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Case report

Characterization of urinary bile acids in a pediatric BRIC-1 patient: Effect of rifampicin treatment

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ABSTRACT

Background: Benign recurrent intrahepatic cholestasis type 1 (BRIC-1), a rare autosomal recessive disorder characterized by recurrent episodes of jaundice and pruritus, is caused by mutations in the *ATP8B1* gene. Rifampicin has been reported to be an effective treatment of jaundice and pruritus in patients with BRIC. Proposed mechanisms of effect for rifampicin include enhancement of multidrug-resistance protein 2 expression, activation of the enzymes of uridine diphosphate glucuronosyltransferase 1A1 and cytochrome P450 3A4, and stimulation of 6α-hydroxylation of bile acids.
Methods: To confirm the diagnosis of BRIC-1 and demonstrate the effect of rifampicin treatment on bile acid metabolism, we analyzed the patient's *ATP8B1* gene and bile acids in urine.
Results: We detected 2 heterozygous mutations in the *ATP8B1* gene, and increasing amounts of unusual bile acids such as 1β-hydroxylated cholic acid, 2β-hydroxylated cholic acid, 4β-hydroxylated cholic acid, 6α-hydroxylated cholic acid, and hyocholic acid in urine during rifampicin treatment.
Conclusions: We diagnosed a jaundiced pediatric patient with BRIC-1 caused by 2 novel mutations (1226delA/2210delA) in the *ATP8B1* gene. Rifampicin was effective in treating cholestasis. Results of urinary bile acid analyses during rifampicin treatment in this patient, suggested that rifampicin might stimulate 1β-, 2β-, and 4β-hydroxylation of bile acids in addition to 6α-hydroxylation.

1. Introduction

Rifampicin (RFP) was reported to be effective for treating jaundice and pruritus in patients with benign recurrent intrahepatic cholestasis (BRIC) [1], a rare autosomal recessive disorder characterized by recurrent episodes of jaundice and pruritus with neither elevation of serum γ-glutamyltransferase (GGT) nor, typically, any progressive liver damage [2]. Recent molecular biologic studies have demonstrated that BRIC type 1 (BRIC-1), and another hereditary disorder, progressive familial intrahepatic cholestasis type 1 (PFIC-1), are caused by mutations in the *ATP8B1* gene, which encodes ATP8B1 protein, a P-type ATPase [3]. Mechanisms underlying the hepatocytic effect of RFP treatment include enhancement of expression of multidrug-resistance protein 2 (MRP2/*ABCC2*) and activation of the enzymes of uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) and cytochrome P450 3A4 (CYP3A4) [4]. We report a pediatric patient diagnosed with BRIC-1 who had 2 novel mutations in the *ATP8B1* gene. We also make clinical correlations with results of urinary bile acid analyses during RFP treatment.

2. Case

2.1. Clinical and laboratory course

A previously healthy 7-year-old Japanese girl presented with an episode of progressive jaundice and pruritus. She was born with an uncomplicated pregnancy and delivery to healthy parents having no consanguinity. Two weeks before the episode, she had an influenza A virus

Abbreviations: RFP, rifampicin; BRIC, benign recurrent intrahepatic cholestasis; GGT, γ-glutamyltransferase; PFIC-1, progressive familial intrahepatic cholestasis type 1; UGT, uridine diphosphate glucuronosyltransferase; CYP, cytochrome P450; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBA, total bile acids; UDCA, ursodeoxycholic acid; BSEP, bile salt export pump; MRP, multidrug-resistance protein; PCR, polymerase chain reaction; Cr, creatinine; GC–MS, gas chromatography–mass spectrometry; CA, cholic acid; HCA, hyocholic acid; CDCA, chenodeoxycholic acid.

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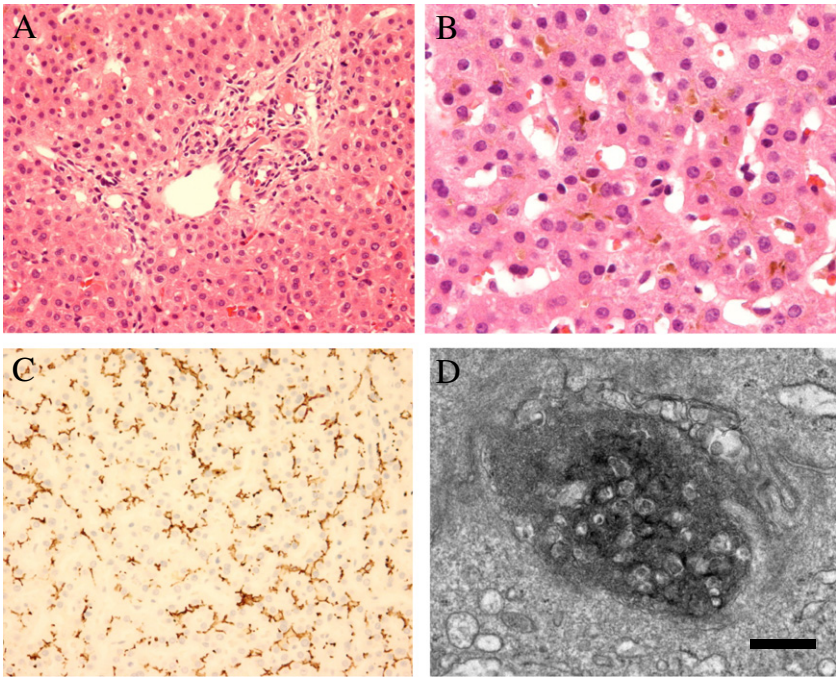

Fig. 1. Pathologic findings in the liver. (A) A portal tract shows no significant change (hematoxylin and eosin, ×200). (B) Intrahepatic cholestasis is evident, without giant cell transformation (hematoxylin and eosin, ×400). (C) Immunohistochemical reactivity for BSEP was strong at the canalicular membrane (brown reaction product, ×200). (D) Electron microscopic examination showed a canaliculus containing granular structures (Byler's bile). Bar, 5 μm.

infection, which was treated orally with oseltamivir phosphate. When progressive jaundice and pruritus became apparent, the patient was referred to Kurume University Hospital.

On physical examination, growth and development were within the normal range. No dysmorphic features were present. Hepatomegaly, jaundice and pruritus were noted. Neurologic findings were normal. Stools were gray. Initial serum chemistry results included aspartate aminotransferase (AST), 43 U/L (normal <33); alanine aminotransferase (ALT), 40 U/L (<30); alkaline phosphatase, 1377 U/L (115 to 359); total/direct bilirubin, 18.5/14.2 mg/dL (<1.2/<0.6); albumin, 3.8 g/dL (4.0 to 5.0); and total cholesterol, 236 mg/dL (128 to 219). Prothrombin time was 12.7 s (10.0 to 13.5); and blood ammonia, 27 μg/dL (<66). Serum GGT and total bile acids (TBA) were 40 U/L (<47) and 323 μmol/L (<10), respectively. Other causes of liver disease such as autoimmune hepatitis, chronic viral hepatitis, and other metabolic conditions were excluded by appropriate investigations. A drug-induced lymphocyte stimulation test using oseltamivir phosphate was negative. Abdominal ultrasonography showed a visible gallbladder and hepatomegaly; no choledochal cyst, bile duct dilation, or ascites were demonstrated. She received ursodeoxycholic acid (UDCA, 10 mg/kg/day), but clinical symptoms and liver dysfunction did not improve. Microscopic findings in a liver biopsy specimen included intrahepatic cholestasis with no giant cell transformation, fibrosis, or bile duct loss (Fig. 1A, B). Strong expression of the bile salt export pump (BSEP/*ABCB11*; Santa Cruz Biotechnology, Santa Cruz, CA; Fig. 1C) was demonstrated by immunohistochemistry. Electron microscopic examination showed finely granular bile materials (Byler's bile) in a dilated bile canaliculus (Fig. 1D). From the above data, we suspected a diagnosis of PFIC-1 or BRIC-1. Accordingly, we started RFP treatment (10 mg/kg/day). Treatment brought a dramatic improvement of jaundice and pruritus with significant reduction of serum bilirubin and TBA. Interestingly, GGT was transiently elevated after starting treatment. RFP was continued for a total of 7 weeks (Fig. 2). At this writing the patient is 8 years old, and has had no further episodes of jaundice or pruritus for 12 months after finishing RFP treatment.

2.2. Genetic analysis

To confirm the diagnosis of PFIC-1/BRIC-1, we analyzed the patient's *ATP8B1* gene with informed parental consent. Genomic DNA was extracted from mononuclear cells in a peripheral sample of whole blood, and the *ATP8B1* gene was amplified by polymerase chain reaction (PCR) using oligonucleotides designed according to the sequence of the *ATP8B1* from GenBank (NG_007148). Amplified PCR products were subcloned and sequenced using the Applied Biosystems 3,730*xl* genetic analysis system (Applied Biosystems, Foster City, CA). Sequencing analyses of all exons disclosed 2 heterozygous mutations in exons 13 and 20. In exon 13 we detected a 1-bp deletion, A of nucleotide 1226, causing a frameshift leading to formation of a stop codon at position 410 (c.1226delA, p.E409fsX2). In exon 20 we detected a 1-bp deletion, A of nucleotide 2210, causing a frameshift leading to formation of a stop codon at position 742 (c.2210delA, p.E737fsX6). Neither of the

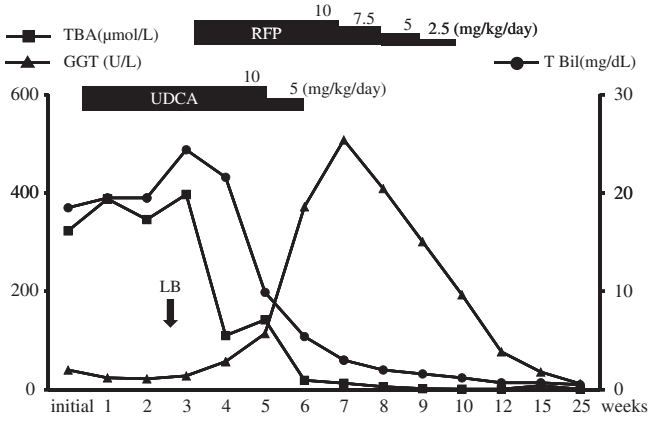

Fig. 2. Clinical course of the patient. Responses of the serum total bile acids (TBA, ■), total bilirubin (T Bil, ●), and γ-glutamyltransferase (GGT, ▲) to treatment with ursodeoxycholic acid (UDCA) and rifampicin (RFP) are shown. LB, liver biopsy.