Case report

Bile salt transport in the Dubin-Johnson syndrome

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SUMMARY Serum bile salt measurements and intravenous clearance of glycocholate were performed in a woman with Dubin-Johnson syndrome. Fasting conjugated cholate concentration was raised and prolonged intravenous clearance of sodium glycocholate revealed a secondary rise in conjugated cholate concentration after two hours. The intravenous clearance of bromsulphthalein also showed a secondary rise. These findings support the proposal that Dubin-Johnson syndrome is not homogeneous and that in some patients an abnormality of bile salt clearance coexists with an abnormality of bilirubin and bromsulphthalein clearance.

The Dubin-Johnson syndrome (DJS)^{1,2} is characterised by benign, familial conjugated hyperbilirubinaemia associated with black pigment in an otherwise normal liver. There is defective biliary excretion of organic anions including conjugated bilirubin, bromsulphthalein, and porphyrins,³ but the excretion of anionic dyes, such as dibromsulphthalein, rose bengal, and indocyanine green, which are not conjugated during transport across the liver is normal.⁴ While fasting and postprandial bile salt concentrations may be normal in a majority of patients with this syndrome, some have raised bile salt concentrations in the absence of an independent abnormality in liver function.⁵ This raises the possibility that the syndrome is not a homogeneous disorder.

We report a 74 year old woman with the Dubin-Johnson syndrome in whom we have measured the fasting conjugated cholate concentration in the plasma and compared the intravenous clearance of an injected bolus of sodium glycocholate with the clearance of a bolus of bromsulphthalein.

Methods

BILE SALT ANALYSES

After an overnight fast, blood was taken for fasting bile salt estimation. An intravenous glycocholate clearance test was then performed.⁶ A prolonged intravenous glycocholate clearance test with blood

samples taken for four hours was performed on another day. Serum conjugated cholate was determined by radioimmunoassay.⁷ The results of the intravenous clearance of glycocholate were subjected to compartmental analysis,⁶ using previously determined reference ranges. The prolonged intravenous clearance test was also performed on an age- and sex-matched control and on the 47 year old daughter of the patient with the Dubin-Johnson syndrome.

BROMSULPHTHALEIN ANALYSES

An intravenous bromsulphthalein (BSP) clearance test was performed as follows: BSP (6 µg/kg body weight) was injected rapidly into an antecubital vein and blood samples were taken at three minutes and at 15 minute intervals for four hours. Total BSP concentration in plasma was measured after alkalisation of portions of the plasma, using plasma diluted with buffer at pH 7.4 as blank.

The BSP in plasma was separated into conjugated and unconjugated fractions using thin-layer chromatography on plates of silica gel G (0.25 mm × 20 cm × 20 cm) developed in a solvent system consisting of butanol; acetic acid; water::4;1;2 (v/v). The conjugated and unconjugated BSP were visualised by spraying the plate with NaOH (10 mmol/l) and the spots quantified using a flying spot densitometer (Vitatron TLD 100, Fisons Instruments, UK).

OTHER ANALYSES

Plasma total bilirubin concentration and the activities of aspartate aminotransferases and of

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alkaline phosphatase were measured on a Sequential Multiple Analysis with Computer (SMAC) System (Technicon Instruments Company Ltd., Basingstoke, UK). Conjugated bilirubin concentration in plasma was also measured.

Case Report

A 74 year old woman with a history of anorexia, tiredness, and weight loss for six months had a haematemesis and was admitted to hospital. She had two episodes of jaundice eight and 23 years before, but extensive investigations, including a laparotomy, had failed to find a cause. She had been treated for hypertension for six years with methyl-dopa, thiazide diuretics, potassium chloride, and digoxin. Abdominal examination revealed only a right paramedian scar. Full blood count was normal and ESR 40 mm in the first hour. Endoscopy showed three small gastric ulcers on the lesser curvature of the stomach which were benign histologically. She was given cimetidine and at endoscopy two months later the ulcers had healed.

On admission she was jaundiced but had no stigmata of chronic liver disease. She remained jaundiced and passed dark brown urine which contained much bilirubin and a trace of urobilinogen. The plasma bilirubin was 100 $\mu\text{mol/l}$ of which 61 $\mu\text{mol/l}$ was conjugated. Plasma alanine and aspartate aminotransferase and alkaline phosphatase activities, the plasma albumin concentration, and the prothrombin time were consistently normal. The hepatitis B surface antigen test was negative. Single and double dose oral cholecystograms and an intravenous cholangiogram failed to show the

gallbladder or bile ducts. Both ultrasound and $^{99\text{m}}\text{Tc}$ sulphur colloid scans of the liver were normal, and percutaneous transhepatic cholangiography demonstrated a normal biliary tree.

Bromsulphthalein clearance showed an initial rapid fall in the plasma BSP concentration but at 45 minutes 24% of the injected dose was still present in the plasma: half of this, however, was conjugated. After 45 minutes there was a steady rise in the plasma BSP concentration, most of which was conjugated (Fig. 1). Liver biopsy yielded black liver tissue which on microscopy had a normal hepatic architecture with intracellular granular brown lipofuscin-like material characteristic of the Dubin-Johnson syndrome.

Results

The fasting conjugated cholate concentration was 4.5 $\mu\text{mol/l}$ (reference range 0.2–2.9 $\mu\text{mol/l}$). The intravenous clearance of sodium glycocholate revealed abnormalities in the decay curve after the first hour. Though the serum cholate concentration was within the normal range 10 minutes after the injection, the 60 minutes sample and the ratio of the 60 minutes to the 10 minute values were abnormally high at 6.2 $\mu\text{mol/l}$ (reference range 0–4.4 $\mu\text{mol/l}$) and 0.34 (reference range 0.001–0.24 respectively). Compartmental analysis of the intravenous clearance of sodium glycocholate indicated that the curve from the patient with the Dubin-Johnson syndrome was not a true biexponential, whereas in normal subjects the data satisfactorily fitted a biexponential curve.

Figure 2 shows the intravenous clearance of

Fig. 1 Prolonged bromsulphthalein clearance (6 $\mu\text{mol/kg}$ body weight) in a patient with the Dubin-Johnson syndrome. ●—●: Total BSP. ○.....○: Unconjugated BSP. ×.....×: Conjugated BSP.

Fig. 2 Prolonged sodium glycocholate clearance ($5 \mu\text{mol/kg}$ body weight). Shaded area is normal fasting range. (x) 75 year old Dubin-Johnson syndrome patient; (o) 75 year old female control; (Δ) 47 year old daughter.

sodium glycocholate over four hours in the Dubin-Johnson syndrome patient and for comparison shows the clearance curves in a 75 year old woman and in the 47 year old daughter of our patient, neither of whom had clinical or biochemical evidence of liver disease. After a fall to a value of $4.3 \mu\text{mol/l}$ at 105 minutes, there was a secondary rise in the serum conjugated cholate concentration to $8.8 \mu\text{mol/l}$ at 180 minutes. No secondary rise in serum conjugated cholate concentration was found in either of the control subjects.

Discussion

There is disagreement regarding the mechanism of excretion of bile salts and other conjugated organic anions such as bilirubin and BSP. The maximum biliary excretion of taurocholate is the same in normal sheep and Corriedale mutant sheep which have a conjugated hyperbilirubinaemia due to a defect in bilirubin transport similar to the Dubin-

Johnson syndrome. Also, the infusion of taurocholate into Corriedale mutant sheep does not alter the biliary excretion of bilirubin or BSP infused at the same time.⁸ These results have been interpreted as showing that bile salts, and bilirubin and BSP are excreted into the bile by different mechanisms. Conversely, excretory competition between bilirubin and BSP in rats⁹ and between bile salts and BSP in mongrel dogs¹⁰ has supported the view that these organic anions are excreted into the bile by the same mechanism.

Our patient had a conjugated hyperbilirubinaemia and, while the clearance of unconjugated BSP from the plasma appeared to be normal, conjugated BSP was regurgitated into the plasma from the liver giving an overall abnormality in the plasma BSP curve. The clearance of conjugated bile salts has not been previously studied in DJS. The sodium glycocholate injected in this study was already conjugated and was therefore indistinguishable from bile salt which may have been regurgitated after hepatic uptake. The normal clearance of glycocholate up to 10 minutes, followed by the secondary rise, would, however, suggest that uptake of conjugated bile salts into the liver was normal but that transport of the conjugated bile salt across the membrane of the biliary canaliculus into bile was defective.

In a study⁵ of 13 patients with the Dubin-Johnson syndrome, fasting and postprandial bile salt concentrations in the plasma were normal in 12 patients: both were abnormal in one. These findings appear to support the view that conjugated bilirubin and conjugated bile salts have separate excretory pathways across the canalicular membrane into bile. Our studies lend additional weight to the proposal that the syndrome is not a homogeneous disorder. The possibility that the abnormalities in bile salt clearance which we have found in our patient are due to an unrecognised abnormality of liver function or to biliary tract disease is small. The data seem to point to the coexistence of an abnormality of excretion of bilirubin and bromsulphthalein, which probably share an excretory pathway, and of conjugated bile salts in some patients with the Dubin-Johnson syndrome.

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