia in the forefront). Any other health disorders interfering with serum lipid profile or oxLDL, CRP, fibrinogen, PAI-1 serum levels were excluded: any inflammatory diseases, decompensated circulatory failure (NYHA III/IV), unstable angina, myocardial infarction or revascularization intervention within 6 months prior to the beginning of the study, hemodynamically significant cardiac defects, heart rhythm and conduction system disorders, arterial hypertension II and III, history of apoplectic stroke episode or transient ischemic attack (TIA), taking drugs interfering with the studied substances' serum levels or affecting lipid metabolism (eg. hypolipemic drugs, niacin, non-selective β -blockers) within 3 months before starting the study. Patients treated with anticoagulants were also excluded. Apart from the patient's history and physical examination, the following tests were performed: total blood cell count, red blood cell count, leucocyte differential count, erythrocyte sedimentation rate, glycosylated hemoglobin level, liver enzyme test, plasma protein and lipoprotein electrophoresis, urine analysis, abdomen ultrasonography, electrocardiography, fasting glucose serum level and specialist consultations if needed. The additional tests were performed if required: creatinine, aminotransferase, bilirubin, platelet count, hematocrit, white blood cell count or pregnancy test. The above-mentioned procedures were conducted to accurately enroll patients to the study.

HLP IIb patients were treated with micronized fenofibrate (Lipanthyl) 267 mg per day along with a low-fat diet and antioxidants for 1 month. No seri-

ous side effects or episodes of intolerance were observed during fibrate therapy. The low-fat diet followed the dietary recommendations of the European Atherosclerosis Society for 3 months (National Education Cholesterol Program I – total fat < 30%, saturated fat < 10% of consumed calories, cholesterol < 300 mg per day).

Lipidograms including TC, HDL, LDL and TG were determined with bioMerieux enzymatic kits (5 ml blood samples) 16 h after the last meal. For oxLDL, PAI-1 and CRP assays, blood samples (2 ml) were collected into heparinized tubes. ELISA method was used to measure oxLDL (Oxidized LDL, Mercodia, Sweden) and PAI-1 (Asserachrom, Diagnostica Stago, France). Plasma CRP levels were measured spectrophotometrically using specific BioSystems kits (Spain).

For fibrinogen and factor VII assays 5 ml of blood was collected into tubes with sodium citrate and automatic coagulometer (OPTION 2 PLUS, bioMerieux, France) was used.

All values are expressed as the mean \pm standard error (AVG \pm SE). A statistical analysis was performed using Mann-Whitney test. A $p < 0.05$ was considered to indicate a significant difference. Correlation analysis was performed with a Spearman test. All procedures were carried out with the GraphPad Prism 2.01 software (GPA-26576-117).

The study was approved by the Ethics Committee of the Medical University of Silesia. All the study participants signed an informed consent form.

Results

Lipid parameters

In the HLPiIb patients lipid parameters: TC, LDL, oxLDL and TG were markedly increased and HDL was significantly diminished comparing with healthy individuals. After 1-month fenofibrate treatment, the following values of the parameters under study were observed: significant decrease in TC (-21.4% ; 277.2 ± 12.3 to 217.8 ± 8.4 mg/dl; $p < 0.05$), LDL (-29.5% ; 183.6 ± 12.7 to 129.4 ± 6.5 mg/dl; $p < 0.05$), TG (-30.3% ; 316.7 ± 27.7 to 220.6 ± 21.8 mg/dl; $p < 0.05$) and marked increase in HDL (19.4% ; 35.1 ± 3.7 to 41.9 ± 3.2 mg/dl, $p < 0.05$) (Tab. 1). Moreover, the

Tab. 1. Lipid parameters in healthy individuals and dyslipidemia IIb patients before and after treatment

	CONTROLS	DYSLIPIDEMIA IIb	1-month TREATMENT
TCh (mg/dl)	187.2 \pm 26.56	277.2 \pm 46.02	217.8 \pm 31.42; $p < 0.05$
LDL (mg/dl)	130.2 \pm 32.55	183.6 \pm 47.51	129.4 \pm 24.32; $p < 0.05$
HDL (mg/dl)	52 \pm 12.34	35.1 \pm 13.84	41.9 \pm 11.97; $p < 0.05$
TG (mg/dl)	135.7 \pm 57.24	316.7 \pm 103.64	220.6 \pm 81.56; $p < 0.05$

plasma oxLDL was normalized to the level comparable with the value obtained in the control group (Fig. 1). There was no correlation between LDL and oxLDL reduction.

C-reactive protein

The mean levels of CRP in dyslipidemic group were markedly raised (on the average, by 24% higher) compared to the control group (5.81 ± 0.25 mg/l vs. 4.68 ± 0.17 mg/l; $p < 0.001$). Fenofibrate significantly decreased CRP levels in the treated patients by 12.6% (5.81 ± 0.26 to 5.08 ± 0.06 mg/l, $p < 0.001$, Tab. 2).

Fibrinogen and factor VII

The mean levels of circulating both fibrinogen and factor VII were higher in HLPiIb patients than in healthy individuals, by 5.9% (3.6 ± 0.17 g/l vs. 3.4 ± 0.14 g/l, ns) and 17.5% ($159.7 \pm 10.15\%$ vs. $135.9 \pm 15.53\%$, ns), respectively. One month later, in HLPiIb patients the decrease in circulating fibrinogen by 6% (3.65 ± 0.17 to 3.44 ± 0.16 g/l, ns) as well as factor VII by 11.7% ($159.7\% \pm 56.7$ to $141\% \pm 42.4$, ns) were observed (Tab. 2).

Plasminogen Activator Inhibitor type 1

Finally, considerably elevated plasma PAI-1 levels in HLPiIb patients were diminished by a monthly treatment, with micronized fenofibrate by nearly 30% (120.4 ± 9.7 to 84.7 ± 5.9 ng/ml, $p < 0.001$) (Fig. 2) (Tab. 2). Further analysis revealed a significant correlation between the reduced PAI-1 concentration and the decrease in lipid parameters: TC ($r = 0.59$; $p < 0.05$) and TG ($r = 0.72$; $p < 0.01$) (Fig. 3).

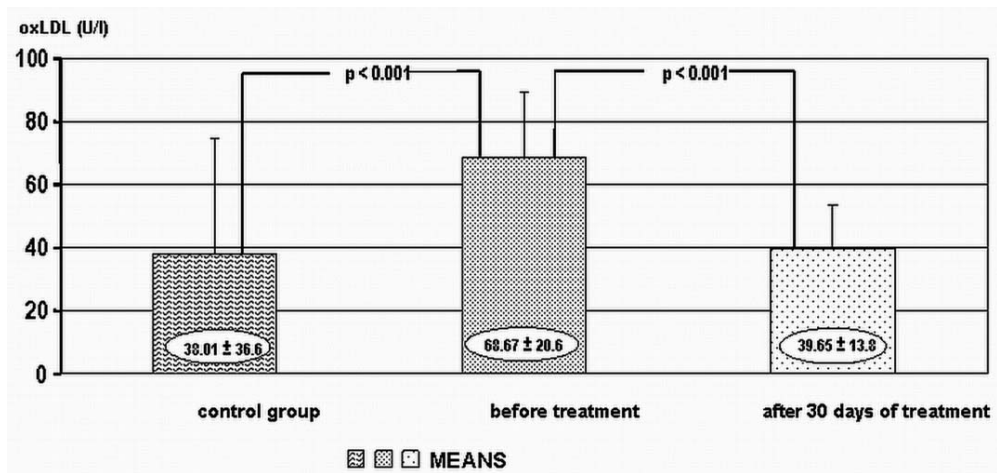


Fig. 1. Plasma levels of oxLDL in HLP1Ib patients before and after 1 month of hypolipidemic therapy (values are means \pm SD)

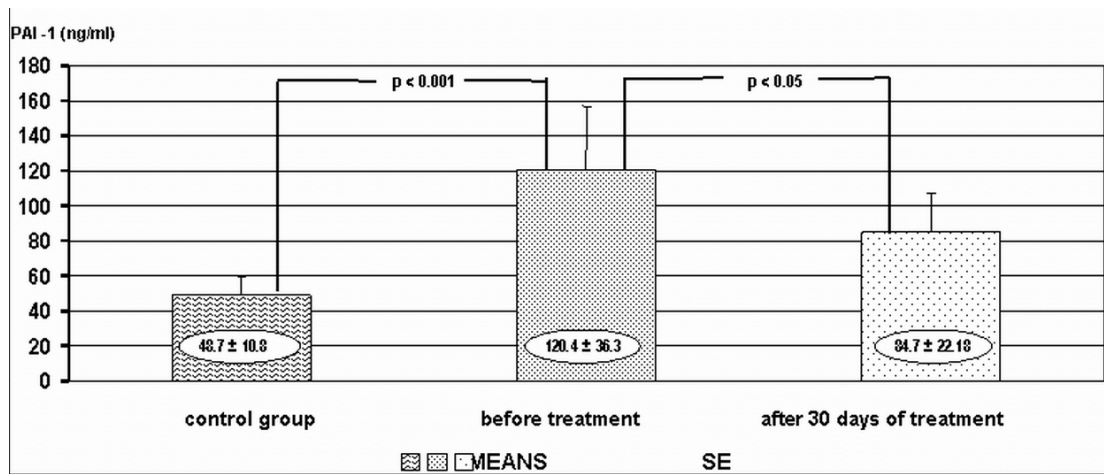


Fig. 2. Plasma levels of PAI-1 in HLP1Ib patients before and after 1 month of hypolipidemic therapy (values are means \pm SD)

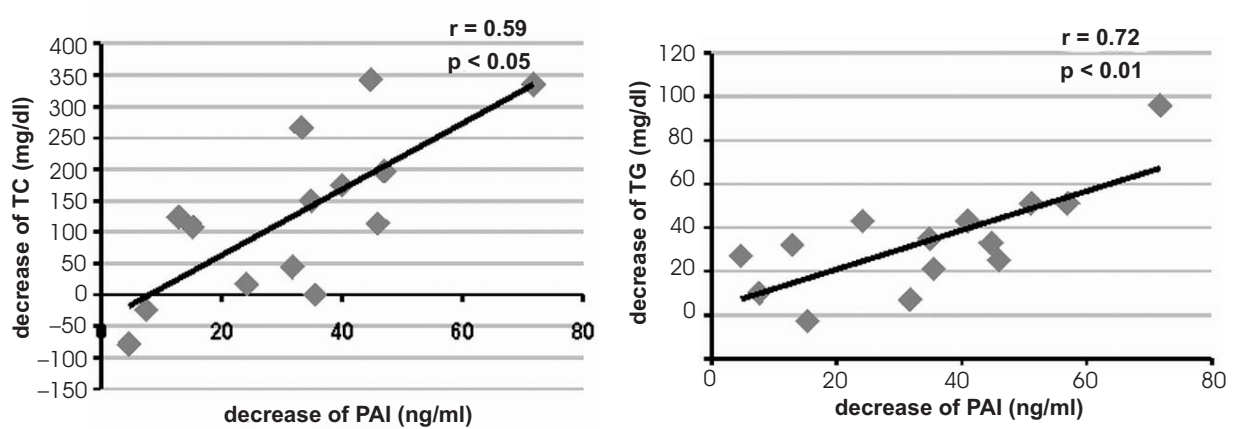


Fig. 3. Correlation between PAI-1 concentration and lipid parameters: TC ($r = 0.59$, $p < 0.05$) and TG ($r = 0.72$, $p < 0.01$) in HLP1Ib patients after 1-month fenofibrate therapy

Tab. 2. Measured parameters in healthy individuals and dyslipidemia IIb patients before and after treatment

	CONTROLS	DYSLIPIDEMIA IIb	1-month TREATMENT
oxLDL (U/l)	38.01 ± 36.66	68.67 ± 20.65	39.65 ± 13.8; p < 0.001
CRP (mg/l)	4.68 ± 0.63	5.81 ± 0.93	5.08 ± 0.22; p < 0.001
Fibrinogen (g/l)	3.4 ± 0.52	3.65 ± 0.64	3.44 ± 0.58; ns
factor VII (%)	135.9 ± 58.1	159.7% ± 98.23	141% ± 73.29; ns
PAI-1 (ng/ml)	48.67 ± 10.85	120.4 ± 36.3	84.7 ± 22.08; p < 0.001

Discussion

In our study, patients with atherogenic dyslipidemia IIb (total cholesterol > 200 mg/dl, LDL cholesterol > 135 mg/dl and TG > 200 mg/dl) after four weeks' administration of 267 mg fenofibrate daily had the following lipid parameters diminished: total cholesterol by 21.4%, LDL cholesterol by 29.5%, triglycerides by 30.3% and their HDL cholesterol increased by 19.4%. Furthermore, an improvement in decreasing other risk factors was observed. The applied fibrate treatment led to reduction in plasma levels of CRP by 12.4%, PAI-1 by 29.6%, fibrinogen by 6% and factor VII by 11.7%. These data suggests a favorable influence of fenofibrate on commonly known atherosclerotic risk factors. Although recent hyperlipidemia panel treatments [2] indicate the use of fenofibrate mainly for improvement in TG and HDL levels, it should be stated that the moderate reduction of TC and LDL were also observed in the presented study. Similar observations were made in other studies based on micronized fenofibrate [9, 10]. Additionally, micronized fenofibrate proved to be generally well tolerated and no serious adverse events were noted.

Oxidized LDL, inevitably accompanying a long-lasting hyperlipidemia, is a major cause of EC and VSMC dysfunction [12]. It is also chemotactic for atherosclerosis key cells – monocytes and may help expand the local inflammatory response [18]. In addition, oxLDL activates expression of macrophages' tissue factor (TF) and stimulates PAI-1 production, which inhibits fibrinolysis. The final result promotes hypercoagulability. This multidirectional activity constitutes a "vicious circle" and promotes atherosclerosis. Therefore, substantial oxLDL level reduction by over 40% in our study is of clear importance. Lack of correlation between oxLDL diminution and LDL de-

crease indicates the pleiotropic activity of micronized fenofibrate.

In clinical application, CRP has currently emerged as one of the strongest predictors of cardiovascular events, even more powerful than LDL. In addition, CRP and LDL may be useful in prediction and identification of different risk groups [16]. The mechanisms of this strong association remain unclear, but it seems that CRP is also a direct participant in the atherogenesis [26]. Although there is currently no certain evidence that lowering CRP serum level will definitively reduce ACS rates, major prospective and randomized trials involving fibrate therapy [25] suggest that clinical outcomes predominant over benefits from lipids normalization. It was observed that hyperlipidemic patients benefited from fenofibrate therapy by an average of 12.6%, whereas greater falls after fenofibrate treatment by 28% [21, 22] or even 50% [11] were observed.

Plasma fibrinogen level is a well-evidenced independent risk factor for cardiovascular disease [8]. Several studies have established fenofibrate efficiency in reducing circulating fibrinogen in hyperlipidemic patients by 7–23% [7]. Steinmetz et al. noted a more pronounced decrease in mixed dyslipidemia than in hypercholesterolemia [23].

Factor VII, the second hemostatic factor measured in HLP IIb individuals, revealed a moderate fall. Idzior-Walus et al. observed 18% reduction of factor VII activity [6].

PAI-1 elevation augments risk of atherosclerosis [5]. PAI-1 has been regarded to affect VSMC migration or proliferation, to predispose to formation of an abnormal fibrin gel structure [4] and to potentiate formation of plaques with lipid-laden cores and thin fibrous caps [20]. Four-week fenofibrate treatment markedly diminished its serum level. However, as VLDL may induce secretion of PAI-1 from cultured

endothelial cells, the strong correlation with TG fall raises the question of exact mechanisms underlying this effect. On the other hand, *in vitro* studies have shown direct influence of fibrates on PAI-1 transcription [13]. Comparable results of nearly 20% decline were obtained in our previous study [14]. The presented study confirmed that fenofibrate has a potent influence not only on a well-recognized atherosclerotic factors: dyslipidemia and coagulation-fibrinolysis disturbances, but also on the recently established inflammatory response elements. Only large-scale, prospective and randomized clinical trials with micronized fenofibrate (FIELD, ACCORD) shall clearly reassess if the multidirectional effects significantly occur in a large population.

The conclusion is that patients with mixed hyperlipidemia exhibit abnormalities as well as increased plasma inflammatory marker concentrations and impaired coagulation-fibrinolysis balance. Therapy with micronized fenofibrate improved not only the lipid parameters but also the above-mentioned atherogenic disturbances.

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