

Review article: mechanisms of action and therapeutic applications of ursodeoxycholic acid in chronic liver diseases

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SUMMARY

Ursodeoxycholic acid (ursodiol) is a non-toxic, hydrophilic bile acid used to treat predominantly cholestatic liver disorders. Better understanding of the cellular and molecular mechanisms of action of ursodeoxycholic acid has helped to elucidate its cytoprotective, anti-apoptotic, immunomodulatory and choleretic effects. Ursodeoxycholic acid prolongs survival in primary biliary cirrhosis and it improves biochemical parameters of cholestasis in various other cholestatic disorders including primary sclerosing cholangitis, intrahepatic

cholestasis of pregnancy, cystic fibrosis and total parenteral nutrition-induced cholestasis. However, a positive effect on survival remains to be established in these diseases. Ursodeoxycholic acid is of unproven efficacy in non-cholestatic disorders such as acute rejection after liver transplantation, non-alcoholic steatohepatitis, alcoholic liver disease and chronic viral hepatitis. This review outlines the present knowledge of the modes of action of ursodeoxycholic acid, and presents data from clinical trials on its use in chronic liver diseases.

INTRODUCTION

History of ursodeoxycholic acid

Ursodeoxycholic acid (ursodiol, UDCA) is a hydrophilic dihydroxylated bile acid (chemical structure: 3 α , 7 β -dihydroxy-5 β -cholanoic acid) which was first identified in the bile of the Chinese black bear and was named after this species (ursus Lat. = bear).¹ UDCA is also present in very small quantities as a secondary bile acid in humans (1–3% of the total bile acid pool) where it is formed by 7 β -epimerization of the primary bile acid chenodeoxycholic acid in the gut by intestinal bacteria.^{2, 3} In contrast to humans, UDCA is a primary bile acid in bears and nutria where it is directly synthesized from cholesterol. Dried bear bile has been used for centuries as a remedy for liver disease in China on an empirical basis,

based on a long-standing belief that bear bile had curative properties.⁴ The structure of UDCA was elucidated in 1936 by Iwasaki⁵ and it was subsequently synthesized and marketed in combination with vitamins as a hepatoprotective agent in Japan.³ Reports from Japan and Europe first revealed that UDCA was able to dissolve gallstones, similarly to chenodeoxycholate, but that it was not hepatotoxic.^{6, 7} Leuschner and coworkers were the first to report in the Western literature their observation that UDCA given for gallstone dissolution in patients with chronic active hepatitis also had improved routine liver function tests.⁸ Similar observations had previously been made in Japan.^{9, 10} These initial reports prompted further studies on the use of UDCA, mainly in chronic cholestatic disorders.^{3, 11–13}

Biochemical properties of bile acids

Bile acids are natural steroids synthesized from cholesterol in the liver. After excretion into bile, 95% of bile

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acids are resorbed in the intestine (mainly in the terminal ileum), returned to the liver, and then re-secreted into bile in an 'enterohepatic circulation'.¹⁴ Primary bile acids (cholic and chenodeoxycholic acid) are deconjugated and then dehydroxylated to secondary bile acids (mainly deoxycholic and lithocholic acid) by colonic bacteria. Under normal circumstances the majority of bile acids (90–95%) are conjugated to glycine or taurine and tightly bound to plasma and intracellular proteins; the free concentration of usually < 5% of the total concentration limits their toxicity.¹⁵

Bile acids differ in number (mono-, di-, tri-), position (C3, 7, 12) and orientation (α , β) of their steroid hydroxyl groups.^{3, 14, 16, 17} The lower the number of hydroxyl groups, the higher the hydrophobicity of a bile acid (e.g. monohydroxy bile acids are more hydrophobic than dihydroxy bile acids). In addition to the hydroxylation site, orientation of the hydroxyl group is another important determinant of hydrophobicity [e.g. 7 β -hydroxylation (e.g. ursodeoxycholic acid) renders a bile acid less hydrophobic than 7 α -hydroxylation (e.g. chenodeoxycholic acid)]. In general, the toxicity of a bile acid is directly related to its hydrophobicity; this in turn is predictive of such biological properties as interaction with biological membranes. A 'hydrophobicity index' of bile acids can be determined by reversed-phase high-performance liquid chromatography. As such, hydrophobicity decreases in the following order: lithocholic acid (3 α -OH) > deoxycholic acid (3 α , 12 α -OH) > chenodeoxycholic acid (3 α , 7 α -OH) > cholic acid (3 α , 7 α , 12 α -OH) > ursodeoxycholic acid (3 α , 7 β -OH).¹⁷ However, depending on the method used (e.g. octyl silane instead of octadecyl silane as the stationary phase), ursodeoxycholic acid (3 α , 7 β -OH) may be more hydrophobic than cholic acid (3 α , 7 α , 12 α -OH).¹⁸

Normal systemic serum bile acid concentrations are in the range of 2 μ M (basal) to 10 μ M (postprandial), while bile acid levels in portal vein plasma may reach 100 μ M following a meal.¹⁵ The mean concentration in the human hepatocyte is estimated to be 50 μ M. Bile acids reach millimolar concentrations in the biliary tree, where they form mixed micelles with phospholipids, preventing destruction of the bile duct epithelium.

Bile acids are amphipathic detergents that are potentially toxic and cholestatic depending on their hydrophobicity and concentration. Bile acids interact with cell membranes in different ways, including binding, insertion into the lipid bilayer, and solubilization of mem-

brane lipids.¹⁴ However, the concentrations required for clear-cut cell lysis are rarely (if ever) reached in cholestatic conditions.¹⁵ Rather, bile acids increase membrane fluidity and permeability to calcium (resulting in increased intracellular calcium levels), inhibit mitochondrial function (oxidative phosphorylation), induce cytoskeletal changes, lipid peroxidation, changes in DNA structure, and finally apoptosis.^{3, 15}

With cholestasis there is intrahepatic and systemic retention of biliary constituents, including bile acids.^{19, 20} Bile acids may be involved in the primary events leading to cholestasis, and may also sustain or worsen a cholestatic process.^{14, 21} In cholestasis, serum levels in the range 500 μ M to 1 mM have been observed; these are clearly toxic. Further alterations of bile acid metabolism in cholestasis include an initial increase, then a decrease of the ratio of trihydroxy to dihydroxy bile acids (due to decreased hepatic hydroxylation reflecting cholestatic liver injury), a decrease in secondary bile acids (due to disruption of the enterohepatic circulation), the appearance of several unusual bile acids, and sulphation and glucuronidation of bile acids as the significant modes of conjugation (making the bile acids more water soluble for urinary excretion).¹⁴

Pharmacodynamics of ursodeoxycholic acid

After oral administration of unconjugated UDCA, 30–60% of the dose is passively absorbed in the small and large intestines, and undergoes efficient hepatic uptake (> 60% of the absorbed dose) and conjugation in the liver with glycine (to a lesser extent with taurine).^{2, 22} Because colonic absorption may account for as much as 20% of an ingested dose, unconjugated UDCA is also absorbed in patients who have had ileal resections, as long as high oral doses (4 g/day) of UDCA are administered.²² Conjugated UDCA is then secreted into the biliary tree and intestine where it undergoes efficient enterohepatic circulation with active reabsorption in the terminal ileum.² Under continuous oral treatment at pharmacological doses (10–15 mg/kg/day) UDCA becomes the predominant bile acid in the liver and the systemic circulation, comprising 40–60% of the circulating bile acid pool^{2, 22} (see below). In contrast to rats,²³ administration of taurine conjugated UDCA (TUDCA) to humans does not result in higher UDCA enrichment in the liver than oral administration of unconjugated UDCA.²⁰

MECHANISMS OF ACTION OF UDCA

Four principal mechanisms of action of UDCA have been recognized:^{3, 4, 18, 24, 25} (i) replacement/displacement of toxic endogenous bile acids through UDCA; (ii) cytoprotective effects on hepatocytes and bile duct epithelial cells; (iii) immunomodulatory effects; and (iv) stimulation of bile secretion by hepatocytes and bile duct epithelial cells.

Initial research interest was focused on changes in bile acid pool composition, hepatocyte membrane protective effects, immunomodulatory effects, and bicarbonate-rich hypercholeresis induced by UDCA. Over recent years, it has become clear that other mechanisms, including anti-apoptotic effects, protection against bile duct injury, and stimulation of hepatocellular secretion may be more important. It must be kept in mind that, depending on the pathophysiology of the underlying liver disease, the predominant mechanisms of action of UDCA may vary. One characteristic feature for all proposed mechanisms is that the beneficial effects of the hydrophilic bile acid UDCA oppose the toxic effects of other, more hydrophobic, bile acids. This observation may explain why chronic cholestatic disorders characterized by the retention of toxic bile acids have become the main therapeutic domain of UDCA.

Replacement/displacement of toxic bile acids through UDCA

During cholestasis bile acids accumulate in the liver and in the systemic circulation, reaching toxic concentrations^{19, 20} and resulting in necrosis, apoptosis, fibrosis, and ultimately liver cirrhosis.^{15, 21} Under continuous oral treatment with UDCA (at doses of 13–15 mg/kg/day), conjugated UDCA becomes the predominant bile acid in serum (up to 60% of total serum bile acids), liver tissue (30%) and bile (30–40%) where it replaces/displaces more hydrophobic and therefore toxic endogenous bile acids.^{20, 26–32} Thus, during UDCA treatment, the liver and peripheral tissues are exposed to lower levels of endogenous bile acids and to an increased concentration of UDCA. The effects of UDCA administration on endogenous bile acid kinetics and bile acid levels in serum, bile and urine have mostly been studied in cholestatic patients with primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).^{20, 26–28, 30, 31}

Under UDCA treatment, serum levels of primary bile acids (cholic acid, and to a lesser extent chenodeoxy-

cholic acid) decrease, while levels of secondary bile acids (which are already low in the presence of cholestasis as a result of impaired enterohepatic circulation) are unaffected (deoxycholic acid) or may even increase (lithocholic acid, as a result of bacterial conversion from UDCA).^{26–31} Deoxycholic acid may decrease in a subgroup of patients with early-stage PBC with normal deoxycholic acid serum levels before UDCA treatment.³³ The reduction in (primary) bile acid levels may be due to an inhibition of intestinal (ileal) bile acid absorption by UDCA^{29, 34, 35} and stimulation of hepatocellular bile acid excretion by UDCA³ (see below).

Conjugated UDCA also becomes the predominant bile acid in the bile of PBC patients; it decreases cholic acid levels, while chenodeoxycholic and deoxycholic acid levels remain unchanged, and lithocholic acid increases.³⁰ Liver tissue also appears to be enriched by 30% in UDCA at the expense of hydrophobic bile acids.²⁰

The effects of UDCA on bile acid pool composition are controversial. In theory, this is an attractive mechanism,² but the pool sizes of primary bile acids (cholic acid, chenodeoxycholic acid) and secondary bile acids (deoxycholic acid) do not change significantly under UDCA treatment in patients with PBC and PSC,^{33, 36, 37} although one study on patients with early-stage PBC (with normal deoxycholic acid pool size before UDCA treatment) showed a further reduction of deoxycholic acid pool size.³⁷

Cytoprotective effects of UDCA

Bile acids are hepatotoxic when administered to cultured hepatocytes, to isolated perfused rat livers and to experimental animals.¹⁵ Administration of the hydrophobic bile acid lithocholic acid to experimental animals induces liver cirrhosis and its taurine conjugate, taurolithocholic acid, is highly cholestatic.^{38, 39} This toxicity can be best explained by the ability of bile acids to interact with biomembranes, ranging from binding to solubilization of the membrane with frank cytolysis (necrosis).³ In addition to necrosis, bile acids can also induce apoptosis.⁴⁰ The hydrophilic bile acid UDCA protects hepatocytes and bile duct epithelial cells (cholangiocytes) against necrosis and apoptosis induced by more hydrophobic bile acids which are retained in cholestasis. These protective effects appear to be mediated through direct membrane-stabilizing and anti-apoptotic effects of UDCA.

Stabilization/protection of cell membranes. UDCA at millimolar concentrations protects against membrane damage induced by more hydrophobic bile acids, as shown in experimental settings using artificial or biomembranes, erythrocytes, as well as cultured rat and human hepatocytes *in vitro*.^{41–45} Similar observations have been made in the isolated perfused rat liver and in rats *in vivo*.^{41, 46–48} The hepatoprotective/cytoprotective effects of UDCA on hepatocytes appear to be specific for bile acid-induced injury, because UDCA does not protect against acetaminophen, CCl₄, α -naphthyl-isothiocyanate or ischaemic reperfusion injury.³ However, some studies suggest that UDCA might also protect against ethanol-induced cell injury in rats and a human Hep G2 cell line.^{49, 50} In addition, UDCA appears to inhibit the fibroproliferative activity of human fibroblasts stimulated by platelet-derived growth factor *in vitro*,⁵¹ which could explain the antifibrotic effects of UDCA observed experimentally.⁴⁸

UDCA conjugates are adsorbed on the interface of the cell membrane at the extracellular space, while unconjugated UDCA is inserted into the apolar domain of the membrane ('cholesterol-like' membrane-stabilizing effect),⁴⁵ which subsequently becomes more resistant to the injurious effect of hydrophobic bile salts. The big caveat of these studies is the unphysiologically high concentrations of UDCA, which was also often used in the unconjugated form. However, human hepatocytes in culture may conjugate more than 80% of bile acids present in the medium.⁵² A recent study suggests that these membrane-stabilizing effects may result from UDCA's effects on mixed micelle formation rather than from direct membrane interaction.⁵³

Although the high concentrations of UDCA conjugates required for these membrane-stabilizing effects are not reached in the systemic circulation under therapeutic conditions *in vivo*, these findings may be important for the bile duct epithelium, which is normally exposed to millimolar bile acid concentrations. Cholangiocytes can 'survive' these high biliary concentrations of bile acids (in the millimolar range) only through formation of mixed micelles of phospholipids with conjugated bile acids in bile. Knockout mice for the canalicular phospholipid export pump (mdr2) develop a chronic non-suppurative cholangitis resembling PBC^{54, 55} and resulting from the toxic effect of bile acids on the biliary epithelium in the absence of protective mixed micelle formation with phospholipids.^{56, 57} UDCA feeding in mdr2 (–/–) knockout mice ameliorates liver pathology

by rendering the bile acid composition of bile less toxic.⁵⁸ A subtype of progressive familial intrahepatic cholestasis (PFIC-3) resembles the phenotype of mdr2 (–/–) mice and is caused by a mutation of the human MDR3-gene (human homologue of rodent mdr2) resulting in a marked reduction in biliary phospholipid levels.⁵⁹ UDCA may benefit some patients with PFIC-3 by exerting its protective effect from the biliary lumen, presumably by counteracting the toxic effects of other (more hydrophobic) bile acids in bile.⁶⁰ The beneficial effects of UDCA in adult cholangiopathies/cholestatic syndromes could be explained by similar mechanisms. In the bile of PBC patients treated with UDCA, conjugated UDCA (glyco-UDCA > tauro-UDCA) becomes the predominant biliary bile acid, accounting for approximately 30–40% of the bile acids in fasting bile compared to 0.4–0.6% in placebo-treated patients.^{30, 32} Therefore, direct membrane-protective effects of UDCA may play a role mainly at the bile duct level, because the required high (millimolar) concentrations are achieved only in this location.

Anti-apoptotic effects. Apoptosis is a 'physiological' cell death that deletes damaged and aged cells from the organism.^{40, 61, 62} Dysregulation of apoptosis in hepatocytes and bile duct epithelial cells (cholangiocytes) contributes to cholestatic liver injury. Hydrophobic bile acids induce apoptosis in hepatocytes by activation of the pro-apoptotic Fas-receptor on the cell surface, followed by activation of caspases and nuclear translocation of cathepsin B.^{63, 64} Cholangiocyte apoptosis is increased in PBC livers and the small interlobular bile ducts affected in PBC overexpress the pro-apoptotic Fas-receptor.^{65, 66} Fas-ligand is expressed on cytotoxic lymphocytes infiltrating the portal tract in many vanishing bile duct syndromes. Tumour necrosis factor- α and oxidative stress may also contribute to cholangiocyte apoptosis under these conditions.⁶²

Tauroursodeoxycholic acid (TUDCA) reduces both bile acid-induced apoptosis and cytolysis in cultured rat hepatocytes *in vitro*,⁶⁷ which may partially explain the cytoprotective effects of UDCA. This finding has recently been confirmed by bile acid feeding in rats *in vivo*.⁶⁸ This elegant study by Rodrigues *et al.* demonstrated that coadministration of UDCA with various pro-apoptotic stimuli, such as hydrophobic bile acids, ethanol, transforming growth factor- β and Fas-ligand, markedly inhibited apoptosis by 50–100% in hepatocytes and various non-hepatocyte cell lines *in vitro*.⁶⁸ UDCA also

reduced the mitochondrial membrane permeability transition associated with toxic bile acid levels, indicating that inhibition of mitochondrial membrane permeability transition may at least be one central pathway by which UDCA protects against apoptosis.⁶⁹ However, the importance of these findings for prevention of apoptosis in cholestasis remains to be determined because cholestasis per se confers resistance to mitochondrial membrane permeability transition through an increase in mitochondrial cardiolipin content.⁷⁰ An interesting recent study has demonstrated a decrease in nuclear DNA fragmentation (as an indicator of apoptotic stress) in biliary epithelial cells from PBC livers under UDCA treatment, indicating that the anti-apoptotic effects of UDCA could play an important role under clinical conditions.⁷¹ Therefore, UDCA may indeed be the 'first FDA-approved drug to inhibit apoptosis'.⁷² Most importantly, the anti-apoptotic effects of UDCA had already been observed at low micromolar concentrations *in vitro*, which can be achieved in the systemic circulation and in the liver under therapeutic conditions.

Immunomodulatory effects of UDCA

Cholestasis per se induces major histocompatibility complex (MHC) class I expression in hepatocytes under experimental conditions (cultured human and rat hepatocytes incubated with bile acids, bile duct ligated rats)^{52, 73–75} and clinical conditions (obstructive cholestasis, PBC, PSC).^{33, 76, 77} Because MHC class I molecules are essential for antigen recognition by cytotoxic T lymphocytes, aberrant expression of MHC class I molecules on hepatocytes may render these cells vulnerable to an (auto)immune attack. In PBC and PSC patients, MHC class I molecules are expressed on hepatocyte membranes and cytoplasm, particularly in the periportal zone within areas of piecemeal necrosis.^{33, 76} The mechanism of increased MHC class I expression on hepatocytes may involve stimulation of *de novo* synthesis by hydrophobic bile acids⁵² and unmasking of the membrane molecules by the detergent action of hydrophobic bile acids. UDCA treatment down-regulates expression of abnormal MHC class I molecules in periportal hepatocytes of PBC and PSC patients,^{33, 76, 77} which may contribute to the anti-inflammatory properties of UDCA, whereas abnormal expression of MHC class II molecules on cholangiocytes does not change in PBC.⁷⁶ These effects on MHC class I expression are probably the result of improved chole-

static liver injury, rather than the direct immunomodulatory effects of UDCA.⁴

UDCA may also have direct immunosuppressive effects through modulation of immunoglobulin and cytokine production by immunocompetent cells.⁷⁸ However, a recent study suggests that bile acids with widely differing hydrophobicities (including UDCA) are incapable of influencing cytokine release by human and murine macrophages, provided they are studied in the presence of physiological amounts of protein.⁷⁹ UDCA has been shown to activate the glucocorticoid receptor,⁸⁰ possibly via activation of protein kinase C.⁸¹ Furthermore, UDCA inhibits induction of epithelial nitric oxide synthase *in vitro* and *in vivo*, effects which may contribute to the anti-inflammatory and cytoprotective actions of UDCA.⁸² Clinically, UDCA treatment lowers serum levels of immunoglobulin M, antimitochondrial antibodies (AMA)^{13, 83} and antibodies against pyruvate dehydrogenase.⁸⁴ The clinical relevance of these findings is unclear because clinical features and course, as well as response to treatment with UDCA, are not different in AMA-positive and AMA-negative PBC cases.^{85, 86} Finally, it is important to keep in mind that toxic levels of hydrophobic bile acids in cholestasis may also have immunosuppressive effects^{74, 75} which might be reversed by UDCA treatment.^{87, 88}

Stimulation of bile secretion

Cholestasis results in intrahepatic and systemic retention of bile acids and other biliary constituents that are hepatotoxic and cholestatic.^{3, 15} Experimentally, UDCA prevents cholestasis induced by more hydrophobic bile acids in rat liver.^{41, 42, 46, 47} In addition, UDCA stimulates the secretion of bile acids and other organic anions (e.g. bilirubin, bromosulphophthalein) in isolated hepatocytes,⁸⁹ isolated perfused rat liver,^{90, 91} bile fistula rats,^{41, 92, 93} and patients with PBC, PSC and cystic fibrosis (CF).^{94–96} In line with these observations, UDCA treatment lowers serum levels of endogenous bile acids and bilirubin in PBC and PSC patients^{32, 83, 97–100} and reduces serum levels of endogenous bile acids and potentially cholestatic progesterone metabolites in patients with intrahepatic cholestasis of pregnancy.^{101–103} Therefore, it is conceivable that some of the beneficial effects of UDCA in the treatment of cholestatic liver disease may be due to improved mobilization of potentially toxic biliary constituents retained in the liver and the systemic circulation of cholestatic patients.

Thus, UDCA is able to re-establish hepatobiliary excretory function and to counteract cholestasis in several ways, including stimulation of vesicular exocytosis, stimulation of transporter gene expression, and stimulation of ductular bicarbonate secretion via cholehepatic shunting and other mechanisms.

Stimulation of exocytosis and insertion of canalicular membrane transporters. TUDCA stimulates Ca^{2+} - and α -protein kinase C (PKC)-dependent hepatocellular exocytosis in the isolated perfused rat liver.^{104–106} Vesicular exocytosis normally regulates the insertion of bile acid transporters and other hepatobiliary transport proteins into the canalicular membrane,^{107, 108} a process that is defective in (experimental) cholestasis.¹⁰⁵ TUDCA (but not other bile acids) at low physiological concentrations (in the low μM range) induces a sustained increase in hepatocellular Ca^{2+} concentrations $[\text{Ca}^{2+}]_i$ which in turn induces translocation of Ca^{2+} -sensitive α -PKC from the cytosol to the membrane, resulting in stimulation of apical exocytosis.^{104, 105} It can be speculated that, in addition to facilitating Ca^{2+} -mediated secretory events, TUDCA might also 'desensitize' the hepatocyte against potentially harmful Ca^{2+} -mediated effects of toxic hydrophobic bile acids.¹⁰⁴ In addition to enhancing $[\text{Ca}^{2+}]_i$ and translocation of α -PKC, TUDCA also induces osmo-dependent hepatocyte swelling with subsequent activation of mitogen-activated protein kinases, which also stimulate vesicular exocytosis with insertion of biliary carriers into the canalicular membrane.^{108–110} Taken together, these studies suggest that physiological concentrations of TUDCA enhance the biliary excretory capacity of the cholestatic liver by activation of hepatocellular $[\text{Ca}^{2+}]_i$, α -PKC, and mitogen-activated protein kinases, resulting in stimulation of vesicular exocytosis (for review see Beuers *et al.*⁴). Whether UDCA also stimulates apical exocytosis in cholangiocytes is currently unknown.

Stimulation of defective gene expression of hepatobiliary transport systems. Expression of the $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger (AE2) is reduced in livers from PBC patients but not from cholestatic and cirrhotic controls.^{111, 112} Because $\text{Cl}^-/\text{HCO}_3^-$ exchange activity contributes to both hepatocellular and ductular bile secretion, decreased hepatic expression of AE2 could contribute to impaired bile flow. UDCA treatment of PBC patients

up-regulates AE2 mRNA and protein expression,^{111, 112} indicating that the beneficial effects of UDCA could in part be mediated by stimulation of defective gene expression of hepatobiliary transport systems that are down-regulated in cholestasis.¹¹³ Increased expression of hepatobiliary transporters under UDCA treatment could be due to stimulation of gene transcription and/or post-transcriptional events such as increased targeting of transport proteins to the canalicular membrane via stimulation of vesicular exocytosis (see above). Future studies will have to investigate whether UDCA also stimulates the expression of other hepatobiliary/canalicular transport systems for bilirubin and bile acids.

Bicarbonate-rich hypercholerisis. Under experimental conditions high pharmacological doses of unconjugated UDCA (exceeding the conjugation capacity of the liver) can induce a bicarbonate-rich hypercholerisis in rats.¹¹⁴ This choleretic effect of UDCA is much more pronounced than that of taurocholic acid or even its own taurine conjugate (TUDCA)¹¹⁴ and is greater than expected from the amount of bile acids (UDCA) excreted into bile, therefore the term 'hypercholerisis'. According to the 'cholehepatic shunt hypothesis' suggested by Hofmann and coworkers, protonation of unconjugated UDCA in the bile ducts results in the generation of a HCO_3^- anion each time an unconjugated UDCA molecule is taken up via passive diffusion and undergoes cholehepatic shunting via the peribiliary plexus.^{2, 115–117} Therefore, the site of origin of UDCA-induced hypercholerisis is the bile duct epithelium and not the hepatocyte. UDCA-induced hypercholerisis has received much attention over recent years, because it is theoretically conceivable that bicarbonate-rich bile might 'flush' mucus-obstructed bile ducts (e.g. in CF) or 'dilute' toxic bile with high concentrations of hydrophobic bile acids. However, biliary levels of unconjugated UDCA do not markedly increase during UDCA therapy of patients with PBC and CF^{30, 118} and intraduodenal administration of UDCA to patients (with cholelithiasis) at a therapeutic dose (15 mg/kg) did not result in hypercholerisis.¹¹⁹ Therefore, UDCA-induced hypercholerisis may not be relevant in humans under clinical conditions. However, this mechanism might be of interest for related bile acid derivatives that are more resistant to endogenous conjugation by the human liver (e.g. nor-UDCA with a modified side chain).²

Table 1. Features of six major randomized, double-blind, controlled trials of ursodeoxycholic acid vs. placebo in the treatment of patients with primary biliary cirrhosis

Author	Number of patients	Follow-up (years)	Improvement of				
			Biochemistry	Symptoms	Histology	Disease progression	OLT-free survival
Heathcote <i>et al.</i> ⁹⁸	222	2	yes	no	yes	no	no
Lindor <i>et al.</i> ⁹⁹	180	4	yes	no	no	yes	yes
Poupon <i>et al.</i> ¹³⁰	146	4	yes	ND	yes	yes	yes
Eriksson <i>et al.</i> ¹²⁸	116	2	no	no	no	no	no
Combes <i>et al.</i> ³²	150	2	yes	yes*	yes	no	no
Poupon <i>et al.</i> ¹³²	548**	4	yes	ND	yes	yes	yes

*Significantly reduced development of severe symptoms (fatigue/pruritus). However, mean scores for pruritus and fatigue were not favourably affected.

**Analysis of combined data from three previously published trials.^{98, 99, 130}

ND: no data available.

Various choleretic effects. TUDCA also stimulates canalicular contractions that normally promote bile flow from pericentral to periportal regions,¹²⁰ a process that is defective in many forms of cholestasis.¹²¹ Furthermore, UDCA stimulates cholangiocyte bicarbonate secretion via Ca²⁺-dependent activation of a cAMP-independent chloride channel, an effect that might be beneficial in patients with cystic fibrosis (with mutations/defects of the cAMP-dependent chloride channel CFTR).^{122, 123}

TREATMENT OF CHOLESTATIC LIVER DISEASES WITH UDCA

Primary biliary cirrhosis

Primary biliary cirrhosis (PBC) is a chronic cholestatic disorder, primarily affecting middle-aged women, and characterized by immune-mediated destruction of small intrahepatic bile ducts. This ongoing destruction of bile ducts results in a failure of bile secretion, with subsequent retention of toxic hydrophobic bile acids causing hepatocyte damage. Thus, treatment of PBC has focused on suppression of this abnormal immune response and reduction of the toxic effects of retained hydrophobic bile acids.

In initial uncontrolled trials, UDCA improved both symptoms (mainly decreased pruritus) and biochemical parameters of cholestasis (bilirubin, gamma-glutamyl transpeptidase, alkaline phosphatase) and inflammation (aminotransferases).^{12, 124, 125} A controlled multicentre trial in France also demonstrated a significant clinical benefit with respect to the onset of hyperbilirubinemia, ascites or bleeding in the UDCA group compared to

placebo.⁸³ Several subsequent controlled studies consistently showed an improvement in biochemical parameters,^{13, 32, 83, 98, 99, 126} while beneficial effects on symptoms were reported only in a subset of patients in one study (severe fatigue and pruritus were less pronounced)³² (Table 1).

Data on histological improvement are controversial. While some studies have failed to show histological improvement,^{99, 127, 128} others have reported improvement of piecemeal necrosis, portal and lobular inflammation, cholestasis, and bile duct paucity.^{32, 83, 98} However, fibrosis and histological stage, according to the Ludwig criteria,¹²⁹ do not appear to be favourably influenced by UDCA therapy.

Most importantly, UDCA slows down the progression of PBC and reduces the need for orthotopic liver transplantation (OLT).^{130–132} Poupon *et al.* demonstrated that the probability of OLT or death was substantially lower in the group assigned to UDCA for 4 years (13–15 mg/kg/day) compared to those receiving placebo for 2 years and later switched to UDCA for 2 additional years.¹³⁰ A study from the Mayo Clinic also reported a three-fold higher mortality or need for OLT in patients randomized to placebo compared to UDCA-treated patients.¹³¹ Analysis of combined data from three large controlled trials with a total of 548 patients showed a significant prolongation of transplant-free survival after 4 years of UDCA treatment (13–15 mg/kg/day).¹³² In addition, sub-group analysis demonstrated significantly improved survival, free of OLT, in medium- and high-risk groups (serum bilirubin level above 1.4 and 3.5 mg/dL, respectively) and in the histological stage IV (cirrhosis) sub-group treated by UDCA.¹³² Bounand

et al. showed that serum bilirubin levels remain a valid prognostic factor in PBC under UDCA treatment, because normalization of serum bilirubin level under UDCA therapy was associated with improved clinical outcome.¹³³ In addition, UDCA treatment may have a positive effect on the development of portal hypertension. Two controlled studies reported a lower incidence of newly developed oesophageal varices in patients treated with UDCA,^{134, 135} although this was not noted in two previous large controlled trials.^{32, 99} Moreover, UDCA is a highly cost-effective therapy that actually reduces the cost of medical care in PBC patients.¹³⁶

The response of AMA-negative and AMA-positive PBC patients to UDCA treatment is similar.⁸⁵ In contrast, patients with PBC and autoimmune hepatitis (overlap syndrome) require combination therapy with UDCA and corticosteroids to obtain complete biochemical response, indicating that overlap syndrome may represent a yet unrecognized cause of resistance to UDCA monotherapy in about 7% of patients with PBC.¹³⁷

Given the modest effect of UDCA on the natural course of PBC, a number of groups have combined UDCA with other medications, such as prednisone, azathioprine, methotrexate and colchicine, in an attempt to augment the positive effects of UDCA. A controlled trial comparing a colchicine/UDCA combination to UDCA alone revealed a slight advantage of colchicine/UDCA over UDCA monotherapy with regard to progression of portal hypertension, symptoms and laboratory findings, while histology was not improved.¹³⁸ Similarly, a combination of UDCA (10 mg/kg/day) and prednisone (10 mg/day) for 9 months improved liver enzymes and reduced hepatic inflammation more effectively than UDCA alone, whereas the degree of fibrosis and bile duct damage did not differ between the two groups.¹³⁹ Controlled studies on combination of UDCA with newer steroids (e.g. budesonide) are currently under way. Lindor *et al.* reported no added benefit in 32 PBC patients treated with UDCA-methotrexate compared to patients who received UDCA monotherapy.¹⁴⁰ This negative finding was confirmed by a study including 25 patients in whom a combination therapy with UDCA-methotrexate was not associated with any additional improvement in biochemical markers, symptoms and histological features.¹⁴¹ In a controlled study including 50 patients, triple therapy with UDCA, prednisone and azathioprine over 1 year revealed an additional beneficial effect with regard to symptoms, biochemical and histological parameters in patients who had previously

not achieved complete biochemical response under UDCA monotherapy.¹⁴² Unfortunately, no information was given on the prevalence of PBC-autoimmune hepatitis overlaps, although these patients might benefit most from triple therapy.

In summary, UDCA monotherapy at a dose of 13–15 mg/kg/day is recommended for the treatment of PBC patients. Lower doses have failed to show any benefit except a mild improvement in serum liver enzymes.¹²⁸ Combination of UDCA with other (immunosuppressive) drugs may have additional benefit, but (with the possible exception of PBC-autoimmune hepatitis overlap syndromes) currently should be limited to controlled clinical studies until risks and benefits are better defined.

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease, occurring mostly in young male patients and frequently associated with inflammatory bowel disease, usually chronic ulcerative colitis. It is characterized by intra- and extrahepatic biliary strictures and bile duct fibrosis associated with inflammatory changes involving the portal and periportal regions of the liver. Given the similarities between PSC and PBC, particularly with regard to the profound cholestasis and the deleterious effects of retained toxic bile acids, several trials were initiated to determine the effectiveness of UDCA in the treatment of PSC.

Biochemical improvement in serum liver tests was observed in UDCA-treated PSC patients in early small and mostly uncontrolled studies.^{97, 143–145} UDCA did not significantly improve symptoms of PSC after 1–2 years of treatment^{97, 145} but improved histological features in some patients.⁹⁷ However, after these initial optimistic reports, more recent studies failed to show a positive effect on clinical symptoms and, more importantly, on OLT-free survival and/or overall survival.^{100, 146, 147} The largest series (105 patients) conducted so far demonstrated that patients treated with UDCA at a dose of 13–15 mg/kg/day experienced some improvement in cholestatic parameters, but that UDCA had no beneficial effect on PSC-related symptoms over a mean follow-up of 2.2 years.¹⁰⁰ Furthermore, UDCA neither seems to halt the histological progression of the disease nor to prolong OLT-free survival regardless of the histological stage. However, UDCA in combination with endoscopic treatment of dominant strictures may substantially improve patient survival, as

shown by Stiehl *et al.* for 65 patients with a mean follow-up of 45 months.¹⁴⁶

In summary, administration of UDCA in PSC patients at doses of 13–15 mg/kg/day may be justified because a biochemical benefit can be achieved and no alternative treatment is currently available. Prolongation of survival, however, has not yet been demonstrated. In contrast to trials in PBC, the number of PSC patients studied and the median follow-up period have been limited. Moreover, the optimal dose of UDCA remains to be determined. In addition, endoscopic treatment of dominant strictures appears to be an essential adjunct to UDCA therapy.

Intrahepatic cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy (ICP) is a liver disorder of late pregnancy (third trimester) characterized by pruritus of the mother coinciding with an increase in serum bile acids, aminotransferases and bilirubin. The main consequences are disturbed maternal well-being due to severe pruritus and an increased risk of premature delivery and stillbirth. An initial open-label study showed that UDCA improved maternal pruritus and biochemical abnormalities in patients with ICP.¹⁴⁸ Several subsequent small series and case reports confirmed these encouraging results.^{149–152}

A controlled study of 24 patients demonstrated improvement of pruritus, jaundice and serum liver tests in mothers during UDCA treatment. Most importantly, relevant aspects of fetal outcome were also significantly improved. All babies of mothers in the UDCA group were delivered at or near term, whereas five of seven babies in the placebo group were born before week 36 and one of them was a stillbirth.¹⁵³ No adverse effects were observed in mothers or babies. In addition, UDCA decreased elevated endogenous bile acid levels in serum, urine and colostrum of mothers with ICP.^{101–103, 154}

Based on these results, UDCA treatment of ICP may be considered safe for relieving pruritus and improving both biochemical parameters and fetal outcome. However, further larger controlled studies are needed before UDCA treatment of patients with ICP can be generally recommended.

Chronic graft vs. host disease of the liver

Marked improvement of serum liver tests was observed after UDCA treatment in patients with cholestasis due to chronic graft vs. host disease (GvHD).^{155, 156} In a

controlled trial, prophylactic treatment with UDCA at a dose of 600–900 mg/day significantly decreased the incidence of veno-occlusive disease (VOD) after allogeneic bone marrow transplantation.¹⁵⁷ Further studies are certainly needed, but UDCA may be considered for prophylaxis of VOD and treatment of GvHD of the liver.

Total parenteral nutrition-associated liver disease

Long-term total parenteral nutrition (TPN) can be associated with a wide spectrum of hepatobiliary disorders, ranging from mild liver function abnormalities to end-stage liver disease requiring OLT. Cholestasis develops most commonly in premature infants and in adults with inflammatory bowel disease, particularly in patients with previous extensive intestinal resection. The pathogenesis of TPN-induced cholestasis is unclear and several factors may play a role, including increased formation of toxic bile acids, translocation of bacterial endotoxin from the intestinal flora, as well as the amount of calories and composition of the TPN.¹⁵⁸

UDCA led to a prompt and sustained improvement in hyperbilirubinemia in a single patient who developed severe jaundice while receiving long-term TPN.¹⁵⁹ In a subsequent uncontrolled study including seven children with TPN-related liver disease, UDCA treatment (30 mg/kg/day) was associated with normalization of biochemical parameters of cholestasis and disappearance of signs of chronic liver disease (i.e. jaundice and hepatosplenomegaly on ultrasound) within 4–8 weeks.¹⁶⁰

These findings suggest that UDCA appears to be an effective and safe treatment in patients with TPN-associated cholestasis. However, larger controlled series are awaited.

Drug-induced cholestasis

The beneficial effect of UDCA on laboratory markers of cholestasis in the setting of cyclosporin-induced cholestasis was demonstrated in 13 heart transplant recipients. Cholestasis recurred after discontinuation of UDCA but resolved again when UDCA was reintroduced.¹⁶¹ Isolated observations suggest that UDCA may be useful in the relief of symptoms and possibly in improving the outcome of patients with severe drug-induced cholestasis, in particular drug-induced vanishing bile duct syndromes.^{162–164} Data from controlled trials are needed to establish the beneficial effect of UDCA in drug-induced hepatopathies.

Cystic fibrosis

Cystic fibrosis (CF) is a genetic disorder characterized by abnormal electrolyte (chloride) transport resulting in thickening of secretions in the bronchial tree, pancreas, intestine and biliary tree; this leads to chronic obstructive pulmonary disease, pancreatic insufficiency, intestinal obstruction and biliary cirrhosis. Abnormal biliary secretions and inspissation of bile with the subsequent development of plugs within small bile ducts cause biliary obstruction (cholestasis) 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.¹⁶⁵

UDCA has been used successfully to treat hepatobiliary complications associated with CF. Early uncontrolled studies showed that UDCA significantly improved laboratory tests and nutritional status in CF patients.^{94, 166, 167} In a controlled trial UDCA distinctly improved nutritional status, pulmonary involvement and patients' general condition after 1 year of treatment.¹⁶⁸ Moreover, histological features improved during UDCA treatment.¹⁶⁹ Two studies suggest that higher doses of UDCA (20 mg/kg/day) may be more efficacious than lower doses (5–15 mg/kg/day).^{94, 170} Thus, UDCA may be considered a safe and efficacious treatment option in patients with CF-related liver disease. Further studies are needed to establish a positive effect of UDCA on the course of the disease and survival.

Other paediatric cholestatic disorders

Several uncontrolled studies have shown beneficial effects of UDCA on biochemical markers of cholestasis and/or cholestasis-related clinical symptoms at doses of at least 15 mg/kg/day in patients with biliary atresia,^{171, 172} syndromic paucity of the intrahepatic bile ducts (Alagille syndrome),¹⁷³ Caroli's disease,¹⁷⁴ progressive familial intrahepatic cholestasis,^{60, 173} and benign recurrent intrahepatic cholestasis.¹⁷⁵

TREATMENT OF NON-CHOLESTATIC LIVER DISEASES

Liver transplantation—acute rejection

Initial animal and small open-label human studies suggested that UDCA may be useful in the prevention of

acute rejection episodes.^{176, 177} Therefore, the possible role of UDCA as an immunomodulator was further explored in four prospective randomized trials.^{178–181} Three studies, including a total of 134 patients, failed to show a beneficial effect of prophylactic UDCA treatment (at a dose of 15 mg/kg/day) on the incidence of acute rejections following OLT.^{178, 179, 181} Only one study, including 52 patients, reported a positive effect of UDCA at a dose of 10–15 mg/kg/day with fewer patients experiencing multiple rejection episodes in the UDCA group, although the number of patients free of rejection did not differ. Nevertheless, UDCA provided a slightly improved survival at 90 days and at 1 year.¹⁸⁰ In summary, these data do not support prophylactic use of UDCA after OLT.

Chronic viral hepatitis

UDCA alone or in combination with interferon was evaluated in a number of controlled trials for the treatment of chronic hepatitis C. Although some biochemical improvement of serum transaminases was achieved, UDCA failed to improve either the virological response rate or histological features.^{182–184} Thus, UDCA (alone or in combination with interferon) cannot be generally recommended for the treatment of chronic hepatitis C,¹⁸⁵ although a subset of patients with high serum γ -GT levels (increased to more than threefold) might benefit from UDCA.¹⁸⁶

Non-alcoholic steatohepatitis

UDCA markedly improved liver function tests as well as the histological grade of steatosis in a small open-label series of patients with non-alcoholic steatohepatitis (NASH).¹⁸⁷ The possible benefit of UDCA therapy in patients with NASH should be further investigated in randomized controlled studies before UDCA is generally recommended for treatment of this condition.

Alcoholic liver disease

In a placebo-controlled crossover study, administration of UDCA for 4 weeks led to a reduction in serum bilirubin levels, as well as serum aminotransferases, gamma-glutamyl transpeptidase and alkaline phosphatase levels in patients with alcoholic liver cirrhosis who continued to drink.¹⁸⁸ These new perspectives for UDCA

in the treatment of patients with alcoholic liver disease deserve further investigation.

CONCLUSIONS

UDCA has been used to treat patients with a wide variety of liver diseases, in particular cholestatic disorders. UDCA is of proven efficacy, as defined by prolongation of (OLT-free) survival, only in the treatment of PBC. However, UDCA improves serum liver tests and thus may be justified in patients with PSC, ICP, CF-related liver disease, various other paediatric cholestatic disorders and chronic GvHD of the liver, although a positive effect on natural course and survival has not yet been proven; this requires further investigation in controlled clinical trials. The efficacy of UDCA is still unproven in the setting of liver transplantation (prevention of acute rejection), NASH, alcoholic liver disease, and drug-induced cholestasis. UDCA cannot be recommended to treat chronic viral hepatitis.

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