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Effect of pravastatin on biliary lipid composition and bile acid synthesis in familial hypercholesterolaemia

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Abstract

Nine patients with heterozygous familial hypercholesterolaemia were treated for eight weeks with either 40 mg pravastatin or placebo under double blind conditions. Six patients received pravastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Treatment with pravastatin resulted in a significant decrease in plasma cholesterol caused by a decrease in low density lipoprotein cholesterol (LDL-c) of 30% ($p<0.005$). We determined the effect of this medication on the lithogenicity of bile. Cholesterol saturation index of fasting gall bladder bile decreased with 23% ($p<0.01$) from 1·06 to 0·75 during treatment with pravastatin. A reduction of 24% ($p<0.01$) in molar percentage of biliary cholesterol was seen. After treatment the total bile acid excretion in faeces and the molar percentage of biliary bile acids were not significantly changed, suggesting that pravastatin does not influence bile acid biosynthesis to a significant extent. These findings indicate that treatment with pravastatin can decrease the incidence and complications of cholesterol gall stones.

Pravastatin (eptastatin, CS-514, SQ 31·000), is a new hypocholesterolaemic agent which effectively lowers total plasma cholesterol, by competitive inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol synthesis.[1,2] Next to lowering plasma cholesterol this drug can also influence biliary cholesterol excretion.[3] A reduction in cholesterol synthesis can theoretically lead to changes in bile acid synthesis, resulting in unpredictable changes in bile composition. Supersaturation of bile with cholesterol predisposes to formation of gall stones.[4] Other systemic lipid lowering drugs, like clofibrate and gemfibrozil increase cholesterol saturation of bile, thereby enhancing the risk of gall stone formation.[5,6] Data on the effect of HMG-CoA reductase inhibitors on bile composition in animals are limited and give conflicting results. Tsujita showed that pravastatin induces a reduction of the cholesterol saturation index (CSI) in dogs by decreasing the biliary neutral sterols and cholesterol, without effects on phospholipids and bile acids.[7] In another study Kempen *et al* found in rats that compactin, another HMG-CoA reductase inhibitor, resulted in a decrease of bile cholesterol. In that study, however, bile acid secretion decreased as well.[8] Duane *et al* recently[9] found that treatment with simvastatin of 10 patients with non-familial hypercholesterolaemia induced a decrease in mean cholesterol saturation index. The present investigation was designed to determine whether patients treated with pravastatin because of familial hypercholesterolaemia, are at risk for developing cholesterol gall stones.

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Methods

SUBJECTS

Nine patients (two postmenopausal women and seven men), aged 22–54 years, with heterozygous familial hypercholesterolaemia gave informed consent and participated in this study. Heterozygous familial hypercholesterolemia was diagnosed by family history, tendon xanthomata and plasma lipid analysis. The mean of two terminations of fasting LDL-cholesterol concentrations obtained at least one week apart was more than 5·0 mmol/l in all patients. The mean plasma triglyceride concentration in the same specimens was less than 4·0 mmol/l. Drugs known to affect plasma lipid levels were prohibited during the study. Treatment with thiazide diuretics and beta-blockers was continued and kept constant from eight weeks before randomisation throughout the study. Patients with diabetes mellitus, hypothyroidism or any other cause of secondary hyperlipidaemia were excluded from the study. The plasma lipid concentration was stabilised for at least eight weeks on a lipid lowering diet. In the last four weeks of this stabilisation period all patients were given placebo. After this period the patients were randomised and given either 40 mg pravastatin or placebo under double blind conditions. Medication was taken before retiring to bed. Six patients received pravastatin, three placebo. Compliance was checked by counting tablets. The study was approved by the Medical Ethics Committee of the University Hospital Rotterdam.

Sample Collection

Fasting blood samples and bile were collected before and after eight weeks of treatment. Blood samples were collected after an overnight fast. Plasma cholesterol and triglyceride concentrations were determined by enzymatic methods (Boehringer-test kit combination). Lipoprotein fractions were separated after 24 hours ultracentrifugation of the plasma at 40·000 rpm at 4°C using a SW-41 rotor in a Beckmann L5–50 UC.[10]

Gall bladder bile was collected after an overnight fast through duodenal intubation. The tip of the intubation tube was placed before the ampulla of Vater as judged by gastroscopy and controlled by *x* ray examinations. Gall bladder contraction was induced by im injection of 0·3 μg/kg bodyweight of ceruletide (Takus,

TABLE I *Plasma lipid concentrations before (B) and after (A) treatment for eight weeks of nine patients with familial hypercholesterolaemia, six patients were treated with 40 mg pravastatin daily, three with placebo*

	Cholesterol	*LDL-c*	*HDL-c*	*TG*
Treated patients				
B	10·3 (2·2)	7·4 (1·7)	1·09 (0·38)	1·59 (1·02)
A	7·5 (1·1)	5·1 (1·0)	1·14 (0·34)	1·46 (0·51)
p-value:	<0·01	<0·01	NS	NS
Placebo treated patients				
B	9·8 (1·5)	7·2 (0·2)	1·11 (0·32)	1·99 (2·0)
A	9·4 (1·4)	6·7 (0·2)	0·94 (0·44)	1·58 (1·0)

Data are presented as mean (standard deviation) and expressed in mmol/l).

Farmitalia). After collection, the gall bladder bile was diluted 1:9 with isopropanol and centrifuged for 10 minutes at 1300 *g*. The supernatant was used for determination of phospholipid concentration.[11] Cholesterol was determined in the supernatant by an enzymatic method (Boehringer-test kit combination). Faecal bile acid excretion was quantified as bile acids deconjugated by alkaline hydrolysis, in the extract of homogenised four days stool collection. Stools were collected after swallowing 25 radioopaque markers. For each patient the distribution pattern and the amount of markers found in the collected faeces before and after treatment was not significantly different. Bile acid concentration in the isopropanol extract of the gall bladder bile as well as in the faeces extract was determined using the 3-hydroxysteroid dehydrogenase method adapted from Bruusgaard,[12] (Sterognost-Flu, Nyegaard, Norway). The cholesterol saturation index was calculated according to Carey.[13]

CLINICAL FOLLOW UP OF PATIENTS

Physical examination was performed every second week. In addition electrocardiographic and ophthalmologic examinations were performed before and after the observation period. Haematologic studies, clinical chemistry profiles and prothrombin time were done every second week.

TABLE II *Gall bladder bile composition, cholesterol saturation index (CSI) and faecal bile acid loss before (B) and after (A) treatment for eight weeks of nine patients with familial hypercholesterolaemia, six patients were treated with 40 mg pravastatin daily, three with placebo. Gall bladder bile composition is expressed as molar percentage*

	Bile composition				*Faecal bile acids in mmol/24*
	Cholesterol	*Bile acid*	*Phospholipids*	*CSI*	
Treated patients					
1B	9·4	66·1	24·5	1·65	1·05
A	6·2	70·1	22·7	0·98	1·65
2B	6·5	72·7	20·8	1·40	1·62
A	3·2	79·5	17·3	0·73	1·43
3B	5·6	73·9	20·5	0·93	0·50
A	4·8	75·2	20·0	0·84	0·49
4B	4·0	81·5	14·5	1·08	0·76
A	3·7	79·4	16·9	0·70	0·61
5B	3·8	80·9	15·3	0·73	1·25
A	3·1	85·1	11·8	0·72	nd
6B	3·4	78·8	17·8	0·58	1·05
A	2·9	79·4	17·7	0·56	0·91
Mean change	−24%	+3%	−1%	−23%	−1%
p value	<0·01	NS	NS	<0·01	NS
Placebo treated patients					
7B	4·0	80·2	15·8	0·84	0·72
A	4·5	77·6	17·9	0·81	1·52
8B	3·4	79·4	17·2	0·69	1·50
A	3·7	80·5	15·8	1·01	1·76
9B	2·5	80·3	17·2	0·49	0·59
A	2·0	85·0	13·0	0·47	0·50

STATISTICAL ANALYSIS

The Wilcoxon's rank-sum test was used for comparisons within the treated groups before and after treatment.

Results

EFFECTS OF PRAVASTATIN ON LIPOPROTEIN CONCENTRATIONS

After treatment with pravastatin for eight weeks a significant decrease of total plasma cholesterol of 24% ($p<0·01$) was seen, which was caused by a lowering in low density lipoprotein cholesterol of 30% ($p<0·005$) as illustrated in Table I. In contrast total plasma triglycerides and high density lipoprotein cholesterol concentrations were not significantly changed. The placebo treated patients did not show any significant change in lipoprotein concentrations.

EFFECTS OF PRAVASTATIN ON COMPOSITION OF GALL BLADDER BILE

The gall bladder bile of six patients treated with pravastatin, showed a significant decrease in cholesterol saturation index from 1·06 to 0·75 (23%, $p<0·01$) (Figure). The most impressive decreases in cholesterol saturation index (48, 40 and 35%) were seen in three patients having indexes above 1·0 before therapy (indicating an increased risk for gall stone formation). In faeces total bile acid excretion was not influenced by pravastatin treatment (Table II). In bile the molar percentage of bile acids was not changed either, while the molar percentage of biliary cholesterol was significantly reduced by 24% ($p<0·001$). As expected the concentration of phospholipids did not change. The placebo treated patient did not show any effect on either the gall bladder bile composition, or the faecal bile acid excretion (Table II). None of the patients showed any side effect.

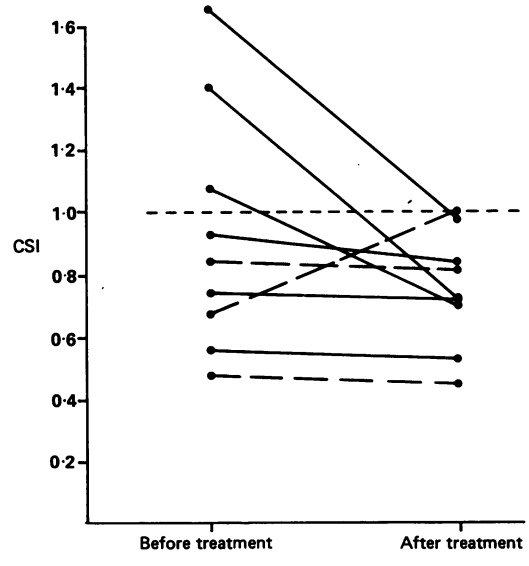

Figure: Cholesterol saturation index (CSI) of gall bladder bile before and after treatment for eight weeks of nine patients with familial hypercholesterolaemia, six patients were treated with 40 mg pravastatin (closed lines), three with placebo (dotted lines).

Discussion

Six patients with familial hypercholesterolaemia were treated with 40 mg pravastatin and showed a significant decrease in plasma cholesterol concentration as a result of a decrease in low density lipoprotein cholesterol concentration. In addition to the effect on plasma cholesterol, we found a significant decrease in cholesterol saturation index of fasting gall bladder bile after treatment with pravastatin. In a steady state the faecal bile acid excretion practically equals total hepatic bile acid synthesis. In our pravastatin treated patients, the faecal bile acid excretion and the molar percentage of biliary bile acids were not significantly changed. This suggests that pravastatin did not alter total bile acid synthesis to a great extent. In contrast, we did see a reduction in molar percentage of biliary cholesterol. The combination of a steady bile acid excretion and a decreased cholesterol saturation index indicates that the altered bile composition can be a result of a decreased biliary cholesterol excretion. Grundy[3] showed a reduction in cholesterol excretion in some familial hypercholesterolaemia patients treated with mevinolin.

The decreases in cholesterol saturation index induced by pravastatin are comparable with those after treatment with chenodeoxycholic acid or ursodeoxycholic acid.[14] Perhaps a combination of pravastatin with those older drugs can even have a more pronounced effect. Our data suggest that pravastatin (and probably other HMG-CoA reductase inhibitors as well) can decrease the incidence of cholesterol gall stones and gall stone complications. If this effect on cholesterol saturation index is confirmed in larger studies of longer duration, HMG-CoA reductase inhibiton may prove valuable in the medical treatment and prevention of patients with recurrent cholesterol gall stones.

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