Profiles of bile acids and their glucuronide and sulphate conjugates in the serum, urine and bile from patients undergoing bile drainage

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SUMMARY Bile acid profiles in serum, urine, and bile from patients undergoing bile drainage and the changes of serum bile acids after bile drainage were studied. Bile acids were separated into non-glucuronidate-non-sulphate, glucuronidated, and sulphated fractions and were measured by mass fragmentography using conjugates of deuterium labelled bile acids as internal standards. Glucuronidated and sulphated bile acids contribute 14–32% and 16–44% of serum bile acids, 4–11% and 61–82% of urine bile acids and 0·2–1% and 0·3–2% of biliary bile acids respectively. After bile drainage the concentration of serum non-glucuronidated-non-sulphated bile acids decreased more rapidly than glucuronidated and sulphated bile acids. There was little biliary excretion of the glucuronidated and sulphated bile acids. Such conjugation appears to have a role in facilitating bile acid excretion by the urinary route.

Bile acid glucuronides have been estimated in plasma, urine, and bile of patients with cholestasis and the malabsorption syndrome. There are few studies of the fate of bile acid glucuronides after biliary drainage. From a study of the changes of serum bile acids after bile drainage, Eklund et al reported that the sulphated bile acids decreased more slowly than the non-sulphated bile acids and the excretion of sulphated bile acids into bile was low. In our previous study, a microassay method for bile acid glucuronide measurements involving highly sensitive mass fragmentography was described. The loss of bile acids during the analytical procedure was compensated for by the use of conjugates of deuterium labelled bile acids as internal standards.

Using this method, bile acid concentrations were studied in simultaneously obtained serum, urine, and bile of patients undergoing bile drainage. The changes in serum bile acids concentration after bile drainage were also studied and their usefulness for clinical evaluation is discussed.

Methods

Patients
Bile acids were measured in serum, urine, and bile simultaneously obtained from the four patients undergoing bile drainage listed in Table 1. Blood samples were collected after overnight fasting and serum was stored at −20°C until analysis. Urine and bile were collected in 24 h regimens and also stored at −20°C. In two patients percutaneous transhepatic bile drainage was performed with an ultrasonically-guided technique and, in the other two cases, T-tubes were placed operatively into the common bile duct.

The four patients with obstructive jaundice described in Table 2 were studied to observe the changes of serum bile acids after percutaneous transhepatic bile drainage. All patients were shown to have complete obstruction of the common bile duct as shown by cholangiography. Serum bilirubin for concentration decreased to the normal range within two or three weeks after percutaneous transhepatic bile drainage.

Bile Acid Analysis
The method for analysis of bile acids has been described previously. Known amounts of (11,11,12-2H₃)deoxycholic acid and its

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Profiles of bile acids and their glucuronide and sulphate conjugates

Table 1  Details of the patients undergoing bile drainage for analysis of bile acids in serum, urine and bile

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (sex)</th>
<th>Clinical diagnosis</th>
<th>Methods of bile drainage</th>
<th>Days after drainage</th>
<th>Serum bilirubin concentration (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69 (M)</td>
<td>Choledochal cancer</td>
<td>PTBD</td>
<td>45</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>54 (M)</td>
<td>Pancreas head cancer</td>
<td>PTBD</td>
<td>10</td>
<td>221</td>
</tr>
<tr>
<td>3</td>
<td>71 (F)</td>
<td>Pancreas head cancer</td>
<td>T-tube</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>65 (M)</td>
<td>Choledochal stone</td>
<td>T-tube</td>
<td>50</td>
<td>7</td>
</tr>
</tbody>
</table>

PTBD: percutaneous transhepatic bile drainage

glucuronidated and sulphated conjugates, and glucuronidated (2,2,4,4–2H₄)lithocholic acid were added to serum (0.5–1 ml), urine (0.5–5 ml) and bile (0.2 ml of 100 × diluted samples) as internal standards, and the bile acids extracted using Sep-Pak C₁₈ cartridges. After enzymatic hydrolysis of amide bonds, bile acids were separated into non-glucuronidated-non-sulphated, glucuronidated and sulphated fractions by piperidino-hydroxypropyl Sephadex LH-20 column chromatography. The glucuronidated bile acids were treated with β-glucuronidase and the sulphated bile acids were svolysed by the method of Parmentier and Eyssen. The bile acids in each fraction were measured by mass fragmentography as the hexafluoroisopropyl-trifluoroacetyl derivatives.

The biliary bile acids determined in case 5 were only non-esterified bile acids, measured by a simplified method using mass fragmentography.

Results

Bile acid concentrations and percentages of each conjugate in serum, urine, and bile of patients undergoing bile drainage (cases 1–4) are shown in Table 3. The glucuronidated and sulphated bile acids in serum comprised 14–32% and 16–44% of the total bile acids. In urine 4–11% of bile acids were glucuronidated and the major proportion were sulphated bile acids (61–82%). The predominant biliary bile acids were non-glucuronidated-non-sulphated, only 0.2–1% and 0.3–2% were glucuronidated and sulphated.

Cholic acid and chenodeoxycholic acid were the major bile acids in serum, urine, and bile. 3β-hydroxy-5-cholenoic acid comprised 5–25% of serum bile acids and was mostly in the glucuronidated or sulphated form. In urine, 3β-hydroxy-5-cholenoic acid was present as 11–60% of the total bile acids, most of which was sulphated. Only a trace amount of 3β-hydroxy-5-cholenoic acid was seen in bile (0.2–0.8%), most of which was sulphated.

The changes of serum bilirubin concentrations, bile acid excretion into bile, and the serum concentrations of each conjugate of total bile acids, chenodeoxycholic acid and 3β-hydroxy-5-cholenoic acid after percutaneous transhepatic bile drainage in case 5, are shown in Fig. 1. Serum bilirubin concentrations gradually decreased after percutaneous transhepatic bile drainage. About 1–6 l of bile was drained daily and the biliary bile acid excretion gradually increased. Almost all of the biliary bile acids were cholic acid and chenodeoxycholic acid.

The concentration of serum bile acids before percutaneous transhepatic bile drainage in case 5 was 130.8 μmol/l, most of which was non-glucuronidated-non-sulphated (98.9 μmol/l). The glucuronidated and sulphated bile acids comprised only a small proportion (7.0 and 24.8 μmol/l). A day after percutaneous transhepatic bile drainage, serum bile acid concentrations had decreased to 16.8 μmol/l largely as a result of a decrease in the non-glucuronidated-non-sulphated bile acids to the normal range (1.4 μmol/l). The glucuronidated and sulphated bile acids showed only a small decrease compared with the non-glucuronidated-non-sulphated bile acids (5.8 and 9.6 μmol/l). Three days after percutaneous transhepatic bile drainage, the glucuronidated and sulphated bile acids had decreased to a concentration similar to that of non-glucuronidated-non-sulphated bile acids. The three fractions of bile acids remained at almost the same concentration after the third day.

The serum concentration of chenodeoxycholic acid before percutaneous transhepatic bile drainage
Table 3  Individual bile acid concentrations in serum, urine and bile of patients undergoing bile drainage

<table>
<thead>
<tr>
<th>Case</th>
<th>Lithocholic acid</th>
<th>Deoxycholic acid</th>
<th>Chenodeoxycholic acid</th>
<th>Ursodeoxycholic acid</th>
<th>Cholic acid</th>
<th>3β-hydroxy-5-cholenoic acid</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum bile acids (μmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>nd</td>
<td>0.08 (0.0/0.100)</td>
<td>0.69 (50.40/10)</td>
<td>nd</td>
<td>0.28 (96/0.4)</td>
<td>0.21 (9/0.6)</td>
<td>1.26 (51.3/0.2)</td>
</tr>
<tr>
<td>2</td>
<td>0.32 (1.5/0.49)</td>
<td>0.23 (0.0/0.100)</td>
<td>12.40 (18/17/2)</td>
<td>0.44 (0.0/0.100)</td>
<td>5.61 (83/9/8)</td>
<td>6.82 (3/38/59)</td>
<td>25.82 (29/27/44)</td>
</tr>
<tr>
<td>3</td>
<td>0.21 (0.9/0.91)</td>
<td>0.75 (28/12/60)</td>
<td>2.05 (48/17/34)</td>
<td>0.38 (0.0/0.100)</td>
<td>0.86 (90/5/6)</td>
<td>0.03 (10/53/37)</td>
<td>4.58 (44/14/41)</td>
</tr>
<tr>
<td>4</td>
<td>0.20 (3/0.97)</td>
<td>nd</td>
<td>0.86 (60/21/19)</td>
<td>nd</td>
<td>0.90 (8/9/0)</td>
<td>0.11 (2/1/99)</td>
<td>2.07 (2/1/99)</td>
</tr>
<tr>
<td>Urinary bile acids (μmol/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.02 (0.0/0.100)</td>
<td>0.04 (35/53/12)</td>
<td>0.25 (7/72/71)</td>
<td>nd</td>
<td>0.11 (69/7/4)</td>
<td>0.67 (7/2/92)</td>
<td>1.09 (14/11/74)</td>
</tr>
<tr>
<td>2</td>
<td>0.61 (0.0/0.100)</td>
<td>0.67 (67/87)</td>
<td>25.74 (60/17/64)</td>
<td>0.25 (0.0/0.100)</td>
<td>7.40 (90/6/5)</td>
<td>7.21 (10/58/95)</td>
<td>41.88 (9/7/82)</td>
</tr>
<tr>
<td>3</td>
<td>1.06 (1/0.99)</td>
<td>2.15 (6/49/0)</td>
<td>1.99 (4/5/91)</td>
<td>0.94 (0/0/100)</td>
<td>1.34 (7/9/9)</td>
<td>1.00 (10/189)</td>
<td>8.48 (17/4/79)</td>
</tr>
<tr>
<td>4</td>
<td>0.12 (0.0/0.100)</td>
<td>0.11 (26/27/22)</td>
<td>0.77 (4/29/4)</td>
<td>0.01 (0/0/20)</td>
<td>0.94 (85/8/7)</td>
<td>0.85 (10/2/88)</td>
<td>2.80 (34/1/61)</td>
</tr>
<tr>
<td>Biliary bile acids (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.08 (45/0.48)</td>
<td>0.18 (0/17/83)</td>
<td>88.66 (99/2/2)</td>
<td>nd</td>
<td>70.40 (99/4/-2)</td>
<td>0.28 (0/18/82)</td>
<td>159.60 (99/3/4)</td>
</tr>
<tr>
<td>2</td>
<td>0.15 (74/0.26)</td>
<td>0.21 (0/69/4)</td>
<td>14.07 (98/4/2)</td>
<td>nd</td>
<td>27.14 (98/1/7)</td>
<td>0.34 (0/50/50)</td>
<td>41.91 (97/1/2)</td>
</tr>
<tr>
<td>3</td>
<td>1.30 (37/0.63)</td>
<td>18.83 (93/4/6)</td>
<td>77.17 (99/-1/-7)</td>
<td>nd</td>
<td>106.02 (99/-1/-5)</td>
<td>0.43 (0/20/80)</td>
<td>203.75 (98/-2/2)</td>
</tr>
<tr>
<td>4</td>
<td>0.78 (83/0.17)</td>
<td>0.25 (0/35/65)</td>
<td>70.89 (99/-2/0)</td>
<td>nd</td>
<td>116.41 (99/-2/0)</td>
<td>0.36 (0/31/69)</td>
<td>188.69 (99/4/-3)</td>
</tr>
</tbody>
</table>

In parentheses are shown the percentages of non-glucuronidated-nond-sulphated, glucuronidated and sulphated bile acids, respectively. nd: not detected.

in case 5 was 83.6 μmol/l and the major proportion (60.4 μmol/l) was non-glucuronidated-non-sulphated. The non-glucuronidated-non-sulphated chenodeoxycholic acid had rapidly decreased to 0.7 μmol/l a day after percutaneous transhepatic bile drainage, whereas the glucuronidated and sulphated chenodeoxycholic acid decreased slowly. The three fractions of chenodeoxycholic acid decreased to almost the same concentrations after the third day of percutaneous transhepatic bile drainage. Before percutaneous transhepatic bile drainage 3β-hydroxy-5-cholenoic acid was mostly glucuronidated or sulphated and the three conjugates of this acid showed a slow decrease after percutaneous transhepatic bile drainage.

The changes of serum bilirubin and bile acid values after percutaneous transhepatic bile drainage in cases 6, 7, and 8 are shown in Figure 2. Bile volumes drained by percutaneous transhepatic bile drainage were about 0.4 1/day in cases 6 and 7, and 0.7 1/day in case 8. In case 6, the non-glucuronidated-non-sulphated bile acids (379.3 μmol/l) comprised a major part of the total bile acids (459.7 μmol/l) before percutaneous transhepatic bile drainage and rapidly decreased to 2.9 μmol/l, although the glucuronidated and sulphated bile acids slowly decreased. Also in case 7, only the non-glucuronidated-non-sulphated bile acids rapidly decreased from 363.1 to 2.1 μmol/l after percutaneous transhepatic bile drainage. In case 8, the decrease of total bilirubin was slower than in the other three cases and serum bile acids also slowly decreased to 67.1 μmol/l three days after percutaneous transhepatic bile drainage. The non-glucuronidated-non-sulphated bile acids also decreased faster than the glucuronidated and sulphated bile acids.

Discussion

Bile drainage reduces jaundice in patients with extrahepatic bile duct obstruction. During bile drainage, the enterohepatic circulation of bile acids remains interrupted, but the bile acid synthesis in
Profiles of bile acids and their glucuronide and sulphate conjugates

Fig. 1  Daily changes of bile volume, serum total bilirubin level (T Bil) and biliary bile acid concentration (upper), and the changes of each conjugate of total bile acids (TBA) (middle), chenodeoxycholic acid (CDC) and 3β-hydroxy-5-cholenoic acid (3β-Δ^5) (lower) in serum of case 5 after PTBD. CA, cholic acid; NG-NS and non-G-non-S, non-glucuronide-non-sulphate; G, glucuronide; S, sulphate.

Fig. 2  The changes of total bilirubin level (T Bil) and each conjugate of total bile acids (TBA) in serum of cases 6, 7 and 8 after PTBD.

Liver decreases because of the lack of bile acids returning to the liver through the portal vein. In the present study, the percentages of glucuronidated and sulphated bile acids in the serum of patients undergoing bile drainage were higher than those of control subjects and patients with hepatobiliary diseases (Table 3). This may be explained by increased bile acid synthesis and only non-esterified bile acids being efficiently excreted into bile.

The sulphated bile acids were excreted into urine. The present study shows that glucuronidated (mostly chenodeoxycholic acid and 3β-hydroxy-5-cholenoic acid) and non-esterified bile acids (mostly cholic acid) are excreted into urine was at almost the same rate. A previous report had shown that the renal clearance of glucuronidated bile acids will be higher than that of non-esterified bile acids.

The glucuronidated and sulphated bile acids in bile were present at very low concentrations compared with in serum. The excretion of these conjugated into bile may occur at a low rate. The excretion of 3β-hydroxy-5-cholenoic acid into bile may occur at a low rate and this may also explain the high serum concentration of 3β-hydroxy-5-cholenoic acid.

The decrease in concentration of non-esterified bile acids was rapid and a normal concentration was reached a day after percutaneous transhepatic bile drainage in three cases, when more than 90% of serum bile acids was either glucuronidated or sulphated. This is of importance for the accurate determination of bile acids, for example, by the enzymatic fluorimetric method.

In case 8, the initial excretion of bile acids into
bile was slower than the other cases and the glucuronidated and sulphated bile acids were effectively excreted into urine. So, the three conjugates apparently decreased almost at the same rate. The non-esterified chenodeoxycholic acid also decreased more rapidly than the glucuronidated and sulphated bile acids (Fig. 1).

From our study of the changes of serum bile acids after bile drainage, not only the sulphated bile acids as reported by Eklund et al., but also the glucuronidated bile acids in serum decreased much more slowly than the non-esterified bile acids. The different rate of decline in the concentration of each conjugate may be because of the different biliary excretion rates of these conjugates. Another explanation might be that glucuronidated and sulphated bile acids preferentially accumulate in tissue and hence reduction to normal serum concentrations takes longer. Another explanation is that the increase in synthesis during drainage is confined to non-esterified bile acids and, therefore, non-esterified biliary bile acids apparently increase.

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