Ursodeoxycholic acid therapy and biliary lipids—a dose-response study

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SUMMARY The effects of four different doses of ursodeoxycholic acid (250, 500, 750, and 1000 mg daily) on biliary lipids were studied in 24 patients with radiolucent gallbladder stones. There was a significant increase in the proportion of ursodeoxycholic acid in bile after all doses, being greatest with 750 and 1000 mg daily. Unsulphated lithocholic acid was also increased in the bile after therapy, while the other major bile acids were reduced. The cholesterol content of bile was reduced by all doses, and this change was most marked after 1000 mg (from 10.9±1.4 to 5.6±0.5 mol %, p<0.01). Biliary cholesterol saturation improved significantly only after 750 and 1000 mg daily.

Ursodeoxycholic acid (UDCA) is the 7β epimer of chenodeoxycholic acid (CDCA). Both agents are known to dissolve cholesterol gallstones. Whereas CDCA has been used in several large trials,1–6 experience with UDCA is less extensive and the two main studies have been in Japanese patients.7,8 Bile in cholesterol gallstone disease contains excess cholesterol, which cannot be held in stable solution by the bile acid-phospholipid micelles.9–14 Drugs which dissolve gallstones reduce the cholesterol content and saturation of bile and this reduction has been used to assess potentially useful agents.15–21 In addition, the extent of changes in bile has been used to estimate optimal dosage of CDCA.2,5,15,16

The data available at present on biliary lipid changes induced by UDCA therapy are either from small numbers of patients who have been treated with increasing doses17 or from single fixed-dose regimens.18,22 There have been no formal dose-response studies to determine the differing efficacies of a range of doses of UDCA.

The present study was designed to compare the effects of four different doses of UDCA on bile chemistry in an attempt to define the minimal and optimal dosage for reducing the cholesterol content and saturation of bile to a level which might be expected to promote the dissolution of gallstones.

Methods

Patients Twenty-four unselected patients with radiolucent stones in functioning gallbladders were included in a dose-response study while undergoing treatment for dissolution of gallstones. The patients were aged from 18 to 76 years and their characteristics are shown in Table 1. The first 12 patients (group A) were allocated to treatment with UDCA 250 and 500 mg daily. The next 12 patients (group B) were allocated to treatment with 750 mg and 1000 mg daily. Each patient received each of the two doses for one month in a predetermined order, so that equal numbers received each dose first. No interruption of therapy was permitted, because this might have prejudiced the eventual outcome, which will be assessed by gallstone dissolution. Compliance was ensured by dispensing capsules in the clinic and inspecting bottles at each visit. All patients were treated with pure UDCA obtained from Tokyo Tanabe Ltd., Japan, which was packed in 250 mg capsules. Patients on 250 mg daily took their dose in the evening, those on 500 and 1000 mg ingested equal twice daily doses, and those on 750 mg daily took one capsule in the morning and two in the evening.

Duodenal intubation was performed after a 12-hour fast, with aspiration of bile-rich fluid after 33 units of cholecystokinin (Boots) IV. A portion of the most concentrated moiety, as judged by bile pigment content, was subjected to Folch extraction for phospholipid analysis immediately after aspiration. The procedure followed thereafter was as described by Murison et al.23 Analysis for bile acids and cholesterol was conducted without solvolysis by a gas-liquid chromatography technique.16 The duodenal aspirate was incubated with cholyl-glycine hydrolase, Sorensen’s buffer and 3(H) glycocholic acid. The product was extracted in ethyl acetate, methy-
lated with freshly prepared diazomethane, and evaporated to dryness under a stream of nitrogen. This residue was incubated with trifluoroacetic anhydride, taken to dryness in the same way, and injected into a GLC column (1% OV 210 on Gas-Chrom Q) with reference standards for the principal bile acids and cholesterol.

The results were expressed as molar percentages of the major lipids. Cholesterol saturation and lithogenic indices were calculated by the formula of Thomas and Hofmann from the data of Admirand and Small, Holzbach, and Hegardt and Dam. To take into account the altered cholesterol-solvent ability of bile enriched with UDCA, the modified lithogenic index of Carey and Ko was also calculated. The proportions of bile acids were expressed as molar percentages of total bile acids. Sulphated bile acids were not measured.

At each intubation, and afterwards at monthly visits to the hospital, blood was taken for estimation of aspartate amino-transferase (AST), \( \gamma \)-glutamyl transpeptidase (\( \gamma \)-GTP), alkaline phosphatase, bilirubin, total protein, albumen, urea, glucose, triglycerides, total and high density lipoprotein (HDL) cholesterol, sodium, potassium, chloride, calcium, phosphate, haemoglobin, white cell count, and platelet count. These were all performed by the routine laboratory service. HDL cholesterol was estimated colorimetrically after manganese-heparin precipitation.

Results were expressed as means ± standard errors. Statistical significance was assessed by use of Student’s paired and unpaired t tests, or by Wilcoxon two-sample and signed-rank tests as appropriate to the distribution of data. Correlation coefficients were calculated between cholesterol content and saturation of bile after therapy, and dosage of UDCA and biliary bile acid ratios.

### Results

There was no difference between the groups of patients studied (Table 1), who were similar for weight, height, and body build.

### Biliary Lipids

There was no significant difference between biliary lipids in group A and group B before UDCA administration (Table 2).

The molar percentage of cholesterol in bile was reduced 28% by 250 mg daily (\( P<0.05 \)), 36% by 500 mg daily, 41% by 750 mg daily (\( P<0.01 \)), and 49% by 1000 mg daily (\( P<0.01 \)) (Table 2). The change after 500 mg daily did not reach statistical significance because of two patients whose biliary cholesterol increased, in one from 9.4 to 17.2 mol%. Total bile acid content of bile was slightly increased after 1000 mg daily (\( P<0.05 \)), but there was no change in phospholipids.

Neither 250 nor 500 mg daily produced a significant reduction in any of the indices of cholesterol saturation, though a trend to decrease was apparent (Table 2). By contrast 750 mg daily produced significant and 1000 mg daily highly significant changes both in the saturation index and the uncorrected lithogenic index. The mean lithogenic index after 1000 mg daily (0.81±0.05) was significantly lower than the mean lithogenic index after 250 mg daily (1.07±0.12, \( P<0.05 \)). However, when the lithogenic index was corrected for the UDCA content of bile, by subtraction from the calculated equilibrium cholesterol of the molar percentage biliary UDCA×0.037 as proposed by Carey and

### Table 1 Characteristics of patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Men</th>
<th>Women</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>Body mass index (wt/ht²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>2:10</td>
<td>71.9±4.5</td>
<td>1.61±0.007</td>
<td>27.8±0.5</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>1:11</td>
<td>72.0±3.9</td>
<td>1.62±0.015</td>
<td>27.4±1.5</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2 Change in biliary lipids and saturation indices after four doses of ursodeoxycholic acid

<table>
<thead>
<tr>
<th>Daily dose (mg)</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>250 (7.5 mg/kg)</td>
</tr>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Cholesterol (mol%)</td>
<td>9.4±1.0</td>
<td>6.8±0.9*</td>
</tr>
<tr>
<td>Bile acids (mol%)</td>
<td>67.7±2.5</td>
<td>72.0±2.3</td>
</tr>
<tr>
<td>Phospholipids (mol%)</td>
<td>22.9±1.8</td>
<td>21.2±1.9</td>
</tr>
<tr>
<td>Saturation index (Admirand and Small)</td>
<td>0.96±0.09</td>
<td>0.71±0.08</td>
</tr>
<tr>
<td>Lithogenic index (Holzbach/Hegardt and Dam)</td>
<td>1.34±0.12</td>
<td>1.07±0.12</td>
</tr>
<tr>
<td>Lithogenic index (Carey and Ko)</td>
<td>1.35±0.12</td>
<td>1.29±0.15</td>
</tr>
</tbody>
</table>

*\( P<0.05 \)  
†\( P<0.01 \)  
‡\( P<0.001 \)  

versus initial values, paired t test.
Table 3  Change in biliary bile acids after four doses of ursodeoxycholic acid (mol% total bile acids)

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>250</td>
</tr>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>2.3±0.3</td>
<td>26±2.2</td>
</tr>
<tr>
<td>Lithocholic acid</td>
<td>2.3±0.4q</td>
<td>3.0±2*</td>
</tr>
<tr>
<td>Cheno deoxycholic acid</td>
<td>43±0.27</td>
<td>30±1.3*</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>29±2.1</td>
<td>21±0.2</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>21±2.2</td>
<td>19±0.3</td>
</tr>
</tbody>
</table>

qExcludes one patient with abnormal initial lithocholate (18.5 mol%). *
*p<0.05 vs all initial values, Wilcoxon signed rank sum test.
†p<0.01 vs all 24 initial values, Wilcoxon two-sample test.

Ko,26 only 1000 mg daily produced a significant fall. Even then the mean value after treatment was not less than 1 (1.15±0.09).

There were striking increases in the UDCA content of bile after therapy, which were most marked (and similar) after 750 and 1000 mg daily (Table 3). The molar percentages of UDCA in bile after these two higher doses were significantly greater than after 250 mg daily (p<0.001 and p<0.001 respectively) or after 500 mg daily (p<0.01 and p<0.05). There was a good correlation between daily dose and molar percentage of UDCA and UDCA + CDCA in bile (r=0.62, p<0.001 and r=0.36, p<0.01 respectively), and this was also seen when daily dose/unit body weight was compared with biliary UDCA and UDCA + CDCA (r=0.73, p<0.001 and r=0.42, p<0.01).

One patient had an inexplicably raised lithocholic acid content of bile before therapy (18.5 mol%, >10 standard deviations from mean of 2.3 mol%). Her values fell after treatment, but were excluded from calculations because she was clearly unrepresentative. Otherwise all doses of UDCA were found to produce increased amounts of biliary unsulphated lithocholic acid (Table 3).

The proportion of chenodeoxycholic acid in bile was reduced by all doses of UDCA, that of cholic acid by doses ≥500 mg daily, and that of deoxycholic acid by doses >750 mg daily.

The molar percentage of UDCA in bile correlated weakly with the uncorrected lithogenic index (r=0.26, p<0.05). There was no other correlation between molar percentage UDCA, molar percentage UDCA + CDCA, daily dose or dose/unit body weight on the one hand and cholesterol content or any index of cholesterol saturation on the other.

**Blood tests**

Results were available over a period of six months and are summarised in Table 4. There was a trend to a fall of about 20% in mean γGTP levels, which did not reach statistical significance. Mean AST levels did not change. Simultaneous isolated peaks in both γGTP and AST were observed on single occasions in two patients who had recurrent biliary symptoms at the time.

There was a reduction in serum creatinine, which was significant at six months (from 89±6.0 to 77±4.6 μmol/l, p<0.02), and an increase in platelets which was also significant at six months (from...
220±20 to 261±24×10^9/l, p<0.05). No other changes were detected in serum electrolytes, liver function tests, lipids, glucose, or haematology.

Three patients treated with UDCA 250 and 500 mg daily had initial hypertriglyceridaemia: this was not improved by therapy and their biliary lipid response was similar to the other patients.

**Discussion**

Chenodeoxycholic acid is known to dissolve radiolucent gallstones in a proportion of patients. However, problems with its use have included the requirement for large doses, a tendency to diarrhoea which has been a major limitation on therapy, and the observation that during therapy there is a rise of mean serum AST levels. We have also found that blood glucose levels are increased, but this has not been reported by others. If UDCA could be shown to be free of any or all of the above effects it might prove to be more useful than CDCA in the treatment of cholesterol-containing gallstones.

The most meaningful index of cholesterol saturation in bile in relation to gallstone dissolution remains to be determined. This issue is important when potential gallstone-dissolving agents are being assessed. The saturation index derived from the data of Admirand and Small includes metastable bile with truly stable solutions. Although gallstones are unlikely to form in metastable bile, they may persist in such bile once they have formed. There is a wide interindividual range in the saturation indices obtained from spot samples of gallbladder bile. Indices less than 1 can be encountered in individual samples from cholesterol gallstone patients, while bile from patients without stones may have a saturation index greater than 1.

The lithogenic index derived from the data of Hegardt and Dam and Holzbach discriminates between a true micellar solution of cholesterol and both metastable and unstable bile. It is usual to find that individual bile samples from gallstone patients have a lithogenic index >1 if allowance is made for the concentration of bile. If the lithogenic index of bile can be reduced consistently below this level by drugs then it would be reasonable to expect that cholesterol from a gallstone will be taken up in micellar solution and stone dissolution should occur. Agents which are known to dissolve gallstones have the ability to produce a decreased cholesterol content and saturation in the bile. It has been suggested that the optimal dosage of CDCA can be inferred from the improvement in cholesterol saturation, but we and others have been unable to confirm the reported correlation between achievement of bile unsaturation and gallstone dissolution in individuals. Therefore our findings that daily doses of 750 and 1000 mg UDCA produce a significant reduction in the indices of cholesterol saturation cannot be taken in themselves as indicating that these are optimal doses for gallstone dissolution for individuals. It will be necessary to relate the various doses to outcome of therapy as judged by gallstone disappearance and this study is at present under way.

The different bile acids have different abilities to solubilise cholesterol. In particular, UDCA has a markedly inferior capacity to form bile acid-cholesterol micelles compared with other bile acids in bile. Thus cholesterol saturation cannot easily be assessed accurately in bile which has been enriched with UDCA. Some correction for the content of UDCA is necessary when calculating the lithogenic index. Ideally this is calculated for each individual bile sample with allowance for total lipid concentration. This is not feasible for duodenal aspirates which contain bile that has been diluted by varying degrees. A simple correction factor for the equilibrium concentration of cholesterol in a given bile acid-phospholipid solution has been proposed. From this a 'UDCA-corrected' lithogenic index can be calculated to give an approximation of the true ability of the bile to hold cholesterol in solution. Whether this newer concept will be more accurate in predicting gallstone dissolution and how closely will be shown to correlate with individual results has yet to be established.

Maton et al. and Makino and Nakagawa, using 5 mg/kg/day and 150 or 600 mg daily, found significant falls in the uncorrected lithogenic indices at lower doses than we report. Although a trend to reduction can be distinguished on the lower doses on our study, it was only the larger doses which produced significant changes. We did not find any evidence that dose/unit body weight correlated better than absolute daily dose with cholesterol content and saturation of bile. The different patterns of bile acids in bile after UDCA and CDCA treatment make direct comparison of the effects of these agents difficult. However, the largest reduction in the molar proportion of cholesterol in bile that we encountered was after 750 and 1000 mg UDCA daily, which was similar to the changes which we have reported with 1000 mg of CDCA daily. Also found 1000 mg UDCA daily as effective as 1000 mg CDCA daily in a crossover study of predominantly non-gallstone patients to whom the bile acid was fed for two weeks; but Stiehl et al. also found 750-1250 mg UDCA daily to be superior to similar doses of CDCA administered to gallstone patients over a three month period.

The progressive increase in biliary UDCA (Table 3) with progressively larger doses (with a concomi-
tant reduction in the other major bile acids) agrees well with the observations of Maton et al., Makino and Nakagawa, and Stiehl et al. Our finding of an increase in unsulphated lithocholic acid in bile was also demonstrated by Makino and Nakagawa after daily doses of both 150 and 600 mg UDCA, but these authors found that the amounts remained small and the changes did not reach statistical significance. By contrast Fedorowski et al. and Stiehl et al. did not detect any increase in either sulphated or unsulphated lithocholic acid after 750–1250 mg daily UDCA. The reason for these differences is not clear. It is probable that there is direct conversion of UDCA to lithocholic acid, which becomes the main faecal bile acid after UDCA therapy.31

Any increase in biliary unsulphated lithocholic acid is potentially hepatotoxic.32,33 Despite this theoretical hazard, there is a reassuring absence in humans of histological abnormality after UDCA therapy17 and serum lithocholic acid levels seem not to increase after UDCA therapy.23 UDCA does not produce the liver damage seen in Rhesus monkeys with CDCA.34 Abnormalities of those blood tests considered to reflect liver function were not seen in Japanese patients treated with UDCA but falls in yGTP, AST, and alkaline phosphatase have been observed in British patients.17 We observed no overall change in mean AST levels, and, while there was a reduction in yGTP concentrations, it did not reach statistical significance. Whereas we found that an alteration in bowel habit frequently required CDCA treatment to be adjusted, none of these 24 patients developed diarrhoea. This difference between the behaviour of dihydroxy bile acids could be because there is rapid conversion of UDCA to lithocholic acid, or possibly because UDCA does not induce the increased colonic water secretion and permeability known to be induced by CDCA.35

UDCA is likely to exert its action by an effect within the enterohepatic circulation of bile acids because it is here that it is concentrated. It could act by increasing the total amount of bile acid in gallbladder bile relative to phospholipids and cholesterol, but this seems unlikely to be important in view of the absence of a decrease in the proportion of phospholipid and the fact that only a very slight increase in biliary bile acid was noted by us and by other groups. A possible mode of action is via an alteration of cholesterol metabolism either from impaired intestinal absorption, or by reduced hepatic excretion after an increased conversion to bile acids, or by reduced synthesis either in the liver or the intestine. There is some evidence to suggest that hepatic cholesterol synthesis is reduced, and this could prove to be the mechanism for the improved cholesterol solubility in bile after UDCA administration.

The role of UDCA for the dissolution of radiolucent gallstones will need to be determined by assessment of its efficacy in long-term clinical studies. This bile acid has been reported to be at least as good as CDCA in Japanese patients and biliary lipid analysis shows that small doses of UDCA have a profound effect on biliary cholesterol. There are insufficient data to make a comparison possible in Caucasians either with CDCA or with the recently publicised agent Rowachol.36 At present, CDCA should be regarded as the agent of first choice for dissolution of gallstones because it has been extensively evaluated, but it is very costly. The manufacture of UDCA is also complex and it is even more expensive to produce. The only inexpensive agent which has been shown to dissolve gallstones is the relatively untried Rowachol.36 However, the acceptability and absence of overt toxicity of UDCA therapy make it an attractive alternative to CDCA for the dissolution of gallstones.

Thanks are due to Mrs N Spain for typing the manuscript, to Miss A McDonald and Mr A Hill for skilled technical assistance, and to Tokyo Tanabe Ltd for generously supplying ursodeoxycholic acid.

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Ursodeoxycholic acid therapy and biliary lipids--a dose-response study.

M C Bateson, P E Ross, J Murison, J H Saunders and I A Bouchier

*Gut* 1980 21: 305-310
doi: 10.1136/gut.21.4.305

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