Use of ursodeoxycholic acid in patients with hypertransaminasemia

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ABSTRACT

Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid deriving from 7β epimerization of primary bile compound chenodeoxycholic acid. It may have antioxidant, immunomodulatory, antifibrotic and cytoprotective effects, therefore it has been extensively used in the treatment of cholestatic chronic liver diseases (primary biliary cirrhosis, primary sclerosing cholangitis, cholestasis of pregnancy, etc.). Due to the effectiveness of UDCA in decreasing serum liver enzyme levels also in non-cholestatic patients with chronic liver damage of various etiologies, this bile acid is now extensively used in clinical practice in combination with standard therapies, or as alternative treatment. In this paper, after a review of the biochemistry and physiology of UDCA, we have analyzed available data from the international literature on the efficacy and safety of UDCA in patients with hypertransaminasemia due to non-cholestatic chronic liver diseases, such as chronic viral hepatitis B and C and liver steatosis, the three main causes of aminotransferase elevation in western countries.

Biosynthesis and functions of bile acids

Bile acids (BAs), the major constituents of bile, are synthesized in the liver from cholesterol.[1] Due to their biochemical characteristics, BAs allow the absorption and transport of dietary lipids and fat-soluble vitamins.[2] They may be classified into primary BAs, which are synthesized in the liver, such as the cholic acid (CA) and the chenodeoxycholic acid (CDCA), and secondary bile acids, such as deoxycholic acid and lithocholic acid (LA), which are formed in the intestine through 7α-dehydroxylation of primary bile acids by intestinal bacteria.[3]

More in details, BAs synthesis starts from cholesterol,[4] following a classic or an alternative pathway. The former, which accounts for 90% of BAs synthesis, leads through a cascade of several enzymatic steps to the synthesis of CA ($3\alpha,7\alpha,12\alpha$-trihydroxy-5β-cholanoic acid) and of CDCA ($3\alpha,7\alpha$-dihydroxy-5β-cholanoic acid), while the latter pathway leads to the formation of CDCA only. Under normal conditions, 70% of the human pool of BAs is composed by CA and its metabolites, while 30% is represented by CDCA.[1]

After their synthesis, BAs are conjugated with glycine and taurine, improving their solubility. These conjugated BAs (glyco- and tauro-conjugated BAs) are present as anionic salts under physiological pH conditions and are therefore called bile salts.[5] After glyco- or tauro-conjugation, primary BAs are excreted in the intestine, where they are de-conjugated and converted by 7α-dehydroxylase in intestinal bacterial flora to the secondary BAs, deoxycholic acid ($3\alpha,12\alpha$-dihydroxy-5β-cholestanoic acid) and lithocholic acid (3α-hydroxy-5β-cholestanoic acid).[6]

The intestinal conservation mechanism of bile salts is highly efficient. From 20-40 g of bile salts excreted daily into the bile, only 0.5 g are lost through fecal excretion and have to be replaced by *de novo* BA synthesis. This conservation is achieved through the enterohepatic circulation of bile salts, which depends on the action of several transporter proteins expressed at the basolateral and apical membrane of liver, biliary and small intestinal epithelial cells.[6]

In addition to their function of absorption and transport of dietary lipids and fat-soluble vitamins, BAs play an important role in decreasing cholesterol supersaturation human bile.[7] It is well-known that the relative concentrations of bile salts, phospholipids, and cholesterol are the major determinants of gallstone

formation.[7] This separation was the result of an increase in the quantity of cholesterol relative to the amounts of bile salts and lecithin contained in the bile from patients with cholesterol gallstones. In other words, BAs and lecithin fully solubilize cholesterol through the formation of the so-called *mixed micelles*. Forty-five years ago, William Admirand and Donald Small[8] plotted these three constituents simultaneously on triangular coordinates (the so-called *triangle of Admirand-Small*), thus achieving a complete separation of the normal and *abnormal* bile. This separation was the result of an increase in the quantity of cholesterol relative to the amounts of bile salts and lecithin contained in the bile from patients with cholesterol gallstones (supersaturated bile).

The enrichment of the BA pool by cholic acid did not desaturate the bile,[7] on the contrary, the other primary BA (CDCA) seemed to be able to reduce cholesterol biliary supersaturation,[9] although in several patients it induces an increase in serum aminotransferase levels.

An outstanding progress in the treatment of gallstone disease came from Japanese studies,[10] showing that ursodeoxycholic acid (UDCA), another BA, might be more effective than CDCA in dissolving gallstones with no relevant side effects. This paved the way to the use of UDCA for gallstone dissolution, which is nowadays very widely used for this purpose.

Ursodeoxycholic acid for the treatment of chronic liver diseases: a fascinating history

UDCA is a BAs derived from CDCA and a highly hydrophilic dihydroxy (3α,7β-dihydroxy-5 β-cholanoic acid) bile acid.[2,11] In humans, UDCA accounts for up to 4-5% of the BA pool. It is not synthesized in the liver, but it probably originates in the colon from bacterial 7*β* epimerization of CDCA.[12] After its formation, UDCA is passively absorbed by the colonic mucosa, thus entering the portal circulation and subsequently it enriches the pool of BAs.

BAs have both hydrophobic and hydrophilic properties that enable them to function as detergents. However, the different BAs have variable degrees of hydrophobicity and hydrophilicity, which are determined by their biochemical and physiochemical properties. LA acid is the least water soluble, whereas UDCA is much more hydrophilic. CA and CDCA acid have intermediate degrees of hydrophilicity.[2] The orientation (α or β) of the hydroxyl groups relative to each other determines the bile acid hydrophilicity in these planar molecules.

Due to its high hydrophilic properties, its ability to reduce supersaturation of human bile and the lack of the hepatotoxicity of other endogenous BAs, UDCA was rapidly marketed globally for the non-surgical treatment of gallstones. Thus, millions of persons in the western countries were treated with the new drug.

After a few years, however, it was clear that UDCA did perform some other therapeutic actions, beyond its biliary litholytic properties. In 1987, Poupon *et al.* suggested that long-term use of UDCA was safe and effective in patients suffering from primary biliary cirrhosis (PBC).[13] These preliminary results were confirmed 4 years later by a multicenter, prospective, placebo-controlled study in patients with PBC,[14] showing that UDCA therapy for 2-years has led to a reduction in clinically overt disease, improvement of liver blood tests and of Mayo risk score, a decrease in serum levels of immunoglobulin M class anti-mitochondial autoantibodies, and of the mean histologic score in the treated group compared with placebo. Subsequent studies have shown that UDCA delays the progression rate of PBC resulting in a decreased need for liver transplantation.[15] Lindor *et al.* reported a lower mortality or need for liver transplantation in the UDCA treatment group compared with patients receiving placebo.[16]

These beneficial effects of UDCA were attributed to several other actions of this bile acid, such as expansion of hydrophilic pool of BAs with displacement of endogenous and more toxic BAs at hepatocyte level, competitive inhibition of the absorption of endogenous BAs at the terminal ileus,[2] choleretic effects, immunomodulatory properties, cytoprotection and stabilization of liver cell structures, anti-apoptotic effects, anti-inflammatory properties.[17]

Several other studies did confirm the efficacy of UDCA in PBC, which is now widely used in clinical practice for the treatment of this and of other cholestatic diseases, such as primary sclerosing cholangitis and cholestatic disease of pregnancy.[7]

However, in 1999, a meta-analysis[18] failed to show some efficacy of UDCA in patients with PBC. More recently, a Cochrane review[19] did conclude that UDCA does not demonstrate any significant benefits on mortality or liver transplantation, pruritus, or fatigue in patients with PBC. UDCA simply seemed to have a beneficial effect on serum liver enzyme levels and on histological progression compared with the control group.

Anyway, due to the apparent beneficial effects of UDCA in cholestatic liver diseases, over the past 20 years, several studies have investigated the safety and efficacy of this BA also in non-cholestatic chronic liver diseases, such as chronic viral hepatitis [hepatitis B virus (HBV)- and hepatitis C virus (HCV)-related], non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), etc., mainly characterized by cytolysis and an increase of serum aminotransferase levels, rather than by cholestasis.

Ursodeoxycholic acid and hypertransaminasemia: myth or fact?

In their everyday clinical practice, physicians frequently must deal with patients showing increased aminotransferase levels, often found by chance in biochemical tests. Several causes for these enzyme alterations should be investigated, such as chronic hepatitis B or C (CHB, CHC), liver steatosis (NAFLD, NASH), autoimmune hepatitis, alcohol abuse, hemochromatosis, drugs, etc. To achieve a correct diagnosis, several parameters should be evaluated, such as the clinical history and age of the patient, lifestyle, body mass index, risk factors, magnitude of enzymatic elevations, duration, alanine transaminase (AST)/aspartate transaminase (ALT) ratio, presence of other biochemical alterations (*e.g.*, gamma-glutamyl transpepidase (GGT), alkaline phosphatase, bilirubin, platelet count, etc.).[20]

Studies on the general population in Italy have shown that approximately 17% of apparently *healthy* individuals have chronically elevated aminotransferase levels, often only slightly increased. In the majority of these patients the main cause is the steatosis of the liver, followed by CHC and CHB.[21]

Due to the effectiveness of UDCA in decreasing serum liver enzyme levels in patients with chronic liver damage of various etiologies, this BA is now extensively used in clinical practice in combination with standard therapies or as an alternative treatment.

We intend to examine here available data on the efficacy and safety of UDCA in the 3 main causes of aminotransferase elevation in our country: CHC, CHB and liver steatosis.

Ursodeoxycholic acid and hepatitis C virus related chronic hepatitis

Since the discovery of HCV in 1989, given the lack of optimal treatment and the severe side effects of the former available therapy with recombinant interferon (IFN) plus ribavirin,[22] several investigators began to evaluate the usefulness of UDCA in patients suffering from HCV-related CHC, non-candidates or non-responders to standard antiviral therapy.[23]

These studies were aimed at evaluating whether UDCA might reduce serum aminotransferase and GGT levels, decrease viral load (serum HCV RNA amounts), improve liver histology, and finally favorably modify the natural history of the disease and the progression to more severe liver damage, such as cirrhosis and hepatocellular carcinoma (HCC).[24] Some trials[25-27] compared UDCA *versus* placebo or no intervention for CHC. The dose of UDCA ranged from 400 to 800 mg/day, and the duration of treatment ranged from 3 to 12 months.[24] Two other trials compared tauro-conjugated UDCA (TUDCA) *versus* placebo[28] or no treatment.[29] Other trials compared UDCA combined with IFN *versus* IFN monotherapy in patients with CHC for chronic hepatitis C.[30]

All these investigations reported a significant reduction in serum aminotransferase and GGT levels during the study (−14% to −40%), and in several cases liver enzymes actually return within the normal range. However this biochemical normalization was lost at follow up, often returning to pre-treatment levels shortly after treatment.[23] Furthermore, it should be underlined that this transient beneficial effects on serum liver enzymes were not seen in all patients treated with UDCA, as at least 30-40% did not show any significant biochemical improvement.[26]

The mechanisms of these biochemical remissions during UDCA treatment are not clearly understood. As mentioned above, it is possible that UDCA might exert some direct cytoprotective effects on the hepatocyte membrane, by replacing more toxic endogenous BAs at this level, or by modifying the hydrophobic/hydrophilic BA ratio.[2-17]

It still remains unclear why UDCA treatment is effective on liver biochemistry in some patients with CHC, but ineffective in others. Some authors showed that a CDCA reduction in the hepatocytes is an important factor or cytoprotection, suggesting that UDCA exerts its cytoprotective action as a consequence of the stimulation of the efflux of cytotoxic BAs, such as CDCA from hepatocytes.[31] Nojiri *et al.*[32] reported that in patients with CHC responding to UDCA treatment the percentage of this BA in the serum and the percentage of CDCA in biliary bile were significantly higher than in the non-responders. This might indicate that, when effective, UDCA favors a decrease of CDCA in hepatocytes, thus contributing to hepatoprotection.

Another important issue is that UDCA treatment fails to eradicate HCV infection, and does not decrease the amount of circulating genomes, as detected by HCV RNA serum levels. These disappointing results have been reported both in studies using only UDCA and in trials of a combined treatment with UDCA and IFN.[24] It is noteworthy that even in the latter, when comparing the efficacy of antiviral therapy with IFN plus UDCA *versus* IFN alone, the decrease of HCV RNA levels and the rate of responders (*i.e.*, HCV RNA negative patients at the end of follow up) did not differ between the two groups. This means that, despite biochemical improvement, UDCA is not able to induce an enhanced clearance of HCV RNA from serum.[29]

The third important question to be answered is whether UDCA treatment can improve liver histology in patients with CHC. Only a few studies actually address this issue, probably due to the difficulties to perform a second liver biopsy shortly after the pre-treatment one. In the study of Attili *et al.*[33] 18 pa-

tients with CHC were treated with UDCA 600 mg/day for 12 months, while 18 others did received placebo. A percutaneous liver biopsy was performed before and after 1 year of treatment. Histological analysis showed an improvement in the biliary features of the liver damage, although no significant modifications of the necroinflammatory scores were reported.[33]

Similar unsatisfactory results were reported in studies comparing the effects of IFN plus UDCA treatment *versus* IFN alone on the portal and periportal inflammation scores or on the Knodell score.[24]

Although no data exists on the ability of UDCA to modify the natural history of CHC, the lack of efficacy on viral replication and the failure to improve liver histology have led to conclude that UDCA treatment *per se* is not able to slowdown or prevent the progression of chronic hepatitis into liver cirrhosis and HCC.[24]

At present, paramount advances in antiviral therapy introduce a new era of treatment for hepatitis C based on directly acting antiviral agents (oral protease inhibitors boceprevir and telaprevir), which are associated with a significant improvement in viral eradication rates in combination with pegylated IFN plus ribavirin.[34] Further antiviral drugs, even more potent and effective, are under study. Therefore the chance to eradicate HCV infection is strikingly increased with respect to past decades, thus decreasing the need for an *alternative* therapy with BAs.[34]

Anyway, several patients with CHC are not candidates to new therapies, because of major contraindications, while others show non-response or relapses. In these cases, UDCA treatment might be taken into account with the aim to maintain low ALT levels, although it is not clear whether ALT normalization might modify the natural history of the disease.[26]

Ursodeoxycholic acid and hepatitis B virus related chronic hepatitis

Antiviral treatment of CHB is more effective than that of CHC, therefore treatment with UDCA has been less used in this setting.[35]

The goal of antiviral therapy for CHB is to improve survival by preventing progression of the disease into cirrhosis and HCC. This goal can be achieved, if HBV replication can be suppressed in a sustained manner.[36]

Two different types of drugs can be administered to patients with CHB, IFN and nucleoside/nucleotide analogues (NAs). IFN is a cytokine with antiviral, antiproliferative, and immunomodulatory effects. It has shown to be effective in suppressing HBV replication and in inducing remission of liver disease.[35,36] NAs act as inhibitors of the HBV polymerase activity. The more recent NAs (entecavir and tenofovir) show high potency, high genetic barrier, minimal side effects, low

risk of viral resistance, oral administration, although they should be administered indefinitely.[36]

For these reasons, trials examining the use of UDCA in CHB are few and less recent than those in CHC. Available data confirms that UDCA decreases aminotransferase levels in patients with CHB, similarly to CHC, although it is not be able to modify liver histology or decrease serum HBV DNA levels.[24]

No data exists on the ability of UDCA treatment to modify clinical outcome and to prevent progression to more severe liver damage.[24]

Ursodeoxycholic acid and steatosis of the liver

In western countries, liver steatosis is the main cause of an apparently unexplained increase of aminotransferases in the general population with a prevalence accounting for around 20-30%.[21] Liver fat derives from dietary free fatty acids (FFA), from liver FFA inflow, and from hepatic *de novo* lipogenesis.[37]

Steatosis might be distinguished in 2 different forms, alcoholic and non-alcoholic. The latter may be secondary to a variety of causes, such as overweight/obesity, insulin resistance (IR), HCV, drugs, diabetes mellitus, disorders of lipid metabolism, rapid and severe weight loss, etc.[37]

NAFLD represents a spectrum of disorders characterized predominantly by macrovesicular steatosis occurring in individuals in the absence of significant alcohol consumption.[38] It is possible to distinguish a condition of simple fatty liver, where the only histologic finding is the presence of steatosis, from a state of NASH, a potentially progressive hepatic disorder leading to end-stage liver disease, characterized by hepatocellular injury/inflammation with or without fibrosis.[37] NAFLD is considered the hepatic manifestation of IR, and is therefore strongly associated with other clinical expressions of IR, such as metabolic syndrome and its features: obesity, type 2 diabetes, dyslipidemia and hypertension.[38] Although NAFLD is a rather benign condition, a fraction of these patients (20-30%) with non-alcoholic fatty liver disease might develop more severe liver damage and liver cirrhosis.[39]

At present most hepatologists attempt to manage NASH using lifestyle changes to reverse the consequences of metabolic disease, such as weight reduction with or without exercise, as well as standard therapeutic interventions to control concomitant associated diseases, hyperlipidaemia, hypertension and type 2 diabetes.[39]

Pharmacological treatment of steatosis is still an unmet medical need. Due to the lack of specific and effective agents, several drugs have been studied: insulin-sensitizing agents, antioxidant therapy, vitamin E, betaine, pentoxifilline, probucol, omega-3 polyunsaturated fatty acids, sartans and lastly UDCA.[40]

In patients with fatty liver UDCA might prevent steatosis by protecting against mitochondrial injury, by inducing a plasma membrane stabilizing effect, and decreasing lipid peroxidation. Moreover, taurine-conjugated bile acids could inhibit the activity of some microsomal enzymes, which are induced by FFAs. UDCA inhibits the activation of Kupffer cells caused by toxic bile salts, thus acting as an antioxidant agent.[41] Furthermore, UDCA increases hepatocyte levels of glutathione and thio-containing protein, therefore protecting hepatocytes against oxidative injury.[42]

UDCA may also reduce the expression of class II HLA inhibitors, the production of interleukin and interferon, thus acting as an immunomodulator. In addition, UDCA seems to reduce reactive oxygen species.[43]

Both UDCA and TUDCA have been tested, alone or in combination with other drugs, *versus* no intervention or in randomized controlled trials *versus* placebo.[40]

Biochemical response was assessed by serum activities of AST, ALT, alkaline phosphatase (ALP), GGT, and serum total bilirubin levels. UDCA or TUDCA treatments actually induce a decrease of AST and ALT serum levels, but there were no significant differences between patients treated with BAs and the control (placebo) group. Similarly, no significant decrease in GGT, ALP and bilirubin were seen.[40]

Conflicting results were reported as to ultrasound (US) response. The majority of trials did not show significant improvement of US features of steatosis after UDCA treatment,[40] only one study reported a slight decrease in the US steatosis score.[44] Therefore, available data allow to exclude any radiological benefit of UDCA *versus* placebo/no intervention in patients with fatty liver.

The major clinical outcome of such treatment should be the possible improvement of histological liver damage. Only a few studies have addressed this issue, failing to show significant modifications of degree of steatosis, inflammation, or fibrosis among treated patients. Lindor *et al.*[43] studied the efficacy of UDCA in 166 patients with biopsy-proven NASH. End-points included changes in liver test results and liver histology at 2 years of therapy. Unfortunately, also this study failed to show any biochemical or histological benefit of UDCA in patients with NASH.

More recently, Ratziu *et al.*[41] investigated the efficacy and safety of high-dose UDCA (HD-UDCA, 28-35 mg/kg per day) in a 12-month, randomized, double-blind, placebo-controlled multicenter trial enrolling 126 patients with biopsy-proven NASH and elevated ALT levels. The primary study end-point was a reduction in ALT levels from baseline in patients treated with HD-UDCA compared with placebo. Secondary study end-points were the proportion of patients with ALT normalization, a relative reduction in the scores of serum markers of fibrosis and hepatic inflammation, and safety and tolerability.

In this study, HD-UDCA significantly reduced mean ALT levels (–28.3% from baseline after 12 months compared with –1.6% with placebo, P<0.001). At the end of the trial, ALT levels normalized in 24.5% of patients treated with HD-UDCA and in 4.8% of patients who received placebo (P=0.003). Both results were not accounted for by changes in weight during the trial.

HD-UDCA significantly reduced the serum fibrosis marker compared with placebo. Furthermore, this BA also significantly improved markers of glycemic control and insulin resistance.[41] The results of this study indicate that probably UDCA should be administered in a dosage higher than that reported in previous trials.

Conclusions

Although UDCA is extensively prescribed in clinical practice for the treatment of patients with hypertransaminasemia of various origins, its use is hampered by the fact that it is not clear whether biochemical remission seen during UDCA treatment might be associated with histological improvement and a favorable modification of the disease progression. Moreover, it should be underlined that in our country UDCA treatment for non-cholestatic chronic liver disease is considered off-label and therefore it is not refunded by our National Health System.

On the other side, absence of evidence does not always mean absence of effect.[40] Furthermore, it should be considered that UDCA is a rather safe and relatively inexpensive drug.[26] The decrease of liver biochemistry during treatment often reduces patient anxiety, thus improving quality of life and mental health status (Elmo MG, unpublished data, 2012).

Lastly, although it is not clear whether ALT levels actually correlate with the severity of liver damage and disease progression, particularly in CHC,[45] we cannot rule out the possibility that persistent ALT improvement or even normalization might favorably modify the clinical outcome and the long-term history of chronic liver diseases.

In conclusion, further prospective studies using higher doses of UDCA and/or longer treatment periods are needed. Patients with CH should be clearly informed that this therapy might merely have cosmetic effects on the liver biochemistry.

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