Antibacterial Drug Treatment Increases Intestinal Bile Acid Absorption via Elevated Levels of Ileal Apical Sodium-Dependent Bile Acid Transporter but Not Organic Solute Transporter α Protein

Masaaki Miyata,*a,b Kenjiro Hayashi,a Hiroki Yamakawa,a Yasushi Yamazoe,a and Kouichi Yoshinari*†

a Division of Drug Metabolism and Molecular Toxicology, Graduate School of Pharmaceutical Sciences, Tohoku University; 6–3 Aoba, Aramaki, Aoba-ku, Sendai 980–8578, Japan; and b Department of Food Science and Technology, National Fisheries University; 2–7–1 Nagatahonmachi, Shimonoseki, Yamaguchi 759–6595, Japan.

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Antibacterial drug treatment increases the bile acid pool size and hepatic bile acid concentration through the elevation of hepatic bile acid synthesis. However, the involvement of intestinal bile acid absorption in the increased bile acid pool size remains unclear. To determine whether intestinal bile acid absorption contributes to the increased bile acid pool size in mice treated with antibacterial drugs, we evaluated the levels of bile acid transporter proteins and the capacity of intestinal bile acid absorption. Ileal apical sodium-dependent bile acid transporter (ASBT) mRNA and protein levels were significantly increased in ampicillin (ABPC)-treated mice, whereas organic solute transporter α (OSTα) mRNA levels, but not protein levels, significantly decreased in mice. Similar alterations in the expression levels of bile transporters were observed in mice treated with bacitracin/neomycin/streptomycin. The capacity for intestinal bile acid absorption was evaluated by an in situ loop method. Increased ileal absorption of taurochenodeoxycholic acid was observed in mice treated with ABPC. These results suggest that intestinal bile acid absorption is elevated in an ASBT-dependent manner in mice treated with antibacterial drugs.

Key words bile acid; apical sodium-dependent bile acid transporter; ampicillin; organic solute transporter

Bile acids are cytotoxic, and the elevation of hepatic bile acid concentration causes hepatobiliary injury. The size of the bile acid pool is strictly regulated by bile acid-mediated negative feedback mechanisms.1–3 The antibacterial drug treatment elevates the total bile acid pool size and bile acid synthesis rate compared with that before treatment in the patients with gallstones.4,5 Ampicillin (ABPC) treatment increases the bile acid pool size and hepatic bile acid concentration in experimental animals.6 This disruption of bile acid homeostasis is in part due to the elevation of bile acid synthesis. The mRNA levels of hepatic rate-limiting enzyme in bile acid synthesis, Cyp7a1, and hepatic cholesterol 7α-hydroxylase activities are increased in mice treated with antibacterial drugs. In contrast, increase in the portal bile acid concentration and ileal apical sodium-dependent bile acid transporter (ASBT) protein levels were observed in mice.7 However, it remains unclear whether the capacity of intestinal absorption is elevated in mice treated with antibacterial drugs.

The intestinal transport of bile acids requires multiple steps. ASBT mediates the initial uptake of bile acids across the ileal enterocyte apical brush border membrane.8,9 After entering the cytosolic compartment, bile acids bind to the ileal bile acid-binding protein (IBABP). IBABP is believed to be involved in the transcellular transport of bile acids.10,11 Bile acids are ultimately shuttled across the ileal enterocyte and are exported across the basolateral membrane into the portal circulation by the organic solute transporter, organic solute transporter (OST)α–OSTβ.12,13 Bile acid pool sizes are significantly decreased not only in Asbt-null mice but also in Ostα-null mice, indicating that both bile acid transporters are essential for the intestinal bile acid transport.14,15 In the present study, we evaluated the expression levels of each transporter and the capacity of intestinal bile acid absorption in mice treated with antibacterial drugs.

MATERIALS AND METHODS

Materials ABPC was purchased from Nacalai Tesque (Kyoto, Japan). Bacitracin (BC) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Neomycin trisulfate (NM) was purchased from MP Biomedicals (Irvine, CA, U.S.A.). Streptomycin sulfate (SM) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Bacitracin (BC) was purchased from Sigma-Aldrich Inc. or Steraloids Inc. (Newport, RI, U.S.A.). Oligonucleotides were commercially synthesized by Fasmac (Atsugi, Japan). Antiserum for mouse OSTα was provided by Dr. Paul A. Dawson (Wake Forest University, School of Medicine, Winston-Salem, NC, U.S.A.).

Animal Treatment and Sample Collection All animal procedures were approved by the Institutional Animal Care and Use Committee of Tohoku University in Sendai, Japan. C57BL/6N male mice (Charles River Japan Inc., Yokohama, Japan) were housed under a standard 12-h light:dark (9:00 a.m. to 9:00 p.m.) cycle. Before experimentation, mice were fed standard rodent chow (CE-2; Clea Japan Inc., Tokyo, Japan) and water ad libitum for acclimatization. Mice were administered per os (p.o.) ABPC (100 mg/kg body weight,
dissolved in saline) or bacitracin/neomycin/streptomycin (BC/NM/SM) (200 mg/kg body weight each agent, dissolved in saline) at 9:00 a.m. for 7 d.

**Ileal Bile Acid Absorption (in Situ Loop Method)**  
In situ loop method was performed as previously described with minor modifications. Mice were anesthetized with ethyl ether. An ileal loop of approximately 10 cm in length was isolated using ligatures at both ends, and 500 µL of dosing solution [5 mM taurochenodeoxycholic acid (TCDCA) in phosphate-buffered saline] was injected into the loop with a syringe. The portal blood was collected once from each mouse at 10 min after the injection. TCDCA concentration in the portal blood was analyzed by high-performance liquid chromatography (HPLC) as previously described. The portal blood was suspended in three volumes (v/v) of ethanol and was centrifuged at 20000 × g for 10 min. Supernatants were subjected to HPLC analysis.

**RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (PCR)**  
Total RNA was extracted using the acid guanidine thiocyanate–phenol–chloroform method, and cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, U.S.A.). Quantitative PCR was performed using GoTaq qPCR master mix (Promega, Madison, WI, U.S.A.) with the TP800 thermal cycler dice real time system (TaKaRa Bio, Otsu, Japan). Relative mRNA levels were calculated using the comparative threshold cycle method. The following specific forward and reverse primers were used for real-time quantitative PCR: Gapdh forward, 5'-TGT GTC CGT CGT GGA TCT GA-3' and reverse, 5'-CCT GCT TCA CCA CCT TCT TGA T-3'; Asbt forward, 5'-TGG GTT TCT TCC TGG CAT GAC T-3' and reverse, 5'-TGG TGT TCT TCC TGG CTA GAC T-3'; Ostα forward, 5'-ATG CAT CTG GGT GAA CAG AA-3' and reverse, 5'-GAG TAG GGA GGT GAG CAG AAC GA-3'; and Ostβ forward, 5'-AGA TGC GGC TCC TTG GAA TTA-3' and reverse, 5'-TGG CAG AAA GAC AAG TGA TG-3'.

**Preparation of Ileal Crude Membrane**  
The preparation of ileal crude membrane was performed as described previously. Intestinal segments were homogenized on ice in 250 mM sucrose, 10 mM triethanolamine HCl (pH 7.6) in the presence of a protease inhibitor mixture (0.1 mM phenylmethylsulfonyl fluoride, 10 µg/mL pepstatin, 10 µg/mL aprotonin, and 10 µg/mL leupeptin) using a Potter–Elvehjem homogenizer. After tissue fragments and nuclei were pelleted at 2500 × g.
membranes were pelleted at 20500×g, resuspended in the homogenization buffer, aliquoted, and stored at 80°C.

**Immunoblotting** Prepared ileal crude membranes (2 μg protein/lane) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 9.5% polyacrylamide gels. After transfer to an Immobilon-P transfer membrane (Millipore, Billerica, MA, U.S.A.), blots were blocked for 2 h in phosphate buffered saline (PBS) containing 5% non-fat dried milk and 0.05% Tween 20. The membranes were incubated for 2 h with the primary antibody, washed with PBS containing 0.05% Tween 20 (PBS-T), and incubated for 1 h with the secondary antibody (Sigma-Aldrich Inc.). The following dilutions of antibodies were used: anti-ASBT, 1:500; anti-OSTα, 1:10000; anti-β-actin (AC-15), 1:10000; alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (IgG) antibody, 1:20000; horseradish peroxidase-conjugated goat anti-rabbit IgG antibody, 1:10000; and horseradish peroxidase-conjugated rabbit anti-mouse IgG antibody, 1:10000. Antibody binding was detected using a Luminata Crescendo Western HRP substrate (Millipore Corporation) and ImageCapture G3 (Liponics Inc., Tokyo, Japan).

**Statistical Analysis** Values are presented as mean±S.D. Data were analyzed using the unpaired Student’s t-test or ANOVA with Dunnett’s post test using Prism 4.0 software (GraphPad Software Inc., San Diego, CA, U.S.A.) for significant differences between the mean values of each group. The p<0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

In the ileum, bile acids are transported across the apical brush border membrane via ASBT and across the basolateral membrane via OSTα/OSTβ. To elucidate the influence of antibacterial drug treatment on the ileal bile acid transport, we measured the ileal expression levels of these two transporters. In the ileum, mRNA levels of Ostα were significantly decreased in mice treated with ABPC (100 mg/kg for 7d), whereas Asbt mRNA levels were significantly increased in mice treated with ABPC (Fig. 1A). To test whether these changes in gene expression were found in mice treated with other antibacterial drugs that are not absorbed from the intestine, mice were orally treated with BC/NM/SM (200 mg/kg) for 7d). The treatment of mice with these antibiotics reduces the levels of enterobacteria to less than 0.001%. Consistent with ABPC-treated mice, Asbt mRNA levels were significantly increased in mice treated with BC/NM/SM, whereas Ostα mRNA levels were significantly decreased in mice.

To measure the ileal protein levels of both ASBT and OSTα in the same sample, we prepared ileal crude membranes and subjected them to Western blot analysis. Consistent with changes in mRNA levels, ASBT protein levels were significantly increased in the ileal crude membrane of mice treated with ABPC or BC/NM/SM (Fig. 1B). OSTα protein levels were not significantly changed in the ileal crude membrane of mice treated with ABPC or BC/NM/SM, although the mRNA levels of Ostα were decreased significantly.

To elucidate the influence of antibacterial drug treatment on ileal bile acid transport, we measured the capacity of ileal bile acid absorption using an in situ loop method. TCDCA is efficiently transported by mouse ASBT and OSTα/OSTβ but is not typically a component of the mouse bile acid pool. TCDCA concentrations in the portal blood were measured after TCDCA was infused into an in situ ileal loop taken from mice treated with ABPC for 7d. TCDCA concentrations in the portal blood depend on the time after TCDCA injection. In subsequent experiments, the ileal bile acid absorption rate was estimated as the TCDCA concentration of the portal blood at 10 min after the injection. TCDCA concentrations in the portal blood were significantly higher in mice treated with ABPC than in mice treated with vehicle (Fig. 2). The concentrations were increased to 193% of control in mice treated with ABPC.

The present study demonstrated that the capacity of ileal bile acid absorption was increased in mice treated with antibacterial drugs. Ampicillin-mediated elevation of bile acid pool size is likely due to increases in not only hepatic bile acid synthesis but also intestinal bile acid absorption. The capacity of ileal bile acid absorption was increased, although there were no significant increases in Ostα protein levels in mice treated with antibacterial drugs. Under conditions of treatment with antibacterial drugs, the capacity of ileal bile acid absorption might be independent of Ostα-mediated bile acid transport in basolateral membrane. Bile acid transport across the apical brush border membrane via ASBT is likely a rate-limiting step for ileal bile acid absorption in mice treated with antibacterial drugs because of the elevation of ileal bile acid absorption and ASBT protein levels in the apical brush border membrane. However, we do not exclude the possibility of the contribution of other bile acid transporters.

There are at least two bile acid-mediated mechanisms for the downregulation of ileal ASBT expression: transcriptional regulation via farnesoid X receptor/small heterodimer partner (FXR/SHP) signaling) and post-translational regulation via the ubiquitin-dependent protein degradation pathway. Decrease in the ileal Ostα mRNA levels in mice treated with antibacterial drug was likely due
to the suppression of ileal FXR signaling because ileal FXR target genes, Osxlf, Shp, Fgfl5, and Ihabp mRNA levels were significantly decreased in mice (data not shown). Changes in Ostα protein levels in mice treated with antibacterial drugs were not consistent with those in Ostα mRNA levels. In addition, the OSTα expression may exert regulatory effects through the bile acid-mediated post-translational mechanism. The present study raises the possibility that OSTα protein expression is also regulated by the bile acid-mediated protein degradation pathway that is found in ASBT protein.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES


