Radioimmunoassay of ursodeoxycholic acid in serum

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Abstract   A sensitive and specific radioimmunoassay for the measurement of serum ursodeoxycholic acid has been developed. Ursodeoxycholic acid bound to bovine serum albumin was used as an antigen, and antiserum to this antigen was raised in the rabbit. \[^{1,12-3H_2}\text{Ursodeoxycholic acid}\ was used as the radioactive tracer, and the radioimmunoassay was carried out by the method of Simmonds et al. (1973. Gastroenterology. 65: 705-711). The percentage of bound radioactivity decreased linearly with a logarithmic increase in unlabeled ursodeoxycholic acid from 10 to 200 pmol. The antiserum showed extremely high specificity for ursodeoxycholic acid (free and conjugated), and the values determined by radioimmunoassay indicated a close correlation with those found by gas-liquid chromatography. In normal Japanese subjects, a small amount of ursodeoxycholic acid in serum was detected, and the level was 0.15 ± 0.11 nmol/ml. This convenient radioimmunoassay will provide useful information about the metabolism of ursodeoxycholic acid in man.

MATERIALS AND METHODS

Reagents

All reagents were analytical grade. Nonradioactive UDCA was synthesized by Tokyo Tanabe Company (Tokyo, Japan) and was better than 99% pure by gas-liquid and thin-layer chromatography. \[^{[1,12-3H_2}]\text{UDCA}\, sp\ act\ 2.36\ mCi\ per\ \mu\text{mol}\], was kindly supplied by Tokyo Tanabe Company and was better than 99% pure by thin-layer chromatography and zonal scanning. Bovine serum albumin (BSA) was obtained from Armour Laboratories (Kankakee, IL.).

Antigen

The UDCA-BSA conjugate was prepared by the method of Erlanger et al. (6). Briefly the procedure was as follows: 392 mg of UDCA was dissolved in 20 ml of dioxane at room temperature, and 203 mg of tri-n-butylamine was added followed by 185 mg of isobutylchloroformate that was added with stirring and cooling (near 12°C). Thirty minutes later, the mixture was added to 1.4 g of BSA in 50 ml of 50% aqueous dioxane containing 2 ml of 1 N NaOH. After 2 hr, another 1 ml of 1 N NaOH was added and stirring was continued overnight at room temperature. Dioxane was removed under reduced pressure at 20°C, and the resulting solution was dialyzed against cold running water for 2 days. Lyophilization of the turbid protein solution gave 1.2 g of conjugate.

Gas-liquid chromatographic methods have usually been employed for analyzing serum bile acids, but these methods are complex and require a relatively large amount of serum (4). Recently, Simmonds et al. (5) established a radioimmunoassay with sufficient sensitivity for estimating serum conjugated cholic acid, and this method seems to be useful for detecting other serum bile acids. This report describes a radioimmunoassay for serum UDCA.

Supplementary key words: serum bile acids · gas–liquid chromatography · hepatic disease

Abbreviations: UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; BSA, bovine serum albumin.

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Antiserum

Antiserum was prepared by the method of Simmonds et al. (5). Freshly prepared UDCA–BSA complex in 0.01 M potassium phosphate buffer was emulsified with an equal volume of Freund’s complete adjuvant (Difco Laboratories, Detroit, MI); the final concentration was 400 μg of UDCA–BSA complex per ml. A known volume (1.2 ml) of this mixture was injected intracutaneously, in divided doses, into the back and each of the four legs of a rabbit. The antigen was injected at 2-week intervals. After 4 months of immunization, antiserum with titer above 40% binding of added radioactive tracer at 1∶320 dilution was obtained for the four rabbits that were immunized.

Radioimmunoassay

[11,12-3H]UDCA was used as a tracer. Bile acid-free serum was prepared by charcoal extraction according to the method of Simmonds et al. (5). Radioimmunoassay was carried out by the procedure described by Simmonds et al. (5); the assay system consisted of 0.1 ml of charcoal-extracted human serum (dilution 1∶2), 0.1 ml of unlabeled UDCA (2.00–0.10 nmol/ml) as standard or unknown serum sample, 0.1 ml of antiserum (dilution 1∶160), 0.1 ml of [11,12-3H]UDCA (0.02 μCi/ml), and 0.6 ml of buffer. The components of the incubation mixture were diluted with or dissolved in 0.01 M potassium phosphate buffer pH 7.4. The tubes were incubated at 42°C for 1 hr, and placed at 4°C for 45 min. To the reaction mixture was added 0.5 ml of 37.5% polyethylene glycol (mol wt 6000) solution (w/v); it was allowed to stand for 10 min, and then the tubes were centrifuged at 1200 g at 4°C for 30 min. The supernatant was decanted into a scintillation vial and 15 ml of a dioxane-based scintillation fluid was added. Radioactivity was measured by liquid scintillation. Bound radioactivity was calculated by subtracting the percentage activity in the supernatant in the presence of antibody from 100% free value for the supernatant in the absence of antibody.

Subjects

Ten healthy persons, 9 patients with gallstone undergoing UDCA treatment (oral, 450 mg/day), and 33 patients with hepatic diseases were used in this study. The normal subjects did not have hepatobiliary diseases; they were selected from medical staff of our clinic. Of the group of patients with hepatic disease, 27 had chronic hepatitis and 6 had compensated liver cirrhosis. Blood samples were obtained in the morning before breakfast after overnight fasting; separated sera were kept frozen at −20°C.

RESULTS

Binding of UDCA to the antiserum

Fifty-six percent of radioactive tracer was bound by a 1∶320 dilution of antiserum. The percentage of bound radioactivity decreased linearly with the dilution of antiserum on a logarithmic scale from 1∶80 to 1∶2560. The percentage of bound radioactivity decreased linearly, with a logarithmic increase in unlabeled UDCA concentration from 10 to 200 pmol (Fig. 1).

Specificity

The percent cross reactivity of antiserum with other bile acids was calculated at 50% displacement of [11,12-3H]UDCA. As shown in Table 1, this UDCA antiserum possessed an extremely high specificity for UDCA, glyco-UDCA, and tauro-UDCA. However, it should be noted that cross reactivity of conjugated UDCA was almost twice as much as that of free UDCA. Therefore, a glyco-UDCA standard curve was used for the determination of serum UDCA because serum bile acid is mainly composed of conjugate.

Precision and accuracy of assay

Two serum samples (0.81 and 0.60 nmol/ml) were diluted with 1, 3, and 7 volumes of buffer, respectively, and the reproducibility of measurement was tested with a glyco-UDCA standard curve. The results are indicated in Fig. 2. The coefficient of variation was
TABLE 1. Cross reactivity of bile acids with the antiserum

<table>
<thead>
<tr>
<th>Bile Acid</th>
<th>Cross Reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ursodeoxycholic</td>
<td>100</td>
</tr>
<tr>
<td>Glycoursodeoxycholic</td>
<td>180</td>
</tr>
<tr>
<td>Tauroursodeoxycholic</td>
<td>180</td>
</tr>
<tr>
<td>Chenodeoxycholic</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Glycochenodeoxycholic</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Taurochenodeoxycholic</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cholic</td>
<td>none</td>
</tr>
<tr>
<td>Glycocholic</td>
<td>none</td>
</tr>
<tr>
<td>Taurocholic</td>
<td>none</td>
</tr>
<tr>
<td>Lithochoic</td>
<td>0.2</td>
</tr>
<tr>
<td>Glycolithochoic</td>
<td>0.2</td>
</tr>
<tr>
<td>Deoxycholic</td>
<td>none</td>
</tr>
<tr>
<td>Progesterone</td>
<td>none</td>
</tr>
<tr>
<td>Cortisol</td>
<td>none</td>
</tr>
<tr>
<td>Estradiol</td>
<td>none</td>
</tr>
<tr>
<td>Testosterone</td>
<td>none</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>none</td>
</tr>
</tbody>
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4% within assay and 10% between assays. Recovery was studied by the addition of various amounts of UDCA (20–500 pmol/ml serum) to two serum samples with a free UDCA standard curve; the recovery ranged from 95 to 120% (Fig. 3).

Assay of human serum

As mentioned above, a glyco-UDCA standard curve was used for the assay of human serum. Thirteen serum samples, four from normal subjects and nine from patients with gallstones during UDCA therapy (oral, 450 mg/day), were analyzed by radioimmunoassay and gas–liquid chromatography [according to the method of Sandberg et al. (4)]. As indicated in Fig. 4, the values by radioimmunoassay showed a close correlation to those by gas–liquid chromatography, and the coefficient of this relation-

Fig. 2. Dilution study. This study was carried out using glyco-UDCA for preparation of the standard curve. Two serum samples (0.81 and 0.60 nmol/ml) were diluted 1:2, 1:4, and 1:8 with buffer, and the coefficient of variation was 4% within assay and 10% between assays.

Fig. 3. Recovery study. This study was carried out using free UDCA for preparation of the standard curve. Several different amounts of UDCA (20–500 pmol/ml serum) were added to two serum samples. Recoveries ranged from 95 to 120%. The original UDCA level of two serum samples measured using a glyco-UDCA standard curve was 0.09 and 0.15 nmol/ml, respectively.

Fig. 4. Relationship between serum UDCA values by radioimmunoassay (RIA) and by gas–liquid chromatography (GLC). Open circles, patients with gallstones, during UDCA treatment (oral, 450 mg/day); solid circles, normal subjects.
ship was 0.91. In all 10 normal subjects, a small amount of UDCA in fasting serum was detected, the level was 0.15 ± 0.11 nmol/ml (Fig. 5). In some patients with hepatic disease an elevation of serum UDCA was observed (Fig. 5). The levels were 0.23 ± 0.22 nmol/ml in 27 patients with chronic hepatitis and 0.51 ± 0.53 nmol/ml in 6 patients with cirrhosis.

DISCUSSION

The development of a rapid and sensitive method for the analysis of individual serum bile acids is needed in clinical studies. In 1973, Simmonds et al. (5) established a radioimmunoassay of conjugated cholic acid and made it possible to measure this serum bile acid in samples of less than 0.1 ml. However, this method (5) and similar applications to the measurement of conjugated cholic acid (8–10) each has the problem of unwanted cross reactivity with other bile acids, so that the specificity of the antiserum must be strictly tested for the assay of serum bile acid, especially in the case of bile acid present as a minor component in serum such as lithocholic acid and UDCA. In the present study, the antiserum showed an extremely high specificity for UDCA (free and conjugated), and the highest percentage of cross

![Graph showing fasting serum UDCA level in normal subjects and patients with hepatic diseases.](image1)

![Graph showing gas chromatogram of trifluoroacetate derivatives of serum bile acid methyl esters in a normal subject (I.M.).](image2)
reaction for other bile acids was 0.2%, with lithocholic and glycolithocholic acid. On the basis of these results, an interference by the other bile acids could be excluded. However, it was surprising that these results, an interference by the other bile acids phenomenon is that the antigenic structure of reaction for other bile acids was more similar to that of conjugated UDCA than that of free UDCA.

The present assay system using a specific antiserum makes it possible to detect 10 pmol of UDCA in 0.1 ml of serum, and no interference by the presence of serum proteins was detected by the dilution test or the recovery test. In addition, the reliability of this assay system was assured by the fact that there was high correlation between the immunoassay values and gas–liquid chromatography values.

In Japan, UDCA has been widely used as a choleagogue (dosage: 150–300 mg/day) and some digestives contain 2 mg of UDCA per tablet. Therefore, normal subjects who had not received any drug for 6 months were selected for the determination of normal UDCA levels in serum; the range was from 0.07 to 0.44 nmol/ml (0.15 ± 0.11). Four out of 10 normal sera assayed by the radioimmunoassay were further analyzed by gas–liquid chromatography, and the presence of UDCA was confirmed (Fig. 6). In some patients with hepatic diseases, an increase of serum UDCA level was observed. It would be interesting to know the clinical significance of UDCA in fasting serum; further investigation concerning this question should consider the possibility of previous administration of drugs containing UDCA.

Much information concerning bile acid tolerance following administration of UDCA in patients with liver diseases and UDCA metabolism in patients with gallstones during UDCA (11) and CDCA (12) therapy will probably be obtained by using this convenient radioimmunoassay for serum UDCA.

REFERENCES