Improved Liver Function and Relieved Pruritus after 4-Phenylbutyrate Therapy in a Patient with Progressive Familial Intrahepatic Cholestasis Type 2

Sotaro Naoi, MS[1,*], Hisamitsu Hayashi, PhD[1,*], Takeshi Inoue, MD[2], Ken Tanikawa, MD[3], Koji Igarashi, PhD[4], Hironori Nagasaka, MD, PhD[5], Masayoshi Kage, MD, PhD[3], Hajime Takikawa, MD, PhD[6], Yuichi Sugiyama, PhD[7], Ayano Inui, MD, PhD[8], Toshiro Nagai, MD, PhD[2], and Hiroyuki Kusuhara, PhD[1]

To examine the effects of 4-phenylbutyrate (4PB) therapy in a patient with progressive familial intrahepatic cholestasis type 2. A homozygous c.3692G>A (p.R1231Q) mutation was identified in *ABCB11*. In vitro studies showed that this mutation decreased the cell-surface expression of bile salt export pump (BSEP), but not its transport activity, and that 4PB treatment partially restored the decreased expression of BSEP. Therapy with 4PB had no beneficial effect for 1 month at 200 mg/kg/day and the next month at 350 mg/kg/day but partially restored BSEP expression at the canalicular membrane and significantly improved liver tests and pruritus at a dosage of 500 mg/kg/day. We conclude that 4PB therapy would have a therapeutic effect in patients with progressive familial intrahepatic cholestasis type 2 who retain transport activity of BSEP per se. *(J Pediatr 2014;164:1219-27).*

Progressive familial intrahepatic cholestasis type 2 (PFIC2), an inherited autosomal-recessive liver disease caused by mutations in *ABCB11* encoding the bile salt export pump (BSEP), is characterized by cholestasis and jaundice in the first year of life.[1] This disease progresses to severe cholestasis with sustained intractable itching, jaundice, diarrhea, and failure to thrive, leading to liver failure and death before adulthood. No medical therapy has been established for PFIC2.[1,2] BSEP is an adenosine triphosphate (ATP)-binding cassette transmembrane transporter located on the canalicular membrane of hepatocytes that mediates the biliary excretion of monovalent bile salts.[3-6] Therefore, in patients with PFIC2, biliary bile salt secretion is impaired, bile salts accumulate in hepatocytes, and consequently the hepatocytes are damaged.

We have published experimental evidence that 4-phenylbutyrate (4PB), a drug used to treat ornithine transcarbamylase deficiency (OTCD), has an additional pharmacologic effect to increase the hepatocanalicular expression of BSEP and the biliary excretion capacity of bile salts when given at a clinically relevant concentration in patients with OTCD.[7-9] Greater BSEP expression levels in liver specimens from patients with OTCD after 4PB therapy compared with before 4PB therapy suggest that 4PB treatment increases BSEP expression in humans.[10] These results indicate the possibility that 4PB may be a potential therapeutic compound for patients with PFIC2 who show a reduced BSEP expression at the canalicular membrane but who have retained transport activity of BSEP per se. To test this hypothesis, we investigated the effects of 4PB therapy in a patient with PFIC2 carrying a homozygous c.3692G>A (p.R1231Q) mutation in *ABCB11*.

[^{3}H]-TC	[^{3}H]-Taurocholate
4PB	4-Phenylbutyrate
ALT	Alanine aminotransaminase
AST	Aspartate aminotransaminase
ATP	Adenosine triphosphate
BSEP	Bile salt export pump
cDNA	Complementary DNA
D-Bil	Direct bilirubin
EV	Empty vector
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GGT	Gamma-glutamyl transferase
GPCR	G protein-coupled receptor
HA	Hemagglutinin
mRNA	Messenger RNA
OTCD	Ornithine transcarbamylase deficiency
PCR	Polymerase chain reaction
PFIC2	Progressive familial intrahepatic cholestasis type 2
P-gp	P-glycoprotein
qPCR	Quantitative polymerase chain reaction
T-Bil	Total bilirubin
TfR	Transferrin receptor
UDCA	Ursodeoxycholic acid
WT	Wild type

From the [1]Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan; [2]Department of Pediatrics, Dokkyo Medical University, Koshigaya Hospital, Saitama, Japan; [3]Department of Diagnostic Pathology, Kurume University Hospital, Fukuoka, Japan; [4]Bioscience Division, Reagent Development Department, TOSOH Corporation, Kanagawa, Japan; [5]Department of Pediatrics, Takarazuka City Hospital, Takarazuka-shi, Japan; [6]Department of Medicine, Teikyo University School of Medicine, Tokyo, Japan; [7]Sugiyama Laboratory, RIKEN Innovation Center, Research Cluster for Innovation, RIKEN, Yokohama, Japan; and [8]Department of Pediatric Hepatology and Gastroenterology, Saiseikai Yokohamashi Tobu Hospital, Yokohama, Japan

*Contributed equally.

Supported by a Grant-in-Aid for Young Scientists (B) (21790146) and Astellas Foundation for Research on Metabolic Disorders (to H.H.). H.H. and Y.S. have applied for a US patent on the effect of 4-phenylbutyrate on bile salt export pump (13/299 989). The other authors declare no conflicts of interest.

 http://dx.doi.org/10.1016/j.jpeds.2013.12.032

Methods

We obtained approval for the study from the institutional ethics review boards. Informed consent was provided by the patient's parents before assessment because the patient was younger than 18 years of age. A detailed description of the materials and methods is presented in the **Appendix** (available at www.jpeds.com). All materials and methods used standard techniques and commercially available reagents.

The patient, a girl who is now 1 year of age, was born to parents of Pakistani descent. She developed hepatocellular cholestasis and jaundice with normal serum gamma-glutamyl transferase (GGT) activity at the age of 2 months and was diagnosed with PFIC2 by the presence of the c.3692G>A (p.R1231Q) mutation in both alleles of *ABCB11* and no detectable immunosignal for BSEP at the canalicular membrane of a liver section sample (**Figure 1**, A and B). Despite treatment with ursodeoxycholic acid (UDCA; 120 mg/day), she continued to have severe cholestasis with sustained intractable itch, jaundice, diarrhea, and failure to thrive. 4PB therapy was started at the age of 4 months. The administration of UDCA was maintained during and after the course of 4PB treatment.

Sequence Analysis of *ATP8B1* and *ABCB11*

Genomic DNA was isolated from peripheral blood leukocytes using a Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin), and all exons of *ATP8B1* and *ABCB11* and flanking intron–exon boundaries were amplified by polymerase chain reaction (PCR). Primer sequences and PCR conditions were designed according to previous reports.[11,12] Both strands were analyzed using BigDye Direct Cycle Sequencing Kit (Applied Biosystems, Foster City, California).

In Vitro Studies

pShuttle (Clontech, Palo Alto, California) containing complementary DNA (cDNA) of human BSEP with a hemagglutinin (HA) tag at the N-terminus (HA-BSEP$^{\text{wild type [WT]}}$) and that of HA-BSEP^{WT} with the c.3692G>A (p.R1231Q) or p.T1210P mutation (HA-BSEP^{R1231Q} and HA-BSEP^{T1210P}) were used for this study.[8] The c.3692G>A (p.R1231Q) and p.T1210P mutations were introduced into pShuttle containing HA-BSEP^{WT} cDNA by site-directed mutagenesis as described previously.[9] HEK293T cells and McA-RH7777 cells transfected with pShuttle containing HA-BSEP^{WT}, HA-BSEP^{R1231Q}, or HA-BSEP^{T1210P} cDNA, or empty vector (EV) (HA-BSEP^{WT}, HA-BSEP^{R1231Q}, HA-BSEP^{T1210P}, or EV HEK293T cells and HA-BSEP^{WT}, HA-BSEP^{R1231Q}, HA-BSEP^{T1210P}, or EV McA-RH7777 cells) were subjected to analysis of quantitative PCR (qPCR), cell surface biotinylation, immunofluorescence, and transport. All in vitro experiments were performed as described previously,[7,9] and a detailed description of the experiments is presented in the **Appendix**. The cells were treated with 1 mM 4PB for 24 hours before the in vitro experiments, which were designed to examine the effects of 4PB on HA-BSEP^{WT}, HA-BSEP^{R1231Q}, and HA-BSEP^{T1210P}.

Treatment of the Patient with PFIC2 with 4PB

Oral administration of 4PB (AMMONAPS; Swedish Orphan Inter AB, Stockholm, Sweden) was started at a daily dosage of 200 mg/kg/day divided into 4 doses a day. After 1 month, the dosage was increased to 350 mg/kg/day, which was maintained for an additional month. Because neither a therapeutic effect nor any side effects were observed, the dosage was increased up to 500 mg/kg/day, which is a clinically relevant dosage for OTCD, and this dosage was maintained for the next 4 months.

A liver biopsy sample was collected before and after the course of 4PB treatment. A part of the sample was preserved in RNAlater (QIAGEN, Hilden, Germany) for RNA preparation and stored at −20°C. Another portion was fixed in 4% formaldehyde at room temperature for histological analysis, and the remaining portion was snap-frozen in liquid nitrogen for preparation of membrane fractions and stored at −70°C in a deep freezer. Serum was collected before, during, and after the course of 4PB treatment. Liver tests were performed using standard methods immediately after collection, and the remaining specimens were preserved at −70°C for further analysis.

The severity of pruritus was scored according to a previous report[13]: 0, none; 1, mild scratching when undistracted; 2, active scratching without abrasion; 3, abrasions; or 4, cutaneous mutilation, with bleeding and scarring. Serum concentrations of total bile acids, histamine, and tryptase were measured by an enzyme cycling method (Wako Pure Chemicals, Osaka, Japan), a competitive enzyme immunoassay (Bertin Pharma, Montigny le Bretonneux, France), and a fluoroenzyme immunoassay (USCN Life Science Inc, Hubei, People's Republic of China), according to the manufacturer's instruction. The concentration and activity of autotaxin in serum were assessed using a specific two-site enzyme immunoassay and the measurement of choline liberation from the substrate lysophosphatidylcholine.[14]

Histologic Analysis of Human Liver Specimens

Liver biopsies were fixed in 10% formalin and embedded in paraffin. Then, 4-μm thick sections from the liver specimens were prepared by a Microm HM340E (Microm International GmbH, Walldorf, Germany), adhered to the glass coverslips, and subjected to hematoxylin and eosin staining and immunohistochemistry followed by microscopic analysis with an Olympus CX41 (Olympus, Tokyo, Japan) to evaluate the degree of cholestasis, giant cell transformation, inflammation in the liver tissues, and BSEP expression at the canalicular membrane.

Statistical Analyses

The data in the **Figures** are presented as the mean ± SE. The significance of differences between 2 variables and multiple variables was calculated at the 95% confidence level by Student *t* test and by one-way analysis of variance with Tukey test, respectively, using Prism software (GraphPad Software, Inc, La Jolla, California).