Clinical Research

Comparison of the Effects of Atorvastatin and Fenofibrate on Apolipoprotein B-Containing Lipoprotein Subfractions in Patients with Combined Dyslipidemia

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Introduction: It has been shown that the profile of LDL subfractions may represent an independent risk factor for the development of cardiovascular disease. Although the impact of hypolipidemic drugs on the conventional lipid risk factors has been extensively studied, their effects on the concentration and relative distribution of lipoprotein subfractions remain ill defined. We undertook the present study to evaluate and compare the effects of atorvastatin and fenofibrate on apolipoprotein B-containing lipoprotein subfractions.

Methods: Forty-four patients with combined dyslipidemia were included. At the end of the dietary lead-in period patients were randomised to receive either atorvastatin (20 mg, n=21) or fenofibrate (200 mg, n=23). Serum lipids and lipoproteins and the concentrations of individual lipoprotein subfractions were determined at baseline as well as after 16 weeks of active treatment.

Results: Both drugs sufficiently reduced the concentrations of total and LDL cholesterol and triglycerides. In addition, fenofibrate increased the values of HDL cholesterol. Atorvastatin and fenofibrate significantly reduced the concentrations of VLDL+IDL as well as of total LDL. These reductions in total LDL mass were due to the reductions in the masses of all individual LDL subspecies. When the changes in the concentrations of LDL subfractions were compared with analysis of covariance taking into account baseline values as a covariate, no significant differences were found between the two hypolipidemic drugs.

Conclusions: Our results support the assumption that adequate doses of atorvastatin have the same effect as fenofibrate on LDL subfraction metabolism, and thus raise important questions concerning the need for combination therapy in patients with mixed dyslipidemia.

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Cardiovascular disease is the leading cause of death in developed countries.[1,2] Despite the recent advances in the identification of high risk patients, a substantial number of ischemic events occur in individuals lacking the classic cardiovascular risk factors. Hence, it is obvious that additional, so far unknown, parameters may play significant roles in the pathophysiology of the atherosclerotic process. In this context, it has been proposed that the LDL subfraction profile may contribute to the determination of total cardiovascular risk. Indeed, experimental studies have shown that small, dense LDL particles are more atherogenic than large, buoyant ones, since they easily penetrate to the arterial intima,[3] have higher affinity for intima proteoglycans[4] and exhibit higher oxidability[5] as well as a lower affinity for LDL receptors.[6] These observations were confirmed in clinical studies, which have shown that patients with pattern B subfraction pro-

file (predominance of small, dense LDL particles) display a significantly higher incidence of ischemic events as compared to patients with pattern A profile (predominance of large, buoyant LDL particles).[7-9] Although the regulation of LDL particle distribution is a complicated process, large scale epidemiological studies have shown that triglyceride values represent the most important single determinant of the LDL subfraction profile.[10,11] Thus, individuals with triglycerides above 120 mg/dl usually display pattern B subfraction profile, whereas subjects with triglycerides below this value usually exhibit pattern A profile.[11] Consequently, interventions that modify the size of the pool of triglyceride-rich lipoproteins can be expected to have significant effects on the distribution of LDL particles.

Large scale clinical studies have shown that HMG-CoA reductase inhibitors (statins) as well as fibric acid derivatives (fibrates) substantially reduce the incidence and the severity of cardiovascular disease.[12,13] However, this reduction is inadequately explained by the modification of classic cardiovascular risk factors.[12.13] Consequently, the evaluation of the impact of hypolipidemic drugs on the distribution of LDL subfractions is of special relevance, since it may give additional information about the mechanisms that underlie the antiatherogenic properties of statins and fibrates. The aim of our study was to evaluate and compare the impact of fenofibrate and atorvastatin on the concentration and relative distribution of LDL subfractions in patients with combined dyslipidemia, i.e. patients that usually exhibit a predominance of the atherogenic small, dense LDL subfractions.

Materials and methods

Patients

Forty-four unrelated patients were included in the study. These patients represented the type IIB dyslipidemia groups of previous studies that tested the effects of hypolipidemic drugs on Platelet Activating Factor-Acetylhydrolase activity.[14,15] None of the study participants were hypertensive, diabetic, or had any clinical or laboratory evidence of coronary artery disease. Patients were excluded if they had a secondary form of dyslipidemia (defined by history, physical examination and appropriate laboratory tests) or if they were taking medications known to affect the metabolism of lipoproteins (including hormonal replacement therapy). All patients were instructed to follow a National Cholesterol Education Program step 1 diet for at least 3 months and then a fasting baseline laboratory analysis was performed. Eligible patients (those with LDL-cholesterol values greater than 160 mg/dl and triglyceride values greater than 200 mg/dl) were then divided into two groups: the atorvastatin group consisted of 21 patients and the fenofibrate group consisted of 23 patients. Both drugs were given in a single daily dose (20 mg for atorvastatin and 200 mg for fenofibrate) at bedtime for 16 weeks. At this time, a second blood analysis was performed. Compliance with treatment was assessed as previously described.[14] All study participants gave their signed informed consent for participation in the study. The study protocol was approved by the Scientific Committee of the University Hospital of Ioannina.

Subfractionation of plasma lipoproteins

Lipoproteins were fractionated by isopycnic density gradient ultracentrifugation, as previously described.[14,15] After the centrifugation of total plasma 30 fractions of 0.4 ml each were collected. Equal volumes of gradient fractions 1 to 12 were pooled to constitute the following apolipoprotein B-containing subfractions: fractions 1 and 2 (VLDL+IDL, d< 1019 g/ml); 3 and 4 (LDL-1, d = 1019-1023 g/ml); 5 and 6 (LDL-2, d = 1023-1029 g/ml); 7 and 8 (LDL-3, d = 1029-1039 g/ml); 9 and 10 (LDL-4, d = 1039-1050 g/ml); 11 and 12 (LDL-5, d = 1050-1063 g/ml).

Analytical methods

Serum total cholesterol, triglycerides, HDL-cholesterol, as well as the concentrations of apolipoproteins B and AI were determined, as previously described.[14,15] Serum LDL-cholesterol was calculated using the Friedewald formula, provided that triglyceride values were lower than 400 mg/dl. In patients with triglyceride concentrations greater than 400 mg/dl LDL-cholesterol values were not determined. The total cholesterol, triglyceride and phospholipid content of each LDL subfraction were measured enzymatically using the BioMerieux kits, while the protein content of each subfraction was determined with the bicinchronic acid method (Pierce). The lipoprotein mass of each subfraction was calculated as the sum of the masses of the individual lipid and protein contents.

Table 1. Characteristics of the study population.

	ATORVASTATIN	FENOFIBRATE
Number	21	23
Sex (male/female)	10 / 11	14 / 9
Age (years)	52.1 ± 8.2	51.5 ± 9.3
Body mass index (Kg/m^2)	26.6 ± 3.2	26.6 ± 2.8
Smokers/non-smokers	12 / 9	10 / 13

Statistical analysis

Data were expressed as mean ± standard deviation. Comparisons between baseline and post-treatment values were performed using Student's paired t-test, whereas comparisons between patients and controls were performed by one-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA).

Results

Effect of hypolipidemic drugs on lipids and lipoproteins

The clinical characteristics of the study population are shown in table 1. There were no significant differences in the age, body mass index, proportion of smokers and sex distribution between study groups. There were no significant differences concerning the values of total cholesterol, triglycerides, or the concentrations of apolipoproteins AI and B between study groups before treatment (Table 2). However, baseline LDL-cholesterol values of the atorvastatin group patients were significantly higher than those of the fenofibrate group (Table 2). Both drugs induced decreases in the concentrations of total and LDL-cholesterol, triglycerides and apolipoprotein B. In addition, fenofibrate induced a significant increase in HDL-cholesterol values as well as in the concentration of apolipoprotein AI, a finding not observed in patients receiving atorvastatin. Comparisons of the proportional changes in lipid parameters by ANCOVA, taking into account baseline values as a covariate, revealed that atorvastatin was more efficient than fenofibrate in reducing total and LDL-cholesterol as well as apolipoprotein B levels. In contrast, fenofibrate-induced changes in HDL-cholesterol and apolipoprotein AI were greater than those observed under atorvastatin treatment. Finally, it must be noted that changes in triglyceride values were similar in the two patient groups.

Effect on apolipoprotein B-containing lipoprotein subfractions

Fenofibrate group patients exhibited higher values of VLDL+IDL as compared to atorvastatin group patients, although these differences were not significant (Table 3). Dense LDL subfractions were the predominant subfractions in both patient groups. Patients in the atorvastatin group displayed significantly higher total LDL mass as compared to fenofibrate group patients. This difference was due to differences in the masses of all individual LDL subfractions (Table 3). Both drugs substantially reduced the concentration of VLDL+IDL subfractions. In addition, fenofibrate and atorvastatin both induced significant reductions in total LDL mass, a phenomenon that was due to the decrease in the concentrations of all LDL subfractions. When the reductions in the masses of LDL subfractions were compared, atorvastatin was found to be more efficient than

Table 2. Effect of fenofibrate and atorvastatin on lipids and lipoproteins (in mg/dl).

	ATORVASTATIN			FENOFIBRATE		
	Before treatment	**After treatment**	**% change**	**Before treatment**	**After treatment**	**% change**
Total cholesterol	318 ± 45	220 ± 43§§	- 30.4	294 ± 32	237 ± 34§§	- 19.5*
Triglycerides	288 ± 83	182 ± 73§§	- 35	313 ± 93	177 ± 70§§	- 43
HDL-cholesterol	44 ± 10	47 ± 16	+ 7	39 ± 5	45 ± 8§	+ 14*
LDL-cholesterol	216 ± 38	135 ± 39§§	- 36	192 ± 27*	157 ± 32§§	- 18*
Apolipoprotein AI	154 ± 27	158 ± 38	+ 2	146 ± 18	159 ± 24§	+ 10*
Apolipoprotein B	170 ± 31	113 ± 24§§	- 32	154 ± 19	127 ± 23§§	- 19*

Values represent mean ± SD. Baseline and post-treatment values were compared with paired t-test, while proportional changes were compared using one-way analysis of variance (ANOVA). §$p<0.05$ and §§$p<0.01$ as compared to baseline values, *$p<0.05$ as compared to atorvastatin group.

Table 3. Effect of fenofibrate and atorvastatin on the concentrations of apolipoprotein B-containing lipoprotein subfractions (in g/l).

	ATORVASTATIN			FENOFIBRATE		
	Before treatment	**After treatment**	**% change**	**Before treatment**	**After treatment**	**% change**
VLDL	1.21 ± 0.47	0.63 ± 0.21§	- 42	1.58 ± 0.42	0.93 ± 0.14§	- 39
Total LDL	4.2 ± 0.72	2.8 ± 0.68§	- 31	3.14 ± 0.71*	2.53 ± 0.49§	- 18*
Large	0.91 ± 0.22	0.54 ± 0.12§	- 36	0.78 ± 0.23*	0.63 ± 0.19§	- 18*
Intermediate	1.27 ± 0.29	0.77 ± 0.24§	- 39	1.08 ± 0.26*	0.87 ± 0.22§	- 19*
Dense	2.09 ± 0.66	1.56 ± 0.57§	- 23	1.37 ± 0.24*	1.06 ± 0.22§	- 22

Values represent mean ± SD. Baseline and post-treatment values were compared with paired t-test, while proportional changes were compared using one-way analysis of variance (ANOVA). §$p<0.05$ as compared to baseline values, *$p<0.05$ as compared to atorvastatin group. When the proportional changes in the concentrations of individual LDL subfractions were compared with ANCOVA, taking into account baseline values as a covariate, no differences were observed between the two drugs.

fenofibrate in reducing the concentrations of the large LDL subfractions as well as of the LDL subfractions of intermediate density. However, when these changes were compared with ANCOVA, taking into account the baseline values as a covariate, no significant differences were found between the two drugs. Finally, the changes induced by the two drugs in the concentrations of dense LDL subfractions were of the same magnitude. Since both drugs simultaneously decreased the concentrations of all LDL subfractions, they did not significantly affect the percentage mass distribution of LDL subfractions (data not shown).

Discussion

Clinical and experimental studies have shown that the distribution of LDL subfractions may play a significant role in the determination of total cardiovascular risk.[3-9] More specifically, small, dense LDL subfractions were found to exhibit higher atherogenic potential as compared to larger and more buoyant ones, and their predominance is now considered by some authors as an independent risk factor for the development of ischemic heart disease.[7-9] Large, prospective clinical trials revealed that statins as well as fibrates substantially reduce the incidence and the severity of cardiovascular disease.[12,13,16] However, this reduction is only partially explained by the modification of the classic lipid risk factors.[12,13] Consequently, the determination of the effects of hypolipidemic drugs on the LDL subfraction profile is of special interest, since it may explain a part of the antiatherogenic potential of these compounds. In this study, we provide some additional evidence concerning the impact of fenofibrate and atorvastatin on the concentration and relative distribution of apolipoprotein B-containing lipoprotein subfractions. Both drugs substantially reduced the concentrations of VLDL+IDL, as well as the concentrations of all LDL subfractions. Although by conventional statistical analysis atorvastatin was found to be more potent than fenofibrate in reducing the concentrations of large and intermediate density LDL subfractions, when the comparison was performed by ANCOVA no differences were found between the two drugs.

Previous studies that compared the impact of atorvastatin and fenofibrate on LDL subfraction profile concluded that fenofibrate administration usually induces a shift toward larger LDL subspecies, whereas the effects of atorvastatin, if any, are usually minor.[17-19] These results are in disagreement with those reported herein. However, our study differs from these studies in several aspects. Firstly, in most of the previously mentioned studies, peak particle diameter was used for the determination of LDL subfraction profile.[18,19] Although gradient gel electrophoresis is by far the most commonly used technique for the estimation of LDL subfraction distribution, it may not accurately reflect the changes induced by drug administration (or other interventions) in the concentrations of individual LDL subspecies. An interesting finding from studies where the concentration of LDL subfractions and the peak particle diameter were determined simultaneously[20] was that the differential effect of some statins on LDL subfraction concentration (for example the reduction in small, dense LDL subfractions without any change in larger subspecies) was not followed by concomitant changes in peak LDL particle diameter.[20] Therefore, the quantitative

determination of the concentration of LDL subfractions seems to be a more precise estimator of the changes in LDL subfraction profile.

Epidemiological studies have shown that triglyceride values represent the most important single determinant of LDL subfractions profile.[10,11] Thus, elevated concentrations of triglycerides are usually accompanied by a predominance of small, dense LDL subfractions, whereas in individuals with normal triglyceride values large LDL subfractions predominate.[10,11] The greater effect of fenofibrate than atorvastatin on LDL size observed in the previously mentioned studies[17-19] may just reflect the higher hypotriglyceridemic potency of fenofibrate. Indeed, in most of these studies atorvastatin was used in a dose of 10 mg/day i.e. a dose that does not significantly reduce the concentrations of triglycerides.[17-19] in contrast, the use of 20 mg of atorvastatin per day in our study resulted in a more prominent triglyceride reduction, which was comparable to that observed after fenofibrate administration. As a consequence, the reduction in the concentrations of small, dense LDL subfractions was similar in the two patient groups.

An important finding of the previous studies that compared the impact of atorvastatin and fenofibrate on LDL subfraction distribution was that fenofibrate usually induced marginal changes in the concentration of LDL-cholesterol.[17-19] As a consequence, the concentrations of the cholesterol-rich LDL subfractions of intermediate density remained unchanged or even increased under fenofibrate treatment. This increase, along with the reduction in the concentrations of small dense LDL subfractions, resulted in a significant increase in peak particle diameter of LDL. In contrast, in our study, fenofibrate administration resulted in a significant decrease in LDL-cholesterol values, and thus in a reduction in the concentrations of intermediate density LDL subfractions. Hence, the simultaneous decrease in the masses of all LDL subfractions under fenofibrate therapy resulted in a subfraction profile that did not differ significantly from that before therapy. Although this discrepancy cannot be easily interpreted, patients' selection criteria as well as individual differences in the response to fenofibrate treatment may play significant roles.

In conclusion, both atorvastatin and fenofibrate substantially reduce the concentrations of VLDL+IDL and LDL in patients with combined dyslipidemia. The two potent hypolipidemic drugs simultaneously reduce the concentrations of all LDL subfractions and thus they do not significantly affect the distribution of LDL particles. Our results support the assumption that adequate doses of atorvastatin have the same effect as fenofibrate on LDL subfraction metabolism, and thus raise important questions concerning the need of combination therapy in patients with mixed dyslipidemia.[18]

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