Effects of Long-Term Treatment With Low-Dose Pravastatin on Biliary Lipid and Bile Acid Composition in Patients With Nonfamilial Hyperlipoproteinemia

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We tested the possibility that pravastatin, a competitive inhibitor of hepatic hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase, would alter cholesterol saturation of gallbladder bile by decreasing its cholesterol saturation index and/or degree of fatty acyl chain unsaturation in lecithin. Eighteen patients with type IIa hyperlipoproteinemia were treated with pravastatin 10 mg/d for 12 months. Gallbladder bile samples were aspirated with a duodenal tube by stimulating gallbladder contraction with intramuscular administration of cerulein before and after treatment. Serum cholesterol level was significantly reduced by 20% after 3 months, and this level was maintained after 12 months. In contrast, the cholesterol saturation index of gallbladder bile was not altered after 3 months (1.52 ± 0.20 *v* 1.70 ± 0.24), but it decreased significantly after 12 months (0.95 ± 0.11, $P < .01$). The degree of fatty acyl chain unsaturation tended to decrease, although this was not statistically significant except for the decrease in molar percent of linoleate after 3 months. These findings suggest that long-term treatment with an inhibitor of HMG CoA reductase improves bile lithogenicity even at a comparatively low dose, and can decrease the incidence and complications of cholesterol gallstones.

PRAVASTATIN is one of a newly developed type of hypocholesterolemic drugs that acts as a competitive inhibitor of hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase, the rate-limiting enzyme for hepatic cholesterol synthesis. High doses (20 mg/d) of this agent may decrease plasma cholesterol by increasing expression of hepatic low-density lipoprotein (LDL) receptors.[1] Chenodeoxycholic acid desaturates biliary cholesterol through a reduction in HMG CoA reductase activity, partly accounting for the dissolution of cholesterol gallstones.[2] Therefore, inhibitors of HMG CoA reductase may be useful in the treatment of cholesterol gallstones.

However, certain hypocholesterolemic agents, ie, clofibrate and gemfibrozil, decrease serum cholesterol by increasing biliary secretion of cholesterol, resulting in increased bile cholesterol saturation.[3,4] This increases the possible risk of cholesterol gallstone formation during treatment with these agents. In fact, cholelithiasis is a side effect of such hypocholesterolemic agents.[5] Inhibitors of HMG CoA reductase may carry a similar clinical risk, namely worsening lithogenicity, although these inhibitors decrease serum cholesterol by a different mechanism from that of clofibrate and gemfibrozil. Although several studies demonstrated that administration of such inhibitors for 4 to 13 weeks decreased the bile cholesterol saturation index in humans,[6-9] none of the studies provided data on the long-term effect. Since cholesterol-lowering drugs are administered for years to prevent coronary diseases, information on the long-term effects of inhibitors of HMG CoA reductase on bile lithogenicity is of clinical importance.

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Furthermore, we recently demonstrated that human bile metastability measured by nucleation time is affected by a high degree of acyl chain unsaturation in lecithin.[10] Also, high-dose pravastatin decreases bile cholesterol saturation and prevents cholesterol gallstone formation in prairie dogs, presumably resulting from reduced unsaturation in biliary lecithin.[11] Thus, the relevance of such an action of pravastatin in humans is another interest.

Therefore, the aim of this study was to evaluate the effect of long-term administration of pravastatin at a low dose of 10 mg/d on bile lithogenicity, including the degree of fatty acyl chain unsaturation, in comparison to that of short-term administration.

SUBJECTS AND METHODS

This study included 18 patients with type IIa nonfamilial hyperlipoproteinemia (two men and 16 women) aged 37 to 78 years, with a mean of 60 years. None of the women received hormonal replacement therapy. Patients ranged from 91% to 128% of ideal body weight, with a mean of 108%. No patient lost or gained more than 3% of his or her body weight during the course of the studies. None had diabetes mellitus, thyroid disease, ethanol overconsumption, or any other causes of secondary hyperlipidemia, and none had undergone cholecystectomy. All patients provided a detailed dietary history and were instructed to maintain their customary diet unchanged throughout the study. Informed consent was obtained from each subject before the study. All study protocols were approved by the Committee of Hiroshima University.

After 4 to 6 weeks of placebo treatment, pravastatin administration was started at 10 mg/d at bedtime. Bile samples were obtained following an overnight fast. The subject swallowed a duodenal tube, and the tip was allowed to pass into the duodenum proximate to the ampulla of Vater as judged by x-ray and pH. Gallbladder contraction was induced by intramuscular administration of cerulein (Kyowa Hakko Kogyo, Tokyo, Japan) 0.2 μg/kg body weight. Bile was collected by this method before and after the drug treatment period (at 3 and 12 months). Blood samples were also drawn when the bile sample was collected.

Serum cholesterol and triglyceride levels were measured by standard enzymatic methods. High-density lipoprotein (HDL) cholesterol level was measured enzymatically in the supernatant

Table 1. Effects of Pravastatin on Serum Lipids

Serum Lipid (mg/dL)	At Entry	3 Months	12 Months
Total cholesterol	282 ± 8	227 ± 10*	223 ± 8*
Triglycerides	110 ± 10	109 ± 15	101 ± 14
HDL cholesterol	55 ± 5	55 ± 7	51 ± 5
LDL cholesterol	201 ± 10	151 ± 8*	152 ± 9*

NOTE. Values are the mean ± SE.

*Significantly different from values at entry ($P < .01$).

after precipitation of LDL and very-low-density lipoprotein with heparin-manganese.

Biliary lipids were simultaneously quantified by gas-liquid chromatography using a OV-1 capillary column (Chromatopackings Center, Tokyo, Japan), with 0.25-mm diameter × 25-m length.[12] The cholesterol saturation index was calculated from a table reported by Carey,[13] assuming a total biliary lipid concentration of 10 g/dL.

Statistical significance was estimated by Wilcoxon's signed-rank test.

RESULTS

Serum Lipids

Table 1 shows the effect of pravastatin on serum lipids. Serum cholesterol level was significantly reduced by 20% after 3 months, and by 21% after 12 months. Also, serum LDL cholesterol was significantly reduced by 25% after 3 months, and this level was maintained after 12 months. In contrast, no significant change was observed in HDL cholesterol or triglyceride levels.

Biliary Lipids

Table 2 shows the effect of pravastatin on biliary lipids, cholesterol, phospholipids, total bile acids, and cholesterol saturation index. The cholesterol saturation index was significantly reduced after 12 months, but no significant reduction was seen after 3 months.

Bile Acid Composition in Bile

Table 3 shows the changes in bile acid composition (mole percent) in bile produced by pravastatin. Bile acid composition was not affected after either 3 or 12 months.

Fatty Acid Composition of Biliary Lecithin

Table 4 shows the changes in fatty acid composition (mole percent) of biliary lecithin produced by pravastatin. After 3 months, the molar percent of linoleic acid (C18:2) was significantly decreased, but that of oleic acid (C18:1) was significantly increased. The degree of fatty acyl chain unsaturation in lecithin as indexed by the molar ratio of saturated to unsaturated fatty acids was increased at 3 and 12 months as compared with the value at entry, but these changes were not statistically significant.

Table 2. Effects of Pravastatin on Biliary Lipids

Biliary Lipid	At Entry	3 Months	12 Months
Cholesterol (molar %)	7.1 ± 0.9	7.5 ± 0.9	5.9 ± 1.1
Phospholipid (molar %)	21.0 ± 1.7	23.2 ± 1.4	19.6 ± 1.6
Total bile acid (molar %)	71.9 ± 2.5	69.4 ± 2.0	74.5 ± 2.2
CSI	1.52 ± 0.20	1.70 ± 0.24	0.95 ± 0.11*

NOTE. Values are the mean ± SE.

Abbreviation: CSI, cholesterol saturation index.

*Significantly different from values at entry ($P < .01$).

Table 3. Effects of Pravastatin on Bile Acid Composition in Bile

Bile Acid (molar %)	At Entry	3 Months	12 Months
CA	25.6 ± 3.2	26.1 ± 2.1	28.6 ± 4.5
CDCA	40.8 ± 3.9	40.2 ± 3.6	37.8 ± 2.1
DCA	29.4 ± 4.2	27.7 ± 4.4	29.9 ± 4.1
UDCA	2.6 ± 0.6	3.6 ± 0.9	4.9 ± 1.5
LCA	1.7 ± 0.2	2.3 ± 0.4	1.3 ± 0.3

NOTE. Values are the mean ± SE.

Abbreviations: CA, cholate; CDCA, chenodeoxycholate; DCA, deoxycholate; UDCA, ursodeoxycholate; LCA, lithocholate.

DISCUSSION

The present study showed that the cholesterol saturation index in bile from patients with type IIa nonfamilial hyperlipoproteinemia was significantly decreased with low-dose (10 mg/d) pravastatin for 12 months, although this change was not observed after 3 months' administration of pravastatin at the same dose. However, serum cholesterol level was significantly reduced by 3 months' administration of pravastatin (−20%), and this level was maintained over 12 months' administration (−21%). Thus, the reduction of serum cholesterol level preceded that of biliary cholesterol level in our study. Duane et al[6] reported that simvastatin administration at 20 or 40 mg/d for 7 to 13 weeks decreased the cholesterol saturation index, and Freeman et al[14] reported a similar effect of lovastatin at 40 or 80 mg/d for 6 to 13 weeks. This effect of such inhibitors was confirmed by others.[7-9] In our study, no change was found in the bile cholesterol saturation index after 3 months' administration of pravastatin, but a reduction occurred after 12 months' administration. Similarly, Okamoto et al[15] failed to find a drastic change in the bile cholesterol saturation index of cholesterol-gallstone patients with a short-term treatment with pravastatin (20 mg/d for 1 to 2 weeks), whereas serum levels of total cholesterol and LDL cholesterol were significantly reduced. These observations taken together suggest the unique action of such inhibitors in modulating cholesterol metabolism in humans. First, the action of such

Table 4. Effects of Pravastatin on Fatty Acid Composition of Bile

Fatty Acid (molar %)	At Entry	3 Months	12 Months
C14:0	2.01 ± 0.41	1.41 ± 0.45	1.32 ± 0.22
C16:0	53.93 ± 2.71	54.68 ± 1.67	54.65 ± 1.65
C16:1	3.87 ± 0.62	3.44 ± 0.39	3.40 ± 0.59
C18:0	7.35 ± 0.58	8.25 ± 0.62	4.60 ± 0.55
C18:1	11.99 ± 0.95	14.98 ± 1.62*	11.17 ± 0.84
C18:2	20.11 ± 2.18	16.35 ± 1.90*	20.63 ± 3.08
C20:0	0.24 ± 0.12	0.37 ± 0.17	0.38 ± 0.08
C20:3	0.50 ± 0.20	0.14 ± 0.09	0.52 ± 0.16
C20:4	0.83 ± 0.42	1.03 ± 0.49	0.32 ± 0.09
C22:6	0.43 ± 0.18	0.41 ± 0.21	0.53 ± 0.27
Saturated/ unsaturated ratio	1.65 ± 0.23	1.78 ± 0.25	1.74 ± 0.13

NOTE. Values are the mean ± SE.

*Significantly different from values at entry ($P < .05$).

inhibitors is apparently dose-dependent, and may also be time-dependent. Second, the serum cholesterol-lowering effect of such agents precedes a reduction of cholesterol level in the bile, especially when administered at a considerably low dose. Since pravastatin somehow increases expression of hepatic LDL receptors,[1] an enhanced uptake of serum cholesterol followed by a possibly induced excretion of biliary cholesterol might mask a decrease of newly synthesized cholesterol followed by a presumably decreased biliary cholesterol excretion in short-term treatment with pravastatin (3 months in the present study). Thereafter, the cholesterol pool in the body reaches a nadir, resulting in a significant reduction in the bile cholesterol saturation index by long-term treatment with pravastatin. Cholesterol-lowering drugs are usually given to patients with hypercholesterolemia for a considerably long period for the important purpose of prevention of coronary diseases. Thus, the long-term effect of such inhibitors on bile lithogenicity is of clinical interest, and the present study has shown that such inhibitors are not risk factors for cholesterol gallstone formation, but may even be advantageous for the treatment of cholesterol gallstones. Although the efficacy of such agents in cholesterol gallstone treatment is yet to be established, this is the first report to state the long-term effect of such an inhibitor on bile metastability.

The effect of long-term treatment with pravastatin on the degree of fatty acyl chain saturation is also of interest, since several investigators, including our group, have demonstrated that an increase in the degree of acyl chain saturation results in an increase in cholesterol-holding capacity of vesicles in model bile systems.[16-19] In fact, our previous study using prairie dogs demonstrated that pravastatin increased this parameter, and consequently inhibited cholesterol gallstone formation.[11] In the present study, the degree of fatty acyl chain saturation in biliary lecithin tended to be increased by pravastatin. This might be partially attributable to the stabilization of cholesterol-carrying particulate species in bile.

The effect of pravastatin on bile acid metabolism is also of clinical interest, but no change was found in bile acid composition in the present study. Reihner et al[1] reported an increase in the proportion of cholic acid and a decrease in that of chenodeoxycholic acid, but failed to demonstrate any change in hepatic 7α-hydroxylase activity. Such alterations in bile acid metabolism by pravastatin need further clarification.

Gallstone incidence is increased after long-term administration of clofibrate.[3] This is due to enhanced secretion of cholesterol from the liver into the bile, resulting in an increase in bile cholesterol saturation. In contrast, long-term administration of pravastatin did not worsen bile lithogenicity, but rather improved bile metastability as reflected by the cholesterol saturation index. Thus, long-term administration of pravastatin may be able to decrease the incidence and complications of cholesterol gallstones, although further studies are necessary to clarify such a preventive action of this agent.

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