Lipoprotein composition and oxidative modification during therapy with gemfibrozil and lovastatin in patients with combined hyperlipidaemia

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Aims To evaluate the resistance to oxidation of human lipoproteins after hypolipidaemic therapy.

Methods VLDL and LDL samples were obtained from patients with Familial Combined Hyperlipidaemia included in a randomized, double-blind, cross-over study, with 8 weeks of active treatment (gemfibrozil, 600 mg twice daily, or lovastatin, 40 mg daily) and a 4-week wash-out period. Oxidation related analytes after Cu-induced oxidation of VLDL and LDL have been investigated. Further, in order to relate possible changes in oxidative behaviour to lipoprotein composition, the proportion of the lipid species transported by lipoproteins (triglycerides, phospholipids, and cholesteryl esters), the molar composition of fatty acids for each lipoprotein lipid, and the content of antioxidant vitamins in plasma (vitamin C) and lipoproteins (vitamin E) have been studied.

Results Both drugs reduced the plasma concentration of apo-B lipoproteins (–23% gemfibrozil, –26% lovastatin), but whereas lovastatin affected mainly LDL-cholesterol (–30%), gemfibrozil reduced triglycerides (–49%) and VLDL-cholesterol (–48%). Lovastatin treatment had no effect on the lipid and protein composition, the fatty acid profile, or the vitamin E content of either VLDL or LDL; likewise, lipoprotein oxidation markers (Cu-induced conjugated dienes, thiobarbituric acid reactive substances formation, and lysine residues) were similar before and after lovastatin treatment. Gemfibrozil therapy also had no effect on lipoprotein oxidation; nevertheless, it consistently: a) decreased the proportion of LDL-triglycerides (–32%), and b) increased the proportion (molar%) of 18:3 n-6 in VLDL triglycerides (+140%), phospholipids (+363%) and cholesteryl esters (+53%).

Conclusions Based on these results, lovastatin and gemfibrozil do not adversely affect lipoprotein oxidation in patients with mixed dyslipidaemia. In the case of gemfibrozil, this occurs in spite of an increased proportion of some polyunsaturated fatty acids in VLDL. In the context of a fixed dietary intake, such modifications suggest that the drug influences liver enzyme activities involved in fatty acid chain synthesis (elongases and desaturases).

Keywords: lovastatin, gemfibrozil, combined hyperlipidaemia, LDL oxidation, fatty acids

Introduction

A growing body of evidence has been collected regarding the crucial role of oxidized LDL in the development of atherosclerosis [1]. Interventions have been aimed at modifying the type of fatty acids present in lipoproteins or the plasma concentrations of antioxidant vitamins able to reduce LDL oxidation, either in experimental animals or in humans [2, 3].

Certain hypolipidaemic drugs display antioxidant activity which is unrelated to changes in plasma lipid levels [4, 5]. Further, there is evidence that fibrates and, to a lesser extent, statins are able to modify the fatty acid composition of circulating lipoproteins in humans [6].

The objective of the present study was to evaluate human

lipoprotein composition and oxidizability after treatment with gemfibrozil (GFB) and lovastatin (LOV). For this reason, blood samples from patients with familial combined hyperlipidaemia (FCHL) were used to measure the levels of antioxidant vitamins, and to isolate VLDL and LDL, evaluate their composition in the main lipid species, including fatty acids, and determine their resistance to a copper-induced oxidative challenge.

Methods

Study design

Fifty-two FCHL patients entered a randomized, active controlled, double-placebo, double-blind, cross-over study, carried out in five phases: phase 1, selection of patients; phase 2, 4-week placebo period; phase 3, 8-week active treatment period after random assignment to treatment with

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either GFB (600 mg twice daily) or LOV (40 mg day⁻¹); phase 4, 4-week placebo washout period; and phase 5, 8-week treatment cross-over [7]. The study protocol was approved by the institutional review board, and all patients gave written informed consent. The last nine patients (seven men and two women, body mass index 27.3 ± 3.3 kg m⁻², age 53 ± 10 years) were considered for the evaluation of lipoprotein composition and oxidizability.

Hypolipidaemic diet

Throughout the study, the patients were instructed to adhere to a standard hypolipidaemic diet. Adherence was determined by means of a 7 day food-frequency questionnaire combined with 24 h recall of 2 weekdays and a weekend day through a personal interview with a trained dietitian. Quantitative nutrient ingestion was calculated with the Nutridiet Pro 3.0 software program (J. & J. Soldevila, Barcelona, Spain).

Analysis

At the beginning and end of phases 3 and 5, 10 ml blood samples were collected from each patient in EDTA-tubes for the measurement of: total plasma triglycerides, total and free cholesterol and phospholipids (enzymatic kits from Boehringer Mannheim, Barcelona, Spain), HDL cholesterol (precipitation of apo B-containing lipoproteins with phosphotungstate/magnesium), apo B levels (immunoturbidimetry), thiobarbituric acid reactive substances (TBARS), and vitamin C content [8].

Further, a portion of the plasma samples was used for obtaining lipoprotein fractions [8]. After dialyzing the lipoprotein fractions against a degassed phosphate-buffered saline, the following parameters were determined: triglycerides, total and free cholesterol, phospholipids, protein [9], and vitamin E content [8]. After VLDL and LDL lipid extraction and separation by thin-layer chromatography, the fatty acid (FA) composition was determined by capillary-gas chromatography [10].

Lipoprotein oxidation in the presence of $5 \mu\text{M}$ CuSO₄·5H₂O was determined by measuring TBARS, conjugated dienes, and lysine content, exactly as described previously [8].

Statistical analysis

The absence of differences between the two baseline placebo values was assessed by means of the Wilcoxon signed rank test. The differences among the three treatment periods (placebo PB, GFB, and LOV) were assessed by means of a Friedman nonparametric repeated measures test, and *a posteriori* Dunn's multiple comparisons test. The significance was fixed for a $P \leq 0.05$ (two tailed). All values are expressed as means $\pm$ s.e.mean.

Results

Therapy with GFB induced significant reductions in VLDL cholesterol (-48% , PB 60 ± 14 mg dl⁻¹), plasma triglycerides (-49% , PB 271 ± 46 mg dl⁻¹), and apo B (-23% , PB

189 ± 38 mg dl⁻¹), while increasing HDL cholesterol ($+24\%$, PB 39 ± 10 mg dl⁻¹). LOV treatment also reduced apo B levels (-26%), in association with a significant decrease of LDL cholesterol (-30% , PB 201 ± 15 mg dl⁻¹). GFB substantially reduced the plasma free cholesterol fraction (-22% , PB 76 ± 5 mg dl⁻¹), while LOV decreased mainly the cholesteryl ester fraction (-24% , 206 ± 14 mg dl⁻¹). GFB also significantly reduced circulating phospholipid levels (-19% , PB 262 ± 17 mg dl⁻¹).

The main constituent of VLDL was the triglyceride fraction, accounting for 53–56% of the lipoprotein mass (Table 1). The main effect observed after drug treatment was a 43% reduction in plasma VLDL protein induced by GFB, and a 28% reduction in VLDL cholesteryl ester after LOV treatment. The degree of unsaturation of the whole VLDL particle was unchanged by drug treatment.

Treatment with LOV induced a 17% reduction in the amount of cholesterol transported by LDL (Table 1). Although GFB treatment did not modify the amount of lipids transported by LDL, it effectively reduced by 32% the weight percent of triglycerides in the LDL particle. No changes in the degree of unsaturation of the whole LDL particle occurred after treatment with either drug.

The basal FA composition (molar %) of lipid fractions in VLDL and LDL is shown in Table 2. Irrespective of the lipoprotein species, cholesteryl esters were mainly enriched in polyunsaturated FA (60–62%), and triglycerides in monounsaturated FA (44–48%), whereas phospholipids contained almost equal proportions of saturated (45%) and polyunsaturated FA (41%).

LOV treatment had little effect on the FA profile of both VLDL and LDL. The only significant change was a 15% increase in the proportion of 20:4 n-6 in the LDL-phospholipid fraction. On the other hand, the FA composition of VLDL was strongly affected by GFB treatment: the proportion of 14:0 was increased by 138% and 330% in the triglyceride and phospholipid fractions, respectively. More striking was the increase induced by GFB in the proportion of 18:3 n-6 in the triglyceride (140%), phospholipid (363%), and cholesteryl ester (53%) fractions, although in this last case the difference did not reach statistical significance (Figure 1).

Plasma TBARS, vitamin C concentration and oxidation related analytes were similar before and after drug therapy (data not shown).

Discussion

In comparison with compositional data of LDLs from normolipidaemic subjects [11, 12], in our FCHL patients the proportion of LDL triglyceride was nearly twice and that of LDL cholesteryl ester was approximately one half. Small, dense LDL particles, enriched in TG and depleted in CE are far more atherogenic than large, buoyant LDL with a normal cholesteryl ester content [13]. Although the VLDL composition in our FCHL patients shows the same trend than LDL, the modifications are of lesser magnitude.

The lipoprotein changes observed in this study after drug administration to patients with mixed dyslipidaemia are in accord with those reported previously [14, 15]. Further, our results generally agree with those reported in the three

Table 1 Lipid and protein composition and fatty acid profile of VLDL and LDL.

	VLDL			LDL		
	Placebo	LOV	GFB	Placebo	LOV	GFB
<i>Protein (mg dl⁻¹ pl)</i>	29.8 ± 2.50	31.4 ± 4.07	17.0 ± 2.20*	79.0 ± 10.1	66.4 ± 8.6	67.6 ± 8.5
TG (mg mg ⁻¹ p.)	5.11 ± 0.39	5.00 ± 0.73	4.40 ± 0.50	0.97 ± 0.17	0.96 ± 0.18	0.55 ± 0.11
TC (mg mg ⁻¹ p.)	1.55 ± 0.13	1.22 ± 0.14	1.41 ± 0.17	2.23 ± 0.15	1.88 ± 0.14*	1.96 ± 0.15
FC (mg mg ⁻¹ p.)	0.61 ± 0.04	0.56 ± 0.06	0.55 ± 0.05	0.61 ± 0.05	0.50 ± 0.04	0.51 ± 0.04
CE (mg mg ⁻¹ p.)	0.92 ± 0.09	0.66 ± 0.09*	0.85 ± 0.1	1.61 ± 0.12	1.40 ± 0.10	1.45 ± 0.11
PL (mg mg ⁻¹ p.)	1.62 ± 0.09	1.47 ± 0.19	1.35 ± 0.13	1.44 ± 0.11	1.15 ± 0.10	1.11 ± 0.12
<i>% (w/w)</i>						
TG	54.2 ± 1.1	56.3 ± 2.1	53.3 ± 2.9	16.7 ± 2.3	18.5 ± 3.6	11.4 ± 1.3*
TC	16.6 ± 1.0	14.3 ± 1.1	17.1 ± 1.6	39.6 ± 1.5	37.6 ± 1.4	42.5 ± 0.9
FC	6.6 ± 0.3	6.6 ± 0.4	6.7 ± 0.4	10.9 ± 0.5	10.0 ± 0.4	10.9 ± 0.3
CE	9.9 ± 0.7	7.6 ± 0.9*	10.5 ± 1.4	28.7 ± 1.2	27.4 ± 1.2	31.5 ± 0.8
PL	17.5 ± 0.4	16.5 ± 0.9	16.9 ± 1.4	25.4 ± 0.9	24.0 ± 1.1	23.6 ± 1.1
Prot.	11.3 ± 0.5	13.0 ± 1.8	12.7 ± 0.9	18.3 ± 1.2	20.8 ± 1.3	21.8 ± 1.4
PUFA/SFA + MUFA	0.39 ± 0.09	0.43 ± 0.16	0.44 ± 0.10	0.76 ± 0.05	0.64 ± 0.04	0.83 ± 0.02

Results are means ± s.e.mean of nine different VLDL or LDL preparations and expressed as mg dl⁻¹ of plasma (pl.) or mg mg⁻¹ of VLDL protein (mg p.). *Values different from placebo ($P < 0.05$). TG: triglycerides; TC: total cholesterol; CE: cholesteryl ester; FC: free cholesterol; PL: phospholipids; PUFA: polyunsaturated; MUFA: monounsaturated and SFA: saturated fatty acids.

Table 2 Basal FA composition (molar %) of lipid fractions in VLDL and LDL.

FA	VLDL			LDL		
	TG	PL	CE	TG	PL	CE
14:0	1.3 ± 0.3	2.0 ± 0.5	11.3 ± 1.3	3.3 ± 0.7	1.6 ± 0.3	5.5 ± 0.8
16:0	25.3 ± 1.4	28.3 ± 0.6	10.4 ± 1.1	22.0 ± 1.7	29.6 ± 0.6	9.7 ± 0.5
16:1	3.7 ± 0.3	0.73 ± 0.17	1.7 ± 0.2	3.1 ± 0.3	0.57 ± 0.10	2.3 ± 0.3
18:0	3.0 ± 0.2	14.9 ± 0.3	0.76 ± 0.08	3.0 ± 0.3	14.8 ± 0.2	0.74 ± 0.04
18:1	44.2 ± 1.7	12.3 ± 0.8	16.5 ± 1.3	41.1 ± 2.6	12.5 ± 0.8	19.3 ± 1.3
18:2 (n-6)	16.7 ± 1.3	21.0 ± 0.8	32.5 ± 2.1	17.0 ± 2.2	21.7 ± 0.9	44.3 ± 1.9
18:3 (n-6)	2.5 ± 0.9	3.0 ± 0.9	20.7 ± 2.5	5.0 ± 0.9	2.5 ± 1.1	9.5 ± 1.9
18:3 (n-3)	1.0 ± 0.2	0.12 ± 0.01	0.31 ± 0.04	0.88 ± 0.24	0.14 ± 0.02	0.35 ± 0.04
21:1 (n-9)	0.32 ± 0.04	0.2 ± 0.03	0.04 ± 0.02	0.30 ± 0.05	0.20 ± 0.02	0.02 ± 0.01
20:3 (n-9)	0.12 ± 0.06	0.14 ± 0.04	0.11 ± 0.07	0.23 ± 0.10	0.14 ± 0.04	0.04 ± 0.02
20:3 (n-6)	0.31 ± 0.02	3.7 ± 0.5	0.50 ± 0.08	0.35 ± 0.05	3.6 ± 0.3	0.76 ± 0.07
20:4 (n-6)	0.97 ± 0.07	9.0 ± 0.7	4.1 ± 0.4	1.5 ± 0.3	8.6 ± 0.6	5.8 ± 0.6
20:5 (n-3)	0.24 ± 0.05	0.91 ± 0.16	0.58 ± 0.16	0.25 ± 0.08	0.89 ± 0.19	0.70 ± 0.18
22:4 (n-6)	0.07 ± 0.02	0.14 ± 0.03	0	0.05 ± 0.02	0.13 ± 0.02	0
22:5 (n-3)	0.2 ± 0.03	0.51 ± 0.10	0	0.14 ± 0.04	0.45 ± 0.05	0
22:6 (n-3)	0.79 ± 0.13	3.3 ± 0.3	0.47 ± 0.16	0.70 ± 0.14	3.3 ± 0.3	0.57 ± 0.08
SFA	29.6 ± 1.5	45.2 ± 0.4	22.4 ± 1.3	28.5 ± 1.7	46.0 ± 0.4	15.9 ± 0.8
MUFA	48.2 ± 1.7	13.2 ± 0.9	18.2 ± 1.4	44.5 ± 2.7	13.2 ± 0.8	21.6 ± 1.5
PUFA	22.1 ± 1.7	41.5 ± 0.8	59.8 ± 1.2	25.4 ± 3.4	40.8 ± 0.8	62.5 ± 1.4
(n-6)	19.7 ± 1.5	36.5 ± 1.0	58.0 ± 1.2	24.7 ± 3.4	35.9 ± 1.0	60.8 ± 1.4
(n-3)	2.2 ± 0.4	4.9 ± 0.4	1.3 ± 0.3	2.0 ± 0.3	4.7 ± 0.5	1.6 ± 0.2
PUFA/SFA + PUFA	0.30 ± 0.03	0.71 ± 0.02	1.5 ± 0.1	0.41 ± 0.1	0.70 ± 0.02	1.7 ± 0.1

Results (expressed as molar %) are means ± s.e.mean of nine different lipoprotein preparations. PUFA: polyunsaturated, MUFA: monounsaturated and SFA: saturated fatty acids.

previous studies comparing lovastatin and gemfibrozil in patients with combined hyperlipidaemia [16–18]. The reduction in LDL cholesterol and apo B levels caused by lovastatin was basically due to a decrease in circulating LDL particles, without modification of their composition (Table 1). The main effect elicited by gemfibrozil in our study seems to be the reduction of hepatic VLDL output

[19], resulting in a strong decrease in the plasma VLDL-protein concentration (43%), without changes in VLDL composition. Regarding LDL, the weight percent composition was shifted towards a more normal pattern, with a 32% reduction of TG content and increases in the proportion of CE (+10%) and protein (+20%). Although such changes ameliorate, but do not normalize, the composition of

Figure 1 Individual values of VLDL 18:3 n-6 content (molar %) in phospholipids a) and triglycerides b) before and after gemfibrozil (GFB) treatment.

LDL particles, they nevertheless should reduce their atherogenicity [13].

To our knowledge, this is the first study reporting the analysis of fatty acid molar composition of triglycerides, phospholipids and cholesteryl esters transported in VLDL and LDL obtained from type IIb dyslipoproteinaemic patients and the effect of drug treatment. Regarding lipoprotein fatty acid composition, comparison of values reported for normolipidaemic subjects [20, 21] with those reported in Table 2 shows no major differences. Because diet was not varied throughout the study, any changes occurring with drug treatment should be directly attributed to specific effects on liver enzyme activities involved in fatty acid chain modification [22]. The only significant modification induced by lovastatin in the lipoprotein fatty acid profile was a modest increase in the content of arachidonic acid in the LDL-PL fraction, although no effect on fatty acid elongation or desaturation has been reported for lovastatin. The LDL fatty acid profile remained unchanged after gemfibrozil administration. On the other hand, noticeable effects were observed on VLDL fatty acids, specially regarding 18:3 n-6. Given the metabolic position of 18:3 n-6 in the biosynthetic pathway of fatty acids [22], the increase in the proportion of 18:3 n-6 could reflect an induction of fatty acid delta-6 desaturase activity, an inhibition of fatty acid elongation or both, produced in human liver by gemfibrozil. Although high cholesterol levels within liver cells have been related to inhibition of delta-6 desaturase activity [23], the hypocholesterolaemic effect of gemfibrozil is unlikely to have modulated enzyme activity

because no increase in 18:3 n-6 was observed with lovastatin despite a more potent cholesterol-lowering effect.

Neither lovastatin nor gemfibrozil appeared to modify the VLDL/LDL resistance to an oxidative challenge. Because lovastatin did not modify the lipid or fatty acid composition of LDL, no antioxidant effect should be expected when oxidation-related analytes are referred to milligrams of LDL-protein as in our study. In accord with the results of Bredie *et al.* [24], gemfibrozil treatment also had no effect on LDL oxidizability in our patients.

Based on these results, lovastatin or gemfibrozil do not appear to adversely affect lipoprotein oxidation in patients with mixed dyslipidaemia. In the case of gemfibrozil, this occurs in spite of an increased proportion of some polyunsaturated fatty acids in VLDL. In the context of a fixed dietary intake, such modifications suggest that the drug influences liver enzyme activities involved in fatty acid chain synthesis (elongases and desaturases).

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