Administration of Ampicillin Elevates Hepatic Primary Bile Acid Synthesis through Suppression of Ileal Fibroblast Growth Factor 15 Expression

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ABSTRACT

Administration of the antibiotic drug ampicillin (ABPC) significantly increased hepatic bile acid concentrations. In the present study, we investigated the mechanisms for the elevation of bile acid levels in ABPC-treated mice. Hepatic microsomal cholesterol 7α-hydroxylation and CYP7A1 mRNA level were increased 2.0-fold in ABPC-treated mice despite higher bile acid levels in the liver and small intestinal lumen. A significant change in hepatic small heterodimer partner (SHP) mRNA level was not observed in ABPC-treated mice, whereas a marked decrease in ileal fibroblast growth factor 15 (FGF15) mRNA level was observed (3% of vehicle-treated mice). These phenomena were also observed in mice cotreated with bacitracin/streptomycin/neomycin, which are barely absorbed from the intestine. Primary bile acid contents in the small intestinal lumen were increased in ABPC-treated mice, whereas secondary bile acid, deoxycholic acid (DCA), contents were reduced to below detection limits (<0.01 μmol). In ABPC-treated mice, cotreatment with tauroDCA reversed reductions in ileal FGF15 mRNA level. Ileal SHP mRNA level was, however, not decreased in ABPC-treated mice. ABPC administration to farnesoid X receptor (Fxr)-null mice also decreased ileal FGF15 mRNA levels and secondary bile acid content in the small intestinal lumen. These results suggest that ABPC administration elevates hepatic primary bile acid synthesis, at least in part, through suppression of ileal FGF15 expression.

Administration of antibacterial drugs for infectious diseases can cause side effects such as diarrhea and hepatic injury (Beaugerie and Petit, 2004; Polson, 2007). Administration of antibacterial drugs decreases enterobacterial populations, which have a crucial role in symbiosis with the host through metabolism of endogenous compounds such as bile acids and exogenous compounds. Antibacterial drug-mediated alteration of enterobacterial populations is therefore an important issue concerning human health and disease.

Some enterobacteria catalyze 7α-dehydroxylation of primary bile acids. This results in the production of more hydrophobic secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid (LCA). In general, killing of enterobacteria by administration of antibacterial drugs reduces secondary bile acids in the bile acid pool (Hofmann, 1977). Administration of ampicillin (ABPC) significantly reduces DCA levels in the bile acid pool of humans (Berr et al., 1996), but the physiological influence of the reduction of DCA production remains unclear.

Bile acids play an essential part not only in the intestinal absorption of lipids and vitamins, but also in the activation of nuclear receptors and cell signaling pathways. It is well recognized that bile acids serve as key signaling molecules that regulate a network of metabolic pathways, including lipid, glucose, and bile acids (Hylemon et al., 2009). The size of the bile acid pool is therefore tightly controlled by negative feedback regulations through several nuclear receptor and cell signaling pathways. The bile acid pool size is mainly regulated by the synthesis of hepatic bile acid and reabsorp-

ABBREVIATIONS: ABPC, ampicillin; SHP, small heterodimer partner; FGF15, fibroblast growth factor 15; FXR, farnesoid X receptor; DCA, deoxycholic acid; CDGA, chenodeoxycholic acid; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]propanesulfonate; LCA, lithocholic acid; TDCO, taurodeoxycholic acid; BDL, bile duct-ligated; BC, bacitracin; NM, neomycin; SM, streptomycin; CA, cholic acid; PON1, paraoxonase 1; αMCA, muricholic acid; TαMCA, tauro-β-muricholic acid; TUDCA, tauroursodeoxycholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid; UDCA, ursodeoxycholic acid; HPLC, high-performance liquid chromatography; PCR, polymerase chain reaction.
tion of intestinal bile acid (Hofmann, 1994). The specific mRNA level of CYP7A1, the rate-limiting enzyme of the synthesis of hepatic bile acid, is negatively regulated by several bile acid-mediated signaling pathways (Chiang, 2009). It was initially reported that CYP7A1 transcription is suppressed by the hepatic FXR/SHP signaling pathway activated by bile acids (Goodwin et al., 2000; Lu et al., 2000). However, the expression of hepatic CYP7A1 and activity of cholesterol 7α-hydroxylase are elevated in bile duct-ligated (BDL) mice in which hepatic bile acid concentration is increased (Dueland et al., 1991). Furthermore, intraduodenal, but not intravenous or portal, administration of bile acid significantly suppresses CYP7A1 expression (Pandak et al., 1991; Nagano et al., 2004). These results suggested a role of the intestine in feedback regulation of the synthesis of hepatic bile acids. Hepatic CYP7A1 levels are also negatively regulated by the endocrine action of intestine-produced FGF15 (human ortholog, FGF19) signaling pathway that is induced by intestinal bile acid-mediated FXR activation (Inagaki et al., 2005). Thus, intestine-produced FGF15 signaling pathway, rather than hepatic FXR pathway, is required for short-term repression of hepatic CYP7A1 (Kim et al., 2007).

In our preliminary experiment, we administered the antibacterial drug ABPC to mice and observed elevation in hepatic bile acid concentration. In the present study, to understand the mechanisms of this elevation, we focused on the intestinal role for elevated primary bile acid synthesis. The present experiments suggest that ABPC administration elevates hepatic primary bile acid synthesis, at least in part, through suppression of expression of ideal FGF15.

Materials and Methods

Materials. ABPC was purchased from Nacalai Tesque (Kyoto, Japan). Bacitracin (BC) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Neomycin trisulfate (NM) was purchased from MP Biomedicals (Irvine, CA). Streptomycin sulfate (SM), cholic acid (CA), tauroursodeoxycholic acid (TUDCA), etiocholan-3β,17β-diol (5αMCA), ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TCDCA), DCA, taurocholic acid (TCA), chenodeoxycholic acid (CDCA), from MP Biomedicals (Irvine, CA). Streptomycin sulfate (SM), cholic acid (CA), clostridium sordellii were purchased from Sigma-Aldrich (St. Louis, MO). β-Muricholic acid (βMCA), tauro-β-muricholic acid (TβMCA), ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), etiocholan-3α,17β-diol (5β-androsten-3α, 17β-diol), and 7α-hydroxycholesterol were purchased from Steraloids (Newport, RI). L-column ODS (2.1 × 150 mm) was obtained from Chemicals Evaluation and Research Institute (Tokyo, Japan). Enzymepak 30-HSD column was purchased from Jasco (Tokyo, Japan).

Animal Treatment and Sample Collection. C57BL/6N male mice (Charles River Japan, Yokohama, Japan), farnesoid X receptor (Fxr)-null mice (Sinal et al., 2000), and wild-type mice were housed under a standard 12-h light/dark (9:00 AM to 9:00 PM) cycle. Before experimentation, mice were fed standard rodent chow (CE-2; Clea Japan, Tokyo, Japan) and water ad libitum for acclimatization. Age-matched groups of 8- to 9-week-old mice were used for all experiments. Mice were orally administered ABPC (100 mg/kg body weight, dissolved in saline) at 9:00 AM, BC/NM/SM (200 mg/kg body weight, dissolved in saline), or ABPC (100 or 500 mg/kg body weight, dissolved in saline) for 3 days. They were fasted for the last 12 h to control bile acid secretion into the duodenum and euthanized 24 h after the last administration (9:00 AM). For the CA-treatment experiment, mice were fed a control diet (CE-2) or control diet supplemented with 1% (w/w) CA for 6 days and euthanized at 9:00 AM. Liver samples were taken for biochemical assays. The small intestinal lumen was washed with phosphate-buffered saline, and the washed solution was collected. All experiments were performed in accordance with the Guidelines for Animal Experiments of Tohoku University.

Quantification of Bacterial DNA in Feces. The fecal content of bacteria in stools collected over the last 24 h in vehicle- and ABPC-treated mice was determined. DNA was extracted from 200 mg of feces by use of a QIAamp DNA Stool Mini Kit (QIAGEN K.K., Tokyo, Japan). The extracted DNA was diluted in Tris-EDTA buffer, and the purity confirmed by measuring absorbance at 260 and 280 nm with a DU800 spectrophotometer (Beckman Coulter, Fullerton, CA). These DNA samples were subjected to real-time polymerase chain reaction (PCR) by use of SYBR Green 1 with an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Relative DNA levels were calculated by the comparative threshold cycle method. The following specific forward and reverse primers were used for real-time quantitative PCR: Bacteroides fragilis sense, 5'-CTGAAACCAGCCAAGTAGCG-3' and antisense, 5'-CCGCAACTTTCAACATTGTACATA-3'; Clostridium scindens sense, 5'-GCAACCTGGCTGACT-3' and antisense, 5'-ACGGAATGCTTGTGACAC-3'; Clostridium sordellii sense, 5'-TCAGGGCCATCTCGG-3' and antisense, 5'-CACCCATGTGCACC-3'.

Bile Acid Composition in the Liver and Small Intestinal Lumen. Bile acid composition in the liver and small intestinal lumen was measured by high-performance liquid chromatography (HPLC) as described previously (Kitada et al., 2003; Miyata et al., 2006). The contents of bMCA, TβMCA, UDCA, TUDCA, CA, TCA, CDCA, TCDCA, DCA, TCA, LCA, and TLCA were measured.

Cholesterol 7α-Hydroxylase Activity. Cholesterol 7α-hydroxylase activity was measured by HPLC. We modified the previously reported method (Chiang, 1991). The reaction mixture consisted of 0.1 M potassium phosphate, pH 7.4, 50 mM NaF, 5 mM dithiothreitol, 0.015% CHAPS, 0.1 mM cholesterol, and 0.5 mg of hepatic microsomes in a final volume of 0.5 ml. The mixture was preincubated at 37°C for 5 min, and 10 μl of 100 mM NADPH was added to initiate the reaction. After incubation at 37°C for 10 min, the reaction was terminated by addition of 10 μl of 25% CA, and 2.5 nmol of 20α-hydroxycholesterol was added as an internal standard. Then, one unit of cholesterol oxidase in 10 mM potassium phosphate buffer, pH 7.4, containing one mM dithiothreitol and 20% glycerol was added to the reaction mixture. After incubation at 37°C for 10 min, the reaction was stopped by the addition of 3 ml of ice-cold hexane. The mixture was then centrifuged at 2000g, and 2 ml of supernatant was collected. The collected supernatant was evaporated to dryness, dissolved in 100 μl of acetonitrile/methanol (92:8), and then analyzed by HPLC. The metabolites were separated at 37°C with a Chemco-Pak Hypersil ODS-5 (4.6 × 250 mm) (Chemco Scientific Co., Osaka, Japan). The metabolites were eluted using acetonitrile/methanol (92:8) at a flow rate of 0.6 ml/min and monitored at 240 nm.

Analysis of mRNA Levels. Total RNA was prepared from the liver and ileum by use of an RNAGents total isolation system (Promega, Madison, WI). RNA concentration was determined by measuring absorbance at 260 nm with use of a DU800 spectrophotometer (Beckman Coulter). Single-stranded cDNA were synthesized by use of an oligo (dT) primer and a Ready-To-Go You-Prime First-Strand cDNA Synthesis Kit (Beckman Coulter). The following specific forward and reverse primers were used for real-time quantitative PCR: CYP7A1 sense, 5'-AGCAACTAACACACACGGCCTAATCTA-3' and antisense, 5'-GTCCGCGATTTTCAAGGATGCA-3'; SHP sense, 5'-AAGATCTAACACATGAGCCTCCTG-3' and antisense, 5'-GTTCGGGTGCTGCAAGCAG-3'; FGF15 sense, 5'-AGGCGAACTGAACTCAG-3' and antisense, 5'-GTGTGGTGCGCTGGATGCTA-3'; and glyceraldehyde-3-phosphate dehydrogenase sense, 5'-TGTTGCTCGCTGGAGTCTGA-3' and antisense, 5'-CTGCTTCCACACCT-3'.
Data are mean ± hydroxylase activities were determined by HPLC.

Hepatic bile acid compositions and microsomes were prepared from mice treated with ABPC (100 mg/kg) for 3 days and fasted for the last 12 h. Hepatic bile acid levels were measured by real-time PCR. CYP7A1 mRNA levels were significantly higher in ABPC-treated mice compared with vehicle-treated mice (2.5-fold relative to vehicle-treated mice) (Fig. 1A). Analysis of hepatic bile acid composition revealed significant increases in primary bile acid (βMCA, TβMCA, and TCA) contents in ABPC-treated mice compared with vehicle-treated mice (4.8-, 2.4-, and 2.3-fold, respectively).

Hepatic bile acid synthesis was estimated to understand the mechanisms of accumulation of hepatic bile acids. Hepatic microsomal activity of cholesterol 7α-hydroxylase, which catalyzes the rate-limiting step in bile acid synthesis, was increased in ABPC-treated mice (2.0-fold relative to vehicle-treated mice) (Fig. 1B). Influence of ABPC or CA Treatment on CYP7A1, SHP, and FGF15 mRNA Levels. To identify the mechanistic basis for increased microsomal cholesterol 7α-hydroxylation in ABPC-treated mice, hepatic CYP7A1 mRNA levels were measured by real-time PCR. CYP7A1 mRNA levels were significantly higher in ABPC-treated mice compared with vehicle-treated mice (Fig. 2A). To further explore the mechanistic basis for the increase in CYP7A1 mRNA levels despite the elevation of hepatic bile acid levels, mRNA levels of the gene involved in the negative regulation of CYP7A1 expression were analyzed by real-time PCR. Significant changes in hepatic SHP mRNA levels were not observed in ABPC-treated mice. For comparison, mRNA levels of the gene were measured in CA-fed mice in which elevation of hepatic bile acid levels and the negative regulation of CYP7A1 expression are found. Consistent with previous reports (Sinal et al., 2000; Miyata et al., 2005), CYP7A1 mRNA levels were markedly reduced in CA-fed mice (7% of control group), whereas mRNA levels of hepatic SHP and ileal FGFI5 were significantly increased in CA-fed mice (3- and 100-fold relative to the control diet, respectively) (Fig. 2B). In ABPC-treated mice, marked decreases in ileal FGFI5 mRNA levels were observed (3% of vehicle-treated mice) (Fig. 2A). Hepatic paraoxonase 1 (PON1) and CYP7A1 are also decreased by bile acids acting via FXR to increase FGFI5 expression. Hepatic paraoxonase 1 (PON1) and CYP7A1 are also decreased by bile acids acting via FXR to increase FGFI5 expression, whereas ileal FGFI5 mRNA levels were markedly reduced in CA-fed mice (7% of control group), whereas mRNA levels of hepatic SHP and ileal FGFI5 were significantly increased in CA-fed mice (3- and 100-fold relative to the control diet, respectively) (Fig. 2B). In ABPC-treated mice, marked decreases in ileal FGFI5 mRNA levels were observed (3% of vehicle-treated mice) (Fig. 2A).

Influence of Bacitracin/Neomycin/Streptomycin Treatment on Hepatic Bile Acid Levels and Gene Expression. 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 each agent for 3 days) (Fig. 3). Treatment of mice with these antibiotics reduces levels of enterobacteria to less than 0.001% (Kinouchi et al., 1993). Consistent with ABPC-treated mice, hepatic bile acid levels were elevated in mice treated with BC/NM/SM (Fig. 3A). Hepatic CYP7A1 mRNA level was increased in BC/NM/SM-treated mice (2.5-fold relative to vehicle-treated mice), whereas ileal FGFI5 mRNA level was markedly decreased in BC/NM/SM-treated mice (10% of vehicle-treated mice) as well as ABPC-treated mice (Fig. 3B).

Influence of Treatment by ABPC or CA on Bile Acid Composition in the Small Intestinal Lumen. Bile acid composition in the small intestinal lumen was measured to identify the reason for the differential response of FGFI5 mRNA levels between ABPC- and CA-treated mice (Table 1).
Fig. 3. Influence of BC/NM/SM treatment on hepatic bile acid level and hepatic CYP7A1, SHP, and ileal FGF15 mRNA levels. A, hepatic bile acid levels. B, mRNA levels. Hepatic bile acid compositions were determined by HPLC. mRNA levels were measured by quantitative real-time PCR. Liver homogenates, liver and ileal total RNAs, were prepared from mice treated with BC/NM/SM (200 mg/kg each) for 3 days and fasted for the last 12 h. Data are mean ± S.D. (n = 5). Significant differences from vehicle-treated group are expressed with an asterisk (**, p < 0.01).

Total bile acid contents were increased in both ABPC- and CA-treated mice (1.6- and 2.3-fold, respectively). Primary bile acid contents were increased in both ABPC- and CA-treated mice (1.7- and 1.8-fold, respectively). A decrease in TβMCA content was observed in CA-fed mice, suggesting reduction of de novo synthesis of primary bile acids. DCA and TDCA contents were reduced to below detection limits (<0.01 μmol) in ABPC-treated mice, whereas they were increased in CA-treated mice (12- and 66-fold, respectively, relative to the control diet).

Fecal bacterial DNA was quantified by real-time PCR to identify the mechanism for the reduction of DCA levels in the small intestinal lumen of ABPC-treated mice. Consistent with the reduction of DCA levels in the small intestinal lumen, the levels of C. scindens, C. sordellii, and B. fragilis that exhibit 7α-dehydroxylase activity of bile acids (Kitahara et al., 2001; Ridlon et al., 2006) were markedly reduced to 8.8, 1.4, and <0.1%, respectively, of vehicle-treated controls compared with ABPC-vehicle-treated mice.

**Influence of TDCA Cotreatment on FGF15 mRNA Levels in ABPC-Treated Mice.** To assess the influence of TDCA cotreatment on ileal FGF15 mRNA level in ABPC-treated mice, ABPC and TDCA were administered to mice. TDCA content in the small intestinal lumen and ileal FGF15 mRNA level were markedly decreased in ABPC-treated mice (<0.01 μmol and 2% of vehicle-treated mice, respectively). Cotreatment with increasing amounts of TDCA reversed reductions in the TDCA content in the small intestinal lumen and ileal FGF15 mRNA level of ABPC-treated mice in a dose-dependent manner (Fig. 4, A and B).

**Influence of ABPC Treatment on FGF15 mRNA Levels in Fxr-Null Mice.** To clarify whether antibiotic-mediated suppression of ileal FGF15 expression depends on FXR signaling, ileal Fxr target gene SHP mRNA levels were measured in ABPC-treated mice. Significant decreases in ileal SHP mRNA levels were not observed in ABPC-treated mice, although marked decreases in ileal FGF15 mRNA levels were noted (data not shown). Furthermore, Fxr-null mice were treated with ABPC to clarify the involvement of FXR-independent signaling. Ileal FGF15 mRNA levels in Fxr-null mice were 10 times lower than those in wild-type mice (Fig. 5A). ABPC administration to Fxr-null mice decreased ileal FGF15 mRNA levels. Secondary bile acid (TDCA, DCA, TLCA, and LCA) contents in the small intestinal lumen were reduced to below detection limits in ABPC-treated Fxr-null mice, despite increases in primary bile acid contents (Fig. 5B).

**Discussion**

In the present study, administrations of antibacterial drug suppressed ileal FGF15 expression, resulting in elevation of synthesis of hepatic primary bile acids. ABPC is absorbed into the small intestine and secreted into bile, whereas BC, NM, and SM are barely absorbed from the small intestine. The influence of BC, NM, and SM are therefore largely restricted within the intestinal lumen. Elevation of the synthesis of hepatic primary bile acids was observed in both ABPC-treated mice and BC/NM/SM-treated mice, so indirect effects through enterobacteria are probably involved in the elevation of hepatic primary bile acid synthesis in antibacterial drug-treated mice. ABPC-treated mice showed increased activity of hepatic cholesterol 7α-hydroxylase and CYP7A1 mRNA levels, and also showed reduced ileal mRNA levels of FGF15. FGF15 was shown to be a potent negative regulator of CYP7A1. In ABPC-treated mice, changes in ileal FGF15 mRNA levels, not hepatic SHP mRNA levels, were inversely related with hepatic CYP7A1 mRNA levels. Hepatic CYP7A1 mRNA levels were increased in Fg15-null mice (Kim et al., 2007) and in BDL mice in which ileal FGF15 mRNA levels were reduced (Inagaki et al., 2005). Antibacterial drug-mediated suppression of ileal FGF15 expression is probably, at least in part, involved in the elevation of hepatic CYP7A1 expression, resulting in increases in hepatic primary bile acid synthesis and bile acid concentration. Elevation of PON1 mRNA levels in ABPC-treated mice also supports this hypothesis. In the present study, the role of SHP in the antibacterial drug-mediated elevation of hepatic CYP7A1 expression has remained unclear. Decreases in hepatic SHP protein level through attenuation of FGF15 signaling might be involved in the elevation of hepatic CYP7A1 expression (Miao et al., 2009).

In patients with gallstones, ABPC treatment elevated total bile acid pool size and CA synthesis rate compared with before the treatment (Carulli et al., 1981; Berr et al., 1996), although the mechanisms have remained unclear. The results of the present study raise the possibility that administration of antibacterial drugs to humans reduces serum levels of FGF19 and elevates the activity of hepatic cholesterol 7α-hydroxylase.

An antibiotic-treated model is similar to a BDL model that elevates hepatic bile acid levels and CYP7A1 expression and reduces ileal FGF15 expression (Dueland et al., 1991; Inagaki et al., 2005). However, it is of interest to note that bile acid contents in the intestinal lumen were increased in ABPC-treated mice, whereas they were decreased in BDL.
mice because of the blocking of bile flow into the intestine. Despite the increase in bile acid contents in the intestinal lumen, ileal FGF15 mRNA levels were reduced in ABPC-treated mice concomitant with decreased levels of DCA contents. FGF15 mRNA levels were elevated in CA-treated mice in which elevation of DCA content in the small intestinal lumen was determined by HPLC. N.D. represents <0.01 μmol. Data are mean ± S.D. (n = 4–5). Significant differences from the vehicle-treated group are expressed with an asterisk (**, p < 0.01; ***, p < 0.001).

**Table 1** Influence of ABPC or CA treatment on bile acid composition in the small intestinal lumen.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>ABPC</th>
<th>Untreatment</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary bile acid (μmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>βMCA</td>
<td>0.32 ± 0.32</td>
<td>N.D.</td>
<td>0.40 ± 0.08</td>
<td>0.02 ± 0.02**</td>
</tr>
<tr>
<td>TtMCA</td>
<td>3.43 ± 1.57</td>
<td>6.63 ± 0.60**</td>
<td>3.40 ± 1.04</td>
<td>0.40 ± 0.22**</td>
</tr>
<tr>
<td>UDCA</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>TUDCA</td>
<td>0.10 ± 0.07</td>
<td>0.32 ± 0.03**</td>
<td>0.14 ± 0.06</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>CA</td>
<td>0.54 ± 0.46</td>
<td>N.D.</td>
<td>0.64 ± 0.30</td>
<td>1.14 ± 1.02</td>
</tr>
<tr>
<td>TCA</td>
<td>3.05 ± 0.86</td>
<td>5.39 ± 0.42**</td>
<td>3.20 ± 0.50</td>
<td>12.98 ± 3.98**</td>
</tr>
<tr>
<td>CDCA</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>TDCDA</td>
<td>0.04 ± 0.03</td>
<td>0.17 ± 0.04**</td>
<td>0.02 ± 0.02</td>
<td>N.D.</td>
</tr>
<tr>
<td>Total</td>
<td>7.49 ± 1.87</td>
<td>12.50 ± 0.95**</td>
<td>7.80 ± 1.82</td>
<td>14.00 ± 4.78*</td>
</tr>
<tr>
<td>Secondary bile acid (μmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCA</td>
<td>0.04 ± 0.06</td>
<td>N.D.</td>
<td>0.02 ± 0.02</td>
<td>0.24 ± 0.38</td>
</tr>
<tr>
<td>TDCA</td>
<td>0.21 ± 0.09</td>
<td>N.D.</td>
<td>0.06 ± 0.02</td>
<td>3.96 ± 1.26**</td>
</tr>
<tr>
<td>LCA</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>TLCA</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Total</td>
<td>0.26 ± 0.09</td>
<td>N.D.</td>
<td>0.08 ± 0.02</td>
<td>4.18 ± 1.14**</td>
</tr>
<tr>
<td>Total bile</td>
<td>7.74 ± 1.81</td>
<td>12.50 ± 0.95**</td>
<td>7.88 ± 1.80</td>
<td>18.20 ± 5.86*</td>
</tr>
</tbody>
</table>

Data are shown as mean ± S.D. (n = 4–5). N.D. represents <0.01 μmol. Significant differences from the vehicle-treated group are expressed with an asterisk (*, p < 0.05; **, p < 0.01; ***, p < 0.001).

In general, it is considered that suppression of FXR signaling is involved in the reduction of ileal FGF15 mRNA levels (Holt et al., 2003). Based on this hypothesis, bile acid composition in the intestinal lumen was determined by HPLC. Primary bile acid (BA), βMCA, TtMCA, UDCA, TUDCA, CA, TCA, CDCA, and TDCDA; Secondary bile acid (BA), DCA, TDCA, LCA, and TLCA. Data are mean ± S.D. (n = 5). Significant differences from the vehicle-treated group are expressed with an asterisk (*, p < 0.05; ***, p < 0.001).
significant decreases in the ileal FGF15 mRNA level in ABPC-treated Fxr-null mice suggest FXR-independent mechanisms for ileal FGF15 expression. Various bile acid-mediated signals independent of FXR have been reported (Nguyen and Bouscarel, 2008). Wistuba et al. (2007) reported that LCA induction of FGF19 promoter in intestinal cells is mediated by FXR. Involvement of secondary bile acid-mediated signaling of other nuclear receptors and cellular kinases on ileal FGF15 expression in vivo may need to be evaluated.

Several studies have provided evidence that bile acid metabolism is implicated in the regulation of not only cholesterol metabolism, but also lipid and glucose metabolisms (Watanabe et al., 2004; Ma et al., 2006; Zhang et al., 2006). FGF19 signaling is also involved in the regulation of glucose, lipid metabolism, and bile acid metabolism (Bhatnagar et al., 2009; Shin and Osborne, 2009). Administration of human FGF19 or transgenic expression of human FGF19 in mice enhanced energy expenditure and fatty acid oxidation and reduced hepatic, serum triglyceride, and glucose levels (Tomlinson et al., 2002; Fu et al., 2004). Thus, long-term administration of an antibacterial drug, which decreases ileal FGF15 expression, may cause disruption of lipid and glucose homeostasis resulting in metabolic diseases such as diabetes and hyperlipidemia. The relationship between enterobacteria and human disease has recently been investigated (Hecht, 2009). Human health and disease may be influenced by antibacterial drug-mediated alteration of the enterobacteria population.

Although the physiological significance of enterobacteria-mediated secondary bile acid production is unclear, the present study suggests that secondary bile acid contents may alter hepatic synthesis of primary bile acids through changes in FGF15 level. Enterobacteria convert primary bile acids in the colon that have escaped ileal reabsorption to secondary bile acids that are signaling molecules to suppress the synthesis of primary bile acids. Enterobacteria may serve as bile acid sensors cooperating with FXR and FGF15 signaling.

The present study suggests that administration of antibacterial drug suppresses ileal FGF15 levels, resulting in the elevation of hepatic primary bile acid synthesis. Elevation in the activity of hepatic cholesterol 7α-hydroxylase is probably involved, at least in part, in the elevation of hepatic bile acid levels in ABPC-treated mice. The mechanism for the reduction of ileal FGF15 mRNA levels in antibiotic-treated mice is unclear. However, the disappearance of enterobacteria-mediated secondary bile acids may be involved in the reduction in FGF15 mRNA levels via the FXR and other signaling pathways. Further studies are necessary to understand the relationship between primary bile acids and secondary bile acids in the regulation of ileal FGF15 expression under physiological conditions.

References


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