Review

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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/flow at the level of the hepatocyte and/or cholangiocyte. 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 *nor*UDCA, 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.

Keywords: UDCA; FXR; PXR; Cholestasis.
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Abbreviations: AE2, anion exchanger 2; ASBT, apical sodium bile salt transporter; BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; CFALD, cystic fibrosis-associated liver disease; CFTR, cystic fibrosis transmembrane conductance regulator; DCA, deoxycholic acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GR, glucocorticoid receptor; IBD, inflammatory bowel disease; ICP, intrahepatic cholestasis of pregnancy; LCA, lithocholic acid; LPAC, low phospholipid-associated cholelithiasis; MAPK, mitogen-activated protein kinase; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PFIC, progressive familial intrahepatic cholestasis; PKA, protein kinase A; PKC, protein kinase C; PPARα, peroxisome proliferator-activated receptor α; PSC, primary sclerosing cholangitis; PXR, pregnane X receptor; UDCA, ursodeoxycholic acid; VDR, vitamin D receptor.

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 *urso*deoxycholic 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].

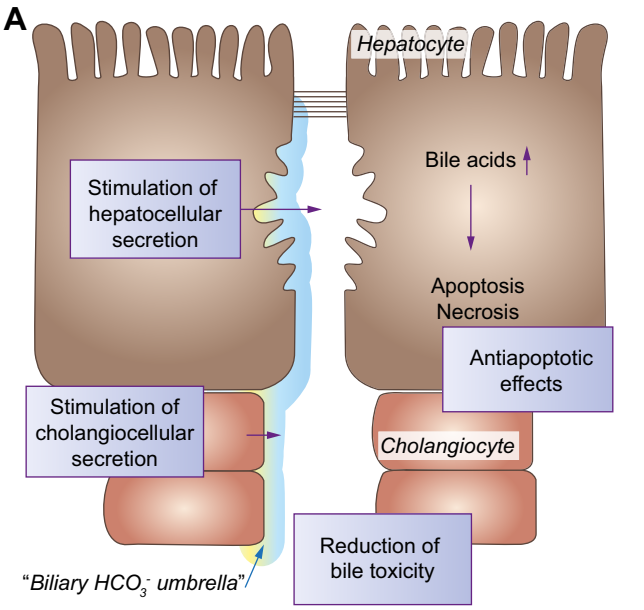
UDCA therapy has been used for a number of other clinical conditions. Efficacy is regarded as likely in ABCB4 deficiency with progressive familial intrahepatic cholestasis type 3 (PFIC-3) and/or low phospholipid-associated cholelithiasis (LPAC syndrome) and cystic fibrosis-associated liver disease (CFALD). Efficacy is regarded as uncertain in various forms of sclerosing cholangitis, drug-induced liver injury, progressive familial intrahepatic cholestasis type 1 & 2, sarcoidosis hepatis, 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 Ca^{2+} agonist [31–34] and an activator of protein kinase C (cPKCα) [35–37], mitogen-activated protein kinases (MAPK: Erk1/2, $p38^{MAPK}$) [38,39] and $\alpha_5\beta_1$ 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 choleretic effects via a dual MAPK- and integrin-dependent mechanism in healthy liver [39,40] and in anticholestatic effects via Ca^{2+}-/type II inositol-1,3,4-triphosphate receptor/cPKCα/PKA-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 choleretic and anticholestatic effects of UDCA in man.

More recently, the 'biliary HCO_3^- umbrella' hypothesis has been introduced as a protective mechanism for hepatocytes and cholangiocytes against the toxic effects of millimolar BA monomers present in bile [47]. This hypothesis indicates that biliary HCO_3^- 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 $pK_a \geqslant 4$. Notably, the experimental proof of concept also unraveled that an intact 'biliary HCO_3^- umbrella' is critically dependent on adequate function of the major HCO_3^- exporter, the Cl^-/HCO_3^- exchanger AE2, and an intact biliary glycocalyx in human cholangiocytes [48]. Functional impairment of this biliary HCO_3^- 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 HCO_3^- secretion under



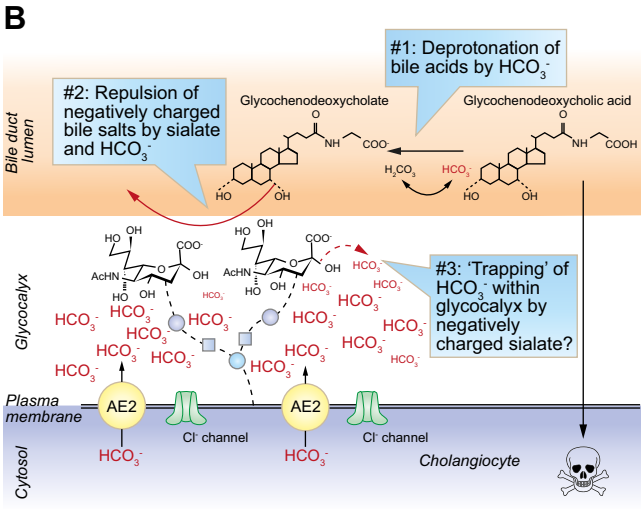


Fig. 1. Major mechanisms and sites of action of UDCA in cholestatic diseases. (A) UDCA conjugates exert anticholestatic effects and stimulate bile acid (BA) and organic anion secretion by mainly post-transcriptional molecular mechanisms at the level of the hepatocytes (for details see text). UDCA also stimulates biliary HCO_3^- secretion at the level of hepatocytes and cholangiocytes leading to stabilization of a 'biliary HCO_3^- umbrella' [47,48]. Antiapoptotic and anti-inflammatory effects are discussed in the text. (Figure modified from [28], courtesy of G. Paumgartner). (b) A closer look into the 'biliary HCO_3^- umbrella' on the apical membrane of a cholangiocyte [47,48]. The 20–40 nm glycocalyx on the apical membrane may trap HCO_3^- molecules in order to create an alkaline milieu which keeps bile acids in a negatively loaded state (bile salts) and, thereby, prevents carrier-independent invasion of apolar hydrophobic BA into cholangiocytes (and hepatocytes) [47,48]. (Figure modified after Maillette de Buy Wenniger *et al.* Dig Dis 2015, in press).

experimental conditions as well as in patients with PBC [28,49]. A defective AE2 expression [50,51] and biliary HCO_3^- secretion [49] have been described in patients with PBC. Thus, stabilization of the 'biliary HCO_3^- umbrella' may be a crucial mechanism of action of UDCA in cholestatic liver diseases (Fig. 1).

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.

*Nor*ursodeoxycholic acid: experimental and clinical effects

24-*nor*ursodeoxycholic acid (*nor*UDCA) 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, *nor*UDCA is passively absorbed from cholangiocytes and undergoes 'cholehepatic shunting' (instead of a full enterohepatic cycle) which generates a HCO_3^- anion resulting in induction of a HCO_3^--rich hypercholeresis [62,65,66] counteracting intrinsic BA toxicity [67] and reinforcing the 'biliary HCO_3^- umbrella' [47,48]. Cholehepatic shunting may also allow 'ductular targeting' of drugs [62,68]. As a proof of principle, taurine-conjugated *nor*UDCA and bis-*nor*UDCA (resulting from additional side chain shortening) lack cholehepatic shunting properties [69], emphasizing the unique properties of *nor*UDCA. In addition, *nor*UDCA 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 (intrinsic) biliary toxicity. Notably, neither *nor*UDCA 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]. *nor*UDCA (but not "conventional" UDCA or bis-*nor*UDCA) reversed sclerosing cholangitis in the experimental *Mdr2/Abcb4* knockout mouse (*Mdr2/Abcb4*$^{-/-}$) cholangiopathy model for sclerosing cholangitis, while the mother compound UDCA even aggravated bile infarcts in cholestatic conditions with biliary obstruction [68–70]. Moreover, *nor*UDCA has anti-lipotoxic, anti-proliferative, anti-fibrotic as well as anti-inflammatory effects which may complement stimulation of HCO_3^- 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 *nor*UDCA had anti-cholestatic and anti-apoptotic properties, suggesting that combination of UDCA and *nor*UDCA may be superior to UDCA or *nor*UDCA monotherapy in biliary disorders in which both hepatocyte as well as cholangiocyte dysfunction are involved in the pathophysiology of the disease [72].

Review

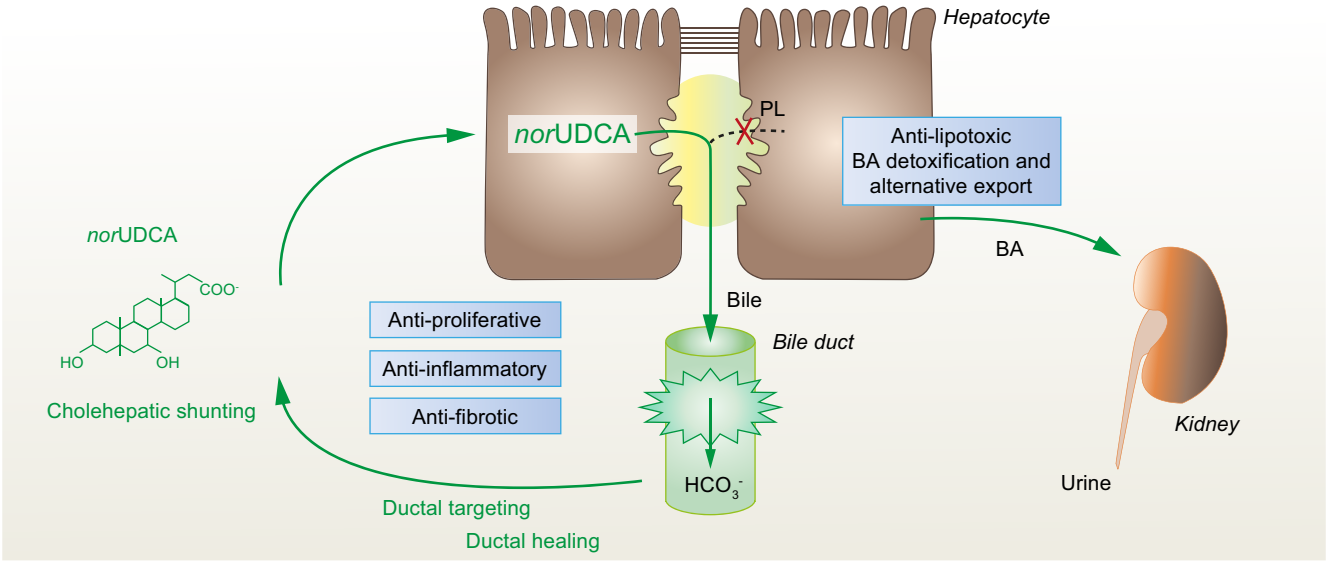


Fig. 2. Proposed mechanisms of action of *nor*UDCA in the *Mdr2 (Abcb4)*$^{-/-}$ model of sclerosing cholangitis. As hydrophilic bile acid (BA) and by generating a bicarbonate-rich (hyper)choleresis due to cholehepatic shunting, *nor*UDCA counteracts intrinsic biliary toxicity resulting from absent phospholipid (PL) secretion with increased free non-micellar bound BAs in this model. Importantly, cholehepatic shunting allows 'ductal targeting' of anti-inflammatory, anti-fibrotic and anti-proliferative effects to injured bile ducts, resulting in 'ductal healing'. *nor*UDCA also counteracts lipotoxic fatty acid composition and promotes BA detoxification and elimination via basolateral efflux pumps facilitating their subsequent renal excretion. Modified after [200].

*nor*UDCA 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 HCO_3^- umbrella may also be involved [47,48]. Notably, *nor*UDCA induces a HCO_3^--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 HCO_3^--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 *nor*UDCA an attractive therapeutic agent for cholangiopathies [73].

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

Pharmacologic agents under study (examples)	Targets	Natural ligands (examples)
	Nuclear receptors	
Obeticholic acid PX-102	FXR	Chenodeoxycholic acid Cholic acid
All-trans retinoic acid	RXR	Retinoic acid (Vit. A)
Bezafibrate Fenofibrate Ciprofibrate	PPARα	Free fatty acids
Budesonide and other corticosteroids	GR PXR	Cortisol
Rifampicin Statins Corticosteroids	PXR	Lithocholic acid
Vitamin D	VDR	1.25-diOH-vitamin D
	Membrane receptors	
FGF19 NGM 282	FGFR4	FGF19
Int-777	TGR5	Chenodeoxycholic acid Lithocholic acid
ASBT inhibitors	ASBT	Conjugated bile acids
	Bile acid derivatives	
*nor*UDCA	?	

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

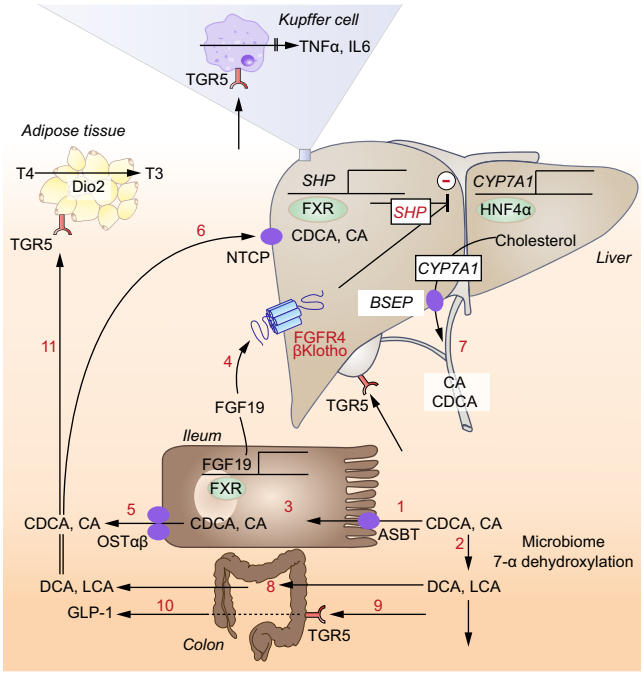


Fig. 3. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are absorbed in the ileum via the apical sodium-dependent bile acid transporter (ASBT) (1). About 5% of bile acids (BA) spill over into the colon where they are converted to the secondary BAs deoxycholate and lithocholate by 7α-hydroxylase-containing bacteria (2). In the mucosa cells of the ileum, BAs bind to FXR (3). Ligand-bound FXR activates the transcription of FGF19 and subsequently FGF19 is secreted into the portal circulation. At the hepatocyte surface, FGF19 binds to FGFR4/βKlotho (4). In the ileum, BAs exit the mucosa cells via the organic solute transporter OSTαβ (5). BAs are taken up in the liver via the Na^+-taurocholate-cotransporting polypeptide (NTCP) (6). In hepatocytes, BAs bind to FXR and this activates transcription of short heterodimer partner SHP. FGF19 via FGFR4/βKlotho–SHP and BA-activated FXR-SHP block HNF4α and LRH-1 - mediated transcription of CYP7A1. CYP7A1 is the rate-limiting enzyme in the conversion of cholesterol to 7α-hydroxycholesterol, 7α-hydroxy-4-cholesten-3-one, CA and CDCA. Bile salts exit the liver via BSEP, the canalicular bile salt export pump (7). Deoxycholic acid (DCA) and lithocholic acid (LCA) are secondary bile salts that enter and exit the colon mucosa passively via the help of transport proteins (8). At the colon mucosa surface DCA and LCA bind to TGR5 (9). This stimulates the production of cyclic AMP (cAMP) which triggers production and secretion of glucagon-like peptide 1 (GLP-1) (10). DCA and LCA are high affinity ligands for TGR5 in a variety of tissues. In these tissues TGR5 has metabolic and anti-inflammatory effects (e.g., in adipose tissue TGR5 stimulates the expression of DIO2 that mediates the conversion of T4 to T3; in Kupffer cells TGR5 inhibits the secretion of cytokines).

short heterodimer partner (SHP) and fibroblast growth factor (FGF)19 (Fgf15 in rodents) and the transporters BSEP, OST α/β, and putatively MRP3 and MRP4 [84–88]. SHP is a protein that in the liver suppresses the HNF4α and LRH-1 mediated transcription of CYP7A1, the rate-determining enzyme in BA synthesis. SHP also plays a complex role in glucose, lipid and energy metabolism [89–91]. Therefore SHP is a crucial effector molecule in the regulation of metabolism inside and outside the liver (Fig. 3).

FGF19 and FGF21 are members of the fibroblast growth factor family [92]. These proteins serve as hormones in inter-organ signaling (FGF19, gut to liver; FGF21, liver to adipose tissues) supporting the actions of FXR and peroxisome proliferator-activated receptor alpha (PPARα) [93–95]. If after a meal the gallbladder contracts, BAs enter the intestine, are re-absorbed in the ileum where they activate FXR before they enter the portal circulation and are taken up in the liver. In the ileum, FXR activation leads to FGF19 expression. FGF19 enters the portal circulation 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 PPARα [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.

FXR 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

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 HCO_3^-/fluid secretion via CFTR and AE2 [122]. Surprisingly, a highly potent TGR5 agonist (INT-777) failed to induce HCO_3^- 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 sclerosing 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 HCO_3^- 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 implied 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.

PPARα: experimental and clinical effects

The PPARs are enriched in tissues with high energy metabolism such as liver (PPARα, NR1C1), skeletal muscle, heart and gastrointestinal tract (PPARβ/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, PPARα has anti-steatotic effects. However, PPARα also has anti-inflammatory actions. It was recently argued that the anti-inflammatory action of PPARα 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 PPARα, 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 PPARα 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 agonists 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 (NR1I2) 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], MDR1 [167], MRP2 [168], MRP3 [169], and OSTβ [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-16α-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. choledocholithiasis, 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. Compared with prednisolone, GR binding activity of budesonide is 15–20 times higher, so its effect on liver inflammation may be greater. In patients with inflammatory bowel disease and autoimmune hepatitis, oral budesonide has been shown to exert fewer systemic side effects than conventional corticosteroids [177].

Recent studies have shed some light on the complex relationships between GR activation and BAs. Glucocorticoids promote hepatic cholestasis in mice by inhibiting the transcriptional activity of the FXR [178] and increase intestinal apical sodium-dependent bile salt transporter (ASBT) activity [179] while promoting PXR-mediated hydroxylation and sulfation of hydrophobic BAs [180]. Conversely, high BA concentrations might promote SHP-mediated inhibition of GR activation [181] that could explain some loss of anti-inflammatory effects of glucocorticoids in cholestatic conditions. Taken together these data suggest that classical glucocorticoids may have negative effects in cholestatic conditions.

In contrast to other natural BAs, UDCA is a GR agonist [57–59]. Moreover, combination of budesonide and UDCA have been shown to promote activity of the Cl^-/HCO_3^- exchanger AE2, the key transporter involved in alkaline-rich choleresis in humans [182].

Two randomized studies showed the GR (and PXR) agonist budesonide (6–9 mg/day) combined with UDCA to be more effective in improving liver biochemistries and histology than did UDCA alone in patients with stages I-III PBC [183,184]. This was not observed in a study including late stage PBC patients who also developed serious side effects [185]. In an open trial involving non-cirrhotic PBC patients with severe inflammation and biochemical cholestasis not responding to UDCA, the combination of UDCA, budesonide and mycophenolate mofetil – as a corticosteroid-sparing agent – achieved biochemical remission and marked improvement of liver histology [186]. Combination therapy (UDCA + budesonide) is currently being evaluated in comparison to UDCA monotherapy in PBC patients with suboptimal response to UDCA alone in a European multicenter randomized, placebo-controlled trial. Because of its high first-pass hepatic clearance, budesonide should not be given to patients with evidence of cirrhosis and portal hypertension as portosystemic shunting may occur and development of portal vein thrombosis has been described [187].

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 (T_H1) type cytokines, while promoting those of the T_H2 subtype. Furthermore, vitamin D inhibits dendritic cell differentiation resulting also in a decrease in T_H1 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]. $1,25(OH)_2D_3$ was also shown to decrease hepatic Cyp7a1 expression by increasing the expression of Fgf15 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].

Review

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 unrepressed 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 stimulate 7α-dehydroxylation of BA and stimulate TGR5. This enhances GLP-1 secretion which will increase insulin secretion by the pancreas and improve insulin sensitivity. Anionic exchange resins such as colesevalam have a similar effect [198,199]. Thus, ASBT inhibitors are potential drugs for treatment of NAFLD. ASBT inhibitors interrupt the enterohepatic circulation of BA and this may reduce the circulating BA pool, given that the loss exceeds the upregulated BA synthesis. This may be beneficial in cholestatic liver disease but convincing clinical data are currently lacking.

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 PPARα 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, *nor*UDCA, 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.

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