New paradigms in the treatment of hepatic cholestasis: From UDCA to FXR, PXR and beyond

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Summary

Cholestasis is an impairment of bile formation and flow. It is the first, and for the moment, most established medical treatment is the natural bile acid (BA) ursodeoxycholic acid (UDCA). This secretagogue improves, e.g. in intrahepatic cholestasis of pregnancy or early stage primary biliary cirrhosis, impaired hepatocellular and cholangiocellular bile formation mainly by complex post-transcriptional mechanisms. The limited efficacy of UDCA in various cholestatic conditions urges for development of novel therapeutic approaches. These include nuclear and membrane receptor agonists and BA derivatives. The nuclear receptors farnesoid X receptor (FXR), retinoid X receptor (RXR), peroxisome proliferator-activated receptor α (PPARα), and pregnane X receptor (PXR) are transcriptional modifiers of bile formation and at present are under investigation as promising targets for therapeutic interventions in cholestatic disorders. The membrane receptors fibroblast growth factor receptor 4 (FGFR4) and apical sodium BA transporter (ASBT) deserve attention as additional therapeutic targets, as does the potential therapeutic agent norUDCA, a 23-C homologue of UDCA. Here, we provide an overview on established and future promising therapeutic agents and their potential molecular mechanisms and sites of action in cholestatic diseases.

Introduction

Bile was first mentioned in the Ebers Papyrus (circa 1550 B.C.) as a useful remedy and purge [1]. Dried black bear's bile rich in ursodeoxycholic acid (UDCA) was recommended in China for treatment of jaundice at times of the Tang dynasty (618-907 A.D.) as documented in the Tang Materia Medica, the first state pharmacopoeia worldwide. In the Western hemisphere, bile had been regarded as a major constituent of the human body by the Corpus Hippocraticum and Galen of Pergamon, but was increasingly seen as a useless excrement by the 16th and 17th century (“cloaca sordium et superfluitatum”) [1]. Only during the last centuries, the digestive function of bile was recognized and during the last 30 years the signaling and therapeutic potential of its major constituents, bile acids (BAs), and the (patho-)physiological role of BAs, phospholipids, bicarbonate and other bile constituents were unraveled. Today, bile formation is regarded as a vital secretory process modulated by complex transcriptional and post-transcriptional mechanisms in hepatocytes, cholangiocytes and ileocytes [2–4].

Cholestasis is an impairment of bile formation and flow. It may result from; (i) hepatocellular and/or cholangiocellular secretory defects; or (ii) obstruction of bile ducts by bile duct lesions, stones or tumours, but may also be related to mixed mechanisms in conditions such as primary biliary cirrhosis/cholangitis (PBC) or primary sclerosing cholangitis (PSC). For adequate treatment of cholestasis and cholestatic injury, identification and targeting of the defective hepatocellular and cholangiocellular secretory mechanisms and/or bile duct lesions (or removal of obstructing stones and tumours) is required. Evolving pathophysiological insights in cholestatic disorders, particularly chronic fibrosing cholangiopathies such as PBC, PSC and (other) secondary forms of cholangitis, provide novel opportunities for the development of therapeutic approaches for these disorders. Currently, treatment with UDCA may slow the
progression of chronic cholangiopathies, but has limited or no proven efficacy in various chronic cholestatic disorders and cannot heal them. Immunosuppressive/immunomodulating interventions aiming to minimize immune-mediated damage in immune-mediated/autoimmune cholestatic disorders such as PBC and PSC have disappointed in the past, but new approaches, which are beyond the scope of this review, are at present under consideration for clinical evaluation. The use of specific modifiers of hepatobiliary secretory and cellular protection mechanisms against BA-mediated cytotoxicity may eventually give rise to new classes of disease-modifying drugs. Here, we provide an overview of transcriptional and post-transcriptional modulators of bile formation which may serve as therapeutic agents in the future for the treatment of cholestatic disorders.

UDCA: clinical use

Therapy with natural BAs arose in the 1970s when it was discovered that oral administration of chenodeoxycholic acid (CDCA) induces the dissolution of cholesterol gallstones. However, CDCA induced biliary cirrhosis in some species and was shown to be mildly hepatotoxic and induced dose-dependent diarrhea in humans [5]. Thereafter, UDCA was shown to have similar efficacy in gallstone disease without any side effects [6]. The markedly different behaviour of the two natural BAs was ascertained by numerous experimental studies in vitro and in vivo.

UDCA was thereafter proposed as a potential therapeutic approach for chronic cholestatic disorders with the following rationale: (a) accumulation of toxic BAs might be at least in part responsible for liver injury in chronic cholestasis; (b) replacement of endogenous BAs by a non-toxic BA (UDCA) could protect the liver and slow down the progression of these disorders. This hypothesis was first tested in PBC [7]. UDCA was shown to provide marked improvement in serum liver tests [7,8]. Placebo-controlled trials showed that UDCA also improves histological features and delays progression to cirrhosis and the time to liver transplantation [9–14]. Today, UDCA therapy is recommended for all patients with PBC provided that they show abnormal serum liver tests [15,16]. The accepted optimal dose is 13–15 mg/kg/day. All patients with PBC do not respond to UDCA in the same way. The transplant-free survival rate among UDCA-treated patients remains significantly lower than that of an age- and gender-matched control population [17], indicating that there is a need for new therapeutic options particularly for patients with a suboptimal biochemical response to UDCA and predictive factors of a poor outcome [18]. Serum bilirubin was shown to be the most potent prognostic marker in PBC as were also serum albumin, prothrombin time and cirrhosis, the traditional prognostic factors in advanced liver disease. More recently, the biochemical response to UDCA has been shown to predict long-term outcomes and, thus, may be applied as a simple selection criterion for clinical trials [18–23].

In PSC, UDCA may lower disease progression but long-term efficacy remains uncertain [24,25]. A placebo-controlled trial using very high doses of UDCA (28–30 mg/kg/day) showed that UDCA was not only ineffective but also harmful in that more patients developed varices or were listed for liver transplantation [26]. Therefore, no evidence-based recommendation can be given for normal doses, but very high-dose regimens should be avoided [16,27]. UDCA therapy has been used for a number of other clinical conditions. Efficacy is regarded as likely in ABCB4 deficiency with progressive familial intrahepatic cholestasis type 3 (PFIC-3) and/or low phospholipid-associated cholelithiasis (LPAC syndrome) and cystic fibrosis-associated liver disease (CFALD). Efficacy is regarded as uncertain in various forms of sclerosing cholangitis, drug-induced liver injury, progressive familial intrahepatic cholestasis type 1 & 2, sarcoidosis hepatitis, prevention of bile duct injury after liver transplantation, and total parenteral nutrition (TPN)-induced cholestasis [16]. In all of these conditions (as in PSC), no clear-cut survival benefit with UDCA has been shown.

UDCA: major molecular mechanisms and sites of action

Dried black bear’s bile was recommended more than a thousand years ago for treatment of jaundice at times of the Tang dynasty in China as mentioned above. UDCA may form up to 60% of black bear’s total BAs [28] whereas it forms only 1–3% of total BAs in human bile, but is enriched to 40% in bile of patients with PBC and healthy volunteers treated with therapeutic UDCA doses (13–15 mg/kg/day) [29]. UDCA has potent anticholestatic and antiapoptotic properties in conditions of hepatocellular (e.g., intrahepatic cholestasis of pregnancy (ICP)) or cholangiocellular cholestasis (e.g., early PBC).

Early after the first peer-reviewed reports on UDCA in PBC [7,8] it was proposed that UDCA exerts its hepatoprotective effects in cholestatic liver disease mainly by stimulating impaired hepatobiliary secretion [30]. In the 1990’s, UDCA was then unraveled as a potent intracellular signaling molecule acting as a Ca2+/mitogen-activated protein kinase (MAPK) [38,39] and α5β1 integrins [40,41] in hepatocytes. It was earlier proposed [33,42] and later experimentally proven that UDCA conjugates as potent signaling molecules that might stimulate secretion of hepatocytes (and cholangiocytes [43]) by activating vesicular exocytosis and carrier insertion into their apical membranes resulting in cholERIC effects via a dual MAPK- and integrin-dependent mechanism in healthy liver [39,40] and in anticholestatic effects via Ca2+-type II inositol-1,3,4-triphosphate receptor/cPKCα/PAK-dependent mechanisms in cholestatic liver [44–46]. It remains to be proven if these complex post-transcriptional molecular mechanisms unraveled in experimental animals may explain the cholERIC and anticholestatic effects of UDCA in man.

More recently, the ‘biliary HCO3 umbrella’ hypothesis has been introduced as a protective mechanism for hepatocytes and cholangiocytes against the toxic effects of millimolar BA mono- mers present in bile [47]. This hypothesis indicates that biliary HCO3 secretion in humans serves to maintain an alkaline pH near the apical surface of hepatocytes and cholangiocytes to prevent the uncontrolled membrane permeation of protonated glycine-conjugated BAs which have a pKα ≥ 4. Notably, the experimental proof of concept also unraveled that an intact ‘biliary HCO3 umbrella’ is critically dependent on adequate function of the major HCO3 exporter, the Cl-/HCO3 exchanger AE2, and an intact biliary glycocalyx in human cholangiocytes [48]. Functional impairment of this biliary HCO3 umbrella or its regulation would lead to enhanced vulnerability of cholangiocytes and periportal hepatocytes towards the attack of apolar hydrophobic BAs. Notably, UDCA stimulates biliary HCO3 secretion under
The original proposal that UDCA may exert anticholestatic effects by removing major hydrophobic BAs such as CDCA or DCA from the circulating BA pool was disproved during short-term UDCA treatment when cholestasis improved, but hydrophobic BA pool sizes remained stable [52]. In contrast to hydrophobic BAs such as CDCA or lithocholic acid (LCA), UDCA also does not markedly affect transport protein expression in vivo at the transcriptional level to modulate transport capacity and probably exerts only limited post-transcriptional modification of carrier expression [53].

Antiapoptotic mechanisms [28,54–56] and effects on endoplasmic reticulum stress may contribute to the cytoprotective action of UDCA in cholestatic liver disease as summarized elsewhere [28]. Of note, UDCA has early been described to act as a glucocorticoid receptor (GR) agonist in a ligand-independent way [57–60]. Interaction of UDCA with the GR has been linked to the antiapoptotic effect of UDCA in TGF-β1-induced liver-cell apoptosis [61]. Thus, antiapoptotic and anti-inflammatory actions of UDCA in the liver and bile ducts may at least in part be secondary to this and the numerous effects described above.

Norousodeoxycholic acid: experimental and clinical effects

24-norousodeoxycholic acid (norUDCA) is a side chain shortened UDCA derivate which lacks a methylene group resulting in a relative resistance to amidation with taurine or glycine compared with UDCA [62–65]. As a result, norUDCA is passively absorbed from cholangiocytes and undergoes ‘cholehepatic shunting’ (instead of a full enterohepatic cycle) which generates a HCO₃⁻ anion resulting in induction of a HCO₃⁻–rich hypercholeresis [62,65,66] counteracting intrinsic BA toxicity [67] and reinforcing the ‘biliary HCO₃⁻ umbrella’ [47,48]. Cholehepatic shunting may also allow ‘ductular targeting’ of drugs [62,68]. As a proof of principle, taurine-conjugated norUDCA and bis-norUDCA (resulting from additional side chain shortening) lack cholehepatic shunting properties [69], emphasizing the unique properties of norUDCA. In addition, norUDCA is more hydrophilic and thereby less toxic for hepatocytes and cholangiocytes in vitro than its mother compound UDCA [70] which may further help to counteract (intrinsically) biliary toxicity. Notably, neither norUDCA nor UDCA have relevant affinities for dedicated BA receptors such as the farnesoid X receptor (FXR) or G protein-coupled plasma membrane receptor TGR5 [71], norUDCA (but not “conventional” UDCA or bis-norUDCA) reversed sclerosing cholangitis in the experimental Mdr2/Abcb4 knockout mouse (Mdr2/Abcb4<sup>−/−</sup>) cholangiopathy model for sclerosing cholangitis, while the mother compound UDCA even aggravated bile infarcts in cholestatic conditions with biliary obstruction [68–70]. Moreover, norUDCA has anti-lipotoxic, anti-proliferative, anti-fibrotic as well as anti-inflammatory effects which may complement stimulation of HCO₃⁻ secretion with BA detoxification and induction of alternative export via overflow systems at the basolateral membrane [68,71] (Fig. 2). Notably, in a hepatocellular model of cholestasis induced by TLCA, taurine-conjugated norUDCA had anti-cholestatic and anti-apoptotic properties, suggesting that combination of UDCA and norUDCA may be superior to UDCA or norUDCA monotherapy in biliary disorders in which both hepatocyte as well as cholangiocyte dysfunction are involved in the pathophysiology of the disease [72].
norUDCA is currently undergoing further clinical development in humans. The final results of a double-blind, randomized, European multicenter, placebo-controlled, comparative, exploratory phase II dose-finding trial in the treatment of PSC are expected for 2015 [73]. Future clinical indications, next to PSC, may include PBC and cystic fibrosis-associated liver disease/cholangiopathy where defects of the biliary HCO3 umbrella may also be involved [47,48]. Notably, norUDCA induces a HCO3-rich choleresis independent of cystic fibrosis transmembrane conductance regulator (CFTR) [69] consistent with the concept of cholehepatic shunting which does not appear to involve active transport processes [62]. Promotion of HCO3-rich bile flow may also beneficially affect sclerosing cholangitis of critically ill patients, non-anastomotic strictures following liver transplantation, or ABCB4 deficiency with progressive familial intrahepatic cholestasis type 3 (PFIC-3) [47,74]. Collectively, these broad and multiple levels of mechanisms make norUDCA an attractive therapeutic agent for cholangiopathies [73].

FXR – FGF19: experimental and clinical effects

The discovery of the nuclear hormone receptors in the 1990s was followed by the notion that BAs serve as their ligands [75–79]. This caused a dramatic turn around in BA research. In addition to understanding the role of BAs in regulating their own synthesis and transport, it is now clear that the post-prandial surge of BAs through intestine and liver, and to a lesser degree through adipose tissues, kidney and muscle, triggers signals that prepare the organism for production or storage of energy [80]. Recent studies also show that BA signaling is a major regulator of the circadian rhythm of metabolism [81,82].

Nuclear hormone receptors act as intracellular ligand-activated receptors (Table 1). Cholic acid (CA) and CDCA bind to the FXR (NR1H4). FXR, as a heterodimer with the all-trans retinoic acid (ATRA) receptor RXR, binds to the LR-1 DNA motive in the promoter region of target genes [75,83]. FXR target genes include

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**Table 1. Potential future therapeutic agents which modulate bile formation and secretion.** See text for potential molecular mechanisms of action and clinical observations.

Fig. 2. Proposed mechanisms of action of norUDCA in the Mdr2 (Abcb4)−/− model of sclerosing cholangitis. As hydrophilic bile acid (BA) and by generating a bicarbonate-rich (hyper)choleresis due to cholehepatic shunting, norUDCA counteracts intrinsic biliary toxicity resulting from absent phospholipid (PL) secretion with increased free non-micellar bound BAs in this model. Important, cholehepatic shunting allows ‘ductal targeting’ of anti-inflammatory, anti-fibrotic and anti-proliferative effects to injured bile ducts, resulting in ‘ductal healing’. norUDCA also counteracts lipotoxic fatty acid composition and promotes BA detoxification and elimination via basolateral efflux pumps facilitating their subsequent renal excretion. Modified after [200].
and binds to the FGFR4/βKlotho receptor on hepatocytes (Fig. 3). This activates a series of MAP-kinases (e.g. ERK-1 and ERK-2) and suppresses CYP7A1 expression [88]. Like FXR, FGF19 has strong metabolic effects as it suppresses the insulin-stimulated expression of the lipogenic enzymes FAS and SREBP1c and stimulates glycogen synthesis and reduces the expression of gluconeogenic enzymes [96–98]. Thus, FGF19 has insulin-like effects except that it inhibits lipogenesis while insulin stimulates it. There is a debate on the importance of direct BA/FXR/SHP signaling in the liver vs. indirect signaling by FGF19 from gut to liver in the repression of CYP7A1 [99,100]. Both actions probably reinforce each other. SHP and additional factors (Shp2 in mice [101]) are required for FGF19/FGFR4 signaling in the liver.

During fasting FGF21 is produced in the liver as a result of free fatty acid-stimulated PPARγ [102]. FGF21 increases fatty acid oxidation and ketogenesis in the liver and in cooperation with PPARγ improves insulin sensitivity and glucose uptake in white adipose tissue [103–105]. Notably, FGF21 stimulates adiponectin secretion in white adipose tissue of mice [106,107].

BAs in excess are cytotoxic and their synthesis is tightly regulated. In liver disease this regulation may be insufficient or may have gone astray and this prompted researchers to design BA analogues and non-BA compounds with high FXR affinity [108–112]. These agents are tested as drugs for the treatment of PBC, PSC and non-alcoholic fatty liver disease (NAFLD). This latter indication rests on the fact that FXR inhibits lipogenesis and gluconeogenesis and improves insulin sensitivity, important factors in the pathogenesis of NAFLD/NASH.

FGX agonists such as the BA derivative obeticholic acid (OCA, 6-ethyl-chenodeoxycholic acid) [113] and the non-BA PX-102 are tested in phase II and phase III trials [114]. In a recent study, PBC patients with an incomplete response to UDCA, received 10 mg, 25 mg, and 50 mg OCA or placebo [113]. UDCA therapy was continued. The results showed a 20% decrease in alkaline phosphatase in 70% of patients. Gamma-glutamyltransferase, CRP and IgM also decreased. However, fifty percent of PBC patients in this study suffered from pruritus at baseline and this did not improve. Pruritus even worsened in patients receiving 25 or 50 mg OCA. In a new phase III trial, pruritus is addressed by using a lower dose (<10 mg) of OCA (NCT01473524). Pruritus as a side effect of OCA was also observed in a NASH trial suggesting that pruritus may be directly OCA- and not disease-related. The mechanism of OCA-related pruritus remains unclear [115].

As potential drugs, FGF19 and FGF21 have the disadvantage that they need to be injected but this may be counterbalanced by different action profiles. The non-tumorigenic FGF19 derivative NGM 282 in combination with UDCA has entered phase II in PBC patients [116] (NCT02135536). For PSC therapeutic possibilities are limited. FXR agonists can be considered but FGF19 induction by these agonists may be a caveat. FGF19 has carcinogenic properties and effects on cholangiocarcinogenesis have to be carefully considered [117]. Nevertheless, ATRA, a ligand of RXR, the heterodimer of FXR, in combination with UDCA, in a short-term small non-randomized trial, has most recently been reported to reduce alkaline phosphatase, serum ALT and BA levels (Assis et al., unpublished).

TGR5: experimental effects

In addition to FXR and other nuclear hormone receptors, BAs can also signal through a membrane-bound BA-specific receptor
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TGR5 (also known as GPBAR1 or M-BAR/BG37) [118]. The most potent endogenous TGR5 activator is LCA followed by DCA [119], while other BAs are less potent. Of note, TGR5 is expressed in various tissues with low or even absent FXR such as spleen, lung or adipose tissue [118,120] with the highest expression in gallbladder and colon [121–123]. In liver, sinusoidal endothelial cells, Kupffer cells and intrahepatic bile ducts express TGR5 (with high expression in human and rat, lower in mouse), while hepatocytes and quiescent stellate cells do not express TGR5 [124–126].

TGR5 activation inhibits pro-inflammatory cytokine production, migration and phagocytic activity of macrophages and Kupffer cells [120,124], in part by suppression of NF-κB signaling [127] [120,128,129]. Accordingly, mice lacking TGR5 display aggravated liver injury after LPS challenge [129]. TGR5 is also involved in modulation of intestinal inflammation, motility, and improves intestinal barrier function thereby protecting from DSS induced colitis in rodents [130–134]. These findings may be of potential relevance for the gut-liver axis in cholangiopathies such as PSC.

Mice lacking TGR5 have a decreased total BA pool size [121], increased CYP7A1 gene expression [123] and a more hydrophobic BA composition [135] which may be due to the impact of BA-mediated TGR5 activation in inhibiting gallbladder contractility. As such, TGR5 activation by hydrophobic BAs inhibits gallbladder smooth muscle contractility [136]. TGR5−/− mice display prolonged cholestasis, exacerbated inflammatory response and more severe liver injury after partial hepatectomy, dietary BA-challenge or bile duct ligation [135,137]. Importantly, TGR5 polymorphisms in PSC patients may imply a potential role in cholangiocyte pathophysiology [138,139]. Cholangiocytes express TGR5 at the apical membrane/cilia where it may sense the luminal BA concentration and regulate cholangiocellular HCO3−/fluid secretion via CFTR and AE2 [122]. Surprisingly, a highly potent TGR5 agonist (INT-777) failed to induce HCO3− output and bile flow in healthy mice as well as in Mdr2−/− model without improvement of bile duct injury [140], findings which could be explained by relatively low TGR5 expression in mouse cholangiocytes. However, mice overexpressing TGR5 showed less liver injury in a mouse model of xenobiotic (DDC)-induced scarring cholangitis, while mice lacking TGR5 showed aggravation of inflammation and fibrosis [141]. Collectively, these findings suggest a critical role of TGR5 for liver protection against BA overload, primarily through the control of bile hydrophobicity and cytokine secretion. Conversely, TGR5 deficient mice are protected against lithogenic diet-induced gallstone formation [123] suggesting that inhibition rather than activation of TGR5 may be beneficial for gallstone disease. On the other hand, TGR5 activation of biliary HCO3− secretion could theoretically counteract gallstone formation, at least at the level of bile ducts.

Importantly, activation of TGR5 may also have some undesired off-target effects. For example, bile reflux-induced pancreatitis has been linked to BA-mediated TGR5 activation in mouse pancreas [142]. Moreover, TGR5 activation promoted oxidative stress in astrocytes [143] and activated AKT signaling in cardiomyocytes possibly contributing to cardiac hypertrophy under cholestatic conditions [144]. More recently, TGR5 has also been implicated in the pathogenesis of pruritus [145,146]. TGR5-activation by BAs was linked to increased hepatocellular apoptosis [147] through activation of c-Jun N-terminal kinase (JNK) signaling pathways while cholangiocytes seem to evade apoptosis via TGR5 [148]. Importantly, TGR5 is also highly expressed in gastric and oesophageal adenocarcinoma as well as gallbladder carcinoma where it promotes cell proliferation in response to BA [149]. Such extrahepatic “off-target effects” may need consideration when developing BA receptor ligands as therapeutics in patients with liver disease.

PPARz: experimental and clinical effects

The PPARs are enriched in tissues with high energy metabolism such as liver (PPARζ, NR1C1), skeletal muscle, heart and gastrointestinal tract (PPARγ/δ, NR1C2) and adipose tissue (PPARγ, NR1C3) [150]. Fatty acids and their derivatives are the natural ligands for these receptors. By stimulating fatty acid oxidation, PPARζ has anti-steatotic effects. However, PPARζ also has anti-inflammatory actions. It was recently argued that the anti-inflammatory action of PPARζ is based on trans-repression of AP1 and NF-κB signaling while its metabolic action depends on direct trans-activation of metabolically active target genes. By introducing a mutation in the zinc-finger domain of PPARζ, a DNA-binding deficient PPAR derivative with maintained anti-inflammatory activity but no metabolic activity was recently produced [151].

For the application of PPAR agonists in cholestatic liver disease the notion that the canalicular phospholipid translocator MDR3 is a PPARz responsive gene is relevant [152,153]. Treatment with PPAR agonists (fibrates) increases MDR3 insertion into the canalicular membrane [153–155]. This stimulates phosphatidylcholine secretion and protects cholangiocytes against bile salt toxicity. This is among the rationales to test fibrates in PBC and PSC. Other actions of PPAR agonists, that underscore their possible therapeutic use, are repression of CYP7A1 and induction of CYP3A4 enzymes that are instrumental for bile salt synthesis and detoxification, respectively [156]. In cholestasis inflammatory and pro-fibrotic genes are activated and it is possible that the anti-inflammatory action of PPAR agonists is equally important [157]. In most trials in which fibrates were tested as treatment of UDCA-refractory PBC, the endpoint has been a decrease of alkaline phosphatase [158]. In a recent meta-analysis on studies comparing patients treated with UDCA plus bezafibrate vs. UDCA alone, combination therapy performed better than monotherapy regarding biochemical parameters but as for symptoms and survival there was no difference (meta-analysis in [159]). In the one study wherein liver stiffness was measured no change was observed [160]. Notably, a significant improvement of pruritus in PBC patients receiving bezafibrate was recently reported [160]. Rigorous testing of histologic endpoints and transplantation-free survival needs to be performed but fibrates may be too weak a PPAR agonist to be successful. Currently stronger PPAR agonists are developed with considerable anti-inflammatory activity [150]. Although primarily developed for the treatment of NASH, these may be promising agents for the treatment of PBC and PSC as well (Table 1).

The pregnane X receptor (PXR): experimental and clinical effects

The PXR (NR112) has a critical role in regulating the expression of genes involved in detoxification and metabolism of BA, drugs and other toxins [161]. PXR modulates expression of CYP3A4 and
CYP7A1 [162,163], SULT2A1 [164], UGT1A1, UGT1A3, and UGT1A4 [165,166], MDRI [167], MRP2 [168], MRP3 [169], and O斯塔 [166]. Cholestatic PXR knockout mice exhibited more hepatic damage than wild-type mice both after bile duct ligation and cholic acid feeding, [170-172]. The potent PXR ligand 5-pregnen-3β-ol-20-one-16β-carbonitrile (PCN) reduced (litho-)cholic acid-induced liver injury in wild-type mice, but not in PXR knockout mice [163,172]. Marked upregulation of the basolateral BA efflux transporter MRP3 (ABCC3) may have been crucial in mediating the beneficial effect of PCN [172].

Human PXR agonists include lithocholic acid and a number of drugs including rifampicin, statins, corticosteroids, phenobarbital, and St. John’s wort. The antibiotic rifampicin is a potent human PXR activator and an evidence-based treatment for pruritus in cholestatic patients [16]. Rifampicin has been reported to improve serum liver tests in PBC [173,174]. In otherwise healthy gallstone patients, rifampicin induced upregulation of UGT1A1 and MRP2 facilitating bilirubin elimination and increased CYP3A4 expression facilitating detoxification of BAs [53]. Rifampicin also markedly induced CYP3A metabolism in patients with early stage PBC and healthy controls [175]. All these effects were not observed with UDCA indicating that the combined use of UDCA and rifampicin might have synergistic beneficial effects in patients with non-obstructive cholestasis [175]. Rifampicin was reported to be safe in cholestatic liver disease during short-term use for up to two weeks [176]. However, severe hepatotoxicity has been reported in up to 13% of patients with cholestatic disorders after use for more than 4 weeks [174]. Strikingly, rifampicin has been shown most recently to completely reverse severe persistent hepatocellular secretory failure [166] induced by drugs (e.g., clavulanic acid, flucloxacilline, estrogen + progesterone, testosterone, total parenteral nutrition) or transient biliary obstruction (e.g. cholechocholitis, pancreatic carcinoma) in formerly healthy individuals, an enormous relief for otherwise desperate patients [166]. A prospective, controlled trial on the promising effect of PXR agonists in severe persistent hepatocellular secretory failure is under preparation.

The GR and UDCA: experimental and clinical effects

The use of glucocorticoids to suppress the inflammation in PBC has been always considered as a very attractive approach, but at the cost of serious side effects, especially aggravating osteopenia. Budesonide is a non-halogenated glucocorticoid mainly absorbed in the small intestine. Of an oral dose, 90% is metabolized during the first liver pass in healthy individuals. Of an oral dose, 90% is metabolized during the first liver pass in healthy individuals.

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The vitamin D receptor (VDR): experimental findings

VDR ligands represent potentially attractive agents for pharmacotherapy of autoimmune cholestatic disorders because they may influence several key processes involved in the pathogenesis such as innate and immune activation, BA metabolism and detoxification, bile duct integrity and fibrogenesis. VDR is expressed in almost all immune cells and mediates the immunoregulatory properties of vitamin D. Indeed, vitamin D through VDR interferes directly with T cells by inhibiting the production of T-helper-1 (Th1) type cytokines, while promoting those of the Th2 subtype. Furthermore, vitamin D inhibits dendritic cell differentiation resulting also in a decrease in Th1 cell development. Taken together, these observations indicate that vitamin D through VDR diminishes the effector T cell response suggesting that the vitamin D-VDR axis may be involved in autoimmune diseases [188]. In bile duct epithelial cells, activation of VDR by BAs or vitamin D induces expression of cathelicidin, an anti-microbial peptide known to be protective against bacterial infection. Cathelicidin is known to neutralize the deleterious effects of LPS that accumulates in the biliary tree in fibrosing cholangiopathies [189].

Treatment with VDR agonists stimulates BA detoxification enzymes (such as CYP3A4 and SULT2A1) in the liver and intestine, and protects against lithocholic acid hepatotoxicity [190]. L25(OH)2D3 was also shown to decrease hepatic Cyp7a1 expression by increasing the expression of fgl15 in the intestine. Consistently, Cyp7a1 expression was increased in mice lacking VDR when compared to wild-type mice, indicating that intestinal VDR activity controls the basal expression of Cyp7a1 [191].
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The anti-fibrotic potential of VDR stimulation has been demonstrated in several models of liver fibrosis, among them the Mdr2 (Abcb4)−/− mice [192,193]. Recently, the vitamin D-VDR axis has been shown to modulate fibrogenesis and hepatic stellate cell activity through a complex mechanism involving epigenetic modifications induced by the SMAD pathway [194].

ASBT inhibitors

The ASBT (SLC10A2) at the luminal surface of ileum enterocytes transports conjugated BA from the gut lumen into the enterocytes [195]. From here BA are secreted into the portal circulation. Under normal physiological conditions ASBT works at maximum capacity since overproduction of BA in the liver leads to increased spill over of BA into the colon [196]. In the colon, BA activate chloride channels and this causes watery diarrhea [197]. Moderate inhibition of ASBT however can have beneficial effects while avoiding diarrhea. ASBT inhibition lowers the intra-mucosal concentration of BA with less activation of FXR, lowered synthesis of FGF19 and unpressed expression of CYP7A1 in the liver. This causes an enhanced conversion of cholesterol to BA and lowers serum cholesterol. Spill over of BA into the colon will involve epigenetic modifications induced by the SMAD pathway [194].

Conclusion

Three decades after the introduction of UDCA as the first anticholestatic agent into clinical practice to treat patients with chronic cholestatic disorders, an enormous progress in the understanding of the molecular pathophysiology of hepatocellular and cholangiocellular cholestasis has led to the development of a variety of novel therapeutic options which are currently under evaluation. Novel immunomodulating approaches are beyond the scope of this review. While UDCA exerts its anticholestatic effects mainly by post-transcriptional mechanisms as a potent intracellular signaling agent and secretagogue, candidates for future combined treatment with UDCA mostly represent transcriptional modulators of secretion and cell protection or membrane receptor agonists. Among these, evaluation of agonists for FXR, GR, and PPARs in combination with UDCA is far advanced to large scale phase III trials in chronic cholestatic disorders such as PBC. The next line of upcoming therapeutic agents, often in combination with UDCA, for cholestatic disorders includes the 23C-analogue of UDCA, norUDCA, as well as PXR agonists, FGF19 derivatives and ASBT inhibitors. While there was 30 years ago no effective treatment available, promising times for patients with cholestatic disorders and their caring physicians are visible on the horizon.

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Conflict of interest

UB signed consultancy agreements (via University of Amsterdam) with Intercept and Novartis and received lecture fees from Falk Foundation, Gilead, Roche.

MT has received research grants from Albireo, Intercept and Falk Pharma, travel support from Falk Foundation and Gilead, and has served as advisor for Albireo, Falk Pharma, Genfit, Intercept and Phenex. MT is listed as co-inventor of patents on the medical use of norUDCA (WO 2006/119803 A1 and WO 2009/013334).

PJ has a consultancy agreement with Shire and has received obeticholic acid from Intercept for basic studies.

RP received fees for an advisory board meeting from Intercept in 2014.

References


Review


