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Oral Cholic Acid Is Efficacious and Well Tolerated in Patients With Bile Acid Synthesis and Zellweger Spectrum Disorders

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ABSTRACT

Objectives: Patients with bile acid synthesis disorders (BASDs) due to single enzyme defects (SEDs) or Zellweger spectrum disorders (ZSDs) accumulate hepatotoxic atypical bile acids resulting in potentially fatal progressive liver disease. We evaluated the efficacy and safety of oral cholic acid in patients with BASD.

Methods: In this phase 3, open-label, single-arm, nonrandomized, noncomparative study conducted over 18 years, patients were administered cholic acid orally 10 to 15 $mg \cdot kg^{-1} \cdot day^{-1}$. The primary efficacy variables were changes from pre- to post-treatment in atypical urinary bile acids, liver chemistries (serum aspartate aminotransferase, alanine aminotransferase), and height and weight. Additional efficacy variables included changes in serum bilirubin and liver histology.

Results: Of the 85 enrolled patients (63 with SED and 22 with ZSD), 79 received at least 1 dose of study medication; 70 patients (50 with SED and 20 with ZSD) were included in the modified intent-to-treat dataset. Cholic acid significantly improved urine bile acid metabolite scores ($P < 0.0001$) and serum aspartate aminotransferase and alanine aminotransferase ($P < 0.0001$) in patients with SED and ZSD. Cholic acid also improved height and weight percentiles in both groups, but only the change in weight was significant ($P < 0.05$). Serum direct bilirubin decreased significantly post-treatment ($P < 0.001$) in the intent-to-treat population, and liver biopsies showed either stable findings or histologic improvement in all parameters except bridging fibrosis. The overall safety profile of cholic acid was favorable, with no study drug-related serious adverse events or drug-related deaths reported.

Conclusions: Oral cholic acid is a safe, efficacious, and well-tolerated treatment for BASD due to SED and ZSD.

Key Words: AKR1D1 deficiency, HSD3B7 deficiency, inborn errors of bile acid synthesis, peroxisomal disorders

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The synthesis of cholic acid and chenodeoxycholic acid from cholesterol involves several steps catalyzed by 15 enzymes located in various subcellular fractions of the hepatocyte (1–4). Rare congenital disorders affecting this biosynthetic pathway are known collectively as bile acid synthesis disorders (BASDs). BASDs are due to a deficiency of or lack of activity in enzymes catalyzing the conversion of cholesterol to bile acids (referred to here as single enzyme defects [SEDs]) or to defects in the oxidation and shortening of the cholesterol side chain (2–5). The most common BASDs involving SEDs are 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (HSD3B7, referred to here as 3β-HSD deficiency) and Δ^4-3-oxosteroid 5β-reductase (AKR1D1, referred to here as 5β-reductase deficiency), in which primary acid synthesis is absent or negligible (4,6). In the group of diseases known as Zellweger spectrum disorders (ZSDs), side-chain oxidation of bile acid precursors, a process that occurs in the peroxisomes, is impaired and these patients synthesize and accumulate long-chain (C_{27}) bile acids (2,5).

The impairment in bile acid synthesis causes hepatocytes to continuously metabolize bile acids from cholesterol in an attempt to establish a normal bile acid pool. The result is the continued production of high concentrations of atypical bile acids and bile acid intermediates, which may cause progressive cellular injury (4–9). Accumulation of these potentially hepatotoxic metabolites and the reduction in bile acid–dependent bile flow from the lack of primary bile acids may lead to liver injury (4,10). Liver disease associated with these conditions is progressive and if untreated may lead to death from cirrhosis and liver failure. In some cases, extrahepatic injury leads to central nervous system disorders with cholestasis and potential liver injury limited to the newborn period. ZSDs are characterized by progressive liver disease and extrahepatic manifestations such as central nervous system dysfunction (4,11).

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It was hypothesized that exogenous administration of bile acids in patients with SED or ZSD would reduce the production of hepatotoxic intermediates and metabolites through downregulation of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid synthesis; promote bile acid–dependent bile flow, thereby resolving cholestasis; and improve growth by facilitating the absorption of fat and fat-soluble vitamins.

Oral bile acid therapy has been shown to reduce production of atypical bile acids, normalize serum liver transaminases, and improve liver histology in patients with 3β-HSD or 5β-reductase deficiencies (12–17) and to improve liver function in patients with Zellweger syndrome (16,17).

In the present study, we evaluated the efficacy and safety of oral cholic acid in the largest cohort of patients with BASD due to SED or with ZSD. This was initially an investigator-initiated, compassionate use program and then transitioned into a formal clinical trial program. This report focuses on data collected over the 18-year period from January 3, 1992 to December 31, 2009.

METHODS

Patients and Study Design

This phase 3, open-label, single-arm, nonrandomized, noncomparative, compassionate treatment study began at the Cincinnati Children's Hospital Medical Center (CCHMC) but was later expanded to enroll patients with BASD or ZSD from other sites. Diagnosis of an inborn error of bile acid synthesis was confirmed based on urine fast atom bombardment ionization mass spectrometry (FAB-MS) analysis and the presence of atypical bile acids characteristic of the specific defect in bile acid synthesis (10). No specific exclusion criteria were defined for the study. Patients were permitted to continue their other medications as indicated.

Signed informed consent by the patient and/or parents or legal guardian was solicited and obtained. The present study was approved by the institutional review board of the CCHMC and conducted under an Investigational New Drug application (45,470) approved by the US Food and Drug Administration. All authors of this manuscript had access to the study data and reviewed and approved the final manuscript.

Treatment

Cholic acid (10 to 15 $mg \cdot kg^{-1} \cdot day^{-1}$) was administered once a day or in divided doses twice daily as capsules emptied into food, or as a liquid formulation (15 mg/mL) for patients who could not swallow capsules (see the Supplemental Digital Content, Material, *http://links.lww.com/MPG/B25*, for discussion of use of ursodeoxycholic acid in the original protocol).

Efficacy Assessments

Urinary Bile Acid Analysis by Fast Atom Bombardment Ionization Mass Spectrometry

Negative ion mass spectra were acquired over 50 to 1000 Da (see the Supplemental Digital Content, Material, *http://links.lww.com/MPG/B25*, for details). In BASD, the FAB-MS negative ion mass spectrum reveals ions that correspond in mass to the accumulated intermediates and/or their metabolites in the biosynthetic pathway proximal to the enzyme block.

Using a scoring system based on signal/noise ratio for the major ions (developed by KDRS) the FAB-MS mass spectra at baseline and post-treatment were assessed as normal (score 0) or as showing slight (score 1), significant (score 2), or marked (score 3) increases in the levels of atypical bile acids. This semiquantitative assessment of the urinary atypical bile acid levels was recently validated for 3β-HSD deficiency and shown to correlate well with quantitative excretion as measured by a targeted tandem MS method (18).

Liver Chemistries

Laboratory assessment of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and serum bilirubin (total and direct) were performed at baseline and at regular intervals during treatment in Clinical Laboratory Improvement Act or College of American Pathologists certified laboratories. A value of 50 IU/L was selected as the upper limit of normal (ULN) for AST and ALT. Multiples above the ULN (1–<2 and ≥2× ULN) were examined.

Body Measurements

Anthropometric measurements (height and weight) were performed at baseline and at intervals determined by the attending physician using standard clinical methods during treatment.

Liver Histology

For patients treated at CCHMC, percutaneous liver biopsies were performed at baseline (if no historical sample was available) and between 1 and 10 months after treatment start. In patients with ZSD, a liver biopsy was performed if there was no contraindication to biopsy that increased the risk of the procedure. Liver biopsies were not required for patients evaluated outside the CCHMC site. Liver biopsy processing was done by standard methods, and sections stained with hematoxylin and eosin and trichrome were routinely used.

An experienced pediatric pathologist (K.E.B.) served as a central reader for review of all liver biopsies and narrative text data available. Pre- and post-treatment liver biopsies were analyzed qualitatively for the presence of inflammation, fibrosis, necrosis, giant cells, and cholestasis using the fibrosis staging (0–4) and grading (0–4) system of Ishak et al (19) In a third step, any liver biopsy results were compared with those of the previous biopsy (if available), and the degree of change (improved or worse) was assessed.

Efficacy Variables

The primary efficacy variables were changes from pre- to post-treatment in urinary bile acid metabolites, liver chemistries, and growth measurements (height and weight). Additional efficacy variables evaluated were changes from pre- to post-treatment in serum bilirubin (total and direct) and liver histology (for patients with liver biopsies).

Safety Assessments

Information on adverse events (AEs) was collected retrospectively and recorded in patient files. The principal investigator (J.E.H.) assessed AE severity (mild, moderate, severe), seriousness, and relationship to study drug based on patient records and memory recall. For analysis, AEs were coded using the Medical Dictionary for Regulatory Activities.

Statistical Analysis

All patients identified as having an inborn error of bile acid synthesis were included in the intent-to-treat (ITT) population.

TABLE 1. Baseline demographic characteristics

Characteristic	ITT population (N = 85)
Sex, n (%)	
Male	50 (59)
Female	31 (36)
Not recorded	4 (5)
Disorder type, n (%)	
Single enzyme defect	54 (64)
3β-HSD	35 (41)
5β-reductase	10 (12)
Sterol 27-hydroxylase deficiency	5 (6)
2-methylacyl-CoA racemase (AMACR) deficiency	1 (1)
Others	2 (2)
Unknown	1 (1)
ZSD	31 (36)
Zellweger syndrome	12 (14)
Neonatal adrenoleukodystrophy	8 (9)
Type unknown	6 (7)
Infantile Refsum disease	4 (5)
Generalized peroxisomal disorder	1 (1)
Age at diagnosis, y	
Mean ± SD	2 ± 4 (n = 74)
Min, max	0, 13 (n = 74)
Age group at diagnosis, n (%)	
<3 mo	23 (27)
3–6 mo	19 (22)
7–12 mo	13 (15)
13–36 mo	12 (14)
>36 mo	18 (21)
Age at treatment start, y	
Mean ± SD	3 ± 4 (n = 77)
Min, max	0, 16 (n = 77)
Height percentile	
Mean ± SD	33 ± 31 (n = 16)
Min, max	0, 92 (n = 16)
Weight percentile	
Mean ± SD	39 ± 36 (n = 16)
Min, max	0, 98 (n = 16)

AKR1D1 = 5β-reductase, Δ^4-3-oxosteroid 5β-reductase; AMACR = 2-methylacyl-CoA racemase; 3β-HSD = 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (HSD3B7); CoA = coenzyme A; ITT = intent-to-treat; SD = standard deviation; ZSD = Zellweger spectrum disorders.

The primary analysis was based on a modified ITT (mITT) dataset, including all patients who received cholic acid and had at least 1 pre- and post-treatment outcome assessment for urine bile acid analysis, liver chemistries, and height and weight. The safety set included all patients who received at least 1 dose of cholic acid. The primary analysis was the worst pretreatment to the best post-treatment response for each efficacy outcome. Sensitivity analyses of the worst pretreatment to the worst post-treatment results and the best pretreatment to the best post-treatment results were also performed (see the Supplemental Digital Content, Material, *http://links.lww.com/MPG/B25*, for details of statistical analysis).

RESULTS

Study Population

A total of 85 patients (63 with SED and 22 with ZSD) were enrolled; 79 (93%) took at least 1 dose of the study medication and were included in the safety set. Mean duration of treatment for patients in the safety set (patients with available treatment start and stop dates) was 145 weeks (range: 0–545 weeks). Seventy patients (50 with SED and 20 with ZSD) were included in the mITT set.

The majority of patients with SED presented with 3β-HSD deficiency (median age, 37 months) or 5β-reductase deficiency (median age, 3 months). Zellweger syndrome (median age, 6 years) and neonatal adrenoleukodystrophy (median age, 2 years) were the most common ZSD (Table 1).

Impact of Cholic Acid Treatment on Urinary Bile Acid Excretion

Treatment with cholic acid significantly improved urine bile acid FAB-MS scores in patients with SED (Fig. 1A) or ZSD (Fig. 1B) in the worst-to-best analysis (see the Supplemental Digital Content, Material, *http://links.lww.com/MPG/B25*, for results of best-to-best and worst-to-worst sensitivity analyses for key efficacy variables). Among patients with SED, the percentage with normal FAB-MS scores (indicating normalized urinary bile acid excretion) increased from 2.3% at baseline to 65.1% post-treatment; the percentage with marked abnormalities decreased from 72.1% to 14.0% post-treatment ($P < 0.0001$). Among patients with ZSD, the percentage with normal FAB-MS scores increased from 33.3% to 85.2% with cholic acid treatment ($P < 0.0001$).

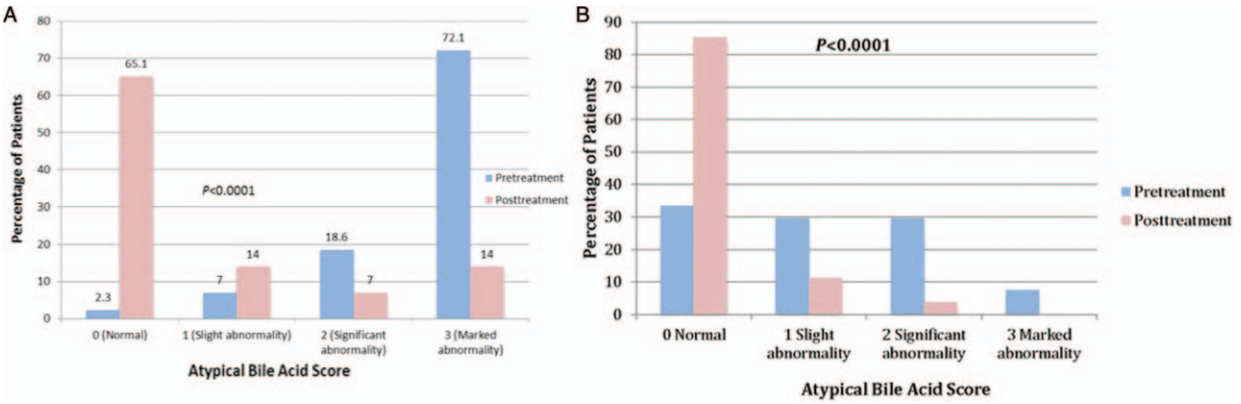

FIGURE 1. Impact of cholic acid treatment on urinary bile acid excretion in (A) patients with single enzyme defects (n = 43) and (B) patients with Zellweger spectrum disorder (ZSD) (n = 27)—worst-to-best analysis, modified intent-to-treat (mITT) population.

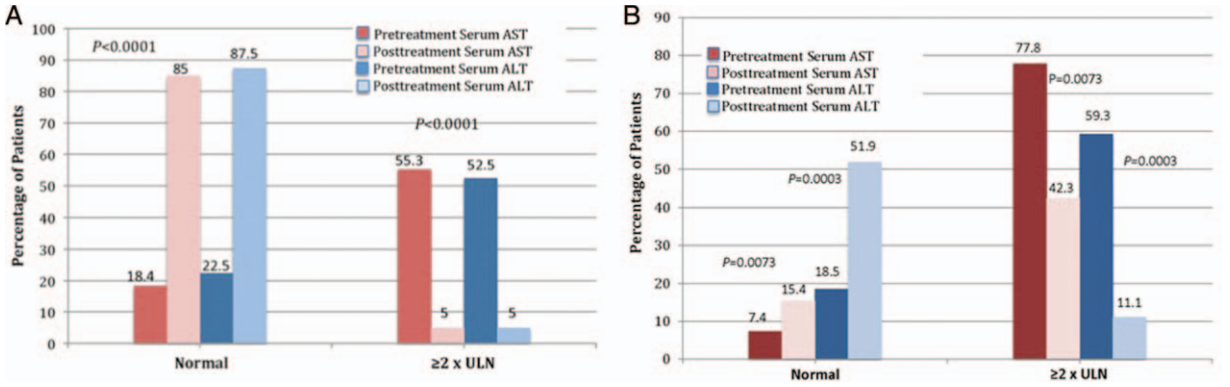

FIGURE 2. Impact of cholic acid treatment on liver chemistries in (A) patients with single enzyme defects (n = 43) and (B) patients with ZSD (n = 27)—worst-to-best analysis, mITT population. *P* values represent pre- versus post-treatment, *P*-value is from a Cochran-Mantel-Haenszel chi-square test with modified ridit scoring. ALT = alanine aminotransferase; AST = aspartate aminotransferase; mITT = modified intent-to-treat; ULN = upper limit of normal; ZSD = Zellweger spectrum disorders.

Impact of Cholic Acid Treatment on Serum Liver Chemistries

Serum Alanine Aminotransferase and Aspartate Aminotransferase

In the worst-to-best analysis, treatment with cholic acid significantly improved liver chemistries in the mITT population ($P < 0.0001$).

For patients with ZSD and those with SED, there was a marked increase in the number of patients with serum ALT and AST values below the ULN and a marked decrease in the number with values ≥2 times the ULN (Fig. 2A and B); changes in serum AST were less pronounced in patients with ZSD.

Serum Bilirubin

Mean serum bilirubin in the ITT population (n = 85) decreased from pretreatment to post-treatment for each bilirubin category (total, direct, indirect, and not otherwise specified), but the decrease (from 3.5 to 0.6 mg/dL) was statistically significant only for direct bilirubin ($P < 0.001$).

Effect of Cholic Acid Treatment on Height and Weight

Treatment with cholic acid improved height and weight percentiles in patients with SED and those with ZSD, as determined by the worst-to-best analysis (Fig. 3). Only the changes in weight were, however, statistically significant ($P = 0.006$ and $P = 0.014$) for patients with SED and ZSD, respectively. The magnitude of changes in weight and height were comparable for patients with SED and those with ZSD.

Effect of Cholic Acid Treatment on Liver Histology

A total of 26 patients in the ITT population had at least 1 liver biopsy for qualitative analysis. Pre- and post-treatment liver biopsies were available for analysis in 4 patients (3 with 3β-HSD and 1 with 2-methylacyl-CoA racemase deficiency). In each patient with 3β-HSD, there was either a reduction or no change in inflammatory infiltrates with cholic acid treatment. In a 13-year-old boy with 3β-HSD, inflammation subsided and fibrosis was stable after 10 months of therapy (see the Supplemental Digital Content, Fig. S1A and S1B, *http://links.lww.com/MPG/B25*). Two of 3 patients had no change in fibrosis, whereas 1 had an increase. In a 10-week-old infant with 2-methylacyl-CoA racemase deficiency, giant multinucleated hepatocytes, and mild portal inflammatory infiltrates disappeared and fibrosis remained absent after 10 months of treatment (see the Supplemental Digital Content, Fig. S1C and S1D, *http://links.lww.com/MPG/B25*). With the exception of bridging fibrosis and unspecified fibrosis, all histopathologic features were less prominent in post-treatment biopsies than in pretreatment biopsies. The numbers of young patients with cholestasis, giant cells, and necrosis decreased from pre- to post-treatment.

Safety Assessments

A higher percentage of patients with ZSD experienced treatment-emergent AEs (TEAEs) and serious AEs (SAEs) compared with patients with SED (see the Supplemental Digital Content, Table S1, *http://links.lww.com/MPG/B25*). TEAEs were predominantly mild or moderate. Only 3 TEAEs in 2 patients were considered treatment related by the investigator. Each AE considered treatment related/unknown occurred in 1 patient only (malaise and jaundice in 1 patient and skin lesion in 1 patient).

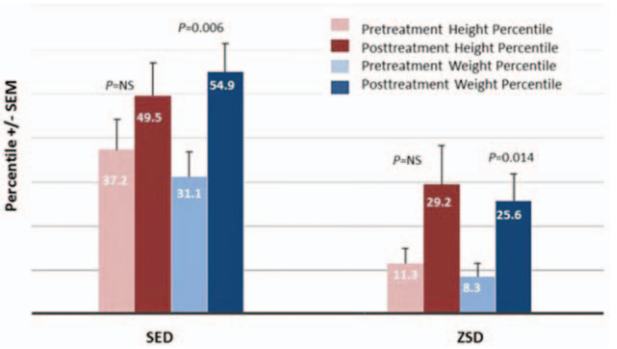

FIGURE 3. Mean height and weight percentiles from pretreatment to post-treatment in the modified intent-to-treat population (N = 70), worst-to-best analysis. Numbers in bars represent absolute percentiles for each group. NS = not significant; SED = single enzyme defect; ZSD = Zellweger spectrum disorders.

A total of 4 patients discontinued the study due to AEs. The AE most frequently leading to study discontinuation was disease progression. All others occurred in single patients only. None of the AEs leading to study discontinuation were considered related to study medication by the investigator.

Ten patients (13%) experienced AEs of severe intensity. These were most frequently related to disease progression (7 patients). The only other severe AE occurring in at least 2 patients was dehydration. Diarrhea is a known adverse effect of excessive dosing with cholic acid. During the 18-year study period, diarrhea was documented in 6 patients. None of the cases of enteritis or diarrhea were assessed as related to study medication and none of the episodes led to discontinuation of treatment.

Serious Adverse Events

Of the 28 SAEs, disease progression was the most frequently reported, followed by diarrhea (3%), urinary tract infection (3%), and dehydration (3%). All other SAEs occurred in single patients only. None of the SAEs were considered related to study treatment. One patient had a bleeding gastric ulcer, and for 1 patient with disease progression, the SAE outcome was unknown. A total of 21 patients died during the study period and 1 patient after the study period (see the Supplemental Digital Content, Material, *http://links.lww.com/MPG/B25*, for details). Disease progression, or an event secondary to worsening of the underlying condition (ZSD), was the most frequently noted AE. Death was not considered related to study medication in any case.

DISCUSSION

Based on the treatment of these 70 patients, oral cholic acid appears to be safe, efficacious, and well tolerated. Our experience in patients with SED is supported by the findings of a case series of 15 patients with SED treated with cholic acid (13). Our experience in 27 patients with ZSD suggests that cholic acid therapy ameliorates liver dysfunction. There is no evidence that treatment with cholic acid has any impact on the extrahepatic disease associated with ZSD; however, there is compelling evidence that it promotes weight gain (16).

Our studies have demonstrated that oral cholic acid treatment of patients with SED leads to reductions in serum bilirubin and serum ALT and AST, suppression of the urinary excretion of bile acid intermediates, and in a limited number of patients, stabilization or improvement in liver histology. The histologic findings in the present study, although limited, are consistent with previous reports, which showed either improved or stabilized inflammation and fibrosis with therapy (5,13,15). It is important to note that the presenting symptoms and signs in patients with SED may be quite variable (20) and may appear from infancy through adulthood (21,22). Patients with 5β-reductase deficiency tended to present at a younger age (median age, 3 months) with marked biochemical abnormalities compared with those presenting with 3β-HSD deficiency, many of whom presented at a later age (median age, 37 months) with evidence of liver fibrosis but less impressive biochemical abnormalities. In a subgroup of patients, particularly those who were identified prospectively due to a previously affected sibling, the biochemical findings were subtle. Patients with a milder phenotype would likely not have the same therapeutic response on their biochemistries, which would explain why not all patients had a biochemical response to therapy. Furthermore, greater awareness of BASD because of a previously affected sibling resulted in a diagnosis being established at an early age and before significant liver dysfunction becomes apparent. Initiating cholic acid therapy would therefore serve to prevent significant advancement in liver disease.

This study was initiated as a compassionate use of cholic acid for the treatment of infants, children, and adolescents with SED and subsequently patients with ZSD. Later, an institutional review board approved study with an Investigational New Drug for use of cholic acid was obtained. The study design therefore was not structured with the rigor now required in contemporary studies. Given the nature of these diseases and understanding of their natural histories, there was never any consideration for performing a placebo-controlled trial because it was considered unethical. Logistically, patients were recruited through an international screening program established at CCHMC. Because patients were identified at sites distant from Cincinnati, we were dependent on local treating physicians to collect laboratory data and submit urine samples for FAB-MS analysis based on our guidelines and their clinical practice; therefore, the collection of laboratory data and urine FAB-MS was not defined at specific and rigid times during the study period.

The overall safety profile of cholic acid was favorable, with no study drug-related SAEs or deaths reported. During the 18-year study period, 48% of patients experienced TEAEs. AEs were considered treatment related by the investigator in only 2 patients. Disease progression was the most common TEAE, followed by diarrhea, urinary tract infection, and dehydration. Diarrhea, recognized as a potential side effect of cholic acid treatment, was reported in only 6 patients over the extended study period. The deaths of patients with SED are concerning and might be misconstrued as cholic acid being ineffective. The 4 patients with 5β-reductase deficiency who died all had end-stage liver disease with ascites and coagulopathy. It is possible that their disease was so advanced that cholic acid was not effective. Alternatively, some of these patients may have had end-stage liver disease from other causes despite their urinary bile acid profile suggesting 5β-reductase deficiency. Because 5β-reductase is the most sensitive of the bile acid biosynthetic enzymes to be affected by advanced liver disease, the atypical urine metabolites observed in this condition may actually be due to terminal liver disease (23,24) rather than due to a primary 5β-reductase deficiency. When this study was conducted, gene sequencing was not available. Indeed, the genes encoding these enzymes had not been cloned when most of these SED were first discovered. A number of Clinical Laboratory Improvement Act/College of American Pathologists certified laboratories now, however, perform gene sequencing for many of these enzymes, including 5β-reductase (AKR1D1), and can determine whether mutations are present in similarly affected patients, permitting either confirmation or clarification of the urine FAB-MS findings in this select population (25).

AEs and deaths were more often reported among patients with ZSD. This was not unexpected, given the additional significant nonhepatic comorbidities typically present in these patients, which would not be expected to be responsive to cholic acid therapy. In addition, some of these patients who died were treated with cholic acid only after advanced liver disease had developed and serious events had occurred and some had been wait-listed for transplantation.

CONCLUSIONS

Orally administered cholic acid at a dose of 15 $mg \cdot kg^{-1} \cdot day^{-1}$ is a safe, efficacious, and well-tolerated treatment for BASD due to SED and ZSD in patients with liver dysfunction. Cholic acid is effective in improving liver biochemical abnormalities and urine bile acid metabolite excretion in patients with SED and ZSD. In a limited number of patients with SED, it has been shown to stabilize or reduce hepatic inflammation and fibrosis. Future follow-up of treated patients will provide additional comprehensive data demonstrating long-term safety and efficacy.

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