PXR Prevents Cholesterol Gallstone Disease by Regulating Biosynthesis and Transport of Bile Salts

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BACKGROUND & AIMS: Cholesterol gallstone disease (CGD) results from a biochemical imbalance of lipids and bile salts in the gallbladder bile. We investigated whether the xenobiotic receptor pregnane X receptor (PXR) has a role in pathogenesis of CGD. **METHODS:** Wild-type, PXR-null ($PXR^{-/-}$), and CGD-sensitive C57L mice were placed on a lithogenic diet and then analyzed for CGD at the biochemical, histological, and gene-regulation levels. **RESULTS:** Loss of PXR sensitized mice to lithogenic diet-induced CGD, characterized by decreases in biliary concentrations of bile salts and phospholipids and an increases in the cholesterol saturation index and formation of cholesterol crystals. The decreased bile acid pool size in $PXR^{-/-}$ mice that received lithogenic diets was associated with reduced expression of cholesterol 7α-hydroxylase, the rate-limiting enzyme of cholesterol catabolism and bile acid formation. The reduced expression of cholesterol 7α-hydroxylase most likely resulted from activation of farnesoid X receptor and induction of fibroblast growth factor 15 in the intestine. In C57L mice given the PXR agonist, pregnenolone-16α-carbonitrile, or the herbal medicine, St John's wort, cholesterol precipitation was prevented by increases in concentrations of biliary bile salt and a reduced cholesterol saturation index. PXR prevented CGD via its coordinate regulation of the biosynthesis and transport of bile salts in the liver and intestine. **CONCLUSIONS: PXR maintains biliary bile acid homeostasis and may be developed as a therapeutic target for CGD.**

Keywords: Fgf15; PCN; Nuclear Receptor; Gene Regulation; Transgenic Mice.

Cholesterol gallstone disease (CGD) is one of the most common gastrointestinal diseases. Precipitation of excess cholesterol in the bile as solid crystals is a prerequisite for cholesterol gallstone formation. Cholesterol is only slightly soluble in aqueous media, but is made soluble in bile through mixed micelles composed of bile salts and phospholipids.[1] Cholesterol precipitation can occur as a result of excessive cholesterol, deficiency of bile salts or phospholipids, or a combination of these factors.[1] If unmanaged, the cholesterol crystals will aggregate, fuse, and ultimately form pathologic gallstones.

Biliary secretion of bile salts and lipids is regulated by an elaborate network of transporters. Cholesterol secretion is mediated by the ABC binding cassette (Abc) transporters, Abcg5 and Abcg8.[2] Secretion of phospholipids is controlled by Abcb4, a P-glycoprotein member of the multidrug-resistance gene family.[3] Biliary bile salts consist of those newly synthesized in the liver and those that have undergone enterohepatic cycling. Cholesterol 7α-hydroxylase (Cyp7a1) is the rate-limiting enzyme of bile acid biosynthesis, catalyzing the conversion of cholesterol to bile acids.[4] The majority of bile acids, once emptied from the gallbladder to the intestine, are reabsorbed as facilitated by the apical sodium-dependent bile acid transporter (Asbt) and organic solute transporter α/β (Ostα/β) in the ileum, and then transported back to the liver through the hepatic uptake transporters sodium-taurocholate cotransport protein (Ntcp) and the organic anion transporting polypeptides (Oatps).[5] Both newly synthesized and reabsorbed bile acids are secreted into the bile by the bile acid export pump (Bsep) and multidrug-resistance protein 2 (Mrp2).[5] Mrp3 and Mrp4 play a role in the sinusoid export of conjugated bile salts and promote their renal secretion.[6]

Genetic background has a major impact on CGD susceptibility. In humans, gallstone prevalence is linked to ethnicity and displays familial clustering.[7] In mice, when challenged with a lithogenic diet, certain strains, such as the C57L mice, formed gallbladder cholesterol crystals within a week, and some other strains were resistant to CGD. Quantitative trait locus (QTL) analysis identified genomic regions that harbored the causative genetic variations in susceptible strains.[8,9] In QTL analysis, several

Abbreviations used in this paper: Abc, ATP binding cassette; Asbt, apical sodium-dependent bile acid transporter; Bsep, bile salt export pump; CAR, constitutive androstane receptor; CGD, cholesterol gallstone disease; CSI, cholesterol saturation index; Cyp7a1, cholesterol 7α-hydroxylase; Fgf, fibroblast growth factor; FXR, farnesoid X receptor; LXR, liver X receptor; Mrp, multidrug resistance protein; Ntcp, Na^+-taurocholate cotransport proteins; Oatp, organic anion transporting polypeptides; Ost, organic solute transporter; PCN, pregnenolone-16α-carbonitrile; PXR, pregnane X receptor; QTL, quantitative trait locus; SHP, small heterodimerization partner; SJW, St John's wort; WT, wild-type.

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nuclear receptors, including pregnane X receptor (PXR), were suggested as potential lithogenic (*Lith*) genes.[9]

PXR, highly expressed in the liver and small intestine, was initially identified as a xenobiotic receptor regulating expression of drug-metabolizing enzymes and transporters.[10] Subsequent studies suggested that PXR is also implicated in bile acid metabolism.[11,12] PXR can be activated by bile acids and their precursors, and activation of PXR plays an essential role in the detoxification of bile acids and relief of cholestasis.[11,12] Knowing PXR has a role in preventing cholestasis, it remains to be determined whether this receptor plays a role in the pathogenesis of CGD.

In this report, we showed that loss of PXR sensitized mice to lithogenic diet-induced CGD, whereas a pharmacological activation of PXR prevented CGD. We conclude that PXR is an antilithogenic receptor.

Methods

Animals, Lithogenic Diet, and Drug Treatment

PXR null ($PXR^{-/-}$) mice have been described previously.[13] $PXR^{-/-}$ and their wild-type (WT) control littermates were maintained on a mixed background of C57BL/6J and 129SvJ. C57L mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The lithogenic diet (Harlan Teklad TD-02189) contains 21% butterfat, 1.5% cholesterol, 0.5% cholic acid, and 23% casein. Male $PXR^{-/-}$ and WT mice were fed with lithogenic diet for 4 weeks, at which time WT mice were largely resistant to CGD and the diet did not cause appreciable hepatotoxicity. Male C57L mice were fed with lithogenic diet for 1 week because they are genetically sensitive to CGD.[8] When necessary, daily intraperitoneal injection of pregnenolone-16α-carbonitrile (PCN; 30 mg/kg) or oral gavage of St John's wort (SJW; 300 mg/kg) was given for 4 days before the lithogenic diet, and the drug treatment was continued until the completion of the experiments. Mice were then sacrificed and gallbladders were removed by ligation of the cystic duct after overnight fasting. Gallbladder bile and its contents were gently squeezed into tubes after cholecystectomy and immediately examined by a polarized microscope for cholesterol crystals and gallstones. The remaining bile was used for biochemical analysis. The use of mice in this study complied with relevant federal guidelines and institutional policies.

Bile Duct Cannulation and Collection of Hepatic Bile

Bile duct cannulation was performed as described previously.[14] Briefly, the lower end of the common bile duct of mice fasted overnight was ligated and the common bile duct was cannulated with a PE-10 polyethylene catheter below the entrance of the cystic duct. The cystic duct was then doubly ligated and a cholecystectomy was performed. Hepatic bile was collected by gravity every 15 minutes for the first hour, and then every hour for 8 hours.

Biochemical Analysis of Bile, Serum, Urine, and Feces

Biliary lipids were extracted as described.[15] Phospholipid, cholesterol, and triglyceride levels were measured with assay kits from Stanbio Laboratory (Boerne, TX). Total bile acids were quantified with an assay kit from Bioquanti (San Diego, CA). The cholesterol saturation index (CSI) was calculated according to Carey's critical tables.[16] Urine and feces were collected with metabolic cages. Serum alanine aminotransferase and aspartate aminotransferase activities were determined with assay kits from Stanbio.

Gas Chromatography-Mass Spectrometry Analysis of Biliary Bile Acids

Biliary bile acid analysis were performed as we have described previously,[17] with minor modifications in sample preparation. Briefly, 10−20 μL gallbladder bile was treated with 0.6 mL acetone/methanol/6M HCl (36:4:0.4, v:v:v) at 37°C for 14 hours to remove the conjugated sulfonyl group, and then with 15% (w/v) NaOH at 120°C for 2 hours in an autoclave to deconjugate taurine or glycine moiety. Samples were then extracted with hexane twice to remove cholesterol and then acidified with 2 M HCl. Deconjugated bile acids were extracted with diethyl ether and converted to methyl esters with trimethylsilyldiazomethane and to trimethylsilyl derivatives with *N*, *O*-bis(trimethylsilyl)trifluoroacetamide plus trimethylchlorosilane. Gas-chromatographic separation was performed as described.[17] Calculation of bile salt hydrophobicity was performed according to a published method.[18]

Western Blot Analysis

Western blot analysis was performed as described previously, with minor modifications.[19] The primary antibodies used include those against Fgf15 (sc-27177), Bsep (sc-74500), and Cyp7a1 (sc-25536) from Santa Cruz Biotechnology (Santa Cruz, CA). The Asbt, Ostα, and Ostβ antibodies were generous gifts from Dr Paul Dawson.[19]

Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction

Total RNAs were extracted with the TRIZOL reagent. Real-time polymerase chain reaction using SYBR Green-based assay was performed with the ABI 7300 real-time PCR system. Primer sequences are listed in Supplementary Table 1.

Statistical Analysis

Data are expressed as mean ± standard error. One-way analysis of variance Tukey's test was performed for statistical analysis using GraphPad Prism version 4.0. P values $<.05$ were considered statistically significant.