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Ursodeoxycholic acid treatment of vanishing bile duct syndromes

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Abstract

Vanishing bile duct syndromes (VBDS) are characterized by progressive loss of small intrahepatic ducts caused by a variety of different diseases leading to chronic cholestasis, cirrhosis, and premature death from liver failure. The majority of adult patients with VBDS suffer from primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Ursodeoxycholic acid (UDCA), a hydrophilic dihydroxy bile acid, is the only drug currently approved for the treatment of patients with PBC, and anticholestatic effects have been reported for several other cholestatic syndromes. Several potential mechanisms of action of UDCA have been proposed including stimulation of hepatobiliary secretion, inhibition of apoptosis and protection of cholangiocytes against toxic effects of hydrophobic bile acids.

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Key words: Cholestasis; Primary biliary cirrhosis; Primary sclerosing cholangitis; Secretion; Signaling; Transport; Ursodeoxycholic acid; Vanishing bile duct syndrome

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INTRODUCTION

Cholestasis is defined as an impairment of bile flow and failure to adequately secrete cholephilic compounds into bile often associated with clinical manifestations such as jaundice and pruritus and biochemical alterations such as an elevation in serum alkaline phosphatase and γ -glutamyl

transpeptidase. The liver damage observed in chronic cholestasis has long been attributed to accumulation and retention of potentially toxic bile acids within hepatocytes. Chronic cholestasis is the main feature of vanishing bile duct syndromes (VBDS) characterized by progressive loss of small intrahepatic ducts caused by a variety of different diseases. In children, biliary atresia and Alagille syndrome are rare ductopenic diseases classified as VBDS. The majority of adult patients with VBDS suffer from primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Other diseases associated with ductopenia include autoimmune cholangitis, chronic hepatic allograft rejection, graft-versus-host disease (GVHD), chronic cholestatic sarcoidosis and ischemic (vascular) cholangiopathies. Among the various causes of VBDS, drugs have an increasing importance. Drug reactions are related especially to antibiotics, phenothiazine derivatives and carbamazepine. It is increasingly clear that immunopathogenetic mechanisms involving innate and adaptive immune responses contribute to ductopenia in most of these diseases. Prognosis varies from hepatic failure and death or liver transplantation to resolution of cholestasis and normal liver function.

Ursodeoxycholic acid (UDCA) is a hydrophilic dihydroxy bile acid (chemical structure: $3\alpha, 7\beta$ -dihydroxy- 5β -cholanoic acid) which was first identified in the bile of the Chinese black bear^[1]. UDCA is a physiologic bile acid also present in man albeit in a low concentration of about 3% of the bile acid pool, where it is formed by 7β -epimerization of the primary bile acid chenodeoxycholic acid in the gut by intestinal bacteria^[2,3]. It has been used for centuries in traditional Chinese medicine for the treatment of liver diseases. Reports from Japan and Europe first revealed that UDCA was able to dissolve gallstones^[4-6]. In 1985, Leuschner and coworkers observed improved serum liver tests in patients with chronic active hepatitis treated with UDCA for gallstone dissolution^[7], and similar observations were made previously in Japan. Since then, various trials have shown the beneficial effect of UDCA for different cholestatic syndromes.

PHARMACOKINETICS AND METABOLISM OF UDCA

After oral administration, UDCA is absorbed by passive nonionic diffusion mainly in the small intestine (~80%)

and less in the colon (-20%) following solubilization in mixed micelles of endogenous bile acids in the proximal jejunum^[2,8]. Its absorption rate is enhanced when it is given with a meal and may be decreased in patients with cholestasis and decreased biliary secretion of endogenous bile acids^[9]. After intestinal absorption, UDCA is taken up from the portal blood by the hepatocytes at their sinusoidal domain *via* specific bile acid transporters namely, NTCP and OATP, conjugated mainly with glycine and to a lesser extent with taurine and is subsequently transported across the canalicular domain into the bile ducts *via* another bile acid carrier molecule, designated BSEP^[2,10]. UDCA conjugates reach the small intestine and are reabsorbed mainly from the distal ileum *via* an active Na⁺-dependent transport mechanism undergoing an effective enterohepatic circulation. Non-absorbed UDCA and UDCA conjugates pass into the colon and, after bacterial deamidation of conjugates, are mostly converted to lithocholic acid by intestinal bacteria and eliminated *via* the feces^[2]. Only minute amounts of the insoluble lithocholic acid are reabsorbed *via* the colonic mucosa, sulphated in the liver, secreted into bile and excreted in the feces. Under continuous oral treatment at pharmacological doses (13-15 mg/kg per day) UDCA becomes the predominant bile acid in the liver and the systemic circulation, comprising 40% to 60% of total bile acid^[2,8].

MECHANISMS OF ACTION OF URSO-DEOXYCHOLIC ACID

The mechanisms underlying the beneficial effects of UDCA in cholestasis are being increasingly unraveled^[11-14]. Initial research interest was focused on changes in bile acid pool composition, hepatocyte membrane protective effects, immunomodulatory effects, and bicarbonate-rich hyperchloresis induced by UDCA. Over the past decade, it has, however, become evident that UDCA is a potent intracellular signaling agent that induces stimulation of impaired hepatocellular secretion, anti-apoptotic effects and may mediate cholangiocyte protection. Depending on the pathophysiology and the stage of the underlying liver disease, the predominant mechanisms of action of UDCA may vary.

Stimulation of hepatobiliary secretion

Cholestatic liver diseases are characterized by an impairment of hepatobiliary secretion. As a consequence, bile acids and other potentially toxic cholephiles accumulate in the hepatocyte and may lead to liver cell injury, apoptosis and necrosis. UDCA stimulates biliary secretion of bile acids and other organic compounds (e.g. bilirubin glucuronides, glutathione conjugates, bromosulphophthalein) in various experimental models such as the isolated hepatocytes, isolated perfused rat liver and bile fistula rat model and counteracts cholestasis induced by hydrophobic bile acids in rat liver^[15-22]. In line with these observations, biliary secretion of bile acids and phospholipids is stimulated and elevated serum levels of the hydrophobic bile acid, chenodeoxycholic acid, and

of bilirubin are decreased in patients with PBC and PSC during UDCA treatment^[23-28]. Thus, the beneficial effects of UDCA in cholestatic liver disease may be partly due to the enhanced elimination of toxic compounds from the hepatocytes. The secretory capacity of the hepatocytes is determined by the number and activity of transporter proteins in the canalicular membrane. UDCA stimulates the expression of transporter proteins for biliary secretion in the liver and the targeting and insertion of transporter molecules into the canalicular membrane at a transcriptional and posttranscriptional level^[15,29-31]. As such, UDCA stimulates the overall gene expression of both canalicular (Mrp2, Bsep) and alternative basolateral carriers (Mrp3, Mrp4) in mouse liver, which facilitates alternative efflux of bile salts and other organic anions into the systemic circulation^[32,33]. UDCA also stimulates murine renal (Mrp2, Mrp4) and intestinal (Mrp2, Mrp3) efflux transport proteins, resulting in an increased overall elimination capacity for potentially toxic biliary compounds^[32]. In addition to these transcriptional effects, UDCA also stimulates vesicular exocytosis and insertion of transporter proteins into the canalicular membrane by modulating complex intracellular signalling cascades including calcium, protein kinase C and different mitogen-activated protein kinases^[15,31,34-36]. Moreover, UDCA may also directly activate canalicular transporters through modification of their phosphorylation status^[37]. While 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. In conclusion, UDCA modulates hepatobiliary secretion by transcriptional and posttranscriptional mechanisms in experimental models *in vivo* and *in vitro*. Thus, upregulation of synthesis, apical targeting and insertion, and activation of key canalicular transporters may represent key mechanisms to explain the anticholestatic action of UDCA in patients with cholestatic liver disease.

Inhibition of apoptosis

Apoptosis, or programmed cell death, is an important mechanism of cell death in cholestatic liver diseases^[14,38,39]. For example, apoptotic features causing bile duct loss have been observed more frequently in liver tissue from patients with PBC than in normal controls^[40]. Toxic bile acids can induce apoptosis in hepatocytes at concentrations comparable to those found in chronic cholestasis and the mechanisms of bile acid-induced apoptosis have increasingly been understood (reviewed by Higuchi *et al*^[41]). Endogenous hydrophobic bile acids such as glycochenodeoxycholic acid (GCDCA) or glycodeoxycholic acid (GDCA) induce apoptosis by ligand-independent activation of the Fas death-receptor, followed by activation of caspase 8 and Bid, a pro-apoptotic member of the Bcl-2 protein family, which chaperones another pro-apoptotic Bcl-2 molecule, Bax, to the mitochondrial membrane inducing mitochondrial membrane permeability transition (MMPT). MMPT causes a sudden increase in permeability of the inner mitochondrial membrane to ions followed by

mitochondrial swelling and release of cytochrome c from the intermembrane space to the cytosol. In the cytosol, cytochrome c interacts with the apoptotic protease-activating factor 1 (APAF-1), which leads to activation of caspase 9 and subsequently causes apoptotic cell death^[40,42].

A number of studies showed that UDCA can block apoptosis *in vitro* as well as *in vivo* in the rat and in human hepatocytes by interrupting classic pathways of apoptosis^[43-45]. Antiapoptotic effects of UDCA were associated with a reduction of the MMPT and mitochondrial cytochrome c release^[14,45]. UDCA protects rat hepatocytes against bile acid-induced apoptosis by preventing bile acid-induced, c-Jun N-terminal kinase-dependent Fas trafficking to the plasma membrane^[46]. Apoptosis can not only be inhibited by blocking pro-apoptotic pathways, but also by activation of intracellular survival signals. Indeed, UDCA was shown to induce a survival signal in hepatocytes *via* activation of the epidermal growth factor receptor (EGFR) and mitogen-activated protein kinase (MAPK) that may contribute to the antiapoptotic effect^[47]. Thus, UDCA appears to be a bile acid that partially activates pro-apoptotic signals, but also simultaneously a strong MAPK-dependent survival signal, which makes UDCA an overall non-toxic bile acid. Although intriguing, the impact of antiapoptotic mechanisms for the beneficial effects of UDCA in cholestatic liver disease remains unclear at present.

Protection of cholangiocytes against toxic effects of hydrophobic bile acids

Hydrophobic bile acids damage cell membranes of hepatocytes and cholangiocytes and exert extracellular cytotoxicity at millimolar concentrations present in bile^[48-50]. Indeed, levels of hydrophobic bile acids are about 1000-fold higher in bile than in serum even under physiological conditions. The proposed mechanisms of bile acid-induced cell damage extend from simply binding to plasma membranes to the induction of apoptosis or even necrosis. UDCA has been shown to counteract hydrophobic bile acid-induced membrane disruption *in vitro* presumably by alteration of the structure and composition of simple and mixed micelles rather than by direct membrane interactions^[50,51]. UDCA treatment at therapeutic doses of 13-15 mg/kg per day renders the bile acid composition of bile less hydrophobic leading to a relative enrichment of UDCA to about 40%-50% of total biliary bile acids^[8]. To achieve this effect in isolated cells or whole organ a high (millimolar) concentration of UDCA is required. Thus, the membrane protective effects of UDCA play a role mainly at the bile duct level, since high concentrations of bile acids are only present within the biliary tree^[52]. UDCA decreases the degree of cholangiocellular injury, portal inflammation and ductular proliferation in the Mdr2-knockout mouse, an animal model of cholestasis which shares morphological features with PSC resulting from the toxic effect of bile acids on the biliary epithelium in the absence of protective mixed micelle formation with phospholipids^[53,54]. In line with these observations, the (peri-) portal inflammatory reaction is less severe in patients with PBC and PSC

under UDCA treatment as compared to those treated with placebo^[27,28,55,56]. The effects of UDCA conjugates on cholangiocytes are apparently mediated by Ca²⁺- and protein kinase C α -dependent mechanisms which have been implicated in stimulation of biliary secretion in cholestatic hepatocytes as outlined above. Although the protection against the consequences of bile duct destruction is likely to be one mechanism of action of UDCA, the underlying pathophysiologic events leading to the ongoing bile duct destruction are probably not influenced by UDCA.

UDCA IN VANISHING BILE DUCT SYNDROMES

Primary biliary cirrhosis

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by chronic inflammation and destruction of intrahepatic bile ductules, which leads to progressive ductopenia, ultimately resulting in fibrosis and biliary cirrhosis. The prevalence differs considerably in different geographic areas, ranging from 40 to 400 per million. It primarily affects women, with a female to male ratio as high as 10 to 1^[57,58]. Current theories on the pathogenesis of PBC favor the hypothesis that the disease develops as a result of an inappropriate immune response following stimulation by an environmental or infectious agent. The pathogenetic mechanism is believed to be caused by a defect in immunologic tolerance, resulting in the activation and expansion of self-antigen specific T and B lymphocyte clones and the production of circulating autoantibodies in addition to a myriad of cytokines and other inflammatory mediators. The serologic hallmark of PBC is the presence of autoantibodies to mitochondria, especially to the E2 component of the pyruvate dehydrogenase complex (PDC)^[59]. 50% to 60% of patients are asymptomatic at diagnosis, and the disease is initially detected on the basis of screening liver-biochemistry profiles. Fatigue and pruritus are the most common presenting symptoms^[60], occurring in up to 78% and 70% of patients, respectively. Jaundice develops usually later, often associated with progression of the disease^[61]. Eventually, complications of cirrhosis and portal hypertension, such as ascites, variceal bleeding, and hepatic encephalopathy, develop.

UDCA is now the mainstay of therapy for PBC. In 1987 Poupon and co-workers reported benefit from UDCA treatment^[62]. Since then, several randomized double-blind placebo-controlled studies have shown that UDCA improves biochemical parameters including serum bilirubin levels which are used as a prognostic marker for PBC^[25,27,55,63-66]. Indeed, it has been shown that the mean survival in patients with serum bilirubin levels above 34 $\mu\text{mol/L}$ is 4 years; in those with values of more than 103 $\mu\text{mol/L}$ only 2.1 years^[67]. This prognostic value of serum bilirubin is similar in UDCA-treated patients as in non-treated patients. The benefit from UDCA therapy on liver fibrosis progression in PBC has been shown by Corpechot *et al.*^[68] using Markov modeling: A fivefold decrease in progression rate from early-stage disease toward extensive fibrosis or cirrhosis was found in UDCA-treated

patients compared with placebo-treated patients. At 4 years, the probability of UDCA-treated patients to remain in early stage disease was 76% (95% confidence interval: 58%-88%), as compared with 29% (15%-52%) in placebo-treated patients. These findings are consistent with recent data showing that a 2-year treatment with UDCA reduces periportal necroinflammatory lesions, improves ductular proliferation, and delays the progression of histologic stage when given at the earlier stages (stages I-II) of the disease^[69]. When started later in the disease course, UDCA can still ameliorate inflammation and ductular proliferation but is not capable of reversing fibrosis.

What is still more debatable is the effect of UDCA treatment on long-term survival. A combined analysis of three larger randomized trials, in which patients with primary biliary cirrhosis were randomly assigned to receive UDCA ($n = 273$) or placebo ($n = 275$) suggested that survival free of liver transplantation was significantly improved in patients who received UDCA treatment for 4 years^[70]. This benefit was seen in patients with moderate to severe disease but not in those with mild disease (serum bilirubin concentration $< 23.9 \mu\text{mol/L}$ or histologic stage I or II) probably because progression to end-stage disease did not occur in the short time interval of the study in patients with mild disease^[70]. Lindor *et al* showed that survival of patients treated up to 6 years with UDCA was increased as compared with a placebo group or the predicted survival from the Mayo model^[71]. In a large cohort of UDCA-treated PBC patients (225 patients) followed for up to 10 years survival free of liver transplantation was significantly higher than survival predicted by the Mayo model and 10-year survival among UDCA-treated patients is slightly lower than that of an age- and sex-matched population, the difference being mainly explained by mortality among cirrhotic patients^[72]. However, the efficacy of UDCA in PBC has also been questioned. The extended follow-up of another clinical trial^[73], as well as a randomized controlled trial with the longest follow-up^[55], did not show a favorable effect of UDCA on the incidence of death or liver transplantation. In addition, a systematic review and meta-analysis of 11 randomized controlled trials, including 1272 patients, and six reports of the switch-over phases did not find a beneficial effect of UDCA on the incidence of liver-related death, liver transplantation, or the development of complications of liver disease^[74]. Similarly, another meta-analysis of 16 randomized clinical trials evaluating UDCA against placebo ($n = 15$) or no intervention ($n = 1$) in 1422 patients did not detect a significant effect of UDCA on mortality^[75]. However, these meta-analyses included trials of different length of follow-up, mostly only up to 24 mo, which were performed with various doses of UDCA, in part less than 13-15 mg/kg per day, a dose currently regarded as optimal for PBC. Thus, these meta-analyses have to be interpreted with caution, because survival analyses in a disease with a very long natural history over decades are ideally based on longer follow-up periods. In a further analysis including five studies with a follow-up of at least 4 years, a 32% reduction in the risk of death or need of liver transplantation was reported in patients treated with UDCA^[76]. Nonetheless, to clarify the true efficacy of UDCA in the long-term treatment of PBC, additional data

are needed. At present, the general recommendation is to treat PBC patients with UDCA at a dose of 13-15 mg/kg per day. Treatment should be started as early as possible, since patients with mild or moderate disease are likely to have a greater benefit from therapy than those with advanced disease.

Primary sclerosing cholangitis (PSC)

Primary sclerosing cholangitis (PSC) is a rare chronic cholestatic disease of the intra- and extrahepatic bile ducts that is generally progressive and leads to end-stage liver disease with a median survival from diagnosis to death or liver transplantation, currently estimated at 12 years^[77-80]. PSC is characterized by diffuse inflammation, concentric obliterative fibrosis and progressive stricturing and dilatation of the biliary tree. The etiology and pathogenesis is unknown, but as in PBC, immune dysregulation plays an important role in the development of the disease. In Western populations, the estimated prevalence of PSC is 8.5 cases per 100 000 persons^[81] and 70%-80% of PSC patients have or will develop inflammatory bowel disease. Of importance, patients with PSC have an increased risk of developing cholangiocarcinoma with an estimated yearly incidence of 1.5% after diagnosis of PSC^[82-85] and patients with both PSC and ulcerative colitis may be at higher risk for developing colorectal aneuploidy, dysplasia, or cancer than UC patients without PSC^[86,87]. Based on the beneficial effect of UDCA in patients with PBC, UDCA was evaluated in a number of small trials for patients with PSC in the early 1990s^[28,88,89]. Although small, these studies observed significant biochemical and histologic improvements for patients receiving UDCA. Similar to the treatment of PBC, no major side effects were reported. In contrast to these promising results, the first large randomized placebo-controlled study including 105 patients with PSC could not find a clinical benefit of UDCA treatment with respect to time to treatment failure or histologic findings in a dose of 13-15 mg/kg per day during a mean follow-up period of 2.2 years^[26]. A meta-analysis of six randomized clinical trials showed no difference between UDCA and placebo in the effects of incidence on death, treatment failure (including liver transplantation, varices, ascites, and encephalopathy), liver histological deterioration or liver cholangiographic deterioration^[90]. However, most trials were small with an average sample size of 37 patients and the follow-up period might have been too short to show a clinical benefit, since PSC has usually a long natural history of over a decade. A large percentage of the patients had advanced disease and as in PBC, these patients might respond less to medical treatment than patients in earlier stages of disease. Finally, UDCA therapy alone will not suffice to treat the bile duct strictures typical for PSC, but may need additional mechanical intervention. Indeed, major bile duct strictures develop during UDCA treatment^[91]. Based on these observation, UDCA treatment was combined with endoscopic dilatation of bile duct strictures in an 8-year prospective study. The survival of patients receiving this combination therapy was significantly better than the calculated survival without treatment^[92]. As biliary enrichment of UDCA is expected to be lower in cholestasis and because of discouraging results with standard doses of UDCA (13 -15

mg/kg per day), use of high doses of UDCA in PSC has a rationale and has been evaluated by three groups^[93-95]. The first study compared high-dose UDCA (20 mg/kg per day) ($n = 13$) to placebo ($n = 13$) for 2 years in 26 patients with PSC and demonstrated a significant improvement in serum levels of AP and γ -GT (no effect on bilirubin and albumin levels), and a significant reduction in progression of cholangiographic features and liver fibrosis as assessed by disease staging without significant side effects. UDCA did not improve symptoms^[94]. Harnois *et al.*^[93] reported similar results in a 1-year open-label study of 30 PSC patients treated with UDCA at a dose of 25-30 mg/kg per day. Changes in the Mayo risk score at 1 year of treatment and projected survival at 4 year were compared with that observed in patients randomized to placebo or UDCA at a dose of 13-15 mg/kg per day in a previous study. A significant improvement in serum alkaline phosphatase activity, AST, albumin and total bilirubin occurred at 1 year of therapy with high-dose UDCA. The expected survival at 4 years was significantly improved for patients in the high-UDCA group when compared with a historical placebo control, but not between the dose of 13-15 mg/kg per day and placebo. As in the first study, high-dose UDCA was well tolerated. Finally, in the biggest placebo-controlled prospective study in PSC ever performed, a total of 219 patients were randomized to 17 to 23 mg/kg per day of UDCA ($n = 110$) or placebo ($n = 109$) for 5 years^[95]. No statistically significant benefit from 20 mg/kg UDCA on survival without liver transplantation or prevention in cholangiocarcinoma in PSC was shown in this study. However, there was a tendency to improved survival in the UDCA-treated patients and the study was underpowered to show a possible positive effect.

UDCA has also been shown to have colon cancer chemopreventive effects in preclinical studies^[96,97]. In addition, two clinical studies suggested that UDCA also decreases the risk for developing colorectal neoplasia in UC patients with PSC^[98,99]. In a cross-sectional study by Tung *et al.* involving 59 patients with ulcerative colitis and primary sclerosing cholangitis who were undergoing colonoscopic surveillance for colonic dysplasia, UDCA use was strongly associated with decreased prevalence of colonic dysplasia (odds ratio, 0.18, 95% confidence interval, 0.05 to 0.61; $P = 0.005$)^[98]. In a blinded, prospective study by Pardi *et al.*^[99], 52 patients with ulcerative colitis and PSC were randomized to receive UDCA or placebo and were followed-up for a total of 355 person-years. Those originally assigned to receive UDCA had a relative risk of 0.26 for the development of dysplasia or cancer (95% confidence interval, 0.06-0.92; $P = 0.034$) suggesting a significant chemoprotective effect for UDCA in these patients.

In conclusion, UDCA therapy (13-20 mg/kg per day) of PSC is warranted and should be initiated as soon as possible and be continued life-long. In addition, major strictures should be dilated regularly in centers with experienced endoscopists. In patients with PSC and ulcerative colitis, who carry a considerable lifetime risk for colorectal neoplasia, UDCA may act as a chemopreventive drug. Due to the unresolved issues of optimal dosing and the true long-term benefit of UDCA in PSC, patients

should be included in trials if possible.

Intrahepatic cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy (ICP) is the most common pregnancy-related liver disorder characterized by pruritus associated with a mild or moderate increase in serum aminotransferases and serum bile acids starting in the second or third trimester of pregnancy and disappearing after delivery. ICP is associated with increased risk of intrauterine fetal death and premature delivery^[100,101]. There is increasing evidence that genetically determined dysfunction in the canalicular ABC transporters bile salt export pump and multidrug resistance protein 3 might be risk factors for ICP development^[102]. In small controlled trials and several observational trials, UDCA improved maternal pruritus and serum liver chemistries such as serum bilirubin and transaminases, and diminished the number of premature deliveries. The medication seems to be well tolerated by pregnant women and no adverse effects in mothers or newborns have been detected^[103-105]. A recent prospective, randomized trial ($n = 84$) comparing cholestyramine (8 g daily) and UDCA (8-10 mg/kg daily) for the treatment of ICP further supported these findings. Pruritus was more effectively reduced by UDCA than cholestyramine. Newborns were delivered significantly closer to term by patients treated with UDCA, than those treated with cholestyramine. Serum alanine and aspartate aminotransferase activities were markedly reduced by 78.5% and 73.8%, respectively, after ursodeoxycholic acid, but by only 21.4%, each, after cholestyramine therapy. Ursodeoxycholic acid, but not cholestyramine was free of adverse effects^[106]. In ICP, the relief of cholestasis by UDCA has been suggested to be due to stimulation of vesicular exocytosis resulting in mobilization of an increased number of transport proteins to the canalicular membrane and, thereby, stimulation of transport systems involved in the biliary secretion of steroid mono- and disulfates. In conclusion, data that are available support the use of UDCA as first-line therapy for ICP in the third trimester.

Hepatic complications of allogeneic bone marrow transplantation

Hepatic graft-versus-host disease (GVHD) is a frequent complication after bone-marrow transplantation usually associated with a cholestatic picture characterized by elevated serum alkaline phosphatase and hyperbilirubinemia attributed to damage of the small bile ducts. UDCA has been reported to be effective in the treatment of manifest GVHD^[107,108]. In an open-label study 12 patients with refractory chronic GVHD of the liver after allogeneic bone marrow transplantation were given UDCA (10 to 15 mg/kg per day) for 6 wk. Serum tests of cholestatic liver injury showed improvement compared with baseline. After discontinuation of UDCA therapy, 11 patients were followed for 6 additional weeks. All showed significant worsening in liver function test results. Symptom scores were unchanged throughout the study. No adverse effects were observed^[107]. In addition, the efficacy of UDCA has also been reported as a prophylaxis for veno-occlusive disease (VOD) of the liver after

allogeneic bone marrow transplantation. In a randomized, double-blind, placebo-controlled study including 67 consecutive patients undergoing transplantation with allogeneic bone marrow with a preoperative regimen of busulfan plus cyclophosphamide were randomly assigned to receive UDCA 300 mg twice daily or placebo. The incidence of VOD was 40% in placebo recipients and 15% in the UDCA-treated recipients ($P = 0.03$)^[109]. Thus, further studies are certainly needed, but UDCA may be considered for prophylaxis of VOD and treatment of GVHD of the liver.

Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene resulting in the secretion of viscous bile. This may lead to the formation of bile duct plugs within small bile ducts, biliary obstruction and ultimately result in (focal to multilobular) biliary cirrhosis. Hepatobiliary complications increase with patient age and up to 7% of children and young adults with CF may present with liver cirrhosis^[110]. Early uncontrolled studies showed that UDCA significantly improved laboratory tests and nutritional status of patients with CF^[111-113]. A small randomized, placebo-controlled study showed clinical, biochemical and nutritional improvement in CF patients treated with UDCA (15 mg/kg per day) for one year^[114]. Improvement of liver histology of CF patients treated with UDCA (10-15 mg/kg per day) for 2 years has been reported as well^[115]. A higher dose of 20 mg/kg per day may be more efficacious than lower doses (5-15 mg/kg per day)^[111,116]. Thus, UDCA may be considered as potentially effective in CF patients. However, whether UDCA affects the natural history of liver disease in CF-particularly amelioration of the complications of portal hypertension, need for liver transplantation, or a measurable survival is unknown and further well-designed, adequately powered, randomized controlled trials should address these issues. Stimulation of biliary secretion may be the major mechanism for the therapeutic effect. Interestingly, it has been hypothesized that the therapeutic effect of UDCA is mediated in part by stimulation of ATP release into bile and subsequent ATP-induced bicarbonate secretion by bile-duct epithelia *via* calcium-dependent chloride channels distinct from the CFTR^[117].

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