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FXR and PXR: Potential therapeutic targets in cholestasis☆

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Abstract

Cholestatic liver disorders encompass hepatobiliary diseases of diverse etiologies characterized by the accumulation of bile acids, bilirubin and cholesterol as the result of impaired secretion of bile. Members of the nuclear receptor (NR) family of ligand-modulated transcription factors are implicated in the adaptive response to cholestasis. NRs coordinately regulate bile acid and phospholipid transporter genes required for hepatobiliary transport, as well as the phases I and II metabolizing enzymes involved in processing of their substrates. In this review we will focus on FXR and PXR, two members of the NR family whose activities are regulated by bile acids. In addition, we also discuss the potential of pharmacological modulators of these receptors as novel therapies for cholestatic disorders.

Keywords

FXR; PXR; Cholestasis; Nuclear receptor; Liver; Review

1. Introduction

Cholestatic liver disorders include a spectrum of hepatobiliary diseases of diverse etiologies that are characterized by impaired hepatocellular secretion of bile, resulting in accumulation of bile acids, bilirubin and cholesterol. Causes of cholestasis include extrahepatic biliary obstruction (*e.g.* stones, tumors, biliary atresia), intrahepatic biliary obstruction (*e.g.* primary biliary cirrhosis, primary sclerosing cholangitis) and intrahepatic cholestasis (*e.g.* drugs, genetic transporter defects, or infections) [1,2]. In patients with cholestasis, the major abnormalities observed are an elevation of circulating levels of primary bile acids and an increase in the formation of sulfated bile acids. The major mechanism for bile acid elimination in severely cholestatic patients is renal excretion, with the relatively hydrophilic tetrahydroxy bile acids found in their urine [3]. In advanced cholestasis, the ratio of cholic

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acid (CA) to chenodeoxycholic acid (CDCA) increases in the serum, the proportion of unconjugated bile acids is reduced, and concentrations of the secondary bile acid deoxycholic acid (DCA) decreases [4]. The physiological consequences of reduced intestinal bile acids include maldigestion of fat and malabsorption of fat-soluble vitamins. In addition, increased circulating bile acids may contribute to pruritis [4], as well as apoptosis or necrosis of hepatocytes [5]. Progressive hepatic fibrosis and cirrhosis can ensue leading to death due to hepatic failure or the complications of portal hypertension.

Hepatobiliary transport of bile acids and phospholipids is mediated by specific transporters expressed at the canalicular membrane of the hepatocyte. Impaired function of these transporters leads to reduced bile formation or cholestasis and mutations in these genes are associated with a variety of hereditary cholestatic syndromes. At the transcriptional level, these transporters and the phases I and II metabolizing enzymes involved in processing of their substrates are coordinately regulated by members of the nuclear receptor (NR) family of ligand-modulated transcription factors. In this review, we will focus on FXR and PXR, two members of the NR family whose activities are regulated by bile acids and which are implicated in the adaptive response to cholestasis.

2. FXR (farnesoid X receptor)

FXR (farnesoid X receptor, NR1H4, also known as bile acid receptor, BAR) was the first nuclear receptor identified to have bile acids as endogenous and physiologically relevant ligands [6,9]. FXR serves as a sensor for bile acids and promotes enterohepatic clearance of bile acids by controlling the expression of genes involved in their transport and metabolism. In addition, FXR counteracts liver X receptor (LXR) in both cholesterol and triglyceride metabolism [7]. FXR is abundantly expressed in the liver and intestine as well as the kidney and adrenal gland [8]. Endogenous bile acids have differing abilities to activate FXR. *In vitro* studies indicate that CDCA is a potent FXR ligand at physiological concentrations (Fig. 1), whereas other bile acids such as lithocholic acid (LCA), DCA, and CA are less effective, and hydroxylated CDCA metabolites (muricholic acids) do not activate FXR [6,9]. Interestingly, although LCA functions as a weak agonist for FXR, it strongly antagonizes CDCA-stimulated activation of FXR [10]. Synthetic FXR agonists have been identified, including GW4064, an isoxazole derivative [11], and fexaramine [12] as well as the semi-synthetic CDCA derivative, 6-ethyl CDCA [13] (Fig. 1). In addition, the natural product guggulsterone is a promiscuous nuclear receptor ligand that antagonizes FXR [14], but activates PXR [15] and the progesterone receptor [16]. FXR binds DNA in most instances as a heterodimer with retinoid X receptor α (RXRα), to response elements consisting of tandem repeats of the AGGTCA hexamer. FXR preferentially binds as a heterodimer to an IR-1 element (inverted repeat with a single nucleotide spacer), but can also activate transcription through other DNA elements, such as DR-3 or DR-4 motifs. Less commonly it can activate transcription by binding to DNA as a monomer, independent of RXR [17,18].

Characterization of mice with targeted deletions of FXR has provided insight into the physiological importance of this nuclear receptor. Sinal et al. [19] showed that *Fxr* knockout (*Fxr* KO) mice display high serum bile acids, cholesterol and triglycerides and decreased fecal excretion of bile acids, a phenotype similar to Byler's disease [19]. Surprisingly,

increased serum bile acid levels were associated with a reduction of total bile acid pool size in the *Fxr* KO animals, suggesting that bile acid synthesis was suppressed in the absence of functional FXR [19]. In another study, however, elevated serum bile acid concentrations in *Fxr* KO mice were associated with an increased biliary bile acid output as well as increased cholate pool size and total bile acid pool size, corresponding to an increase in bile acid synthesis [20]. *Fxr* KO mice fed with high concentrations of dietary CA exhibited increased hepatocyte necrosis and increased mortality as compared to wild-type mice, most likely due to liver failure secondary to toxic bile acid accumulation [21]. A shorter duration of 1% CA feeding resulted in a marked increase in serum, liver and urine bile acid concentrations, and severe hepatotoxicity in *Fxr* KO mice, associated with the loss of expression of the apical hepatocyte bile acid transporter, *Abcb11*, and reduced biliary excretion of bile acids [19–22]. Compensatory increases in expression of the basolateral bile acid efflux transporters, *Mrp3* and *Mrp4* have been demonstrated in these mice [23]. *Fxr* KO mice also fail to repress *Cyp7a1* appropriately in response to bile acid (BA) feeding, resulting in increased bile acid synthesis, consistent with loss of induction of short heterodimer partner (*Shp*) [19,24].

3. Regulation of bile acid transport and metabolism by FXR

Activation of FXR *in vivo* is associated with increased hepatobiliary circulation of bile acids, inhibition of hepatic bile acid biosynthesis, and a reduction in plasma triglycerides [25]. Below we will discuss the roles of FXR in bile acid transport and metabolism and the implications for cholestatic disorders (Fig. 2).

3.1. Regulation of bile formation

FXR coordinately controls secretion of bile acids and phospholipids, the main constituents of bile (72% and 24% of solutes by weight, respectively), which together form mixed micelles for the emulsification of dietary lipids and lipid-soluble vitamins. Hepatobiliary transport of bile acids and phospholipids is mediated by dedicated transporters expressed at the canalicular (apical) membrane of the hepatocyte. Impaired function of these transporters leads to impaired bile formation or cholestasis and mutations in these genes are associated with progressive familial intrahepatic cholestasis (PFIC) syndromes, a heterogeneous group of autosomal recessive disorders, which can be classified into three subtypes [26–28]. PFIC1 and PFIC2 usually appear in the first months of life, whereas onset of PFIC3 may also occur later in infancy, in childhood or even during early adulthood. The predominant clinical manifestations are cholestasis, pruritus and jaundice. PFIC patients usually develop fibrosis and end-stage liver disease before adulthood. Serum gamma-glutamyltransferase (GGT) activity is usually normal in PFIC1 and PFIC2 patients, but is elevated in PFIC3 [26–28].

PFIC1 or Byler disease is characterized by the appearance of intrahepatic cholestasis in the first months of life, with recurrent episodes of jaundice and pruritus, and progression to chronic liver failure in later stages [29]. PFIC1 is caused by mutations in the *ATP8B1* (FIC1) gene, encoding a phospholipid flippase that transports aminophospholipids from the outer to the inner leaflet of the canalicular membrane [29,30]. Mutations in *ATP8B1* are also associated with benign recurrent intrahepatic cholestasis (BRIC), a milder form of hereditary cholestasis [29]. PFIC1 is associated with decreased FXR activity and nuclear translocation [31,32], which is caused by impaired protein kinase C zeta (PKCzeta)-mediated

phosphorylation of cytosolic FXR and subsequent absence of nuclear translocation [33,34]. Studies with *ATP8B1* KO mice revealed that *Atp8b1* deficiency affects the canalicular phospholipid membrane asymmetry, rendering the canalicular membrane less resistant toward hydrophobic bile salts and subsequently resulting in impaired BA transport and cholestasis [35].

PFIC2 (Byler Syndrome) is characterized by early onset intrahepatic cholestasis, jaundice, pruritus and progression to hepatic fibrosis, cirrhosis and endstage liver disease before adulthood. PFIC2 patients exhibit a 100-fold reduction in bile acid secretion into bile resulting in the accumulation of bile acids within hepatocytes, cholestatis and liver injury. Unlike patients with PFIC3, serum levels of cholesterol and GGT are usually normal or only mildly elevated. PFIC2 is caused by mutations in the *ABCB11* gene (bile salt export protein (BSEP) or sister of P-glycoprotein (S-PGP)). Shortly after the identification of ABCB11 as a bile acid transporter, the human ortholog was cloned and found to be mutated in PFIC2 [36]. Mutations in *ABCB11* have also been associated with two milder cholestatic syndromes: (1) benign recurrent intrahepatic cholestasis type 2 (BRIC2), which is characterized by intermittent episodes of cholestasis without progression to liver disease and (2) intrahepatic cholestasis of pregnancy (ICP), which typically presents with pruritus and abnormal liver tests in the third trimester of pregnancy and is associated with increased risk of intrauterine fetal death and prematurity [37,38]. Although the functional consequences of most mutations in *ABCB11* are still unknown, several mutations have been demonstrated to result in impaired activity, stability or trafficking to the membrane [39,40]. The severity of the different cholestatic phenotypes has further been demonstrated to correlate with activity and levels of expression of ABCB11 [41]. In contrast to humans with PFIC2, targeted inactivation of *Abcb11* in mice resulted only in a mild non-progressive cholestasis [42]. Surprisingly, although secretion of CA, the major bile acid in mice, was greatly reduced (to 6% of wild-type), total bile acid output in mutant mice was still about 30% of wild-type. Also, secretion of an unexpectedly large amount of tetrahydroxylated bile acids, which were not present in wild-type mice, was observed. These results suggested that hydroxylation and an alternative canalicular transport mechanism for bile acids could compensate for the absence of Abcb11 and protect the mutant mice from severe cholestatic liver injury [43]. *Abcb11* KO mice fed with a CA supplemented diet displayed a more severe PFIC2 phenotype, indicating that with bile acid loading this compensatory transport was not sufficient [43]. Further analysis of the *Abcb11* KO mice showed that expression of Abcb1 (Mdr1) was markedly increased, especially after CA feeding, while Abcb4 (Mdr2), Abcc2 (Mrp2), and Abcc3 (Mrp3) were increased only to a moderate extent [44]. Moreover, plasma membrane vesicles isolated from a cell line overexpressing ABCB1 exhibited ATP-dependent bile salt transport, albeit with a 5-fold lower affinity compared to ABCB11. These findings suggested that, in mice Abcb1 may act as a compensatory bile acid transporter, and could explain the relatively mild phenotype of *Abcb11* KO mice [44]. In keeping with the more severe phenotype in humans, no upregulation of ABCB1 was found in PFIC2 patients [45]. In addition to its role in PFIC2, *Abcb11* has been mapped to the *Lith1* locus for gallstone susceptibility in mice [46]. Interestingly, the *Lith1* locus also harbors the nuclear receptor LXRα, which is associated with gallstone formation through the regulation of cholesterol transport by ABCG5/8. The role of Abcb11 in the formation of

gallstones was confirmed by the finding that gallstone-susceptible C57L/J mice (Lith1 mice) displayed increased levels of Abcb11 as compared to gallstone-resistant AKR/J mice [47,48] and further by the fact that transgenic mice overexpressing hepatic Abcb11 rapidly developed cholesterol gallstones [49]. FXR transcriptionally activates ABCB11 [50–52], and bile acids increase ABCB11 expression in primary hepatocytes or HepG2 cells with the same rank order of potency that activates FXR [23]. Conversely, the secondary bile acid LCA decreases ABCB11 expression by antagonizing FXR activation [10]. An important mechanism of drug-induced cholestasis is inhibition of ABCB11, with accumulation of bile acids in hepatocytes and subsequent liver injury. Examples of such drugs include cyclosporin A, rifampicin and glibenclamide. Reductions in ABCB11 have also been implicated in sepsis-induced cholestasis [53]. Recently, a liver receptor homologue-1 (LRH-1) response element (LRHRE) was identified in the human *ABCB11* promoter and overexpression of LRH-1 was shown to induce expression of ABCB11, suggesting that the NR LRH-1 supports FXR in the regulation of bile acid levels [54].

PFIC3 is characterized by reduced or absent phospholipid excretion into bile [28]. Phospholipids are an essential constituent of the bile and act to reduce the detergent activity of bile acid micelles, thereby protecting the membranes of cells lining the biliary tree from damage. In the absence of phospholipids, bile acid toxicity results in damage to cholangiocytes and progressive cholestatic liver injury accompanied by increased serum levels of GGT. PFIC3 is caused by mutations in the *ABCB4* gene (MDR2/3 P-glycoprotein). ABCB4 was originally isolated based on homology with ABCB1 [55,56]. However, unlike ABCB1, ABCB4 was not involved in conferring the MDR phenotype and its function remained unknown for several years until KO mice were generated [57]. *Abcb4* KO mice displayed progressive liver damage at an early age, which was accompanied by hyperbilirubinemia and increased liver enzymes in plasma. Further analysis revealed the absence of phospholipids and dramatically reduced levels of cholesterol and glutathione in bile, whereas bile flow itself was about 2-fold increased [57]. A link with a human disease was made when de Vree et al. showed that *ABCB4* is mutated in patients with PFIC3 [58]. Later it was established that ABCB4 functions as a phospholipid floppase, promoting the ATP-dependent transfer of phosphatidylcholine from the inner to the outer leaflet of the plasma membrane lipid bilayer [59]. In humans, ABCB4 is induced in cholestasis [21], and is regulated by FXR [60]. Trans-activation of *ABCB4* by FXR has been demonstrated through direct binding of FXR/RXRα heterodimers to a highly conserved inverted repeat element (FXR response element) in the distal promoter [60]. In rats, Abcb4 is induced by the FXR agonist GW4064 [61], but can still be induced in *FXR* KO mice fed a CA diet [19], suggesting that several bile acid-responsive regulatory mechanisms must be capable of inducing this gene.

3.2. SHP-mediated repression

FXR also downregulates many target genes indirectly via transcriptional induction of another NR, small heterodimer partner (SHP, NR0B2) [24,62]. SHP is an atypical member of the NR subfamily as it lacks a DNA-binding domain. SHP can interact with and negatively affect the transcriptional activation activity of several other members of the NR subfamily, including LXR, LRH-1, HNF4α as well as transcription factors belonging to the

basic-helix-loop-helix family [63,64]. It has been suggested that SHP represses nuclear hormone receptor-mediated target gene trans-activation via two mechanisms; competition with nuclear receptor coactivator proteins as well as its direct transcriptional repressor function [65,66]. In addition, SHP-mediated gene repression is associated with chromatin remodelling through recruitment of histone deacetylases (HDACs), the Swi/Snf-Brm complex and G9a methyltransferase to the CYP7A1 promoter [67,68].

FXR represses transcription of CYP7A1, encoding the rate-limiting enzyme in the classic (neutral) bile acid synthesis pathway, and CYP8B1, which is required for synthesis of CA [6]. CYP7A1 expression is controlled by a variety of factors, including hormones, oxysterols, bile acids, drugs and diurnal rhythms [69]. CYP7A1 is positively regulated by the orphan nuclear receptors Hepatocyte Nuclear Factor 4α (HNF4α) and Liver Receptor Homolog 1 (LRH-1). HNF4α is a key regulator of hepatic gene expression and a major activator of HNF1α, which in turn activates the expression of a large number of liver-specific genes, including those involved in glucose, cholesterol, and fatty acid metabolism [70–72]. LRH-1 is highly expressed in liver and is required for the hepatic expression of CYP7A1 and CYP8B1 [73,74]. In rodents, CYP7A1 is also positively regulated by LXR which binds to a direct repeat NR motif (DR-4) in the *Cyp7a1* promoter when activated by oxysterols, and strongly induces *Cyp7a1* transcription [75]. This regulation is not present in other species and explains why humans develop hypercholesterolemia on a diet high in cholesterol whereas in rodents cholesterol can be converted to bile acids by LXR-mediated stimulation of CYP7A1 transcription [76].

Bile acids repress CYP7A1 through FXR-induced expression of SHP, which in turn negatively interacts with several other members of the NR subfamily, including LXR, HNF4α and LRH-1 [22,58,77]. The importance of SHP in the feedback regulation of bile acid synthesis was demonstrated in *Shp* KO mice, which have increased Cyp7a1 and Cyp8b1 expression and activity and a corresponding increase in the bile acid pool size [78,79], and in SHP transgenic mice, which have reduced expression of Cyp7a1 and a smaller hepatic bile acid pool size [80]. In addition to blocking cholesterol catabolism, FXR also promotes lipid clearance through inducing genes involved in lipoprotein metabolism and inhibiting hepatic lipogenesis via SHP-mediated repression of SREBP-1c [7,81,82].

The FXR-SHP axis also negatively regulates bile acid uptake systems in the gut and liver, including the reabsorption of bile acids in the ileal enterocytes by ASBT (apical sodium-dependent BA transporter, *SLC10A2*) [83], and in the hepatocytes by NTCP (Na^+-taurocholate cotransporting polypeptide, *SLC10A1*) and OATP-C (*SLC21A6*) [25]. Additionally, FXR stimulates transcription of the ileal bile acid-binding protein (I-BABP) and the heterodimeric organic solute transporters OSTα/OSTβ, which are involved in trafficking bile acids across the enterocyte into the portal circulation [6,19,84] and it has also been implicated in the regulation of OATP8 (*SLC21A8*) in the liver [85].

3.3. Endocrine regulation of bile acid homeostasis

In addition to repression by SHP, an alternative SHP-independent pathway to repress CYP7A1, mediated by the c-Jun N-terminal kinase pathway (JNK), has been described [79,86] (Fig. 3). This pathway was linked to FXR activation by the finding that, in cultured

hepatocytes, FXR directly induced the transcription of the fibroblast growth factor 19 (FGF-19), a secreted growth factor that signals through the FGFR4 cell-surface receptor tyrosine kinase and subsequent activation of the intracellular JNK pathway [87]. *In vivo*, FGF15 (the murine ortholog of FGF19) however, was found to be primarily induced in the ileum after administration of the FXR agonist GW4064 to mice [88]. *FGF15* KO mice displayed increased hepatic CYP7A1 mRNA and protein levels and corresponding increases in CYP7A1 enzyme activity, suggesting that FGF15 functions as an enterohepatic signal to regulate bile acid homeostasis [88] (Fig. 3). A similar phenotype is observed in *FGFR4* KO mice [10]. It was further found that the FXR agonist GW4064 could significantly repress CYP7A1 in liver specific *FXR* KO mice but not in intestinal specific *FXR* KO mice, demonstrating that activation of FXR in intestine but not liver is required for short-term repression of CYP7A1 in liver [89]. The existence of alternative pathways to repress CYP7A1 also explains observed differences in feedback repression between CYP7A1 and CYP8B1. In comparison to CYP7A1, FXR-mediated repression of CYP8B1 was more dependent on the presence of FXR in liver (through SHP) and less dependent on its presence in intestine (FGF15). Consistent with these findings, recombinant FGF15 repressed CYP7A1 mRNA levels without affecting CYP8B1 expression. FXR-mediated repression of bile acid synthesis thus requires the complementary actions of FXR in both liver and intestine [89]. Alternatively, FGF19 can also repress CYP7A1 by increasing the stability of SHP through inhibiting its proteasomal degradation in a mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK-ERK) dependent manner [90] (Fig. 3). FGF15/19 has also been demonstrated to stimulate gallbladder filling, suggesting a postprandial feedback loop opposing the actions of cholecystokinin (CCK), which stimulates gallbladder emptying [91]. In addition to its roles in bile acid metabolism, FGF15/19 has been demonstrated to lower serum glucose and triglycerides in diabetic mice by activation of the MAPK-ERK pathway and subsequent induction of hepatic protein and glycogen synthesis [92–94].

4. PXR (pregnane X receptor)

PXR (NR1I2, also known as the steroid and xenobiotic receptor (SXR) or the pregnane-activated receptor (PAR)), is a promiscuous nuclear receptor that is activated by structurally unrelated xenobiotics, steroids, drugs and bile acids [95,96]. In response to a diverse array of compounds, PXR coordinately regulates a suite of genes involved in the metabolism, transport, and ultimately, elimination of these molecules. PXR is highly expressed in the liver, small intestine, and colon [95,96]. Notably, these are the same tissues where cytochrome P450 3A (*CYP3A*) genes are most highly expressed. In rodents, lower levels of PXR mRNA have also been detected in the kidney, stomach, lung, ovary, and placenta [96]. In humans, PXR mRNA has been detected in both normal and neoplastic breast tissue [97]. PXR is most closely related to CAR (NR1I3, Constitutive Androstane Receptor); the two receptors share ~70% amino acid identity in their LBDs, and they also have an overlapping target gene pattern [98,99]. PXR has promiscuous, often low-affinity, ligand specificity. Orthologous receptors from human, rat, mouse and rabbit have been cloned and characterized and share approximately 95% identity in their DNA binding domains. In contrast, they share only 75–80% identity in the amino acid sequences in their LBDs [100].

This results in species-specific variations in PXR ligand specificity, which has an impact on the activation of target genes (particularly CYP3As) by different xenobiotics. For example, rifampicin is a potent activator of rabbit and human PXRs [95] while pregnenolone 16α-carbonitrile (PCN) activates mouse and rat PXRs [101]. The species-specific nature of PXR ligand specificity was further illustrated using mice where mouse PXR was replaced with human PXR. These mice displayed a human PXR-mediated xenobiotic response profile and represent a unique tool for the exploration of the impact of xenobiotics, including therapeutic drugs, on hepatic and intestinal function [102,103].

The spectrum of PXR ligands is large and includes xenobiotics such as rifampicin, natural and synthetic steroids, such as pregnenolone, progesterone, phytoestrogens, dexamethasone, and antiglucocorticoids, as well as drugs and plant products, such as hyperforin in St. John's wort (Fig. 1). The crystal structure of the PXR ligand-binding domain has revealed several unique characteristics that account for its promiscuous ligand binding properties, including a large flexible, elliptical ligand binding pocket, and a relative lack of specific binding interactions, allowing PXR to bind ligands that are diverse in both their size and their structure [104,105].

5. Regulation of bile acid transport and metabolism by PXR

Although PXR was initially characterized as a xenosensor, the discovery that certain bile acids such as LCA can serve as ligands for both human and mouse PXR provided a link between PXR and bile acid regulation [106,107]. Below we will discuss the role of PXR in the detoxification of bile acids and the implications in cholestatic disorders (Fig. 4).

5.1. Activation of PXR by bile acids

A function for PXR in bile acid homeostasis was first suggested by the demonstration that it can be activated by the secondary bile acid LCA [106,107]. PXR was found to be directly activated by bile acids as low affinity ligands, with LCA and its major metabolite in mice, 3-keto LCA, both being efficacious activators of human and mouse PXR at concentrations between 10 and 100 μM. The rank order of potency (3-keto-LCA > LCA > DCA = CA) differs from that of FXR [106,107]. However neither conjugated LCA, nor any of the other conjugated bile acids activate PXR. In addition to direct activation by bile acids, PXR itself is a transcriptional target of bile acid-activated FXR [108] (Fig. 4).

5.2. Regulation of bile acid metabolism (phases I and II)

PXR can mitigate the harmful effects of toxic bile acids such as LCA by activation of two hepatic detoxification pathways, namely hydroxylation by members of the cytochrome P450 subfamily (phase I) and conjugation by glutathione *S*-transferases (GSTs), UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) (phase II) (Fig. 4). These reactions make bile acids more hydrophilic and facilitate their transporter-mediated elimination (phase III) through bile or urine. The potential importance of this function was supported by the observation that non-bile acid PXR activators, such as the steroid PCN, significantly decreased *CYP7A1* expression [106], with competition between PXR and

PGC-1α for binding to HNF4α, thereby blocking PGC-1α-stimulated activation of CYP7A1 by HNF4α [109].

It was proposed that PXR acts as a physiological sensor of LCA and its metabolites, inhibiting *Cyp7a1*, which blocks BA synthesis, inducing *Oatp2*, and presumably increasing uptake of LCA and other bile acids from sinusoidal blood into the hepatocyte where hydroxylation by Cyp3a11 or other Cyp3a subfamily members could take place. These more hydrophilic and less toxic hydroxylated bile acid metabolites could then be excreted in the urine or feces. However, as LCA is a secondary bile acid, formed in the intestine from bacterial 7-dehydroxylation of CDCA, it would not be expected to accumulate significantly in some forms of clinical cholestasis, such as biliary obstruction, where the enterohepatic circulation of bile acids is interrupted. Moreover, while bile duct ligation of mice markedly induced a human CYP3A4 reporter transgene, intraperitoneal injection of LCA had little effect on the reporter, despite causing hepatic necrosis [110]. Therefore the relevance of PXR as a pathophysiological sensor for LCA is uncertain in respect of CYP3A gene regulation.

5.3. Regulation of bile acid elimination (phase III)

Some toxic bile acids such as conjugates of LCA can also be eliminated by the canalicular ABC-transporter ABCC2 (previously known as MRP2 or canalicular multispecific organic anion transporter (cMOAT)). Deficiency in ABCC2 is associated with the Dubin–Johnson syndrome, a recessive disorder, characterized by conjugated hyperbilirubinemia [111]. ABCC2 can transport a variety of compounds including bilirubin diglucuronide, sulfates, some bile acids (*e.g.* conjugates of LCA), xenobiotics (*e.g.* cisplatin, anthracyclines, vinca alkaloids, methotrexate), as well as glutathione conjugates into bile, and is therefore a major determinant of bile acid-independent bile flow [112]. It has been suggested that bile acids can regulate ABCC2 expression, since CDCA, an FXR ligand, can induce the expression of *ABCC2* mRNA in human and rat hepatocytes [113]. An atypical promoter everted repeat element (ER-8) has been identified within the rat *Abcc2* promoter that is involved in the ligand-mediated induction of Abcc2 by FXR, PXR and CAR in cultured cells [114]. Subsequently, *in vivo* studies of mice with cholestasis induced by common bile duct ligation have found regulation of Abcc2 to be independent of FXR [115]. Induction of Abcc2 in the liver of WT but not *PXR* KO mice has been reported after administration of PCN or CA [116,117], implying a significant *in vivo* role for PXR in regulation of Abcc2.

PXR is also an important activator of the ABC-transporter ABCB1 (MDR1, P-glycoprotein). ABCB1 is best known for its role in multidrug resistance and is a key player in the defence of the body against xenotoxins [111]. The transcriptional regulation of ABCB1 is complex, and numerous transcription factors have been implicated in its regulation [118]. A large number of drugs have been identified as either substrates or inhibitors of ABCB1, and a range of exogenous stimuli can increase transcription of the *Abcb1* promoter [119]. Bile acids and their conjugated metabolites are not substrates for ABCB1; however certain substrates for ABCB1, including drugs, may cause drug induced cholestasis by interacting with bile acid homeostasis such as inhibition of ABCB11 [113]. *In vitro* studies have implicated rifampicin- and paclitaxel-mediated activation of PXR in the induction of

ABCB1 expression in human colon carcinoma cell lines, and induction of *Abcb1b* by a range of xenobiotics has also been shown to be dependent on PXR [99,120]. However, in a study involving administration of CA to mice, *Abcb1a* expression was induced independently of PXR and FXR [22], suggesting that induction by endobiotics differs from induction by xenobiotics. *In vivo*, *Abcb1a* and *Abcb1b* are induced by physiological concentrations of bile acids via FXR, independently of PXR [121], demonstrating the complexity of *in vivo* regulation of these transporters.

6. Implications for cholestatic liver disorders

Pharmacological therapy for cholestasis is limited, and ursodeoxycholic acid (UDCA) is the only disease-modifying drug therapy with evidence of efficacy, improving symptoms, hepatic enzyme abnormalities, and reducing death and liver transplantation in patients with primary biliary cirrhosis (PBC), when commenced sufficiently early in the course of the disease [122,123], and improving both maternal and fetal outcomes in cholestasis of pregnancy [124]. However, a majority of PBC patients are incomplete responders to UDCA [125], and UDCA has not been demonstrated to be efficacious in other forms of chronic cholestasis, such as primary sclerosing cholangitis (PSC). There is a pressing need for effective therapies for PSC, as no pharmacological agents have shown benefit in randomized controlled trials. Studies to date show that UDCA at the dose used to treat PBC (15 mg/kg/day) is ineffective, while high dose UDCA (30 mg/kg/day) is deleterious [126]. In addition, there are several potential problems with designing trials for novel therapies in PSC. Firstly, the course of the condition is frequently one of intermittent flares and remissions, making it difficult to separate out drug effects in short to medium term clinical trials. Secondly, dominant strictures of large bile ducts leading to significant biliary obstruction can occur, leading to a mixed picture of small and large duct cholestasis, so careful patient selection is crucial.

Thus, there is a need for novel therapies for treatment of cholestasis, both to delay progression of liver disease and relieve associated symptoms. The physiological response to cholestasis generally involves downregulation of the hepatocyte basolateral uptake transporters [21] and upregulation of the basolateral efflux transporters [127]. Interestingly, the apical transporter function is often preserved. Abcb11 expression is only modestly impaired, or preserved, both in animals with bile duct obstruction [115] and in humans with cholestasis [21,128]. ABCB4 and ABCB1 are both induced in humans with cholestasis [21], suggesting that bile acids that are specific ligands for FXR may help to maintain expression of these transporters during cholestatic injury.

FXR KO mice display an altered bile acid and lipid homeostasis [19,20], and an altered response to various animal models of cholestasis. High concentrations of dietary CA cause a marked increase in serum, liver and urine bile acid concentrations, and severe hepatotoxicity in *FXR* KO mice, associated with the loss of expression of Abcb11, and reduced biliary elimination of bile acids [19–22]. However in a BDL model of complete biliary obstruction, *FXR* KO mice had a mortality and morbidity advantage, and were protected from developing hepatic bile infarcts, even with concurrent deletion of PXR. This protection is probably secondary to downregulation of the FXR-regulated apical transporters ABCB11,

ABCB4 and ABCB1, reducing pressure in obstructed bile ducts, as well as other effects including upregulation of the sinusoidal ABC-transporter MRP4 (ABCC4) that mediates transport of bile acids back into the circulation [121,129]. Indeed, FXR was shown to repress expression of MRP4 through competition for binding to an overlapping binding site with CAR [130]. These findings suggest a role for targeted therapy for different cholestatic syndromes, and specifically, a clinical role for FXR antagonists in the treatment of obstructive cholestasis.

PXR-mediated regulation also has a marked impact on the development of hepatic damage in cholestasis as demonstrated in *PXR* KO mice subjected to various models of cholestasis and/or bile acid overload. *Pxr* KO mice display an increase in the areas of hepatic necrosis and bile infarcts after injection of LCA [106,107], or BDL [131] and increased sensitivity to lithogenic diet-induced cholesterol gallstone disease (CGD) [132]. Mechanisms for this include loss of PXR-mediated bile acid detoxification mechanisms, encompassing both metabolism and transport. Conversely, PXR activation by PCN or the herbal medicine St. John's wort protected WT mouse livers against LCA-induced necrosis and CGD [106,107,132]. When *PXR* KO mice were fed with LCA, they had elevated urinary concentrations of LCA compared with controls, associated with failure to induce hepatic Cyp3a11 and Oatp2 [106,107], and increased liver damage. In contrast, PXR activation by PCN protected WT mouse livers against necrosis caused by LCA. These findings suggest that PXR can function as a receptor for LCA or one of its metabolites, and regulate LCA detoxification *in vivo*.

7. Current and novel FXR and PXR based drug therapies

7.1. FXR agonists

FXR agonists would be expected to provide a positive therapeutic effect where cholestasis is present in the absence of obstruction of large bile ducts. As covered in the preceding section, FXR-mediated up-regulation of apical hepatocyte bile acid transporters and increased bile production is counterproductive when bile cannot be delivered to the intestine. Recently, a potent selective FXR agonist has entered clinical phase II trials for PBC. 6-ethyl CDCA (6-ECDCA) is a modified bile acid with an EC_{50} for FXR activation of 99 nM [133] (Fig. 1). When 6-ECDCA was administered as an additional agent for 12 weeks to patients with PBC with an incomplete biochemical response to UDCA, significant improvements in serum alkaline phosphatase and GGT were observed [134]. At higher doses 6-ECDCA caused dose-limiting pruritus, consistent with it being a substituted bile acid, so future studies of small molecule non bile acid-based FXR agonists will be interesting. The efficacy of 6-ECDCA as monotherapy for PBC and its place in long-term management of this chronic condition remains to be explored and phase III trials designed to address these questions should commence soon.

7.2. FXR antagonists

As covered earlier in this review, FXR antagonists may have therapeutic value for attenuating liver injury where there is segmental or total biliary obstruction. While the focus of medical management should be aimed at alleviating the obstruction by surgery or biliary

stenting, there may be an adjunctive role for a pharmacological therapy that diverts bile acids to the circulation for elimination via the kidney, as has been demonstrated to occur in BDL *Fxr* KO mice [121,129]. The natural compound guggulsterone (Fig. 1) has been identified as an FXR antagonist but its propensity to modulate other NRs (covered in Section 2) is a limiting factor for this molecule as a starting point for drug development. More selective natural [135] and synthetic [136] FXR antagonists have been reported but there have been no published *in vivo* studies to date.

7.3. PXR agonists

Given the pivotal role of PXR in the regulation of therapeutic drug metabolism and its propensity to mediate drug–drug interactions, it is not surprising that PXR has been largely neglected as a drug development target for cholestatic liver disorders. Long before the discovery of PXR, the macrocyclic antibiotic rifampicin, now recognized to be a human PXR agonist (Fig. 1), had been used to treat the intractable pruritus associated with severe cholestasis [137]. However, long-term therapy with rifampicin is not always well tolerated and drug-related hepatitis has been reported [138], a complication that is undesirable in a patient with severe existing liver disease.

The herbal remedy St. John's wort is a widely used alternative therapy for anxiety and depression. It contains the high affinity PXR agonist hyperforin [139] (Fig. 1), which explains the propensity of St. John's wort to cause herb–drug interactions. In a pilot study of St. John's wort administered for 20 weeks to patients with PBC already receiving UDCA we found that this agent was well-tolerated and improved pruritus (C. Stedman, S. Coulter, C. Liddle; unpublished observations). Thus, while PXR agonists are unlikely to be used as first line treatment for cholestatic liver disorders, their role as an adjunctive therapy is worthy of further study.

8. Future perspectives

Cholestatic liver disorders cover a wide spectrum of diseases of diverse etiologies. However, they all share the consequences of retention of bile constituents, especially bile acids. As covered in this review, the nuclear receptors FXR and PXR are activated by bile acids and in turn regulate aspects of the enterohepatic cycling and metabolism of bile acids. It follows that any disease process in which either of these factors is important could potentially benefit from pharmacological manipulation of these nuclear receptors. As covered above, FXR agonists inhibit bile acid synthesis and promote bile acid excretion while PXR agonists promote bile acid detoxification, predominantly by the induction of CYP enzymes that mediate bile acid hydroxylation. Manipulation of both FXR and PXR, either by administration of pharmacological ligands or genetic abrogation, have been shown to influence cholestatic liver injury in animal models to the point of significantly impacting on liver injury and survival after complete bile duct ligation or other models of bile acid accumulation, such as bile acid feeding. Research priorities now include the development of additional pharmacologic tools, such as non-bile acid FXR agonists and FXR antagonists, for the manipulation of these receptors and exploration of their effects in a more diverse range of *in vivo* models, particularly in view of the observed differences between rodents and man in some aspects of transporter regulation. High affinity, non-bile acid FXR ligands

such as GW6064 [11] and fexaramine [12] (Fig. 1) have been developed, but both compounds suffer from poor pharmacokinetic profiles, especially poor oral bioavailability. However, these molecules demonstrate the feasibility of this approach and compounds with suitable characteristics for human clinical trials are expected in the near future. Another related path of drug discovery could center on ligands for FGFR4, the receptor for FGF19, which also suppresses bile acid production through repression of CYP7A1 (Fig. 3), and could potentially be administered in combination with FXR agonists. This approach is worthy of exploration in animal models.

Early phase human studies of existing bile acid-derived FXR agonists are in progress in PBC and clinical trials in PSC, the PFIC syndromes and PFIC-associated syndromes, such as BRIC and BRIC2, are likely to follow. Therapeutic targeting of PXR is likely to remain as a second line therapy, given the profound effects of PXR activation on the disposition of co-administered drugs. Still, in an area of therapeutics where choices are either limited or non-existent, further evaluation of selective, well-tolerated, PXR agonists is worthwhile.

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Abbreviations

BDL	bile duct ligation
CA	cholic acid
CDCA	chenodeoxycholic acid
DCA	deoxycholic acid
FXR	Farnesoid X Receptor
LCA	lithocholic acid
LXR	liver X receptor
NR	nuclear receptor
PBC	primary biliary cirrhosis
PCN	pregnenolone 16α-carbonitrile
PFIC	progressive familial intrahepatic cholestasis
PSC	primary sclerosing cholangitis
PXR	pregnane X receptor
UDCA	ursodeoxycholic acid

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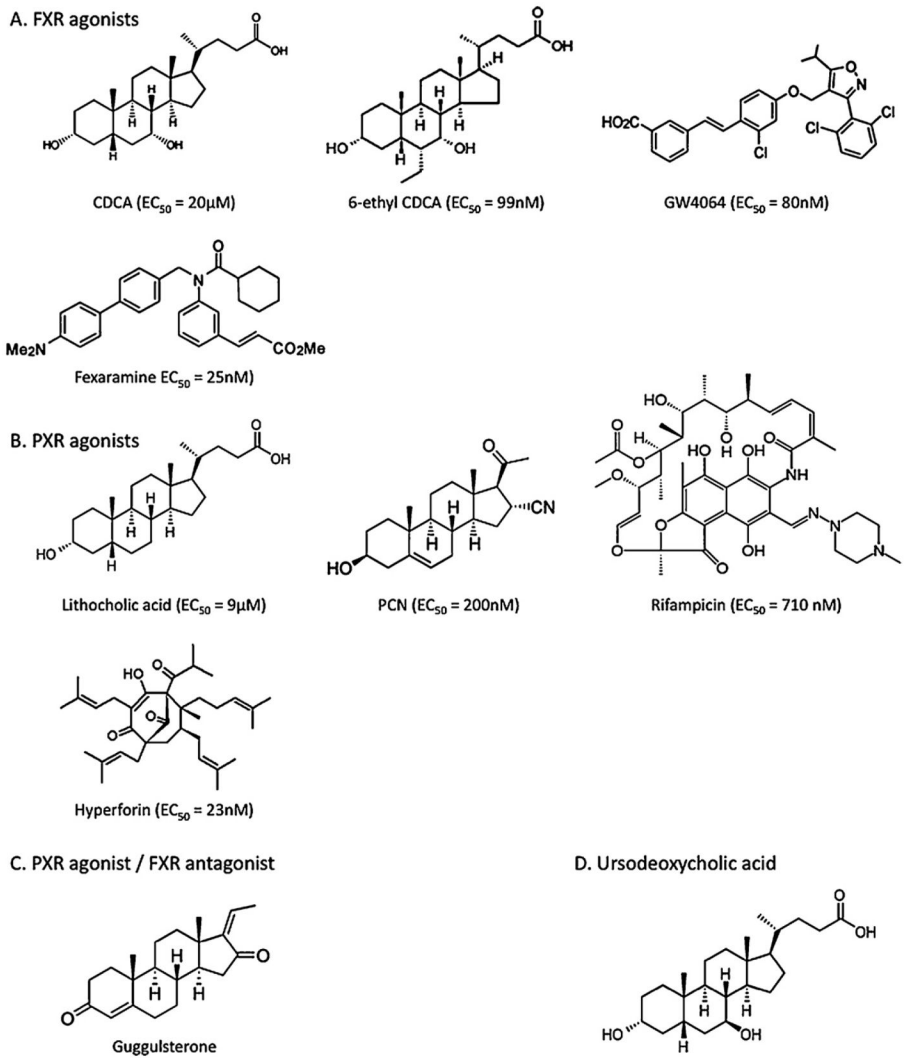

Fig. 1.

Structures of ligands that interact with FXR and/or PXR. Compounds that are agonists for FXR or PXR are shown in panels (A) and (B), respectively. The effective agonist concentration that elicits 50% of maximum activation of the respective human receptor (EC_{50}) is provided, with the exception of PCN, a selective mouse PXR ligand, where the EC_{50} for mouse PXR is shown. Guggulsterone, a promiscuous nuclear receptor ligand that acts as both an FXR antagonist and a PXR agonist, is shown in (C). Ursodeoxycholic acid, a naturally occurring epimer of chenodeoxycholic acid that is used in the therapy of some cholestatic liver disorders, but is not thought to significantly interact with nuclear receptors, is shown for comparative purposes in (D). CDCA, chenodeoxycholic acid; PCN, pregnenolone 16α-carbonitrile.

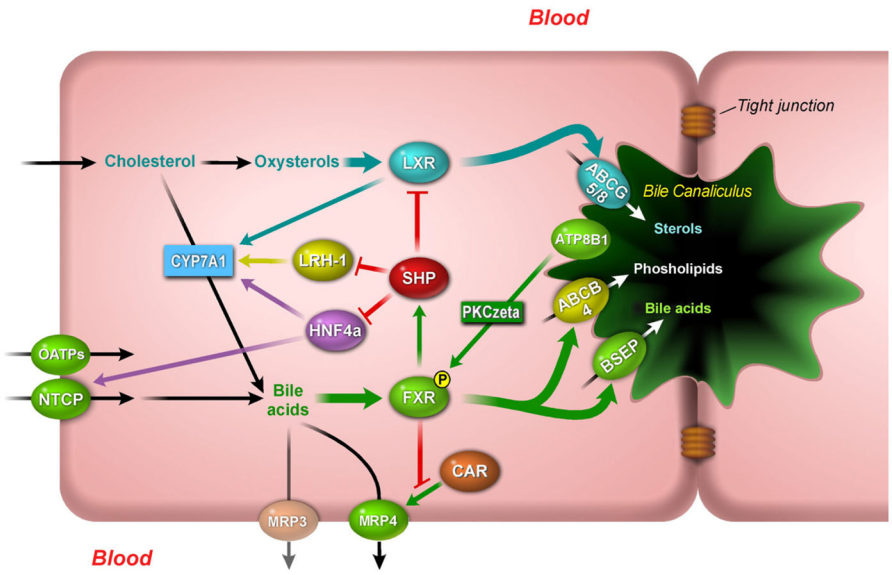

Fig. 2.

FXR-mediated bile acid transport and metabolism in the hepatocyte. Hepatic uptake of bile acids from the circulation takes place at the sinusoidal (basolateral) membrane of the hepatocyte and is mediated by NTCP and several members of the OATP family. Within the hepatocyte, bile acids can be converted to cholesterol or eliminated via bile. In addition, cholesterol can be converted to bile acids by CYP7A1. Bile acids can activate FXR which in turn induces the expression of the bile acid transporter ABCB11 (BSEP) and the phospholipid transporter ABCB4 (MDR2/3). Bile acid secretion is also stimulated by the phospholipid flippase ATP8B1. Loss of ATP8B1 results in reduced activity and nuclear translocation of FXR which is caused by impaired protein kinase C zeta (PKCzeta)-mediated phosphorylation of FXR. Negative feedback on bile acid metabolism and secretion is mediated by SHP, which is induced by FXR and inhibits the action of several NRs including LXR, HNF4α and LRH-1. Via an alternative pathway, cholesterol is converted to oxysterols that can activate LXR which in turn induces the expression of the sterol transporters ABCG5/8 at the canalicular membrane. During cholestasis bile acids can also be excreted back into the circulation via the sinusoidal ABC-transporters MRP3 and 4. MRP4 in turn is repressed by FXR through competition for binding to an overlapping binding site with CAR.

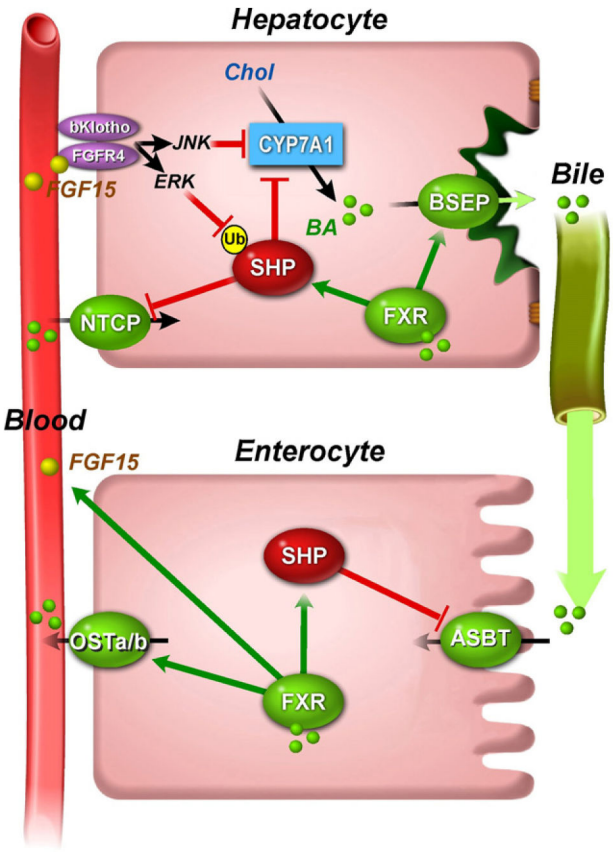

Fig. 3.

Regulation of enterohepatic circulation of bile acids by FXR. FXR regulates the enterohepatic circulation of bile acids in the hepatocyte and enterocyte. After a meal, bile is released from the gallbladder into the duodenum. Bile acid re-uptake in the ileum is mediated by ASBT in the brush border membrane. In the enterocyte, FXR induces the expression of OSTα/β, which mediate bile acid transport from the enterocyte into the portal circulation to hepatocytes where they are taken up via NTCP. In the hepatocyte, FXR induces the expression of BSEP, which mediates bile acid excretion into bile. Negative feedback on the enterohepatic circulation of bile acid is mediated by SHP, which is induced by FXR and inhibits CYP7A1 and NTCP in the hepatocyte, and ASBT in the enterocyte. Alternatively, in the endocrine pathway, FXR in the enterocyte induces the expression and secretion of FGF15/19, which binds in the liver to the FGFR4/βKlotho receptor complex and in turn activates JNK and ERK signaling pathways. Activation of the JNK and ERK pathways results in repression of CYP7A1 and stabilization of SHP by inhibition of its proteasomal degradation, respectively.

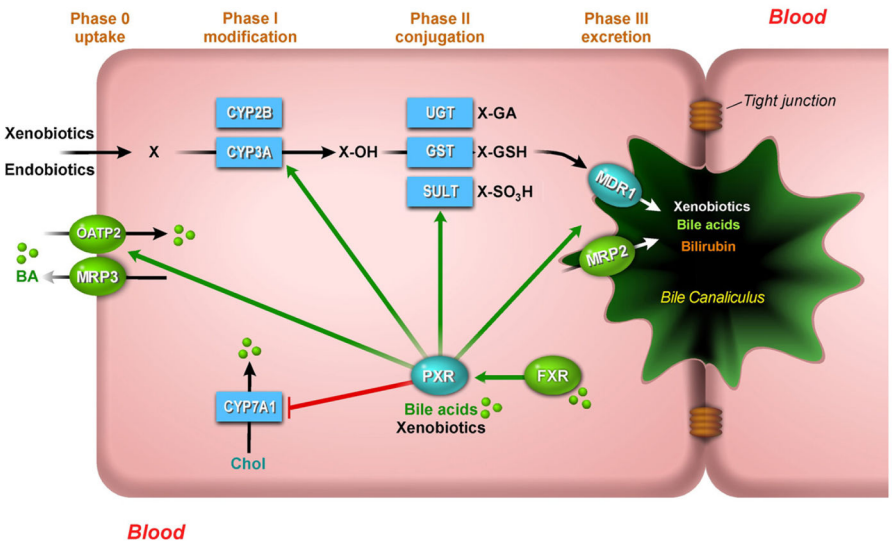

Fig. 4.

PXR-mediated bile acid transport and metabolism in the hepatocyte. PXR can mitigate the harmful effects of toxic bile acids (BA) such as LCA by activation of hepatic detoxification pathways. Activation of PXR induces the uptake of xeno- and endobiotics (phase 0), their modification by members of the cytochrome P450 subfamily (phase I), conjugation by glutathione *S*-transferases (GSTs), UDP-glucuronosyl-transferases (UGTs) and sulfotransferases (SULTs) (phase II) and elimination (phase III) by MRP2 (excretion of bilirubin and some bile acids), and the multidrug transporter MDR1 (excretion of a wide variety of xenobiotics and endobiotics). PXR can be directly activated by certain bile acids or indirectly via transcriptional regulation by FXR. Negative feedback on bile acid metabolism is mediated by inhibition of CYP7A1. During cholestasis bile acids can also be excreted back into the circulation via the sinusoidal ABC-transporters MRP3 and 4.