Nuclear Receptors CAR and PXR; therapeutic targets for cholestatic liver disease

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1. ABSTRACT

Cholestasis results in the intrahepatic retention of cytotoxic bile acids (BA) and it can thus lead to liver injury. Hydrophilic BA ursodeoxycholic acid (UDCA) is currently used to treat cholestasis but its efficacy is limited. Nuclear receptors are key regulators of various processes including metabolism of xenobiotics and endobiotics such as BA and drugs. Recent studies have made significant progress in elucidating the mechanisms which regulate the BA metabolism by nuclear receptors. The nuclear receptor FXR plays the role of master regulator of BA homeostasis and is a promising drug target for cholestatic liver disease. In addition to FXR, the nuclear receptors CAR and PXR function as sensors of toxic byproducts and regulator of BA homeostasis. Ligands for both receptors including phenobarbital have been used to treat cholestatic liver diseases before the mechanisms of these receptors were revealed. Novel compounds targeting CAR and PXR with specific effects and fewer side effects will therefore be useful for the treatment of cholestatic liver diseases. This article will review the current knowledge of the xenobiotic-sensing nuclear receptors CAR and PXR, while also discussing their potential role in the treatment of cholestatic liver diseases.

2. INTRODUCTION

Cholestasis is defined as an impairment of bile secretion and flow followed by a lack of bile in the intestine and accumulation of potentially toxic bile acids (BA) in the liver and the systemic circulation (1-3). Cholestasis results in the intrahepatic retention of cytotoxic BA which can thus lead to liver injury and/or liver fibrosis (1-3). It can be grossly classified into two categories: 1) a functional defect in bile formation and/or secretion at the hepatocyte level, 2) an impaired bile secretion and flow at the bile duct level (1-3). Examples of functional defects in bile formation at the level of hepatocytes are various hereditary transporter defects, exposure to procholestatic drugs and hormones as well as inflammation (1-3). An impaired bile secretion and flow at the ductular/ductal level can be hampered by obstruction and also by either inflammatory processes or the loss of bile ducts (3).

Cholestasis is various associated with clinical features including pruritus, malabsorption, vitamin deficiencies with subsequent coagulation disorders and bone disease (1-3). Persistent cholestasis can cause biliary fibrosis to progress to liver cirrhosis and end-stage liver disease. Hydrophilic BA ursodeoxycholic acid is currently used to...
treat cholestasis but its efficacy is limited. In the human body, cholestasis is counteracted by a variety of intrinsic hepatoprotective mechanisms, including a complex network of drug metabolizing enzymes and transporters (4, 5). Nuclear receptors (NR) regulate the expression of these drug metabolizing enzymes and transporters involved in BA synthesis and detoxification. Recent studies have made significant progress in dissecting the mechanisms in regulating the hepatic xenobiotic and endobiotic metabolism by nuclear receptors (6-9).

Farnesoid X receptor (FXR) plays the role of master regulator of BA homeostasis by modulating BA synthesis, conjugation, secretion and absorption (10). FXR was the first nuclear bile acid receptor identified and it was originally named after its activation by farnesol (11, 12). Physiologic ligands for FXR are the primary BAs, chenodeoxycholic acid (CDCA), cholic acid (CA) as well as the secondary BAs, deoxycholic acid (DCA) and lithocholic acid (LCA) (13, 14). FXR might therefore represent the most promising drug target in cholestatic liver diseases because of its central role in the regulation of BA homeostasis. Indeed, FXR ligands including 6-ethylchenodeoxycholic acid (6-ECDCA; INT-747) are currently being investigated for the treatment of human cholestatic diseases in phase II studies for patients with primary biliary cirrhosis (PBC) (15).

In addition to FXR, the NR1I subfamily members, the constitutive active/androstane receptor (CAR) and the pregnane X receptor (PXR) function as sensors of toxic byproducts and regulators of BA homeostasis (6-9). They regulate numerous genes which are involved in drug and xenobiotic metabolisms, including phase I and II drug metabolizing enzymes, and transporters (6-9). Although these receptors were first identified as xenobiotic sensors, recent studies have revealed these receptors to play many critical roles in the lipid metabolism, glucose homeostasis, and inflammation (8, 16, 17). The ligands for both receptors including phenobarbital (PB) and rifampicin have already been used to treat cholestatic liver diseases before the mechanisms of these receptors were revealed (4). The UDP-glucuronosyltransferase, UGT1A1, the critical enzyme responsible for the detoxification of bilirubin, is induced by PB via regulation by CAR and/or PXR (6-9). However, PB also induces side effects, such as somnolence, skin eruption and intoxication, and furthermore, their effects in the treatment of cholestatic liver diseases are still insufficient. Therefore, novel compounds targeting CAR and PXR with specific effects, while inducing fewer side effects, are thus expected to be useful for the treatment of cholestatic liver diseases. This article reviews the current knowledge on the ability of xenobiotic-sensing nuclear receptors CAR and PXR to regulate BA homeostasis, while describing their potential role in the treatment of cholestatic liver diseases.

3. THE OVERALL VIEW OF THE NUCLEAR RECEPTORS: CAR AND PXR

CAR and PXR are master regulators of phases I/II detoxification and transporters, and regulate numerous hepatic genes in response to a large group of xenobiotics
and endobiotics (6-9). CAR and PXR belong with vitamin D receptor (VDR) to the NR1I subfamily and they are expressed primarily in the liver (18). The ligands and target genes of NR1I subfamily including VDR are shown in Table 1. These two receptors share some common ligands and regulate an overlapping set of target genes. Both CAR and PXR form heterodimers with retinoid X receptor (RXR; Figure 1) and bind to the enhancer region of target genes (19, 20).

3.1. CAR (Constitutive Active/androstane Receptor, NR1I3)

The constitutive active/androstane receptor (CAR, NR1I3) is an approximately 358-amino acid, 40-kDa protein, primarily expressed in the liver and intestine (18). Unlike ligand-activated classical nuclear receptors, CAR is constitutively active (18). Phenobarbital and 1,4-bis (2-(3,5-dicholoropyriyloxy))benzene (TCPOBOP) are the best known activators of CAR (6-9, 21-23) (Table 1). Conversely, androstanol and androstenol are inverse agonists that repress the constitutive activity of CAR (23). CAR regulates numerous genes associated with drug detoxification, BA detoxification and BA transport pathways (Table 1) (6-9).

The PB induction of CYP2B causes CAR to bind to PB responsive enhancer modules (PBREM) in human, mouse, and rat CYP2B gene promoters (24). A general schematic drawing of the CAR pathway and its components is shown in Figure 1. PB-induced, CAR-mediated induction of the CYP2B genes is the activation of cytosolic CAR upon PB exposure, thus resulting in its dissociation from its co-chaperone partners, cytoplasmic CAR retention protein (CRP) and heat shock protein (HSP) 90 (25, 26). The translocation of CAR to the nucleus, which is presumably dependent on the activity of the protein phosphatase PP2A, is followed by an association with the RXR-α and binding to the PBREM (25, 26). Transcriptional activation occurs upon CAR binding to the PBREM, which contains two nuclear receptor DR4 sites (NR1 and NR2). Steroid co-activator 1 (SRC-1), and specificity protein 1 (Sp1) are examples of coactivators of CAR (18), whereas nuclear receptor corepressor protein (NCoR) is an example of a corepressor of CAR (27). The major target genes of CAR are shown in Table 1 including cytochromes P450 2B (CYP2B), CYP3A, sulfotransferase 2A1 (SULT2A1), UDP-glucuronosyltransferase 1A1 (UGT1A1), and multidrug-resistance-associated protein 2 (MRP2).

3.2. PXR (Pregnane X Receptor, NR1I2)

The pregnane X receptor (PXR, NR1I2) is an approximately 434-amino acid, 50-kDa protein, primarily expressed in the liver and intestine (28). Unlike CAR, PXR does not show any significant constitutive activity and it must be activated by cognate ligands to elicit its effects (28). The best known agonist ligands of PXR (Table 1) include many pharmaceutical drugs, including rifampicin in humans and pregnenolone 16α-carbonitrile (PCN) in rodents (28-30). Its role in the induction of phases I and II enzymes and transporters was elucidated concurrently with CAR. The features of PXR signaling

Table 1. Nuclear receptor II subfamily in cholestasis and drug detoxification

<table>
<thead>
<tr>
<th>NR</th>
<th>Ligands</th>
<th>Target genes</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR (NR1I3)</td>
<td>Phenobarbital, TCPOBOP, Yin Chin, Dimethylesculetin, Bilirubin, CITCO, androstanol/androstenol</td>
<td>CYP3A4, Sult2a1, UGT1A1</td>
<td>BA detoxification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRP2/Mrp2, Mrp3, MRP4/Mrp4</td>
<td>BA transport</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP2A6, CYP2B6, CYP2B1, Cyp2b2, Cyp2b10, CYP2C9, CYP2C19, Cyp2c29, CYP3A4, Sult1a1, Sult2a1, Sult2a9, UGT1A1</td>
<td>Drug detoxification</td>
</tr>
<tr>
<td>PXR (NR1I2)</td>
<td>Rifampicin (human), Dexamethasone, St. John's wort, Clotrimazole, LCA</td>
<td>CYP7A1</td>
<td>BA synthesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP3A4, SULT2A1/Sult2a1, UGT1A1, UGT1A3</td>
<td>BA detoxification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRP2/Mrp2, Mrp3, Oatp1a4, MDR1</td>
<td>BA transport</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP1A2, CYP2B6, Cyp2b10, CYP2C9, CYP2C19, CYP3A4, CYP3A7, Cyp3a11, Cyp3a13, Cyp3a23, UGT1A1, GSTs</td>
<td>Drug detoxification</td>
</tr>
<tr>
<td>VDR (NR1I1)</td>
<td>1α,25-dihydroxyvitamin, LCA</td>
<td>CYP3A4/Cyp3a11, SULT2A1/Sult2a1</td>
<td>BA detoxification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mrp3, ASBT</td>
<td>BA transport</td>
</tr>
</tbody>
</table>

of bile acid and bilirubin detoxification systems |

Induction of BA canalicular secretion; induction of alternative basolateral BA excretion. Minor increase in bile flow |

Induction of BA canalicular secretion; induction of alternative basolateral BA excretion |

Induction of bile acid and bilirubin detoxification systems |

Induction of bile acid and bilirubin detoxification systems |

Induction of alternative basolateral bile acid excretion and induction of biliary and intestinal bile acid reabsorption
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TCPOBOP, 1,4 bis[2-(3,5-dichloropyridyloxy)]benzene; PCN, pregnenolone 16α-carbonitrile; LCA, lithocholic acid; CITCO, 6-(4-chlorophenyl) imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl) oxime; BA, bile aid

(Figure 1) are similar to those for CAR (30, 31), particularly when comparing the mouse forms of the receptors. However, in contrast to CAR which is primarily found in cytosol (25, 26), the localization of PXR varies among species (32, 33). In humans, PXR is primarily found in the nucleus and inactive PXR may form repressor complexes and thereby inhibit transcription (32-34). Ligand activation releases co-repressors and initiates the recruitment of co-activators and promotes transcription (34). PXR shares many target genes with CAR including CYP3A4, CYP2B6, SULT2A1, UGT1A1 and MRP2 (35). PXR has also been established as a xenosensor and master regulator of xenobiotic responses. The extent of PXR-mediated gene transcription increases by coactivators, such as SRC-1, and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), while it decreases by corepressors, such as NCoR, sterol regulatory element binding protein 1 (SREBP-1), and the silencing mediator of retinoid and thyroid hormone receptors (SMRT) (36).

4. BILE FORMATION AND CHOLESTASIS

4.1. Bile formation

Bile acids are synthesized from cholesterol by a complex pathway (37) and the cholesterol 7-α-hydroxylase (CYP7A1) initiates the first, rate limiting step in bile salt formation biosynthesis (38). Cholic acid (CA) and chenodeoxycholic acid (CDCA) are produced in equal amounts in the classical BA synthesis pathway (38). Sterol 12-α-hydroxylase (CYP8B1) controls the ratio of CA to CDCA in this pathway (38). The alternative pathway is initiated by sterol 27-hydroxylase (CYP27A1), thus leading to the production of CDCA (39). The driving force for hepatocellular bile formation is the active transport of BAs from sinusoidal blood into the canaliculus. Specific transport proteins are localized in the basolateral (sinusoidal) and canalicular (apical) membrane of hepatocytes and cholangiocytes (2).

Bile is primarily formed by the canalicular excretion of BAs and non-bile acid organic anions via ATP-binding cassette (ABC) transporters (1-3). The osmotically active compounds induce the passive movement of water into the canaliculus, and being bile acids are the major osmotic driving force in the generation of bile flow. Bile acids undergo enterohepatic circulation and are reabsorbed in the terminal ileum and then return to the liver via the portal blood (Figure 2). Bile acids that escape first pass clearance by the liver or are actively excreted by hepatocytes into sinusoidal blood are filtered in the glomerulus from plasma into urine (40). Thereafter, bile acids are actively reabsorbed at the proximal renal tubular cells and then in turn are excreted into the systemic circulation (41). Bile acid homeostasis is the result of a tight balance between BA uptake, efflux and biosynthesis. Maintenance of this balance is essential, since most BAs are cytotoxic and can cause liver injury.
Figure 1. Schematic illustration of the signaling pathway for CAR and PXR. PB-induced, CAR-mediated induction of the target genes is the activation of cytosolic CAR upon PB exposure, thus resulting in its dissociation from its co-chaperone partners, CCRP and HSP90. The translocation of CAR to the nucleus, which is presumably dependent on the activity of the protein phosphatase PP2A, is followed by an association with the RXR and binding to the PBREM. Transcriptional activation occurs upon CAR binding to the PBREM, which contains two nuclear receptor DR4 sites (NR1 and NR2). The features of PXR signaling are similar to those for CAR, particularly when comparing the mouse forms of the receptors. PXR shares many target genes with CAR including CYP3A4, CYP2B6, SULT2A1, UGT1A1 and MRP2. PXR is localized in the cytoplasm in a complex with HSP90 and CCRP. The resultant ligand-bound PXR translocates to the nucleus where it forms a heterodimer with RXR. The ligand–PXR–RXR complex binds to DNA response elements of a PXR target gene, thus resulting in increased gene transcription. CAR; constitutive active/androstane receptor, PXR; pregnane X receptor, RXR; retinoid X receptor, CCRP; cytoplasmic CAR retention protein, HSP90; heat shock protein 90, PP2A; protein phosphatase 2A, PBREM; phenobarbital-responsive enhancer module, XREM; xenobiotic responsive enhancer module.

4.2. Cholestasis
Cholestasis is defined by an impaired of bile secretion with the failure of bile to reach the duodenum in adequate amounts (42). Cholestasis may either result from a functional defect in bile formation at the level of the hepatocyte or from impaired bile secretion and flow at the bile duct level. Examples of functional defects in bile formation at the level of hepatocytes include various hereditary transporter defects (e.g., progressive familial intrahepatic cholestasis), exposure to procholestatic drugs and hormones (e.g., cyclosporine, 17β-estradiol glucuronide) as well as inflammation (e.g., sepsis) (1). The bile flow at the ductular/ductal level can be hampered by obstruction (e.g., stones, tumors) and by inflammatory processes or the loss of bile ducts (e.g., vanishing bile duct syndromes as primary biliary cirrhosis and primary sclerosing cholangitis) (3).

4.3. Drug metabolizing enzymes and transporters in bile homeostasis
The hepatic phase I and II detoxification enzymes, and hepatobiliary transporters related to BA clearance are summarized in Figure 2. The BA uptake is mediated via basolateral Na+/taurocholate cotransporter (NTCP) and organic anion transporter (OATP) (43). Bile acids are able to induce a negative feedback regulating their hepatic uptake in order to maintain BA concentrations at constant, non-toxic levels within hepatocytes. The CYP7A1 initiates the first, rate limiting step in bile salt formation biosynthesis (38). CYP8B1 controls the ratio of CA to CDCA in the classical BA synthesis pathway (38). The alternative pathway is initiated by CYP27A1, thus leading to the production of CDCA (39).

Phase I (hydroxylation) and phase II reactions (conjugation of BAs with sulfate or glucuronidate) render BA more hydrophilic, less toxic and more amenable for urinary excretion. The hydroxylation of BAs is mediated by CYP3A and potentially CYP2B (44, 45). The phase II conjugation is mediated by sulfotransferase 2A1 (SULT2A1), UDP-glucuronosyltransferases (UGT) 1A, UGT2B4 and UGT2B7 (43, 46-48).

Canalicular BA export is mediated by the bile salt export pump (BSEP) and to a lesser extent by MRP2 (43). Multidrug resistance protein (MDR) acts as a
CAR and PXR in cholestatic liver disease

Figure 2. Bile acids metabolizing enzymes and hepatobiliary transport systems in the liver, kidney and intestine. Bile acids are taken up by the NTCP and OATPs at the basolateral membrane of hepatocytes and are exported into bile by the canalicular bile salt export pump (BSEP) and the canalicular conjugate export pump (MRP2). MRP2 also mediates excretion of various anions such as bilirubin into the canaliculus. MRP3, MRP4 and OSTα/β, at the basolateral membrane of hepatocytes provide an alternative excretion route for BA and other anions into the systemic circulation. Bile acids secreted into bile are reabsorbed in the terminal ileum via ASBT and effluxed by OSTα/β. Bile acids are reabsorbed in the kidney by apical ASBT in proximal renal tubuli to minimize urinary bile acid loss under normal conditions. MRP2 and MRP4 are also expressed in the kidney on the apical membrane and might actively excrete bile acids into the proximal tubuli under cholestatic conditions. NTCP; Sodium-taurocholate cotransporting polypeptide, OATP; organic anion-transporting polypeptide, CYP; cytochrome P450, UGT; UDP-glucuronosyltransferase, SULT; sulfotransferase, MRP; multidrug-resistance-associated protein, BSEP; bile salt export pump, ABCG; ATP-binding cassette subfamily G, MDR; multidrug resistance protein, OST; organic solute transporter, ASBT; apical sodium-dependent bile salt transporter.

canalicular phospholipid flippase, while the two-half transporters AGCG5/8 are responsible for canalicular cholesterol export (43). Retrograde BA secretion via the basolateral membrane represents an alternative elimination route to reduce the accumulation of toxic BAs within hepatocytes when orthograde biliary BA output is either blocked or reduced during cholestasis. The key transport systems mediating this alternative excretory pathway include the heteromeric organic solute transporter (OST) α/β, MRP3 and MRP4 (43).

5. CAR AND PXR IN THE BILE ACID HOMEOSTASIS

5.1. CAR and PXR in the bile acid homeostasis

Limiting hepatic BA synthesis during cholestasis is thought to be a protective mechanism to reduce hepatocellular BA overload. In vitro studies suggest that activation of PXR inhibits CYP7A1 gene transcription by reducing the interaction of PGC-1α (peroxisome proliferative activated receptor gamma coactivator 1α) with HNF4α (Hepatocyte nuclear factor 4α) (49). As a result, PXR is thus considered to have a protective effect on cholestatic liver disease by inhibiting BA synthesis (Figure 3).

The hydroxylation of BAs is mediated by members of CYP3A and potentially by those of CYP2B (44, 45). Both PXR and CAR are key regulators of CYP3A expression and toxic BAs including LCA can induce CYP3A expression (50-54). Transgenic mice expressing a human PXR are protected from BA toxicity with LCA (50, 54). As a result, PXR senses toxic bile acid, LCA, while eliminating toxic BAs by up-regulating its metabolism as a xenobiotic sensing receptor. The PXR response elements of the CYP3A promoter also mediate the transactivation of
The role of CAR and PXR in the hepatic defense mechanism against cholestasis. CAR and PXR agonists do not affect major basolateral uptake (NTCP, OATP) and canalicular export (BSEP) systems for bile acid. PXR reduce the expression of CYP7A1, the rate limiting enzyme in bile synthesis. CAR stimulate bile acid/bilirubin phase I hydroxylation (CYP3A, CYP2B), phase II conjugation (UGT1A1, SULT1A1), and coordinated alternative phase III export for bile acids (MRP3, Mrp4, potentially OATP2) and bilirubin (MRP2). PXR agonists stimulate phase I/II enzymes (CYP3A, CYP2B, UGT1A1, SULT2A1) and phase III transport systems (MRP3, OATP2).

CYP3A by CAR (30). The CAR response elements of the CYP2B promoter are also shared by PXR (7).

Phase II conjugation reactions of BAs with sulphate or glucuronidate are catalyzed by SULT2A1 and by the UGTs, respectively (47, 55). SULT2A1 is regulated by not only CAR but also FXR and VDR (56-60). However, CAR appears to be a central regulator of BA sulfation since CAR transgenic mice are resistant to LCA toxicity due to increased LCA sulfation (61). FXR and peroxisome proliferator-activated receptor (PPAR) α can activate UGT2B4 transcription (62, 63). PPARα and liver X receptor (LXR) α induce the UGT1A3-dependent glucuronidation of BA, and PXR and CAR also mediate the induction of UGT1A enzymes (4). The regulation of phase II glutathione S-transferases (GST) depends on PXR, CAR and glucocorticoid receptor (GR) (64, 65). Taken together, PXR and CAR are master regulators of the detoxification pathways, but other NRs also play a role in orchestrating a coordinated response to toxic BAs.

CAR and PXR not only induce the detoxification of toxic BAs but also regulate the elimination of BAs by regulating the transporters. CAR and PXR share the same response element in the MRP2 promoter together with FXR, and ligands for CAR and PXR induce MRP2 expression (64, 66-68). Both CAR and PXR also positively regulate basolateral MRP3 expression while only CAR but not PXR ligands induce MRP4 (59, 64, 67). Therefore, CAR and PXR play a central role in regulating the elimination of phase I and II detoxification products from hepatocytes via the regulation of transporters.

6. CAR AND PXR IN CHOLESTATIC LIVER DISEASES

6.1. Intrahepatic cholestasis

Intrahepatic cholestasis is caused by many factors including drug induced liver injury, sepsis, autoimmune biliary diseases, neonatal jaundice, and pregnancy, etc. The ligands for both receptors were used to treat intrahepatic cholestasis before their mode of action was explored. The CAR agonist PB reduces the serum BA concentrations in intrahepatic cholestasis (69, 70). Rifampicin is a ligand for PXR and it is effectively used to treat pruritus of cholestasis, but it also reduces the serum concentrations of alkaline phosphatase and BAs (70, 71). The traditional Chinese herbal medicine, Yin Zhin Huang has been used in Asia to prevent and treat neonatal jaundice (4). Yin Chin has been identified as a CAR ligand and it also accelerates bilirubin clearance (4). CAR and PXR activation induces bilirubin detoxification and clearance via the induction of its glucuronidation and export (72, 73). Therefore, CAR and PXR induction reduces either the serum BA or bilirubin concentrations in intrahepatic cholestasis, while also playing a protective role in intrahepatic cholestasis.

The importance of both CAR and PXR in protecting against cholestasis has been revealed in knockout mouse studies (50, 54, 74). PXR knockout mice develop increased liver injury after LCA feeding due to absent Cyp3a11 induction (50, 73). In addition, the combined loss of PXR and CAR results in an increase sensitivity to cholestasis in comparison to the loss of PXR.
or CAR alone (74). Similar findings are observed in FXR/PXR double knockout mice displaying more severe toxicity than mice lacking FXR or PXR alone (68). These data indicate, that PXR, CAR and FXR protect against hepatic BA-induced toxicity in a complementary manner, thereby regulating the redundant, but distinct defense pathways.

The administration of PXR and CAR ligands reduces the serum parameters of cholestasis, such as the bilirubin and serum BA levels, in patients with obstructive cholestasis by the induction of the phase I and II detoxification and transport systems (75). However, the increased transaminase levels indicate potential hepatotoxic side effects of the substances at least under increased transaminase levels indicate potential hepatotoxic side effects of the substances at least under conditions when bile flow is completely blocked (75). Therefore, PXR and CAR ligands should be used with care in cases presenting with obstructive jaundice.

6.2. Intrahepatic cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy (ICP) is a liver disorder characterized by pruritus and raised serum BA levels, most often occurring during the third trimester of pregnancy (76, 77). Intrahepatic cholestasis of pregnancy poses little maternal risk, however, it can lead to prematurity, fetal distress and intrauterine death (76, 77). While the pathogenesis of ICP remains unknown, several lines of evidence show that the etiology of the disease is likely to involve environmental, hormonal and genetic factors (78, 79). Most of the reports about genetic loci associated with ICP have so far mainly focused on hepatobiliary transporters. In addition, functional variants in the FXR have also been implicated in the genetic predisposition to ICP, thus suggesting a putative role of the genes controlling BA homeostasis (80, 81).

Castano et al. (82) compared a total of 101 ICP patients and 171 healthy pregnant women in the third trimester of their pregnancies. They suggested that PXR polymorphisms (rs2461823 G allele) are significantly associated with ICP, and also with several disease-associated traits (82), thus suggesting a putative role of PXR variants in individual susceptibility to the disease. PXR polymorphisms are significantly associated with a low birth weight and Apgar score, thus indicating that this variant may also be implicated in some fetal complications, either indirectly by modulating susceptibility to ICP in their mothers, or directly by modulating the expression of the PXR and the PXR network of enzymes that catalyses drugs and xenobiotics (82).

6.3. Constitutional hyperbilirubinemia

Constitutional hyperbilirubinemias are conditions in which there is an abnormal serum bilirubin level without any other abnormalities that can be observed in routine liver biochemistry tests (83). They are divided into two groups; the unconjugated hyperbilirubinemia group (Crigler-Najjar syndrome, Gilbert’s syndrome) and the conjugated hyperbilirubinemia group (Dubin-Johnson syndrome and Rotor syndrome). Crigler-Najjar syndrome and Gilbert syndrome are caused by the genetic defects of the UGT1A1 gene, while Dubin-Johnson syndrome is caused by the MRP2 gene (84), respectively. The gene responsible for Rotor syndrome has not yet been identified although Ligandin is one of the candidate genes (85).

Crigler-Najjar syndrome type II is caused by a partially defect in the UGT1A1 enzyme activity and CAR agonist, PB was had been used for treatment for long time. PB induces UGT1A1 enzyme activity by activating receptor CAR via a CAR binding site named gtPBREM (86). Sugatani et al. (87) found a polymorphism in the gtPBREM (T-3263G) in the individuals with Gilbert's syndrome. Only one case report has up to now indicated that PB is useful for the treatment of Dubin-Johnson syndrome (88), because the number of patients with Dubin-Johnson syndrome is small.

6.4. Primary biliary cirrhosis and primary sclerosing cholangitis

Primary biliary cirrhosis (PBC) is an inflammatory liver disease of unknown etiology (89, 90). It is characterized by inflammation of the portal tracts and the progressive destruction of the intra-hepatic bile ducts (89, 90). The resultant cholestasis leads to chronic liver damage, fibrosis and cirrhosis over many years. The patients with advanced stages of PBC show a down-regulation of the basolateral uptake systems (NTCP, OATP2) and either the preservation or even up-regulation of the canalicular bile salt and bilirubin export pumps (BSEP, MRP2, MDR) and the basolateral efflux pump (MRP3) (91, 92). Zollner et al. (92) reported the expression levels of PXR and CAR to be moderately reduced in 11 patients with late stage PBC without reaching statistical significance. Rifampicin, PXR ligand induces the hepatic metabolism in PBC patients to ameliorate the symptoms of pruritus (93) and lower the serum concentrations of alkaline phosphatase, a marker of cholestatic liver disease, and BAs (94, 95). However, liver enzymes should be monitored to detect any adverse effects of rifampicin (96). The adverse effects of rifampicin are not associated with its interaction with the PXR, but are likely due to its effects on mitochondria. Therefore, other PXR activators which do not have any adverse effects could possibly be more effective. Wallace et al. (97) reported that PXR activation is anti-inflammatory in the liver and the effects of cyclosporin A may be mediated in part via PXR in PBC disease recurrence, thereby suggesting that PXR may be an excellent drug target for the treatment of chronic inflammatory liver disease, since PXR activation promotes hepatocyte growth and is also anti-fibrogenic (97). Although further investigation is needed to determine the roles of CAR and PXR in PBC patients, CAR and PXR ligands may be useful for the treatment of advanced stage PBC patients.

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by progressive inflammation and fibrosis of the bile ducts, thus resulting in biliary cirrhosis. The majority of patients tend to be young males who also have coexisting inflammatory bowel disease (IBD). Karlsen et al. (98)
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7. Treatment for cholestatic liver disease

Ursodeoxycholic acid (UDCA) is a nontoxic BA, which is normally present in human bile albeit at a low concentration of about 3% of total BAs (15). UDCA is widely used for the treatment of a variety of chronic cholestatic liver diseases (74). UDCA stimulates the expression and function of hepatobiliary transporters and enzymes involved in BA synthesis and detoxification at multiple transcriptional and posttranscriptional levels (100-103). UDCA stimulates the overall gene expression of both canalicular (Mrp2, Bsep) and alternative basolateral efflux pumps (Mrp3, Mrp4) (104, 105). Moreover, UDCA also stimulates renal (Mrp2, Mrp4) and intestinal (Mrp2, Mrp3) efflux pumps in mice, thus demonstrating changes that may coordinately result in an increased overall elimination capacity for potentially toxic biliary constituents from the body (106).

Glucocorticoids are sometimes used for the treatment of cholestatic liver diseases. Dexamethasone induces CAR nuclear translocation. Furthermore, glucocorticoids also induce PXR expression and its target gene expression, such as CYP3A4 (107, 108). Glucocorticoids have also been reported to induce MRP2 and BSEP and counteract its downregulation after endotoxin treatment in vitro (109-111). These effects could possibly be mediated by targeting GR, although this has so far only been shown for the apical sodium-dependent bile salt transporter (ASBT) and NTCP (112).

7.2. CAR and PXR as therapeutic targets for cholestatic liver disease

The induction of detoxification enzymes, including Cyp2b10, Cyp3a11, Sult2a1, Ugt1a1 and 3'-phosphoadenosine 5'-phosphosulfate synthase 2 (PAPSS2), an enzyme that generates the sulfate donor for the sulfation reaction, and transporters such as Mrp2, Mrp3 and Mrp4 reduces BA-induced liver injury and serum BA levels in experimental cholestasis followed by an increased elimination of polyhydroxylated BAs into the urine (50, 61). Furthermore, CAR and PXR stimulation induces bilirubin glucuronidation, along with its hepatocellular export and clearance from the body. In addition to the induction of detoxification, PXR also plays a role in modulating liver fibrosis.

Zucchini-Pascal et al. (113) described the cytoprotective effect of PXR activation against BA-induced apoptosis and highlighted the molecular pathways that could be targeted in the treatment of cholestasis. They showed that an activator of PXR, namely clotrimazole protected rat hepatocytes against DCA- and glycocchenodeoxycholic acid (GCDCA)-induced apoptosis, preventing the morphological aspects changes of this process (113). This effect was attributable, at least partly, to the inhibition of caspases, Bcl-xl (B-cell lymphoma xl) induction, the activation of both extracellular-signal-regulated kinase (ERK) and serine/threonine protein kinase Akt signaling, as well as p38 inhibition (113).

The PXR ligand rifampicin and the CAR ligands PB ameliorate not only pruritus as a clinical manifestation of cholestasis (70). Yin Zhin Huang and a number of other herbal decoctions containing Yin Chin have been used in Asia for centuries to prevent and treat neonatal jaundice (4). However, both rifampicin and PB can cause significant adverse effects ranging from fatigue and somnolence (PB) to hepatotoxicity and liver failure (rifampicin) (71), and hepatoma formation has been observed with CAR activators in animal studies (114). The accumulation of these NR ligands in hepatocytes in patients with obstructive cholestasis may cause BA-independent hepatocellular injury despite the beneficial effects on cholestasis. Novel compounds targeting CAR and PXR with specific effects and fewer side effects are also expected be useful for the treatment of cholestatic liver diseases.

8. THE NUCLEAR RECEPTORS FXR AND VDR IN CHOLESTATIC LIVER DISEASE

8.1. FXR (Farnesoid X Receptor, NR1H4)

FXR is a major intracellular BA receptor that regulates the expression of a wide variety of genes involved in BA synthesis, metabolism and transport (Figure 4) (13). Therefore, FXR is a promising therapeutic target for cholestatic liver disease. The physiologic ligands for FXR are BAs, including CDCA, DCA, LCA and CA (13). The preferred DNA-binding motifs are inverted repeat elements separated by one nucleotide (IR-1) and FXR binds to its response element as a heterodimer with RXR (13). Upon ligand binding, FXR undergoes conformational changes to release corepressor such as NCoR and recruit coactivators such as coactivator-associated arginine methyltransferase 1 (CARM1). FXR shares the target genes involving BA regulation with CAR and PXR, including CYP3A4, SULT2A1, MRP2, etc.

FXR activation in rodent models of cholestasis results in the negative regulation of the genes involved in BA synthesis and uptake, whereas the genes involved in BA detoxification and excretion appear to be induced by FXR activation. FXR represses CYP7A1 gene transcription by the induction of the nuclear repressor SHP (small heterodimer partner), and it also negatively regulates the main BA uptake system NTCP via SHP. Fibroblast growth factor 15 (Fgf15) and its human orthologue FGF19 also

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regulate the CYP7A1 expression. Fgf15 senses bile acid concentrations in the intestine in an FXR-dependent manner, and then in turn is secreted from the enterocyte
Figure 4. The role of FXR and VDR in the hepatic defense mechanism against cholestasis. FXR activation results in the negative regulation of the genes involved in BA synthesis and the uptake of BA, whereas the genes involved in BA detoxification and excretion appear to be induced by FXR activation. FXR represses CYP7A1 gene transcription by the induction of the nuclear repressor SHP, and it also negatively regulates the main BA uptake system NTCP via SHP. In the BA detoxification pathways, FXR positively regulates human CYP3A4. BSEP, Mrp2 and MDR3 are transactivated by FXR. The induction of Mrp2 is also known to increase the BA-independent bile flow, and the FXR-dependent up-regulation of MDR3 thereby augments the canalicular phospholipid output. FXR also transactivates the basolateral efflux system OSTαβ.

VDR stimulates the expressions of CYP3A, SULT2A1 and MRp3, while inhibiting the expression of CYP7A1. and signals to the liver where it finally binds to Fgf receptor 4 (FgfR4) and thereby represses Cyp7a1 (115, 116). In the BA detoxification pathways, FXR positively regulates human CYP3A4 (117), while it is not required for the up-regulation of the mouse orthologue, Cyp3a11 (118, 119). BSEP, Mrp2 and MDR3 are transactivated by FXR (49, 120, 121). The induction of Mrp2 will also increase BA-independent bile flow, and FXR-dependent up-regulation of MDR3 will augment canalicular phospholipid output. FXR also transactivates the basolateral efflux system OSTαβ. Finally, FXR activation in the kidney reduces expression of ASBT and thus limits BA reabsorption from the tubular lumen. Besides the regulation of transporter and detoxification enzyme expression, FXR prevents bacterial overgrowth in the ileum and bacterial translocation in bile duct-ligated animals (122). Moreover, FXR has potent anti-inflammatory properties since it inhibits hepatocellular NF-κB (Nuclear Factor-κB) activation and the induction of classical pro-inflammatory NF-κB target genes, including tumor necrosis factor α (TNF-α), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (Cox2) in hepatocytes (123). The FXR-dependent induction of intestinal Fgf15 has also been reported to reduce liver injury in various cholestatic mouse models (124).

These findings indicate that FXR is, therefore, a highly interesting target for drug therapy in the treatment of patients with cholestatic liver disease. Indeed, the beneficial effects of pharmacologic FXR activation have been observed in estrogen-induced cholestasis in rodents (125). The administration of 6-ECDCA increased the expressions of Shp, Bsep, Mrp2, and Mdr2 in the liver, whereas it caused a reduction in the expressions of Cyp7a1 and Cyp8b1 and Ntcp mRNA (125). 6-ECDCA is currently under investigation for the treatment of patients with early stage PBC patients (5). As such, a clinical phase II study is currently underway which addresses the effects of 6-ECDCA with/without UDCA in PBC patients, but the results are still pending (5). Therefore, FXR ligands may be the most promising drug target for intrahepatic cholestasis at present.

However, the stimulation of the biliary bile flow by FXR may have a detrimental effect on obstructive cholestasis (126, 127). FXR has been demonstrated to worsen cholestatic injury in obstructive cholestasis in bile duct-ligated mice (126, 127). FXR agonists should therefore be used with caution in human cholestasis with either an obstructive component or with bile duct loss (e.g. PSC, late stage PBC). The stimulation of PXR and CAR therefore does not increase the bile flow. However, these substances should be used with care because of their potential hepatotoxicity when biliary elimination is hampered (76). A combination therapy consisting of FXR ligand with CAR, PXR ligand or both may therefore be a potentially effective therapeutic treatment for cholestatic disorders.

8.2. VDR (Vitamin D Receptor, NR1I1)

The vitamin D receptor (VDR) regulates calcium homeostasis, cell proliferation and differentiation, and exerts immunomodulatory as well as antimicrobial functions (128). Calcitriol (1α, 25-dihydroxyvitamin D3) is a natural VDR ligand, and VDR mediates its effects on calcium homeostasis after forming a heterodimer with
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The role of VDR as a promising drug target in cholestatic liver disease is now emerging since it plays a role in the regulation of BA synthesis, detoxification and transport (Figure 4) (129). However, at higher doses the calcemic effect of 1α, 25-dihydroxyvitamin D3 and its derivatives may limit their clinical application, and therefore less-calcemic VDR ligands are hypothesized to be potentially more suitable for use in future therapeutic approaches (130).

VDR polymorphisms have been implicated in the development of PBC underlying its role in cholestasis (131). Associations of VDR polymorphisms with mineral density or osteoporosis have been clearly demonstrated (132, 133), and association studies with PBC have been performed (134-136). Interestingly, a Hungarian study supported a positive association between susceptibility to PBC and polymorphism at the VDR locus (134), while both German and Chinese studies disclosed a protective polymorphism association (135, 136). Tanaka et al. (131) investigated the genetic association of VDR gene polymorphisms with PBC in large and well characterized series from both Japan and Italy. They indicated a significant association between VDR gene polymorphisms and the susceptibility of PBC, and their findings were consistent with two published studies from Hungary (134).

9. CONCLUDING REMARKS AND FUTURE DIRECTIONS

This review highlighted the transcriptional regulatory role of the nuclear receptors CAR and PXR in the maintenance of BA and xenobiotic metabolism and clearance. FXR, a major BA receptor, has become the focus of many experimental studies which led to the development of specific ligands currently tested in the clinical setting for the treatment of cholestasis. CAR and PXR represent attractive targets for drug therapy of cholestasis, because of their central role in BA detoxification and transport. Novel compounds targeting CAR and PXR with fewer side effects are needed for the treatment of cholestasis. Intrahepatic cholestasis is a good therapeutic target for both the CAR and PXR ligands. Clinical trials have already investigated, or are currently investigating the effects of various NR ligands other than CAR and PXR (e.g. FXR, PPARs and GR ligands) in human cholestatic disorders (5). Some clinical results have been disappointing and have thus failed to fulfill the expectations that were raised based on animal experimental findings (5). However, the initiation of such studies would not have been possible without the increasing knowledge on NR function in cholestasis derived from in vitro experiments, animal models of cholestasis as well as from human liver disease. Further basic research in this field will probably identify other, more potent substances with lower rates of side effects, which hopefully can eventually be applied to treat human cholestatic diseases (5).

Although CAR and PXR were initially characterized to be xenosensors, CAR and PXR also trigger many pleiotropic effects on either the physiological or pathological function (137). The evaluation of the pathophysiological roles of CAR and PXR is still ongoing. Further studies will hopefully reveal the pathophysiological roles of CAR and PXR in many diseases. Revealing the precise mechanism of CAR and PXR in such diseases should therefore make it possible to develop new therapeutic modalities. It is therefore an exciting possibility that the identification of potent and specific new agonists/antagonists for human CAR/PXR may thus make it possible to establish clinically useful therapeutic regimens to treat many diseases in the near future, including cholestatic liver disease (138).

In summary, CAR and PXR represent attractive targets for the development of new drug therapies for the treatment of cholestasis. Novel compounds targeting CAR and PXR with fewer side effects are needed for the successful treatment of cholestasis and intrahepatic cholestasis is therefore expected to be a good therapeutic target for both the CAR and PXR ligands.

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Abbreviations: CAR, constitutive active/androstane receptor; PXR, pregnane X receptor; RXR, retinoid X receptor; FXR, farnesoid X receptor; GR, glucocorticoid receptor; SHP, small heterodimer partner; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α; CCRP, cytoplasmic CAR retention protein; HNF4α, hepatocyte nuclear factor 4α; HSP90, heat shock protein 90; SRC-1, Steroid co-activator 1; Sp1, specificity protein 1; NCoR, nuclear receptor corepressor protein; SREBP-1, sterol regulatory element binding protein 1; SMRT, silencing mediator of retinoid and thyroid hormone receptors; CAR1M, coactivator-associated arginine methyltransferase 1; Fgf, fibroblast growth factor; Fgfr, Fgfr receptor; CYP, cytochrome P450; UGT, UDP-glucuronosyltransferase; GST, glutathione S-transferase; SULT, sulfotransferase; MRP, multidrug-resistance-associated protein; MDR, multidrug resistance protein; NTCP, Sodium-taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptide; BSEP, bile salt export pump; OST, organic solute transporter; ABCG, ATP-binding cassette subfamily G; ASBT, apical sodium-dependent bile salt transporter; PBREM, phenobarbital-responsive enhancer module; XREM, xenobiotic responsive enhancer module; PB, phenobarbital; TCPOBOP, 1,4 bis (2,3,5-dichloropyridyloxy))benzene; PCN, pregnenolone 16α-carbonitrile; RIF, rifampicin; CITCO, 6- (4-chlorophenyl) imidazo (2,1-b) (1,3)thiazole -5-carbaldehyde-O- (3,4-dichlorobenzyl) oxime; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; 6-ECDCA, 6-ethyl chenodeoxycholic acid; GCDDA, glycochenodeoxycholic acid; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis

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