Long-Term Ursodeoxycholic Acid Therapy Does Not Alter Lithocholic Acid Levels in Patients with Cystic Fibrosis with Associated Liver Disease

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Objective To evaluate the fasting and postprandial serum bile acid composition in patients with cystic fibrosis-associated liver disease (CFLD) after chronic administration of ursodeoxycholic acid (UDCA) (20 mg/kg/day). The aim was to specifically focus on the extent of biotransformation of UDCA to its hepatotoxic metabolite, lithocholic acid, because of recent concerns regarding the safety of long-term, high-dose UDCA treatment for CFLD.

Study design Twenty patients with CFLD (median age 16 years, range: 2.4-35.0) prescribed UDCA therapy for at least 2 years were studied. Total and individual serum bile acids were measured by stable-isotope dilution mass spectrometry, in fasting and 2-hour postprandial samples taken during chronic UDCA (20 mg/kg/day) administration.

Results During chronic UDCA administration (median duration 8 years, IQR: 6-16), UDCA became the predominant serum bile acid in all patients (median, IQR: 3.17, 1.25-5.56 μmol/L) and chenodeoxycholic acid concentrations were greater than cholic acid (1.86, 1.00-4.70 μmol/L vs 0.40, 0.24-2.71 μmol/L). The secondary bile acids, deoxycholate and lithocholate, were present in very low concentrations in fasted serum (<0.05 μmol/L). After UDCA administration, 2-hour postprandial concentrations of both UDCA and chenodeoxycholic acid significantly increased ($P < .01$), but no significant changes in serum lithocholic acid concentrations were observed.

Conclusion These data do not support recent suggestions that enhanced biotransformation of UDCA to the hepatotoxic secondary bile acid lithocholic occurs when patients with CFLD are treated with relatively high doses of UDCA. *(J Pediatr 2016;177:59-65)*.

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Ursodeoxycholic acid (UDCA) is currently the only treatment available for cystic fibrosis-associated liver disease (CFLD)[1]; however, it remains to be established whether the administration of UDCA halts the progression to severe disease in patients with cystic fibrosis (CF) with signs of liver inflammation, because data thus far only show improvements in endpoints indirectly associated with prognosis.[2] Findings from a retrospective long-term, follow-up, case-control study suggest that early introduction of UDCA treatment, at a dose of 20 mg/kg/day in patients with CF with clinical, ultrasonographic, or biochemical signs of liver disease, is effective in limiting its progression.[3] This dose is greater than that usually prescribed (10-15 mg/kg/day) and approved for primary biliary cirrhosis (PBC) or used "off-label" for other cholestatic conditions.

European guidelines for the clinical management of CFLD recommend the use of UDCA at a dose of 20 mg/kg/day as soon as the diagnosis is established,[4] but there is no consensus about this recommendation, and more recently concerns have been expressed regarding its safety in this patient population.[5] These concerns are based on negative outcomes, a 2-fold increase in severe adverse events reported in a randomized, double-blind, placebo-controlled trial of adults with primary sclerosing cholangitis (PSC) who were treated with very high doses of UDCA (28-30 mg/kg/day).[6] It was speculated that changes in bile acid composition caused by biotransformation of UDCA to more toxic hydrophobic bile acids, such as lithocholic acid (LCA), might be responsible for the observed adverse events.[7-9] More than 2 decades have passed since concerns were first raised over LCA toxicity with UDCA usage,[10] but this issue is yet to be fully resolved.

In our study we sought to determine, by using tandem mass spectrometry, the extent to which chronic administration of high doses of UDCA (20 mg/kg/day) to patients with CFLD induces changes in the circulating bile acid pool composition, specifically with regard to LCA levels.

CA	Cholic acid
CDCA	Chenodeoxycholic acid
CF	Cystic fibrosis
CFLD	Cystic fibrosis-associated liver disease
DCA	Deoxycholic acid
LCA	Lithocholic acid
PBC	Primary biliary cirrhosis
PSC	Primary sclerosing cholangitis
UDCA	Ursodeoxycholic acid

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We hypothesized that LCA levels are relatively low even after long-term therapy and not significantly altered by the administration of UDCA. Therefore, the negative effects seen in previous studies of high-dose UDCA in PSC, or proposed in patients with CF treated with UDCA, are unlikely to be the consequence of the biotransformation of UDCA to more toxic hydrophobic bile acids.

Methods

Twenty patients with CF and CFLD (13 with cirrhosis and 7 with less severe disease) who had been treated with UDCA at the dose of 20 mg/kg/day for at least 2 years were enrolled in the study.

Patients with CF were considered to have liver disease based on at least 2 consecutive examinations spanning a 1-year period that included: (1) clinical hepatomegaly confirmed by ultrasonography scanning; (2) abnormal serum liver enzymes, consisting of an elevation above the upper limit of normal for 2 of the following: aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyltranspeptidase; and (3) abnormalities on ultrasound scanning other than hepatomegaly (ie, increased, heterogeneous echogenicity, nodularity, irregular margins, or splenomegaly). Other possible causes of liver disease were excluded. Liver biopsy is not performed routinely at our Center and therefore was not included among the diagnostic investigations.

Evidence of cirrhosis was based on the following ultrasonographic criteria: presence of irregular margins and nodular hepatic pattern or signs of portal hypertension. The following ultrasound signs were considered to reflect presence of portal hypertension: splenomegaly, enlarged portal vein in the absence of respiratory variations, and the presence of collateral veins. Patients with cirrhosis underwent upper digestive endoscopy to assess the presence of esophageal varices if they developed signs of hypersplenism, unexplained anemia, or gastrointestinal bleeding. Endoscopy was repeated at least every 2 years.[4,11]

At the clinic visit, 2 blood samples were obtained from each patient: the first draw (baseline) was made after an overnight fast, and the second draw was made 2 hours (postprandial) after the morning dose of UDCA was administered with breakfast. The patients were not given a specific standardized meal but consumed their normal breakfast, which consisted mostly of milk with or without coffee and a croissant. Fasting blood samples also were obtained in 9 patients with CF without liver disease. Normal serum bile acid data for healthy adults determined by tandem mass spectrometry (n = 49) also were included for comparison.

To evaluate the safety and tolerability of UDCA treatment at each clinic visit (generally every 2 months), the patients were specifically asked to describe any adverse events or symptomatology related to UDCA therapy. These included questions about presence or absence of diarrhea, itching, or abdominal cramps. The occurrence of jaundice or worsening of markers of liver inflammation was determined from biochemical markers. Any reported adverse events were documented in the patient records. The study protocol was approved by the Ethical Committee of the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, and all patients/guardians signed the informed consent form.

Serum was obtained and stored at −80°C and samples batched for analysis. Serum concentrations of the principal bile acids, UDCA, cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), LCA, and their corresponding glycine and taurine conjugates were measured by stable-isotope dilution analysis with a validated liquid chromatography-electrospray ionization-mass spectrometer. Serum samples were analyzed in the Division of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, according to a standard operating procedure (SOP# PATH.CMS.1033). Quality control samples were prepared at concentrations of 100, 200, and 500 ng/mL and the intra- and interassay imprecision of the method for the 16 individual bile acids measured was within the accepted Good Laboratory Practice quality assurance guidelines of <15% coefficient of variance for these quality control samples. The lower limit of quantification of the assay was set at 20 ng/mL, and the imprecision at this concentration was <20%. The limit of detection of the assay was 5 ng/mL (0.01 μmol/L). Serum bile acid concentrations are expressed as μmol/L, and the total serum bile acid concentration is represented by the sum of the individual bile acid species measured.

Statistical Analyses

Data on serum bile acid concentrations were expressed as median and IQR. The Kruskal-Wallis 1-way ANOVA by ranks was used to test for differences in serum bile acid concentrations across groups (patients with CF and cirrhosis, patients with CF and liver disease but no cirrhosis, patients with CF without liver disease, and healthy subjects). If significant differences were detected by the omnibus Kruskal-Wallis test, pairwise multiple comparisons by Tukey and Kramer test with χ^2 approximation for independent samples were performed.

To compare the serum bile acid concentrations before and 2 hours after UDCA administration, we used the Wilcoxon signed-rank test. All statistical tests were 2-tailed, and significance threshold was set at P value <.05.

Results

The clinical and demographic characteristics of the patients with CF enrolled are reported in Table I. The median age of the patients was 16 and range 2.4-35.0 years. Five patients had surgical intervention for neonatal meconium ileus, of whom 2 had an intestinal resection. Patients had been on UDCA therapy for a median period of 8 years (range: 2-22 years, IQR: 6-16 years). The dose of UDCA was normalized to body weight throughout the treatment period.