Effects of pravastatin and cholestyramine on gonadal and adrenal steroid production in familial hypercholesterolaemia

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- 1 Adrenal and gonadal steroids are derived from cholesterol, which may be derived from plasma lipoproteins or *de novo* synthesis.
- 2 Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate limiting enzyme in cholesterol synthesis, may therefore affect steroidogenesis when used as lipid-lowering agents in hypercholesterolaemia.
- 3 We have assessed gonadal and adrenal function in subjects with heterozygous familial hypercholesterolaemia (FH) before and after 12 weeks treatment with pravastatin, an HMG CoA reductase inhibitor, or cholestyramine as a control in maximal recommended doses.
- 4 No changes in measured plasma cortisol responses to tetracosactrin injection were seen in 11 patients on cholestyramine or 12 on pravastatin.
- 5 No changes were seen in testosterone, sex hormone binding globulin, androstenedione, dehydroepiandrosterone sulphate, oestradiol or 17 α -hydroxyprogesterone.
- 6 Gonadotrophin levels were unaffected in 10 male subjects on cholestyramine and 7 on pravastatin.
- 7 Measurements on a subset of subjects continuing to 24 weeks treatment also showed no changes.
- 8 No adverse effect on adrenal or gonadal function could be demonstrated in patients with familial hypercholesterolaemia on maximal recommended doses of pravastatin.

Keywords pravastatin cholestyramine familial hypercholesterolaemia steroid hormones

Introduction

Inhibitors of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, are the most effective agents available for the lowering of plasma cholesterol (Grundy, 1988). Two drugs of this class, simvastatin and pravastatin, are now available in the United Kingdom, and lovastatin has been used in the United States of America since 1987.

Steroid hormone synthesis depends on a supply of cholesterol, which may be derived from the uptake of plasma lipoproteins or from endogenous synthesis (Borkowski *et al.*, 1972a, b; Brown *et al.*, 1979; Carr & Simpson, 1981). Patients with the homozygous forms of FH and hypobetalipoproteinaemia, in which delivery of LDL to the adrenal cortex is impaired, have demonstrable deficiencies of adrenal steroid production under maximal stimulation, although not in the basal state (Illingworth *et al.*, 1982, 1984).

The possibility that inhibition of cholesterol synthesis may interfere with steroid production has been examined by several investigators. In 1960 apparent reductions in plasma and urinary steroids were described in atherosclerotic subjects treated with triparanol (MER-29), an inhibitor of a late step of cholesterol synthesis which is no longer available (Ford, 1960). Tobert *et al.* (1982) noted small decreases in urinary 17-hydroxy and 17-ketosteroids, plasma testosterone and cortisol in normal males treated with lovastatin (mevinolin) for a month although the changes were not statistically significant. Mol *et al.* (1989) found that simvastatin significantly reduced the peak plasma cortisol after ACTH infusion and increased basal levels of ACTH in patients with severe hypercholesterolaemia. By contrast, other studies have shown no adverse effects of lovastatin or simvastatin on adrenal or testicular function (Candrina

et al., 1990; Farnsworth *et al.*, 1987; Illingworth & Corbin, 1985; Laue *et al.*, 1987).

The effect of pravastatin on steroid hormone levels in man has not been reported. We have therefore studied adrenal and gonadal function in patients with heterozygous FH taking pravastatin or cholestyramine for up to 24 weeks.

Methods

Twenty-three patients from two centres who were participating in a multicentre trial comparing the efficacy and safety of cholestyramine, pravastatin and placebo as lipid-lowering therapy in heterozygous FH were studied. Diagnostic criteria for FH were a fasting total plasma cholesterol of greater than 7.5 mmol l⁻¹ and low density lipoprotein (LDL) cholesterol over 5.0 mmol l⁻¹ with the presence of tendon xanthomata in the patient or a first degree relative. Patients gave informed consent and the study received ethics committee approval at both centres.

Previous lipid-lowering therapy was withdrawn for 4 weeks (bile acid sequestrants), 8 weeks (fibrates and nicotinic acid derivatives) or 12 weeks (probucol) prior to baseline measurements and randomisation. Patients were stabilised on a lipid-lowering diet corresponding to the American Heart Association phase I recommendations (Ad Hoc Committee, 1984) at the beginning of the run-in period and dietary advice reinforced at regular intervals during treatment. For the first 12 weeks patients were randomly assigned to three groups, taking cholestyramine 16–24 g day⁻¹ in divided doses (aiming for 24 g in patients able to tolerate this) with placebo pravastatin, pravastatin 20 mg twice daily with placebo cholestyramine, or double placebo. Since prolonged placebo treatment was considered unethical, at 12 weeks those taking double placebo were re-randomised to one or other active treatment, still double-blind, while those already on active treatment continued unchanged. Assessments of adrenal and gonadal function were made at 0, 12 and 24 weeks, and grouped for analysis according to the length of active treatment. There were no significant differences between the 0 and 12 week data from patients receiving double placebo therapy, and these are therefore not reported.

Measurements were made between 08.30 and 10.30 h, individual patients being studied at the same time at each visit. Venous blood was withdrawn for measurement of cortisol, follicle stimulating hormone (FSH), luteinising hormone (LH), testosterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone (DHEA) sulphate, androstenedione, 17 α -hydroxy-

progesterone and oestradiol. Further samples for cortisol were taken at 30 and 60 min after 250 μ g tetracosactrin (Synacthen, CIBA Laboratories, Horsham, UK) intramuscularly. Semen samples were obtained from 12 subjects after 4 days abstinence, and analysed within 2 h of production by one technician at each centre.

Laboratory methods

Total cholesterol and triglycerides were measured by enzymatic-colorimetric methods, using the CHOD-PAP method for cholesterol and GPO-PAP for triglycerides (Boehringer Mannheim, Lewes, UK). High density lipoprotein (HDL) cholesterol was measured after precipitation with heparin and manganese (Warnick & Albers, 1978) and low density lipoprotein cholesterol was calculated using the Friedewald formula (Friedewald *et al.*, 1972). An interlaboratory quality control scheme was in operation.

Hormone measurements were made on serum samples stored at -20° C, and performed in one centre. LH and FSH were measured using Chelsea solid phase immunoassay kits (WHO Collaborating Centre for Immunoassay, Hammersmith Hospital, London). 17 α -hydroxyprogesterone and androstenedione were measured by 'Coat-a-Count' radioimmunoassay kits (Diagnostic Products (UK) Ltd., Abingdon, Oxon). Methods for testosterone, SHBG, oestradiol, DHEA sulphate and cortisol have been previously described (Adamopoulos *et al.*, 1984; Darne *et al.*, 1989). Intra-assay coefficients of variation were between 4.6% and 10.7%.

Statistical methods

The distributions of variables were tested for approximation to the Gaussian distribution using the Wilk-Shapiro test. Comparisons between treatment and baseline were tested for significance using the two-tailed Student's *t*- or Wilcoxon tests for paired samples. Comparisons between treatment groups employed the equivalent unpaired tests. Bonferroni's correction for multiple samples was applied.

Results

Twelve patients (five female) on pravastatin and 11 (one female) on cholestyramine completed 12 weeks active treatment. Nine patients (three female) on pravastatin and 7 (one female) on cholestyramine completed 24 weeks, the remainder having taken double placebo for the initial 12 weeks. Lipid and lipoprotein data are shown in Table 1. Both agents lowered total and LDL

Table 1 Mean (s.d.) concentrations of lipids and lipoproteins (mmol l⁻¹)

	Cholestyramine			Pravastatin		
	Baseline	12 weeks	24 weeks	Baseline	12 weeks	24 weeks
Total cholesterol	9.7(1.1)	7.6(1.6)**	7.3(1.2)**	10.2(1.8)	7.7(1.1)**	8.0(1.2)*
LDL cholesterol	7.9(1.2)	5.6(1.8)*	5.4(1.3)**	8.2(1.8)	5.7(1.2)**	5.9(1.3)*
HDL cholesterol	1.09(0.32)	1.12(0.32)	1.17(0.40)	1.28(0.17)	1.27(0.34)	1.32(0.37)
Triglycerides	1.63(0.73)	1.91(0.87)*	1.62(0.42)	1.63(0.61)	1.44(0.60)*	1.46(0.61)

Significance of differences from baseline: * *P* < 0.01; ** *P* < 0.001.

Figure 1 Cortisol responses to 250 µg intramuscular tetracosactrin. There were no significant differences from baseline (●) nor between treatment groups (■ 12 weeks, ▲ 24 weeks).

cholesterol by similar amounts. Triglycerides rose on cholestyramine, significantly at 12 weeks, but fell non-significantly on pravastatin.

Cortisol levels before and after tetracosactrin stimulation are shown in Figure 1. All results were within the laboratory normal range. Confidence intervals for percent changes from baseline are shown in Table 2.

Concentrations of other steroid hormones and sex hormone binding globulin, whose levels differ between sexes, are displayed individually in Figure 2, and percent changes from baseline shown in Table 2. There were no significant differences between treatment and baseline, nor between treatment groups.

Gonadotrophin results for male subjects only are shown in Figure 3, with percent changes from baseline in Table 2. Inclusion criteria for the study permitted only postmenopausal women, whose gonadotrophins are already maximally stimulated and were therefore not analysed. Again, no significant differences were seen.

Three comparisons showed statistically significant differences before correction for multiple testing. At 24 weeks the mean $\pm$ standard deviation (s.d.) unstimulated cortisol was 304 ± 61 nmol l⁻¹ on cholestyramine and 381 ± 68 nmol l⁻¹ on pravastatin ($P < 0.05$). Median oestradiol after 24 weeks pravastatin was 117 pmol l⁻¹ compared with 100 pmol l⁻¹ in the same group at baseline ($P < 0.05$). After 12 weeks pravastatin mean $\pm$ s.d. follicle stimulating hormone was 4.0 ± 1.3 u l⁻¹ compared with 3.5 ± 1.0 u l⁻¹ at baseline ($P < 0.05$). There

is no *a priori* explanation for this pattern of changes and their statistical significance was lost after correction for multiple testing. They are therefore thought to arise due to the play of chance.

Semen for analysis was obtainable only in a minority. The sperm count (mean $\pm$ s.e. mean) was 109 ± 22 nl⁻¹ at baseline and 88 ± 18 nl⁻¹ at 12 weeks in six subjects on cholestyramine. In six men on pravastatin it was 48 ± 24 nl⁻¹ at baseline and 46 ± 10 nl⁻¹ after 12 weeks.

Discussion

We have demonstrated that neither pravastatin nor cholestyramine in full doses significantly affect steroid hormone levels after 3 to 6 months treatment in heterozygous FH. The apparently conflicting results in earlier reports using simvastatin or lovastatin may be due to a number of differences between studies. Firstly, the intrinsic variability of hormone levels may mask small but genuine differences with the small numbers of patients observed, although these are unlikely to be clinically significant.

Secondly, the subjects have varied from normal volunteers (Tobert *et al.*, 1982), mixed severe hypercholesterolaemic patients (Fojo *et al.*, 1987; Mol *et al.*, 1989), and heterozygous FH patients (Candrina *et al.*, 1990; Illingworth & Corbin, 1985; Thompson *et al.*, 1986) to homozygous FH (Laue *et al.*, 1987; Thompson *et al.*, 1986). These groups differ in their ability to take up cholesterol from the circulation, and therefore their hormonal responses may theoretically vary. However the expected pattern is not followed as the only statistically significant hormone changes reported were in a mixed group of severe hypercholesterolaemic patients (Mol *et al.*, 1989).

Thirdly the actual measurements have differed. Illingworth and coworkers found that prolonged maximal stimulation of the adrenal by ACTH infusion was necessary to uncover abnormalities in homozygous hypobetalipoproteinaemia and homozygous FH (Illingworth *et al.*, 1982, 1983, 1984). Using this method, they were unable to demonstrate abnormalities in subjects with heterozygous FH (Illingworth *et al.*, 1984) or differences when FH heterozygotes were treated with

Table 2 Mean (95% confidence limits) of % changes from baseline

	Cholestyramine		Pravastatin	
	12 weeks	24 weeks	12 weeks	24 weeks
Cortisol 0 min	-4 (-24, +17)	-4 (-24, +16)	-9 (-33, +15)	+4 (-22, +31)
30 min	+3 (-11, +17)	+10 (-8, +27)	-7 (-16, +2)	+13 (-23, +49)
60 min	+3 (-12, +18)	-4 (-17, +9)	-1 (-8, +6)	+15 (-16, +46)
Testosterone	-8 (-19, +2)	-8 (-26, +9)	-8 (-24, +7)	-9 (-31, +12)
Sex hormone binding globulin	+3 (-27, +33)	+4 (-40, +47)	+16 (-7, +40)	-14 (-34, +6)
Dehydroepiandrosterone sulphate	-11 (-30, +7)	-16 (-35, +3)	+8 (-18, +33)	-9 (-21, +14)
Androstenedione	+3 (-26, +32)	-10 (-27, +6)	-10 (-34, +14)	-4 (-19, +12)
17 α -hydroxyprogesterone	-2 (-17, +13)	-3 (-21, +15)	-9 (-24, +7)	+2 (-32, +36)
Oestradiol	-8 (-17, +1)	-6 (-14, +3)	+1 (-10, +12)	+13 (+2, +24)
Follicle stimulating hormone	+6 (-4, +16)	0 (-28, +27)	+12 (0, +24)	+2 (-45, +49)
Luteinising hormone	+6 (-32, +44)	+6 (-41, +53)	+19 (-8, +45)	-5 (-20, +10)

Figure 2 Sex hormones and sex hormone binding globulin concentrations at baseline and after 12 and 24 weeks treatment. Circles – male; squares – female. No significant differences were seen with treatment.

Figure 3 Gonadotrophin concentrations in male subjects at baseline and after 12 and 24 weeks treatment. No significant differences were seen with treatment.

lovastatin (Illingworth & Corbin, 1985). In spite of this, simvastatin significantly reduced the peak cortisol after only a single bolus of 250 µg synacthen in 24 patients with severe hypercholesterolaemia (Mol *et al.*, 1989). The effect was small and not considered clinically important, the authors speculating that it may relate to the greater cholesterol-lowering effect of simvastatin compared with lovastatin. They achieved a 35% fall in total cholesterol and 40% in LDL cholesterol compared with 25% and 30% respectively in the present study after 12 weeks of pravastatin.

On the basis of animal and *in vitro* studies pravastatin, a hydrophilic molecule, is thought to be more liver-selective in its action than its hydrophobic relatives, simvastatin and lovastatin (Koga *et al.*, 1990; Mosley *et al.*, 1989; Tsujita *et al.*, 1986), although this view has been disputed (Germerhausen *et al.*, 1989). The clinical importance of tissue selectivity is unknown, but preliminary results of sleep laboratory studies suggest that simvastatin is associated with mild sleep disturbances while pravastatin is not (J. Barth, unpublished observations).

In the context of hormone production the importance of tissue selectivity may be expected to be greater in homozygous FH. Cholesterol-lowering effects of HMG CoA reductase inhibition have been demonstrated in such subjects who have defective LDL receptors with some residual function (Laue *et al.*, 1987; Thompson *et*

et al., 1986), but not those with complete absence of receptors (Uauy *et al.*, 1988). Laue *et al.* (1987) studied adrenal function in six subjects with homozygous FH with receptor activities between 3 and 40% and found that, while there were detectable differences from normal subjects, treatment with simvastatin had no additional adverse effect. This may reflect the fact that the inhibition of cholesterol synthesis at therapeutic doses is incomplete (Grundy & Bilheimer, 1984; Reihner

et al., 1990). Therefore tissue-selectivity probably offers only theoretical advantages in terms of hormone production.

The safety of earlier HMG CoA reductase inhibitors with respect to gonadal and adrenal function appears established in a range of patients. Our results show that, in common with related drugs, pravastatin in maximal doses in heterozygous FH does not interfere significantly with steroid hormone production.

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