Rotor Syndrome: Glucuronidated Bile Acidemia From Defective Reuptake by Hepatocytes

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Organic anion transporting polypeptide (OATP) 1B1 (gene, solute carrier organic anion transporter family member 1B1 [*SLCO1B1*]) and OATP1B3 (*SLCO1B3*) serve as transporters for hepatic uptake of important endogenous substances and several commonly prescribed drugs. Inactivation of both proteins together causes Rotor syndrome. How this OATP1B1/1B3 defect disturbs bile acid (BA) metabolism is largely unknown. In this study, we performed detailed BA analysis in 3 patients with genetically diagnosed Rotor syndrome. We found that BAs glucuronidated at the C-3 position (BA-3G) accounted for 50% or more of total BAs in these patients. In contrast but similarly to healthy controls, only trace amounts of BA-3G were detected in patients with constitutional indocyanine green excretory defect (OATP1B3 deficiency) or sodium-taurocholate cotransporting polypeptide (NTCP; gene, solute carrier family 10 member 1 [*SLC10A1*]) deficiency. Therefore, substantial amounts of BA-3G are synthesized in hepatocytes. The cycling pathway of BA-3G, consisting of excretion from upstream hepatocytes and uptake by downstream hepatocytes by OATP1B1/1B3 may exist to reduce the burden on upstream hepatocytes. *Conclusion:* Detailed BA analysis revealed glucuronidated bile acidemia in patients with Rotor syndrome. Further exploration of the physiologic role of glucuronidated BAs is necessary. (*Hepatology Communications* 2021;5:629-633).

Organic anion transporting polypeptide (OATP) 1B1 (gene, solute carrier organic anion transporter family member 1B1 [*SLCO1B1*]) and OATP1B3 (*SLCO1B3*) are hepatic uptake transporters for endogenous substances, such as conjugated bilirubin, bile acids (BAs), eicosanoids, prostaglandins, and hormones, as well as several commonly prescribed drugs. Inactivation of both proteins causes Rotor syndrome, which manifests as conjugated hyperbilirubinemia.[1,2] How OATP1B1/1B3 defects disturb BA metabolism is largely unknown. In this study we performed detailed BA analysis in patients with Rotor syndrome.

Materials and Methods

CASE DESCRIPTION AND BA ANALYSIS

We analyzed a total of 83 BA species (Supporting Tables S1 and S2), including 17 glucuronidated

Abbreviations: ABCB/C, adenosine triphosphate binding cassette subfamily B/C; BA, bile acid; BA-3G, 3-glucuronidated bile acid; BA-3S, 3-sulfated bile acid; BSEP, bile salt export pump; MRP, multidrug resistance-associated protein; NTCP, sodium-taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; SLC10A1, solute carrier family 10 member 1; SLCO1B1/1B3, solute carrier organic anion transporter family member 1B1/1B3; UGT, uridine 5'-diphospho-glucuronosyltrasferase.

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species, using liquid chromatography-electrospray ionization tandem mass spectrometry[3] in 3 patients with genetically diagnosed Rotor syndrome.[2] Our control subjects included patients with constitutional indocyanine green excretory defect (OATP1B3 deficiency),[4] sodium-taurocholate cotransporting polypeptide (NTCP; gene, solute carrier family 10 member 1 [*SLC10A1*]) deficiency, Dubin-Johnson syndrome (multidrug resistance-associated protein 2 [MRP2; gene, adenosine triphosphate binding cassette subfamily C member 2 [*ABCC2*] deficiency), parents of a patient with Rotor syndrome, and 8 healthy individuals. Two of 3 patients with Rotor syndrome and the patient with Dubin-Johnson syndrome had mild hypercholanemia, while the patient who was NTCP deficient exhibited considerable hypercholanemia (Table 1).

Results

In BA analyses of sera, BAs glucuronidated at the C-3 position (BA-3G) accounted for 50% or more of total BAs in patients with Rotor syndrome and consisted mostly of three species: glycochenodeoxycholic acid-3G, glycodeoxycholic acid-3G, and glycolithocholic acid-3G. In analyses of urine from these patients, BA-3G accounted for 20% to 30% of total BAs (Table 1; Supporting Table S3). The serum BA-3G concentration was slightly elevated in the patient with Dubin-Johnson syndrome (3.9 μmol/L) (Table 1; Supporting Table S3), while no predominance of BA-3G was observed in patients with isolated OATP1B3 or NTCP deficiency. Serum concentrations of BAs sulfated at the C-3 position (BA-3S) were slightly higher in patients with Rotor syndrome (range, 2.4-4.3 μmol/L) than in healthy controls (range, 0.1-0.8 μmol/L) (Table 1; Supporting Table S3).

Discussion

The patients with Rotor syndrome manifested remarkable increases of BA-3G in sera and urine. On the other hand, BA-3G concentrations in patients with isolated OATP1B3 deficiency or NTCP deficiency were comparable to those in healthy controls. The parents of the youngest patient with Rotor syndrome (No. 1 in Table 1), who were heterozygous for the *SLCO1B1* and *SLCO1B3* null allele, did not exhibit BA-3G elevation (Supporting Table S3). Accordingly, inactivation of OATP1B1 and OATP1B3 proteins in the same individual would cause BA-3G accumulation in the circulation due to defective hepatic uptake.

The present study of Rotor syndrome indicates that substantial amounts of BA-3G are synthesized. BA glucuronidation at C-3 appears to be catalyzed by hepatocytic uridine 5'-diphosphoglucuronosyltransferase (UGT) 1A4 and UGT2B7.[5] Theoretically, BA-3G can arise from two routes (Fig. 1): by enterohepatic circulation after excretion into bile canaliculi and by direct excretion from hepatocytes into the sinusoid, mediated by MRP3 (*ABCC3*). As OATP1B1/1B3 proteins are expressed exclusively in the pericentral area (zone 3), BA-3G is efficiently taken up by these downstream hepatocytes by OATP1B1/1B3 and subsequently is excreted into the canaliculus by MRP2 or the bile salt export pump (BSEP; *ABCB11*). This cycling of BA-3G serves to reduce the burden on upstream hepatocytes by

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TABLE 1. ANALYSIS OF BAS, INCLUDING 3-GLUCURONIDES, IN PATIENTS WITH ROTOR SYNDROME, CONSTITUTIONAL ICG EXCRETORY DEFECT, NTCP DEFICIENCY, AND DUBIN-JOHNSON SYNDROME

Patient Number	1	2	3	4	5	6	
Diagnosis	Rotor syndrome	Rotor syndrome	Rotor syndrome	Constitutional ICG excretory defect	NTCP deficiency	Dubin-Johnson syndrome*	Healthy controls (n = 8)
Genotype							
SLCO1B1 c.1738C>T (p.R580X)	homozygous	homozygous	homozygous	wild-type	NA	NA	NA
SLCO1B3 L1 insertion	homozygous	homozygous	homozygous	homozygous	NA	NA	NA
SLC10A1 c.800C>T (p.S267F)	NA	NA	NA	NA	homozygous	NA	NA
Age, years	11	61	60	66	1 month	80	40-63
Sex	M	M	M	M	F	M	M/F = 3/5
Direct bilirubin, mg/dL	2.3	4.5	3.8	0.2	0.6	2.1	NA
ALT, U/L	14	19	20	13	17	13	NA
ICG-R15, %	51.4	87.6	75.0	79.8	NA	11.0	NA
Serum total BAs, μmol/L	5.9	17.5	23.6	4.9	532.1	31.5	2.9 (1.3-4.9)†
GCA-3G, μmol/L	ND	ND	ND	ND	ND	trace	trace
GCDCA-3G, μmol/L	0.8 (13.2)‡	2.8 (15.8)	5.3 (22.5)	0.1 (1.1)	0.4 (<0.1)	0.9 (2.9)	0 (0-0.2)
GDCA-3G, μmol/L	1.1 (19.0)	3.4 (19.2)	7.0 (29.5)	0.1 (1.4)	0.1 (<0.1)	0.9 (2.9)	0.4 (0-0.8)
GLCA-3G, μmol/L	0.9 (15.2)	4.3 (24.5)	3.9 (16.3)	ND	ND	0.1 (0.3)	0 (0-0.4)
Other BA-3G, μmol/L	0.2 (2.5)	0.4 (2.3)	0.6 (2.5)	0.1 (0.6)	0.1 (<0.1)	2.0 (6.3)	0 (0-0.5)
Total BA-3G, μmol/L	3.0 (49.9)	10.9 (62.0)	16.8 (71.1)	0.2 (3.1)	0.6 (0.1)	3.9 (12.4)	0.1 (0-1.0) [2.0 (1.5-19.7)]§
Total BA-3S, μmol/L	2.4 (40.5)	3.2 (18.2)	4.3 (18.0)	0.3 (5.1)	12.0 (2.2)	1.9 (5.9)	0.2 (0.1-0.8) [8.7 (1.9-20.2)]
Other BAs, μmol/L	0.6 (9.5)	3.4 (19.7)	2.6 (10.9)	4.6 (91.9)	519.6 (97.7)	25.8 (81.7)	2.1 (1.0-4.1) [88.6 (64.3-95.9)]
Urinary total BAs, mmol/mol Cre	3.6	4.0	NA	NA	66.0	NA	0.47 (0.29-1.68)
Total BA-3G, mmol/mol Cre	0.8 (23.2)	1.1 (28.0)	NA	NA	0.3 (0.4)	NA	0.1 (0-1.0) [5.2 (2.0-13.2)]
Total BA-3S, mmol/mol Cre	2.7 (74.8)	2.8 (68.4)	NA	NA	13.2 (19.9)	NA	0.3 (0.2-1.4) [59.3 (39.9-80.4)]
Other BAs, mmol/mol Cre	0.1 (2.8)	0.2 (3.9)	NA	NA	52.5 (79.6)	NA	0.2 (0.1-0.3) [34.0 (12.6-52.0)]

*The Dubin-Johnson syndrome patient was diagnosed from the presence of black liver discoloration and pigment granules in hepatocytes.
†Median (range).
‡Percentage in total BAs.
§Values in brackets represent median (range) of percentage in total BAs.
Abbreviations: ALT, alanine aminotransferase; cre, creatinine; F, female; GCA-3G, glycocholic acid 3-glucuronide; GCDCA-3G, glycochenodeoxycholic acid 3-glucuronide; GDCA-3G, glycodeoxycholic acid 3-glucuronide; GLCA-3G, glycolithocholic acid 3-glucuronide; ICG, indocyanine green; ICG-R15, indocyanine green retention rate at 15 minutes; M, male; NA, not available; ND, not detected.

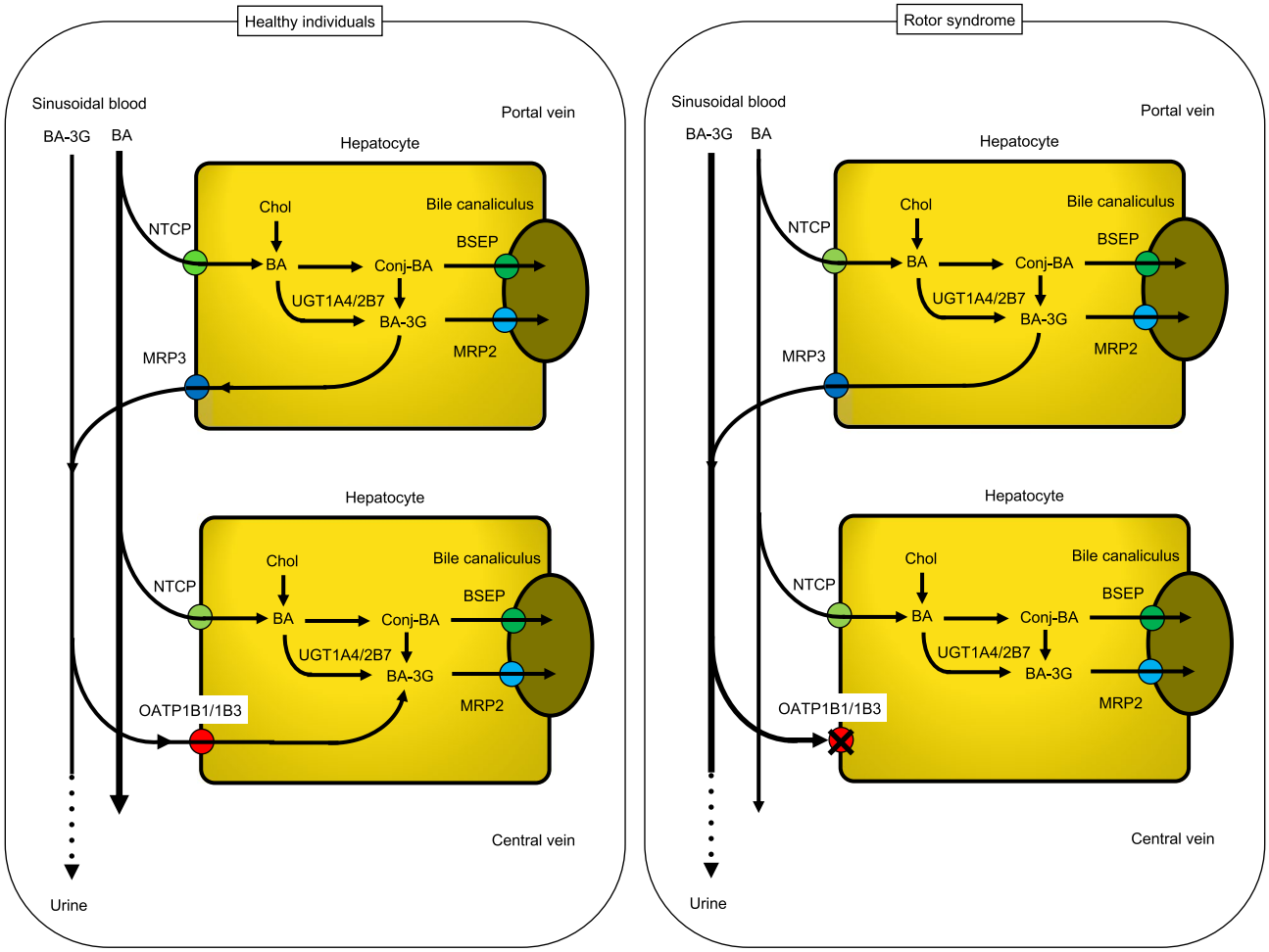

FIG. 1. Hepatic transport of glucuronidated BAs in patients with Rotor syndrome. In healthy individuals, unconjugated BAs arise in hepatocytes by two routes: synthesis from cholesterol and transport from the sinusoidal blood by NTCP. Most BAs undergo conjugation with glycine or taurine and are secreted into the bile canaliculus by BSEP. As much as 10% of BAs are conjugated with glucuronide at the C-3 position by UGT1A4 and UGT2B7 and are excreted into the bile canaliculus by MRP2. A portion of BAs is transported back into the sinusoid by MRP3. BA-3G are taken up by hepatocytes by OATP1B1 and OATP1B3, which are exclusively expressed in the pericentral area (zone 3). In Rotor syndrome, BA-3G are unable to enter hepatocytes due to lack of expression of both OATP1B1 and OATP1B3, so they subsequently accumulate in the circulation, with preferential excretion through the urine. Abbreviations: Chol, cholesterol; Conj-BA, conjugated bile acid.

preventing saturation of BA export capacity, as suggested by metabolism of bilirubin.[1] The clinical significance of glucuronidated BAs in pathologic conditions remains largely unknown. Their hydrophilic property might alleviate the cytotoxicity of excess hydrophobic BAs in cholestasis. Notably, chenodeoxycholic acid (CDCA)-3G and lithocholic acid-3G can activate the farnesoid X receptor (FXR; gene nuclear receptor subfamily 1 group H member 4 [*NR1H4*]) to an extent equivalent to CDCA, a potent endogenous FXR agonist.[6] Furthermore, a recent study demonstrated that fenofibrate, which lessens cholestasis in primary biliary cholangitis, increases BA-3G by up-regulating UGT1A4.[5] These results suggest a significant role of BA-3G in the regulation of BA metabolism.

Interestingly, the serum BA-3G concentration was slightly elevated in our patient with Dubin-Johnson syndrome, but the elevation was relatively small compared to that in patients with Rotor syndrome. This might be explained by a considerable contribution of BSEP to BA-3G export into the canaliculus. Although we did not analyze BAs glucuronidated at the C-24

position in this study, amounts of this species are considered negligible compared to those of BA-3G.[5]

Although BA-3S could be transported by OATP1B1/1B3[7] similarly to BA-3G, elevation of serum BA-3S concentrations in patients with Rotor syndrome remained slight, possibly because of their efficient excretion into the urine.

To date, glucuronidated BAs have attracted little attention. However, our detailed BA analysis in Rotor syndrome indicated that substantial amounts of glucuronidated BAs are synthesized. Further exploration of their physiologic role is necessary.

REFERENCES

1) van de Steeg E, Stránecký V, Hartmannová H, Nosková L, Hřebíček M, Wagenaar E, et al. Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. J Clin Invest 2012;122:519-528.
2) Kagawa T, Oka A, Kobayashi Y, Hiasa Y, Kitamura T, Sakugawa H, et al. Recessive inheritance of population-specific intronic LINE-1 insertion causes a rotor syndrome phenotype. Hum Mutat 2015;36:327-332.
3) Muto A, Takei H, Unna A, Murai T, Kurosawa T, Ogawa S, et al. Detection Δ^4-3-oxo-steroid 5β-reductase deficiency by LC-ESI-MS/MS measurement of urinary bile acids. J Chromatogr B Analyt Technol Biomed Life Sci 2012;900:24-31.
4) Kagawa T, Adachi Y, Hashimonto N, Mitsui H, Ohashi T, Yoneda M, et al. Loss of organic anion transporting polypeptide 1B3 function causes marked delay indocyanine green clearance without any clinical symptoms. Hepatology 2017;65:1065-1068.
5) Trottier J, Perreault M, Rudkowaka I, Levy C, Dallalre-Theroux A, Verreault M, et al. Profiling serum bile acid glucuronides in humans: gender divergences, genetic determinants, and response to fenofibrate. Clin Pharmacol Ther 2013;94:533-543.
6) Mostarda S, Passeri D, Carotti A, Cerra B, Colliva C, Benicchi T, et al. Synthesis, physicochemical properties, and biological activity of bile acids 3-glucuronides: novel insights into bile acid signalling and detoxification. Eur J Med Chem 2018;144:349-358.
7) Meng LJ, Wang P, Wolkoff AW, Kim RB, Tirona RG, Hofmann AF, et al. Transport of the sulfated, amidated bile acid, sulfolithocholyltaurine, into rat hepatocytes is mediated by Oatp1 and Oatp2. Hepatology 2002;35:1031-1040.

Supporting Information

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