

# Mechanisms of action and therapeutic efficacy of ursodeoxycholic acid in cholestatic liver disease

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Ursodeoxycholic acid (UDCA) is widely used for the treatment of a variety of chronic cholestatic liver diseases. At present, it is the only drug approved by the United States Food and Drug Administration for the treatment of primary biliary cirrhosis (PBC). UDCA is a dihydroxy bile acid (3 $\alpha$ ,7 $\beta$ -dihydroxy-5 $\beta$ -cholanic acid), which is normally present in human bile albeit in a low concentration of about 3% of total bile acids. It is the major bile acid of the bile of black bears and has been used for centuries in traditional Chinese medicine as a remedy for liver diseases [1]. First reports of beneficial effects on serum liver tests in cholestatic disorders appeared in the 1980s in the Western literature [2–4], but similar observations were made previously in Japan [5,6].

## **Absorption, enterohepatic circulation, and metabolism of UDCA**

After oral administration, UDCA is absorbed by passive non-ionic diffusion mainly in the small intestine. Dissolution of UDCA in the proximal jejunum occurs by solubilization in mixed micelles of endogenous bile acids [7]. Absorption of UDCA may be enhanced when it is given with a meal and may be diminished in patients with decreased biliary secretion of endogenous bile acids (eg, in patients with cholestasis). After its uptake into the liver, UDCA is conjugated, mainly with glycine and to a lesser extent with taurine, and is actively secreted into the bile undergoing enterohepatic circulation. UDCA conjugates are absorbed mainly from the distal ileum where they compete with endogenous bile acids for active transport. Nonabsorbed UDCA passes into the colon and is converted by intestinal bacteria to lithocholic acid. Most of the

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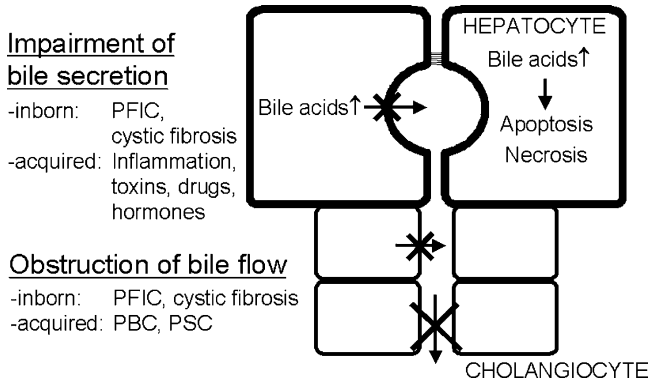


Fig. 1. Pathogenetic processes of cholestatic liver diseases.

*Abbreviations:* PBC, primary biliary cirrhosis; PFIC, progressive familial intrahepatic cholestasis; PSC, primary sclerosing cholangitis.

lithocholic acid is insoluble in the colonic content and is eliminated in the feces. The fraction of lithocholic acid absorbed is sulfated in the liver, secreted into the bile, and excreted in the feces.

In patients with PBC, a daily dose of 13–15 mg/kg UDCA increases the percentage of UDCA in bile to about 40% to 50% of total bile acid. Up to a certain dosage of UDCA, the percentage of UDCA in bile increases in a dose-dependent fashion. In patients with early-stage PBC and normal bilirubin levels receiving 25 to 35 mg/kg/day of UDCA, the percentage of UDCA in biliary bile acids was high as 69% [8]. There seems to be a maximal dose beyond which enrichment does not increase. This dose has not been adequately defined and may depend on the degree of cholestasis and the underlying disorder (eg, PBC or primary sclerosing cholangitis [PSC]).

### Pathogenetic processes in cholestatic liver disease

Because the cause of most chronic cholestatic liver diseases is not known, therapy must aim at inhibiting pathogenetic processes of cholestatic liver disease (Fig. 1). These processes include

1. Aggravation of disease-specific lesions of biliary epithelia by bile acids. Bile with its high concentration of hydrophobic bile acids has an extracellular cytotoxic potential [9]. It may aggravate cholangiocellular injury and loss of bile ducts caused by the cholestatic disease (eg, PBC).
2. Impairment of bile secretion at the level of the hepatocyte, the cholangiocyte, or both. The underlying cause may be an inherited (gene mutation) or acquired (inflammation, toxic injury, drugs, or hormones) malfunction of transporter proteins such as the bile salt export pump (BSEP), the conjugate export pump (MRP2) or the anion exchanger 2 (AE2) that are responsible for

- bile formation. The consequence is retention of bile acids and other potentially toxic substances in the hepatocytes.
- Hepatocyte injury, apoptosis, and necrosis. These processes are mainly caused by retained hydrophobic bile acids (intracellular cytotoxicity) and may be followed by an inflammatory reaction and fibrosis.

### Mechanisms of action of UDCA

Multiple mechanisms of action of UDCA have been described aiming at one or more of the pathogenetic processes described previously [10] and shown in Fig. 2: (1) protection of injured cholangiocytes against toxic effects of bile acids, (2) stimulation of impaired biliary secretion, (3) stimulation of detoxification of hydrophobic bile acids, or (4) inhibition of apoptosis of hepatocytes. It is not clear which of these mechanisms plays a primary role for the beneficial therapeutic effects of UDCA in cholestatic liver diseases. Most likely, this effect depends on the specific cholestatic liver disease and the stage of the disease. In early-stage PBC where excretory function is not yet impaired, protection of injured cholangiocytes against toxic effects of bile acids may be more important than stimulation of biliary secretion, whereas in later stages stimulation of biliary secretion may be important to prevent retention of hydrophobic bile acids and other toxic substances in the hepatocytes. In drug-induced cholestasis with impaired function of transporters, the stimulation of biliary secretion may be essential at the beginning of the cholestatic process. Finally, when there is retention of bile acids within the hepatocyte, inhibition of bile acid-induced hepatocyte apoptosis may become important.

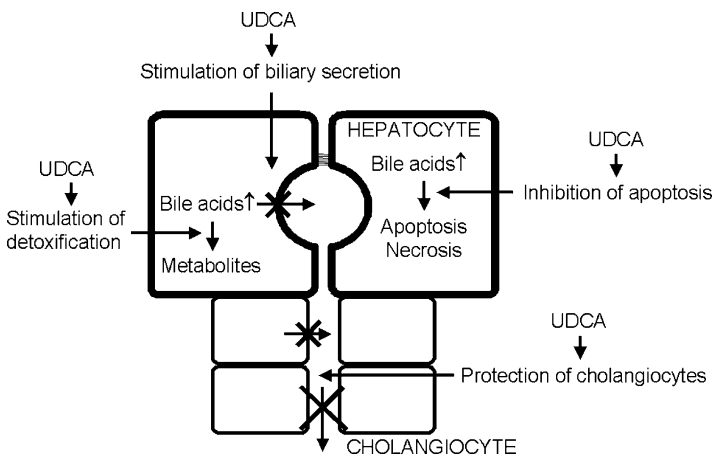


Fig. 2. Mechanisms of action of ursodeoxycholic acid (UDCA) in cholestatic liver diseases.

### *Protection of injured cholangiocytes against toxic effects of bile acids*

Hydrophobic bile acids damage cell membranes and exert extracellular cytotoxicity at concentrations that are present in bile [9,11,12]. UDCA treatment renders the bile acid composition of bile less hydrophobic and more hydrophilic and reduces the toxic potential of bile that may aggravate any primary disease-specific duct lesion. By this mechanism, UDCA seems to decrease the degree of cholangiocellular injury, portal inflammation, and ductular proliferation (eg, in the Mdr2-knockout mouse [13,14], an animal model in which the bile duct lesions resemble PSC). Similarly, the inflammatory reaction around bile ducts is less severe in patients with PBC and PSC receiving UDCA treatment than in patients receiving placebo [15–18]. UDCA may also protect cholangiocytes by diminishing apical uptake and/or stimulating basolateral efflux of bile acids from cholangiocytes and decreasing the intracellular concentration of hydrophobic bile acids, thus reducing intracellular toxicity. This process might explain why UDCA feeding prevented ductular proliferation thought to be induced by hydrophobic bile acids in bile duct–ligated rats [19]. The effects of UDCA on cholangiocytes are apparently mediated by  $\text{Ca}^{++}$ - and protein kinase C  $\alpha$  (PKC $\alpha$ )-dependent mechanisms, which have been shown to stimulate biliary secretion in hepatocytes [20–23].

### *Stimulation of hepatobiliary secretion*

All forms of cholestasis are characterized by an impairment of secretion or flow of bile. As a consequence, bile acids and other potentially toxic biliary constituents accumulate in the hepatocyte and may lead to hepatocyte injury, apoptosis, and necrosis. In experimental models, UDCA stimulates biliary secretion of bile acids and other organic anions (eg, bilirubin glucuronides, glutathione conjugates) and counteracts cholestasis induced by hydrophobic bile acids in rat liver [22,24,25]. In patients with PBC and PSC, UDCA stimulates biliary secretion of bile acids [26] and decreases elevated serum levels of the endogenous, hydrophobic bile acid, chenodeoxycholic acid [27] and of bilirubin [15,17,28,29]. Thus, UDCA may exert beneficial effects in cholestatic liver disease by stimulating the elimination of toxic compounds from the hepatocytes.

The secretory capacity of the hepatocytes is determined by the number and activity of carrier proteins in the canalicular membrane, which are regulated at a transcriptional and post-transcriptional level. It has recently been shown that UDCA stimulates the expression of transporter proteins for biliary secretion in the liver [30,31] and the targeting and insertion of transporter molecules into the canalicular membrane [22,32]. Although the effects of UDCA on mRNA and protein levels of transporters may be important for long-term regulation, the effects on the insertion into the canalicular membrane and the activity of transporters may determine short-term regulation of secretion. By increasing synthesis, apical insertion, and activation of the BSEP, the MRP2, and the AE2, UDCA might enhance bile salt–dependent and bile salt–independent bile flow.

### Increased expression of transporter proteins

The transcriptional regulation of canalicular transporter proteins by UDCA and cholic acid (CA) has recently been addressed [31]. In hepatocytes of mice fed a UDCA- or CA-supplemented diet, both UDCA and CA increased expression of Bsep and Mrp2 mRNA. Hydrophilic bile acids are thought to transactivate the Bsep promoter by the farnesoid X receptor (FXR) [33]. Because this effect was not specific for UDCA, its role for the anticholestatic action of UDCA remains unclear.

### Apical insertion of transporter proteins

In cholestasis, vesicle-mediated targeting of proteins to the canalicular membrane is impaired. Experimental evidence suggests that the taurine conjugate of UDCA (TUDCA), by a complex network of signals, stimulates hepatobiliary vesicular exocytosis and insertion of carrier proteins into the apical membrane of the hepatocyte (Fig. 3) [20–22,32,34,35]. As recently demonstrated in cholestatic rat liver, TUDCA significantly enhances the density of Mrp2 in canalicular membranes of the hepatocyte and thereby stimulates biliary secretion of potentially toxic compounds [20,22].

Cytosolic free calcium  $[Ca^{++}]_i$  seems to be critical for TUDCA-induced exocytosis in the model of the perfused rat liver [20]. TUDCA, but not the trihydroxy bile acid taurocholic acid (TCA), induces a sustained elevation of  $[Ca^{++}]_i$  in isolated hepatocytes [20,36]. TUDCA also selectively induces translocation of the  $Ca^{++}$ -sensitive  $\alpha$ -isoform of PKC, a key mediator of regulated exocytosis, to hepatocellular membranes, and activates membrane-bound PKC [21,37]. Inhibition of PKC $\alpha$  by the PKC inhibitor bisindolylmaleimide-I markedly impairs TUDCA-induced secretion of the model Mrp2 substrate dinitrophenyl-

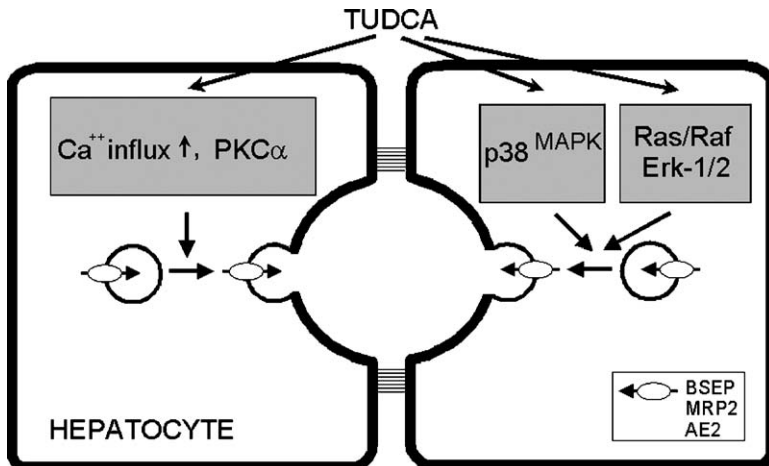


Fig. 3. Stimulation of insertion of transporter proteins into the canalicular membrane of the hepatocyte by tauroursodeoxycholic acid (TUDCA).

S-glutathione (GS-DNP) in experimental models of cholestasis, strongly supporting the concept that TUDCA exerts anticholestatic effects, at least in part, by  $\text{Ca}^{++}$ - and  $\text{PKC}\alpha$ -dependent mechanisms [20,38].

Canalicular bile acid secretion may be increased by TUDCA through alternative signaling pathways independent of PKC in normal liver [32,35]. Activation of the small guanosine triphosphate-binding protein Ras and the mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase (Erk)-1 and Erk-2, on one hand, and the Ras/Raf-independent  $\text{p38}^{\text{MAPK}}$ , on the other hand, mediate TUDCA-induced bile acid secretion in the perfused rat liver [32,35]. TUDCA induced a transient and concentration-dependent activation of  $\text{p38}^{\text{MAPK}}$  and of Erk-2. This process was accompanied by enhanced insertion of Bsep into the canalicular membrane and by an increase of taurocholic acid excretion [32]. Thus, UDCA conjugates may improve the impaired secretory capacity of the cholestatic liver by modulating complex intracellular signaling cascades including calcium,  $\text{PKC}\alpha$ , and different MAP kinases (Fig. 3).

#### *Activation of transporter proteins*

Phosphorylation and dephosphorylation of transporter proteins at their site of action may be a third mechanism by which UDCA modulates apical secretion in hepatocytes. The mouse Bsep is phosphorylated by  $\text{PKC}\alpha$  [39]. Recent evidence indicates that the transport capacity of Bsep is increased by  $\text{PKC}\alpha$ -mediated phosphorylation [39] a process inhibited by PKC [40]. Because taurolithocholic acid (TLCA) has been shown to translocate  $\text{PKC}\epsilon$  selectively to canalicular membranes [41] and to exert cholestatic effects by phosphatidylinositol-3 kinase (PI3K) and putatively  $\text{PKC}\epsilon$ -dependent mechanisms [42], it is attractive to speculate that cholestatic (eg, TLCA) and anticholestatic (eg, TUDCA) bile acids, by differential modulation of PKC isoforms [22,41,42], affect transporter activity in the canalicular membrane in an opposite fashion [39,40].

Cholangiocytes contribute to bile formation by secreting a  $\text{HCO}_3^-$ -rich fluid. In cystic fibrosis,  $\text{Cl}^-$ -dependent  $\text{HCO}_3^-$  secretion is typically impaired as the result of a mutation of the *CFTR* gene that encodes for a  $\text{Ca}^{++}$ -independent  $\text{Cl}^-$  channel. UDCA is known to stimulate  $\text{HCO}_3^-$  secretion in rats and in humans. It has been speculated that UDCA may stimulate cholangiocyte  $\text{HCO}_3^-$  secretion by  $\text{Ca}^{++}$ -dependent mechanisms by activation of a  $\text{Ca}^{++}$ -dependent  $\text{Cl}^-$  channel and concomitant stimulation of  $\text{Cl}^-/\text{HCO}_3^-$  exchange through the AE2. UDCA increases  $[\text{Ca}^{++}]_i$  in cholangiocytes and induces membrane binding of  $\text{Ca}^{++}$ -dependent  $\text{PKC}\alpha$  [19], mechanisms similar to those observed in hepatocytes [20,21,36,37,43]. UDCA conjugates also increase  $[\text{Ca}^{++}]_i$  in cholangiocytes, possibly by stimulation of biliary adenosine triphosphate (ATP) secretion, which then may induce  $\text{Ca}^{++}$ -dependent  $\text{Cl}^-$  secretion through apical P2Y ATP receptors [44]. AE2 expression and bicarbonate secretion are impaired in patients with PBC, and their expression is increased after treatment with UDCA [30,45]. Thus, stimulation of cholangiocyte  $\text{HCO}_3^-$  secretion may contribute to the anticholestatic effect of UDCA, at least in certain biliary diseases in which  $\text{HCO}_3^-$  secretion is impaired.

### *Stimulation of detoxification of hydrophobic bile acids*

Only recently, it has been recognized that UDCA stimulates drug and steroid metabolism [46,47]. In mice it has been shown that UDCA, TUDCA, and taurohyodeoxycholic acid induce cytochrome P-450 (CYP) in the liver [46,47]. In parallel, certain CYP3A-linked enzyme activities such as testosterone 6-hydroxylase were increased [47]. By contrast, deoxycholic acid (DCA) decreased these activities. Concurrent administration of UDCA reduced the CYP-dependent inactivation of mixed function oxidases by DCA [47]. The expression of CYP enzymes is regulated by the pregnane X receptor/steroid and xenobiotic receptor (PXR/SXR). It has been reported that UDCA activates PXR/SXR in primary human hepatocytes and induces CYP3A4, which is a bile acid-metabolizing enzyme [48]. Theoretically, metabolism of hydrophobic, toxic bile acids to hydrophilic, less toxic compounds could be a hepatoprotective mechanism. In this context, the observation that UDCA stimulates CYP3A4-dependent metabolism in patients with gallstones is of great interest [49]. It is still unclear whether UDCA exerts this effect directly by binding to PXR/SXR or possibly by its metabolite lithocholic acid (LCA). LCA belongs to the strongest ligands for PXR/SXR [50]. In any case, the interaction of UDCA and other bile acids with nuclear receptors regulating bile acid metabolism has become an exciting avenue of research, which it is hoped will lead to a more complete understanding of the mechanisms of action of UDCA in cholestatic liver disease.

### *Protection against bile acid-induced apoptosis*

Apoptosis is an important mechanism of cell death in cholestatic liver diseases [51–53]. It has been attributed to the accumulation of hydrophobic bile acids in cholestatic hepatocytes [51]. In rat hepatocytes, glycochenodeoxycholic acid or glycodeoxycholic acid induce apoptosis by ligand-independent activation of the Fas death receptor [54], followed by activation of caspase 8 and the proapoptotic molecule, Bid. Bid chaperones another proapoptotic molecule, Bax, to the mitochondrial membrane. This process induces mitochondrial membrane permeability transition (MMPT), which causes a sudden increase in permeability of the inner mitochondrial membrane to ions (Fig. 4). MMPT is followed by mitochondrial swelling, release of cytochrome c to the cytosol, interaction of cytochrome c with the apoptotic protease-activating factor 1 (APAF-1), subsequent activation of caspase 9, and apoptotic cell death [51]. Antiapoptotic effects of UDCA have been demonstrated *in vitro* and *in vivo* in the rat [55,56] and in human hepatocytes [57]. UDCA was associated with a reduction of the MMPT and mitochondrial cytochrome c release [51,56]. UDCA diminishes Fas ligand-induced apoptosis in mouse hepatocytes, possibly by direct effects on the mitochondrial membrane [58]. It protects rat hepatocytes against bile acid-induced apoptosis by preventing bile acid-induced, c-Jun N-terminal kinase-dependent CD95 (Fas) trafficking to the plasma membrane [59]. Another mechanism by which UDCA inhibits apoptosis is activation of the epidermal growth factor receptor (EGFR), which

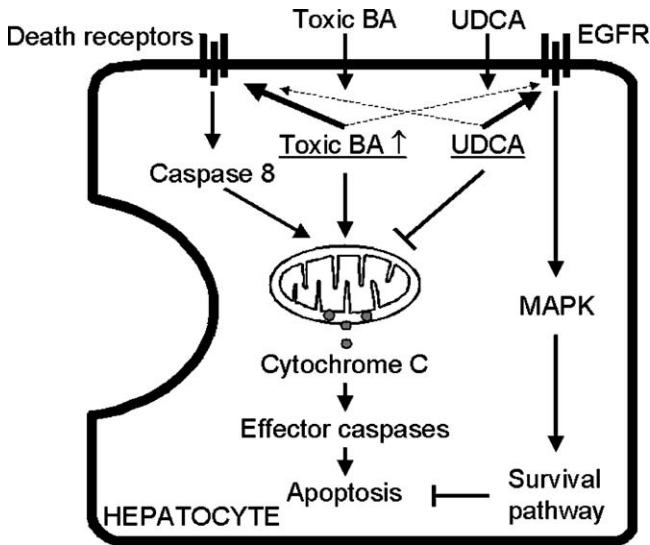


Fig 4. Effects of bile acids on hepatocellular death and survival pathways. For details, see text and Refs. [53,60]. BA, bile acid; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinases; UDCA, ursodeoxycholic acid.

inhibits bile acid–mediated apoptosis in rat hepatocytes. EGFR stimulates MAPK that induce a survival signal and inhibit apoptosis (Fig. 4) [60]. Blockage of this MAPK survival pathway renders UDCA toxic [60]. Although intriguing, the significance of antiapoptotic mechanisms in the beneficial effects of UDCA in cholestatic liver disease remains unclear at present.

A number of other potential sites of action of UDCA in chronic cholestatic liver diseases have been discussed. Modulation of cell-mediated immunity by UDCA, such as reversal of aberrant expression of HLA class I molecules on hepatocytes has been observed [17,61] but seems to be secondary to the anticholestatic effect of UDCA. In vitro studies suggested direct immunomodulating effects of UDCA on cytokine secretion of peripheral monocytes; the physiologic relevance of these studies, however, has been questioned because of methodologic concerns [62].

## Therapeutic efficacy of UDCA

### Primary biliary cirrhosis

PBC is characterized by an inflammatory lesion of interlobular bile ducts, which results in bile duct destruction and may progress to fibrosis and cirrhosis. Because the cause of the disease is unknown, presently available therapies aim at inhibiting the underlying pathogenetic processes (see Fig. 1) and delaying the progression of the disease.

In randomized, double-blind, placebo-controlled trials, UDCA at doses of 13 to 15 mg/kg/day improved serum liver tests, including bilirubin levels and other serum markers of cholestasis [15,16,28,63], the Mayo risk score [63], and liver histology [15,28]. It delays the progression of the disease to severe fibrosis or cirrhosis [64]. In line with this finding is the observation that UDCA delays the onset of esophageal varices [65]. Doses of UDCA lower than 10 mg/kg/day were of little benefit in PBC [66]. A combined analysis of three of the largest trials showed that treatment with UDCA at doses of 13 to 15 mg/kg/day for up to 4 years increased the time to liver transplantation or death [67]. Within the first 2 years of treatment, however, a survival benefit was not seen. A meta-analysis of eight randomized trials showed no difference between UDCA and placebo in the effects on incidence of death, liver transplantation and death, or liver transplantation [68]. In six of the eight studies, however, treatment was evaluated only up to 24 months, and the dose of UDCA was 10 mg/kg/day or lower in two of the studies. Therefore, a benefit of UDCA as shown in the combined analysis with doses higher than 13 mg/kg/day and a follow-up of 4 years [67] may have not been detectable in the meta-analysis. Similarly, another meta-analysis of 16 randomized trials that included studies with a follow-up as short as 3 months (3 months to 5 years) and UDCA doses as low as 8 mg/kg/day (8–15 mg/kg/day) did not detect a survival benefit for patients treated with UDCA [69]. An inadequate dose may often be responsible for nonresponse or suboptimal response to UDCA. It has been suggested that increasing the dose may improve the therapeutic effects [8].

### *Primary sclerosing cholangitis*

PSC is characterized by chronic periductal inflammation of intra- and extra-hepatic bile ducts leading to obliterative fibrosis, duct loss, and biliary cirrhosis. Primary therapeutic targets seem to be the injured cholangiocytes, but the effects of UDCA on the secondary alterations of cholestasis seem to play a role (see Fig. 1).

Randomized, controlled trials showed that UDCA at doses of 13 to 15 mg/kg/day improved serum liver tests and bilirubin levels but did not find an effect on disease progression or transplant-free survival [17,29]. Even the larger of these studies was probably too small and the follow-up period too short to allow evaluation of survival [29]. A small, randomized trial [70] suggested that a higher dose of UDCA, namely 20 mg/kg/day, may delay progression of histologic stage, improve cholangiographic appearance, and prolong projected survival. Thus, therapy of patients with PSC with high doses of UDCA is promising but must be evaluated by larger, controlled trials. In PSC, alkalinization of bile may be impaired because of decreased bicarbonate secretion by the diseased epithelium of the bile ducts and may diminish intestinal UDCA absorption. This impairment may explain why higher doses of UDCA may be required in PSC. Another situation that may lead to failure of medical therapy is the presence or the development of dominant bile duct strictures. Bile duct strictures are not prevented

by UDCA treatment and require endoscopic intervention with dilatation or temporary stenting [71,72].

### *Intrahepatic cholestasis of pregnancy*

In a small, controlled trial, 1 g/day of UDCA improved pruritus and serum liver tests, including serum bilirubin and transaminase levels, and diminished the number of premature deliveries [73]. An uncontrolled study showed that 1.5 to 2.0 g/day of UDCA (20–25 mg/kg/day) improves biochemical liver tests such as transaminase and serum bilirubin levels in intrahepatic cholestasis of pregnancy. These effects seem to be mediated primarily by the anticholestatic effect of UDCA, which is related to stimulation of biliary secretion. An effect on fetal or maternal outcome remains to be proven [74]. No side effects of UDCA have been reported in children or women treated during pregnancy. UDCA may be considered a safe treatment of intrahepatic cholestasis of pregnancy in the third trimester, but further controlled trials are needed before treatment of intrahepatic cholestasis of pregnancy with this drug can be generally recommended.

### *Liver disease in cystic fibrosis*

In a randomized, double-blind, placebo-controlled trial for 1 year, UDCA improved biochemical markers of cholestasis, nutritional status, and general condition [75]. Histologic improvement has been reported as well [76]. A higher dose of UDCA (20 mg/kg/day) seems to be more effective than a lower dose (10 mg/kg/day), because the intestinal absorption of UDCA and, as a consequence, enrichment of bile with UDCA may be impaired when pancreatic insufficiency is present [77]. The prognostic significance of these findings remains unclear, however, and the effect on survival has still to be proven. Because cholestasis in this genetic disease is caused by defective cholangiocellular bicarbonate secretion and diminution of cholangiocellular bile formation resulting in viscous bile, bile plugs, and biliary obstruction, stimulation of biliary secretion may be the major mechanism for the therapeutic effect.

### *Progressive familial intrahepatic cholestasis*

Patients with progressive familial intrahepatic cholestasis (PFIC) have been treated with 20 to 30 mg/kg/day of UDCA for periods of 2 to 4 years [78]. Liver function tests normalized in about 40% and improved in about 25% of the patients. In patients who responded, nutritional state, as evidenced by weight gain, improved. The factors responsible for response to UDCA are not yet clear. In patients with normal  $\gamma$ -glutamyl transpeptidase (GGT), who presumably had mutations of the *BSEP* or *FIC* gene, response may have been related to residual activity of BSEP or FIC. Similarly, patients with elevated serum GGT and a mutation of the *MDR3* gene who responded may have had only a partial defect and residual phospholipids in bile. In these patients UDCA may have reduced bile acid toxicity in bile sufficiently to inhibit the progression of the disease. In

contrast, patients who did not respond may have had a complete defect in phospholipid secretion.

### *Chronic graft-versus-host disease*

A randomized, placebo-controlled trial showed that prophylactic administration of UDCA in patients undergoing bone marrow transplantation with a preparative regimen of busulfan plus cyclophosphamide decreased the incidence of cholestasis and hepatic complications [79].

### *Drug-induced cholestasis*

Small case series suggest that UDCA treatment may be beneficial in some forms of drug-induced cholestasis [51]. The major mechanism of action in these patients may primarily be stimulation of biliary secretion.

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