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Nuclear receptors in bile acid metabolism

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Abstract

Bile acids are signaling molecules that activate nuclear receptors, such as farnesoid X receptor, pregnane X receptor, constitutive androstane receptor, and vitamin D receptor, and play a critical role in the regulation of lipid, glucose, energy, and drug metabolism. These xenobiotic/endobiotic-sensing nuclear receptors regulate phase I oxidation, phase II conjugation, and phase III transport in bile acid and drug metabolism in the digestive system. Integration of bile acid metabolism with drug metabolism controls absorption, transport, and metabolism of nutrients and drugs to maintain metabolic homeostasis and also protects against liver injury, inflammation, and related metabolic diseases, such as nonalcoholic fatty liver disease, diabetes, and obesity. Bile-acid-based drugs targeting nuclear receptors are in clinical trials for treating cholestatic liver diseases and fatty liver disease.

Keywords

Nuclear receptors; bile acid synthesis; cholestasis; drug metabolism

Introduction

Bile acids are physiological detergents that play important roles in facilitating hepatobiliary secretion of endogenous and xenobiotic metabolites as well as intestinal absorption of dietary fats, fat-soluble vitamins, and drugs (Chiang, 2009). Bile acids also are signaling molecules that regulate liver metabolism (Lefebvre et al., 2009) and drug disposition (Eloranta and Kullak-Ublick, 2005). Therefore, maintaining bile acid homeostasis is important for liver metabolic function. Abnormal bile acid metabolism has been associated with cholestatic liver diseases, fatty liver disease (FLD), dyslipidemia, diabetes, obesity, and cardiovascular disease (CVD) (Chiang, 2009; Claudel et al., 2011; Porez et al., 2012).

Nuclear receptors are a group of ligand-activated transcriptional factors. Upon ligand binding and activation, nuclear receptors regulate the transcription of genes involved in various biological processes, ranging from development, differentiation, and metabolism (Mangelsdorf et al., 1995). The ligands for nuclear receptors are usually lipophilic molecules, such as hormones, endogenous metabolites, or xenobiotics. Farnesoid X receptor (FXR; NR1H4) was the first nuclear receptor identified as a bile acid receptor and was shown to regulate the transcription of key genes in bile acid metabolism, from synthesis and transport to detoxification (Makishima et al., 1999). Subsequently, three closely related xenobiotic nuclear receptors in the NR1I subfamily, pregnane X receptor (PXR; NR1I2) (Staudinger et al., 2001), vitamin D receptor (VDR; NR1I1) (Makishima et al., 2002), and

constitutive androstane receptor (CAR; NR1I3) (Guo et al., 2003), were shown to be activated by bile acids or bile acid metabolites. These xenobiotic nuclear receptors play critical roles in regulation of the transcription of genes involved in phase I oxidation, phase II conjugation, and phase III transport in drug metabolism and also bile acid metabolism (Figure 1). More-recent studies have uncovered that activation of CAR and PXR also affect glucose and energy metabolism (Wada et al., 2009; Gao and Xie, 2012). Therefore, drug and bile acid metabolism cross-talk to regulate lipid, glucose, and energy metabolism and significantly affect inflammatory and metabolic diseases, such as nonalcoholic fatty liver disease (NAFLD), diabetes, and obesity. FXR, PXR, CAR, and VDR are promising therapeutic targets for the treatment of liver and metabolic diseases (Lefebvre et al., 2009; Claudel et al., 2011; Porez et al., 2012). This review will summarize recent advances in our understanding of the roles of bile-acid-activated nuclear receptors in regulating bile acid homeostasis as well as the therapeutic potential of bile acids and their derivatives for the treatment of metabolic and liver diseases, including cholestasis, NAFLD, diabetes, and obesity.

Bile acid metabolism

Similar to drug metabolism, bile acid metabolism includes phase I bile acid synthesis in the liver, phase II bile acid conjugation in the liver and intestine, and phase III bile acid transport in the liver, kidney, and intestine. Bile acid synthesis is a complicated pathway involving three hydroxylation reactions catalyzed by cytochrome P450 (CYP) monooxygenases, epimerization, isomerization, and oxidative cleavage reactions.

Phase I: bile acid synthesis

Bile acids are synthesized from cholesterol solely in the liver (Chiang, 1998, 2009; Russell and Setchell, 1992). Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the two major primary bile acids synthesized in human livers (Figure 2A). There are two bile acid biosynthetic pathways: the classic pathway and the alternative pathway. In humans, the classic pathway accounts for synthesis of more than 90% of total bile acid synthesis, producing CA and CDCA in approximately equal amounts. Cholesterol 7 α -hydroxylase (CYP7A1) is a microsomal cytochrome p450 enzyme that catalyzes the first and rate-limiting step in the classic bile acid biosynthetic pathway. Microsomal sterol 12 α -hydroxylase (CYP8B1) is required for synthesis of CA. Therefore, CYP7A1 regulates the overall rate of bile acid synthesis, whereas CYP8B1 regulates the ratio of CA to CDCA in the bile acid pool. Mitochondrial sterol 27-hydroxylase (CYP27A1) catalyzes steroid oxidation in the classic pathways and initiates the alternative pathway, which mainly produces CDCA. Oxysterol 7 α -hydroxylase (CYP7B1) is a nonspecific enzyme that metabolizes steroid intermediates in macrophages and other tissues to produce 7 α -hydroxylated metabolites that may be used for the synthesis of bile acids in the liver. There are other hydroxylases, such as microsomal steroid 25-hydroxylase and a brain-specific steroid, 24-hydroxylase (CYP46A1), that are involved in bile acid metabolism.

Phase II: bile acid conjugation

After synthesis, most bile acids are immediately conjugated to amino acids (glycine or taurine) through amidation at the carboxyl group by bile acid coenzyme A synthase (BACS) and bile acid amino acid transferase (BAAT) (Figure 2B). In humans, the ratio of glycine to taurine conjugates is approximately 3 to 1, whereas in mice most bile acids (95%) are taurine conjugated and the remaining are free bile acids. Bile acids also are conjugated to sulfate at the 3-hydroxyl group by sulfotransferase (SULT2A1). Some bile acids are glucuronidated at 3, 6, and 24 positions by UDP-glucuronosyl N-transferases (UGT1A1, 2B4, and 2B7). Conjugation of bile acids increases ionization and solubility at acidic pH,

prevents Ca^{2+} precipitation, minimizes passive absorption, and prevents cleavage by pancreatic enzymes in the intestine. Like drug conjugation, bile acid conjugation greatly prepares bile acids for efficient transport and detoxification. In the intestine, some conjugated bile acids are deconjugated, and, subsequently, 7α -dehydroxylase activity in bacterial flora converts CA and CDCA to secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. These secondary bile acids are highly insoluble and toxic and may cause cholestatic liver injury or promote colon cancer. DCA is reabsorbed in the colon, whereas most LCA is excreted into feces. A small amount (1–4%) of LCA is circulated to the liver and rapidly conjugated by sulfation and amidation to form diconjugated LCA, which is excreted into bile. Sulfation by *SULT2A1* is the major pathway for detoxification of hydrophobic bile acids in humans (Hofmann, 2004).

Bile acids undergo biotransformation in the liver and intestine (Figure 2C). In mice, *Cyp3a11* (a homolog of human *CYP3A4*) plays a key role in metabolism of LCA and CDCA to a highly soluble and nontoxic bile acid (β -muricholic acid), which is epimerized to α -muricholic acid. *Cyp3a11* also hydroxylates CDCA to hyocholic acid. CDCA is epimerized to ursodeoxycholic acid (UDCA), a highly soluble, nontoxic bile acid. UDCA levels are high in bears and mice, but low in humans. UDCA can be converted to LCA. In mice, but not humans, LCA can be converted to UDCA, hyodeoxycholic acid, and murideoxycholic acid. In humans, the bile acid pool consists of CA, CDCA, and DCA in an approximate ratio of 40:40:20 and is highly hydrophobic. In contrast, the mouse bile acid pool consists mostly of CA and muricholic acids (MCAs; 95%) and is highly hydrophilic.

Phase III: bile acid transport and enterohepatic circulation of bile acids

Bile formation is essential for the elimination of toxic metabolites and drugs/xenobiotics from the liver (Trauner and Boyer, 2003). Bile secretion is also the major route for excretion of endogenous compounds and metabolites, such as drugs, phospholipids, cholesterol, and bilirubin. Bile acids are major components in bile and play a pivotal role in bile formation. Conjugated bile acids are secreted into bile through specific transporters located in the canalicular membrane and are stored in the gallbladder. After each meal, bile acids are released from the gallbladder into the intestine, efficiently reabsorbed in the ileum, and transported back to the liver by portal circulation. The transport of bile acids between the liver and intestine is referred to as enterohepatic circulation of bile acids (Figure 2A). This physiological pathway not only inhibits bile acid synthesis, but also plays a major role in absorption and transport of nutrients from the intestine to the liver for metabolism and distribution to other organs and tissues. In humans, enterohepatic circulation of bile acids is highly efficient; most bile acids (95%) are reabsorbed in the ileum and only 5% of bile acids are excreted into feces and are replenished by *de novo* synthesis. Bile acids are recycled 4 to 12 times a day. The bile acid pool size is defined as the total amount of bile acids circulating in the enterohepatic circulation.

The enterohepatic circulation of bile acids is a process that involves several bile acid and drug transporters (Trauner and Boyer, 2003). Hepatocytes are polarized epithelial cells with basolateral (sinusoidal) and apical (canalicular) membrane domains (Figure 3). Hepatocytes take up bile acids through the basolateral membrane, which is in direct contact with the portal blood, and excrete bile acids at the canalicular membrane into the bile (Boyer, 1980). Because biliary bile acid concentration is significantly higher in bile than in hepatocytes, canalicular bile acid transport is an active transport process requiring energy provided by hydrolysis of adenosine triphosphate (ATP) or cotransportation with other molecules. Efflux of bile acids to bile is the rate-limiting step in bile formation and provides the driving force for bile flow. Several members of the ATP-binding cassette (ABC) transporter family are responsible for transporting bile acids and other charged compounds across the canalicular membrane against their concentration gradients. The bile salt export pump (BSEP;

ABCB11), originally identified as the sister of P-glycoprotein, is the principal bile acid efflux transporter at the canalicular membrane (Childs et al., 1995). Mutations in the *BSEP* gene were first identified in patients with progressive familial intrahepatic cholestasis subtype 2 (PFIC-2) (Strautnieks et al., 1997). The multidrug resistance associated protein (ABCC2, Mrp2) effluxes a wide range of conjugated metabolites, including bile acids, bilirubin, and drugs (Kullak-Ublick et al., 2000). Phospholipids are excreted by the phospholipid flippase, MDR2 (ABCB4) (Smit et al., 1993). Biliary cholesterol secretion by ABCG5/G8 heterodimer transporters requires MDR2 (Langheim et al., 2005). Bile acids, phospholipids, and cholesterol form mixed micelles in bile to increase cholesterol solubility and reduce bile acid toxicity in the bile duct. Bile acids are stored in the gallbladder. After a meal, cholecystokinin secreted by the intestine causes the gallbladder to contract and release bile into the digestive tract for absorption of dietary fats, fat-soluble vitamins, and drugs. Most bile acids are reabsorbed in the ileum by the apical sodium-dependent bile acid transporter (ASBT), located in the brush border membrane. Once absorbed, bile acids bind the ileum bile-acid-binding protein (I-BABP) and are transported to the basolateral membrane for secretion into portal blood by heterodimeric organic solute transporters (OST α and OST β) (Dawson et al., 2005). NPC1L1 is the dietary cholesterol absorption transporter. Bile acids, especially CA, facilitate absorption of fats, cholesterol, and lipid-soluble vitamins into enterocytes. ABCG5/G8 transporter in the apical membrane of enterocytes effluxes plant sterols and cholesterol. Mutations of ABCG5/G8 genes have been identified in sitosterolemia patients (Berge et al., 2000; Lee et al., 2001).

In hepatocytes, Na⁺-dependent taurocholate cotransport peptide (NTCP), located in the sinusoidal membrane, takes up most of the reabsorbed bile acids. Sodium-independent organic anion transporters (OATPs) also uptake bile acids into hepatocytes. The sinusoidal membranes also express bile acid efflux transporters, including OST α /OST β and MRP3/MRP4, which serve to efflux bile acids into the blood circulation. These sinusoidal bile acid efflux transporters are induced during cholestasis and may play critical roles in the protection of liver injury when bile acids are accumulated abnormally in hepatocytes.

Bile-acid-activated nuclear receptors

Bile acid homeostasis is maintained by a combined balance of bile acid synthesis, metabolism, and transport. Bile acids activate several nuclear receptors to regulate bile acid homeostasis. These nuclear receptors act as sensors of bile acid levels in hepatocytes and enterocytes and coordinately regulate transcription of a network of genes in bile acid synthesis, conjugation, and transport. In chronic liver diseases, such as cholestasis, high levels of hepatic bile acids activate these nuclear receptors to increase bile acid metabolism to protect against liver injury.

Bile acids directly activate three nuclear receptors (FXR, PXR, and VDR). FXR and PXR are highly expressed in tissues that are exposed to bile acids, including liver and intestine, whereas VDR is widely expressed in most tissues. Among all bile acids, CDCA is the most efficacious ligand of FXR, with a half-maximal effective concentration (EC₅₀) of ~10 μ M, whereas one of the secondary bile acids, LCA, is the most potent activator of PXR and VDR, with an EC₅₀ of ~100 nM. Bile acids do not bind or activate CAR. FXR is a master regulator of bile acid metabolism by regulating several key genes involved in bile acid synthesis, conjugation, and transport. PXR, CAR, and VDR all play roles in the three phases of drug and bile acid metabolism in the liver and intestine. FXR, PXR, VDR, and CAR target genes identified in bile acid metabolism are shown in Table 1.

The role of FXR in regulation of bile acid homeostasis

The first identified FXR target gene involved in bile acid metabolism is *I-BABP* (Grober et al., 1999). FXR/RXR heterodimer binds to an inverted repeat in the *I-BABP* gene promoter and stimulates *I-BABP* gene transcription in response to bile acid treatment. It is well established that the *CYP7A1* gene is repressed by bile acids, a mechanism that allows the liver to decrease bile acid synthesis in response to an increase in bile acid levels and thus maintains a constant bile acid pool (Chiang, 1998). FXR is the key mediator in this bile acid feedback repression mechanism. It was first discovered that hepatic FXR induced a negative nuclear receptor, small heterodimer partner (SHP), which interacts with and represses the transcriptional activator, liver related homolog 1 (LRH-1), that binds to the *CYP7A1* gene promoter, thus inhibiting *CYP7A1* gene transcription (Goodwin et al., 2000). However, bile acids and FXR agonists still are able to inhibit *CYP7A1* in SHP-deficient mice, suggesting that a SHP-independent pathway also exists (Makishima et al., 2002, Wang et al., 2002). More recently, FXR was shown to induce intestinal fibroblast growth factor 15 (FGF15), which acts as an endocrine factor to repress *CYP7A1* gene transcription in hepatocytes (Inagaki et al., 2005). Direct infusion of recombinant FGF15 into mouse blood circulation or overexpression of FGF15 in mouse liver by an adenovirus expression vector caused marked repression of *CYP7A1* messenger RNA (mRNA) expression. FGF15 activates the cell-surface receptor, FGFR4, leading to activation of extracellular signal-related kinase (ERK)1/2 signaling to inhibit *CYP7A1*, but the exact molecular mechanism of FGF15 repression of *CYP7A1* has yet to be elucidated (Song et al., 2009). The identification of an intestine-initiated bile acid feedback mechanism is consistent with a long-observed phenomenon that intraduodenal, but not intravenous, infusion of taurocholic acid repressed *CYP7A1* mRNA expression in rats (Pandak et al., 1991). In mice lacking functional *Osta/Ostβ* heterodimer, bile acid transportation to the liver was reduced and bile acids were accumulated in the intestine. Interestingly, these mice showed increased intestinal FGF15 expression and reduced liver *CYP7A1* mRNA and total bile acid pool (Rao et al., 2008). Further, intestine-specific FXR knockout, but not liver-specific FXR knockout, prevented GW4064 (an FXR agonist) repression of liver *CYP7A1* gene expression in mice (Kim et al., 2007; Kong et al., 2012). These studies collectively suggest that intestinal FXR plays a dominant role in mediating bile acid feedback repression of bile acid synthesis. FGF19, the human ortholog of mouse FGF15, has been shown to repress *CYP7A1* expression and bile acid synthesis in human hepatocytes (Song et al., 2009). FGF19 mRNA and protein are detectable in human liver and hepatocytes (Song et al., 2009; Schaap et al., 2009). FGF19 protein is also present in human blood circulation (Lundasen et al., 2006). It is likely that bile acid accumulation in the human liver may induce the FXR/FGF19 pathway to repress *CYP7A1* in an autocrine manner.

In addition to its role in mediating bile acid inhibition of bile acid synthesis, FGF15 was shown to stimulate gallbladder refilling (Choi et al., 2006). Both *fgf15*^{-/-} mice and *fgfr4*^{-/-} mice had depleted gallbladder volume, which was rapidly reversed by FGF15 or FGF19 treatment. Because FGF15 does not seem to be expressed in mouse hepatocytes or gallbladder, it is likely that intestinal bile acids may play a role in FGF15-regulated gallbladder refilling in mice. On the other hand, FGF19 is highly expressed in human gallbladder and biliary tract, and human bile contains high levels of secreted FGF19, indicating a potentially important function of FGF19 in the biliary tract (Zweers et al., 2012). Currently, the importance of FGF15 signaling in hepatobiliary transport of bile acids and other organic compounds remains to be determined. Beyond bile acid metabolism, FGF15 has been shown to repress hepatic lipo- and gluconeogenesis as well as to stimulate hepatic glycogen and protein synthesis (Potthof et al., 2011; Bhatnagar et al., 2009; Kir et al., 2011). A recent study showed that FGF19 treatment ameliorated hepatic lipid disorders in mice lacking FXR (Miyata et al., 2011).

FXR also plays a key role in regulation of bile acid transport in the enterohepatic system. It was first demonstrated that bile acid activation of FXR induced BSEP (Ananthanarayanan et al., 2001) and MRP2 in hepatocytes (Kast et al., 2002). On the other hand, FXR “indirectly” repressed NTCP by induction of SHP (Denson et al., 2001). Thus, activation of liver FXR protects hepatocytes from toxic bile acid accumulation by stimulating bile acid secretion at the canalicular membrane and limiting bile acid uptake from the portal circulation. FXR/RXR heterodimer binds to the OST α and OST β gene promoters and induces their expression in the sinusoidal membrane to efflux bile acids to systemic circulation (Lee et al., 2006) and, subsequently, are eliminated by renal excretion. Several basolateral drug transporters (MRP1, MRP3, and MRP4) have substrate specificity for bile acid conjugates (Trauner and Boyer, 2003; Hirohashi et al., 2000; Zelcer et al., 2003; Kullak-Ublick et al., 2000). These MRPs are induced during cholestasis in mice and humans (Zollner et al., 2007; Chai et al., 2011). Stimulation of basolateral bile acid efflux in cholestasis and primary biliary cirrhosis may be an important protective response against cholestatic liver injury (Mennone et al., 2006; Marschall et al., 2006; Zollner et al., 2006; Boyer et al., 2006).

In the intestine, FXR is involved in limiting canalicular bile acid uptake and basolateral bile acid secretion. Mouse and human, but not rat, brush border ASBT is repressed by bile acids (Neimark et al., 2004; Chen et al., 2003; Arrese et al., 1998). ASBT is positively regulated by the RAR/RXR heterodimer and LRH that bind to its gene promoter (Chen et al., 2003; Neimark et al., 2004). It was hypothesized that FXR-mediated induction of SHP might repress RAR or LRH transactivation of ASBT transcription. A more-recent study provides additional evidence that FGF15 may play a role in mediating bile acid repression of ASBT (Sinha et al., 2008). FXR induces the major basolateral bile acid efflux transporters, OST α and OST β (Lee et al., 2006). Thus, increased bile acid levels in enterocytes may activate FXR to limit bile acid absorption and promote basolateral efflux of bile acids for subsequent biliary or renal excretion.

Given the role of FXR in regulating the bile acid transport system, numerous studies have been carried out to evaluate the role of FXR in regulating bile acid homeostasis and detoxification in *Fxr*-null mice under bile duct ligation (BDL), bile acid loading, or drug-induced intrahepatic cholestasis (Wagner et al., 2003, 2011; Cui et al., 2009). Mice lacking FXR showed elevated CYP7A1 mRNA expression and enlarged bile acid pool size, but otherwise were phenotypically normal (Kok et al., 2003; Sinal et al., 2000). When fed a cholic-acid-containing diet, *Fxr*-null mice showed more-severe hepatotoxicity, which was accompanied by the lack of CYP7A1 repression and induction of FXR-regulated transporters. Similarly, *Fxr*-null mice were more susceptible to bile-acid-induced liver injury after BDL, despite induction of the PXR-regulated bile acid detoxification network.

The role of PXR, CAR, and VDR in regulation of bile acid metabolism and toxicity

A potential role of PXR in regulating bile acid synthesis was first implicated by the observation that treating rodents with pregnenolone 16 α -carbonitrile (PCN), a PXR agonist, repressed liver CYP7A1 activity (Mason and Boyd, 1978; Stahlberg, 1995), which provides a molecular basis for cross-talk between bile acids and drug metabolism. A regulatory role of PXR in bile acid synthesis was subsequently confirmed by studies showing that PCN repressed CYP7A1 mRNA expression and biliary bile acid secretion, but failed to do so in PXR-deficient mice (Staudinger et al., 2001). In human hepatocytes and liver cell line models, two groups showed that rifampicin-activated PXR repressed human *CYP7A1* gene transcription by inhibiting the nuclear receptor, hepatocyte nuclear factor 4 alpha (HNF4 α), and coactivator peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1 α) complex that is required for *CYP7A1* gene transcription (Li and Chiang, 2005;

Bhalla et al., 2004). More recently, activation of PXR in the intestine was shown to induce FGF15 expression, and a PXR response element was identified in the promoter of the FGF15 gene (Wistuba et al., 2007). These results suggest that drug or bile acid activation of intestinal PXR may mediate inhibition of CYP7A1 and bile acid synthesis. Whether PXR induces FGF19 in human hepatocytes remains to be determined. PXR induction of intestinal FGF19 was recently associated with promotion of colon tumor growth, implicating a link between PXR activation and anticancer drug resistance (Wang et al., 2011). Interestingly, a recent study showed that mice lacking PXR fed a lithogenic diet had higher susceptibility to development of cholesterol gallstones, which is associated with decreased *Cyp7a1* gene expression, reduced biliary bile acid secretion, and higher intestinal FGF15 expression (He et al., 2011).

The hydroxylation of bile acids is mediated mainly by CYP3A enzymes that are induced upon PXR activation (Staudinger et al., 2001). In addition, the bile acid conjugation enzymes, SULT2A1 and UGTs, the canalicular transporter, MRP2, and the basolateral transporter, OATP2, are also induced by PXR activation (Kliwer and Willson, 2002). Therefore, LCA activation of PXR is clearly an adaptive protection to reduce bile acid toxicity during cholestasis. Indeed, PXR target genes involved in bile acid detoxification were induced in mice treated with LCA or BDL, whereas mice lacking PXR were more susceptible to hepatotoxicity caused by LCA treatment or BDL, supporting the notion of adaptive activation of the PXR-signaling network to reduce bile acid toxicity during cholestasis (Staudinger et al., 2001; Stedman et al., 2005). In addition, numerous studies have collectively demonstrated that activation of PXR protects against bile-acid-induced liver injury in experimental cholestasis models (Stedman et al., 2005). When phenobarbital and 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP)-treated FXR and PXR knockout (KO) mice were administered CA, they were protected from bile acid toxicity and CAR target genes *Ctp2b*, *Cyp3a*, *Mrp2*, *Ugt1a1*, and *Gsta* were induced, suggesting that FXR, PXR, and CAR protect against hepatic bile acid toxicity in a complementary manner (Guo et al., 2003). In humans, rifampicin has been used to reduce pruritus associated with cholestasis (Hofmann, 2002). The pathophysiological cause of pruritus is not clear, though it may be related to high tissue and serum bile acid accumulation. Thus, PXR repression of bile acid synthesis and promotion of bile acid detoxification may provide benefits in reducing bile acid cytotoxicity in cholestasis. The effectiveness of rifampicin treatment in reducing pruritus has been demonstrated in several clinical studies, but the degree of relief varied among individuals (Hofmann, 2002). However, hepatotoxicity has been reported in patients treated with rifampicin, suggesting that the development of an improved PXR agonist is needed for treating pruritus associated with cholestasis.

Similar to PXR, CAR is constitutively active and is considered to be another master regulator of drug metabolism in the liver (Stanley et al., 2006). CAR and PXR recognize the same xenobiotic response elements in target gene promoters and thus regulate an overlapping set of target genes in xenobiotic and bile acid metabolism and detoxification (Table 1). One study reports that activation of CAR also repressed *CYP7A1* gene transcription in hepatocytes (Miao et al., 2006). Activation of CAR is beneficial for protecting against bile acid toxicity during cholestasis in mice (Beilke et al., 2009a, 2009b; Saini et al., 2004, Stedman et al., 2005, Guo et al., 2003). There is no evidence that bile acids bind or activate CAR. One study showed that mice lacking CAR had a higher degree of liver injury than wild-type (WT) mice during LCA- or BDL-induced cholestasis, and such liver damage was aggravated in CAR/PXR double KO mice, suggesting that PXR and CAR may play overlapping, but also complementary, roles in bile acid detoxification (Stedman et al., 2005). Because PXR is thought to be the major mediator of CYP3A induction in bile acid hydroxylation, CAR is thought to play a central role in inducing LCA sulfation,

because the resistance of CAR transgenic mice to LCA toxicity was associated with increased LCA sulfation independent of CYP3A induction (Saini et al., 2004).

VDR is primarily activated by $1\alpha,25$ -dihydroxyvitamin D_3 . Recent studies suggest that besides its well-known role in regulating calcium homeostasis, VDR also regulates drug metabolism, bile acid metabolism, and inflammation (Nagpal et al., 2005). $1\alpha,25$ -dihydroxyvitamin D_3 , acting through VDR, was first shown to induce CYP3A4, CYP2B, and CYP2C in intestinal cells, suggesting a role for VDR in drug and bile acid metabolism (Schmiedlin-Ren et al., 2001; Thummel et al., 2001). VDR mainly acts as an intestinal bile acid sensor and protects the gut from bile acid toxicity. In addition to VDR induction of CYP3A, later studies identified that activation of VDR also induced *SULT2A1* and thus simultaneously stimulated bile acid sulfation (Chatterjee et al., 2005). Further, MRP3 and ASBT, two bile acid transporters, have been shown to be direct VDR targets in the intestine (McCarthy et al., 2005; Chen et al., 2006). Induction of MRP3 in the intestine is thought to stimulate excretion of conjugated and unconjugated bile acids, whereas stimulation of ASBT may facilitate bile acid absorption and transport to the liver. Because high levels of LCA in the gut are thought to promote colon cancer, accumulation of LCA in the intestine may activate VDR to induce CYP3A, which metabolizes LCA to less-toxic intermediates for excretion. Treating primary human hepatocytes with $1\alpha,25$ -dihydroxyvitamin D_3 induces known VDR target genes, including CYP3A, CYP2B, and CYP2C (Drocourt et al., 2002). VDR mRNA and protein were detected at low levels in primary human hepatocytes (Han et al., 2010). Because it was shown that cholestasis could result in a significant elevation of LCA levels in the liver and LCA is a potent endogenous ligand for VDR, studies were thus carried out to investigate the effect and mechanism of VDR activation on hepatic bile acid synthesis. It was first shown that treating primary human hepatocytes with $1\alpha,25$ -dihydroxyvitamin D_3 repressed CYP7A1 mRNA expression (Han and Chiang, 2009). Mechanistic studies suggest that VDR may interact with HNF4 and compete for PGC-1 α in transactivation of the *CYP7A1* gene. Nuclear VDR repression of *CYP7A1* gene transcription is further augmented by a cell-surface VDR-initiated, cell-signaling pathway that led to activation of ERK signaling (Han et al., 2010). Another recent study reported that mice lacking VDR had elevated hepatic *CYP7A1* gene expression and enlarged bile acid pool size, whereas $1\alpha,25$ -dihydroxyvitamin D_3 treatment repressed hepatic *CYP7A1* gene expression in mice (Schmidt et al., 2010). It was shown that *vdr*^{-/-} mice expressed lower levels of FGF15 in the intestine, whereas $1\alpha,25$ -dihydroxyvitamin D_3 treatment increased intestinal FGF15 in WT mice. A VDR-binding site was identified in the FGF15 promoter (Schmidt et al., 2010). Further, $1\alpha,25$ -dihydroxyvitamin D_3 failed to repress CYP7A1 in *fgf15*^{-/-} mice, supporting that activation of intestinal VDR represses hepatic CYP7A1 by FGF15 signaling in mice. Taken together, studies conducted in primary human hepatocytes and mouse models provide evidence that VDR represses hepatic *CYP7A1* gene transcription and bile acid synthesis.

Despite the proposed role of VDR activation in the repression of bile acid synthesis and activation of bile acid detoxification, existing studies on potential effects of VDR ligands in the protection of cholestatic liver injury are still very limited. One study showed that $1\alpha,25$ -dihydroxyvitamin D_3 treatment did not alter hepatic or serum bile acid levels in mice after BDL, suggesting a minimal role of VDR in modulating bile acid metabolism in cholestasis (Ogura et al., 2009). However, this study showed that VDR activation in BDL mice reduced proinflammatory cytokine expression and release, consistent with the known role of VDR in immunity. These results suggest that the anti-inflammatory properties of VDR could provide some benefits during cholestasis. The role of VDR in regulating innate and adaptive immunity is reviewed in detail elsewhere (Nagpal et al., 2005). $1\alpha,25$ -dihydroxyvitamin D_3 treatment generally reduces hepatic and serum bile acids, which is accompanied by increased renal MRP2, MRP3, and MRP4 mRNA expression and increased renal bile acid

secretion (Nishida et al., 2009). However, this study showed that in mice fed a chow diet, $1\alpha,25$ -dihydroxyvitamin D_3 treatment markedly increased hepatic CYP7A1 mRNA without altering total bile acid pool size or serum bile acid concentration. Further studies are needed to evaluate the potential effects of pharmacological activation of VDR in modulating bile acid metabolism and cholestasis liver injury.

Conclusion

This review summarizes the current knowledge of the roles of major bile acid and xenobiotic-sensing nuclear receptors in the regulation of bile acid metabolism and detoxification. The identification of these regulatory mechanisms provided the molecular basis for developing bile acid receptor-based therapies to treat human liver diseases. Several potent FXR agonists have been tested in clinical trials for treating cholestasis (Pellicciari et al., 2004; Rizzo et al., 2005). In addition, FXR agonists also show great promise for the treatment of FLD, diabetes, and CVDs as a result of the roles of FXR in regulating lipid and glucose metabolism (Fiorucci et al., 2010; Mencarelli and Fiorucci, 2010; Porez et al., 2012). PXR and CAR are also attractive drug targets for treating cholestasis, based on their key function in the activation of the bile acid detoxification network. Recent studies indicated that these xenobiotic sensors play important roles in many other physiological aspects, including lipid metabolism and inflammation (Modica et al., 2009). Future advances in the field are needed to improve our understanding of the control of bile acids over metabolism, which is also critical in developing effective nuclear receptor modulators for the treatment of liver diseases.

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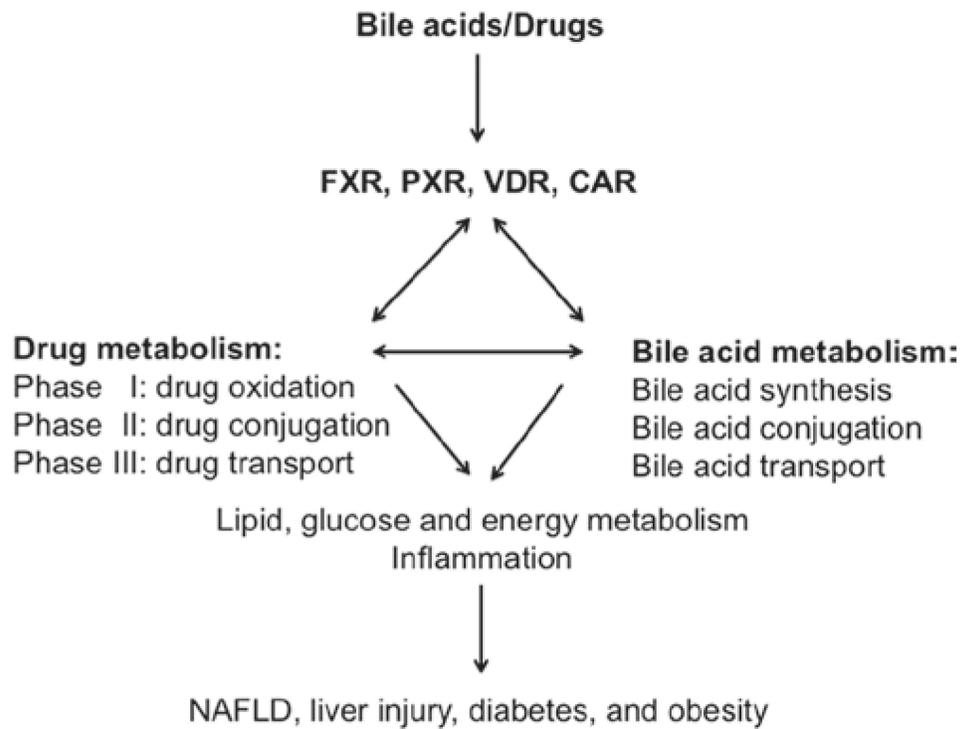
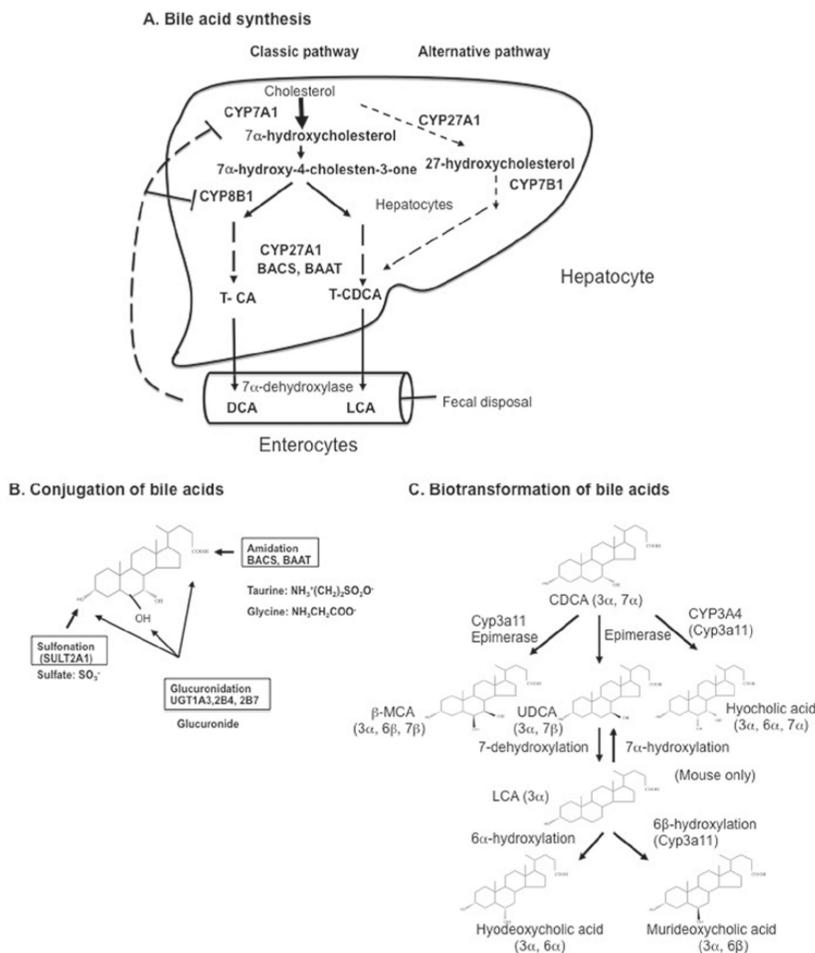


Figure 1.

Roles of FXR, PXR, VDR, and CAR in regulation of drug and bile acid metabolism. Bile acids are endogenous ligands of FXR, PXR, and VDR. Bile acids also activate CAR by an indirect mechanism. These four bile-acid-activated nuclear receptors play a critical role in the regulation of phase I hydroxylation (oxidation), phase II conjugation, and phase III transport in both drug and bile acid metabolism and coordinately regulate lipid, glucose, and energy metabolism and inflammation. Bile acid metabolism plays a key role in maintaining metabolic homeostasis and preventing NAFLD, diabetes, and obesity.

**Figure 2.**

Bile acid synthesis, conjugation, and biotransformation. Bile acid synthesis in the liver converts cholesterol to bile acids through the classic and alternative pathways. (A) Bile acid synthesis. The classic pathway involves 17 enzymes located in the endoplasmic reticulum, cytosol, mitochondria, and peroxisomes. Four CYP monooxygenases (CYP7A1, CYP8B1, CYP27A1 and CYP7B) are involved in hydroxylation reactions. In the classic pathway, a steroid nucleus undergoes hydroxylation, isomerization, and epimerization first, then oxidative cleavage of the side chain by CYP27A1, and cleavage of the 3-C side chain by peroxisomal β oxidation. CYP7A1 is the first and rate-limiting enzyme in the classic pathway, which synthesizes two primary bile acids (CA and CDCA) in the human liver. CYP8B1 is required for synthesis of CA. CYP27A1 catalyzes side-chain oxidation reactions. The alternative pathway is initiated by CYP27A1, followed by CYP7B1, and then further metabolized as the classic pathway to produce CDCA. (B) Conjugation of bile acids. Amidation (BACS and BAAT), sulfation (SULT2A1), and glucuronidation (UGT1A1, 2B4, and 2B7) at specific positions by specific enzymes are shown. (C) Biotransformation of bile acids. Secondary bile acids CDCA and LCA can be converted to more hydrophilic bile acids in the hepatocytes and intestine. Hydroxylation (by human CYP3A4 or mouse Cyp3a11), epimerization, and dehydroxylation interconverts CDCA to UDCA to LCA as well as other bile acids.

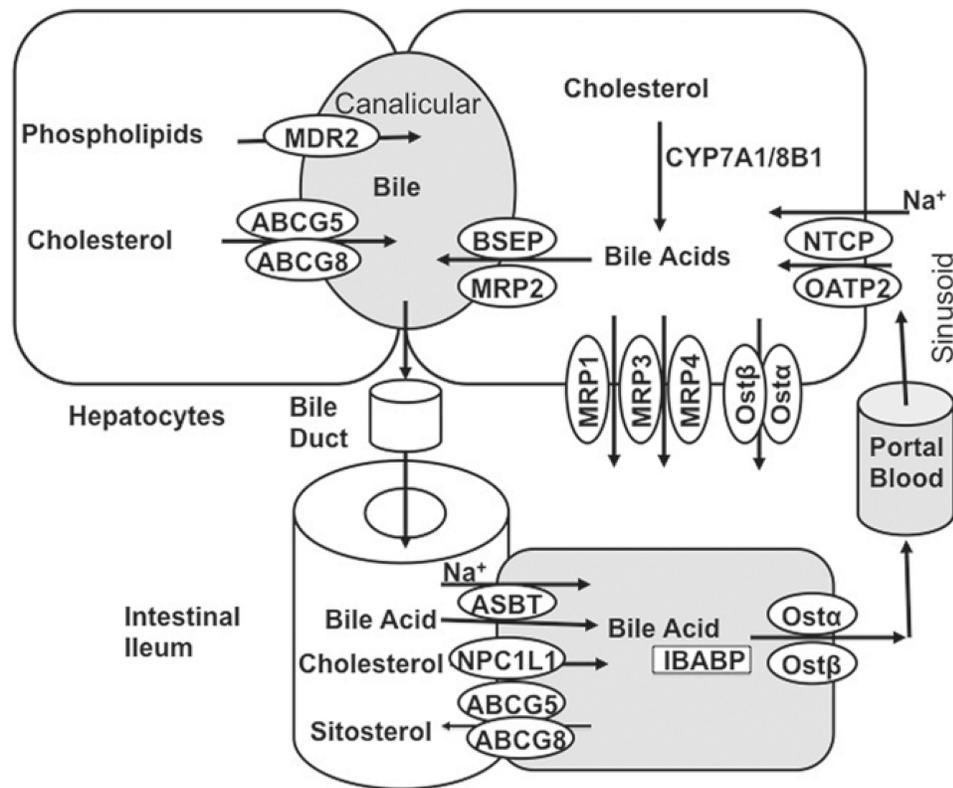


Figure 3.

Enterohepatic circulation of bile acids. Bile acids are synthesized from cholesterol in hepatocytes. Bile acids are secreted into the gallbladder by BSEP and MRP2. Phospholipids are transported by MDR2, and cholesterol is transported by ABCG5/G8 transporters into bile. In the gallbladder, bile acids, phospholipids, and cholesterol form mixed micelles to solubilize cholesterol and reduce bile acid toxicity. After meal intake, the gallbladder releases bile into the small intestine, where bile acids facilitate the absorption of dietary lipids and vitamins. At the terminal ileum, most of the bile acids are reabsorbed by ASBT into the enterocytes and are secreted into the portal circulation by the basolateral bile acid transporters, Ostα/Ostβ. At the basolateral membrane of the hepatocytes, bile acids are taken up by the NTCP transporter for resecretion into the gallbladder, whereas Ostα/Ostβ and MRPs are responsible for basolateral bile acid efflux.

Table 1
Nuclear receptor target genes in bile acid and drug metabolism

NR	Ligand	Target Gene	Tissue	Regulation	Function
FXR	CDCA	CYP7A1	Liver	Down	Encodes the rate-limiting enzyme in classic bile acid synthetic pathway
	CA				
	DCA				
	LCA	BSEP	Liver	Up	Rate-limiting step in canalicular bile acid transport into the gallbladder
	GW4064				
	INT-747				
		NTCP	Liver	Down	Basolateral bile acid uptake into the hepatocytes
		OST α OST β	Liver Intestine	Up	Basolateral bile acid secretion into the portal blood
		I-BABP	Intestine	Up	Intracellular bile acid transport
		FGF15/19	Liver Intestine	Up	Bile acid synthesis inhibition
		SHP	Liver	Up	Bile acid synthesis inhibition
PXR	Rifampicin (human) PCN (mouse)	CYP3A, CYP1, CYP2 isoforms	Liver Intestine	Up	Phase I bile acid and drug metabolism
	LCA	SULT2A1, UGT1A	Liver Intestine	Up	Phase II bile acid and xenobiotic detoxification
	John's wort	MRP1,MRP2, MRP3, MRP4 OATP1A4, MDR1	Liver, Intestine Kidney	Up	Canalicular or basolateral bile acid and drug conjugates transport
		CYP7A1	Liver	Down	Bile acid synthesis
		FGF15/19	Intestine	Up	Bile acid synthesis inhibition
CAR	Phenobarbital TCPOBOP	CYP2 isoforms, CYP3A, SULT2A, UGT1A1	Liver Intestine	Up	Phase I/II bile acid and drug detoxification
	Androstanol	MRP2/MRP3/MRP4	Liver	Up	Canalicular or basolateral bile acid and drug conjugates transport
VDR	1 α , 25-OH VD3 LCA	CYP3A	Intestine	Up	Phase I bile acid and drug metabolism
		SULT2A1	Intestine	Up	Bile acid conjugation
		CYP7A1	Liver	Down	Bile acid synthesis
		FGF15	Intestine	Up	Bile acid synthesis inhibition