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Simvastatin in severe hypercholesterolaemia: a placebo controlled trial

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The effect of simvastatin in 27 patients with severe primary hypercholesterolaemia was assessed by a double-blind placebo controlled parallel group trial. Total serum cholesterol, LDL-cholesterol and apoprotein B (ApoB) were significantly reduced by simvastatin 40 mg daily. Reductions in triglyceride and VLDL-cholesterol and an increase in HDL-cholesterol levels were only significant when calculated as a percentage of baseline, because of wide inter-individual variability. No changes in apoprotein A1, lipoprotein (a), fibrinogen, viscosity or blood pressure were observed. Leucocyte HMG-CoA reductase activity was unchanged after 4 weeks of active treatment but increased by 87% after 3 months ($n = 21$, $P < 0.05$). No severe adverse effects or changes in CK or AST levels were noted. We conclude that simvastatin is effective in the treatment of severe and resistant hypercholesterolaemia, and well tolerated in the short term.

Keywords simvastatin hypercholesterolaemia

Introduction

Simvastatin is a potent inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, a rate limiting enzyme for cholesterol synthesis (Grundy, 1988a). This placebo controlled trial was designed to assess the effect of simvastatin on low density lipoprotein (LDL) cholesterol levels in patients with severe hypercholesterolaemia. We also report measurements of other lipoproteins, apoproteins, leucocyte HMG-CoA reductase activity, plasma fibrinogen, plasma viscosity, blood pressure and adverse events.

Part of this study was presented at the Third Annual Meeting of the British Hyperlipidaemia Association and published in abstract form: *Atherosclerosis* (1989), **79**, 96.

Methods

All patients had primary hypercholesterolaemia due to raised LDL-cholesterol (Fredrickson Type 11a) with total serum cholesterol in excess of 8.0 mmol l^{-1}. The major exclusion criteria were: age greater than 70 or less than 18 years; premenopausal women unless there was a history of at least 3 years successful experience with a medically approved method of contraception, myocardial infarction or coronary bypass surgery within 3 months; diabetes mellitus, impairment of liver function with any serum liver function test more than 20% above the reference range and recent treatment with probucol, on account of its prolonged retention in body fat stores. Of the 27 patients who took part in the trial, 14 were classified as having familial hypercholesterolaemia on the basis of tendon xanthomata in the patient or a first degree relative. Eight patients had been treated with diet alone and 19 had been resistant or intolerant of other drugs.

Trial design

All lipid-lowering drugs were stopped 8 weeks before entry and all patients took a placebo tablet (single-blind) for this run-in period. Dietary supervision was maintained throughout. Patients were randomised on a double-blind basis to receive simvastatin 10 mg daily or matching placebo from week 0. At each subsequent clinic visit, the dose of tablets was doubled so that patients on active treatment would receive simvastatin 10 mg daily for the first month, 20 mg daily for the second month and 40 mg daily for the third month, and patients on placebo would receive a corresponding increase in tablets. All tablets were taken once daily after the evening meal and patients were reviewed at a

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morning clinic having fasted overnight. Blood was collected at weeks 0, 4, 8 and 12 for lipoprotein, viscosity and fibrinogen measurements, and at weeks 0, 4 and 12 for measurement of HMG-CoA reductase activity. Blood pressure was recorded at each clinic visit after 5 min in the seated position using a mercury sphygmomanometer, measuring diastolic pressure at phase V. The protocol of this study had been approved by the Ethics Committee of The Queen's University of Belfast.

Laboratory methods

Lipid assays employed enzymatic methods for cholesterol and triglyceride and heparin-manganese precipitation for high density lipoprotein (HDL)-cholesterol determination. Very low density lipoprotein (VLDL) was separated by ultracentrifugation ($d < 1.006$). LDL-cholesterol was calculated as total cholesterol minus VLDL-cholesterol minus HDL-cholesterol. Apoprotein-A1 (apoA1) and apoprotein-B (apoB) were assayed by immunoturbidimetry using an RA 500 analyser with reagents supplied by Technicon Ltd. Apoprotein (a) levels were measured by a two site immunoradiometric assay (Pharmacia Ltd., Uppsala, Sweden). Assay coefficient of variation was less than 10% over the range 17–840 ul^{-1}. Fibrinogen was analysed using reagents supplied by Boehringer-Mannheim Ltd (Clauss, 1957). Plasma viscosity was measured using a Coulter-Harkness viscometer (Harkness, 1963).

HMG-CoA reductase activity was measured in mononuclear leucocytes isolated from 40 ml blood anticoagulated with EDTA using Ficoll-Paque (Boyum, 1968). Enzyme activity was determined in a microsomal fraction by a radioenzymatic assay with separation of the product by thin layer chromatography (Harwood *et al.*, 1984). This method measures total enzyme activity.

Non parametric statistics were employed. Wilcoxon ranked paired test and rank sum test were applied as appropriate.

Results

The two groups consisted of 15 patients on simvastatin and 12 on placebo. The effect of treatment on lipids and apoproteins is shown in Table 1. There were no significant changes within the placebo group. The following changes occurred within the simvastatin group (mean value week 12 compared to mean value week 0); total cholesterol fell by 32%, LDL cholesterol fell by 39%, VLDL cholesterol fell by 43%, triglyceride fell by 33%, apoB fell by 33%, HDL-cholesterol rose by 23%, but apoA1 and apo(a) did not change. There was a significant fall ($P < 0.05$) in total cholesterol, LDL-cholesterol and apoB in the simvastatin group when compared to the placebo group at weeks 4, 8 and 12. The changes in VLDL-cholesterol, triglyceride and HDL-cholesterol were not statistically significant when compared to the placebo group. However, the baseline values (week 0) for these measurements were not closely matched between the simvastatin and placebo groups and there was a wide degree of intersubject variability. When each patient's results are expressed as a percentage of the baseline for that patient, the mean percentage decrease in VLDL-cholesterol and triglyceride and increase in HDL-cholesterol are significantly different (simvastatin *vs* placebo $P < 0.05$ at all time periods).

Table 1 Lipids and apoproteins in patients treated with simvastatin (S) $n = 15$, and placebo (P) $n = 12$, at weeks 0, 4 (10 mg day^{-1}), 8 (20 mg day^{-1}) and 12 (40 mg day^{-1}). Leucocyte HMG Co-A reductase activity results in patients treated with simvastatin ($n = 13$) and placebo ($n = 8$) at weeks 0, 4 and 12. Mean ± s.e.mean. * = $P < 0.05$ simvastatin *vs* placebo

		Week 0	*Week 4*	*Week 8*	*Week 12*
Total cholesterol ($mmol\ l^{-1}$)	P	11.7 ± 0.6	11.5 ± 0.6	11.3 ± 0.5	11.7 ± 0.6
	S	11.4 ± 0.6	8.3 ± 0.5*	7.7 ± 0.4*	7.7 ± 0.5*
LDL cholesterol ($mmol\ l^{-1}$)	P	9.5 ± 0.6	9.3 ± 0.6	8.7 ± 0.6	9.5 ± 0.6
	S	9.1 ± 0.6	6.4 ± 0.5*	5.6 ± 0.4*	5.6 ± 0.4*
VLDL cholesterol ($mmol\ l^{-1}$)	P	0.73 ± 0.15	0.77 ± 0.14	1.05 ± 0.25	0.66 ± 0.11
	S	0.93 ± 0.17	0.40 ± 0.05	0.54 ± 0.08	0.53 ± 0.06
HDL cholesterol ($mmol\ l^{-1}$)	P	1.46 ± 0.09	1.45 ± 0.08	1.54 ± 0.09	1.53 ± 0.09
	S	1.36 ± 0.11	1.50 ± 0.12	1.59 ± 0.12	1.64 ± 0.12
Triglyceride ($mmol\ l^{-1}$)	P	2.0 ± 0.5	2.0 ± 0.4	2.2 ± 0.5	2.0 ± 0.4
	S	2.6 ± 0.5	1.6 ± 0.2	1.4 ± 0.2	1.6 ± 0.2
ApoB ($g\ l^{-1}$)	P	1.69 ± 0.13	1.61 ± 0.09	1.56 ± 0.09	1.66 ± 0.09
	S	1.55 ± 0.11	1.24 ± 0.09*	1.10 ± 0.09*	1.13 ± 0.09*
ApoA1 ($g\ l^{-1}$)	P	1.45 ± 0.07	1.39 ± 0.07	1.37 ± 0.03	1.40 ± 0.06
	S	1.41 ± 0.07	1.37 ± 0.06	1.36 ± 0.05	1.43 ± 0.05
Apo(a) ($u\ l^{-1}$)	P	445 ± 107	446 ± 111	438 ± 105	448 ± 110
	S	402 ± 109	436 ± 131	491 ± 144	492 ± 149
HMG-CoA reductase Activity ($pmol\ min^{-1}\ mg^{-1}$ protein)	P	7.7 ± 1.4	6.8 ± 1.4	–	5.9 ± 0.8
	S	7.0 ± 1.2	7.0 ± 1.1	–	13.1 ± 3.3*

Table 2 Non-lipid measurements in patients treated with simvastatin (S) $n = 15$ (except for creatine kinase results where $n = 14$) and placebo (P) $n = 12$. Mean ± s.e. mean

		Week 0	*Week 4*	*Week 8*	*Week 12*
Blood pressure Systolic (mm Hg)	P	130 ± 6	128 ± 5	130 ± 4	137 ± 5
	S	133 ± 5	129 ± 4	127 ± 4	136 ± 5
Blood pressure Diastolic (mm Hg)	P	76 ± 3	78 ± 3	80 ± 3	80 ± 2
	S	80 ± 3	75 ± 3	79 ± 3	78 ± 3
Viscosity (centipoise)	P	1.76 ± 0.03	1.78 ± 0.03	1.78 ± 0.03	1.81 ± 0.04
	S	1.80 ± 0.03	1.77 ± 0.02	1.79 ± 0.03	1.79 ± 0.03
Fibrinogen ($g\ l^{-1}$)	P	3.3 ± 0.2	2.9 ± 0.3	2.9 ± 0.2	2.7 ± 0.2
	S	3.1 ± 0.2	3.2 ± 0.3	3.2 ± 0.2	2.8 ± 0.4
Creatine kinase ($iu\ l^{-1}$)	P	74 ± 7	76 ± 7	95 ± 23	86 ± 52
	S	90 ± 13	111 ± 26	88 ± 13	92 ± 49
Aspartate transaminase ($iu\ l^{-1}$)	P	16 ± 1	15 ± 1	17 ± 1	15 ± 1
	S	19 ± 1	19 ± 1	20 ± 1	18 ± 1

Results for leucocyte HMG Co-A reductase activity are shown for 21 patients in Table 1. Results from six patients are not included because of technical failure with one batch of samples. The activity at 4 weeks was not significantly different from baseline (week 0), but activity at week 12 was increased by 87% compared with baseline, which was significant when compared with placebo ($P < 0.05$).

There were no significant differences in blood pressure, plasma viscosity or plasma fibrinogen levels between the simvastatin and placebo treatment groups (Table 2).

The group receiving simvastatin reported the following symptoms: muscle ache (two patients), joint pains, impaired visual acuity, erythematous rash and increased urinary frequency (one patient each), whereas the group receiving placebo reported muscle ache (two patients), abdominal pain (two patients), headaches and impaired visual acuity (one patient each). Most of these symptoms were minor and none were associated with any disturbance of laboratory tests. The tablets were continued in all but one case when a patient on placebo stopped taking tablets during the third month of the trial on account of abdominal pain. No cause for the abdominal pain was found and it resolved without treatment. The results from this patient have been included in the statistical analysis.

Liver transaminases did not change (Table 2) either in terms of mean value for the whole group or in terms of clinically significant changes for any individual patient. One patient who had a persistently elevated creatine kinase (CK) level (564 $iu\ l^{-1}$), probably on the basis of a mild inherited proximal myopathy, was entered into the trial and randomised to receive simvastatin. No significant change in CK level occurred. The results from this patient are not included in Table 2.

Discussion

The reduction in total cholesterol observed in this study was largely due to a marked reduction in LDL-cholesterol with probably a small additional contribution from VLDL-cholesterol. Most of the cholesterol lowering effect of simvastatin was achieved during the first month on a dose of 10 mg daily. The 32% overall reduction in total serum cholesterol is of the same order as that reported in previous uncontrolled studies (Leclercq & Harvengt, 1989; Molgaard *et al.*, 1988) and a placebo-controlled study in familial hypercholesterolaemia (Mol *et al.*, 1986). The trend towards an increase in HDL-cholesterol is probably beneficial as regards atherosclerotic risk. The lack of increase in apoA1, the major apoprotein of HDL, suggests that the cholesterol content of HDL has increased without an increase in the number of HDL particles. This is consistent with some studies (Mol *et al.*, 1986) but not others in which a small increase in apoA1 was reported (Leclercq & Harvengt, 1989; Molgaard *et al.*, 1988). Further studies with larger numbers are required to clarify this point. The reduction in triglyceride can be attributed to a reduction in both LDL and VLDL particles.

Apo(a) is a component of lipoprotein(a) (Lp(a)), a lipoprotein particle which is an independent risk factor for atherosclerosis (Houlston & Friedl, 1988) and may be an important determinant of risk in patients with familial hypercholesterolaemia (Wiklund *et al.*, 1990). Apo(a) is covalently bound to ApoB-100 by disulphide bonds within the Lp(a) particle. ApoB-100 is also the major protein component of LDL, acting as the ligand for the LDL receptor. The lack of change in apo(a), despite the marked fall in apoB, provides evidence that Lp(a) is synthesised and metabolised independently from LDL, despite having ApoB in common (Houlston & Friedl, 1988). Two small uncontrolled studies of the effect of lovastatin on Lp(a) have been conflicting, either reporting a rise in Lp(a) (Jurgens *et al.*, 1989) or no change (Berg & Leren, 1989). In a more recent larger survey, apo(a) levels were unchanged after either cholestyramine or pravastatin (Wiklund *et al.*, 1990). The effect of simvastatin treatment has not previously been described.

Fibrinogen, which has also been identified as an independent atherosclerosis risk factor (Meade *et al.*, 1986) was also unaffected by simvastatin. This is in contrast to decreases in fibrinogen which have been reported in patients treated with bezafibrate (Niort *et al.*, 1988) or gemfibrozil (Avellone *et al.*, 1988). Fibrate class drugs

may inhibit HMG-CoA reductase activity (Stewart *et al.*, 1982). Our results suggest that the fibrinogen lowering activity of fibrate drugs is independent of any inhibitory effect on HMG-CoA reductase.

HMG-CoA reductase is a rate limiting enzyme in the intracellular synthesis of cholesterol (Goldstein & Brown, 1990) which under physiological conditions is only partially activated (Beg & Brewer, 1982). It is assumed that leucocyte enzyme activity reflects activity in other tissues such as the liver (Hagemenas *et al.*, 1990; Harwood *et al.*, 1984). In our study, leucocyte HMG-CoA reductase levels measured at 4 weeks showed no change from baseline levels, despite a marked decrease in serum cholesterol levels. However, our *in vitro* assay measured total enzyme activity. It is therefore not possible by this means to exclude different degrees of enzyme activation *in vivo* at weeks 0 and 4. The observed increase in total activity at 12 weeks may be explained by a compensatory induction in enzyme synthesis, but the design of our study does not permit us to distinguish between a time related and a dose related phenomenon.

Previous experience with simvastatin and lovastatin have indicated that these drugs are generally well tolerated. Some cases of myositis have occurred associated with a marked increase in plasma levels of creatine kinase (Grundy, 1988a,b). Elevations in liver transaminases have also been reported (Leclercq & Harvengt, 1989; Mol *et al.*, 1986). The lack of change in liver transaminases in the present study suggests that the previously reported increases may be idiosyncratic rather than dose-related effects. Similarly, we had no evidence of myositis despite the inclusion of one patient who had a mild inherited myopathy with a moderately raised CK level.

In conclusion, our results show that simvastatin is an effective drug for lowering LDL cholesterol in severe Type II hypercholesterolaemia and is well tolerated in the short term. It is suitable for patients who have failed on standard drug treatment.

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