Effects of simvastatin and cholestyramine on bile lipid composition and gall bladder motility in patients with hypercholesterolaemia


Abstract
Although the effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors and bile acid sequestrants on bile lipid composition have been studied separately, no data are available on combination therapy of these drugs. Moreover, the effects of prolonged (four weeks) administration of these drugs on gall bladder motility, an important determinant of cholesterol gall stone formation, have not been studied so far. A prospective study was therefore performed with eight patients who had hypercholesterolaemia (age 53 (5) SEM, body mass index 27·4 (1·1) kg m⁻², low density lipoprotein cholesterol 5·9 (0·3) mmol/l). They received treatment during three periods of four weeks with simvastatin 20 mg/day, cholestyramine 4 g twice daily, and a combination of both in random order, each treatment period separated by a two week wash out period. Before treatment and after each treatment period, postprandial gall bladder motility was studied with ultrasound, followed by duodenal bile sampling. Serum cholesterol decreased in all subjects in any treatment period illustrating good compliance. Molar percentages in duodenal bile of cholesterol, phospholipids, and bile salts were unchanged during simvastatin and cholestyramine treatment. During combined therapy percentage bile salts was lower (72·5 (2·9)% v 77·8 (1·7)% at baseline, p<0·05) whereas phospholipids were higher (21·2 (2·4)% v 16·4 (1·3)% at baseline, p<0·05). As a result cholesterol saturation index (CSI) did not change in any treatment period. No cholesterol crystals were detected in any bile sample, taken at baseline and after each treatment period. Bile salt hydrophobicity index during cholestyramine (0·19 (0·02)) and combined treatment (0·22 (0·01)) decreased strongly compared with baseline (0·34 (0·01), p<0·001, p<0·01, respectively), resulting from increased proportions of glycocholate (59·4 (3·9)% (cholestyramine), 55·6 (2·4)% (combination) and 28·2 (2·2) (baseline), p<0·001) and decreased proportions of deoxycholic acid and chenodeoxycholic acid. Fasting gall bladder volume was increased during simvastatin (28·7 (2·8) ml v baseline (23·2 (2·3) ml, p<0·01) whereas, residual volume did not differ (5·7 (0·9) ml (simvastatin) v 5·9 (0·7) (baseline). During cholestyramine and combined treatment, no significant differences in gall bladder motility were seen. In conclusion, this study suggests that HMG-CoA reductase inhibitors alone and combined with cholestyramine do not affect major determinants of cholesterol gall stone formation, for example, CSI and gall bladder emptying. In addition cholestyramine alone and combined with simvastatin leads to a strong decrease of bile salt hydrophobicity, which may be beneficial in the prevention of nucleation of cholesterol crystals.

Keywords: simvastatin, cholestyramine, bile, lipids, gall bladder, motility.

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, are now widely used for treatment of hypercholesterolaemia. They reduce the cellular pool of cholesterol, thereby enhancing the synthesis of receptors for low density lipoprotein cholesterol, which leads to decreased values of low density lipoprotein cholesterol.1-4 In severe hypercholesterolaemia, HMG-CoA reductase inhibitors are commonly combined with bile acid sequestrants such as cholestyramine, which has an additional cholesterol lowering effect.5-8 Interest has grown in the effects of HMG-CoA reductase inhibitors on biliary lipid composition, a determinant of cholesterol gall stone formation. Other hypolipidaemic drugs like clofibrate or gemfibrozil increase cholesterol secretion into bile thus giving rise to an increased biliary cholesterol saturation index (CSI) and an increased risk of gall stones.9-11 In a number of studies on the effects of HMG-CoA reductase inhibitors on bile composition, generally performed in hypercholesterolaemic patients, CSI of duodenal bile was found to be reduced.12 13 Although controversial,14 a role for HMG-CoA reductase inhibitors in the dissolution therapy of cholesterol gall stones has been suggested.15 In contrast with HMG-CoA reductase inhibitors, cholestyramine treatment is not known to influence CSI.16 17 No data are available on the effects of combined treatment with HMG-CoA reductase inhibitors and cholestyramine on bile lipid composition.

Apart from bile lipid composition, impaired gall bladder motility plays an important part in...
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the pathogenesis of cholesterol gall stone disease, providing the time necessary for the precipitation of cholesterol crystals from supersaturated bile and for their subsequent aggregation and growth to stones. No studies on the effects of HMG-CoA reductase inhibitors on gall bladder motility have been performed so far. Although a short-term dose of cholestyramine has been described to enhance gall bladder emptying, no data on long term treatment with this drug on gall bladder motility are available. As bile lipid composition and gall bladder motility are important determinants in cholesterol gall stone formation, we performed a prospective study in eight subjects with primary hypercholesterolaemia, investigating the effects of four weeks’ treatment with simvastatin and cholestyramine, alone and in combination on biliary lipid composition, presence of cholesterol crystals in fresh bile as parameter for lithogenicity, and gall bladder motility.

Methods

Patients

Eight patients (four females, four males; age 53 (5) years (mean (SEM); body mass index 27.4 (1-1) kg m⁻², per cent ideal body weight 120 (4%)) with recently diagnosed primary hypercholesterolaemia (low density lipoprotein cholesterol ≥4.14 with standard lipid lowering diet, triglycerides <2.6 mmol/l) were recruited from the outpatient clinic of the University Hospital Utrecht, the Netherlands. Apart from hypercholesterolaemia, the participants had to be healthy, according to a medical questionnaire, physical examination, and routine laboratory screening including liver biochemistry and creatine kinase values. They had no previous operations of the gastrointestinal tract. All had a normal gall bladder and common bile duct at ultrasonography. No potentially interfering treatment was allowed.

Protocol

The participants received treatment during three subsequent periods of four weeks with each of the following treatment regimens: 20 mg simvastatin/day alone taken at bedtime (Zocor, Merck Sharp and Dohme, Haarlem, the Netherlands), cholestyramine 4 g twice daily, taken at breakfast and dinner (Questran, Bristol Myers Squibb, Woerden, the Netherlands) alone, or a combination of both treatment regimens. The sequence of treatment regimens was assigned randomly. The treatment periods were separated by a two week wash out period. Patients were carefully instructed to have an interval of at least four hours between cholestyramine and simvastatin during the combined treatment period. At baseline, and at the end of each treatment period, fasting blood samples and duodenal bile were collected and gall bladder motility was studied. These procedures were performed at 8.00 am, in the fasting state, the last dose taken the evening before. The duodenal sampling was always performed after the motility study, to avoid any potential influence of bile salt depletion resulting from biliary drainage on gall bladder motility. Patients were asked to adhere to their lipid lowering diet throughout the study. Compliance was checked by a tablet count at the end of each treatment period.

Approval of the protocol was obtained from the ethical committee of the University Hospital Utrecht. All patients gave written informed consent.

Duodenal bile collection

Duodenal bile sampling was performed after an overnight fast at 8.00 am. After duodenal intubation, bile was sampled after induction of gall bladder contraction by slow intravenous administration of ceruleide (Takus, Farmitalia, Milan, Italy). Three ml of the most concentrated bile was preserved. Two ml of gall bladder bile was mixed immediately with 6 ml chloroform/methanol (2/1 vol) in ice chilled tubes, extracted according to Bligh and Dyer, and stored at −20°C until further analysis. From the remainder of the bile, 5 μl was analysed microscopically for cholesterol monohydrate crystals, the rest was stored immediately at −20°C until further analysis for total bile salt concentration and bile salt composition.

Bile lipid composition

In the bile extract, cholesterol and phospholipid were measured colorimetrically (Monotest, Boehringer Mannheim, Germany and Sopachem Phospholipids, Sopar Biochem, Brussels, Belgium, respectively). Total bile salt concentration was measured in whole bile using 3α-hydroxyxysteroid dehydrogenase according to Turley. The CSI was determined using Carey’s critical tables assuming a total lipid concentration of 10 g/dl. Conjugated bile salt species were analysed in whole bile by isotopic high performance liquid chromatography on a Waters Bondapak C-18 10 μm column (using methanol/phosphate buffer as solvent, pH 5.2, flow rate 1 ml/min) and detection at 200 nm. Cumulative bile salt hydrophobicity index was calculated according to Heuman.

Gall bladder motility

After an overnight fast, gall bladder volume was measured by real time ultrasonography (Pie Medical 250, Maastricht, the Netherlands: 5-0 MHz transducer) at 8.00 am. Sagittal and transverse scans of the gall bladder at its largest dimensions were obtained. Subsequently, after consumption of a test meal, gall bladder images were made at regular intervals during two hours. The ultrasound equipment contained software for calculation of gall bladder volume according to the sum of cylinders method. The test meal consisted of one slice of bread, 5 g margarine, 50 g cheese, one boiled egg, 200 ml yogurt, and...
50 g glucose. This equals 30 g fat, 30 g protein, and 70 g carbohydrate and gives a physiological stimulation of gall bladder emptying. As characteristics of gall bladder motility, fasting volume (mean of measurements at 15 and 0 minutes before test meal; \( V_0 \) in ml), and residual postprandial volume (\( V_{\text{min}} \) in ml) were measured. As parameters for postprandial gall bladder emptying, maximal decrement of gall bladder volume in ml and percentage (\( \Delta V_{\text{max}} \) ml and \( \Delta V_{\text{max}} \% \)) were calculated.

Serum lipid analysis and laboratory safety parameters

Serum lipid concentrations were measured at baseline and after four weeks of each treatment period in blood samples taken after 12 hours of overnight fasting. Total cholesterol and triglycerides were determined by enzymatic colorimetric methods (Boehringer Mannheim CHOD-PAP and GPO-PAP). High density lipoprotein cholesterol was determined in the supernatant after precipitation of low density lipoprotein and very low density lipoprotein cholesterol. Low density lipoprotein cholesterol was calculated using the Friedewald formula. Laboratory safety parameters were determined by standard methods.

Statistical analyses

Results are shown as mean (SEM) unless stated otherwise. Serum lipids, gall bladder motility parameters, and bile lipid parameters at baseline and at the end of each treatment period were evaluated by repeated measures of one way analysis of variance. When a statistically significant difference was detected, results were further analysed by Fisher’s LSD test. A two tailed probability of less than 0.05 is considered significant.

Results

Patients

One patient could not endure the cholestyramine treatment during the second treatment period and left the study after completing the first (simvastatin) treatment period. Baseline parameters and results after simvastatin treatment for this patient were included in the analysis. The other patients completed the study. No adverse events were seen.

Serum lipid parameters

Table I gives the serum lipid concentrations. All treatment regimens induced decreases in low density lipoprotein cholesterol in all patients confirming good patient compliance. High density lipoprotein cholesterol rose significantly in all treatment periods, whereas triglycerides increased during cholestyramine treatment.

Bile lipid analysis

As Table II shows, molar percentages of biliary cholesterol, phospholipid, and bile salts were not different from baseline at the end of the simvastatin and cholestyramine treatment periods, whereas after combined treatment, bile salts were decreased significantly compared with baseline, whereas phospholipids were increased significantly. As a result, CSI did not differ between any treatment regimen and baseline.

Biliary bile salt composition (Table III) differed from baseline in the cholestyramine and combined treatment periods: percentage of glycocholate was increased strongly in both treatment periods compared with baseline whereas both taurine and glycine conjugates of deoxycholic acid and chenodeoxycholic acid were decreased. As a consequence, hydrophobicity index was decreased strongly after treatment with cholestyramine alone or in combination with simvastatin. Simvastatin alone had no influence on bile salt composition. The ratio of taurine/glycine conjugated bile salts decreased strongly during cholestyramine monotherapy and combined treatment. As taurine and glycine conjugates of lithocholic acid together constituted <1% of total bile acids at baseline and during all treatment regimens, these data were omitted in Table III.

Cholesterol crystals in fresh bile

No cholesterol crystals were present in any bile sample, taken at baseline and after each treatment period.

Gall bladder motility

Table IV shows parameters of gall bladder contraction and the Figure shows gall bladder contraction curves. Fasting gall bladder volume increased in seven of eight patients during simvastatin monotherapy, compared with baseline volume (28-7 (2-8), 23-2 (2-3) ml respectively, \( p<0.01 \)). Despite this increase, minimal postprandial volume (\( V_{\text{min}} \) 5-7 (0-9), 5-9 (0-7) ml respectively) did not change. Therefore, both absolute and relative gall bladder emptying (\( \Delta V_{\text{max}} \) ml and \( \Delta V_{\text{max}} \% \)) increased significantly during simvastatin monotherapy. During cholestyramine monotherapy and combined therapy, no significant differences in fasting gall bladder volumes, minimal postprandial volume, and gall bladder emptying were seen.

Discussion

This study was performed to assess the effects of simvastatin and cholestyramine, alone and in combination on biliary lipid composition.
The unchanged CSI under simvastatin in this study is probably not the result of type II error, as CSI decreased in only one patient. The absence of an effect on CSI of cholestyramine monotherapy is in agreement with earlier studies. Under combined treatment with simvastatin and cholestyramine, a decreased percentage of bile salts in duodenal bile was seen. In the case of interruption of the enterohepatic circulation, as under treatment with bile salt sequestrants, maintenance total bile salt pool size depends on de novo bile salt synthesis, which is reflected by enhanced activity of 7α hydroxylase (the rate limiting enzyme in bile salt synthesis) in this situation. HMG-CoA reductase inhibitors have been shown in vitro to reduce the activity of 7α hydroxylase caused by decreased substrate for this enzyme. Therefore bile salt synthesis and secretion may be susceptible for HMG-CoA reductase inhibitors in the case of interrupted enterohepatic circulation. Indeed, in patients with bile fistula, decreased bile salt secretion was seen. Therefore, increased susceptibility of bile salt synthesis for HMG-CoA reductase inhibitors during treatment with cholestyramine may explain the effects on bile salts as found in this study. Despite this decrease in molar percentage of bile salts, CSI under combination therapy was unchanged because of the increased molar percentage of phospholipids. The absence of effects on CSI in any of the treatment regimens is in agreement with the finding that no cholesterol crystals were seen in the bile samples during any treatment.

Simvastatin monotherapy did not affect bile salt pool composition, whereas profound changes in bile salt composition were found under treatment with cholestyramine alone and in combination with simvastatin. In both groups percentage of glycocholic acid was decreased substantially, whereas both tauro and glyco conjugates of chenodeoxy and deoxycholic acid were decreased, resulting in an important reduction of bile salt hydrophobicity index. These findings obviously relate to the preferential binding by cholestyramine of hydrophobic bile salts (deoxycholic acid and chenodeoxycholic acid) as reported in earlier studies and to the preferential neosynthesis of cholic acid over chenodeoxycholic acid by the liver in the case of interrupted enterohepatic circulation. To our knowledge the effects of combination therapy on bile salt composition have not been studied previously. The changes in bile salt composition as seen in this study may be beneficial as hydrophilic bile salts have weaker detergent capacity than more hydrophobic bile salts and as a result may induce less nucleation of cholesterol crystals from biliary vesicles. Moreover, cholesterol absorption from the gut may be decreased during cholestyramine treatment probably related to decreased micellar formation by less hydrophobic bile salts. The increased ratio of glycine/taurine conjugated bile salts may result from loss of taurine in the case of interrupted enterohepatic circulation.

Under simvastatin and combination treatment, fasting gall bladder volume was

### Table II

<table>
<thead>
<tr>
<th>Table II</th>
<th>Biliary lipid composition in eight patients with primary hypercholesterolaemia, treated for four weeks with simvastatin and cholestyramine, alone or in combination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Simvastatin</strong> (20 mg/day)</td>
</tr>
<tr>
<td>CSI</td>
<td>0.99 (0.08)</td>
</tr>
<tr>
<td>Total percentages</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>58 (0.6)</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>16.4 (1.3)</td>
</tr>
<tr>
<td>Bile salts</td>
<td>77.6 (1.7)</td>
</tr>
</tbody>
</table>

*p<0.05 vs baseline. Data shown as mean (SEM).

### Table III

<table>
<thead>
<tr>
<th>Table III</th>
<th>Bile salt composition in eight patients with primary hypercholesterolaemia, treated for four weeks with simvastatin and cholestyramine, alone or in combination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Simvastatin</strong> (20 mg/day)</td>
</tr>
<tr>
<td>Tauroursodeoxycholate (%)</td>
<td>0.49 (0.16)</td>
</tr>
<tr>
<td>Glycoeuroursodeoxycholate</td>
<td>1.91 (0.58)</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>9.67 (0.96)</td>
</tr>
<tr>
<td>Glycocholate</td>
<td>2823 (2.23)</td>
</tr>
<tr>
<td>Taurochenodeoxycholate</td>
<td>9.16 (1.06)</td>
</tr>
<tr>
<td>Taurodeoxycholate</td>
<td>4.69 (1.05)</td>
</tr>
<tr>
<td>Glycochenodeoxycholate</td>
<td>2741 (2.2)</td>
</tr>
<tr>
<td>Glycochenodeoxycholate</td>
<td>1844 (2.73)</td>
</tr>
<tr>
<td>Hydrophobicity index</td>
<td>0.34 (0.01)</td>
</tr>
<tr>
<td>Taurine/glycine conjugated bile salts</td>
<td>0.33 (0.05)</td>
</tr>
</tbody>
</table>

*p<0.001 vs baseline; †p<0.01 vs baseline; †p<0.05 vs baseline; †see text. Data shown as mean (SEM).

### Table IV

<table>
<thead>
<tr>
<th>Table IV</th>
<th>Postprandial gall bladder motility in eight patients with primary hypercholesterolaemia, treated for four weeks with simvastatin and cholestyramine, alone or in combination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Simvastatin</strong> (20 mg/day)</td>
</tr>
<tr>
<td>Fasting volume (Vf) (ml)</td>
<td>22.2 (2.3)</td>
</tr>
<tr>
<td>Minimal volume (Vmin) (ml)</td>
<td>5.9 (0.7)</td>
</tr>
<tr>
<td>Maximal decrease (%)</td>
<td>73 (3)</td>
</tr>
<tr>
<td>Maximal decrease (ml)</td>
<td>17.3 (2.2)</td>
</tr>
</tbody>
</table>

*p<0.01 vs baseline; †p<0.05 vs baseline. Data shown as mean (SEM).
increased compared with baseline, without affecting contractility. The increased fasting gall bladder volume cannot be explained by biliary lipid composition. Also a relation with serum cholesterol concentrations seems unlikely. An explanation could be that simvas-
tatin increases bile salt independent of bile flow by an osmotic effect because after being metabolized it is excreted in the bile for approximately 90%. The absence of effects with four weeks of treatment with cholestyra-
mine on fasting gall bladder volume is in agreement with findings from our group in seven healthy volunteers, treated with 8 g cholestyra-
mine/day. After an initial decrease, fasting gall bladder volume was restored to its pretreat-
ment value after seven days of cholestyramine administration (unpublished data). Appar-
ently, the interference with the negative feed-
back control of intraduodenal bile salts on cholestokinin release, which is the proposed mechanism of gall bladder contraction during short-term administration of cholestyramine, does not occur during longer treatment.

In conclusion, this study suggests that HMG-CoA reductase inhibitors alone and combined with cholestyramine do not affect major determinants of cholesterol gall stone formation, for examples, biliary cholesterol saturation and gall bladder emptying. In addi-
tion cholestyramine alone and combined with simvastatin leads to a strong decrease of bile salt hydrophobicity, which is considered ben-
eficial for the prevention of nucleation of chole-
terol crystals in gall bladder bile.

These data suggest that simvastatin and cholestyramine alone or in combination do not increase the risk of cholesterol gall stone for-
mation.

Dr von Erpeccum is a fellow of the Royal Netherlands Academy of Arts and Sciences.

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