URSODEOXYCHOLIC ACID THERAPY OF CHRONIC CHOLESTATIC CONDITIONS IN ADULTS AND CHILDREN

RAOUL POUPON*† and RENÉE E. POUPON†

*Unité d'Hépato-Gastroentérologie, Hôpital Saint-Antoine, 184 rue du Faubourg Saint-Antoine, 75571 Paris Cedex 12, France,
†INSERM Unit 21, 16 Ave Paul-Vaillant-Couturier, 94807 Villejuif Cedex, France

Abstract—Cholestasis can be defined as the manifestation of defective bile acid transport from the liver to the intestine. Most chronic cholestatic conditions can progress towards cirrhosis. At this stage, liver transplantation is the treatment of choice. Most of the drugs so far evaluated show some degree of efficacy but have major side effects. Given that ursodeoxycholic acid (UDCA) has no apparent toxicity in humans, it was postulated that long-term treatment with this drug might displace endogenous bile acids and thus reverse their suspected toxicity. We demonstrated that long-term UDCA therapy slows the progression of primary biliary cirrhosis and reduces the need for liver transplantation. In this review, we give the rationale for the use of UDCA in cholestasis and discuss its possible mechanisms of action. We also give an overview of current data on UDCA therapy of chronic cholestatic disorders in adults and children.

Keywords—Cholestasis, primary biliary cirrhosis, primary sclerosing cholangitis, paediatric cholestasis, bile acids, ursodeoxycholic acid.

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†Corresponding author.

Abbreviations—GVHD, chronic graft-versus-host disease of the liver; HLA, human leucocyte antigen; PBC, primary biliary cirrhosis; UCDA, ursodeoxycholic acid.
1. INTRODUCTION

Cholestasis can be defined as the clinical, biochemical and histological manifestations of defective bile acid transport from the liver to the intestine. Chronic cholestatic conditions of the liver form a homogeneous group of diseases with several points in common. Most result from the destruction of intrahepatic or extrahepatic bile ducts, or a developmental defect. Most chronic cholestatic conditions can progress towards cirrhosis (biliary cirrhosis) and hepatocellular insufficiency (Biour et al., 1993; Desmet, 1987, 1992; Larrey and Michel, 1993; Phillips and Poucell, 1987; Woolf and Vierling, 1993).

Table 1 lists the chronic cholestatic conditions of adults that can progress towards biliary cirrhosis and liver failure. In the case of primary biliary cirrhosis (PBC), graft rejection, graft-versus-host disease (GVDH), most types of drug-induced cholestasis and liver involvement by sarcoidosis, the initial abnormality is inflammation and destruction of the interlobular bile ducts. In primary sclerosing cholangitis, the initial abnormality is sclerosis of the extrahepatic or intrahepatic bile ducts. Long-term parenteral feeding can lead to chronic cholestasis. Cholestasis of pregnancy, some cases of drug-induced cholestasis (especially those due to combined oral contraceptives) and recurrent benign cholestasis are clinical situations associated with 'pure' cholestasis, with no bile duct lesions. These forms do not progress towards cirrhosis or hepatocellular failure. The main causes of chronic cholestatic conditions in children are a congenital or acquired lack of bile ducts, the latter sometimes resulting from metabolic or infectious insult. As in adults, most of these pathological conditions can progress towards biliary cirrhosis.

Schematically, the progression of these chronic cholestatic conditions can be divided into four major phases. In the first, clinical and histological manifestations are absent. The main abnormalities are diminished Brome Sulfone-Phthaleine clearance with a characteristic plateau of plasma concentrations from 20 to 60 min after injection of the anion, together with an augmentation of serum alkaline phosphatase and γ-glutamyl transpeptidase activities. At this stage, histological abnormalities are absent or minimal and can only be identified as 'subcellular cholestasis'. In the second stage, patients can develop pruritus; the serum concentration of bile acids, particularly primary bile acids, increases, as do the concentrations of other normal bile components such as cholesterol and certain markers of connective tissue turnover such as the terminal amino peptide of procollagen III and hyaluronic acid. At this stage, histological examination reveals, in addition to inflammation and fibrous expansion of the portal spaces, a ductular reaction (periportal ductular proliferation) associated with fibrosis and periportal inflammation. The third stage is characterized by extensive porto-portal lobular fibrosis associated with early nodular regeneration of the parenchyma. At this stage, serum concentrations of bile acids and bilirubin are clearly abnormal. In addition, the first signs of portal hypertension appear on ultrasonography or endoscopy. The lesions at this stage are partly reversible, as intrahepatic vascular connections are still present. The terminal stage is defined by the onset of bilirubinemia above 100 μmol/L, histological signs of cirrhosis (regenerative nodules with vascular disconnection) and clinical signs of decompensation, ascites and complications of portal hypertension. The only treatment at this stage is liver transplantation.

The rate of progression towards the terminal stage varies from patient to patient and depends on the nature of each disease and probably on the degree of bile duct destruction. For example, in PBC, follow-up studies of patients in controlled trials have shown that within about 4 years, approximately
Ursodeoxycholic acid therapy

40% develop cirrhosis and 20% reach the terminal stage requiring transplantation (Christensen et al., 1980). Very similar results have been reported in sclerosing cholangitis (Dickson et al., 1992; LaRusso et al., 1988; Wiesner et al., 1989). Most children with bile duct atresia develop liver failure before the age of 3 years if transplantation is not done.

Terminal-stage chronic cholestatic conditions are an ideal indication for liver transplantation. This transforms the prognosis, giving a 5 year survival rate of about 70-80% if it is done in good conditions.

Apart from ursodeoxycholic acid (UDCA), the main drugs used in cholestasis are cholestyramine and colestipol for the treatment of pruritus and hyperlipidemia, and vitamins to correct osteopenia and the malabsorptive syndrome due to the absence of intestinal bile acids. Portal hypertension is treated with drugs (mainly β-blockers), surgery (portacaval shunt) or endoscopic techniques (sclerosis of esophageal varices). S-adenosyl-methionine plays a key role in transmethylation and transulfuration and an important role in the generation of cysteine, a precursor of glutathione. Preliminary clinical trials have shown that S-adenosyl-methionine can be effective in cholestasis of pregnancy and cholestasis induced by oral contraceptives (Friedel et al., 1989). Further trials are required to determine the place of this drug in cholestatic diseases.

2. RATIONALE FOR THE USE OF UDCA IN CHOLESTATIC DISORDERS

Bile acids are natural steroids, which differ in the number, position and orientation of the steroid hydroxyl group. This difference has a radical effect on their metabolism, distribution and biological and chemophysical properties.

UDCA (3α,7β-dihydroxy-5β-cholanic acid) is a natural bile acid in many mammals, but is only present in very small quantities in humans. UDCA is formed by 7β-epimerisation of chenodeoxycholic acid, one of the two principal human bile acids, by certain intestinal organisms. Given its structural similarities with chenodeoxycholic acid, it was postulated that it might also dissolve cholesterol gallstones, and this was confirmed in therapeutic trials. Two lessons were learned from these trials: first, UDCA was capable of modifying the composition of circulating bile acids in humans, becoming the predominant bile acid species; and second, it was found to have no toxicity whatsoever, in contrast to chenodeoxycholic acid (Bachrach and Hofmann, 1982a,b; Poupon and Poupon, 1992).

There are several lines of evidence that the defective bile acid elimination in cholestatic conditions could be responsible for a major part of the clinical, biochemical and histopathological manifestations, as well as their progression towards cirrhosis and hepatocellular failure. During the course of chronic cholestatic conditions, bile acids accumulate in the liver, the systemic circulation and peripheral tissues (Akashi et al., 1987; Dupont et al., 1987; Greim et al., 1973; Hendenborg et al., 1986). In the liver, concentrations close to 500-600 nmol/g are sometimes observed. Since the pioneering work by Holsti (1956), it has been shown repeatedly that bile acids administered to animals, the isolated perfused liver or hepatocyte cultures are hepatotoxic. When given chronically to certain animal species, they induce ductular lesions, fibrosis and even cirrhosis (Palmer, 1972). In humans, the administration of chenodeoxycholic and deoxycholic acids can increase transaminase activity and lead to histological changes in the liver (LaRusso et al., 1977; Schoenfield and Lachin, 1981). Because of their chemophysical properties, bile acids are able to act on biological membranes in a number of different ways, including binding, insertion into the lipid bilayer and solubilization of lipids. The biological and toxic effects of bile acids probably result from such interactions. The toxic effects of bile acids include a series of phenomena observed during cholestasis and in vitro: increased membrane fluidity, increased membrane permeability (especially to calcium), impaired oxidative phosphorylation, decreased mono-oxygenase activities, inhibition of γ-glutamyl transpeptidase and glutathione transferase activities, changes in cytoskeleton organization, induction of free radical injury, changes in DNA superstructure and, finally, cell apoptosis and necrosis. These deleterious effects apparently are influenced by three main factors: (1) the chemical structure and the degree of hydrophobicity of the bile acid, (2) the nature of the cells or tissue exposed to bile acids and (3) the concentration–time product in or around the cells.
Contrary to endogenous human bile acids, and particularly, chenodeoxycholic acid, UDCA is strongly hydrophilic and is not toxic in vitro or in humans. In particular, UDCA has no toxicity up to concentrations of 500 μmol/L in several in vitro models. In humans, UDCA is at least partly transformed into lithocholic acid. However, humans rapidly detoxify and excrete lithocholate, which, therefore, does not accumulate in the enterohepatic circulation (Cowen et al., 1975).

3. TREATMENT OF ADULT CHOLESTATIC DISORDERS

3.1. Primary Biliary Cirrhosis

Immunosuppressive treatments have been used in controlled trials for the treatment of PBC (A European Multicentre Study Group, 1993; Bassendine et al., 1982; Bodenheimer et al., 1985; Christensen et al., 1985; Crowe et al., 1980; Dickson et al., 1985; Epstein et al., 1981; Heathcote et al., 1976; Hoofnagle et al., 1986; Lombard et al., 1993; Matloff et al., 1982; Minuk et al., 1988; Mitchison et al., 1989; Neuberger et al., 1985; Taal et al., 1983; Triger et al., 1980; Wiesner et al., 1990). Colchicine has been used for its anti-fibrotic and anti-inflammatory properties (Bodenheimer et al., 1988; Kaplan et al., 1986; Warnes et al., 1987). Most of these drugs, especially cyclosporin (Lombard et al., 1993, Minuk et al., 1988, Wiesner et al., 1990), had a certain effect on laboratory measures, but not on the prognosis of the disease.

In 1987, we postulated that long-term treatment with UDCA might displace endogenous bile acids and thus, reverse their suspected cytotoxicity. In a pilot study of patients with PBC, UDCA indeed led to major sustained improvements in liver function tests (Poupon et al., 1987). To determine whether UDCA would slow the progression of PBC towards the terminal phase, we conducted a 2 year multicenter, double-blind, controlled trial in which patients with PBC were randomized to receive either UDCA or a placebo (Poupon et al., 1991). Patients were admitted to the trial regardless of the duration of the symptoms and histological severity. The end-point chosen to define failure of UDCA treatment was one of the following criteria: hyperbilirubinemia, clinical complication (variceal bleeding, ascites, encephalopathy) or a major adverse effect. Following randomization, 73 patients with PBC received UDCA (13–15 mg/kg/day) and 73 the placebo. At entry, the two groups were well matched for age, sex, time since initial diagnosis and histological severity: in the UDCA group 50% of the patients had Stage I–II disease, compared with 56% in the placebo group. At the end of the trial, the risk of failure was significantly (about 3-fold) higher in the placebo group than in the UDCA group. One patient in each group withdrew because of adverse effects. After 2 years of treatment, the proportion of treated patients with clinically overt disease had fallen significantly; in particular, there was a clear improvement in terms of the severity of pruritus. Patients receiving UDCA showed significant improvements in serum bilirubin level, alkaline phosphatase, transaminases; γ-glutamyl transpeptidase activities, cholesterol and immunoglobulin M levels, the anti-mitochondrial antibody titre and the Mayo risk score. There was a significant improvement in the mean histological score and in all the histological features, except fibrosis, only in the group given UDCA.

Because of the patient selection and the short duration of the follow-up, there were few liver transplantations, preventing any comparison of the two groups of treatment on the basis of this criterion (Poupon et al., 1991). Given the benefit of UDCA, patients completing the study received UDCA in an open fashion and were monitored for a further 2 years. At 4 years, there had been four liver transplantations in the original UDCA group, compared with 13 in the original placebo group. The incidence of liver transplantation was significantly lower in the patients who received UDCA for 4 years than in those initially receiving the placebo (P = 0.003, relative risk 0.21, (CI 0.09–0.76)). Thus, long-term UDCA therapy slows the progression of the disease (Poupon et al., 1994).

UDCA treatment failures have been reported (Hadziyannis et al., 1991). During the study period, other trials of UDCA in PBC were set up (Combes et al., 1991; Leuschner et al., 1989; Oka et al., 1990; Podda et al., 1989). The data so far available consistently show an improvement in biochemical parameters. By contrast, improvements in clinical and histological parameters are inconsistently found (Turner et al., 1994); however, it must be underlined that these trials lack the power required to conclude that UDCA has no effect on these parameters. The recent results of two large North-American trials (Heathcote et al., 1994; Lindor et al., 1994) indicate that UDCA delayed the
progression of the disease, but without reducing the need for liver transplantation. The apparent discrepancies with our results could be related to the duration of the treatment and/or the selection of the patients; a long-term follow-up is needed to show a potential effect on liver transplantation occurrence and UDCA is not expected to reverse the terminal phase of PBC. Further studies are required to better define the patients who are relevant of medical or surgical treatments and to assess the additional benefit of combined medical treatments.

3.2. **Primary Sclerosing Cholangitis**

Since sclerosing cholangitis and PBC share certain features, we studied the clinical and biochemical effects of UDCA administration in patients with sclerosing cholangitis (Chazouillères et al., 1990). In an uncontrolled study (15 patients given 8–16 mg/kg/day UDCA), the proportion of patients suffering from fatigue or pruritus after 6 months of treatment fell from 60% to 20% and from 33% to 20%, respectively. Serum levels of alkaline phosphatases, γ-glutamyl transpeptidase and transaminases also fell significantly. No exacerbation of associated disorders or side effects of UDCA were observed. The efficacy of UDCA was suggested by (a) the clear improvement in patients in whom the cholestasis had been stable for at least 6 months, (b) the absence of similar spontaneous improvements during the natural history of the disease and (c) aggravation of liver test results following the discontinuation of UDCA in three patients.

Two other pilot studies have confirmed these results. One (O’Brien et al., 1991) in which 12 patients were treated for a mean period of 30 months with 10 mg/kg/day UDCA, confirmed the improvement of biochemical test results after 6 months of treatment, and the further improvement with long-term treatment. The other (Stiehl et al., 1994), in which 22 patients received UDCA for 1 year and were then randomized to receive either UDCA or a placebo for a further year, was interrupted for ethical reasons, i.e. the aggravation of the disease in patients receiving the placebo. A placebo-controlled study led to the conclusion that UDCA reduces disease activity in patients with sclerosing cholangitis (Beuers et al., 1992). After 1 year of treatment, the patients in the UDCA group showed improvements relative to the placebo group with respect to serum levels of bilirubin, alkaline phosphatase, γ-glutamyl transpeptidase and transaminases. Histological features, evaluated with a multi-parametric score, also improved.

Although the effectiveness of UDCA in sclerosing cholangitis has been demonstrated, it is less valuable in this condition than in PBC. Indeed, patients with sclerosing cholangitis frequently develop a cholangiocarcinoma and liver transplantation is more widely indicated than in PBC; thus, UDCA therapy is aimed mainly at obtaining an improvement in the quality of life and in the patient’s condition, with a view to transplantation.

3.3. **Intrahepatic Cholestasis of Pregnancy**

Intrahepatic cholestasis of pregnancy is a condition of unknown origin, characterized by pruritus and abnormal liver test results, with a predominantly cholestatic pattern. It appears during the second half of pregnancy in previously healthy women. After delivery, the pruritus resolves rapidly, and biochemical parameters return to normal. Besides the discomfort for the mothers, this disease causes an increased rate of foetal distress, premature delivery and perinatal mortality. There is no effective treatment.

In an open study, UDCA administration to eight patients with intrahepatic cholestasis of pregnancy led to an improvement in pruritus and laboratory test results. UDCA was well tolerated, and had no apparent toxicity for either the mother or the child (Palma et al., 1992). Unfortunately, data concerning bile acid composition before and during UDCA therapy were lacking.

In a case of triple pregnancy complicated by early cholestasis, UDCA administration normalised maternal liver function (Marpeau et al., 1991). The marked improvement in clinical and biochemical parameters, including serum bile acid levels, suggests a role of endogenous bile acids in the onset of this form of cholestasis.
3.4. Other Settings

3.4.1. Liver Graft Rejection

Acute and chronic liver graft rejection is a frequent complication of liver transplantation, affecting 50–75% of patients and justifying aggressive treatment. UDCA could be effective in this setting for the following reasons: (a) there is a striking similarity between the characteristic lesions of PBC and those of liver rejection, (b) in both cases, there is abnormal expression of Class I and II human leucocyte antigen (HLA) molecules in the liver and (c) both conditions are associated with cholestasis and the destruction of interlobular bile ducts. In PBC, UDCA reduces HLA Class I expression in the liver (Calmus et al., 1990), prevents duct destruction and improves the cholestasis.

The results of a pilot study (Svanvik et al., 1990) are in favour of such an action. During the first month after liver transplantation, two episodes of rejection were observed in 18 consecutive patients who received UDCA, compared with six out of eight previous patients who had received the usual immunosuppressive treatments. However, these results were not confirmed subsequently (Sama et al., 1991). During the 6 months after liver transplantation, 12 rejection episodes were observed among eight of 15 patients treated with UDCA, while 10 episodes occurred in seven of 14 untreated patients. Unfortunately, the interpretation of these results is limited by the absence of randomization and the lack of data concerning the bioavailability of UDCA during the post-operative period.

3.4.2. Chronic Graft-Versus-Host Disease of the Liver

The natural history of GVHD of the liver suggests that biliary cirrhosis may be the end-stage. On the basis of biochemical, clinical and histological similarities between GVHD and PBC, the efficacy of UDCA was tested in the therapy of GVHD of the liver (Fried et al., 1992). UDCA (10–15 mg/kg/day) was given for 6 weeks to 12 patients who had failed to respond to immunosuppressive therapy. Biochemical parameters were improved by UDCA and returned to pre-treatment values after discontinuation of the treatment. This preliminary study requires confirmation in longer term controlled trials.

3.4.3. Cyclosporin-Induced Cholestasis

Cholestasis induced by cyclosporin A following liver transplantation may be improved by the administration of UDCA (Kalinowski et al., 1991). The study concerned 13 heart-transplant recipients, five of whom developed cholestasis during immunosuppressive therapy including cyclosporin A. All five patients received UDCA; although no change in blood cyclosporin levels was observed, alkaline phosphatase, γ-glutamyl transpeptidase and transaminase activities, as well as bilirubinemia, were markedly reduced. When UDCA was discontinued, all five patients redeveloped cholestasis, which again resolved when on UDCA.

3.4.4. Parenteral Nutrition-Associated Cholestasis

Cholestasis can occur during long-term parenteral nutrition, and no treatment is known to be effective. A case report and, very recently, a cross-over study, suggest that UDCA could prevent or improve abnormalities in liver tests in this setting (Beau et al., 1994; Lindor and Barnes, 1991).

3.4.5. Benign Recurrent Intrahepatic Cholestasis

Benign recurrent intrahepatic cholestasis is characterized by the abrupt onset of severe cholestasis, which spontaneously subsides after several weeks, in otherwise healthy subjects. These acute attacks have been attributed to unknown factors impairing bile acid transport at the canalicular level in genetically susceptible subjects. All treatments so far evaluated have proven unsatisfactory. Recently, a contracted bile acid pool size was reported in these patients (Bijleveld et al., 1989) and thus, the use of UDCA was suggested. On the basis of a detailed clinical report, it appears that UDCA is unable to prevent acute cholestatic episodes (Crosignani et al., 1991b). In contrast, in a case report, UDCA was found to have a beneficial effect over 4 years (Maggiore and De Giacomo, 1992). However,
because the course of this disease is highly variable, it is difficult to prove or disprove the efficacy of UDCA.

4. TREATMENT OF CHILDHOOD CHOLESTATIC DISEASES

4.1. Cystic Fibrosis

With the improved efficacy of treatments for pulmonary conditions, the survival of patients with cystic fibrosis has increased considerably. In contrast, hepatobiliary complications are more and more frequent: about 25% of adult patients have biliary abnormalities which, in 5–10% of the cases, progresses to cirrhosis.

In young patients with cystic fibrosis and severe cholestasis, UDCA not only improved conventional liver tests, but also improved nutritional parameters (Colombo et al., 1990; Cotting et al., 1992). As in other cholestatic diseases, it seems likely that the beneficial effect of UDCA on cholestasis involves similar mechanisms, including an improvement in hepatic excretory function (Colombo et al., 1992; Cotting et al., 1992) and, as a possible consequence, an improvement in biliary drainage. In contrast, why UDCA should act on nutritional status is less clear. An improvement in intestinal fat absorption can be ruled out because of the lack of change in faecal fat excretion (Bittner et al., 1990), and the fact that UDCA inhibits intestinal cholesterol absorption. The most reasonable hypothesis is that nutritional status improves following improvement in liver function.

4.2. Other Childhood Cholestatic Diseases

Controlled studies are currently underway to evaluate the effects of UDCA in intrahepatic bile-duct paucity syndromes, including Alagille’s syndrome and Byler’s disease (Balistreri et al., 1990). Preliminary results show that UDCA has a spectacular effect on pruritus, laboratory test results, jaundice and nutritional status. Similar effects have been reported in patients with extrahepatic bile duct atresia (Nittono et al., 1988; Ullrich et al., 1987), in whom treatment with UDCA enabled Kasai’s operation to be performed in excellent conditions.

4.3. Inborn Errors of Bile Acid Metabolism

Childhood cholestatic diseases characterized by errors of bile acid synthesis (3β-hydroxysteroid dehydrogenase and Δ5-3-oxosteroid-5α-reductase deficits, Zellweger’s syndrome) manifest clinically as neonatal cholestasis, which can progress towards cirrhosis and death, and biologically as a predominance of atypical bile acids (Setchell et al., 1992). Cholestasis could result from either the inability of the liver to synthesise primary bile acids (essential for bile secretion) or from the toxicity of the atypical bile acids. Treatment with UDCA together with cholate and/or chenodeoxycholate could be effective (Daugherty et al., 1993).

5. MECHANISMS OF ACTION OF URSODEOXYCHOLIC ACID

Bile acids are synthesised and conjugated in the liver and are secreted into the biliary tree and the small intestine. They are absorbed throughout the intestine and return to the liver via the portal circulation. This bile acid cycling process is named the enterohepatic circulation. The pattern of enterohepatic circulation is explained by two driving forces: the first is localised in the terminal ileum, where most of the bile salts are absorbed through high-affinity receptors; intestinal conservation of bile acids is highly efficient: approximately 95% of circulating bile acids are absorbed on each pass through the intestine and are returned to the liver. The second driving force is in the liver, where bile salts are normally efficiently extracted by hepatocytes and, as a result, are present in the systemic circulation at low concentrations.

In cholestatic conditions, bile acid circulation and metabolism are altered. Hepatic clearance is diminished and blood and tissue concentrations are increased; the formation of deoxycholic and lithocholic acids is decreased. Cholic and chenodeoxycholic acids become the main circulating bile
species. Urinary excretion of bile acids, which is negligible in physiological conditions, increases as certain atypical bile acids emerge (hydroxylated bile acids and 3-hydroxy-Δ5-cholanoic acid). The proportion of sulphated and glucuronidated bile acids is strongly increased.

Although we do not know precisely how chenodeoxycholic and cholic acid accumulation damages the liver, it is now possible to sketch a simplified mechanism of action of UDCA in cholestasis. UDCA (a) decreases the accumulation of cholic and chenodeoxycholic acids, (b) inhibits their intestinal absorption and (c) increases their canalicular excretion.

5.1. Serum Bile Acids in Cholestasis: Effects of Ursodeoxycholic Acid Therapy

Continuous administration of UDCA induces marked changes in plasma bile acid composition and distribution in PBC (Batta et al., 1989a, b; Chretien et al., 1989; Crosignani et al., 1991a; Poupon et al., 1993, Stiehl et al., 1990b). In our randomized study, we found that irrespective of the histological stage of the disease, UDCA became the major circulating bile acid. The levels and proportions of cholic acid and, to a lesser extent, chenodeoxycholic acid, decreased markedly. The observation that UDCA lowered cholic acid levels more than chenodeoxycholic acid levels could be explained by the fact that chenodeoxycholic acid absorption is less dependent on ileal absorption, as it can be absorbed throughout the small intestine by non-ionic passive diffusion. In addition, UDCA is partly converted into chenodeoxycholic acid during UDCA feeding (Federowski et al., 1977). Levels of atypical bile acids, such as 3-mono-hydroxy-Δ5-cholanoic acid, also fall significantly. In contrast, levels of total secondary bile acids are not modified. These changes are independent of the histological stage of PBC. However, certain differences in absolute concentrations observed before UDCA administration persist during treatment. For example, in advanced stages, bile acid concentrations before and during UDCA administration were higher than in early stages. Changes in urinary bile acid excretion have also been described: levels of 1- and 6-hydroxylated endogenous bile acids are reduced, and these species are replaced by hydroxylated derivatives of UDCA (Batta et al., 1989b; Stiehl et al., 1990b). Thus, during UDCA administration, the liver and peripheral tissues are exposed to a lower level of endogenous bile acids and to an increased concentration of UDCA and its hydroxylated metabolites.

Levels of cholic acid and chenodeoxycholic acid fall, due to an effect of UDCA on their active intestinal reabsorption and their intrinsic hepatic clearance.

5.2. Inhibition of Intestinal Bile Acid Absorption by Ursodeoxycholic Acid

Active ileal absorption is the main mechanism controlling the bile acid pool in health and cholestasis. Experimental studies have shown a natural inhibition of this active intestinal transport between pairs of bile acids in vitro and in vivo (Wilson, 1981).

In contrast to chenodeoxycholic acid, UDCA does not suppress bile acid synthesis in humans (Tint et al., 1986), and it increases the rate of cholic acid and chenodeoxycholic acid catabolism (Hardison and Grundy, 1984; Nilssell et al., 1983; Roda et al., 1989, 1990). This has also been observed in patients with PBC (Roda et al., 1989, 1990). To test the hypothesis that UDCA might promote intestinal excretion of primary bile acids by inhibiting active ileal transport of endogenous bile acid conjugates, we and others (Eusufzai et al., 1991; Marteau et al., 1990) used 35S-homotaurocholic acid, a structural analog of taurocholate that is not deconjugated by bacteria and that is highly sensitive and specific in the investigation of active ileal absorption of bile acids. During UDCA therapy, the fractional catabolic rate and the percentage retention of 35S-homotaurocholic acid fell significantly, while no significant change occurred in subjects receiving the placebo. Similar findings have been observed in cholestatic patients, regardless of the aetiology (unpublished results). Finally, similar effects have also been obtained in patients with ileostomies, who served as a model to investigate the effects of UDCA and chenodeoxycholic acid on ileal excretion of primary bile acids (Stiehl et al., 1990a). Several arguments, however, suggest that decreased intestinal absorption is not the only explanation for the hepatoprotective effect of UDCA in cholestatic liver diseases. First, cholestyramine, which also reduces bile acid concentrations by impairing their intestinal absorption, does not appear to have a frank beneficial effect in terms of biochemical parameters in chronic cholestatic conditions (Datta and Sherlock, 1966; Schaffner et al., 1965). Second, in experimental
models (the isolated and in situ perfused rat liver), i.v. infusion of UDCA protects against chenodeoxycholic acid- and lithocholate-induced liver injury (Heuman et al., 1991; Kitani and Kanai, 1982; Schmucker et al., 1990; Schölmerich et al., 1990). These findings indicate that UDCA has a direct effect on liver cells.

5.3. Effects of Ursodeoxycholic Acid on Hepatobiliary Transport Pathways

On the basis of the above-mentioned studies, it has been suggested that UDCA has a cytoprotective effect on liver cells, which appears to be specific for bile acid-induced injury. Indeed, UDCA does not protect against acetaminophen, CCl₄, α-naphthyl-isothiocyanate or ischaemic reperfusion injury (Ando et al., 1991; Chazouillères et al., 1991; Michael et al., 1991).

The vectorial transport of bile acids across hepatocytes is dependent on the polarized distribution of transporters located on the basolateral and canalicular membranes. The maximal uptake capacity greatly exceeds the maximum hepatic excretory capacity, indicating that excretion across the canalicular membrane is rate-limiting for overall hepatic bile acid transport. Detailed reviews of this uptake process have been published recently (Nathanson and Boyer, 1991; Suchy, 1993). The uptake of bile acids across basolateral membranes requires the intervention of one or more transport systems. Following uptake, intracellular transport involves specific binding proteins and a transcytotic vesicular pathway. Canalicular secretion occurs via one of several transport proteins and probably through a vesicular mechanism. In physiological (non-cholestatic) conditions, periportal hepatocytes and, thus, the perportal canalculus are mainly responsible for extraction of bile acids from the blood and their excretion into the bile. In cholestatic conditions due to the destruction of a certain proportion of interlobular bile ducts, recruitment of medio- and peri-lobular hepatocytes probably permits bile acids to by-pass cholestatic portal and periportal areas.

The capacity of the liver to take up bile acids varies between species and bile acids. In general, maximal transport and intrinsic clearance is inversely proportional to the degree of bile acid hydrophobicity. Thus, tauro-UDCA has the highest intrinsic clearance, at least in the rat (Poupon et al., 1988).

Several lines of evidence suggest that UDCA protects liver cells through an increase in the intrinsic ability of hepatocytes to secrete bile acids into bile. When taurocholate is simultaneously infused with UDCA to rats, not only is the cholestasis due to excess taurocholate prevented, but canalicular excretion of both taurocholate and total bile acids increases (Kitani and Kanai, 1982). The same observations have been reported using UDCA plus chenodeoxycholate or deoxycholate (Heuman et al., 1991; Schmucker et al., 1990) or lithocholate (Schölmerich et al., 1990). In vitro, the cytoprotection provided by tauro-UDCA was related to its ability to reduce the intrahepatocytic taurochenodeoxycholate content (Ohiwa et al., 1993). Again, in vitro, tauro-UDCA increased the efflux of chenodeoxycholate from preloaded hepatocytes (Ohiwa et al., 1993). In the isolated perfused rat liver, tauro-UDCA increased the clearance $V_{max}/K_m$, i.e. intrinsic hepatic clearance of taurocholate (Deroubaix et al., 1991; Häussinger et al., 1992).

The mechanism(s) by which UDCA, or its conjugates, augment the uptake of other bile acids has rarely been studied. It has been proposed that this effect could be mediated by an increase in hepatocyte volume. In the intact liver, taurocholate excretion into bile is stimulated following hypotonic amino acid- or insulin-induced cell swelling (Hallbrucker et al., 1992; Häussinger et al., 1993). The excretory $V_{max}$ doubles when the intracellular space increases by 10–15% (cell swelling does not affect cellular ATP levels or membrane potential). Tauro-UDCA (10–50 µmol/L) induces hepatocyte swelling. Stimulation of taurocholate excretion by tauro-UDCA strongly depends on the extent of tauro-UDCA-induced swelling rather than the tauro-UDCA concentration. The stimulatory effect of cell swelling on taurocholate excretion into bile is abolished in the presence of colchicine, suggesting a microtubule-dependent mechanism. From these findings, it has been proposed that the swelling-induced stimulation of taurocholate excretion into bile is due to a microtubule-dependent insertion of bile acid transporters into the canalicular membrane (Häussinger et al., 1993). Recent evidence that the targeting of transporters to the canalculus could be mediated through a sustained increase in cytosolic calcium induced by tauro-UDCA has also been provided (Boyer et al., 1993). Bile acid flux throughout the canalculus towards periportal zones and the interlobular canal could be facilitated by the contractions induced by tauro-UDCA (Oda et al., 1990).
Hepatobiliary transport of organic anions, such as bilirubin and Brome Sulfone Phthaleine, can be facilitated by most bile salts, including UDCA. UDCA increases the maximal biliary secretion of bilirubin in the rat (Galan et al., 1990), showing that its effect is not restricted to hepatobiliary bile acid transport pathways.

5.4. Other Possible Mechanisms

5.4.1. Immune Modulation

Our controlled trial unexpectedly showed that some of the immunological markers of PBC improved during UDCA therapy, suggesting that bile acids and/or UDCA interfere with immune regulation. This led us to explore the effect of cholestasis and bile acids on the immune system. HLA molecules, specifically Class I species, are the main targets of the cytotoxic reaction. Abnormal expression of Class I molecules on hepatocytes is a salient feature of PBC and primary sclerosing cholangitis. We (Calmus et al., 1990) and others (Beuers et al., 1992) have found that long-term administration of UDCA reduces both cholestasis and the abnormal expression of HLA Class I molecules on hepatocytes. It has also been reported that such a reduction can occur on biliary cells. Experimental data indicate that this effect is mediated by a decrease in serum and tissue bile acid accumulation. Whatever the mechanism, the reduction in hepatocyte or biliary cell major histocompatibility complex Class I antigen expression induced by UDCA could block periportal and lobular cell necrosis by suppressing the cytotoxic T-cell target; this would provide a rational explanation for the improvement in bile duct paucity and periportal necrosis observed in patients on UDCA.

5.4.2. Stabilization of Hepatocellular Membranes

There is some evidence that UDCA conjugates can stabilize plasma membranes directly against disruption by more toxic bile salts. UDCA has anti-cytolytic properties in erythrocytes and model membranes (with a cholesterol-lecithin molar ratio \( \geq 0.5 \)) exposed to high bile acid concentrations (Güldünama et al., 1993; Heuman, 1993). However, the clinical relevance of these findings is unclear, since most studies have been performed with very high concentrations of bile acids.

5.4.3. Hypercholeresis

Unconjugated UDCA can induce bicarbonate-rich hypercholeresis in rats, and it has been postulated that this property might be related to its therapeutic effect (Dumont et al., 1980). Biliary levels of unconjugated UDCA do not markedly increase during UDCA administration to patients with PBC (Crosignani et al., 1991a) or cystic fibrosis (Nakagawa et al., 1990). As the hypercholeresis induced by UDCA administration to rats was linearly related to the recovery of unconjugated UDCA in the bile, it seems unlikely that the choleresis induced by cholehepatic circulation of unconjugated UDCA plays a role in its action in cholestatic patients.

6. CONCLUSION AND PERSPECTIVES

Until the 1980s, the role of bile acids in the initiation of liver injury in humans was only suspected on the basis of the toxicity of whole bile and bile salts and studies showing elevations in serum and tissue levels of bile salts in liver diseases. The beneficial effects of UDCA in PBC have provided the first firm evidence that in some way, bile acids may be related to liver injury in humans. These observations raise a series of questions and challenges in clinical, cellular and molecular research. For example, is UDCA capable of modifying the progression and prognosis of other cholestatic conditions of children and adults and, more generally, liver diseases in which the enterohepatic bile acid circulation is disturbed? Is it possible and warranted to design UDCA analogs with stronger activities and more specific targets? And how might UDCA and these analogs modulate fundamental cell or organelle functions and cell-to-cell relationships in the normal and cholestatic liver?
REFERENCES


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