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Individual bile acids have differential effects on bile acid signaling in mice ☆

Peizhen Song, Cheryl E. Rockwell, Julia Yue Cui, Curtis D. Klaassen *

Department of Internal Medicine, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160, USA

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ABSTRACT

Bile acids (BAs) are known to regulate BA synthesis and transport by the farnesoid X receptor in the liver (FXR-SHP) and intestine (FXR-Fgf15). However, the relative importance of individual BAs in regulating these processes is not known. Therefore, mice were fed various doses of five individual BAs, including cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), and ursodeoxycholic acid (UDCA) in their diets at various concentrations for one week to increase the concentration of one BA in the enterohepatic circulation. The mRNA of BA synthesis and transporting genes in liver and ileum were quantified. In the liver, the mRNA of SHP, which is the prototypical target gene of FXR, increased in mice fed all concentrations of BAs. In the ileum, the mRNA of the intestinal FXR target gene Fgf15 was increased at lower doses and to a higher extent by CA and DCA than by CDCA and LCA. Cyp7a1, the rate-limiting enzyme in BA synthesis, was decreased more by CA and DCA than CDCA and LCA. Cyp8b1, the enzyme that 12-hydroxylates BAs and is thus responsible for the synthesis of CA, was decreased much more by CA and DCA than CDCA and LCA. Surprisingly, neither a decrease in the conjugated BA uptake transporter (Ntcp) nor increase in BA efflux transporter (Bsep) was observed by FXR activation, but an increase in the cholesterol efflux transporter (Abcg5/Abcg8) was observed with FXR activation. Thus in conclusion, CA and DCA are more potent FXR activators than CDCA and LCA when fed to mice, and thus they are more effective in decreasing the expression of the rate limiting gene in BA synthesis Cyp7a1 and the 12-hydroxylation of BAs Cyp8b1, and are also more effective in increasing the expression of Abcg5/Abcg8, which is responsible for biliary cholesterol excretion. However, feeding BAs do not alter the mRNA or protein levels of Ntcp or Bsep, suggesting that the uptake or efflux of BAs is not regulated by FXR at physiological and pharmacological concentrations of BAs.

Introduction

Bile acids (BAs) generate bile flow, and as physiological detergents facilitate intestinal absorption of lipids and vitamins (Russell, 2003; Chiang, 2009). BAs are also critical signaling molecules that mediate nutrient homeostasis and energy expenditure. The BA concentrations in the body are dependent on hepatic synthesis, biliary secretion, intestinal re-absorption, and hepatic uptake of BAs. The BA-biosynthetic enzyme in liver, cytochrome-P450 7a1 (Cyp7a1), is the rate-limiting enzyme in the classic pathway of BA synthesis. Two critical enzymes responsible for the synthesis of BAs in the alternative pathway are Cyp27a1 and Cyp7b1 (Russell, 2003; Chiang, 2009).

Various transporters are important in mediating the disposition of BAs in the body (Zhang and Klaassen, 2010). In liver, conjugated BAs are thought to be actively taken up into hepatocytes by the sodium-dependent taurocholate co-transporting polypeptide (Ntcp/Slc10a1), which is located on the sinusoidal membrane of hepatocytes. In addition, the organic anion transporting polypeptide 1b2 (Oatp1b2/Slco1b2) is responsible for the sodium-independent uptake of unconjugated BAs into liver (Csanaky et al., 2011). BAs in hepatocytes are excreted into bile at the canalicular membrane of hepatocytes by an ATP-dependent transporter, the bile salt export pump (Bsep, Abcb11) (Wang et al., 2001). The heterodimer Abcg5/Abcg8, which is also located on the canalicular membrane of hepatocytes, mediates biliary excretion of cholesterol into bile. There are also BA-efflux transporters located at the sinusoidal membrane of hepatocytes. The multidrug resistance-associated proteins (Mrp3/Abcc3 and Mrp4/Abcc4) and the heterodimer organic solute transporter (Ostα/β) transports BAs and other solutes from hepatocytes back into the systemic circulation (Ballatori et al., 2008; Rao et al., 2008). In the intestine, the apical sodium dependent bile acid transporter (Asbt, Slc10a2) is important for bile acid uptake from the ileal lumen, and it represents a highly efficient mechanism for the conservation of bile acids. Mutation of human ASBT leads to abolished taurocholate transport (Wong et al., 1995), and targeted disruption of Asbt in mice eliminates enterohepatic cycling of bile acids (Dawson et al., 2003).

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* Corresponding author at: 2617 W 112th Street, Leawood, KS 66211, USA.
E-mail addresses: songacad@gmail.com (P. Song), rockwelc@msu.edu (C.E. Rockwell), juliacui@uw.edu (J.Y. Cui), curtisklaassenphd@gmail.com (C.D. Klaassen).

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BAs are thought to regulate their own homeostasis via their sensor, the farnesoid X receptor (FXR/Nr1h4) (Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999). Activation of FXR increases the expression of its target gene SHP/Nr0b2, which represses the key BA-biosynthetic enzyme Cyp7a1 (Goodwin et al., 2000). This is often referred to as the hepatic FXR-SHP signaling pathway. A second mechanism underlying the regulation of Cyp7a1 mRNA expression is through the intestinal FXR-fibroblast growth factor 15 (Fgf15) pathway. Fgf15 is released from the intestine, and thought to travel via the portal vein to the liver, where it activates the cell surface fibroblast growth factor receptor 4 (Fgfr4) (Inagaki et al., 2005). It has been suggested that activation of Fgfr4 triggers the phosphatidylinositol-3-kinase (PI3K) signaling pathway, which inhibits the transcription factor forkhead box protein O1 (FoxO1), ultimately causing repression of hepatic Cyp7a1 (Shin and Osborne, 2009); whereas another study shows that deficiency of both Erk and Jnk prevents the Fgf15-mediated suppression of Cyp7a1 and Cyp8b1 (Kong et al., 2012).

In addition to the major bile acid sensor FXR, this laboratory has also shown that lithocholic acid (LCA) is a ligand for PXR, and activation of PXR is critically involved in the protection against LCA-mediated hepatotoxicity (Staudinger et al., 2001). In addition, Nrf2, a well-known sensor for oxidative stress and electrophiles, has been shown recently to be a major determinant of bile acid homeostasis in liver and intestine during bile acid-mediated cholestatic injuries in mice (Weerachayaphorn et al., 2012). Therefore, the signaling of FXR, PXR and Nrf2 will be examined in the present study.

FXR activation in cholestatic animal models has been shown to repress the expression of BA uptake transporters, such as Ntcp, Oatp1a1, and Oatp1b2 (Gartung and Matern, 1997; Gartung et al., 1997) and enhance the expression of the BA efflux transporter, Bsep (Fickert et al., 2001; Plass et al., 2002; Cui et al., 2009; Tanaka et al., 2009). These studies have provided mechanistic insights into the regulation of bile acid homeostasis during pathological conditions. However, the regulation of bile acid homeostasis during physiologically relevant concentrations remains to be determined. Considering the clinical relevance that bile acids and their sequestrants have been used as dietary supplements to treat diabetes (Kobayashi et al., 2007) and promote cholesterol absorption (Woollett et al., 2004), it is important to investigate the effect of dietary supplementation of bile acids below hepatotoxic concentrations on bile acid signaling pathways in vivo.

This laboratory has recently developed a highly sensitive and quantitative UPLC–MS/MS analytical method to determine various individual BAs in their conjugated and unconjugated forms in liver and other compartments of mice (Alnouti et al., 2008; Zhang and Klaassen, 2010; Zhang et al., 2011; Cui et al., 2012; Fu et al., 2012; Zhang et al., 2012b). Using this method, we have 1) demonstrated that during bile acid dietary supplementation (as utilized in the present study), bile acid feeding increased the hepatic and serum concentrations of that bile acid administered in a dose-dependent manner, as well as their known and novel metabolites; 2) unveiled the minimal concentrations of each BA in the feed that cause hepatotoxicity and compared the relative hepatototoxicity of different BAs; and 3) demonstrated that although individual BA feeding increased that particular BA in liver, the total hepatic BAs in general remained unchanged compared to mice fed a control diet (Zhang and Klaassen, 2010; Song et al., 2011).

Thus, during the last fifteen years, a marked increase in knowledge has accumulated about the regulation of BA synthesis and transport. However, these data have generally been done with a mixture of BAs and with high doses of BAs. Therefore, the purpose of this study is to use an experimental model, which is to feed mice multiple doses of various individual BAs, to determine whether there are differences in individual BAs to activate FXR to decrease BA synthetic enzymes and the uptake transporter Ntcp, as well as increase the BA efflux transporter Bsep in liver.

Materials and methods

Animal experiments. Male C57BL/6 mice (22 ± 2 g), at 8-weeks of age, were obtained from Charles River Laboratories, Inc. (Wilmington, MA). Mice were housed with a 12:12 h light:dark cycle and fed mouse chow and water *ad libitum* according to the American Animal Association Laboratory Animal Care Guidelines. Mice were acclimated to the housing facility and placed on ground rodent diet (Teklad Rodent Diet #8064, Harlan Teklad, Madison, WI) for one week. The diet was then replaced with one supplemented with various BAs, including cholic acid (CA, 0.03–0.1%, w/w), chenodeoxycholic acid (CDCA, 0.03–0.3%), deoxycholic acid (DCA, 0.03–0.3%), lithocholic acid (LCA, 0.01–0.3%), or ursodeoxycholic acid (UDCA, 1.0–3.0%) (Sigma, St. Louis, MO) in their diets (n = 5 per group). After 7 days of feeding the BA-supplemented diets, livers and ilea were removed, frozen in liquid nitrogen, and stored at −80 °C before RNA and protein assays. These diets did not affect body weight of the mice (Song et al., 2011). Serum and liver concentrations of the various BAs in these mice as well as measures of hepatotoxicity have been reported in a previous publication (Song et al., 2011). All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at KUMC.

Total RNA extraction. Total RNA was extracted from liver and ileum tissues using RNABee reagent (Tel-test Inc., Friendswood, TX) as suggested by the manufacturer's protocol. RNA was quantified by UV spectrophotometry at 260 nm and diluted to 25 ng/μl in diethyl pyrocarbamate (DEPC)-treated double distilled water.

QuantiGene Plex assay. Messenger RNAs for mouse genes involved in BA synthesis and hepatic BA transporters were quantified using the QuantiGene Plex 2.0 assay (Panomics/Affymetrix, Fremont, CA). Individual bead-based oligonucleotide probe sets were designed by Panomics/Affymetrix, Inc. using previously published NCBI gene accession numbers (panel IDs: 21140, 21065, and 21150). Assays were performed following the manufacturer's protocol (Panomics/Affymetrix, Fremont, CA). Briefly, isolated RNA (250 ng) was hybridized overnight at 54 °C to specific oligonucleotide sequences, corresponding to the genes of interest that were bound to capture beads. A linearity test was done prior to the analysis. The following day the hybridized RNA, oligos, and capture beads were pipetted onto a filter plate. The complex was then sequentially hybridized to the pre-amplifier, followed by the amplifier, and lastly the biotinylated label probe (each for 1 h at 50 °C on a shaking platform). Streptavidin–phycoerythrin was then allowed to bind for 30 min at room temperature on a shaking platform. Fluorescence was detected and analyzed with a Bioplex 200 system array reader with Luminex xMAP technology (Bio-Rad, Hercules, CA). The housekeeper gene, Gapdh, was used to normalize the data. The data are expressed as a ratio of the relative light units of the target gene mRNA relative to Gapdh mRNA.

Membrane protein preparation and Western blotting. One hundred milligrams of liver were homogenized in 900 μl of ST buffer (150 mM sucrose, 10 mM Tris, proteinase inhibitor 1 tablet/50 ml), followed by centrifugation at 100,000 *g* at 4 °C for 1 h. The supernatant was removed and the membrane pellet was re-suspended in ST buffer. Fifty micrograms of membrane proteins from each sample was mixed with an equal volume of loading buffer (Laemmli sample buffer containing 5% β-mercaptoethanol). The proteins were resolved by 10% SDS-PAGE and transferred to PVDF membranes. Membranes were blocked using 5% non-fat milk for 10 min each, followed by 3 washes of 10 min each with PBST. The membranes were incubated with primary antibodies, rabbit anti-rat/mouse Ntcp (K4), or rabbit anti-rat/mouse Bsep (K44) (1:2000 dilution in 2% milk/PBS) for 2 h. These primary antibodies were obtained from Dr. Bruno Steiger (University Hospital, Zurich, Switzerland). After washing three times for 10 min each, the membranes were incubated with HRP-coupled secondary antibody (donkey