Δ^4-3-Oxosteroid 5β-reductase deficiency: failure of ursodeoxycholic acid treatment and response to chenodeoxycholic acid plus cholic acid

P T Clayton, K A Mills, A W Johnson, A Barabino, M G Marazzi

Abstract

***Background*—In some infants with liver disease, 3-oxo-Δ^4 bile acids are the major bile acids in urine, a phenomenon attributed to reduced activity of the Δ^4-3-oxosteroid 5β-reductase required for synthesis of chenodeoxycholic acid and cholic acid. These patients form a heterogeneous group. Many have a known cause of hepatic dysfunction and plasma concentrations of chenodeoxycholic acid and cholic acid that are actually greater than those of the 3-oxo-Δ^4 bile acids. It is unlikely that these patients have a primary genetic deficiency of the 5β-reductase enzyme.**

***Aims*—To document the bile acid profile, clinical phenotype, and response to treatment of an infant with cholestasis, increased plasma concentrations of 3-oxo-Δ^4 bile acids, low plasma concentrations of chenodeoxycholic acid and cholic acid, and no other identifiable cause of liver disease.**

***Patients*—This infant was compared with normal infants and infants with cholestasis of known cause.**

***Methods*—Analysis of bile acids by liquid secondary ionisation mass spectrometry and gas chromatography – mass spectrometry,**

***Results*—The plasma bile acid profile of the patient was unique. She had chronic cholestatic liver disease associated with malabsorption of vitamins D and E and a normal γ-glutamyltranspeptidase when the transaminases were increased. The liver disease failed to improve with ursodeoxycholic acid but responded to a combination of chenodeoxycholic acid and cholic acid.**

***Conclusion*—Treatment of primary 5β-reductase deficiency requires the use of bile acids that inhibit cholesterol 7α-hydroxylase.**

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Keywords: inborn error, bile acid synthesis, giant cell hepatitis, cholestasis.

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Diagnosis of inborn errors of bile acid synthesis is important because specific treatment is available in the form of oral supplements of bile acids and such treatment is often dramatically effective.[1 2] Rapid diagnosis is made possible by the use of fast atom bombardment mass spectrometry (FAB-MS) or liquid secondary ion mass spectrometry (LSI-MS). These techniques can be used to analyse the cholanoids (bile acids and bile alcohols) in a urine sample.[2–6] Most children with cholestatic liver disease excrete increased amounts of normal bile acids (principally the taurine and glycine conjugates of cholic acid and chenodeoxycholic acid) but in a few, one of four unusual patterns of cholanoid excretion can be recognised.[6] One of these is characterised by the presence of ions attributable to the glycine and taurine conjugates of 7α-hydroxy-3-oxo-4-cholenoic acid and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid.[5–10] It is generally accepted that this pattern arises as a result of reduced activity of the enzyme that converts 7α-hydroxy-cholest-4-en-3-one to 7α-hydroxy-5β-cholestan-3-one and 7α,12α-dihydroxy-cholest-4-en-3-one to 7α,12α-hydroxy-5β-cholestan-3-one (Fig 1). As a result of the Δ^4-3-oxosteroid 5β-reductase deficiency, 3-oxo-Δ^4 intermediates undergo side chain oxidation to produce the corresponding 3-oxo-Δ^4 bile acids, which are conjugated with glycine and taurine but cannot be secreted into the bile and appear in the urine.[6] The activity of Δ^4-3-oxosteroid 5α-reductase is preserved, leading to the appearance of increased amounts of 5α[H]-or allo-bile acids in plasma. The difficulty in an individual patient is deciding whether the 5β-reductase deficiency is the primary cause of the liver disease or whether reduced activity of the enzyme is a consequence of hepatocyte damage. This has also made it difficult to define the phenotype, natural history, and response to treatment of primary Δ^4-3-oxosteroid 5β-reductase deficiency.

Patients with 5β-reductase deficiency have been treated with various combinations of bile acids.[11] There is little information on the use of ursodeoxycholic acid alone. This is important because this is the bile acid that is most widely used in the treatment of cholestatic disorders of childhood. In this paper we present a child with chronic cholestatic liver disease who failed to respond to ursodeoxycholic acid treatment but improved rapidly with a combination of chenodeoxycholic acid and cholic acid. The low concentrations of primary bile acids in the patient's plasma, urine, and duodenal juice before treatment suggested that this patient probably had primary Δ^4-3-oxosteroid 5β-reductase deficiency.

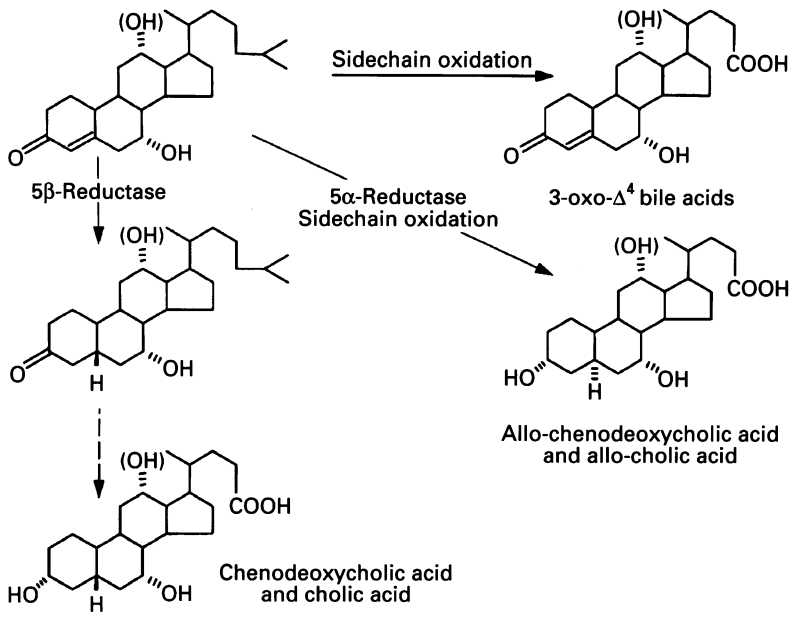

Figure 1: The effect of reduced activity of Δ4-3-oxosteroid 5β-reductase: reduced synthesis of conjugates of chenodeoxycholic acid and cholic acid and increased synthesis of (a) conjugates of 7α-hydroxy-3-oxo-4-cholenoic acid and 7α,12α-dihydroxy-4-cholenoic acid, (b) allo-*chenodeoxycholic acid and* allo-*cholic acid.*

Case report

Our patient was the second child of unrelated Sardinian parents. Her mother took carbamazepine throughout the pregnancy. The infant was born at 37 weeks gestation and was breast fed. She developed mild jaundice but remained well until the third week of life when she developed fever, pronounced jaundice, and drowsiness. Investigations showed: bilirubin 316 μM (conjugated 145 μM), aspartate aminotransferase (AST) 2279 IU/l (normal 20–60), alanine aminotransferase (ALT) 1123 IU/l (normal 5–45), γ-glutamyltranspeptidase (γ-GT) 102 IU/l (normal 5–51), prothrombin time 15·4 seconds (control 12 seconds), α fetoprotein 680 000 μg/l. Screening tests for hepatitis viruses were negative. The plasma carbamazepine concentration was 1·4 μg/l and consequently breast feeding was stopped. The patient's liver function tests showed some improvement but there was persistent cholestasis with steatorrhoea, failure to thrive, and clinical rickets. At the age of 3 months, investigations in the Gaslini Institute of Genoa showed a normal serum $\alpha_1$antitrypsin concentration, red cell galactose-1-phosphate uridyl transferase, and UDP-galactose-4-epimerase. Plasma tyrosine was 390 μM (normal 40–130), plasma threonine 450 μM (normal 65–185), and plasma methionine 10 μM (normal 15–31). The urine organic acid analysis showed increased excretion of 4-hydroxyphenyl-pyruvic and -lactic acids but no succinyl acetone. Plasma analysis showed a low vitamin E concentration (1·4 μM; normal 11·5–35) but a normal vitamin A concentration. Serum ferritin was increased. Ultrasound showed a normal gall bladder and no dilatation of extrahepatic bile ducts. A liver biopsy at 4 months showed lobular disarray resulting from extensive giant cell transformation and necrotic foci with granulocyte accumulation. The portal spaces were of normal size and shape and the interlobular bile duct could always be visualised. Some portal tracts were infiltrated by lymphocytes and some bile duct epithelial cells were vacuolated. The parenchyma showed considerable macrovesicular steatosis and bile pigment accumulation. Granules of haemosiderin were localised almost exclusively to Kupffer cells.

Between the ages of 3 and 8 months our patient had persistent jaundice with failure to thrive and steatorrhoea when fed a normal formula. She continued to fail to thrive when fed with Pregestemil, partly because of poor intake. Treatment with ursodeoxycholic acid at a dose of 12 mg/kg/d and then at a dose of 20 mg/kg/d did not lead to any clinical improvement or to significant improvement in liver function tests (Fig 2). The alkaline phosphatase fell in response to parenteral vitamin D. Vitamins A, E, and K were also given parenterally. The urine bile acids were first analysed when the patient was 0·3 years, the ursodeoxycholic acid treatment having been stopped for two weeks.

At the age of 0·69 years the patient was seen at Great Ormond Street. She had been receiving ursodeoxycholic acid treatment at a dose of 20 mg/kg/d for 0·4 years. Examination showed a weight well below the third percentile, length just below the third percentile, and head circumference just below the second percentile. She was wasted and jaundiced but there were no stigmata of chronic liver disease. The liver edge was palpable 5·5 cm from the costal margin in the mid-clavicular line and 5 cm from the xiphisternum in the midline. Investigations showed: haemoglobin 96 g/l with normal indices, ferritin 245 μg/l (normal 7–150), serum iron 13·7 μM (normal 14–22), total iron binding capacity 54·9 μM (normal 42–66), copper 17·3 μM (normal 12·6–26·8), manganese 254 nM (normal 73–210). Clotting studies were normal as were plasma calcium, phosphate, alkaline phosphatase, and albumin. Plasma amino acids were normal with the exception of threonine (284 μM; normal 70–220). The total bilirubin was 88 μM (conjugated 35 μM); the AST 511 IU/l

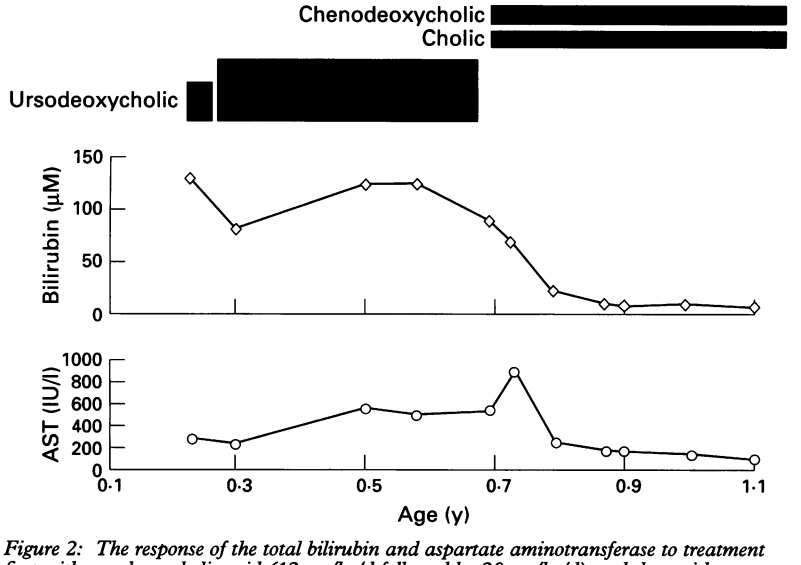

Figure 2: The response of the total bilirubin and aspartate aminotransferase to treatment first with ursodeoxycholic acid (12 mg/kg/d followed by 20 mg/kg/d) and then with chenodeoxycholic acid plus cholic acid (8 mg/kg/d of each).

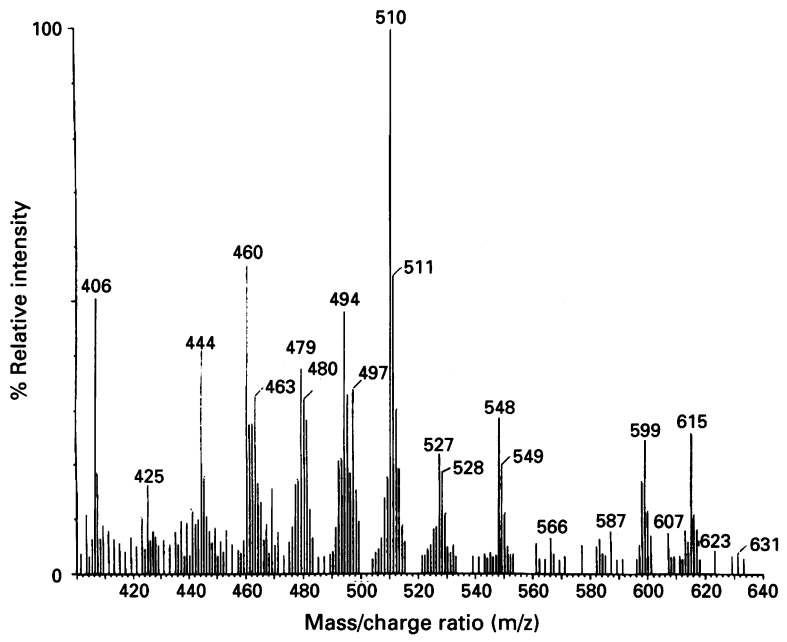

Figure 3: Negative ion liquid secondary ionisation mass spectrum obtained from the urine of our patient before commencement of bile acid therapy. The identities of the major ions were shown to be: m/z 444, glycine-conjugated 7α-hydroxy-3-oxo-4-cholenoic acid; m/z 460 glycine-conjugated 7α12α-dihydroxy-3-oxo-4-cholenoic acid; m/z 494, taurine-conjugated 7α-hydroxy-3-oxo-4-cholenoic acid; m/z 510 glycine-conjugated 7α12α-dihydroxy-3-oxo-4-cholenoic acid.

(normal 20–60); the ALT 252 IU/l (normal 5–45); γ-GT 36 IU/l (normal 5–51); cholesterol 4·6 mM (normal 2·5–4·9). Because of the parenteral therapy the plasma vitamin D was slightly increased but the vitamin E was still low at 4·8 μM (normal 11·5–35). An abdominal ultrasound showed hepatomegaly only. The liver biopsy showed preservation of the architecture but lobular disarray with numerous giant hepatocytes and infiltration by polymorphs, particularly around areas of cholestasis. The portal tracts were infiltrated by inflammatory cells including numerous neutrophils and showed periportal fibrosis. There was extensive fatty change but no iron accumulation. The appearances were those of a giant cell hepatitis. Electron microscopy (Professor B Lake) showed that the hepatocytes contained abundant bile pigment containing some lamellar bodies. Mitochondria were generally of normal structure with a dense matrix. Peroxisomes were plentiful and slightly larger than normal (diameter >1 μM). The canalicular microvilli did not appear stunted. Overall, the liver biopsy showed no evidence of improvement during the period of ursodeoxycholic acid treatment and some progression of fibrosis.

At 0·7 years our patient started treatment with chenodeoxycholic acid and cholic acid at a dose of 8 mg/kg/d of both. There was an initial rise in AST followed by a fall, and a steady fall in bilirubin (Fig 2) to normal values over a period of three months. The steatorrhoea resolved and the patient showed dramatic catch up weight gain so that she was on the third percentile for weight at one year. In contrast with the transaminases, the γ-GT continued to rise during the first two months of treatment to a maximum value of 225 IU/l but fell thereafter to within the normal range. At the age of 1·1 years the patient was asymptomatic with normal liver function tests (apart from a minimally increased alkaline phosphatase). By the age of 1·4 years she was above the third centile for height and weight.

Methods

The sample preparation used in the analysis of urinary cholanoids by negative ion FAB-MS or LSI-MS has been described previously.[3] In this study we used LSI-MS (using a caesium ion gun operated at 10 kV) rather than FAB-MS; the spectra obtained with the two ionisation techniques do not differ significantly.[12] The methods used for the analysis of bile acids in plasma, urine, and duodenal juice by GC-MS[2-4 13 14] and for the analysis of plasma sterols (including cholestanol) by GC-MS[15] have been described previously. The results of plasma and urine analyses from our patient were compared with samples from infants and children who were being investigated in our laboratories for a possible bile acid synthesis defect or peroxisomal disorder. Thus the 'normal' controls came largely from children with neurological symptoms such as hypotonia, developmental delay, fits, deafness, etc; this group had no clinical or biochemical evidence of liver disease. The 'cholestatic controls' consisted of infants with clear evidence of liver disease; the patient was assigned to the control group when the cause of the liver disease was discovered. This group included children with biliary atresia, galactosaemia, α_1 antitrypsin deficiency, severe coarctation of the aorta, etc.

Results

Samples obtained before ursodeoxycholic acid treatment

Negative ion LSI-MS analysis of the urine sample obtained from our patient at 0·3 years produced the result shown in Fig 3. The major ions in the range mass/charge (m/z) ratio 400 to m/z 640 were m/z 444, 460, 494, and 510, which correspond to the quasimolecular $(M-H)^-$ ions for the glycine and taurine conjugates of 7α-hydroxy-3-oxo-4-cholenoic acid and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid. The glycine and taurine conjugates of chenodeoxycholic acid and cholic acid (m/z 448, 464, 498, and 514) were not detectable above the background. In the normal controls, bile acid peaks were just detectable in some cases; the 3-oxo-Δ^4 bile acid peaks were never bigger than the normal bile acid peaks. In the cholestatic controls, 3-oxo-Δ^4 bile acids were sometimes detected, particularly in children with severe liver dysfunction but the normal saturated bile acids were always present and accounted for 30–97% of the total. The urine sample from our patient showed a number of additional peaks in the LSI mass spectrum (Fig 3). The identities of these are being investigated.

Table I shows results of the plasma sample obtained at 0·3 years. The 3-oxo-Δ^4 bile acids, 7α-hydroxy-3-oxo-4-cholenoic acid, and

TABLE I *Concentrations of 3-oxo-Δ^4 bile acids,* allo *bile acids, and normal bile acids (chenodeoxycholic acid and cholic acid) in the plasma of our patient and in plasma samples from infants with normal liver function and infants with cholestasis of known cause*

Bile acid	Plasma concentration (μM)		
	Patient	Normal infants (n=38)	Infants with cholestasis (n=30)
7α-hydroxy-3-oxo-4-cholenoic acid	1·94	ND	0–6·52
7α,12α-dihydroxy-3-oxo-4-cholenoic acid	2·07	ND	0–4·36
Allochenodeoxycholic acid	ND	0–0·1	0–5·2
Allocholic acid	0·76	0–0·15	0–3·1
Chenodeoxycholic acid	ND	0·2–12·7	13·4–181
Cholic acid	ND	0·4–6·68	4·7–403

ND=not detectable (<0·05 μM).

TABLE II *Percentage composition of the mixture of bile acids in plasma of our patient, infants with normal liver function, and infants with cholestasis of known cause*

Bile acid	% Total plasma bile acid concentration		
	Patient	Normal infants (n=38)	Infants with cholestasis (n=30)
7α-hydroxy-3-oxo-4-cholenoic acid	41	0	0–6·5
7α,12α-dihydroxy-3-oxo-4-cholenoic acid	43	0	0–4·4
Allochenodeoxycholic acid	0	0–2·3	0–2·0
Allocholic acid	16	0–2·3	0–14
Chenodeoxycholic acid	0	31–91	11–88
Cholic acid	0	8–69	10–88

7α,12α-dihydroxy-3-oxo-4-cholenoic acid, were the major bile acids and allo-cholic acid (3α,7α,12α-trihydroxy-5α-cholanic acid) was readily detectable whereas the concentrations of cholic acid and chenodeoxycholic acid were below the limit of detection of the method (<0·05 μM). The two 3-oxo-Δ^4 bile acids were undetectable in the plasma of normal infants but they were occasionally detected in the plasma of infants with severe liver dysfunction. Concentrations as high as 6·5 μM and 4·4 μM (Table I) were seen in two infants who had severe coarctation and, as a result, both liver failure and a reduced urine output. In all patients apart from the case described in detail above, however, an increase in the plasma concentration of 3-oxo-Δ^4 bile acids was associated with an increase in the concentration of chenodeoxycholic acid and cholic acid. Thus, as Table II shows, the 3-oxo-Δ^4 bile acids accounted for <15% of the total plasma bile acid mixture in all but the patient described in this report for whom the figure was 84%.

The plasma bile acid chromatogram from our patient showed some additional minor peaks, in particular one with a mass spectrum containing the ions m/z 414, 378, 363, 294, 267, 145, 129, suggestive of the trimethylsilyl either of a side chain hydroxylated derivative of 7α,12α-dihydroxy-cholest-4-en-3-one. The urine LSI mass spectrum also suggested that a 3-oxo-4 cholestenetriol may be present both as the sulphate (m/z 511) and as the glucuronide (m/z 623). Sjövall has also reported the urinary excretion of bile alcohols with a 3-oxo-Δ^4 structure in patients with 5β-reductase deficiency.[16]

TABLE III *Plasma bile acid concentrations in our patient (a) before bile acid treatment; (b) during treatment with ursodeoxycholic acid, and (c) during treatment with chenodeoxycholic acid plus cholic acid*

Bile acid	Plasma concentration (μM)		
	Age 0·3 y No treatment	Age 0·69 y Ursodeoxycholic acid	Age 0·81 y Chenodeoxycholic and cholic acid
7α-hydroxy-3-oxo-4-cholenoic acid	1·94	2·74	ND
7α,12α-dihydroxy-3-oxo-4-cholenoic acid	2·07	3·05	ND
Allochenodeoxycholic acid	ND	ND	ND
Allocholic acid	0·76	0·43	ND
Chenodeoxycholic acid	ND	0·06	24·84
Cholic acid	ND	0·26	7·72
Ursodeoxycholic acid	ND	14·02	2·03

ND=not detectable (<0·05 μM).

Samples obtained during ursodeoxycholic acid treatment

A plasma sample obtained when the patient was 0·69 years and receiving ursodeoxycholic acid treatment showed that there had been no fall in the plasma concentration of 7α-hydroxy-3-oxo-4-cholenoic acid and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid (Table III). Ursodeoxycholic acid was the major plasma bile acid and metabolites of ursodeoxycholic acid were also readily detectable (for example, the compound tentatively identified by Koopman *et al* as 21-hydroxy-ursodeoxycholic acid[17]). The plasma concentration of cholestanol was normal. The LSI-MS analysis of urine showed that 7α-hydroxy-3-oxo-4-cholenoic acid and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid were still present in urine although the major peak was m/z 471 (possibly sulphated ursodeoxycholic acid). Table IV shows the results of analysis of the urine by GC-MS (after enzymatic deconjugation of bile acids). The major bile acids in the urine were 7α,12α-dihydroxy-3-oxo-4-cholenoic acid, 7α-hydroxy-3-oxo-4-cholenoic acid and ursodeoxycholic acid. Several metabolites of ursodeoxycholic acid were present; the major ones were identified as 21-, 6β-, 1β-, and 2β-hydroxy-ursodeoxycholic acid. Chenodeoxycholic acid and cholic acid were undetectable.

Bile samples obtained after stopping ursodeoxycholic acid treatment

Duodenal bile from our patient contained a very low cholanoid concentration of 19 μM (normal 2·5–23 mM). The major cholanoids present were cholic acid (13 μM), 7α,12α-dihydroxy-4-cholenoic acid (1·1 μM), and 3β,7α-dihydroxy-4-cholenoic acid (0·73 μM). Several unidentified compounds were present.

Samples obtained during treatment with chenodeoxycholic acid and cholic acid

A urine sample obtained when the patient had been receiving chenodeoxycholic acid and cholic acid treatment for five days produced the result shown in Table IV, column 2. There had been a small fall in the urinary excretion of 7α,12α-dihydroxy-3-oxo-4-cholenoic acid and a larger fall in the urinary excretion of 7α-hydroxy-3-oxo-4-cholenoic acid. A plasma sample obtained when the patient was 0·8 years and receiving chenodeoxycholic acid and cholic acid treatment showed plasma concentrations of chenodeoxycholic acid and cholic acid that were slightly above the normal ranges and undetectable concentrations of 7α-hydroxy-3-oxo-4-cholenoic acid

TABLE IV *Urinary excretion of bile acids by the patient while taking ursodeoxycholic acid and while taking chenodeoxycholic acid and cholic acid*

Bile acid	Urinary excretion (μmol/mmol creatinine)		
	Age 0·69 y Ursodeoxycholic acid	*Age 0·7 y Chenodeoxycholic and cholic acid*	*Age 0·81 y Chenodeoxycholic and cholic acid*
7α-hydroxy-3-oxo-4-cholenoic acid	22	9·1	1
7α,12α-dihydroxy-3-oxo-4-cholenoic acid	94	82	22
Chenodeoxycholic acid	ND	2·6	1·5
Cholic acid	ND	32	4·3
Ursodeoxycholic acid	37	ND	0·9

ND=not detectable.

and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid (Table III). The urine analysis showed that the 3-oxo-Δ^4 bile acids were still present in urine but the amounts being excreted (measured in μmol/mmol creatinine) were much lower that they had been when the patient was receiving ursodeoxycholic acid treatment (Table IV).

Discussion

In the future, the gold standard for the diagnosis of primary 5β-reductase deficiency will be the finding of a gene mutation that leads to an inactive enzyme. This may not be far off as the cDNA for the human enzyme has been cloned and the enzyme expressed in COS cells.[18] For the time being it is necessary to use other criteria. We believe that the patient described in this report had primary 5β-reductase deficiency for the following reasons: (a) no other cause of chronic cholestatic liver disease was identified; (b) the clinical features were similar to those caused by deficiency of the enzyme 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase, which catalyses a step in bile acid synthesis that just precedes the 5β-reductase reaction.[1] That is, firstly, the chronic cholestatic liver disease was associated with steatorrhoea, rickets (because of malabsorption of vitamin D), a low plasma vitamin E concentration, and a γ-GT that was normal, or only slightly raised at a time when the transaminases were considerably increased.[1 19] Secondly, the abnormality of bile acid concentrations in bile, plasma, and urine was similar to that seen in 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency; the concentrations of chenodeoxycholic acid and cholic acid in bile were <50 μM, the concentrations in plasma were $<3{\cdot}5$ μM (that is, in the low or low-normal range), and the concentrations of abnormal (unsaturated) bile acids were considerably increased. We have analysed plasma bile acids from 30 infants and children who had a known cause of cholestatic liver disease but who were also excreting 3-oxo-Δ^4 bile acids in their urine (secondary 5β-reductase deficiency). These patients had increased plasma concentrations of chenodeoxycholic acid, increased or high normal plasma concentration of cholic acid, and plasma concentrations of 3-oxo-Δ^4 bile acids, which were lower than those of chenodeoxycholic acid and cholic acid. (c) The patient described in this report showed a complete response to treatment with chenodeoxycholic acid and cholic acid. This is strong evidence that defective bile acid synthesis is the cause and not the consequence of liver disease.

The cause(s) of liver cell damage in Δ^4-3-oxosteroid 5β-reductase deficiency and 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency are not known. Two major hypotheses have been proposed. The first suggests that loss of the bile acid dependent component of bile flow leads to accumulation of toxic compounds in the hepatocyte. The second suggests that it is the accumulation of bile acid precursors or unsaturated bile acids, or both, which cannot be secreted into bile that is responsible for liver cell damage.[20 21] Stieger *et al* have shown that the accumulation of 3-oxo-Δ^4 bile acids in 5β-reductase deficiency can be expected to produce adverse effects. They showed that taurine conjugated 7α-hydroxy-3-oxo-4-cholenoic acid strongly inhibits the transport of taurocholate by the ATP dependent canalicular bile acid transporter.[22] Ursodeoxycholic acid treatment can be expected to fuel bile flow but ursodeoxycholic acid does not inhibit the first step in bile acid synthesis, cholesterol 7α-hydroxylase, and therefore cannot be as effective as chenodeoxycholic acid and cholic acid at reducing the synthesis of bile acid precursors and unsaturated bile acids.[23] The patient described in this report failed to respond to ursodeoxycholic acid treatment but showed a clear response to primary bile acid (chenodeoxycholic acid and cholic acid) treatment. This suggests that inhibition of cholesterol 7α-hydroxylase is important for successful treatment of Δ^4-3-oxosteroid 5β-reductase deficiency. From a previous report of bile acid treatment for 5β-reductase deficiency[10] it was not possible to say whether ursodeoxycholic acid had a beneficial effect as it was only used alone for four days. The combination of chenodeoxycholic acid and cholic acid led to reduced excretion of 3-oxo-Δ^4 bile acids, however, it was abandoned because it seemed to be producing diarrhoea. Longterm treatment with a combination of ursodeoxycholic acid and cholic acid led to normalisation of liver function tests and resolution of liver biopsy abnormalities. It took a year for the bilirubin to normalise unlike our patient who had a normal bilirubin within three months of starting treatment with chenodeoxycholic acid and cholic acid. However, the cases are not strictly comparable; the patients described by Daugherty *et al* had more severe cholestasis at the onset of treatment and we know, from our experience of treating 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency, that patients with severe cholestasis take longer to respond.[1] Such patients may also show a deterioration in liver function tests when treated with chenodeoxycholic acid alone but improve quickly with a combination of chenodeoxycholic acid and cholic acid.

While chenodeoxycholic acid plus cholic acid is probably the treatment of choice for a patient such as the one described in this report (who had a low plasma concentration of chenodeoxycholic acid), we would urge caution in its use in patients who have a high

plasma concentration of chenodeoxycholic acid (and thus we would not use it in a patient with secondary 5β-reductase deficiency). The reason for this is that, if the plasma concentration of chenodeoxycholic acid is increased, it is probable that the hepatocyte concentration is also increased and hydrophobic dihydroxy bile acids such as chenodeoxycholic acid are thought to contribute to the liver cell damage that occurs in cholestasis.

Although we have emphasised the similarities between our patient and infants with 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency, the initial presentation with profound liver dysfunction at two weeks has not been described in the latter inborn error of bile acid synthesis. An association of profound liver dysfunction, the histological features of neonatal haemochromatosis, and excretion of 3-oxo-Δ^4 bile acids has been described recently and the authors suggested that primary 5β-reductase deficiency can cause accumulation of iron and neonatal liver failure.[8] We have argued that it is more probable that these patients represent another example of secondary 5β-reductase deficiency.[9] We did not find evidence of iron storage in the liver biopsy specimen of our patient, serum iron was at the lower end of the normal range and iron binding capacity was not reduced. Thus on these criteria there was no evidence of iron overload. The serum ferritin concentrations was slightly increased but within the range expected from a wide range of hepatic insults and not in the range seen in haemochromatosis. Thus this patient provides further evidence that disorders of bile acid synthesis do not produce haemochromatosis.

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