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Treatment of type IIb familial combined hyperlipidemia with the combination pravastatin-piperazine sultosilate

Lluís Masana[a], Jesús Villoria[b,*], Mariano Sust[c], Emilio Ros[d], Nuria Plana[e], Francisco Pérez-Jiménez[f], Miguel Franco[g], Josefina J. Oliván[h], Xavier Pintó[i], Sebastián Videla[c]

[a] *School of Medicine, Rovira i Virgili University, Reus, Spain*
[b] *PRA International, Parque Empresarial "Cerro de los Gamos", Building #1, 1st Floor, 28224 Pozuelo de Alarcón, Madrid, Spain*
[c] *Laboratorios Dr. Esteve, S.A. Avda, Mare de Déu de Montserrat, 221, 08041 Barcelona, Spain*
[d] *Hospital Clínic, Villarroel, 170, 08036 Barcelona, Spain*
[e] *Hospital Sant Joan, Sant Joan s/n, 43201 Reus, Spain*
[f] *Hospital Reina Sofía, Menéndez Pidal, s/n, 14004 Córdoba, Spain*
[g] *Hospital Sant Pau, Sant Antoni María Claret, 167, 08025 Barcelona, Spain*
[h] *Hospital Virgen Macarena, Doctor Fedriani, 3, 41071 Seville, Spain*
[i] *Hospital Bellvitge, Feixa Llarga, s/n, 08907 Barcelona, Spain*

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Abstract

The risk of coronary heart disease is increased for any given low-density lipoprotein (LDL) cholesterol level in patients with high levels of triglycerides because some triglyceride-rich lipoproteins are atherogenic. This paper reports the results of a pilot clinical trial aimed to evaluate a novel triglyceride-lowering drug in combination with pravastatin to treat combined hyperlipidemia. Twenty-six patients with type 2b hyperlipoproteinemia were randomized to receive pravastatin 40 mg/day or pravastatin 40 mg/day plus piperazine-sultosilate 1000 mg/day for 12 weeks if their cholesterol levels, but not triglyceride levels, had responded to therapeutic lifestyle changes and treatment with 40 mg/day of pravastatin. Concentrations of triglycerides, cholesterol and apolipoproteins A and B were measured in duplicate before and after the intervention. There were no significant differences between groups in the change from baseline in the concentration of serum triglycerides. Conversely, significant differences were found for LDL cholesterol, which increased slightly with pravastatin alone but decreased with the combination (12.605 ± 22.777% vs. −6.396 ± 13.157%, respectively; $p = 0.022$). Apolipoprotein-B levels increased with pravastatin alone but remained stable with the combined treatment (10.464 ± 8.446% vs. 0.767 ± 12.335%; $P = 0.028$). The increase in the pravastatin group was significant. Although sultosilate was not efficacious in reducing triglycerides, it helped to decrease the concentration of small, dense, atherogenic LDL particles that are less receptor-sensitive and which could accumulate during long-term statin therapy in patients with high levels of triglycerides.

Keywords: Benzenesulfonate; Familial combined hyperlipidemia; Hypercholesterolemia; Lipoprotein

1. Introduction

Very strong evidence available from both observational and experimental animal, pathological, clinical, genetic and epidemiological studies indicates that an elevated serum cholesterol concentration is an important cause of coronary heart disease and complications, and that lowering serum cholesterol concentrations decreases this risk (Gotto et al., 2000; McGill et al., 2000; Law, 1999; Wilson et al., 1998; LIPID Study Group, 1998; Sacks et al., 1996; Shepherd et al., 1995; Scandinavian Simvastatin Study Group, 1994; Wong et al., 1991; Rossouw et al., 1990; Stamler et al., 1986; Brown and Goldstein, 1986; Lipid Research Clinics Program, 1984). As generally most serum total cholesterol is contained in low-density lipoproteins (LDL), the latter are the most abundant and clearly evident atherogenic lipoprotein.

In addition, there is growing evidence for a strong association between elevated levels of triglycerides and risk of

* Corresponding author. Tel.: +34-91-708-11-15; fax: +34-91-708-11-11.
E-mail address: villoriajesus@praintl.com (J. Villoria).

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coronary heart disease (Austin et al., 1998; Assmann et al., 1998). Some triglyceride-rich lipoproteins are atherogenic; therefore, the risk of coronary heart disease may be greater than that predicted by LDL cholesterol alone in hypercholesterolemic patients who have high levels of serum triglycerides (Austin et al., 1990). Serum total apolipoprotein B has been shown to have a strong predictive power for severity of coronary atherosclerosis and coronary heart disease events (Gotto et al., 2000; Westerveld et al., 1998; Lamarche et al., 1996; Levinson and Wagner, 1992; Kwiterovich et al., 1992; Reinhart et al., 1990; Sniderman, 1988). This has led to the NCEP to adopt lower cut-off levels for triglycerides than in former guidelines and to consider the level of non-high density (non-HDL) cholesterol as a secondary target of therapy in subjects with hypertriglyceridemia (NCEP, 2002).

Although statins are effective in lowering levels of non-HDL cholesterol (Stein et al., 1998), fibric acid derivatives and nicotinic acid are more specific in decreasing levels of triglycerides and are the most effective way to cope with all of the lipoprotein abnormalities in atherogenic dyslipidemia (Leaf et al., 1989; Luria, 1988). Thus their combination with statins is preferred over therapy with statins alone to reduce the risk of coronary heart disease in this setting (Athyros et al., 1997; Brown et al., 2001).

Piperazine sultosilate (2-hydroxy-5-(((4-methylphenyl)-sulfonyl)oxy)benzenesulfonic acid piperazine salt) was introduced 20 years ago and has hypolipidemic effects, and especially for triglycerides (Vinazzer and Farine, 1983; Balaguer and Duarte, 1980; Casellas et al., 1981), but does not provoke the side effects that occur with fibric acid derivatives and nicotinic acid. Although it is not structurally linked to statins, it has been shown to reduce the activity of microsomal hydroxymethylglutaryl-CoA (HMG-CoA) reductase in the liver (Esteve Laboratories, 1998). Peroxisomal proliferation was not observed. To date, no adverse reaction has been reported spontaneously through the post-marketing surveillance system in Spain. However, only a few data on its efficacy are available and clinical experience with this drug is limited. It is not considered in the reports of the NCEP. This profile led the authors to hypothesize on the efficacy of a novel combination with statins to manage combined hyperlipidemia.

This paper deals with the results of a multicentre, randomized, double-blind, pilot clinical trial to evaluate the efficacy and safety of the combination of piperazine sultosilate plus pravastatin versus that of pravastain alone in the treatment of familial combined hyperlipidemia (type 2b hyperlipoproteinemia).

2. Materials and methods

2.1. Patient population

Subjects of either sex aged between 18 and 70 years old diagnosed with familial combined hyperlipidemia (serum total cholesterol >240 mg/dl; triglycerides >300 mg/dl) who had not received any pharmacological treatment or diet to normalize the lipoprotein abnormalities in the previous 2 weeks or whose serum levels had not normalized after 5 weeks of therapeutic lifestyle changes were eligible for the study. Prior to randomization, patients had to remain hypertriglyceridemic (>250 mg/dl) after 9 weeks of therapeutic lifestyle changes and pravastatin (40 mg/day). Exclusion criteria were a major coronary event in the previous 6 months; unstable angina or uncontrolled heart failure, arrhythmias, or hypothyroidism; blood pressure over 160/100 mm Hg; any type of diabetes; alcohol abuse; treatment with lipid-lowering agents, oral hypoglycemic agents, anticoagulants or antineoplastics; renal or hepatic insufficiency; nephrotic syndrome; and body mass index higher than 32. The protocol was in compliance with the recommendations stated in the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to enrolment and the study was approved by independent ethics committees and regulatory authorities in Spain. The study was conducted in the internal medicine department of six general hospitals in Spain.

2.2. Treatment and procedures

Patients enrolled in the clinical trial stayed on therapeutic lifestyle changes (standardized anti-atherogenic diet) for 5 weeks, followed by a 9-week open-label run-in period in which they were treated with pravastatin 40 mg/day in addition to the therapeutic lifestyle changes. After this period, those not showing a triglyceride response (defined as a serum concentration equal or lower than 250 mg/dl) were randomized 1:1 to receive either pravastatin 40 mg/day at night before going to bed, or pravastatin with the same dosing schedule plus piperazine sultosilate 500 mg twice a day (breakfast and dinner), in addition to the therapeutic lifestyle changes in a double-blind manner. This later period lasted for 12 weeks. Treatment with lipid-lowering agents, oral hypoglycemic agents, anticoagulants, corticosteroids or antineoplastics was forbidden throughout the study. Compliance with study medications was evaluated by comparing the number of tablets dispensed and the number returned.

Efficacy endpoints of the study were based on the serum lipid profile. A central laboratory determined the serum concentrations of total cholesterol, VLDL, intermediate density level (IDL), LDL and HDL cholesterol; total triglycerides, VLDL, IDL, LDL and HDL triglycerides; and apolipoprotein A and apolipoprotein-B lipoproteins in each sample. Cholesterol and triglyceride fractions were determined individually using ultracentrifugation techniques. Non-HDL cholesterol was calculated as total cholesterol minus HDL cholesterol. These data were not communicated to the investigators, who received only qualitative information regarding the eligibility of each patient during the screening and randomization visits. Baseline and end-of-

treatment values were the arithmetic mean of the results of two determinations separated by 7 days at the beginning and at the end of the 12-week double-blind treatment period. All patients had to complete a dietary record that was evaluated by the same nutritionist who prescribed the therapeutic lifestyle changes for each patient individually. These records were used to control compliance with the therapeutic lifestyle changes.

2.3. Statistics

Demographic and baseline continuous variables were compared among treatment groups by means of an unpaired *t* test, and the distribution of treatment allocation per sex was analyzed by means of a Fisher's exact test.

The primary efficacy endpoint was the percent change from baseline of the serum total triglyceride concentration. The analysis was performed on an intention to treat basis, using data from all randomized patients in whom there was at least one post-baseline efficacy assessment available. The null hypothesis was the absence of differences between groups; the alternative hypothesis was that the intervention was superior to the control condition. An analysis of covariance model, with percent change from baseline as the dependent variable, treatment as factor and the baseline value as covariate, was used to test the hypothesis. The same approach was used for the remaining secondary endpoints: percent change from baseline of the serum concentrations of triglycerides fractions, total and fractions of cholesterol and apolipoprotein-A and -B lipoproteins. Since the sample size of the study was small, non-parametric tests were also performed to test all efficacy endpoints. To analyze the occurrence of adverse events, a safety population was defined, comprising all patients who received at least one dose of the double-bind study medication.

Sample size was calculated to detect a minimum difference of 25% between groups for the primary efficacy endpoint. A common standard deviation of 20% and a normal distribution of the variable were assumed. In order to achieve a 0.05 significance level with a power of 0.80, a sample size of 11 patients per group was needed. In order to account for up to 20% drop-out during the study, and up to 25% loss as a result of screening failures, 38 patients were to be included.

3. Results

Fifty-seven patients had to be recruited finally to randomize 26 to double-blind treatment. Pre-randomization withdrawal was mostly due to failure to comply with serum lipid criteria. All randomized patients received the study drug and had postbaseline efficacy assessments available and were included in the safety and in the intention to treat populations.

Most of the patients were male. Anthropometric variables and the physical examination findings did not differ among groups at baseline, as neither did mean serum concentrations of triglycerides, cholesterol or apolipoproteins A and B, although serum LDL cholesterol was higher in the pravastatin plus sultosilate group. Table 1 summarizes the baseline characteristics of the study population.

There were no significant differences between groups in the percent change from baseline of serum concentration of triglycerides, although patients in the pravastatin plus sultosilate group showed a slight, but not significant, increase. The mean percent change from baseline in the pravastatin group was (mean ± standard deviation, S.D.) 6.419 ± 69.058% (95% confidence interval from 43.959 to − 31.120) while in the pravastatin plus sultosilate group it was 20.413 ± 50.056% (95% confidence interval from 47.623 to − 6.798) (p value for the comparison of mean

Table 1
Baseline characteristics of the patients in the intention to treat population

	Pravastatin ($n = 13$)	Pravastatin + sultosilate ($n = 13$)	*p*-value
Gender: percentage of males	92.31	76.92	–
Age (years: mean [S.D.])	45.08 [7.29]	47.54 [10.74]	0.501
Years from diagnosis (years: mean [S.D.])	9.14 [7.64]	8.82 [8.00]	0.916
Serum total triglycerides (mg/dl: mean [S.D.])	661.52 [506.06]	426.01 [507.10]	0.147
Serum VLDL triglycerides (mg/dl: mean [S.D.])	542.29 [440.90]	325.03 [442.91]	0.122
Serum IDL triglycerides (mg/dl: mean [S.D.])	31.27 [22.75]	23.34 [22.81]	0.264
Serum LDL triglycerides (mg/dl: mean [S.D.])	23.68 [7.10]	25.52 [6.51]	0.550
Serum HDL triglycerides (mg/dl: mean [S.D.])	22.14 [7.12]	16.56 [7.12]	0.985
Serum total cholesterol (mg/dl: mean [S.D.])	239.86 [69.53]	225.47 [67.32]	0.502
Serum VLDL cholesterol (mg/dl: mean [S.D.])	97.51 [66.80]	63.53 [67.62]	0.119
Serum LDL cholesterol (mg/dl: mean [S.D.])	93.28 [25.69]	111.64 [26.24]	0.074
Serum IDL cholesterol (mg/dl: mean [S.D.])	11.59 [4.22]	12.81 [3.81]	0.508
Serum HDL cholesterol (mg/dl: mean [S.D.])	37.72 [8.86]	37.65 [9.01]	0.985
Serum non-HDL chol. (mg/dl: mean [S.D.])	202.14 [66.43]	187.82 [28.88]	0.448
Apolipoprotein A (mg/dl: mean [S.D.])	118.15 [16.79]	123.89 [16.71]	0.548
Apolipoprotein B (mg/dl: mean [S.D.])	90.54 [17.76]	91.96 [18.93]	0.827

Differences among groups did not reach statistical significance for any parameter.

differences: 0.560, Table 2). There was less variability in the pravastatin plus sultosilate group than in the pravastatin alone group (width of the 95% confidence interval from the origin: 27.210 and 37.539, respectively). The 95% confidence interval for the difference in means ranged from − 62.816 to 34.829 (Fig. 1).

Similarly, there were no significant differences between the groups in the percent change from baseline of the fractions of triglycerides contained in the various types of lipoproteins (VLDL, IDL, LDL and HDL triglycerides) because these fractions did not change significantly within either group. Although in some instances the differences in means between groups were large, the high variation in these variables in the sample precluded the occurrence of significant differences. Table 2 and Fig. 1 summarize the results of the analyses for all endpoints related to triglycerides.

Conversely, there were marked differences between groups in the cholesterol-related endpoints. Total cholesterol increased slightly in the pravastatin group but remained stable in the pravastatin plus sultosilate group (percent changes from baseline (mean ± S.D.): 7.866 ± 18.612% and 2.572 ± 14.084%, respectively), although the difference between groups was not significant. IDL cholesterol increased significantly in the pravastatin group, but not in the pravastatin plus sultosilate group; differences among groups were not significant with the classical approach using the *t*-test, but reached significance with the non-parametric test (*p*-value = 0.033, Mann–Whitney rank sum test). The opposite changes were seen in LDL cholesterol, which increased with pravastatin and decreased with pravastatin plus sultosilate (mean ± S.D. percent change: 12.605 ± 22.777% and − 6.396 ± 13.157%, respectively); the difference between the groups was also significant with the classical approach ($p = 0.022$, *t*-test), but not with the non-parametric approach ($p = 0.053$, Mann–Whitney rank sum test). VLDL cholesterol remained stable in the pravastatin group, but it increased nonsignificantly in the pravastatin plus sultosilate group. HDL cholesterol increased nonsignificantly in both groups but more markedly in the pravastatin group. Non-HDL cholesterol did not increase significantly in either group, but the increase was more pronounced in the pravastatin group. Table 2 and Fig. 1B summarize these data.

Apolipoprotein-A and -B increased significantly in the pravastatin group, but remained stable in the pravastatin plus sultosilate group. The percent change from baseline of apolipoprotein-A concentration (mean ± S.D.) in the pravastatin and in the pravastatin plus sultosilate groups was 6.378 ± 7.346% and − 0.483 ± 9.560%, respectively. For apolipoprotein-B this change was 10.464 ± 8.446% and 0.767 ± 12.335%, respectively. Differences between groups reached statistical significance for apo-B ($p = 0.028$ *t*-test; $p = 0.040$, Mann–Whitney rank sum test). The 95% confidence intervals for the mean changes from baseline within groups as well as for the difference of means between groups are depicted in Fig. 1C.

Both treatments were very well tolerated. The mean duration of treatment was 14.5 weeks. Six adverse events were reported in the pravastatin plus sultosilate group versus 9 in the pravastatin group. All were mild, except for pneumonia occurring in one patient receiving sultosilate

Table 2
Results in the intention to treat population in the 12-week randomized double-blind treatment period

	Mean percent change from baseline					
	Pravastatin	Pravastatin + sultosilate	Difference of means			
	Mean percent change [S.D.]	Mean percent change [S.D.]	Difference	95% CI	*p*-value (parametric)	*p*-value (non-parametric)
Triglycerides						
Total	6.42 [69.06]	20.41 [50.06]	− 13.99	− 62.82–34.83	0.560	0.383
VLDL	− 13.47 [49.80]	21.76 [53.08]	− 35.22	− 82.08–11.63	0.132	0.205
IDL	1.73 [38.67]	17.56 [55.82]	− 15.83	− 60.76–29.11	0.471	0.815
LDL	20.04 [44.89]	13.57 [68.19]	6.47	− 47.77–60.71	0.806	0.367
HDL	− 1.24 [35.29]	24.04 [62.14]	− 25.29	− 73.27–22.70	0.285	0.229
Cholesterol						
Total	7.87 [18.61]	2.57 [14.08]	5.29	− 8.07–18.65	0.422	0.644
VLDL	− 0.04 [55.23]	20.53 [46.40]	− 20.57	− 65.90–24.77	0.355	0.463
IDL	64.83 [57.28]	16.27 [69.34]	48.56	− 10.04–107.16	0.099	0.033
LDL	12.61 [27.78]	− 6.40 [13.16]	19.00	3.04–34.96	0.022	0.053
HDL	9.36 [11.27]	5.21 [13.26]	4.15	− 7.16–15.46	0.453	0.463
Non-HDL	9.09 [23.63]	2.15 [17.73]	6.94	− 8.26–22.15	0.439	–
Apolipoproteins						
Apolipoprotein-A	6.38 [7.35]	− 0.48 [9.56]	6.86	− 0.04–13.76	0.051	0.112
Apolipoprotein-B	10.46 [8.45]	0.77 [12.34]	9.76	1.14–18.25	0.028	0.040

Treatment was pravastatin 40 mg/day or pravastatin 40 mg/day plus sultosilate 500 mg twice in day.
Percent changes were calculated as 100 multiplied by the quotient between end of treatment value minus the baseline value, divided by the baseline value.
S.D. = standard deviation.

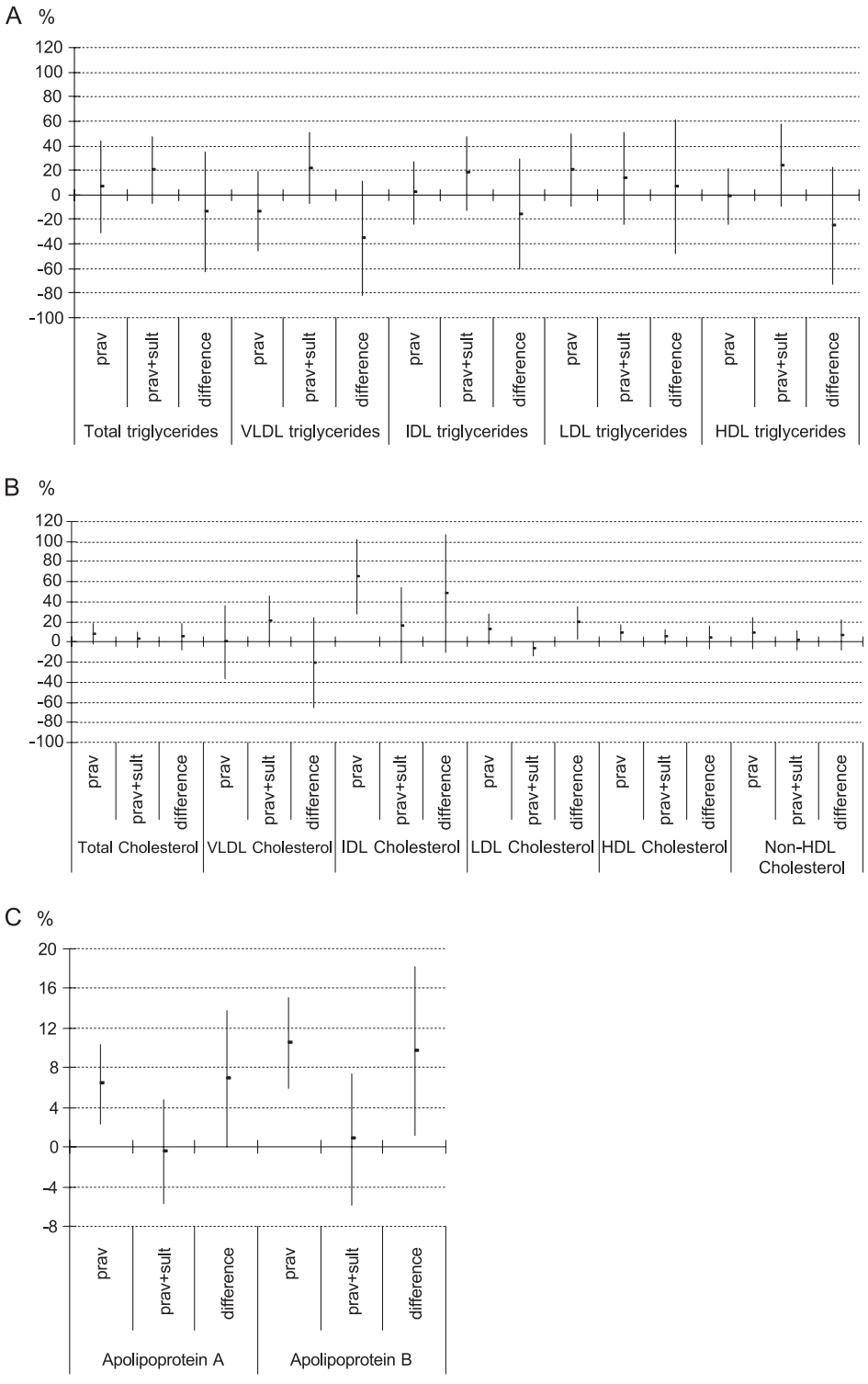

Fig. 1. Ninety-five percent confidence intervals for the results of the triglyceride-related (A), cholesterol-related (B), and apolipoprotein-A/B (C) endpoints in the intention to treat the population during the 12-week randomized double-blind treatment period. Treatment was pravastatin 40 mg/day ("prav") or pravastatin 40 mg/day plus sultosilate 500 mg twice in day ("prav + sult"). Positive changes from baseline indicate increases. Difference is calculated as the mean in the prav group minus the mean in the prav + sult group. A positive difference indicates larger increases (or smaller decreases) in the prav group. Changes from baseline and differences are expressed as percentages.

plus pravastatin, which was not considered treatment related, and epigastric pain in a patient treated with pravastatin alone, which was also not considered treatment related. Only one adverse event (mild epigastric pain) occurring in the sultosilate plus pravastatin group was considered treatment related.

4. Discussion

Although the link between risk of coronary heart disease and cholesterol is well established, studies performed in the statin era have found little evidence for a relationship between variations in serum lipid levels and the reduction of risk generated by therapy (Gotto et al., 2000; WOSCOPS Study Group, 1998), indicating that although the reduction in LDL cholesterol is necessary for risk reduction, it alone does not account entirely for the benefits of therapy. The lipoprotein phenotype has a key role in the atherogenicity of a given cholesterol concentration.

Triglyceride-rich lipoproteins and their remnants are highly atherogenic, given their low affinity for the LDL receptor, which confers on them a long plasma half-life, and their sensitivity to oxidative stress. Some of these particles can be taken up by macrophages by a novel receptor that recognizes species of apolipoprotein-B and not apolipoprotein-E (Havel, 2000), thereby contributing to progression of the atherosclerotic lesion. When triglyceride levels are elevated, a high number of apolipoprotein-B-containing lipoproteins lead to an elevated number of pro-atherogenic small, dense LDL particles even in the absence of significant hypercholesterolemia. These particles may increase the risk of coronary heart disease further than that associated with the absolute concentration of LDL and its reduction by treatment (Chapman et al., 1998).

The increased expression of LDL receptors as a result of the inhibition of HMG-CoA reductase is thought to be the main mechanism by which statins reduce plasma LDL cholesterol levels (Bilheimer et al., 1983). The reduction of the biosynthesis of cholesterol by the liver is also accompanied by a decrease in the production of VLDL. Novel VLDL particles may also be captured intracellularly by LDL receptors within hepatocytes, further reducing their secretion. The final outcome is the stabilization of atherosclerotic plaques. However, enhanced LDL catabolism prevails over reduced VLDL production, and therefore long-term statin therapy could lead to the accumulation of less receptor-sensitive, small, dense atherogenic LDL particles when hypertriglyceridemia is present.

The results of this study point in this direction because LDL cholesterol concentrations decreased in the sultosilate plus pravastatin group but increased in the pravastatin group. IDL cholesterol concentrations also increased strongly in the latter group. While it was possible to measure the concentration of cholesterol and triglycerides contained in all lipoprotein populations as well as that of total apolipoprotein A and B, it was not possible to measure the subpopulations of LDL or the fractions of apolipoproteins within each lipoprotein population. Thus, dense LDL cholesterol was measured in the whole LDL fraction, and this could explain their increase during double-blind treatment in the group continuing on pravastatin alone, while they decreased with the addition of sultosilate. The change in apolipoprotein-B (and non-HDL cholesterol) also reflects this circumstance. The hypothesis that an atherogenic small dense LDL subpopulation increased cannot be directly confirmed, but the IDL population did increase in the group treated with pravastatin alone. The accumulation of small dense LDL particles is thus likely in the absence of co-therapy because IDL will tend to be converted into LDL. Fibrate therapy is reported to normalize the transformation from hepatic VLDL to receptor-active LDL (Chapman et al., 1998). The addition of sultosilate may have a similar effect, increasing the ability of the statin to clear LDL cholesterol.

Although there was not a clear difference between the groups in terms of non-HDL cholesterol, apolipoprotein-B levels (which may serve as a surrogate of non-HDL) clearly increased during double-blind treatment in the group receiving only pravastatin while they remained stable in the group receiving the combination; the baseline values were very similar.

There is not an explanation for the slight, nonsignificant increase in triglyceride levels observed in the combined treatment group, but it should be noted that the variation in serum total triglyceride concentrations among these patients diminished. Given the high biological variability, this decrease might be interpreted as a response, because when there is a small effect, the random variation tends to decrease.

This short-term study has the obvious limitation that its endpoints were changes in lipoprotein profile rather than clinical outcomes, but this was intended as a pilot study of the biochemical effects of a novel combination for the treatment of combined hyperlipidemia. The benefits of this combination should be compared with that of the mostly used statins plus fibrates because there is less concern about the potential for myopathy.

Like fibric acid derivatives, piperazine sultosilate might act by up-regulating genes for fatty acid oxidation and the catabolism of VLDL, thus hampering the formation of VLDL triglycerides and facilitating the shift of LDL subpopulations from small dense to intermediate density and larger size. There are some older animal studies showing the ability of piperazine sultosilate to inhibit HMG-CoA reductase as well as glucose 6 phosphate dehydrogenase and 6 phosphogluconate dehydrogenase at a time when fibric acid derivatives induce changes in the rough endoplasmic reticulum of hepatocytes, but not the proliferation of peroxisomes. Modern investigations would be now of interest to elucidate which of these two actions is most important. In addition, a further clinical trial should fine-tune the research of the effects of this drug by directly measuring the LDL subpopulations in patients with combined hypercholesterolemia or atherogenic dyslipidemia, and by directly comparing the effects of statins alone and a combination of statin plus fibrate.

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