An inborn error of bile acid synthesis (3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency) presenting as malabsorption leading to rickets

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Abstract

Deficiency of 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase (3β-HSDH), the enzyme that catalyses the second reaction in the principal pathway for the synthesis of bile acids, has been reported to present with prolonged neonatal jaundice with the biopsy features of neonatal hepatitis. It has also been shown to present between the ages of 4 and 46 months with jaundice, hepatosplenomegaly, and steatorrhoea (a clinical picture resembling progressive familial intrahepatic cholestasis). This paper reports two children with 3β-HSDH deficiency who developed rickets during infancy and did not develop clinically evident liver disease until the age of 3 years. Bile acid replacement resulted in considerable clinical and biochemical improvement. The importance of thorough investigation of fat soluble vitamin deficiencies in infancy is emphasised.

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Case 1

An Asian girl, the only child of a consanguinous marriage, born at full term presented at the age of 9 months with a fractured rib and osteopenia. Investigations at the time showed plasma alkaline phosphatase of 4515 IU/l (normal, 150–1100), and serum calcium and phosphate of 1.8 nmol/l (normal, 2.25–2.7) and 0.73 nmol/l (normal, 1.2–2.2), respectively. Radiography showed severe rickets and she was treated with vitamin D. Subsequently, her bone chemistry returned to normal and the rachitic changes improved.

She was seen again in hospital at the age of 3 years with jaundice, pale stools, dark urine, and hepatomegaly. Her liver function tests at this time were as follows: total bilirubin, 66 μmol/l (normal, < 17); direct bilirubin, 30 μmol/l; alanine transaminase, 95 IU/l (normal, 5–45); and alkaline phosphatase, 549 IU/l. Other investigations included: serum α_1 antitrypsin concentrations and phenotype, normal; serum copper and caeruloplasmin, normal; autoantibody screen, negative; serum retinol, 0.2 mg/l (normal, 0.2–0.8); serum tocopherol, 0.1 mg/l (normal, 5–15); vitamin D (25-OH-cholecalciferol), 2.1 ng/ml (normal, 5–30); clotting screen, normal; white cell and plasma lysosomal enzymes, normal; hepatitis screen, negative; urinary organic and amino acids, normal; and very long chain fatty acids, normal. Her liver function tests remained persistently abnormal with increasing alkaline phosphatase. The histology of a liver biopsy performed at the age of 3½ years showed cholestasis with expansion of portal tracts, some fibrosis, and bile duct proliferation. A HIDA scan (99mTc-N-substituted-2,6-dimethylphenyl carbamoylethyl iminodiacetic acid) showed a patent extrahepatic biliary system.

Analysis of her urine by electrospray ionisation mass spectrometry showed major peaks of sulphated dihydroxycholenoic acids and trihydroxycholenoic acids (mass/charge ratio 469, 485, 526, and 542). Analysis of her plasma by gas chromatography/mass spectrometry (after solvolysis and enzymatic deconjugation of bile acids) showed very low concentrations of cholic and chenodeoxycholic acids and high concentrations of 3β,7α-dihydroxy-5-cholenoic acid and 3β,7α,12α-trihydroxy-5-cholenoic acid. These findings were consistent with a diagnosis of 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase (3β-HSDH) deficiency. Treatment with chenodeoxycholic acid was commenced at a dose of 12 mg/kg/day and within three months she had become jaundice free, with normal coloured stools and urine, and normal liver function tests.

Case 2

A Jordanian boy was born at full term, birth weight 3550 g. He had no neonatal jaundice but developed steatorrhoea with failure to thrive at the age of 7 months. Physical examination and laboratory investigations confirmed rickets, which responded to vitamin D. At the age of 1 year he developed pruritus and frequent nose bleeds. He continued to grow poorly, and by the age of 3 years, his steatorrhoea was worse. Pancreatic enzyme supplementation was tried with little improvement. A liver biopsy showed fibrosis with some disturbance of architecture, and portal triaditis. Several portal tracts lacked a bile duct but the total number of portal tracts available for examination was insufficient to allow a diagnosis of paucity of interlobular bile ducts. A cousin with hepatomegaly was reported to have a very similar liver biopsy.

Physical examination at the age of 3 years and 4 months showed height on the third centile, and weight on the 10th; liver edge was palpable 4 cm from the right costal margin in the midcla-

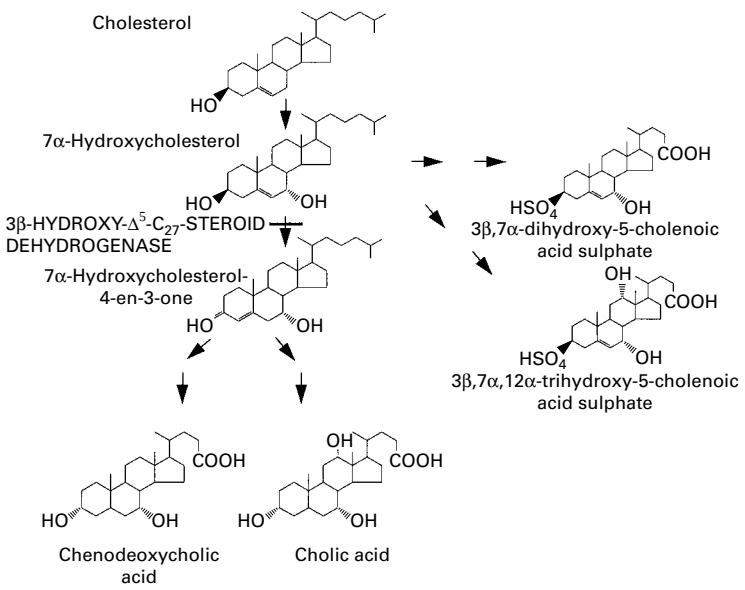

Figure 1 Simplified version of the major pathway involved in the synthesis of bile acids from cholesterol, showing how deficiency of 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase leads to synthesis of unusual unsaturated bile acids that are sulphated and excreted in the urine.

vicular line and 8 cm from the xiphisternum in the midline. Investigations included: alanine transaminase, 114 U/l (normal, 5–45); aspartate transaminase, 87 U/l (normal, 20–60); alkaline phosphatase, 266 U/l; γ-glutamyl transpeptidase, 24 U/l (normal, 6–19); bilirubin, 10 μmol/l (normal, < 7); prothrombin time raised at 20 seconds (control, 14 seconds), normalised by parenteral vitamin K; cholesterol 3.08 mmol/l (normal, 3.1–5.4); vitamin A, 361 μg/l (normal, 200–430); vitamin E, 1.5 μmol/l (normal, 11.5–35); 25-OH-cholecalciferol, 22 nmol/l (normal, 15–100); and α_1 antitrypsin, normal.

Analysis of his urine by fast atom bombardment mass spectrometry showed intense peaks of mass/charge ratio 469, 485, 526, and 542, indicating a diagnosis of 3β-HSDH deficiency. He was treated with chenodeoxycholic acid 9 mg/kg/day. Within a few days he was scratching less and was less irritable. The transaminases became normal within six weeks and, when reviewed 15 months later, he was asymptomatic.

Discussion

The primary bile acids, chenodeoxycholic acid and cholic acid are synthesised from cholesterol by a series of reactions involving modifications to the cholesterol nucleus and oxidation of the cholesterol side chain (fig 1). In the early 1970s, Eyssen and colleagues[1] and Hanson and colleagues[2] described infants with cholestatic liver disease and accumulation of an intermediate in the conversion of cholesterol to cholic acid, trihydroxycholestanoic acid (THCA). This was the first indication that defective bile acid synthesis could cause cholestasis. THCA accumulates when there is a defect in β oxidation of the cholesterol side chain; most patients accumulating THCA have a peroxisomal disorder such as Zellweger syndrome.[3] A defect in cholesterol side chain oxidation, which causes neonatal cholestasis but does not lead to accumulation of THCA, has also been described.[4] In the late 1980s, the first defects in enzymes catalysing the modifications to the steroid nucleus were described in infants with cholestatic liver disease, namely 3β-HSDH deficiency[5] and Δ^4-3-oxosteroid 5β-reductase deficiency.[6 7]

3β-HSDH is the second enzyme in the major pathway for the synthesis of bile acids from cholesterol. Deficiency of this enzyme leads to the accumulation of 7α-hydroxycholesterol, which can then undergo side chain oxidation and hydroxylation to produce 3β,7α-dihydroxy-5-cholenoic acid and 3β,7α,12α-trihydroxy-5-cholenoic acid (fig 1). These abnormal bile acids undergo sulphation and they are found in high concentrations in the plasma and urine.[3] Definitive diagnosis of 3β-HSDH deficiency is established by demonstrating the abnormal bile acids in the urine and plasma using gas chromatography/mass spectrometry and fast atom bombardment or electrospray ionisation mass spectrometry techniques. 3β-HSDH activity can also be measured in cultured skin fibroblasts. Affected patients show very low activity. 3β-HSDH deficiency is inherited as an autosomal recessive trait and there is a high incidence of consanguinity among parents of affected children.[3]

The cause of the liver damage in 3β-HSDH deficiency is uncertain. The abnormal metabolites produced from 7α-hydroxycholesterol may be hepatotoxic and/or failure to synthesise normal primary bile acids may lead to failure of the component of bile flow that is brought about by bile acid secretion; impairment of bile flow (cholestasis) may then lead to retention of toxic compounds that are normally excreted in bile. The occurrence of steatorrhoea and fat soluble vitamin malabsorption in 3β-HSDH deficiency are explained more easily. Duodenal concentrations of normal bile acids are extremely low and this prevents effective micellar solubilisation and absorption of lipids.

The first patients described with 3β-HSDH deficiency presented with prolonged neonatal jaundice and steatorrhoea.[8] Rickets (owing to malabsorption of vitamin D) were often apparent before the age of 6 months and one patient developed a bleeding diathesis caused by vitamin K deficiency at 9 months. All patients had very low plasma vitamin E concentrations (< 4 μM) and normal or only minimally raised γ-glutamyl transpeptidase, despite significantly raised transaminases. In 1994, Jacquemin *et al* described a group of patients who presented between the ages of 4 and 46 months with jaundice, hepatosplenomegaly, and steatorrhoea. Their clinical picture resembled progressive familial intrahepatic cholestasis.[9] Pruritus was absent in these children in contrast to other children with severe cholestasis. The authors noted normal γ-glutamyl transpeptidase activities in plasma, low serum cholesterol values, and low vitamin E concentrations. Patients with 3β-HSDH deficiency comprised 17% of 30 children with chronic cholestatic liver disease in whom all known causes of childhood cholestasis had been excluded. Finally, Setchell *et al* have described a case of

3β-HSDH deficiency presenting in the 2nd decade of life with chronic hepatitis.[10]

The case reports presented in our paper describe a new presentation of 3β-HSDH deficiency. Both our patients presented with rickets in infancy; at this time, liver disease was not evident and their rachitic changes responded well to vitamin D. The first patient developed clinically apparent jaundice more than two years after presenting with rickets; the second was never jaundiced but did have hepatomegaly and deranged liver function tests at the age of 3 years. Although the first patient's bone chemistry initially responded very well to treatment, tests performed when she developed jaundice at age 3 years revealed that she was again profoundly vitamin D deficient. In common with previously described patients, plasma vitamin E concentrations were very low in both patients and one patient also had evidence of vitamin K malabsorption. Liver biopsy findings were non-specific.

In patients with 3β-HSDH deficiency whose bilirubin was < 120 μM and aspartate aminotransferase < 260 μM, treatment with chenodeoxycholic acid has resulted in dramatic improvement in symptoms and in liver function tests,[8 11] and has also led to an improvement in the histological appearance of liver biopsies.[12] The treatment regimens described have used a dose of 12–18 mg/kg/day, reducing to a maintenance dose of 9–12 mg/kg/day after two months. The dose is probably not crucial in patients with mild liver dysfunction and is in part dictated by the availability of chenodeoxycholic acid (125 mg tablets or 250 mg capsules). Doses of 9 mg/kg/day and 12 mg/kg/day were effective in our patients. Chenodeoxycholic acid is retained effectively in the enterohepatic circulation and can promote bile flow and facilitate micellar solubilisation of fats and fat soluble vitamins. It also suppresses the activity of 7α-hydroylase, the enzyme that converts cholesterol to 7α-hydrocholesterol, thereby reducing the synthesis of the abnormal metabolites of 7α-hydrocholesterol. When the disorder is untreated, it can lead to death from complications of cirrhosis in the 1st few years of life.[5]

3β-HSDH deficiency should be considered in the differential diagnosis of any child with chronic hepatitis with features of cholestasis, in whom routine investigations reveal no obvious cause. It should be considered in children with clinical features suggesting progressive familial intrahepatic cholestasis. Routine laboratory investigations may show similar results (including a normal or low γ-glutamyl transpeptidase), but the two disorders are distinguishable on the basis of a high plasma 3α-hydroxy bile acid concentration in the progressive familial intrahepatic cholestasis syndromes but not in 3β-HSDH deficiency. 3β-HSDH should also be suspected in infantile rickets, particularly if there is clinical evidence of steatorrhoea or hepatomegaly, or laboratory evidence of malabsorption of vitamin E, vitamin A, or vitamin K, in addition to the vitamin D malabsorption. Early diagnosis is essential because bile acid replacement is effective in normalising liver function tests, improving symptoms, and preventing the development of cirrhosis and its complications.

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