Nuclear receptor ligands in therapy of cholestatic liver disease

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1. ABSTRACT

Cholestasis is a clinical syndrome resulting from disturbed bile formation. The etiology includes different diseases ranging from genetic defects in hepatocellular bile formation to inflammatory diseases of the bile ducts. Many cholestatic diseases are progressive and ultimately fatal. Whatever the cause, cholestasis results in intrahepatic accumulation of cytotoxic bile acids which lead to liver injury reflected by disruption of hepatocellular integrity, inflammation, fibrosis, cirrhosis and increased risk for development of cancer. Determinants of bile secretion undergo an adaptive response during cholestasis aiming to minimize hepatic injury. This adaptation occurs by modification of transport and metabolism of bile acids and other organic solutes in liver, kidney and intestine. The underlying molecular mechanisms are mediated mainly at a transcriptional level by a complex network involving ligand-activated nuclear receptors. However, the adaptive response to accumulation of bile acids cannot fully prevent or repair liver injury in cholestasis. Therefore, novel therapeutic strategies have to be developed involving nuclear receptor ligands which may intensify the protection of the hepatobiliary system in cholestatic disease.

2. INTRODUCTION

Cholestasis is the result of a broad array of pathophysiological derangements. Non-obstructive impairment of hepatocellular bile formation may develop due to inherited disease, such as progressive familial cholestatic disease, or acquired disorders such as sepsis. A striking set of bile duct diseases leads to eventual destruction of the intrahepatic or extrahepatic biliary tree with ensuing substantial hepatic injury due to retained bile constituents. Because of the central role of bile formation and the toxicity of relentless bile secretory failure it is imperative that in each of these conditions the hepatocyte
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Figure 1. Simplified schematic overview over ligand-activated regulation that determines the hepatic clearance of bile acids and bilirubin in hepatocytes as a basis for therapy of cholestatic liver disease. Influence of NR on specific phases of hepatic clearance is depicted as (+) for induction and (-) for repression. Adapted from reference 292. For effect on specific genes and references please see table 1 and sections in text.

along with other parenchymal cells attempts to adapt to cholestasis with a coordinated protective response. For this purpose the hepatocyte employs multiple layers of regulation in order to perform its functions even in states of accumulation of potentially toxic products, e.g., components of bile such as bile acids.

A major level of hepatic self-regulation is executed by members of the nuclear receptor (NR) superfamily. The activity of these transcriptional regulators is controlled by the intracellular concentration of specific ligands. These ligands bind to their specific NRs, activating the receptor that then in turn binds to specific elements in a gene promoter resulting in stimulation or inhibition of gene expression (1). In liver, many drugs, metabolites, and herbal or synthetic compounds exert their biologic properties as ligands for NRs (2, 3).

The hepatic clearance of bile acids and bilirubin can be divided into 4 phases that include the following: phase 0, hepatic uptake; phase I, metabolism (e.g., hydroxylation); phase II, detoxification (e.g., conjugation); and phase III, excretion. NRs and their ligands are the major determinants of the expression of genes that determine these pathways (4). By adaptation of these pathways, the hepatocyte can provide the appropriate response in physiological or pathophysiological conditions in a timely fashion.

Excellent summaries have addressed the mechanisms of cellular adaptation in cholestasis in detail (4-7) and several chapters in this issue address specifics of these mechanisms. It is the purpose of this review to provide an overview of how selective synthetic or natural ligands may activate members of the NR superfamily as regulators in the expression of a wide variety of metabolically important hepatic target genes which are also relevant in cholestasis. The following sections will address specific changes of NRs and consequences for target genes in disease models and disease states in humans with cholestatic liver disease, as well as potential therapeutic implications of these observations.

3. NUCLEAR RECEPTORS IN CHOLESTATIC DISEASE

3.1. General mechanisms of adaptation

Cholestasis may be the consequence of reduced expression and/ or function of transport systems of the hepatocyte (primary, causative changes). In addition it may induce a protective secondary response with the aim to maintain cellular homeostasis in a situation of metabolic stress, e.g., systemic accumulation of cholephils such as bile acids in biliary obstruction. It is important to emphasize that the defence machinery which is activated in response to cholestasis involves multiple metabolic functions in different organs and cell types. In cholestasis, expression of transporters for biliary constituents in hepatocytes is altered (2). Transporter adaptation is complex and is regulated by ligand-activated and orphan NRs (see Figure 1 for schematic overview). The leading regulatory role in this adaptation process is taken by the farnesoid X-activated receptor (FXR, NR1H4), pregnane X
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receptor (PXR, NR12) and constitutive androstane receptor (CAR, NR13) (for detailed review see refs. 4, 8 and chapter by Modica et al. in this issue). Transporter inhibition may limit the phase 0 import of bile acids into the hepatocyte (e.g., suppression of Na (+)-taurocholate-cotransporting peptide (NTCP, SLC10A2) and Organic Anion Transport Polypeptides (OATPs) in obstructive cholestasis) or may activate canalicular bile acid export (phase III) by BSEP induction or activation of alternative basolateral transporters (e.g., multidrug resistance-associated proteins 3 or 4 (MRP3 (ABCC3) or MRP4 (ABCC4)), organic solute transporter alpha/organic solute transporter beta (OSTa/OSTb) serving as overflow systems. As such, vitamin D receptor (VDR) has been shown to be involved in Mrp3 upregulation in mice (9). Depending on the type of cholestasis, this alteration of transporter expression may be beneficial or detrimental to homeostasis of the liver cell. In hepatocellular cholestasis, (temporal) bile salt export pump protein (BSEP, ABCB11) induction may counteract bile acid retention in hepatocytes, but in obstructive forms of cholestasis this mechanism may even add to liver damage through stimulation of bile secretion against a high pressure gradient in the biliary system created by obstruction (10). Moreover, it has to be kept in mind that these adaptive responses are usually too weak and late for efficient prevention of cholestasis inflicted liver injury. Therapeutic approaches using NR receptor ligands may therefore be needed to adapt to the type of cholestasis which is present and ideally should achieve gene specific modulation. Activation of metabolic pathways in the hepatocyte which includes enzymes active in bile acid hydroxylation (phase I, cytochrome P450 3A (CYP3A11)) and in conjugation and sulfation of bile acids (phase II, UDP-glucuronosyltransferase (UGT2B4), sulfotransferase 2A1 (SULT2A1)) leads to increased excretion of these conjugates into plasma and elimination via the kidneys (11). Adaptation of phase I and II enzymes is regulated by multiple ligand activated NR: FXR, PXR, VDR, peroxisome proliferator-activated receptors α (PPARα) and CAR (12-20). Bile acid synthesis by cholesterol 7α-hydroxylase (CYP7A1) in cholestasis is decreased by transcriptional and posttranscriptional mechanisms. As such, FXR, the orphan receptors SHP and FGF and the c-Jun/JNK pathway contribute to CYP7A1 suppression (21, 22; for detailed information see ref. 8).

In kidney cells, MRP1 as a tubular export system for bile acids is induced resulting in enhanced urinary bile acid excretion (23). The apical expression of MRP4 (24) in kidney cells is reduced in obstructive cholestasis (25). It is of yet unclear, how transporter adaptation to cholestasis is regulated in kidney cells.

Other cells in which transport mechanisms can adapt to cholestasis include the intestinal and bile duct epithelia. In enterocytes, the mechanisms of adaptation to cholestasis are not entirely known and may depend on species-specific regulation (26). Whether ileal bile acid absorption in humans is positively or negatively regulated in cholestasis remains controversial (see section on primary biliary cirrhosis) (8). Human ASBT is regulated by the hepatocyte nuclear factor 1-alpha (NF1-1α), PPARα, glucocorticoid receptor (GR), and retinoid X receptor: retinoid acid receptor (RXRα:RARα) (27-29). Bile duct epithelial cells may be able to adapt by inducing bile acid reuptake and delivery to the liver for detoxification in conditions with biliary obstruction. However, increased expression of ASBT and transporters MRP3 and MRP4 may be also due to the increase of total membrane mass as a consequence of bile duct proliferation in the models studied (e.g., common bile duct-ligated rodent models) and not due to transcriptional mechanisms (30, 31).

Studies in different animal models and humans have addressed the role of NRs in the pathogenesis of cholestatic disease. The majority of the data summarized above however originate from studies in rodents. For understanding the potential of NR receptor ligands in cholestatic liver disease, detailed knowledge of the role of each receptor in disease models and human diseases is essential.

3.2. Animal models of cholestatic liver disease
3.2.1. Animal model for obstructive cholestasis: bile duct ligation in mice

In the bile-duct ligation (BDL) mouse model, the roles of Fxr, Pxr, Car and small heterodimer partner (Shp) for cellular adaptation and modulation of cholestatic injury were studied. Fxr knockout (KO) mice were found to be protected from obstructive cholestasis in BDL (32, 33). Decreased expression of Bsep in Fxr null mice may lead to lower intrabiliary hydrostatic pressure (10) and may therefore reduce parenchymal damage as reflected by less pronounced bile infarcts. Whether Fxr plays a direct role in compensatory activation of bile acid transporters Mrp3 and Mrp4 on the basolateral domain of the hepatocyte is a matter of debate (10, 33). Intracellular bile acid accumulation in bile duct ligated mice in turn limits bile acid import by Ntcp repression via activation of Fxr and Shp (34). Fxr null mice consistently exhibit increased liver injury in BDL (35, 36). This injury has been attributed to the loss of various detoxification mechanisms, because concentrations of bile acids are not increased in these mice (33). In addition, Fxr null mice show increased signs of inflammation which may contribute to liver damage (37). Concurrent deletion of Fxr also could ameliorate an increase in liver injury that is seen usually in Fxr null mice with cholestasis. Mechanisms proposed for this protection include the lowering of bile acid concentrations and altered expression of the hepatic transporters multidrug resistance proteins 1 and 3 (Mdr1 (Abcb1) , Mdr2 (Abcb4, human ortholog MDR3), Bsep, and Mrp4 (33). Car confers protective effects in the setting of BDL through regulation of bile acid synthesis, as well as multiple detoxification pathways (36). Maintenance of Sult2a2 expression in female BDL mice was also shown to prevent BA toxicity and consequently cholestatic damage upon liver x receptor (Lxr) activation (38). The role of Shp has been studied in comparison to Fxr in BDL mice (39).The increased sensitivity of the Shp null mice to BDL provides a dramatic contrast with the decreased sensitivity of Fxr null mice (32, 33), which at first sight appears contradictory in light of the coordinated function of these two receptors in the negative regulation of bile acid synthesis. However, liver damage
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observed in Shp null mice may be due to an increased hepatic bile acid level as a direct result of BDL and not a consequence of defective repression of Cyp7a1 or other targets (39).

In kidney of BDL mice no changes were observed in the expression levels of renal Mrp2, Asbt and Oatp1. However, renal Mrp4 was significantly induced in both wild-type and Fxr null mice. Mrp3 was neither expressed nor induced in the kidney of any of the genotypes (32). As part of intestinal adaptation in BDL mice, expression of fibroblast growth factor 15 (Fgf15), Shp, and ileal bile acid-binding protein (Ibabp, Fabp6) was reduced, suggesting a role for Fxr (40). Interestingly, Fxr-mediated repression of bile acid synthesis may require the complementary actions of Fxr in both liver and intestine, the latter involving Fgf15/19 (41). In summary, in the setting of obstructive cholestasis, ligand-mediated modulation of genes responsible for induction of alternative bile acid exporters, together with activation of bile acid detoxification and enhanced secretion in the kidneys may be beneficial.

3.2.2. Cholestasis due to an inflammatory response after lipopolysaccharide exposure

Following lipopolysaccharide (LPS) exposure, NRs in hepatocytes are almost uniformly repressed. This effect has been demonstrated for Fxr, Hnf1-alpha, Hnf4-alpha, Rxr, Shp, Ppars, Car and Pxr in LPS treated animals (34, 42-45). Transcriptional and posttranscriptional alterations of nuclear receptors may lead to deranged expression of genes relevant to bile metabolism and transport.

Posttranscriptional alterations include activation of the Jnk/c-Jun pathway and interaction with nuclear receptors, e.g., Shp, and decrease of nuclear levels of transcription factors, e.g., Rxr (46, 47). Alterations of transporters include suppression of Ntcp in an Fxr independent fashion (34). Mrp2 and Bsep transcription is decreased and subcellular distribution is altered in rodents (48, 49). However, in humans, both transporters are affected posttranscriptionally (50). Involvement of Fxr, Shp and the Jnk/c-Jun pathway may lead to transcriptional inhibition of Cyp7a1, the rate-limiting enzyme of bile acid synthesis (46, 51, 52). Based on these observations, one may speculate whether NR ligands effective in counter-regulation of the inflammatory response may have advantageous effects regarding maintenance of hepatocyte function in inflammation-associated cholestasis (see also section on inflammation).

3.2.3. Bile acid induced cholestasis in rodents

The cholic acid (CA)-fed mouse model resembles a condition which mimics retention of potentially toxic bile acids which accumulate in cholestasis but which, in contrast to cholestasis, stimulate bile flow. In mouse liver, CA feeding has transcriptional effects on transporters located on the basolateral and canalicular domain of the hepatocyte. CA feeding stimulated hepatic Mrp2, Mrp3, Bsep and renal Mrp2 as well as intestinal Mrp2 and Mrp3 expression (53). Increase in Bsep expression strictly

depends on Fxr whereas induction of hepatic Mrp3 and Mrp2 and renal Mrp2 probably are independent of Fxr as these changes are also observed in Fxr null mice (53). Higher intrahepatic bile acid levels and the development of marked liver cell necroses in CA-fed Fxr null mice, despite an even stronger adaptive overexpression of hepatic Mrp3 and renal Mrp2 than in Fxr+/−, implies that these mechanisms may not appropriately compensate for the lack of Bsep induction in Fxr null animals. Therefore, adaptive overexpression of Bsep represents one of the main therapeutic targets to protect hepatocytes from the toxic effects of accumulating bile acids in hepatocellular cholestasis (8). Additionally, Fxr dependent induction of Osta/Ostβ may serve as a compensatory mechanism counteracting bile acid overload (54). Effects on Mrp2 may be mediated by Pxr (55, 56) whereas Car may be responsible for induction of Mrp3 (57). Upregulation of intestinal Mrp2 and Mrp3 may be aimed at protecting the enterocyte from bile-acid overload (58, 59). Mechanisms aimed at reducing bile acid toxicity (e.g. bile acid hydroxylation, sulfation or glucuronidation) may also contribute to the adaptive response in cholestatic liver injury (23, 35, 55, 56, 60). As such, bile acid hydroxylation via Cyp3a may reduce toxicity and facilitate sulfation and glucuronidation, rendering them better substrates for Mrp3 and Mrp2 (61-63). However, the Fxr independent induction of bile acid hydroxylation is unlikely to counteract liver toxicity sufficiently (54).

The lithocholic acid (LCA)-fed mouse model resembles the situation of toxic damage in cholestasis more closely than the CA fed mouse (64). LCA feeding leads to bile infarcts, destructive cholangitis, and periductal fibrosis (65). The adaptive response includes induction of bile acid detoxifying enzymes and bile acid overflow transporters on the basolateral membrane (65). Pxr has been suggested to have a protective role in LCA induced cholestasis (56). The work of Kitada and colleagues shows that the key event in prevention of LCA toxicity is phase II sulfating activity and not induction of transport for LCA (60). Fxr null mice have an advantage in LCA induced cholestasis, probably due to higher Sult2a expression levels (60). Activation of Lxrs may also prevent LCA toxicity in female mice through the same mechanism (38). A key element in prevention of non-obstructive cholestatic damage may be BA detoxification in which Pxr, Car, or Lxr activation may be of benefit.

3.2.4. Oestradiol induced cholestasis in a mouse model

Experimental intrahepatic cholestasis induced by 17-ethynylestradiol (EE2) treatment in rodents is a widely used in vivo model to examine the mechanisms involved in estrogen induced cholestasis (66). Activation of ER by estrogen receptor agonist EE2 leads to repression of bile acid (Ntcp, Oatp1/2, and Bsep) and cholesterol (Abcg5/8) transporters, and alteration of bile acid biosynthesis enzymes (Cyp7a1, Cyp7b1, and Cyp8b1). This results in repression of biliary lipid secretion and alteration of bile acid composition causing hepatocyte degeneration and inflammation comparable to other forms of intrahepatic cholestasis. Antagonization of Hnf4 may play a key role in the repression of Ntcp and Oatp1 (67) in this model. A clear role for Fxr, Pxr and Car in this model of
hepatotoxicity could not yet be determined. However, treatment of EE2 exposed rats with synthetic or semisynthetic Fxr ligands increased liver expression of Shp, Bsep, Mrp2, whereas it reduced Cyp7a1 and Cyp8b1 and Ntcp mRNA (68).

3.3. Human Disease

3.3.1. Primary biliary cirrhosis

Primary biliary cirrhosis (PBC) is characterized by a T-lymphocyte-mediated damage of small interlobular bile ducts. Chronic destruction of bile duct epithelial cells leads to their eventual disappearance (vanishing bile duct syndrome). The sustained loss of small interlobular bile ducts causes the signs and symptoms of cholestasis comparable to other chronic cholestatic disease, such as biliary atresia in childhood, and eventually results in cirrhosis and liver failure (69). Zollner et al. demonstrated moderately reduced expression levels of FXR, RXR, SHP, PXR, CAR, HNF1α and HNF4α in liver tissue sampled from patients with late-stage PBC (70). Of the genes involved in bile acid metabolism, CYP7A1 mRNA was repressed in PBC to 10–20% of controls, while CYP27 and CYP8B1 mRNA remained unchanged. Repression of CYP7A1 in PBC contrasts unchanged or even increased CYP7A1 expression and function observed in patients with obstructive cholestasis (71) and in common bile duct-ligated rodents (10, 72). SULT2A1 and CYP3A4 mRNA levels are unaltered or only mildly reduced in PBC livers whereas MRP4 protein levels are induced three-fold in PBC, although mRNA levels remain unchanged (70). MRP3 and OSTα/ Ostβ expression is induced, indicating that renal excretion of bile acid conjugates may play a role in compensatory mechanisms during PBC associated cholestasis (31, 73, 74). OSTα/ OSTβ expression in response to cholestasis is highly regulated by FXR (31). Compared to controls, BSEP and MRP2 protein expression remain unchanged on the canalicular membrane (53). The bile acid uptake transporters NTCP and OATP1 on the basolateral membrane are repressed, counteracting a potential bile acid overload of the hepatocyte in PBC (53). Interestingly, PBC patients have been shown to have increased intestinal bile acid reabsorption (75). In contrast, decreased ASBT expression was found in humans with obstructive cholestasis (76). NR ligands activating alternative bile acid export and detoxification genes as well as ameliorating inflammatory pathways may be beneficial in the setting of PBC.

3.3.2. Cholestasis of pregnancy

Pregnancy is a physiological cholestatic-prone condition with functional alterations in bile secretory function related to downregulation of key hepatobiliary transporters, such as NTCP and MRP2, and functional impairment of BSEP. These changes are probably due to the effects of oestrogens in the liver, although transinhibition of BSEP by progesterone metabolites may play a role. Genetic variants, rather than mutations, in canalicular transporters (mainly MDR3 (77), BSEP (78), ATP8B1 (79, 80) and MRP2 (81)), FXR (82) or other genes, may provide a genetic background that exaggerates the cholestatic-prone state and determines the development of a phenotypic continuum, ranging from subclinical cholestasis (asymptomatic hypercholanemia of pregnancy) to cholestatic jaundice (83).

3.3.3. Biliary atresia

Biliary atresia is characterized by progressive inflammatory bile duct damage in early infancy. Chronic cholestasis develops in early stages and may persist despite surgical treatment. Chen and co-workers have recently analysed hepatocyte transport and bile acid metabolism genes in early and late-stage biliary atresia patients (84). Adaptation to chronic cholestasis of biliary atresia show consistent downregulation of bile acid import proteins on the sinusoidal membrane and striking upregulation of OSTα/ OSTβ as a mechanism to adapt to increasing intracellular bile acid levels and excrete bile acids via the basolateral membrane. The CYP7A1 and CYP 3A4 enzymes and FXR are also consistently suppressed. Interestingly, BSEP is repressed in early stages, however returns to near normal levels in later stages of the disease. Compensatory MRP4 expression increases solely in later stages. Low PXR levels were indicative for a bad outcome. A concept of therapeutic strategies for cholestatic disease such as PBC and biliary atresia using NR ligands may have to be adapted to different disease states.

3.3.4. Progressive familial intrahepatic cholestasis

Progressive familial intrahepatic cholestasis (PFIC) syndromes represent a diverse group of liver disorders in humans (85). These diseases are characterized by severe intrahepatic cholestasis, of familial or genetic origin, and defects of BA biosynthesis and/or transport (86, 87). Initial data in animal models and studies in humans have made clear that FXR function has strong relevance to pathophysiology of familial cholestatic disease (88). In ATP8B1 deficiency (PFIC1) evidence has been found for FXR down-regulation and concomitant suppression of SHP and BSEP. Gene-profiling revealed that several genes involved in synthesis, conjugation and transport of bile acids were down-regulated (89). In addition, posttranslational effects may alter FXR function in ATP8B1 deficient states, e.g., by impaired translocation into the nucleus (90). Defects in BSEP (PFIC 2) may involve failure of mRNA to be formed, translation of a functionally impaired transporter, or translation of a transporter that is formed but not delivered to the canalicular membrane (91, 92). Hypothetically, in selected cases with residual function, FXR ligand-activation could lead to induction of increased BSEP expression with amelioration of defective canalicular BA export. MDR3 deficiency (PFIC3) is characterized in parallel, mutations of the ABCB4 gene may lead to expression of MDR3 with partial dysfunction. As FXR can induce human MDR3 expression (93), subtypes of MDR3 deficiency may be amenable to improvement of residual phospholipid secretion by FXR ligand therapy (see section 4.5). Whether activation of peroxisome proliferator-activated receptor-α (PPAR-α) or FXR/ PPAR-α interaction play a role to this regard in humans remains to be shown (see section 4.5). NR ligands activating BA detoxification and alternative routes of BA efflux (basolateral hepatocyte membrane and kidney) may also be of benefit in PFIC induced hepatocellular bile acid overload.
3.3.5. Sepsis associated cholestasis

Jaundice is a well-known complication of sepsis or extrahepatic bacterial infection. Sepsis and bacterial infection are responsible for up to 20% of cases of jaundice in patients of all ages and up to 60% of cases in newborns and infants (94, 95). Sepsis is more likely to manifest with jaundice in infants and children than in adults. In this population, males have a higher incidence of jaundice (96). However, in adults, no gender predilection has been reported. Pathogenetic factors for jaundice in sepsis include hemolysis, deranged bilirubin transport and bile acid transport (97). The bilirubin transporters on the basolateral membrane (OATP) and on the canalicular membrane (MRP2) are functionally impaired (98, 99). Kupffer cells take up bacteria by phagocytosis and when activated secrete large quantities of inflammatory mediators, such as tumor necrosis factor, interleukin 1 and interleukin 6, superoxides, lysosomal enzymes, procoagulants, and platelet-activating factor (99, 100). Kupffer cells play a critical role in the cascade leading from endotoxemia to cholestasis as many of the inflammatory mediators influence transporters for bile constituents (101, 102). NTCP is repressed by transcriptional means whereas reduction of BSEP and MRP2 is mainly due to posttranscriptional mechanisms in humans (50). Several studies have observed endotoxin-induced inhibition of basolateral membrane Na-K-ATPase activity (103, 104). Endotoxin may cause decreased function of the Na-gradient dependent transporters at the basolateral membrane such as NTCP (105). It has also been reported that endotoxin affects membrane fluidity, which could reduce Na-K-ATPase activity after endotoxin administration (103). Bile acid-dependent and -independent flows are reduced in disease models of sepsis compared to controls (106). The main evidence for this is the inhibition of biliary excretion of GSH and, to a lesser extent, of HCO₃⁻ after LPS administration (99, 106, 107). In sepsis-associated cholestasis in humans, NR ligands with anti-inflammatory properties would potentially provide benefits, as mechanisms affecting bile transport are dominantly influenced by the effect of cytokines and not by the effects of bile acids (108).

3.4. The role of nuclear receptors in sequelae of cholestasis

3.4.1. Fibrosis

Chronic liver injury leading to fibrosis occurs in response to a variety of insults, including cholestatic disease. Studies on pathophysiology of fibrosis have focussed primarily on events that lead to the early accumulation of scar tissue hoping to identify therapeutic targets to slow its progression (109). The critical event in liver fibrosis development is the stellate cell changing phenotype from a retinoid storing to a proliferating, fibrogenic and contractile cell (110). The role of nuclear receptors in this process has been investigated recently. Information has become available on the role of RXR, RAR, PPARs as well as FXR in hepatic stellate cell biology. Retinoid receptors modulate a number of target genes in stellate cells including cellular retinoid binding protein (111) and collagen I (112). Natural and synthetic retinoids elicit a range of activities (113): RXR agonists downregulate stellate cell proliferation and synthesis of collagen I and fibronectin. In contrast, RAR agonists both reduce collagen I, collagen III, and fibronectin but have no effect on stellate cell proliferation. Finally, RAR-specific antagonists provoke stellate cell mitogenesis. RAR-α transactivation shows plasminogen activation and TGF-β-dependent procollagen synthesis in in vitro studies (114). The PPAR-γ ligands 15-deoxy-prostaglandin J₂ (15d-PGJ₂) and ciglitizone decrease proliferation of activated stellate cells and inhibit alpha-smooth muscle actin (α-SMA) expression during stellate cell activation (115). PPAR-α ligands are antifibrotic in cultured stellate cells (116, 117) and in experimental animals (118-120). PXR has been identified in stellate cells and PXR ligands inhibit cellular activation in culture (121) and in vivo (122). Stellate cells also express the vitamin D receptor (123), suggesting a potential role of these cells in vitamin D homeostasis and responsiveness. Estrogens, and in particular, 17β-estradiol (124) and estradiol (125, 126) are antifibrotic in liver. Glucocorticoid receptor is also expressed by stellate cells (127), but its contribution to stellate cell physiology has not been explored. Surprisingly, FXR is also expressed by stellate cells, where it may have antifibrotic activity through upregulation of its downstream target SHP (128, 129).

3.4.2. Inflammation

Chronic cholestasis is invariably linked to activation of inflammatory pathways in the liver (130). Certain nuclear receptor family members are responsible for negative regulation of inflammatory responses. They have the potential to ameliorate effects of inflammatory activation in the liver independently of cholestasis being the cause or consequence of inflammation (see chapter by Mulder et al. for extensive review). Mechanisms of beneficial action of NR in inflammation include a ligand-dependent inhibition of inflammatory gene expression by specific DNA binding of NRs to negative regulatory elements, a positive regulation of inhibitory genes, and a specific DNA binding of NRs to negative regulatory elements, a positive regulation of inhibitory genes, and a specific DNA binding of NRs to negative regulatory elements, a positive regulation of inhibitory genes, and a specific DNA binding of NRs to negative regulatory elements, a positive regulation of inhibitory genes. Estrogens, and in particular, 17β-estradiol (131, 131-134). Anti-inflammatory properties have been described in detail for the receptors LXR, the PPAR isoforms, GR and PXR. The ligand-dependent transrepression mechanism has been studied mostly for PPARγ. It involves a complex posttranslational modification of bound ligands leading to maintenance of repression of target genes (SUMOylation (135, 136)). LXR has been shown to regulate macrophage activation most likely by inhibition of the NFκB pathway. Transrepression may play a role in this process (137). Glucocorticoids have been widely used as anti-inflammatory drugs and glucocorticoid receptors have been most extensively studied. Glucocorticoid-mediated inhibition of inflammatory gene expression is mediated by induction of the inhibitory protein IκBα, implicating that inhibition of NF-κB activity is through a negative feedback loop (138-140). The synthetic GR-agonist, dexamethasone was reported to induce IκBα, concomitantly NF-κB DNA binding decreased (141). This mechanism is suggested to be responsible for repression by the GR on several inflammatory target genes (142). In mouse models of inflammation, Teng and colleagues observed a role of Pxr in downregulation of several hepatic proteins (143). Rifampicin, a well known PXR ligand, has been shown to
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suppress several immunological responses in liver cells (144) and to have immunosuppressive properties in humans (145, 146). PXR ligands downregulate a number of NFκB target genes such as prostaglandin-endoperoxidase synthase 2 (COX-2), tumor necrosis factor alpha (TNFα), and intracellular adhesion molecule 1 (ICAM-1 or CD54) and several interleukins (37). Conversely, several important observations have been made in Pxr null mice. Hepatocytes from these mice show elevated NFκB target gene expression and, surprisingly, the small bowel in these animals was also affected by inflammation. Intestinal inflammation could occur due to lack of NFκB repression or lack of detoxification function of Pxr. In conjunction with these observations it is an intriguing finding that low expression levels or polymorphisms of PXR are associated with inflammatory bowel disease (147). In summary, PXR may play a key role in gastrointestinal counter-inflammatory regulation. An intriguing new role for Fxr in regulation of gut barrier function and prevention of bacterial translocation has been presented by Inagaki and colleagues (40). They showed that Fxr reduces bacterial overgrowth and translocation as well as inflammatory mucosal injury presumably by inducing genes with antimicrobial properties (40). Similar findings were reported from experiments in cirrhotic BDL rats (148). Interestingly, FXR may also have functions in inflammation induced by hepatitis virus replication (149, 150). In an in vitro model, Scholtes and colleagues demonstrated that FXR activation induces hepatitis C virus (HCV) replication independently of interaction with interferon whereas FXR inhibition was associated with a decrease in replication (150). Hepatitis B virus (HBV) replication is also influenced by multiple NR such as HNF4α or the PPARα/RXRα heterodimer (151). Recently, SHP has been described to have a role among other NR in regulating HBV replication (152).

3.4.3. Cancer

Chronic cholestatic disease in adults and children and in animal models is associated with an increased risk for development of hepatocellular carcinoma (HCC) (61, 92, 153-162). Nuclear receptors and their respective ligands regulate transcriptional activity of target genes involved in tumor development and suppression. In hepatocellular carcinoma, nuclear receptors have been investigated for their role in pathophysiology and therapy. Research has concentrated on PPARs, ER and VDR. Long-term administration of Ppara ligands to rodents causes accelerated hepatocyte proliferation, increased reactive oxygen species (ROS) generation and development of HCC (163, 164). Interestingly, it was shown in animal models that HCV-core protein related HCC development is dependent on presence and activation of Pparu (165). In contrast, Pparδ has been implicated in the differentiation and growth inhibition of cancer cells. PPARγ therefore represents a putative molecular target for chemopreventive therapy or inhibition of liver cancer growth (166). The human liver expresses estrogen and androgen receptors and experimentally both androgens and estrogens have been implicated in stimulating hepatocyte proliferation and may act as liver tumor inducers or promoters (167). ER polymorphisms have been associated with increased risks for HCC (168). Vitamin D receptor ligands such as 1,25-Dihydroxyvitamin D3 have been shown in vitro and in vivo to inhibit proliferation of liver cancer cells (169). A new role for nuclear receptors in hepatocarcinogenesis was recently described by Kim and colleagues (170). They found that, at 12 months of age, male and female Fxr null mice had a high incidence of degenerative hepatic lesions, altered cell foci and liver tumours including hepatocellular adenoma, carcinoma and hepatocelangiocellular carcinoma. In Fxr null mice, elevated bile acid levels induce IL-1β. IL-1β is an important inflammatory signal that has been demonstrated to mediate cell proliferation, differentiation and apoptosis (171). Hypothetically, IL-1β is responsible for the observed induction of genes responsible for tumorogenesis in this model (170). Although CAR and PXR have also been found to be expressed in human hepatoma cells (172) only preliminary data is available on their promoting role in tumor development. Following exposure to a carcinogen, ligand activated Car is an essential mediator for development of hepatoma in mice (173). In humans, altered CAR expression due to transcriptional regulation of a HNF4α isof orm was found in human HCC (174). PXR has been found to promote development of endometrial cancer (175).

In addition to the multiple effects on hepatic transport, metabolism, inflammation and proliferation (see table 1 for summary), NR also play a key role in the control of other critical hepatocyte functions (e.g., glucose and fatty acid/triglyceride metabolism) Recent reviews have addressed these issues (176-186).

4. NUCLEAR RECEPTOR LIGANDS IN THERAPY OF CHOLESTATIC LIVER DISEASES

Therapeutic strategies may be a multi-level approach targeting impaired bile acid/bilirubin transport and metabolism and reinforcing adaptive rescue pathways for accumulating, potentially toxic biliary compounds. Such pharmacological approaches may be aimed at nuclear receptors and their target genes which affect “orthograde” biliary excretory routes, bile acid phase I and II detoxification systems but also “retrograde” alternative/basolateral overflow and renal elimination systems. Moreover activation of nuclear receptors may also directly target consequences of cholestasis such as inflammation, fibrosis and cancer.

4.1. Bile acids and other FXR ligands

Traditional oriental medicine has taught for centuries that bile of various animals is useful in the treatment of biliary stone disease and jaundice (187). From a pathophysiological point of view, rationales using bile acids as therapy include replacement therapy to correct for bile acid deficiency (e.g. rare inborn errors of bile acid biosynthesis, short bowel syndrome) or as displacement therapy, when the composition of the endogenous bile acid pool is changed via replacement of endogenous cytotoxic bile acids (Chenodexoycholic acid (CDCA) and Deoxycyclic acid (DCA)) by exogenous administration of non-toxic bile acids (e.g. Ursodeoxycholic acid (UDCA) for the treatment of cholestatic liver disease) (6). Moreover,
Table 1. Selected ligand activated NR and target genes involved in bile acid transport, fibrosis, inflammation and cancer

<table>
<thead>
<tr>
<th>NR</th>
<th>Function</th>
<th>Gene</th>
<th>Regulatory effect</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXR</td>
<td>BA uptake</td>
<td>NTCP</td>
<td>Repression</td>
<td>SHP mediated</td>
</tr>
<tr>
<td></td>
<td>BA synthesis</td>
<td>CYP7A</td>
<td>Repression</td>
<td>SHP mediated</td>
</tr>
<tr>
<td></td>
<td>BA conjugation</td>
<td>UGT1A1B4</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SULT2A1</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canaliclar BA, bilirubin export</td>
<td>BSEP</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRP2</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biliary phospholipid secretion</td>
<td>MDR3</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basolateral BA efflux</td>
<td>OSTα/OSTβ</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrosis</td>
<td></td>
<td>Antifibrotic</td>
<td>SHP mediated</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td></td>
<td>Antiproliferative?</td>
<td>IL-1β Repression? In mice.</td>
</tr>
<tr>
<td></td>
<td>Inflammation, immune response</td>
<td>IL18</td>
<td>Induces antimicrobial genes, inhibits intestinal bacterial overgrowth and translocation .</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>iNOS</td>
<td>Induces HCV replication in vitro.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANG1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PXR</td>
<td>BA uptake</td>
<td>OATP1</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA hydroxylation</td>
<td>CYP3A1</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA conjugation</td>
<td>UGT1A1B4</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SULT2A1</td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSTs</td>
<td></td>
<td>Induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canaliclar BA export</td>
<td>MRP2</td>
<td>Induction</td>
<td></td>
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<tr>
<td></td>
<td>Basolateral BA efflux</td>
<td>MRP4</td>
<td>Induction</td>
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<tr>
<td></td>
<td>LXR</td>
<td>BA conjugation</td>
<td>SULT2A</td>
<td>Induction</td>
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<td></td>
<td></td>
<td>BA synthesis</td>
<td>CYP7A</td>
<td>Induction</td>
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<tr>
<td></td>
<td>Basolateral BA import</td>
<td>NTCP</td>
<td>Induction</td>
<td>In female mice</td>
</tr>
<tr>
<td></td>
<td>RAR</td>
<td>BA uptake</td>
<td>NTCP</td>
<td>Induction</td>
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<tr>
<td></td>
<td></td>
<td>Canaliclar BA export</td>
<td>MRP2</td>
<td>Induction</td>
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<tr>
<td></td>
<td>Fibrosis</td>
<td>Collagen1+III</td>
<td>Repression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VDR</td>
<td>BA hydroxylation</td>
<td>CYP3A1</td>
<td>Induction</td>
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<tr>
<td></td>
<td></td>
<td>BA conjugation</td>
<td>SULT2A1</td>
<td>Induction</td>
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<td></td>
<td>Basolateral BA efflux</td>
<td>MRP3</td>
<td>Induction</td>
<td>In mice</td>
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<tr>
<td></td>
<td>Cancer</td>
<td></td>
<td>Antiproliferative</td>
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<td></td>
<td>Inflammation, immune response</td>
<td>IL18</td>
<td>Induces antimicrobial genes, inhibits intestinal bacterial overgrowth and translocation .</td>
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<tr>
<td></td>
<td></td>
<td>iNOS</td>
<td>Induces HCV replication in vitro.</td>
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<td></td>
<td></td>
<td>ANG1</td>
<td></td>
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<tr>
<td></td>
<td>PPARγ</td>
<td>Fibrosis</td>
<td>α-SMA</td>
<td>Repression</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Decreases stellate cell proliferation</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td></td>
<td>Differentiation and growth inhibition of cancer cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PPARα</td>
<td>Biliary phospholipid secretion</td>
<td>MDR3 (mdr2 in mice)</td>
<td>Induction</td>
</tr>
<tr>
<td></td>
<td>Fibrosis</td>
<td></td>
<td>Antifibrotic</td>
<td>In mice</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td></td>
<td>Hepatocyte proliferation, increased ROS generation and development of HCC</td>
<td>In Mice</td>
</tr>
</tbody>
</table>

For details and references see text sections. NOTE: CAR and FTF activators are not true ligands, the NR also have important functions in regulation of glucose and fat metabolism.

with the discovery of bile acids as natural ligands of the nuclear receptor FXR, an additional rationale for bile acid therapy may be targeting FXR. From a teleological point of view, FXR is a highly interesting target for drug therapy, because human and rodent data suggest that FXR expression and activity in cholestasis (especially in inflammatory cholestasis) is low (42, 70). Moreover, Fxr-deficient mice show increased liver injury when challenged with bile acids (53, 88) and low expression of FXR and FXR target genes (i.e. BSEP) is associated with various human cholestatic disorders (i.e. progressive familial intrahepatic cholestasis, intrahepatic cholestasis of pregnancy) (82, 188, 189).

### 4.1.1. Ursodeoxycholic acid

The only approved drug for treatment of cholestatic disorders is the hydrophilic 7ß-epimer of CDCA, UDCA. UDCA has been shown to improve biochemical and clinical parameters in a variety of cholestatic syndromes and positively effects survival free of liver transplantation in PBC (190). The effects of UDCA are mediated via transcriptional and post-transcriptional mechanism. In contrast to CDCA, however, no definite nuclear receptor for UDCA has been identified. Several studies have shown that UDCA was not a FXR ligand (191, 192), while one study reported UDCA as a gene-selective partial FXR agonist (193). As a matter of fact, UDCA may
even exert FXR antagonistic properties by changing the bile acid pool composition and reducing the relative amounts of stronger FXR ligands like CDCA and CA. Moreover, it was speculated that UDCA might activate PXR (194) and the glucocorticoid receptor (GR) (195, 196), the latter being able to increase CAR transcription via a glucocorticoid response element in the CAR promoter (197). Proposed transcriptional anti-cholestatic mechanisms of UDCA action include stimulation of canalicular efflux pumps (i.e. MRP2, BSEP) for “orthograde” biliary excretion and stimulation of basolateral export pumps (i.e. MRP3, MRP4) for adaptive excretion of bile acids back from hepatocytes to the systemic circulation (53, 54, 198). Moreover, at least in mice, UDCA induces expression of renal (Mrp2, MRP4) and intestinal (Mrp2, Mrp3) efflux pumps, changes which may result in increased elimination of potentially toxic bile constituents (34). In line with its effects on PXR and CAR, UDCA stimulates bile acid hydroxylase CYP3A4/Cyp3a11 in human and rodents, respectively (194, 199) and, in accordance with its potential role as partial FXR agonist, down-regulates the key enzyme of bile acid synthesis CYP7A1 in vitro (193). Thus, UDCA appears to act on the metabolic level by induction of detoxification pathways and on transporter level by restoring defective transporters and generating alternative overflow-systems for accumulating biliary compounds. At a post-transcriptional level UDCA may also directly activate canalicular transport function by inducing phosphorylation of ABC transporters and stimulation of vesicular exocytosis and insertion of canalicular transport systems into the canalicular membrane (200, 201). In addition, UDCA also has antiapoptotic and antifibrotic properties contributing at least to some of the beneficial effects under UDCA treatment (190).

4.1.2. Nor-Ursodeoxycholic acid

Nor-Ursodeoxycholic acid (NorUDCA) is a side chain shortened UDCA derivate, which may be more effective than UDCA in the treatment of cholangiopathies such as sclerosing cholangitis (202). In contrast to UDCA, NorUDCA is able to heal sclerosing cholangitis in the Mdr2 knockout animal model (202). It is not amitiated and therefore undergoes enhanced cholehepatic shunting from the bile duct lumen back to the hepatocytes, thereby inducing a bicarbonate rich and potentially less toxic bile flow (203). In addition, NorUDCA is enriched in the hepatocytes by this mechanism and markedly induces phase I and phase II detoxification enzymes and alternative basolateral overflow systems (202). As for UDCA, no definite nuclear receptor for the action of NorUDCA has been defined and parts of its effect may be mediated via posttranscriptional mechanisms. However, these encouraging studies in animals are waiting for their confirmation also in human cholestatic liver disease.

4.1.3. Chenodeoxycholic acid

CDCA is the strongest endogenous FXR agonist (191, 192) and widely used in vitro and in animal models to investigate the effects of FXR activation. In contrast to humans, however, CDCA in rodents is highly hepatotoxic. In human, the efficacy and safety of CDCA for dissolution of gallstones was established in the National Cooperative Gallstone Study already in 1981 (204) and is FDA approved for this indication. However, because of a slight hepatotoxicity in humans, it was subsequently replaced by UDCA, which completely was devoid of any toxic effects (205). Currently, CDCA is used off label for the treatment of cerebrotendinous xanthomatosis and some rare inborn errors of bile acid biosynthesis (205). However, given its FDA approval for the administration in humans and the promising beneficial effects of (synthetic) FXR agonists in disorders of lipid metabolism and glucose homeostasis, a revival for CDCA in the treatment of the metabolic syndrome, hypertriglyceridemia, type 2 diabetes, cholesterol gall stone disease and steatohepatitis can be envisioned (206).

4.1.4. 6-ethyl Chenodeoxycholic acid and other synthetic FXR agonists

The semisynthetic 6-ethyl derivative of CDCA (6-ECDCA) is a selective FXR agonist and approximately 100 fold more potent than CDCA in activating FXR in vitro (207). In vivo administration of 6-ECDCA protects against cholestasis induced by estrogen and LCA in rats providing evidence that development of potent FXR agonists might represent a novel approach for the treatment of cholestatic disorders (68, 207). In fact, phase II studies exploring the effects of 6-ECDCA have already been initiated in PBC. The synthetic FXR agonist GW6046 has shown promising in vivo effects in α-naphthylisothiocyanat-treated animals as model for intrahepatic cholestasis and also marginal beneficial effects in bile duct-ligated animals (208). From a teleological point of view, FXR agonists could restore the reduced bile flow in cholestasis via stimulation of BSEP (increasing bile acid-dependent bile flow) and MRP2 (increasing bile acid-independent bile flow) (5, 68, 207-209). However, it may be simplistic to treat cholestasis merely by stimulation of bile flow, particularly in the presence of bile duct injury and/or biliary obstruction. FXR agonists may also support some adaptive reactions of the cholestatic hepatocyte which would be predicted to limit the hepatocellular bile acid burden, such as down-regulation of bile acid import via NTCP and OATP1A1, upregulation of basolateral overflow systems via OSTα/OSTβ as well as reducing endogenous bile acid synthesis via down-regulation of CYP7A1 and CYP8B1 (5, 7). In addition, stimulation of the canalicular phospholipid flipase MDR3 (mouse ortholog Mdr2) is predicted to change the intrabiliary bile composition rendering bile less aggressive (93, 210). At least in rats, Fxr agonists were also shown to display antifibrotic effects in vitro and in vivo, suggesting that Fxr (via Shp and AP-1) may suppress fibrogenesis in HSC (128). This mechanism, however additionally seems to involve FXR dependent PPARγ induction, which may mediate at least to some degrees the antifibrotic effects of FXR agonists (68). However, first experiments in human HSC indicate a rather low expression of the NRs such as FXR, SHP, PPARs and PXR in human HSC. Bile acid uptake systems are not expressed in human as well as rodent HSC (5, 211, 212). Therefore, the impact of bile acids on HSC biology in humans remains to be determined. Concerning antibacterial effects in the
distal small intestine, which is mediated by cellular pathways involving activation of FXR by FXR agonists induced the expression of genes (e.g. iNOS, IL18) whose products prevent bacterial overgrowth and promote epithelial integrity (see previous section 3.4.2 and (40)). Since bile acid secretion is decreased in liver cirrhosis in humans and bacterial overgrowth can occur in this condition, the use of FXR agonists in this setting (e.g. as prevention of spontaneous bacterial peritonitis) can also be envisioned.

However, use of FXR agonists in cholestatic conditions with an obstructive component (e.g. advanced ductopenic PBC, PSC with (dominant) biliary strictures) should be considered cautiously. Stimulation of bile flow in a mouse model of sclerosing cholangitis and in bile duct ligated mice (using the hydrophilic bile acid UDCA) increased liver injury, aggravated bile infarcts and induced hepatocyte necroses whereas mice lacking Fxr are protected from cholestasis and the development of bile infarcts after bile duct ligation (10, 33, 213). In addition, serum bile acid levels in bile duct-ligated mice lacking Fxr were lower and urinary bile acid output was increased, indicating enhanced adaptation to cholestasis in Fxr-deficient mice (32). This suggests that the bile flow stimulating effect of FXR agonists could be detrimental in obstructive cholestasis and rather FXR antagonists or gene selective FXR agonists (lacking the stimulatory effects on canalicular bile salt transporters) might be helpful. In this regard, the fibroblast growth factor FGF19 (Fgf15 as mouse ortholog) might be involved in this process. FGF19 signals from the gut to the liver and efficiently represses endogenous bile acid synthesis via CYP7A1 (involving SHP and the JNK-pathway) (21). In obstructive cholestasis with impaired bile flow the intestinal bile acid content can be too small for sufficient FXR and subsequent FGF19 induction, thereby leading to paradoxically increased endogenous bile acid synthesis even in the presence of cholestasis (21). Selectively stimulating intestinal FXR without affecting hepatic FXR could therefore reverse bile acid overproduction without affecting bile flow and aggravation of liver injury in obstructive cholestasis. This mechanism might explain the apparent discrepancy in bile duct ligated rodents between the beneficial effects of the synthetic FXR agonist GW4064, which is only poorly absorbed after gavage and the beneficial effects of Fxr knock out in this model (10, 33, 208). The broad therapeutic use of FXR agonists could be additionally jeopardized by interfering with FXR functions in lipid homeostasis such as HDL and triglyceride metabolism. FXR activation lowers HDL cholesterol levels by decreasing apolipoprotein A I levels, the major lipoprotein of HDL (209). Cardiovascular side effects therefore should be taken into consideration, when treating patients with FXR agonists for longer periods, especially in chronic cholestatic disorders such as PBC which are typically associated with hypercholesterolemia (214).

4.2. Enzyme inducers

Traditionally, for a long time, cholestatic conditions and related symptoms such as jaundice and pruritus as well as gallstone disease have been treated empirically with a variety of herbal remedies, bile of various animals (see above) or in more recent time’s synthetic drugs (e.g. phenobarbital, rifampicin) without knowing their exact mode of action. Recent molecular investigations of these traditional remedies, have, however, revealed individual components that target nearly all classes of signalling molecules and turned out to function via defined molecular NR-mediated mechanisms. NRs are frequent biological targets of active compounds contained in traditional remedies, since a large set of nuclear receptors are regulated by lipophilic molecules derived from diet, the environment or molecules mimicking endogenous ligands.

Since centuries, Yin Zhih Huang and a number of other herbal decoctions containing Yin Chin (Artemisia capellaris) are widely used for the treatment of neonatal jaundice in Asia and are well known to enhance bilirubin clearance. Recently, an active compound of Yin Chin (dimethylsculetin) was found to be a selective CAR agonist (215). Intravenously administered bilirubin in rodents was efficiently cleared after Yin Chin treatment in wild type mice, but this effect was completely abolished in mice lacking Car. Likewise, the active compound dimethylsculetin only accelerates bilirubin clearance in the presence of Car via transactivation of enzymes and transporters of bilirubin metabolism (215). Similarly, St. John's wort, which has been used for centuries as a tonic for liver disorders, including cholestasis, turned out to be an agonist for PXR (216). In the pre-UDCA area phenobarbital and rifampicin have been widely used in cholestasis long before their mode of action was known. Phenobarbital, which turned out to be a CAR agonist improves pruritus and also reduces serum bile acid concentrations in cholestasis in addition to alleviating pruritus (217-219). Rifampicin, a ligand for PXR was effectively used to treat pruritus of cholestasis but also ameliorates elevated liver function tests (217, 220, 221). However, both drugs can also cause significant side effects ranging from fatigue and somnolence (phenobarbital) to hepatotoxicity and liver failure (rifampicin) (217, 222).

As classical xenobiotic sensors, PXR and CAR agonists induce a battery of phase I and phase II metabolizing enzymes and drug transporters, including enzymes and transporters for bilirubin and bile acids. In BDL mice, pretreatment with rodent Pxr and Car ligands led to a significant reduction of elevated serum bilirubin and bile acid levels, which were accompanied by increased levels of polyhydroxylated bile acids in serum and urine (223). These findings may be explained by a coordinated stimulation of phase I (e.g. Cyp2b10, Cyp3a11) and phase II (e.g. Sult2a1, Ugt1a1) detoxification enzymes together with alternative basolateral overflow systems (e.g. Mrp3, Mrp4), while classical orthograde bile acid and organic anion transporters (Ntcp, Oatp1a1, Oatp1a2, Bsep) remained unaffected (223). A study exploring CA toxicity (the major retained bile acid species in cholestasis) in Fxr- and Pxr-knockout mice revealed that Car agonists can mitigate bile acid toxicity, even when both classical BA receptors are knocked-out, strengthening the fundamental
role of Car in bile acid detoxification (224). In human, administration of the “old-fashioned” PXR agonist rifampicin to healthy volunteers significantly induced CYP3A4, UGT1A1 and MRPI expression resulting in increased bile acid hydroxylation and reduced serum bilirubin levels (198). Given the side effects of the “old-fashioned” NR agonists, the use of specific PXR or CAR agonists for treatment of human cholestasis may be promising. However, one has to keep in mind that these drugs not only detoxify but also can act as a toxin by interacting with several other endogenous metabolic pathways (e.g. thyroid hormone metabolism pathway or influencing drug metabolism, e.g. acetaminophen toxicity) (225-227). A caveat is also the liver tumor promoting effects of glucocorticoids may not only be direct but may be attributed to modulation of bile acid transport and metabolism (11, 233). Glucocorticoids activate the ubiquitously expressed glucocorticoid receptor (GR) and thereby can directly transactivate several transporters (e.g. ASBT, NTCP, MRPI, AE2, and BSEP) (5). However, effects of glucocorticoids may not only be direct but may also be modulated indirectly by other NRs. As such, CAR has been identified as a primary GR response gene with a glucocorticoid responsive element in its promoter region and dexamethasone can increase the translocation of CAR into the nucleus (234). In addition, glucocorticoids increase the levels of PXR and RXRα mRNA and protein (197, 235). Interestingly, UDCA has been reported to activate GR (195, 196, 236, 237), which could in part contribute to beneficial effects of UDCA treatment. In this respect, a combination of UDCA and dexamethasone enhanced transcriptional expression and activity of cholangiocellular anion exchanger AE2, predicted to induce bicarbonate rich choleresis, while UDCA or dexamethasone alone failed to increased AE2 expression and function (238). This may explain some of the beneficial effects of the combination of UDCA and glucocorticoids in PBC patients with inadequate response to UDCA monotherapy.

4.3. Glucocorticoids

Glucocorticoids are used in the treatment of various cholestatic disorders especially in addition to the standard treatment with UDCA, when a single UDCA treatment is not sufficient enough (228-232). Beneficial effects of glucocorticoids on serum parameters of cholestasis (termed ‘steroid whitewash’) and liver histology have been noted, however it remains unclear whether these effects are only the consequence of the anti-inflammatory properties of corticosteroids or whether they can in part be attributed to modulation of bile acid transport and metabolism (11, 233). Glucocorticoids activate the ubiquitously expressed glucocorticoid receptor (GR) and thereby can directly transactivate several transporters (e.g. ASBT, NTCP, MRPI, AE2, and BSEP) (5). However, effects of glucocorticoids may not only be direct but may also be modulated indirectly by other NRs. As such, CAR has been identified as a primary GR response gene with a glucocorticoid responsive element in its promoter region and dexamethasone can increase the translocation of CAR into the nucleus (234). In addition, glucocorticoids increase the levels of PXR and RXRα mRNA and protein (197, 235). Interestingly, UDCA has been reported to activate GR (195, 196, 236, 237), which could in part contribute to beneficial effects of UDCA treatment. In this respect, a combination of UDCA and dexamethasone enhanced transcriptional expression and activity of cholangiocellular anion exchanger AE2, predicted to induce bicarbonate rich choleresis, while UDCA or dexamethasone alone failed to increased AE2 expression and function (238). This may explain some of the beneficial effects of the combination of UDCA and glucocorticoids in PBC patients with inadequate response to UDCA monotherapy.

4.4. Glitazones and nonsteroidal anti-inflammatory drugs

Glitazones are a new class of agents originally approved for the use of type 2 diabetes. The first member of glitazones, troglitazone, had to be withdrawn from the market in 2000, because of several cases of severe acute hepatitis and liver failure (239, 240). The other glitazones, rosiglitazone and pioglitazone, however do not appear to have the same degree of hepatotoxicity, although a class-dependent inhibiting effect on Na+- and ATP-dependent bile acid transport have been reported (241, 242) which could contribute to toxic side effects, especially under cholestatic conditions. Glitazones improve insulin sensitivity via mechanisms, which increase muscle glucose uptake, decrease central adiposity, promote adipocyte differentiation and alter mitochondrial mass and thermogenesis (243). In this setting glitazones (i.e. pioglitazone and rosiglitazone) were promising in pilot experiments in biopsy-proven nonalcoholic steatohepatitis (244-247). However, the use and potential benefit of glitazones in cholestatic disorders has so far supported only experimentally.

Glitazones and non steroidal anti-inflammatory drugs (NSAIDs) (e.g. ibuprofen, indomethacin and naproxen) are potent activators of PPARα (248, 249). PPARα agonists can induce expression of cholesterol transporters ABCA1 and ABCG1 and ABCG2 (250, 251), but a direct role for PPARα in the regulation of bile acid homeostasis has not yet been reported. Rosiglitazone can induce Shp via a Ppar response element in the Shp promoter in rat hepatocytes in vitro (252). Whether PPARα ligands might affect bile acid homeostasis via this pathway also in vivo remains to be determined. Rosiglitazone was demonstrated to reverse LPS-mediated down-regulation of hepatic transporters (e.g. NTCP, BSEP and CYP3A11 without affecting cytokine levels), which implies a role for its potential use in inflammation-mediated liver diseases (253). Moreover, PPARα agonists inhibit HSC activation and counteract liver fibrosis in models of cholestasis (see section 3.4.1 and references. 118, 129, 254).

4.5. Fibrates and statins

Another promising therapeutic approach in cholestasis (in particular chronic vanishing bile duct disorders and cholangiopathies such as PBC and PSC) is the use of PPARα agonists or stimulators. Non-randomized controlled pilot studies with fibrates in patients with PBC, which included only small numbers of patients, showed biochemical and in part also histological improvement (255-260). Statins are also PPARα activators (261) and have been successfully tested in small uncontrolled trials in patients with PBC (262-264). However, a recent study using increasing dosages of atorvastatin over a period of 12 weeks was unable to demonstrate improvement of cholestasis in PBC patients with a prior incomplete response to UDCA, although atorvastatin was safe and efficiently reduced cholesterol levels (265). Whether long-term application of PPARα ligands improves cholestasis and disease outcome in a larger cohort of patients remains to be demonstrated.

From a molecular point of view, the mode of action of PPARα agonists in cholestasis could be linked to the stimulation of the canalicular phospholipid flippase MDR3 (266-269). Increased phospholipid secretion into bile may reduce the aggressiveness of bile by counteracting the detergent effects of bile acids which may protect cholangiocytes. In addition, PPARα positively regulates ASBT expression in cholangiocytes and intestine (28), which increases bile acid absorption from bile ducts and the
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intestine; reabsorption of bile acids from stagnant bile in obstruction might minimize cholangiocyte damage. Indeed, in animal models of obstructive cholestasis fenofibrate administration to bile duct-ligated rats moderately reduced serum markers of cholestasis and histological parameters of liver injury (270). However, atorvastatin in this setting (i.e. common bile duct-ligated mice) efficiently reduced only serum bile acid levels, but did not improve markers of liver injury (223, 271). Some of the positive effects of statins however, may also be attributed to activation of PXR, since statins were also shown to be ligands for PXR (272). Moreover, PPARα is involved in the regulation of bile acid metabolism which may contribute to potential therapeutic effects of PPARα agonists in cholestasis. Bile acid glucuronidation via UGT2B7 and UGT1A3 as well as bile acid sulfatation via SULT2A1 is enhanced by PPARα rendering bile acids more hydrophilic for urinary excretion (12, 273, 274). Also repression of bile acid synthesis via reduced HNF4 binding to the CYP7A1 is mediated by PPARα (275-279). In addition, there is nuclear receptor cross-talk between PPARα and FXR transcriptional pathways since PPARα is an FXR target gene (280). Thus, FXR agonists may add to their therapeutic spectrum by additionally activating PPARα pathways.

4.6. Others

Recently, several other nuclear receptors were found to activate genes involved in bile acid metabolism and transport. Data are, however, largely obtained in vitro or in animal models and effects of agonists on bile acid metabolism and cholestasis in human are almost entirely missing.

Besides FXR and PXR, VDR is the third nuclear receptor which is activated by (mostly hydrophobic) bile acids (i.e., LCA) (281). In contrast to high expression in intestine, VDR expression in liver is low in human hepatocytes and is mainly restricted to Kupffer cells, endothelial cells, biliary epithelial cells and HSC. Rather, VDR seems to be an intestinal sensor and defender against the highly hydrophobic and toxic secondary bile acid LCA (9, 123). In line with this concept, activation of VDR by vitamin D or LCA in vitro induces expression of CYP3A4 which can detoxify LCA via phase I hydroxylation (281). Also SULT2A1 is a target for VDR, which mediates phase II sulfation of LCA (20, 282). In addition, induction of Mrp3 in colon and Asbt in the ileum after targeting Vdr has been reported in vivo in rodents and in vitro data indicate that VDR negatively interacts with FXR, since calcitriol inhibits FXR transactivation (9, 283, 284). However, conclusive experiments in animal models of cholestasis, which investigate effects of Vdr agonists on clinical parameters of cholestasis, are missing, so far. Moreover, given the role of LCA for carcinogenesis of colon cancer, the induction of intestinal detoxification mechanisms for LCA via activation of VDR rather explain the proposed protective effects of vitamin D and its receptor agonists in colon cancer (285-287).

Recently, also LXRα which is activated by oxysterols, unsaturated fatty acids and 6α-hydroxylated bile acids (288-290) and primarily acts as cholesterol sensor to keep cholesterol homeostasis in balance, has been reported to reduce cholestatic liver injury. Lxrα transgenic mice and mice treated with a synthetic Lxrα agonist were resistant to liver damage induced by LCA feeding and bile duct ligation (38). In contrast, Lxrα knockout animals displayed severe liver injury after bile duct ligation. Enhanced cholestatic liver injury was attributed to reduced urinary bile acid elimination via reduced expression of phase II sulfotransferase Sult2a9 and alternative export via Mrp4 in knockout animals lacking the Lxr-mediated induction of these target genes (38). Also UGT1A3, which is able to glucuronidate bile acids has been identified as LXRα target (291). Moreover, LXR agonists in vivo were shown to have anti-inflammatory properties and reduce the expression levels of TNFa and IL1β (132). Thus, beside its role for cholesterol homeostasis, targeting LXRα should be further evaluated as a target also in cholestatic liver disease, especially in those forms with an inflammatory component (see also Mulder et al. in this issue).

5. CONCLUSIONS AND OUTLOOK

NRs play a key role in regulating several key aspects of normal bile acid, bilirubin and biliary lipid transport and metabolism. Thus, alterations in NR function contribute to the pathogenesis of cholestasis and may explain several secondary, mostly adaptive changes in bile acid transport and metabolism. The central role of NRs in hepatobiliary physiology and pathophysiology makes them attractive candidates for pharmacological treatment of cholestasis and many drugs already used in cholestatic liver diseases are effective NR ligands (e.g., glucocorticoids, glitazones). However, a major drawback of several currently available NR ligand drugs (e.g., rifampicin) is their low grade of specificity and considerably high risk for side effect with long-term treatment. The future should bring us more effective and specific (e.g., gene-selective) NR ligands.

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**Abbreviations:** α-SMA, alpha smooth muscle actin; BA, bile acids; BDL, bile duct ligation; BSEP, bile salt export
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pump; CAR, constitutive androstane receptor; CYP3A1, cytochrome P450 3A; CYP7A, cholesterol 7α-hydroxylase; FTF, fetal transcription factor; FXR, farnesoid X-activated receptor; GR, glucocorticoid receptor; GST, glutathione-S-transferase; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HNF1α, Hepatocyte Nuclear Factor 1-alpha; HSC, hepatic stellate cells; IBABP, ileal bile acid-binding protein, FABP6; LXR, liver X receptor; MRP3, multidrug resistance-associated protein 3; MRP4, multidrug resistance-associated protein 4; NR, nuclear receptor; NTCP, sodium-bile acid cotransporter, SLC10A1; OATP, organic anion transporting polypeptide ; OSTα/OSTβ, organic solute transporter alpha/beta; PBC, primary biliary cirrhosis; PFIC, progressive familial intrahepatic cholestasis; PPARα, γ, peroxisome proliferator-activated receptors α, γ; PXR, pregnane X-receptor; RAR, retinoid acid receptor; RXR, retinoid X receptor; ROS, reactive oxygen species; SHP, small heterodimer partner; SULT2A1, sulfotransferase 2A1; UGT, UDP-glucuronosyltransferase; VDR, vitamin D receptor

Key Words: Nuclear Receptors, Cholestasis, Farnesoid X-activated Receptor, Pregna X-receptor, Constitutive Androstanee Receptor, Review

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