Effects of an HMG-CoA Reductase Inhibitor, Pravastatin, and Bile Sequestering Resin, Cholestyramine, on Plasma Plant Sterol Levels in Hypercholesterolemic Subjects

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To study exogenous sterol metabolism during the suppression or stimulation of cholesterol biosynthesis induced by treatments for hyperlipidemia, we determined plasma plant sterol concentrations before and after administration of an HMG-CoA reductase inhibitor, pravastatin, and compared these with changes in these plasma sterol levels by the bile-sequestering resin, cholestyramine. The effects of the drugs were also studied in a sitosterolemic patient who has had increased plasma levels of plant sterols. Plasma cholesterol levels determined by the HPLC method were decreased significantly after administration of pravastatin. Plasma plant sterol(sitosterol and campesterol) as well as cholestanol concentrations were also significantly reduced. Cholestyramine administration decreased plasma levels of cholesterol, but did not change those of plant sterols in the hypercholesterolemic subjects. Pravastatin had little effect in a sitosterolemic patient on plasma levels of sterols, where cholestyramine decreased the plasma levels of both cholesterol and cholestanol. These results indicate that treatment with the HMG-CoA reductase inhibitor decreases plasma plant sterol concentrations, and suggest that the increased plasma plant sterol levels in sitosterolemia might not be due to the decreased cholesterol biosynthesis in vivo.


Kew words : Hyperlipidemia, Phytosterolemia, Cholestyramine, Pravastatin, Plant sterols

Plant sterols are little absorbed by the small intestine compared with cholesterol despite the similarities in their structures (1). However, small amounts of these sterols are present in the plasma, and Miettinen et al. (2) proposed the use of these plasma concentrations as a marker for sterol absorption from the gut, since the plant sterols are not synthesized in humans (3).

A rare inherited disorder, sitosterolemia, is characterized by increased plasma levels of plant sterols, xanthomatosis, and premature atherosclerosis (4). The increased plasma plant sterols appears to be mainly due to over-absorption from the gut along with decreased excretion of the sterols (5). Therefore, the pathophysiological mechanisms of the disease may give us new insight concerning cholesterol absorption, which is one of the major causes of hypercholesterolemia in the general population. Recently, Salen et al. (6, 7) proposed a hypothesis for the etiology of sitosterolemia. They suggested that the decreased biosynthesis of cholesterol in the patients may induce increased sterol absorption and lead to the lack of sterol-specificity in terms of intestinal absorption. They demonstrated not only reduced activity of cholesterol biosynthesis in tissues from sitosterolemic patients, but also that the inhibition of cholesterol biosynthesis by an HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitor, lovastatin, did not change the increased plant sterol concentrations in the patients and may increase the sterol levels in control subjects (8, 9).

It is of interest whether the changes of cholesterol biosynthesis in vivo induced by treatment for hyper-
lipidemia may alter the plasma plant sterol concentrations to speculate on the pathophysiology of sitosterolemia especially in relation to cholesterol synthetic activity. Therefore, we studied the effects of two cholesterol lowering drugs, pravastatin, an HMG-CoA reductase inhibitor, and cholestyramine, a bile-sequestering resin, on plasma plant sterol levels. It has been shown that the former drug decreases the cholesterol biosynthetic rates and the latter increases those in vivo. We also determined the effects of the HMG-CoA reductase inhibitor on the pathologically elevated plasma levels of plant sterols in a patient with sitosterolemia.

Subjects and Methods

Seven subjects (5 male and 2 female) aged between 37 and 60 years old with hypercholesterolemia were treated with 10 mg/day pravastatin (Mevalotin®, Sankyo Co. Ltd, Tokyo, Japan). None of the subjects had a family history of xanthomas. They had been treated for more than 3 months on cholesterol-lowering diets before drug administration. Blood was drawn after overnight fasting into tubes containing 1 mg/ml EDTA. Plasma was separated immediately, and prepared for lipoprotein analysis by ultracentrifugation using a Beckman tabletop ultracentrifuge TL-100 with a TL-100.3 rotor as described previously (10). Eight hypercholesterolemic subjects (4 males and 4 females, age between 21 and 62 years old) were also treated with 8 g/day cholestyramine. Blood was taken before and 2 weeks after the treatment. The effects of these drugs on the elevated plasma levels of plant sterols were also studied in a sitosterolemic patient. The clinical and biochemical characteristics of this sitosterolemic patient have been reported in a previous article (11) as case 4. Briefly, the patient (45-year-old female) presented with marked xanthomatosis and mild anemia. The plasma of the patient contained large amounts of plant sterols as well as cholestanol. The patient had been treated with cholestyramine, but unfortunately could not tolerate the treatment because of her associated hemorrhoids. The patient was treated with probucol and pravastatin with her informed consent.

Plasma lipid levels were determined by enzymatic methods unless otherwise specified. Plasma sterol

![Fig. 1. Elution profiles of benzoate derivatives of plasma sterols from a sitosterolemic patient (left) and a control subject (right). Peak 1, internal standard (desmosterol); peak 2, cholesterol (relative retention time (RTT) 1.481); peak 3, campesterol (RTT 1.69); peak 4, cholestanol (RTT 1.778); peak 5, sitosterol (RTT 1.989).]
determination in subjects with hypercholesterolemia as well as sitosterolemia was performed according to the method described previously (11) with a minor modification. Briefly, 200 μl of plasma was saponified with 10 μg of desmosterol as an internal standard. Plasma desmosterol concentrations, determined by an original method (12) in which epicoprostanol was used as an internal standard, were 0.11±0.03 mg/dl before the pravastatin administration and 0.08±0.05 mg/dl after 4-week treatment. Elution patterns of benzoate derivatives of unsaponifiable fraction in plasma of a healthy control (42-year-old male) and the sitosterolemia patient are shown in Fig. 1, and the samples were used as standards in each assay. Data were expressed as mean±SD.

Results

Plasma cholesterol levels in 7 hyperlipidemic subjects determined by an enzymatic method decreased significantly from the initial value of 257±22 mg/dl to 207±23 and 201±26 mg/dl at 2 and 4 weeks, respectively, with pravastatin administration. Concentrations of plasma triglycerides and HDL cholesterol were not changed by the treatment. Plasma lipoprotein analysis showed a significant decrease in cholesterol concentrations in the LDL fraction from 156±39 mg/dl to 112±27 mg/dl after pravastatin administration for 4 weeks, but no change in other lipoprotein fractions.

Baseline levels of plasma sterols determined by HPLC were 224±30 mg/dl cholesterol, 1.39±0.21 mg/dl sitosterol, 0.67±0.25 mg/dl campesterol, and 0.42±0.07 mg/dl cholestanol. Pravastatin administration decreased these sterol concentrations significantly (Table 1). Ratios of the plasma sitosterol to cholesterol concentrations were slightly increased after the 4-week administration of pravastatin (4.82±0.31 to 5.06±0.42×10⁻³, p<0.05). Another lipid-lowering drug, cholestyramine, however did not alter the plant sterol concentrations after 2 weeks of administration despite marked decreases in plasma cholesterol as well as cholestanol levels (Table 2).

The patient with sitosterolemia who could not tolerate cholestyramine treatment underwent treatment with other drugs for more than 3 years (Fig. 2). Plasma sterol levels in the patient were extremely variable even though a low plant sterol diet was recommended by a trained dietitian. All plasma sterol levels seemed to change in parallel to plasma cholesterol concentrations during all periods. However, cholestyramine (4.8 g/day) decreased plasma concentrations of cholesterol as well as cholestanol. Pravastatin (10-20 mg/day) administration had little effect on plasma sterol concentrations when data from 1991, with the treatment exception of the sample obtained just after cessation of pravastatin treatment (Sep. 6, 1991), were used for evaluation (cholesterol, 196±25 mg/dl in control period, 180±10 during the treatment with pravastatin; sitosterol, 31.8±4.34, 31.0±1.91; campesterol, 21.5±3.4, 20.5±1.57; cholestanol, 2.03±0.23, 2.03±0.20 respectively). Cholestyramine seemed to be the most effective drug in terms of reducing the plasma sterol concentrations, especially of cholesterol and cholestanol in the sitosterolemic patient.

Discussion

We studied the effects of an HMG-CoA reductase inhibitor, pravastatin, on plasma plant sterol levels to investigate the metabolism of exogenous sterols under the suppression of cholesterol biosynthesis by determining the plasma plant sterol levels using an HPLC method in hypercholesterolemic subjects, and found that the drug lowered plasma plant sterol concentrations. No such

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Table 1. Effects of pravastatin on plasma sterol levels in 7 hypercholesterolemic subjects determined by HPLC.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After pravastatin administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 weeks</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>224±30</td>
<td>180±27*</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>1.39±0.21</td>
<td>0.95±0.17*</td>
</tr>
<tr>
<td>Campesterol</td>
<td>0.67±0.25</td>
<td>0.53±0.20*</td>
</tr>
<tr>
<td>Cholestanol</td>
<td>0.42±0.07</td>
<td>0.35±0.06*</td>
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</tbody>
</table>

Plasma lipid levels were determined by HPLC. Numbers in parentheses are percent of baseline values. Results were expressed mean±SD. *p<0.01 vs baseline, by paired t-test.

Table 2. Effects of cholestyramine administration for 2 weeks on plasma sterol levels in 8 hypercholesterolemic subjects determined by HPLC.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol (mg/dl)</th>
<th>Sitosterol (mg/dl)</th>
<th>Campesterol (mg/dl)</th>
<th>Cholestanol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>246±43.7</td>
<td>1.03±0.31</td>
<td>0.81±0.43</td>
<td>0.44±0.15</td>
</tr>
<tr>
<td>Cholestyramine</td>
<td>200±34.3*</td>
<td>1.01±0.40</td>
<td>0.74±0.36</td>
<td>0.33±0.12*</td>
</tr>
<tr>
<td>8 g/day</td>
<td>(81±7.7)</td>
<td>(98±27.2)</td>
<td>(91±26.8)</td>
<td>(75±14.3)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percent of sterol concentrations before treatment. *p<0.01 vs before treatment, by paired t-test.
decreases in plasma sterol levels were observed in hypercholesterolemic subjects treated with another anti-hyperlipidemic drug, cholestyramine, which interrupts the entero-hepatic circulation of bile acids and increases bile acid synthesis as well as cholesterol biosynthesis.

HMG-CoA reductase inhibitors are now widely used for the treatment of hypercholesterolemia (13), and these drugs have been shown to increase the number of low-density lipoprotein receptors in the liver, thus lowering plasma cholesterol levels (14). Treatment with these drugs also results in an increase in HMG-CoA reductase activity in the liver (15) as well as mononuclear cells (16) as determined in vitro because of the compensatory mechanisms induced by the competitive inhibitors. However, cholesterol biosynthesis in vivo during the drug treatment seems to be reduced, since the plasma concentrations of cholesterol precursors such as mevalonate (17) and lathosterol (18) were reported to be decreased. Therefore, mechanisms of the decreases in plasma levels of plant sterols as well as cholestanol in hypercholesterolemic subjects induced by the HMG-CoA reductase inhibitor can be accounted for by the increased clearance of plasma sterols through increased LDL pathway especially in the liver (15). However, this is not supported by the evidence that the bile-sequestrating drug, cholestyramine, did not change the plasma plant sterol concentrations in this study, since this drug has been reported to decrease plasma cholesterol levels also through increasing LDL receptor concentrations (14). It is possible that the altered micellar environments of the lipids in the gut lumen may change the absorption efficacy, including that of sterols, since the resin binds bile acids which play important roles in the absorption of lipids (19). It is also possible that the HMG-CoA reductase inhibitors have other mechanisms for the decrease in plasma plant sterol levels in addition to the increased lipoprotein clearance through the up-regulation of LDL receptor expression. Miettinen et al. (18, 20) recently reported that HMG-CoA reductase inhibitors decreased absorption of cholesterol in hypercholesterolemic subjects, and that plasma plant sterol levels tended to be increased during combined treatment with lovastatin and cholestyramine (21). Although the mechanisms of the changes in plasma plant sterol levels are not clear at this point, alteration of cholesterol biosynthesis seems not to be the only factor in their regulation.

Recently, Nguyen et al. (7, 9) reported that plasma sterol levels were not lowered by an HMG-CoA reductase inhibitor, lovastatin, in sitosterolemic patients. Our results in a patient with this rare disease mostly agree with theirs, although the effects of the drug on plasma sterol levels was difficult to evaluate because of their extraordinary fluctuations in the subject as shown in Fig. 2. Nguyen et al. (9) also reported that lovastatin treatment increased or did not change the plasma plant sterol concentrations in control subjects with concomitant decreases in plasma cholesterol levels, although the number of subjects in their study was small. The discrepancy in the effects of the drug between the results in this study and those of Nguyen et al. (9) in control subjects is difficult to explain.
It is possible that differences in pharmacological effects of two HMG-CoA reductase inhibitors may have induced the different results. However, we have observed a similar decrease in plasma plant sterol levels induced by another HMG–CoA reductase inhibitor, simvastatin (data not shown). More recently, Uusitupa et al. (21) observed a decrease in plasma concentrations of plant sterols after administration of lovastatin in a large number of hypercholesterolemic subjects. Therefore, HMG–CoA reductase inhibitors can decrease plasma plant sterol levels, indicating that these drugs lower exogenous sterol concentrations, since plant sterols are not synthesized in humans (3).

The ineffectiveness of an HMG–CoA reductase inhibitor in lowering plasma sterol concentrations in our sitosterolemic patient as well as in the subjects reported by Nguyen et al. (9) is also difficult to explain. Salen et al. (22) speculated that maximally up-regulated LDL receptor activities due to the decreased HMG–CoA reductase activity can explain this ineffectiveness. However, the primary defect (s) of sitosterolemia is still unknown. The observations in this study that pravastatin administration, which decreases cholesterol biosynthesis (13), resulted in a decrease in plasma plant sterol concentrations in hypercholesterolemic subjects, and that cholestyramine therapy, which increases cholesterol biosynthesis, did not change plant sterol levels suggest that the etiology of the increased plasma levels of these compounds in sitosterolemia might reflect an abnormal mechanism of absorption of sterols in the gut rather than reduced cholesterol biosynthesis. The inherited abnormality of the disease may also be a deficit in sterol elimination, since subjects heterozygous for sitosterolemia have been reported to hyper-absorb sitosterol but not to show marked increases in plasma plant sterol levels (23).

In summary, we determined plasma plant sterol concentrations using a high performance liquid chromatography method in hypercholesterolemic subjects before and after administration of an HMG–CoA reductase inhibitor, pravastatin, and compared changes in these plasma sterol levels with those induced by the bile-sequestrating resin, cholestyramine. Both drugs decreased plasma levels of cholesterol, however, changes in plasma plant sterol concentrations were different. Pravastatin had little effect on plasma sterol levels in a sitosterolemic patient. These results indicate that the inhibition of cholesterol biosynthesis by an HMG–CoA reductase inhibitor decreases plasma plant sterols, and also suggest that the cholesterol synthetic rate is not directly linked to the plasma concentrations of plasma plant sterols.

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Plant Sterol Levels during Treatments


