Effects of pravastatin and cholestyramine on products of the mevalonate pathway in familial hypercholesterolemia

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Abstract Patients with heterozygous familial hypercholesterolemia (n = 12) were treated either with pravastatin, a specific inhibitor of HMG-CoA reductase, or cholestyramine, followed by a period of combined treatment with both drugs. Initially, these patients had increased serum levels of low density lipoprotein (LDL) cholesterol (8.77 ± 0.48 mmol/l; SEM), lathosterol (5.32 ± 0.60 mg/l), and ubiquinone (0.76 ± 0.09 mg/l), while the serum dolichol concentration was in the normal range. Cholestyramine treatment (n = 6) decreased the levels of LDL cholesterol (−32%) and increased lathosterol (+125%), but did not change dolichol or ubiquinone levels in a significant manner. Pravastatin treatment (n = 6) decreased LDL cholesterol (−27%), lathosterol (−46%), and ubiquinone (−29%). In this case, the amount of dolichol in serum also showed a small but statistically insignificant decrease (−16%) after 12 weeks of treatment. Combined treatment with cholestyramine and pravastatin (n = 6) resulted in changes that were similar to, but less pronounced than, those observed during pravastatin treatment alone. In no case was the ratio between ubiquinone and LDL cholesterol reduced. Possible effects on hepatic cholesterol, ubiquinone, and dolichol concentrations were studied in untreated (n = 2), cholestyramine-treated (n = 2), and pravastatin-treated (n = 4) gallstone patients and no consistent changes could be observed. The results indicate that treatment with pravastatin in familial hypercholesterolemia decreases serum ubiquinone levels in proportion to the reduction in LDL cholesterol. — **Elmberger, P. G., A. Kalén, E. Lund, E. Reihnér, M. Eriksson, L. Berglund, B. Angelin, and G. Dallner.** Effects of pravastatin and cholestyramine on products of the mevalonate pathway in familial hypercholesterolemia. *J. Lipid Res.* 1991. **32:** 935–940.

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The mevalonate pathway involves a large number of enzymatic steps, starting with the condensation of acetyl-CoA units, leading to the final common precursor farnesyl-PP, and thereafter branching to yield several end products (1–3). The most well-studied enzyme of this sequence is 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the conversion of HMG-CoA to mevalonate. This reaction is one of the important regulatory steps of the pathway. Most of the farnesyl-PP in the liver and other tissues is converted to cholesterol. Condensation of isopentenyl-PP with farnesyl-PP gives three important polyisoprenoid substances. One of these is ubiquinone, which is a redox component of the mitochondrial respiratory chain and an important endogenous antioxidant (4). The other main products of the condensation reactions are dolichol and its activated derivative dolichyl phosphate (5). Dolichol appears to regulate the biophysical properties of membranes, including stability, fluidity, and permeability, and also has membrane fusogenic properties (6). Dolichyl-P is an obligatory intermediate in the biosynthesis of N-glycosidically linked oligosaccharide chains involved in the production of many important proteins, such as enzymes, receptors, and antigens (7). Both dolichol and ubiquinone are discharged from the liver into the circulation, but their concentrations in blood are much lower than that of cholesterol (8). Recent investigations have found that several cellular proteins, such as oncogene products and proteins that regulate cellular growth, are isoprenylated and contain covalently bound farnesol or geranyl-geranyl moieties (9).

Lathosterol or mevalonate concentration in plasma and the 24-h urinary excretion of mevalonic acid have recently been demonstrated to be good indicators of the in vivo rate of cholesterol biosynthesis (10–13). Thus, increased levels of these substances are seen in response to treat-

Abbreviations: FH, familial hypercholesterolemia; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; HMG, 3-hydroxy-3-methylglutaryl; -PP, pyrophosphate; HPLC, high performance liquid chromatography.

TABLE 1. Basal data on the patients

Patient No.	Sex	Age	Body Weight	Relative Body Weight[a]	Serum Cholesterol	Serum Triglyceride	Clinical Remarks[b]
		yr	*kg*	*%*	*mmol/l*	*mmol/l*	
1.	F	53	52	108	11.4	1.6	TX
2.	F	61	70	123	13.0	1.9	TX,
3.	M	44	92	100	10.2	1.9	TX, MI, AP, BB
4.	F	62	59	102	11.6	1.8	TX, MI, AP, BB
5.	M	38	69	91	11.3	0.8	TX, epilepsy; phenytoin
6.	M	35	74	93	9.2	1.2	TX, brother of patient no. 5
7.	F	65	68	108	11.2	1.4	TX,
8.	F	56	69	128	9.1	2.0	TX, AP, HT, BB
9.	M	19	70	93	9.4	2.1	TX
10.	M	40	102	131	12.2	1.1	TX, AP, CBP;
11.	F	60	64	100	9.4	1.1	TX, MI, AP; BB, nifedipine
12.	F	58	55	102	9.8	1.5	TX, MI, AP, HT; BB
Mean ± SEM		49 ± 4	70 ± 4	107 ± 4	10.7 ± 0.4	1.5 ± 0.1	

[a]Calculated as body weight (kg)/[height (cm) − 100].
[b]TX, tendon xanthoma; MI, myocardial infarction; AP, angina pectoris; CBP, coronary by-pass surgery; BB, beta-blocker therapy.

ment with drugs such as cholestyramine, which increases the rate of cholesterol production in the liver, whereas decreased levels are observed during therapy with specific inhibitors of HMG-CoA reductase, such as pravastatin (14, 15).

Less information is, however, available on the effects of such drugs on the synthesis of polyisoprenoid end products of the mevalonate pathway. In a preliminary report, no significant effect on serum ubiquinone levels was observed during treatment with compactin (16). In the present study, we have therefore investigated the effects of induction and inhibition on HMG−CoA reductase activity in patients with familial hypercholesterolemia (FH) on serum levels of ubiquinone and dolichol as well as on lathosterol and LDL-cholesterol. In addition, we also studied the effect of combination treatment with these two drugs.

MATERIALS AND METHODS

Patients

Altogether, 12 patients with heterozygous FH were studied. They were participants of a larger treatment study described earlier (17, 18). Some basal data on the subjects are presented in **Table 1**. The age of the patients ranged between 19 and 65 years, and in five patients body weight moderately exceeded normal values. All had one or several of the clinical manifestations of FH, i.e., tendon xanthoma, myocardial infarction, angina pectoris, or presence of symptoms in close relatives (19), and some of them had been previously subjected to coronary by-pass surgery or beta-blocker therapy. For determination of liver polyisoprenoid concentrations, 8 normolipidemic patients with uncomplicated gallstone disease were also studied. Informed consent was obtained from all subjects and these studies were approved by the hospital ethics committee.

Study design

The patients had been randomized in a double-blind fashion for treatment with either pravastatin or cholestyramine for a total of 12 weeks (17). During the first 6 weeks, pravastatin was administered at 10 mg b.i.d., a dose that was increased to 20 mg b.i.d. during the following 6 weeks. Cholestyramine was given at a dose of 24 g daily, or the highest tolerable dose, for 12 weeks. After the double-blind treatment period of 12 weeks, the patients were given combined therapy with pravastatin (20 mg twice daily) and cholestyramine (24 g daily) for another 12 weeks.

For studies of liver concentrations of cholesterol, ubiquinone, and dolichol, liver samples were obtained from a total of 8 gallstone patients who had been treated with either cholestyramine (16 g daily, n = 2) or pravastatin (40 mg daily, n = 4), as well as from untreated controls (n = 2). The final dose of the drug was administered approximately 12 h before operation. Data on LDL receptor expression in some of these patients have been reported previously (14).

Biochemical analysis

Serum total, VLDL, LDL, and HDL cholesterol, and total triglyceride were determined before randomization and at weeks 12 and 24. Cholesterol and triglyceride levels were determined with standard enzymatic techniques using autoanalyzers. HDL cholesterol was determined after precipitation of apoB-containing lipoproteins (20). Lipoprotein fractionation using a quantitative procedure based on ultracentrifugation was carried out as described by Carlson (21). In this procedure, VLDL is isolated as

TABLE 2. Baseline values for products of the mevalonate pathway in 12 patients with heterozygous familial hypercholesterolemia (FH) and comparison with references values[a]

Product		FH Patients		Reference Values	
Lathosterol	(mg/l)	5.32 ± 0.58[b]	(n = 12)	3.07 ± 0.23	(n = 21)
Ubiquinone	(mg/l)	0.755 ± 0.093[c]	(n = 12)	0.465 ± 0.23	(n = 31)
Dolichol	(μg/l)	112 ± 9	(n = 12)	110 ± 4	(n = 42)

[a]Values are given as means ± SEM. Numbers of subjects are given in parentheses. Reference values are from determinations in healthy controls.
[b]Significantly different from controls, $P < 0.001$; [c]$P < 0.01$.

the fraction floating at d 1.006 g/ml. Both the infranate (LDL + HDL) and the supernate (VLDL) were assayed for cholesterol, and LDL cholesterol was thereafter calculated as total serum cholesterol minus VLDL and HDL cholesterol.

Lathosterol was determined in serum by isotope dilution-mass spectrometry after addition of $^2H_3$-labeled lathosterol, exactly as described earlier (22). The coefficient of variation of this assay procedure is 4%.

For quantitation of dolichol and total cholesterol, the samples were supplemented with an internal standard of dolichol-23 and ergosterol and, after alkaline hydrolysis, the lipids were extracted using the Folch procedure as described earlier (23-25). This extract contained total dolichol, i.e., both the free alcohol and esterified form and total cholesterol. The extract was washed with theoretical upper phase and purified using two sequential HPLC runs with a C-18 reversed phase system. In the first preparative run the bulk of cholesterol was separated from dolichol and in the following analytical runs the individual isoprenes of the dolichol family and total cholesterol were isolated and quantitated, using two different gradient systems. The coefficents of variation for dolichol and cholesterol quantitation were 5.5% and 2.5%, respectively.

In the case of ubiquinone, the serum samples were supplemented with ubiquinone-6 as internal standard and lipids extracted with chloroform-methanol 2:1, at 37°C for 1 h with continuous magnetic stirring. This extraction procedure was repeated once. The extracts were combined, washed three times with Folch theoretical upper phase, and evaporated under nitrogen. The lipids were then dissolved in chloroform and passed through a Silica Sep-Pak cartridge (Waters). After elution with chloroform, the eluate was evaporated, dissolved in 400 μl chloroform-methanol 2:1, supplemented with 10 ml methanol, and applied to a C18 Sep-Pak cartridge. This cartridge was then eluted with an additional 25 ml methanol, the eluate was evaporated, and the lipids were dissolved in 100 μl chloroform-methanol 2:1. HPLC analyses were performed by injecting the samples onto a Hewlett-Packard Hypersil ODS (C18) 3-μm reversed phase column. A linear gradient from the initial (A) methanol-water 9:1, to (B) methanol-isopropanol 4:1, was run during a period of 20 min. Elution was monitored at 275 nm. The coefficient of variation in this assay is 4.1%.

Statistical methods

Data are presented as means ± SEM. The significance of differences was evaluated by Wilcoxon's paired and unpaired test.

RESULTS

In addition to the elevated levels of total and LDL cholesterol, the patients with heterozygous FH also demonstrated elevated levels of serum lathosterol and ubiquinone (**Table 2**). However, since these two compounds are transported primarily in association with LDL (8), we also calculated the lathosterol/free cholesterol and ubiquinone/free cholesterol ratios (μg/mg) and these values were not different in the FH patients compared to the reference population (5.5 ± 0.6 vs. 5.1 ± 0.5 and 1.4 ± 0.2 vs. 1.4 ± 0.1, respectively). Dolichol, which is predominantly transported in HDL (24), was not present at a different concentration in the serum of FH patients compared to controls.

Upon treatment with cholestyramine for 12 weeks, the LDL-cholesterol levels decreased by 20-50%, but they were still above control levels (**Table 3**). As expected, no changes were observed in the levels of cholesterol associated with HDL and VLDL. The increased rate of cholesterol synthesis was clearly reflected in a twofold increase in serum lathosterol levels. Ubiquinone levels tended to decrease towards normal values, but this decrease was not statistically significant. During cholestyramine treatment, dolichol levels were not consistently affected.

Similar to cholestyramine treatment, administration of pravastatin decreased the elevated LDL-cholesterol levels in FH patients by 20-40% (Table 3) without influencing the levels of HDL or VLDL cholesterol. The inhibited cholesterol biosynthesis was mirrored by a distinct decrease in serum lathosterol levels in all cases. The levels of ubiquinone were significantly decreased by 20-40%, approaching normal values and dolichol levels tended to decrease in response to therapy.

When the two drugs were combined, LDL-cholesterol values were decreased 20-50% (Table 3), whereas the

TABLE 3. Effects of hypolipidemic treatment on lipoprotein cholesterol and products of the mevalonate pathway in patients with heterozygous familial hypercholesterolemia[a]

Treatment	Baseline	12 Weeks Treatment	Difference
			%
Cholestyramine (n = 6)			
LDL cholesterol (mmol/l)	8.82 ± 0.45	6.02 ± 0.33[b]	− 32
HDL cholesterol (mmol/l)	1.10 ± 0.12	1.10 ± 0.15	± 0
VLDL cholesterol (mmol/l)	0.53 ± 0.13	0.60 ± 0.11	+ 13
Lathosterol (mg/l)	5.37 ± 1.03	12.1 ± 2.49[b]	+ 125
Ubiquinone (mg/l)	0.694 ± 0.153	0.602 ± 0.130	− 13
Dolichol (μg/l)	103 ± 9	106 ± 9	+ 3
Lathosterol/LDL cholesterol (mg/mmol)	0.597 ± 0.107	2.00 ± 0.338[b]	+ 225
Ubiquinone/LDL cholesterol (mg/mmol)	0.077 ± 0.018	0.099 ± 0.021[b]	+ 29
Pravastatin (n = 6)			
LDL cholesterol (mmol/l)	8.72 ± 0.81	6.38 ± 0.44[b]	− 27
HDL cholesterol (mmol/l)	1.20 ± 0.12	1.35 ± 0.16	+ 13
VLDL cholesterol (mmol/l)	0.28 ± 0.10	0.30 ± 0.11	+ 7
Lathosterol (mg/l)	5.63 ± 0.46	3.06 ± 0.43[b]	− 46
Ubiquinone (mg/l)	0.861 ± 0.099	0.576 ± 0.059[b]	− 29
Dolichol (μg/l)	121 ± 15	102 ± 8	− 16
Lathosterol/LDL cholesterol (mg/mmol)	0.646 ± 0.098	0.490 ± 0.083	− 24
Ubiquinone/LDL cholesterol (mg/mmol)	0.093 ± 0.008	0.090 ± 0.007	− 3
Combination (n = 6)			
LDL cholesterol (mmol/l)	8.55 ± 0.51	6.60 ± 0.28[b]	− 23
HDL cholesterol (mmol/l)	1.20 ± 0.12	1.17 ± 0.15	− 2
VLDL cholesterol (mmol/l)	0.53 ± 0.15	0.47 ± 0.14	− 11
Lathosterol (mg/l)	5.75 ± 0.46	5.66 ± 0.82	− 2
Ubiquinone (mg/l)	0.927 ± 0.125	0.711 ± 0.122[b]	− 23
Dolichol (μg/l)	120 ± 15	117 ± 11	− 2
Lathosterol/LDL cholesterol (mg/mmol)	0.577 ± 0.118	0.869 ± 0.141	+ 51
Ubiquinone/LDL cholesterol (mg/mmol)	0.106 ± 0.012	0.106 ± 0.018	± 0

[a]Values are given as means ± SEM.
[b]Significantly different from baseline, $P < 0.05$.

serum levels of the two other lipoproteins were not changed. Lathosterol concentrations were not significantly affected nor did the lathosterol/cholesterol ratio change substantially. Ubiquinone levels were decreased 10–40%. In addition, the amount of serum dolichol was not affected by combined therapy. Since the changes in ubiquinone paralleled those in LDL-cholesterol levels, it is of interest to note that the ratio between ubiquinone and LDL-cholesterol was not reduced by treatment in any of the groups studied.

In order to directly assess whether the reduced serum levels of ubiquinone observed during treatment with the reductase inhibitor actually reflected a reduced tissue concentration of this important compound, we measured the concentrations of ubiquinone, dolichol, and cholesterol in liver samples obtained from patients pretreated with pravastatin or cholestyramine. However, as shown in **Table 4**, there was no reduction of the concentrations of these products of the mevalonate pathway in human liver, indicating that no major changes occur in response to treatment.

DISCUSSION

FH is associated with high LDL-cholesterol levels requiring appropriate drug treatment. In this study two different principles for treatment were used: cholestyramine administration leads to an increased metabolism of cholesterol by continuous removal of bile acids, while pravastatin inhibits the biosynthetic pathway at the levels of HMG-CoA reductase.

Cholestyramine treatment resulted in a lowering of serum LDL-cholesterol and also a somewhat decreased serum level of ubiquinone. This latter finding may be explained by the flux-diversion mechanism known to occur

TABLE 4. Concentrations of some products of the mevalonate pathway in liver biopsies of gallstone patients

Patient (Sex/Age)	Cholesterol	Ubiquinone	Dolichol
	mg/g	*μg/g*	*μg/g*
Untreated controls			
1. F/75	2.52	35.3	428
2. F/32	1.88	44.6	498
Cholestyramine treatment			
3. F/66	1.93	61.8	605
4. F/44	2.02	51.2	256
Pravastatin treatment			
5. F/53	2.00	35.3	500
6. F/63	1.79	54.7	ND[a]
7. F/54	2.04	54.0	412
8. F/65	1.76	52.0	292

[a]ND, not determined.

during elevated cholesterol synthesis (26). Pravastatin was equally effective in decreasing LDL-cholesterol, but also led to a lowering of serum ubiquinone. During pravastatin treatment, serum dolichol also showed a tendency to decrease (– 16%), an effect that was, however, not significant with the present limited number of samples.

Unselective inhibition of the common mevalonate pathway may decrease the concentrations of all other end products derived from farnesyl-PP in addition to cholesterol, e.g., dolichol, dolichyl-P, ubiquinone, and isoprenylated proteins. On the other hand, it has been established that regulation of the biosynthesis of all polyisoprenoid compounds occurs beyond the site of HMG-CoA reductase, so that partial inhibition of the reductase does not necessarily inhibit the synthesis of all end products to the same degree (27–29). However, even in this case extensive inhibition of the reductase activity must lead to an inhibition of both ubiquinone and dolichyl-P biosynthesis, interfering with important cellular functions such as mitochondrial respiration and protein glycosylation.

Ubiquinone and dolichol are synthesized in all tissues in order to satisfy local cellular requirements for these lipids (25). In contrast to cholesterol, these lipids are taken up from the diet to only a very limited extent and, furthermore, redistribution of these lipids via the circulation does not appear to occur to any appreciable extent (30). For these reasons, the serum levels of these two polyisoprenoid lipids reflect secretion from the liver and are not directly related to the nonhepatic tissue levels.

If drug treatment inhibits the biosynthesis of all mevalonate lipids in various tissues, it could influence important metabolic functions. We were able to obtain liver biopsies and determine the effects of treatment on hepatic dolichol, ubiquinone, and cholesterol levels. Definitive conclusions cannot be drawn from this limited number of samples, but it appears that the liver levels of the polyisoprenoid lipids were unchanged by the treatments used. This finding indicates that the treatment used in this study with an HMG-CoA reductase inhibitor or cholestyramine has no profound effect on total liver concentrations of cholesterol, ubiquinone, or dolichol. However, total tissue lipid concentrations probably change slowly and one cannot at present exclude important changes in biosynthetic rates or lipid function during intensive and prolonged treatment. It seems probable that changes in mevalonate-derived serum lipids other than cholesterol may be more sensitive indicators of adverse metabolic effects on tissues than are tissue concentrations themselves. If this is the case, it will be of great importance to study further the effects of various doses and administration regimens on all end products of the mevalonate pathway.

Dietary administration of dolichol and ubiquinone results in very limited uptake of these lipids into the circulation and various organs. For this reason there are at present no possibilities of supplying tissues with selected products of the mevalonate pathway upon extensive inhibition of HMG-CoA reductase. FH is associated with an increased serum level of ubiquinone, but no change in dolichol level. This fact is in agreement with the lipoprotein distribution pattern of these two lipids: ubiquinone is a component of both LDL and HDL, while dolichol is present only in HDL (8). Furthermore, a net increase of serum ubiquinone may result in an increase in blood antioxidant capacity, since the reduced form of this lipid is a well-established antioxidant and radical scavenger (31). This is of interest in view of the observed changes in LDL upon oxidation, resulting in an increased macrophage uptake via the scavenger receptor (32). Consequently, the increase in ubiquinone may serve as a compensatory mechanism to supply the circulation with protective endogenous antioxidants. For this reason, a decrease in ubiquinone, particularly in the LDL fraction, could affect its susceptibility to oxidative modification and might thus influence the atherosclerotic process. In our study pravastatin treatment did not decrease the serum ubiquinone level below the control value and the optimal concentration of ubiquinone for antioxidant activity is not known. On the other hand, a high level of reduced ubiquinone in blood and tissues may be required under many physiological and pathophysiological conditions (33).

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