Medical treatment of cholestatic liver diseases: From pathobiology to pharmacological targets

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Abstract

Bile secretion is dependent on the coordinated functions of a number of hepatobiliary transport systems. Cholestasis may be caused by an impairment of bile secretion, an obstruction of bile flow or a combination of the two. The common consequence of all forms of cholestasis is retention of bile acids and other potentially toxic compounds in the hepatocytes leading to apoptosis or necrosis of hepatocytes and eventually to chronic cholestatic liver disease. In certain cholestatic disorders there is also leakage of bile acids into the peribiliary space causing portal inflammation and fibrosis. The following pharmacological targets for treatment of intrahepatic cholestasis can be identified: stimulation of orthograde biliary secretion and retrograde secretion of bile acids and other toxic cholephils; inhibition of fibrosis caused by elevated levels of cytotoxic bile acids; inhibition of apoptosis caused by elevated levels of toxic compounds in the hepatocytes leading to apoptosis or necrosis of hepatocytes and eventually to chronic cholestatic liver disease. In certain cholestatic disorders there is also leakage of bile acids into the peribiliary space causing portal inflammation and fibrosis. The common consequence of all forms of cholestasis is retention of bile acids and other potentially toxic compounds in the hepatocytes leading to apoptosis or necrosis of hepatocytes and eventually to chronic cholestatic liver disease. In certain cholestatic disorders there is also leakage of bile acids into the peribiliary space causing portal inflammation and fibrosis. The common consequence of all forms of cholestasis is retention of bile acids and other potentially toxic compounds in the hepatocytes leading to apoptosis or necrosis of hepatocytes and eventually to chronic cholestatic liver disease. In certain cholestatic disorders there is also leakage of bile acids into the peribiliary space causing portal inflammation and fibrosis. The common consequence of all forms of cholestasis is retention of bile acids and other potentially toxic compounds in the hepatocytes leading to apoptosis or necrosis of hepatocytes and eventually to chronic cholestatic liver disease. In certain cholestatic disorders there is also leakage of bile acids into the peribiliary space causing portal inflammation and fibrosis. The common consequence of all forms of cholestasis is retention of bile acids and other potentially toxic compounds in the hepatocytes leading to apoptosis or necrosis of hepatocytes and eventually to chronic cholestatic liver disease. In certain cholestatic disorders there is also leakage of bile acids into the peribiliary space causing portal inflammation and fibrosis. The common consequence of all forms of cholestasis is retention of bile acids and other potentially toxic compounds in the hepatocytes leading to apoptosis or necrosis of hepatocytes and eventually to chronic cholestatic liver disease.

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INTRODUCTION

Great progress has been made in the last decade in our understanding of the molecular basis of bile formation and the pathobiology of cholestasis[1-3]. Targets for medical therapy of cholestasis have been identified which help to understand the established treatments and facilitate the development of new drugs for cholestatic liver disease. In this short review, present concepts of bile formation and cholestasis are briefly summarized and medical treatment of cholestatic liver diseases is illustrated using primary biliary cirrhosis (PBC), the model disease for chronic cholestatic liver disease, as an example.

MOLECULAR MECHANISMS OFBILE FORMATION

Hepatocellular bile is formed by active transport of solutes into the bile canaliculi. Thereby, a local osmotic gradient is established between canalicular bile and sinusoidal plasma. This causes a flow of water, electrolytes and small solutes into the bile canaliculi, mainly via a paracellular pathway through the tight junctions which exhibit perm selectivity, and are impermeable for large and negatively charged solutes[4].

The most important driving force for hepatocellular bile formation is the secretion of bile acids from the sinusoidal blood into the bile[3]. Conjugated bile acids, which represent the major fraction of bile acids in the blood, are transported across the basolateral membrane of hepatocytes together with sodium by the sodium-taurocholate cotransporter (NTCP, SLC10A1). Unconjugated bile acids and a large variety of other organic anions including bilirubin are taken up by the hepatocytes via the organic anion-transporting polypeptide 2 (OATP2, SLC21A6). The rate limiting step for bile formation is the active transport of bile acids and other solutes across the canalicular membrane of hepatocytes. This concentrative step is driven by a number of ATP-dependent export pumps (ATP-binding-cassette-transport proteins also known as ABC-transporters). Bile salts are transported by the bile salt export pump (BSEP, ABCC11), whereas bilirubin diglucuronide, glutathione, divalent bile acids conjugates and a large variety of other conjugated organic anions are transported by the multidrug resistance associated protein 2 (MRP2, ABCC2)[3].

A special ABC-transporter, namely the multidrug resistance P-glycoprotein 3 (MDR3, ABCB4), flipps...
phospholipids from the inner to the outer leaflet of the canalicular membrane. This flippase provides phosphotidylcholine for bile which forms mixed micelles with bile acids and cholesterol[2].

The formation and final composition of bile depends on additional transporters in the canalicular membrane of hepatocytes as well as transporters in cholangiocytes which add cholangiocellular bile to hepatocellular bile. Among these, the chloride-bicarbonate anion exchanger 2 (AE2, SLC16A2) is present in the apical membrane of both hepatocytes and bile duct epithelial cells, whereas the cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7), a chloride channel, is located in the apical membrane of bile duct epithelial cells only[3].

The basolateral membrane of hepatocytes possesses a number of transporters which are expressed during cholestasis and transport solutes in a retrograde fashion back into the blood (see below). These are MRP4 (ABCC4) which transports bile acids together with glutathione[4], MRP3 (ABCC3) which transports conjugated bilirubin and other organic anions[5,6] and OSTα/OSTβ, a heteromeric organic solute transporter which transports bile acids[7]. During cholestasis, MRP3[8] and OSTα/OSTβ are also upregulated in the basolateral membrane of cholangiocytes[9].

Molecular Mechanisms of Cholestasis

Cholestasis can be defined as an impairment of bile flow. The consequences are retention of bile acids, bilirubin and other cholephils in the liver and blood and a deficiency of bile acids in the intestine. Various forms of cholestasis can be caused by an impairment of bile secretion, an obstruction of bile flow or a combination of the two (Figure 1).

Impairment of bile secretion can be inborn, for instance in different forms of progressive familial intrahepatic cholestasis (PFIC), benign recurrent intrahepatic cholestasis (BRIC), or cystic fibrosis, and it also can be acquired by inflammation, toxins, drugs or hormones[8,9].

Inborn defects of bile secretion: If BSEP is defective because of a gene mutation, PFIC2 or BRIC2[10] can occur. PFIC2 can be identified by immunostaining of BSEP in liver biopsies[11].

Mutations of MRP2 cause the Dubin Johnson syndrome, which is not a complete cholestasis, but a more selective defect of biliary secretion of organic anions such as bilirubin glucuronide. Mutations of MDR3 cause PFIC3 and mutations of CFTR cause cystic fibrosis[12].

Acquired impairment of bile secretion: In inflammatory disorders such as sepsis, bacterial infections, viral hepatitis as well as toxin or drug-induced hepatitis, inflammatory cytokines can impair bile secretion. Thus, TNFα and IL-1β down regulate NTCP and BSEP which are responsible for bile acid transport, as well as OATP2 and MRP2 which are responsible for transport of bilirubin and a variety of other organic ions[13,14].

Figure 1. Causes of cholestasis. PBC: Primary biliary cirrhosis; PFIC: Progressive familial intrahepatic cholestasis; PSC: Primary sclerosing cholangitis; VBDS: Vanishing bile duct syndrome. For details see text.

Drugs can cause cholestasis by inhibiting the function of hepatobiliary transport proteins. Some drugs are known to inhibit BSEP directly from the inside of hepatocytes, which is called cis-inhibition. Examples are cyclosporine A, glibenclamide, troglitazone and bosentan[15,16]. Other drugs, such as estradiol 17β-D-glucuronide, must first be transported into the canalicular lumen by MRP2 and then act on BSEP from the luminal side, which is called trans-inhibition[17].

Obstruction of bile flow can also be caused by inborn disorders, e.g. in cystic fibrosis or in PFIC3, and it can be acquired for instance in PBC, primary sclerosing cholangitis (PSC) or the vanishing bile duct syndrome (VBDS). Much more often obstructive cholestasis is caused by stones or tumours.

In cholestatic disorders caused by an initial injury of cholangiocytes (e.g. an immunological injury in the case of PBC), hydrophobic bile acids in bile (in millimolar concentrations) may aggravate the bile duct lesion and contribute to the destruction and loss of bile ducts resulting in progressive obstructive cholestasis. This may be called extracellular bile acid cytotoxicity in contrast to intracellular bile acid toxicity when bile acids accumulate in hepatocytes (in micromolar concentrations). Extracellular bile acid toxicity also occurs towards normal biliary epithelium when phospholipids in bile are low, as in the inborn defect of PFIC3 or in other “low phospholipid syndromes”, in low phospholipid gallstone disease[18] or in bile acid phospholipid imbalance in bile after liver transplantation[19].

Adaptive Responses to Cholestasis

In order to compensate for the loss of biliary excretory function in cholestasis and to limit hepatocellular accumulation of potentially toxic biliary constituents, adaptive responses to cholestasis occur in the liver[20,21], the kidney[22,23] and the intestine[24,25]. In the following only the adaptive changes in the liver are discussed.

Down regulation of NTCP and OATP2 reduces the uptake of bile acids and other organic anions in cholestasis and thus protects the hepatocytes against an overload
of bile acids and bilirubin. At the same time there is upregulation of MRP3 and MRP4 in the basolateral membrane. These transporters normally are expressed at a low level only. MRP4 pumps bile salts and bile salt conjugates together with glutathione from the cells into the blood and thus decreases bile acid retention in cholestatic hepatocytes. MRP3 mainly exports other organic anions. Prior to their extrusion from hepatocytes, hydrophobic bile acids and many xenobiotics are metabolized to more hydrophilic and less toxic compounds by cytochrome P-450(CYP) 3A enzymes. A large fraction of bile acids is sulphated by the enzyme sulfotransferase 2A1 (Figure 2).

The major players in these adaptive regulations are the nuclear receptors FXR, PXR and CAR. The farnesoid X-receptor (FXR), a bile acid sensor, is mainly involved in the down regulation of NTCP, in the maintenance of BSEP function and in the up-regulation of MRP4 and MDR3. The pregnane X receptor (PXR), to which many xenobiotics bind, is mainly responsible for the upregulation of MRP3 and various CYP enzymes, especially the family of CYP3A enzymes. There is evidence that more than one of these nuclear receptors can act on the same transporter. Recently, it has been demonstrated that the constitutive androstane receptor (CAR) up-regulates sulfotransferase 2A1 and MRP4 in a coordinated fashion facilitating the conjugation and export of hydrophobic bile acids. In addition to PXR, the peroxisome–proliferator-activated receptor α (PPARα) up-regulates MDR3 (Figure 2).

It is of considerable interest that besides natural bile acids, bile acid derivatives such as ethyl-chenodeoxycholic acid are ligands for FXR. Ligands for PXR are many xenobiotics and drugs like rifampicin. Bilirubin and phenobarbital are ligands for CAR and fibrates as well as statins (e.g., pravastatin) bind to PPARα.

These findings open an avenue for the development of drugs which bind to nuclear receptors which enhance normal compensatory mechanisms in cholestasis for the elimination of toxic compounds via alternative excretory routes.

**TARGETS FOR PHARMACOLOGICAL THERAPY**

The common consequence of all forms of cholestasis is retention of bile acids in hepatocytes. Elevated levels of bile acids can lead to apoptosis or necrosis of hepatocytes and eventually to chronic cholestatic liver disease. In certain cholestatic disorders there is also leakage of bile acids into the peribiliary space, causing portal inflammation and fibrosis via induction of chemokines and cytokines. Accordingly, the following pharmacological targets for treatment of intrahepatic cholestasis can be identified (Figure 3): stimulation of orthograde biliary secretion and retrograde secretion of bile acids and other toxic cholephils into the systemic circulation for excretion by the kidneys to reduce their retention in the hepatocytes; stimulation of the metabolism of hydrophobic bile acids and other toxic compounds to more hydrophilic but less toxic metabolites; protection of injured cholangiocytes against toxic effects of bile; inhibition of apoptosis caused by elevated levels of cytokotoxic bile acids; inhibition of fibrosis caused by leakage of bile acids into the peribiliary space.

**Stimulation of secretion**

Secretion of bile acids and other potentially toxic compounds into the bile and blood may be stimulated by enhancing transporter expression and/or function at different levels, namely the levels of transcription, translation, targeting and protein activation.

In mice both cholic acid (CA) and UDCA stimulate the expression of Bsep and Mrp2 mRNA. One must, however, be aware that these findings may not hold true for men, because considerable species differences exist with regard to binding of bile acids to nuclear receptors and regulation of transporter expression by nuclear receptors. Rifampicin, a ligand of PXR, stimulates the expression of Mrp2 at the transcriptional level in man.

Ursodeoxycholic acid (UDCA) stimulates targeting of the transporters Bsep and Mrp2 to the canalicular membrane in the rat via at least two different signalling cascades. Immuno-electronmicroscopy with gold particles is employed to assess localization of Bsep in the canalicular membrane and in a subapical compartment of the bile canalicular membrane.
rat liver. Bsep and Mrp2 in the canalicular membrane are markedly reduced when taurolithocholic acid (TLCA) is administered in the perfused rat liver, but is maintained when tauroursodeoxycholic acid (TUDCA) is added \[48,49\]. Enhanced expression of BSEP under UDCA treatment in men may contribute to a better elimination of bile acids from the blood. As shown by Poupon et al \[41\], in collaboration with our group, UDCA decreases serum levels of the hydrophobic bile acid, chenodeoxycholic acid (CDCA) in PBC. As shown by Zollner et al \[14\], expression of MRP2 mRNA and protein increases with the enrichment of UDCA in the liver during treatment of patients with PBC and UDCA. Accordingly, as shown by Poupon et al \[43\], UDCA improves excretory function in PBC. Thus, in a randomised, placebo controlled study over two years, in patients with PBC, serum bilirubin was significantly lower in the UDCA group than in the placebo group.

Activation of transporters in the canalicular membrane by UDCA and phosphorylation may also occur \[40\], which has not yet been sufficiently studied.

**Stimulation of metabolism**

Stimulation of the metabolism of hydrophobic bile acids produces more hydrophilic and less toxic compounds. Rifampicin, a drug used for the treatment of cholestatic pruritus, stimulates the expression of CYP3A4 mRNA in patients with gallstones. In line with this, Dilger et al \[44\] showed that in patients with early stage PBC, rifampicin stimulates CYP3A metabolic activity as assessed by urinary 6β-hydroxy cortisol, whereas UDCA has no effect.

**Protection of cholangiocytes**

Protection of cholangiocytes by making the bile more hydrophilic and less toxic appears to be an important therapeutic target. UDCA fulfills this requirement because it renders bile acid composition of bile more hydrophilic and increases biliary phospholipid secretion \[46\].

**Inhibition of apoptosis**

Inhibition of apoptosis caused by elevated levels of hydrophobic bile acids \[46,47\] may also be a therapeutic target in cholestasis. As shown by Rodrigues et al \[48,49\], feeding of the hydrophobic bile acid deoxycholic acid (DCA) to rats increases hepatocyte apoptosis as assessed by the number of tunnel positive hepatocytes. Addition of UDCA inhibits this effect. Toxic bile acids such as CDCA can cause apoptosis of hepatocytes via the CD95 receptor with formation of a death inducing signalling complex (DISC) and activation of caspase 8. Caspase 8 then causes mitochondrial membrane permeability transition (MMPT) which leads to activation of effector caspases and apoptosis. In addition, UDCA stabilizes the mitochondrial membrane and inhibits MMPT and apoptosis \[48,49\]. The antipapoptotic effect of UDCA has also been demonstrated in human hepatocytes \[48\].

**Inhibition of fibrosis**

Inhibition of fibrosis may become an important therapeutic target in the future. In the rat with common bile duct ligation, fibrosis can be inhibited by 6-ethyl CDCA (6-ECDCA). The antifibrotic effect of 6-ECDCA appears to be mediated via FXR and SHP \[53\]. Recently, an antifibrotic effect of NOR-UDCA has been described in the Mdr2 knock-out mouse \[51\]. It remains to be shown whether these findings are relevant to human cholestatic liver diseases, but they point towards a promising new way for the development of drugs to inhibit cholestatic fibrosis.

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**PHARMACOLOGICAL TREATMENT OF CHRONIC CHOLESTATIC LIVER DISEASES**

In the following, primary biliary cirrhosis (PBC), the model disease for chronic cholestatic liver disease, is used as an example for the treatment of chronic cholestatic liver diseases by UDCA. PBC is characterized by an inflammatory lesion of interlobular bile ducts, which results in bile duct destruction and may progress to fibrosis and cirrhosis. Since the etiology of the disease is unknown, presently available therapies aim at inhibiting the underlying pathogenetic processes and delaying the progression of the disease.

The pathogenesis of this slowly progressive disease involves a still unknown immunologic injury of small interlobular bile ducts; aggravation of the bile duct lesion by cytotoxic bile acids; obstruction and loss of small bile ducts followed by cholestasis and retention of bile acids; hepatocyte injury, apoptosis, necrosis, fibrosis and eventually cirrhosis with liver failure.

UDCA, at present, is the only approved drug for PBC. It appears to exert its beneficial effects by rendering bile composition less toxic for the injured biliary epithelium, reducing the retention of bile acids in hepatocytes and inhibiting apoptosis \[10,34\]. Immunosuppressive agents have met with limited success. They have been found useful in combination with UDCA in selected patients \[32,33\].

In randomized, double-blind placebo-controlled trials UDCA at doses of 13-15 mg/kg body weight per day could improve serum liver tests including serum bilirubin and other serum markers of cholestasis \[52,54,59\], the Mayo risk score \[56\] and liver histology \[42,50\]. As shown by Pares et al \[40\] and Poupon et al \[47\], UDCA inhibits histological progression in early stage PBC. As shown by Corpechot et al \[48\], UDCA inhibits progression to severe liver fibrosis or cirrhosis in early stage PBC. In line with this is the observation that UDCA delays the onset of esophageal varices \[59\]. A combined analysis of three of the largest trials showed that treatment with UDCA at doses of 13-15 mg/kg per day for up to 4 years can delay the time of liver transplantation or death \[64\]. Within the first 2 years of treatment, however, a survival benefit was not seen. Doses lower than 10 mg/kg per day of UDCA are of little benefit in PBC. A meta-analysis of 8 randomized trials which showed no difference between UDCA and placebo in the effects on incidence of death, liver transplantation and death or liver transplantation \[62\] has a number of shortcomings. In 6 of the 8 studies treatment was evaluated up to 24 mo only and the dose of UDCA was 10 mg/kg per
day or lower in two of the studies. Therefore, improvement of transplant free survival by UDCA as shown in the combined analysis of the three largest studies with doses of 13-15 mg/kg per day and a follow-up of 4 years may not have been detectable in this meta-analysis.

CONCLUSION

Better insight into the pathobiology of cholestasis has provided new concepts for pharmacological therapies of cholestatic liver diseases. Among those, therapy with UDCA has been studied most extensively. In PBC, the model disease for cholestatic liver diseases which has been highlighted in this review, the beneficial effects of UDCA have been documented by randomized controlled trials. Treatment with UDCA appears to be beneficial also in a number of other cholestatic disorders, such as primary sclerosing cholangitis (PSC) [63-65], intrahepatic cholestasis of pregnancy [66,67], liver disease in cystic fibrosis [68-70], progressive familial intrahepatic cholestasis (PFIC) [71] and some forms of drug-induced cholestasis [60].

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