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Enterobacteria-mediated deconjugation of taurocholic acid enhances ileal farnesoid *X* receptor signaling

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ABSTRACT

Enterobacteria are known to deconjugate amino acid-conjugated bile acids in the intestine. Administration of ampicillin (ABPC; 3 days, 100 mg/kg) decreased the expression of ileal farnesoid *X* receptor (Fxr) target genes, and increased the levels of total bile acids in the intestinal lumen. The primary tauro-conjugates of cholic acid (TCA) and beta-muricholic acid (TβMCA) levels were increased, whereas the primary unconjugates, cholic acid (CA) and beta-muricholic acid (βMCA), levels decreased to below detectable levels (<0.01 μmol) in ABPC-treated mice. The effects of individual bile acid on expression of the ileal farnesoid *X* receptor target genes were examined in ABPC-treated mice. The expression of ileal farnesoid *X* receptor target genes in ABPC-treated mice was clearly enhanced after CA (500 mg/kg), but not TCA (500 mg/kg) cotreatment. Their levels in control mice were enhanced after either CA or TCA-cotreatment. Unconjugated CA levels in the intestinal lumen and portal vein were increased in both ABPC-treated and control mice. Reduced ileal Fgf15 and Shp mRNA levels in ABPC-treated mice were also increased after CA (100 mg/kg) cotreatment at which luminal CA levels was restored to the level in controls, but was unaffected by βMCA (100 mg/kg) cotreatment. In addition, no increase in ileal Shp, Ibabp or Ostα mRNA levels was observed even after CA (500 mg/kg) cotreatment in ABPC-treated farnesoid *X* receptor-null mice despite increased CA levels in the intestinal lumen. These results suggest the role of enterobacteria in bile acid-mediated enhancement of ileal farnesoid *X* receptor signaling by TCA deconjugation.

1. Introduction

Bile acids are synthesized from cholesterol in the liver and conjugated with amino acids (taurine or glycine) before excretion into the bile. They are stored in the gallbladder, and are secreted postprandially into the small intestine. They serve as detergents to promote efficient absorption of dietary lipids in the intestine (Russell, 2003). The secreted bile acid conjugates are partly deconjugated by enterobacteria, and most of the deconjugated and conjugated bile acids ($>95\%$) are reabsorbed from the terminal ileum for transport back into the liver through the portal vein. The bile acids are reconjugated after returning to the liver. Unconjugated bile acids are more cytotoxic and less efficient for intestinal absorption of dietary lipids than conjugated bile acids (Vessey et al., 1977). However, the physiological significance of bacterial deconjugation of bile acids is currently unclear.

The farnesoid *X* receptor (FXR; NR1H4) is a nuclear receptor that is highly expressed in the intestine and liver, and is activated by several bile acids with high potency, including chenodeoxycholic acid, deoxycholic acid, lithocholic acid and cholic acid (CA), as well as conjugates of these bile acids (Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999). Farnesoid *X* receptor activation plays a crucial role in the regulation of bile acid homeostasis (Lee et al., 2006). Recent studies suggest that farnesoid *X* receptor signaling in the intestine plays a critical role in the regulation of bile acid homeostasis rather than in the liver (Kim et al., 2007). Ileal farnesoid *X* receptor activation enhances gene transcription of fibroblast growth factor (FGF) 19 and mouse ortholog FGF15, a secreted factor from the intestine that acts on the liver to repress bile acid synthesis (Inagaki et al., 2005; Miyata et al., 2012; Potthoff et al., 2012), and of small heterodimer partner (SHP), a nuclear orphan receptor that is involved in bile acid absorption in the terminal ileum (Neimark et al., 2004). In addition, ileal farnesoid *X* receptor activation enhances gene transcription of ileal bile acid binding protein (IBABP) facilitating bile acids trafficking the basolateral membrane (Grober et al., 1999), and of organic solute transporter alpha–beta (OST α–OSTβ), the principal bile acid transport carrier into the portal vein from the ileum (Frankenberg et al., 2006).

We recently demonstrated administration of antibiotics altered bile acid composition in the intestinal lumen and increased total

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bile acid levels in the intestinal lumen and liver, which was caused by disruption of bile acid homeostasis (Miyata et al., 2009, 2011). Reports using farnesoid *X* receptor-null mice indicate that farnesoid *X* receptor signaling played a crucial role in regulating bile acid homeostasis (Lee et al., 2006; Sinal et al., 2000). These reports raise the possibility that antibiotic treatment may disrupt ileal farnesoid *X* receptor signaling through the alteration of bile acid composition in the intestinal lumen.

We evaluated the effects of predominantly existing individual amino acid conjugates or unconjugates of CA and beta-muricholic acid (βMCA) in mice (Claus et al., 2011; Moschetta et al., 2005) on farnesoid *X* receptor signaling. The present study shows the physiological significance of enterobacteria-mediated deconjugation on bile acid-mediated ileal farnesoid *X* receptor signaling.

2. Materials and methods

2.1. Chemicals

Ampicillin (ABPC) was purchased from Nacalai Tesque (Kyoto, Japan). CA, tauro-cholic acid (TCA), chenodeoxycholic acid (CDCA), tauro-chenodeoxycholic acid (TCDCA), deoxycholic acid (DCA) and tauro-deoxycholic acid (TDCA) were purchased from Sigma-Aldrich (St. Louis, MO). β-Muricholic acid (βMCA), tauro-β-muricholic acid (TβMCA), ursodeoxycholic acid (UDCA), tauro-ursodeoxycholic acid (TUDCA) and etiocholan-3,17-diol (5-androstan-3, 17-diol) were purchased from Steraloids (Newport, RI). L-column ODS (2.1–150 mm) was obtained from the Chemicals Evaluation and Research Institute (Tokyo, Japan). The Enzymepak 3α-HSD column was purchased from Jasco (Tokyo, Japan).

2.2. Animal treatment

C57BL/6N male mice (Charles River Japan, Yokohama, Japan) and farnesoid *X* receptor-null mice (Sinal et al., 2000) were housed under a standard 12-h light/dark cycle (9:00 am to 9:00 pm). During acclimatization, mice were fed standard rodent chow (CE-2; Clea Japan, Tokyo, Japan) and water ad libitum. Age-matched groups of 8- to 9-week-old mice were used for all experiments. Bile acids (CA and TCA, 500 mg/kg weight, dissolved in saline) were orally administered (gastric gavage) to the mice at 9:00 am. For ABPC-cotreatment experiments, ABPC (100 mg/kg body weight, dissolved in saline) was orally administered (gastric gavage) to mice at 9:00 am for 3 days. Bile acids (βMCA and TβMCA, 100 mg/kg body weight, CA and TCA, 100, 500 mg/kg, dissolved in saline) were co-administered (gastric gavage) with the final dose of ABPC. All mice were euthanized 3 h after final administration (12:00 am). All experiments were performed in accordance with the Guidelines for Animal Experiments of Tohoku University. The protocol was approved by the Institutional Animal Care and Use Committee at Tohoku University (Permission No. 22-Pharm-Animal-6 and 2011-Pharm-Animal-23).

2.3. RNA extraction and quantitative real time PCR

Total RNA was prepared from the liver and whole ileum by using RNAgents Total Isolation System (Promega, Madison, WI). RNA concentration was determined by measuring the absorbance at 260 nm with a DU800 spectrophotometer (Beckman Coulter). cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantitative PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) with ABI PRISM 7000 Sequence Detection Systems (Applied Biosystems). The following forward and reverse primers were used to detect gene expression for quantitative real time PCR. β-Actin forward: 5′-ACCCTGTGCTGCTCACCGA-3′ and reverse, 5′-CTGGATGGCTACGTACATGGCT-3′; Fgf15 forward, 5′-GAGGACCAAAACGAACGAAATT-3′ and reverse, 5′-ACGTCCTTGATGGCAATCG-3′; Shp forward, 5′-AAGATACTAACCATGAGCTCCG-3′ and reverse, 5′-GTCTTGGCTAGGACATCCAAG-3′; Ibabp forward, 5′-AGATCATCACAGAGGTCCAGC-3′ and reverse, GGTAGCCTTGAACTTCTTGCC; Ostα forward: 5′-ATGCATCTGGGTGAACAGAA-3′ and reverse, GAGTAGGGAGGTGAGCAAGC; GAPDH forward; 5′-TGTGTCCGTCGTGGATCTAG-3′ and reverse, 5′-CCTGCTTCACCACCTTCTTGAT-3′; Bsep forward: 5′-GGAAGGCGCTCGAGTTGGCT-3′ and reverse, 5′-ATGCCTGGCTGGGCCATTCC-3′.

2.4. Bile acid composition in the small intestinal lumen, portal vein, and liver

Bile acid composition in the small intestinal lumen, portal vein and liver was determined by high performance liquid chromatography (HPLC) as described previously (Kitada et al., 2003; Miyata et al., 2006). Samples were prepared from the small intestinal lumen, portal vein, and liver of ABPC and/or bile acid-treated mice. The small intestinal lumen was washed with the 6 mL of phosphate-buffered saline (PBS), and the washed solution was collected and homogenized in an equal volume (v/v) of 50% tert-butanol, using a Polytron. Homogenates were centrifuged at $20{,}400 \times g$ for 10 min. Supernatants were subjected to HPLC analyses. Plasma from the portal vein and liver homogenates was prepared and denatured with special grade ethanol and was vortexed. The samples were centrifuged at $20{,}400 \times g$ for 10 min. Supernatants were subjected to HPLC analyses. The quantities of βMCA, TβMCA, UDCA, TUDCA, CA, TCA, CDCA, TCDCA, DCA and TDCA were determined.

2.5. Statistical analysis

Values are presented as mean ± SD. Data were analyzed by the unpaired Student's *t* test or by analysis of variance with Dunnett's post-test using Prism 4.0 software (GraphPad Software Inc., San Diego, CA) for significant differences between the mean values of each group. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of ABPC treatment on ileal farnesoid X receptor target gene mRNA levels and bile acid profiles in the intestinal lumen

To investigate the effects of ABPC treatment on ileal farnesoid *X* receptor signaling, the ileal mRNA levels of Fgf15, Shp, Ibabp, and Ostα were measured and found to decrease to approximately 12, 46, 66 and 60%, respectively, of the levels in the vehicle-treated group (Fig. 1). CA and βMCA levels were below the limit of detection in the intestinal lumen (< 0.01 μmol), whereas TCA and TβMCA levels were significantly higher than those in the vehicle-treated group (approximately 1.4-fold and 1.3-fold, respectively) (Table 1). The bile acid pool size in the intestinal lumen was increased significantly (approximately 1.3-fold compared with the vehicle-treated group) (Table 1). These results indicate the possibility that CA or βMCA, produced by enterobacteria-mediated deconjugation of TCA or TβMCA, respectively, have the potential to activate ileal farnesoid *X* receptor signaling.

3.2. Expression of farnesoid X receptor target genes and bile acid profiles

We raised the possibility that CA or βMCA, but not their tauroconjugates, have the potential to activate ileal farnesoid