The effect of micronised fenofibrate on paraoxonase activity in patients with coronary heart disease

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SUMMARY

Objective: To evaluate the effect of micronised fenofibrate on serum paraoxonase (PON) and lipoprotein levels in coronary heart disease patients with type IIb hyperlipidaemia.

Patients and methods: Fifty-two patients were investigated for the three-month effect of 200 mg per day micronised fenofibrate on the serum enzyme activity and concentration of PON and their relationship with serum lipids, high-density lipoprotein (HDL-C) parameters.

Results: Serum paraoxonase activity was lower in CHD patients with type IIb hyperlipoproteinemia. During the three-month study it was observed that following treatment with micronised fenofibrate, serum triglyceride and cholesterol levels decreased, while HDL-C increased significantly (p < 0.001). Low-density lipoprotein (p < 0.05) and apolipoprotein B-100 (p < 0.01) decreased, while HDL constituent apolipoprotein A-I (p < 0.05) increased after micronised fenofibrate treatment. The HDL-associated paraoxonase specific activity increased significantly (p < 0.05). To assess whether the increased PON activity was due to elevated HDL and apoA-I level, we standardized PON activity for HDL and apoA-I concentrations. The standardized values for HDL (PON/HDL) increased (p < 0.05) while the PON/apoA-I ratio did not change significantly.

Conclusion: Three months of treatment with micronised fenofibrate is thought to normalize lipid profile and improve antioxidant status by increasing serum paraoxonase activity in these patients.

Key-words: Paraoxonase · Micronised Fenofibrate · Hyperlipidemia · Coronary Heart Disease.

RÉSUMÉ

Effet du fénofibrate micronisé sur l’activité paraoxonase chez des patients coronariens

Objectif : Évaluer l’effet du fénofibrate micronisé sur la paraoxonase sèrique (PON) et sur les niveaux de lipoprotéines chez des patients coronariens atteints d’hyperlipidémie de type IIb.

Patients et méthodes : L’étude a porté sur 52 patients traités pendant 3 mois par 200 mg par jour de fénofibrate micronisé, avec mesure de l’activité enzymatique sérique, des concentrations de PON et de leur relation avec les lipides circulants et les paramètres du cholestérol-HDL.

Résultats : L’activité paraoxonase sérique est plus basse chez les patients coronariens avec hyperlipidémie de type IIb. Pendant les 3 mois de l’étude, sous traitement par fénofibrate micronisé, les taux sériques de triglycérides et de cholestérol ont diminué, et ceux de HDL-C ont augmenté significativement (p < 0.001), ceux de LDL-C (p < 0.05) et d’apolipoprotéine B-100 (p < 0.01) ont diminué, tandis que l’apolipoprotéine A-I constitutive des HDL (p < 0.05) a augmenté. L’activité paraoxonase spécifique associée aux HDL a augmenté significativement (p < 0.05). Pour évaluer si l’augmentation d’activité PON était due à l’augmentation du taux de HDL et d’apoA-I, nous avons standardisé l’activité PON en fonction des concentrations de HDL et d’apoA-I. Les valeurs standardisées pour les HDL (PON/HDL) ont augmenté (p < 0.05) tandis que le ratio PON/apoA-I n’a pas significativement changé.

Conclusion : Trois mois de traitement par fénofibrate micronisé semblent normaliser le profil lipidique et améliorer le statut antioxydant en augmentant l’activité paraoxonase sèrique chez ces patients.

Mots-clés : Paraoxonase · Fénofibrate micronisé · Hyperlipidémie · Coronaropathie.

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Previous epidemiological studies have proved that individuals with elevated plasma cholesterol level (mainly elevated LDL level) have increased risk for cardiovascular disease [1-3]. The connection between triglyceride concentration in serum and coronary events has been debated for a long time. Recently, many studies have discussed the role of elevated triglyceride level in the atherosclerotic process [4-6]. Fibrates with marked triglyceride reducing effect are widely used in type IIb hyperlipidemia. The beneficial effect of second generation fibrates on cholesterol level is smaller when compared to third generation fibrates [7, 8]. Decreased HDL level is also an important factor in the development of atherosclerosis [4, 9]. HDL is able to intervene at different stages of atherosclerosis, such as removal of excess cholesterol by reverse cholesterol transport, direct protective effect on the endothelium and prevention of LDL oxidative modification by an HDL associated antioxidant system [10]. Oxidized LDL plays an important role in the initial stage of atherosclerosis. Oxidatively modified circulating LDL is taken up by scavenger receptors of macrophages migrating in the subepithelial space. This pathway does not activate the receptor-linked regulating mechanism, which leads to an increased uptake of cholesterol turning the macrophages into foam cells [11, 12]. In the Helsinki Heart Study, the role of fibrates in elevation of HDL level was found to be beneficial in reducing the incidence of ischaemic heart disease [13]. In previous studies using second generation fibrates, we found that in addition to more favourable lipid profile, these drugs have advantageous effect on HDL-associated, antioxidant paraoxonase activity [14]. The goal of the present study was to investigate changes in lipid profile and HDL-associated paraoxonase activity in type IIb hyperlipidemic patients with definitive coronary heart disease, using a third generation fibrate: micronised fenofibrate.

Methods

Patients

Fifty-two type IIb hyperlipidemic patients with coronary heart disease were enrolled in the study. We classified hyperlipidemic phenotypes by plasma total cholesterol (TC) and triglyceride (TG) concentrations (type IIa, TC > 5.7 mmol/L and TGs < 1.7 mmol/L; type IIb, TC > 5.7 mmol/L and TGs > 1.7 mmol/L; type IV, TC < 5.7 mmol/L and TGs > 1.7 mmol/L). All of them had one or more of the following conditions in their past medical histories: documented acute myocardial infarction, coronary artery stenosis diagnosed by angiography, stable angina pectoris, positive ECG and thallium scintigraphy findings. Between the last cardiac event and the beginning of this study, 6-24 months had passed. Triglyceride levels were between 2.3 and 4.6 mmol/L. According to WHO criteria of 1999, 8 patients had IFG; based on our standard (75 g glucose) OGTT, no patient had diabetes or IGT. After six weeks on step 1 diet of the National Cholesterol Education Program (NCEP), patients received 1 × 200 mg micronised fenofibrate daily (1 tablet Lipidil® Fournier). Physical examination, BMI, ECG and laboratory tests were performed at the beginning of the study and repeated after 3 months of drug treatment. Patients with liver or kidney disease, alcoholism, drug dependence, gallstones, malignancy, pregnancy or lactation, anticoagulant or statin therapy were excluded from the study.

Blood Sampling

After overnight fasting a 5 ml blood sample was drawn. Haemoglobin, haematocrit, white blood cell count, liver enzymes, BUN, creatinine, CK, fibrinogen, CRP, bilirubin, uric acid, serum glucose, cholesterol, HDL-C, triglyceride, Apo A1 and Apo B100, lipoprotein (a) and serum paraoxonase activity levels were determined. The lipid parameters were measured from fresh serum. Sera for paraoxonase activity tests were stored at –20 °C before analysis.

Lipid measurements

Serum cholesterol and triglyceride were assayed using a Boehringer Mannheim GmbH Diagnostic enzyme kit, while HDL cholesterol was investigated by the phosphotungstic-magnesium precipitation method. The LDL cholesterol fraction was calculated indirectly using the Friedewald equation. Apolipoprotein measurement was performed by an immuno-nephelometric assay.

Analysis of paraoxonase activity

Paraoxonase activity was determined using paraaxon (O, O-diethyl-O-p-nitrophenylphosphate(Sigma Chemical Co.) as substrate by measuring the increase in absorbance at 412 nm due to formation of 4-nitrophenol. Activity was measured by adding 50 µl serum to 1 ml Tris/HCl buffer (100 mmol/l, pH = 8.0) containing 2 mmol/l CaCl₂ and 5.5 mmol/l paraaxon. The rate of generation of 4-nitrophenol was determined at 412 nm, 25 °C, using a Hewlett-Packard 8453 UV-visible spectrophotometer. Enzyme activity was calculated from the molar extinction coefficient 17100 M⁻¹ cm⁻¹. One unit of paraoxonase activity is defined as 1 nmol of 4-nitrophenol formed per minute under the above described assay conditions [15].

Arylesterase assay

Arylesterase activity was measured spectrophotometrically. The assay contained 1 mM phenylacetate in 20 mM Tris/HCl pH = 8.0. The reaction was started by the addition of serum and the increase in absorbance was read at 270 nm as already described [16, 17]. Blanks were included to correct for spontaneous hydrolysis of phenylacetic. Enzyme activity was calculated using a molar extinction coefficient of
phenotype distribution did not differ significantly before activity was investigated by determining phenotypes. The distribution of paraoxonase activity (454 ± 142 U/L; p = 0.48) did not change significantly. The distribution of paraoxonase activity were logarithmically transformed to give a gaussian distribution before analysis. Spearman correlation coefficients were used to test the strength of correlation between variables.

Statistical analysis

Statistical analysis was performed by the SAS for Windows 6.12 computer program. Data were presented by descriptive analysis (mean ± SD). For comparison between baseline and posttreatment values, paired t test was used for normally distributed variables. Values for triglycerides and paraoxonase activity were logarithmically transformed to give a gaussian distribution before analysis. Spearman correlation coefficients were used to test the strength of correlation between variables.

Results

Lipid parameters of patients before and after 3 months of therapy with 200 mg of micronised fenofibrate once a day are shown in Table I. Serum triglyceride (p < 0.001), cholesterol (p < 0.001) and LDL-C (p < 0.05) levels were significantly reduced while HDL level was significantly increased (p < 0.001). The main apolipoprotein of HDL, apoA-I was significantly increased (p < 0.05), while apoB100 — main apolipoprotein of LDL-C — was significantly reduced (p < 0.01) by micronised fenofibrate therapy (Tab I). Fasting blood glucose was 4.97 ± 1.71 mmol/L. HDL-associated paraoxonase activity significantly increased (p < 0.05) (Tab II), while the concentration of the enzyme did not change significantly. Paraoxonase specific activity was significantly increased during treatment with micronised fenofibrate (p < 0.05). Arylesterase activity (103 ± 30 U/L vs. 142 ± 28 U/L; p < 0.01) significantly increased, while salt stimulated paraoxonase activity (454 ± 225 U/L vs. 457 ± 166 U/L; p = 0.48) did not change significantly. The distribution of activity was investigated by determining phenotypes. The phenotype distribution did not differ significantly before and after micronised fenofibrate treatment (Tab II). Elevated PON activity could be the result of significantly increased HDL level, thus the PON/HDL ratio was determined: This activity was significantly elevated after therapy (Fig 1). Apolipoprotein A-I is an important component of HDL for activation of PON, thus PON/apoA-I ratio was also determined. There was no significant elevation in this ratio (Fig 1). To answer the question of whether the increase in PON activity levels correlated with the increase in serum HDL-C and apoA-I concentrations, the respective differences of PON activity, HDL-C, apoA-I values before and after therapy were calculated (deltaPON, deltaHDL, deltaapoA-I). A slight, positive correlation (r = 0.34, p < 0.05) was observed between deltaPON activity and deltaapoA-I concentration (data not shown).

Discussion

Effect of fibrates on lipids and lipoproteins is accomplished via triglyceride and cholesterol rich particles since fibrates are able to decrease free fatty acid level — the substrate of triglyceride formation — and enhance the metabolism of triglyceride rich lipoproteins [19]. Blane et al. found that fenofibrate produced a 40-60% reduction of triglyceride level in type IIb hyperlipidemia [20]. In this study we found a 43% reduction. The same authors found a 10-30% reduction in cholesterol level after 300 mg of fenofibrate administration, while Kornitzer et al. showed a 20.5% reduction in cholesterol level using 200 mg of fenofibrate daily [21]. In our study we found a 13% reduction in cholesterol and LDL cholesterol levels. Recent studies showed that this effect manifested via receptor PPARα, resulting in increased lipoprotein lipase activity and HDL level and reduced triglyceride level [22]. Different derivatives of fibrates have different
effects. In type IIb hyperlipidemia apolipoprotein A-I and A-II level reduction and HDL level reduction have been described. Reduced HDL formation and enhanced HDL degradation can explain these abnormalities [23]. Earlier studies verified that fibrates could increase the level of apolipoprotein A-I [24, 25]. Compared to other lipid parameters, HDL showed the strongest association with ischaemic heart disease, it was a three times more predictive indicator of acute myocardial infarct than other lipid parameters [26]. It is well known that the incidence of cardiovascular diseases is 2-4 times lower in premenopausal females. The higher level of HDL in this group could explain these data. Assmann et al. in the PROCAM study found that 20,000 patients with CHD had HDL levels under 0.95 mmol/l, in other words, a lower HDL cholesterol level predicts a higher risk of coronary heart disease [4]. Kornitzer et al. proved that micronised fenofibrate is more effective in patients with low HDL concentration compared to patients with normal HDL parameters, a 37.9% vs. 15.2% increase in HDL levels was found [21]. In the present study, the increase of the HDL level was 22%. This increase was higher than 15% described by Brown et al. [27]. The preventive role is due to the antioxidant effect of HDL. Mainly paraoxonase, an HDL-associated enzyme is responsible for the inhibition of oxidative processes [28]. The PON gene is localized on the 7. chromosome. Previous studies showed that the population can be subdivided into three phenotypic groups: AA represents low; AB intermediate; and BB high enzyme activity [29]. The genetic polymorphism at codon 192 and 55 is responsible for the changes in enzyme activity [30]. Recent studies found that in diabetes mellitus, chronic renal failure, renal transplantation and familial hypercholesterinemia, the activity of the enzyme is reduced [31-33]. Some studies investigated the effect of lipid lowering drugs on enzyme activity [14]. Previously we found that gemfibrozil was able to increase the activity of paraoxonase in hyperlipidemia [14]. Durrington et al. proved that bezafibrate and gemfibrozil therapy did not influence the activity of paraoxonase in type IIb hyperlipidaemic patients [34]. The more favourable effect of third generation fibrates on lipids compared to gemfibrozil is well known, therefore, we were interested in whether they change the activity of the HDL-associated antioxidant enzyme, paraoxonase. We found that the lipid lowering effect and the effect on paraoxonase activity were

<table>
<thead>
<tr>
<th>Phenotype distribution (%)</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
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<tbody>
<tr>
<td>AA</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>AB</td>
<td>39</td>
<td>41</td>
</tr>
<tr>
<td>BB</td>
<td>9</td>
<td>11</td>
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Table II

Paraoxonase activity, concentration and phenotype distribution before and after micronized fenofibrate treatment.

<table>
<thead>
<tr>
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<th>PON activity (U/L)</th>
<th>PON concentration (µg/ml)</th>
<th>PON specific activity (U/mg)</th>
<th>Phenotype distribution (%)</th>
</tr>
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<tbody>
<tr>
<td>Before treatment</td>
<td>189 ± 104</td>
<td>48.8 ± 8.6</td>
<td>3.87 ± 0.44</td>
<td>AA 52, AB 39, BB 9</td>
</tr>
<tr>
<td>After treatment</td>
<td>260 ± 64*</td>
<td>45 ± 9.4</td>
<td>5.77 ± 0.41*</td>
<td>AA 48, AB 41, BB 11</td>
</tr>
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* p < 0.05, values are mean ± SD.

Figure 1

PON/HDL and PON/ApoA1 ratio changes before and after micronised fenofibrate treatment * p < 0.05.
more favourable. The effect of fibrates on lipids is manifested via receptor PPARδ activation resulting in increased lipoprotein lipase, and HDL level and reduced triglyceride level. Similarly to lipoprotein lipase and apoA-I, there may be a special proliferation response element of PON, responsible for increased enzyme production on the transcripational level secondary to treatment. To examine this theory, we measured the quantity of PON before and after treatment. There was no significant change in serum PON concentration after therapy, suggesting that other mechanisms cause the increase in PON activity. Another possibility is the effect of fenofibrate on lipid metabolism (apoA-I, lipoprotein lipase), which contributes to increase in enzyme activity via frbrate-induced structural changes in HDL.

Conclusion

We conclude that micronised fenofibrate is able to normalize lipid profile in our patients and may reduce the incidence of cardiovascular diseases via the LDL oxidation inhibitor effect.

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References


