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).

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...
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, glycochenodeoxycholic 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 SLC01B1 and SLC01B3 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'-diphospho-glucuronosyltransferase (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...
### 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 | Diagnosis                  | Rotor syndrome | Rotor syndrome | Rotor syndrome | Constitutional ICG excretory defect | NTCP deficiency | Dubin-Johnson syndrome* | Healthy controls (n = 8) |
|----------------|----------------------------|----------------|----------------|----------------|-----------------------------------|----------------|-------------------------|--------------------------|
| Genotype       |                            | homzygous      | homzygous      | homzygous      | wild-type                         | NA             | NA                      | NA                       |
| SLC10A1 c.1738C>T (p.R580X) |                | homozygous      | homozygous      | homozygous      | NA                                | NA             | NA                      | NA                       |
| SLC10A1 L1 insertion |                | NA             | NA             | NA             | homozygous                        | NA             | NA                      | NA                       |
| SLC10A1 c.800C>T (p.S267F) |                | NA             | NA             | NA             | NA                                | NA             | 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            | 0.2                     | 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                               | trace          | 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 (0.2)                  |
| 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 (0.8)                |
| GLCA-3G, μmol/L |                           | 0.9 (15.2)     | 4.3 (24.5)     | 3.9 (16.3)     | ND                               | ND             | 0.1 (0.3)               | 0 (0.4)                  |
| Other BA-3G, μmol/L |                         | 0.2 (2.5)     | 0.4 (2.5)      | 0.6 (2.5)      | 0.1 (0.6)                        | 0.1 (0.1)      | 2.0 (6.3)               | 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) [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             | 0.3 (0.4)                        | 0.5 (0.1)      | 0.4 (0.1-0.3)           | 0.1 (0.2-1.68)          |
| Total BA-3G, mmol/mol Cre |                    | 0.8 (23.2)    | 1.1 (28.0)     | NA             | 0.3 (0.4)                        | NA             | 0.1 (0.1-0.3)           | [5.2 (2.0-13.2)]       |
| Total BA-3S, mmol/mol Cre |                    | 2.7 (74.8)    | 2.8 (68.4)     | NA             | 13.2 (19.9)                      | NA             | 0.3 (0.1-1.4)           | [59.3 (39.9-80.4)]      |
| Other BAS, mmol/mol Cre |                        | 0.1 (2.8)     | 0.2 (3.9)      | NA             | 0.5 (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).

§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.
preventing saturation of BA export capacity, as suggested by metabolism of bilirubin. 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. Furthermore, a recent study demonstrated that fenofibrate, which lessens cholestasis in primary biliary cholangitis, increases BA-3G by up-regulating UGT1A4. 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.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1660/suppinfo.