CONJUGATED CHOLIC ACID FOR THE TREATMENT OF INBORN ERRORS IN BILE ACID SYNTHESIS INVOLVING SIDE-CHAIN CONJUGATION

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To: Institutional Review Board on Studies in Human Beings and the Scientific Advisory Committee, GCRC
Cincinnati Children's Hospital Medical Center

From: Kenneth D.R. Setchell, Ph.D., Division of Pediatric Pathology and James E. Heubi, M.D./ Division of Gastroenterology, Hepatology and Nutrition, Co Principal Investigators
Kevin Bove.M.D., Division of Pediatric Pathology, Coinvestigator
Nadine Brown, Ph.D., Division of Pediatric Pathology

Abstract

Inborn errors of bile acid metabolism have been established as a well recognized cause of neonatal cholestasis and fat-soluble vitamin malabsorption. Although there is extensive experience with metabolic defects in the biosynthetic pathway, few patients have identified with defects in conjugation with taurine or glycine that allows bile acids to become effective detergents. This proposal is designed to study the effect of defects of conjugation of bile acids on growth and fat-soluble vitamin malabsorption. Study subjects will have liver function studies performed, serum and urinary bile acid measurements, vitamin levels, growth measurements, bile acid pool size measurements made by stable isotope dilution mass-spectrometry, and measurements of absorption of two fat-soluble vitamins, tocopherol and vitamin D. Subjects will be treated orally with conjugates of cholic acid with follow-up laboratories performed as an outpatient and then subjects will have all of the initial studies repeated during an inpatient stay 3-12 months after starting treatment.

I. Purpose/Specific Aims

The purpose of this proposal is to define the pathophysiology of these newly described genetic defects of bile acid biosynthesis related to impaired conjugation of bile acids, and to examine the effect of oral bile acid therapy with conjugated bile acids on the clinical course of these disorders. Specifically, we propose to address the following interrelated hypotheses:

1. Unique alterations in bile acid metabolism, specifically affecting the conjugation of primary bile acids during early life, lead to reduced intraluminal bile acids concentrations and associated reductions in absorption of fats and fat-soluble vitamins.

2. Treatment of affected infants/children with conjugated bile acids will lead to resolution of liver disease, if present, and improvements in fat and fat-soluble vitamin absorption

In order to address the above interrelated hypotheses we will perform the following:

1. We will determine the extent of alteration in biliary bile acid composition and measure the primary bile acid pool sizes by the administration of stable-isotope labeled primary bile acids, and correlate these with the concentration of unconjugated bile acids and with markers of liver dysfunction.
2. We will administer the conjugate of the primary bile acid, glycocholic acid, to patients with disorders in bile acid conjugation, monitor its metabolic fate and determine the effect of bile acid therapy on biliary bile acid composition and it ability to correct the clinical manifestation of fat and fat-soluble vitamin malabsorption.
3. Where liver dysfunction is present at diagnosis and liver biopsies have been obtained during the evaluation of the patient, a single follow-up liver biopsy will be performed after treatment to assess the effect of therapy on liver histology and ultrastructural pathology.

II. Significance of the study in relation to human health

Liver disease in infancy has many definable causes, including known infections, anatomic defects, well delineated metabolic errors, and inherited or acquired defects in hepatobiliary structure and function. However, "idiopathic" neonatal hepatitis syndromes make up by far the largest proportion (35-40%) of cases of liver disease in neonates and children [1]. The frequent pattern of intrafamilial recurrence for many of these cases suggested to us a genetic basis, and this was subsequently substantiated by our description some years ago of two inborn errors in bile acid synthesis presenting as progressive cholestatic conditions. More recently we have described three additional unique defects in bile acid synthesis.

Because of the essential and multiple roles that bile acids play in gastrointestinal physiology, it was our contention that specific defects in the conversion of cholesterol into the primary bile acids, cholic and chenodeoxycholic acids, contribute to or are causal in the pathogenesis of idiopathic cholestasis. Such defects would lead to an overproduction of potentially hepatotoxic "atypical" bile acids that would be synthesized from intermediates accumulating proximal to the enzyme defect, exacerbated by inadequate production of the primary bile acids that are essential for promoting bile-flow, and consequently would be expected to present as progressive cholestatic conditions.

The two "primary" bile acids synthesized by the human liver, cholic and chenodeoxycholic acids, serve a number of important physiological functions. In addition to facilitating fat absorption from the gastrointestinal tract, primary bile acids provide the major driving force for the promotion and secretion of bile [2] and are therefore essential to the maintenance of a normal enterohepatic circulation. The pathways for bile acid synthesis from cholesterol are complex [3]. At least 15 enzymes, located within various subcellular fractions of the hepatocyte, participate in bile acid synthesis [4, 5]. Prior to initiating this research program, only one primary enzyme defect in bile acid synthesis was known, and this was the rare lipid-storage disease of Cerebrotendinous Xanthomatosis, shown to be due to point mutations in the gene encoding the cholesterol

C-27 hydroxylase enzyme, a condition that until recently was not associated with liver disease. Seven genetic defects involving enzymes in the pathway for bile acid synthesis from cholesterol are now known, six of which were discovered by the Principal Investigator and coworkers [6,7,8 and unpublished data]. These disorders are manifest as progressive cholestatic conditions and indicate that inborn errors in bile acid synthesis should now be classified as a new category of metabolic liver disease. As a consequence, our laboratory has become an important international resource for screening for these defects and referral of affected patients.

These conditions were identified as a result of our application of the technique of liquid secondary ionization mass spectrometry (LSIMS, formerly FAB-MS), which affords definitive identification of derangements in bile acid synthesis, and consequently, identification of genetic defects in bile acid synthesis. By this approach the first two new defects in bile acid synthesis, manifest as neonatal and late-onset chronic cholestasis, were discovered in infants and children and later inborn errors in bile acid synthesis became associated with unexplained fat-soluble vitamin malabsorption syndromes in some patients

(a) 3β-Hydroxy-C_{27}-steroid dehydrogenase/isomerase deficiency [6]

(b) Δ^4-3-Oxosteroid 5β-reductase deficiency [7]

(c) 2-Methylacyl CoA racemase deficiency (8), accounting for chronic liver disease in a 3 week old infant, initially presenting as fat-soluble vitamin malabsorption.

(d) Oxysterol-7α-hydroxylase presenting as severe and fatal liver disease in infancy

(e) Sterol 27-hydroxylase deficiency, accounting for transient cholestatic liver disease in early life and later presenting as the rare lipid-storage disease of cerebrotendinous xanthomatosis

(f) Defects of bile acid conjugation (see Preliminary Results)

(g) Cholesterol 7α-hydroxylase deficiency described by Pugelli et al and presenting in adults as a condition of dyslipidemia that is unresponsive to statin treatment

In addition to the above defects, bile acid synthesis is severely compromised in conditions in which there are abnormalities in peroxisomal assembly, structure, or function, because the peroxisome packages key enzymes responsible for β-oxidation of the side-chain of cholestanoic acid intermediates in the pathway for primary bile acid synthesis. These peroxisomopathies, particularly when severe, as in Zellweger's syndrome, present with progressive cholestatic liver disease. Peroxisomal disorders can be subdivided into several groups [9, 10]. In disorders of peroxisome biogenesis, the organelle is not formed normally and multiple peroxisomal functions are defective. Included in this category are the Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum syndrome, and hyperpipecolic acidemia. A second group is characterized by a single enzyme defect, without disturbance of peroxisomal structure or other peroxisomal functions. In all of the above described conditions, primary bile acid synthesis is markedly reduced or absent. Instead, the liver of these patients synthesizes increased amounts of the intermediates in the pathway proximal to the enzyme defect, and these are subsequently metabolized to bile acids retaining the nuclear structure of the substrates for the missing enzyme. We have speculated that the cholestasis and liver disease result from the lack of primary bile acids that are essential for promoting bile-flow

and/or the accumulation of atypical bile acids that are potentially cholestatic and hepatotoxic.

III. Previous Work done in the Area By our Group

3β-Hydroxy-C_{27}-steroid dehydrogenase/isomerase deficiency

We first described this defect in 1987 [6], but since then it has been recognized in >25 patients. Since 1986, 2 subjects have been studied on the GCRC at Children's Hospital and an additional 6 subjects have been investigated by KDRS/JEH in Paris. The condition is fatal if not diagnosed at an early enough stage to permit therapeutic intervention with primary bile acids. As a result of our efforts at least three children have avoided the need for liver transplantation (all were on transplant waiting lists) and have seen remarkable resolution of liver histology and normalization of serum liver enzymes. This enzyme defect involves the second step in primary bile acid synthesis from cholesterol. Age at onset and preliminary diagnosis have been variable, ranging from 3 mos - 15 yrs, confirming that cases of late-onset chronic cholestasis can be explained by inborn errors in bile acid synthesis [11]. Interestingly, this inborn error is frequently but not exclusively associated with an elevated serum bilirubin and transaminases, or a normal serum gamma-GT concentration, and this biochemical picture is often a useful clinical marker for the defect [12, 13]. Furthermore, serum bile acid concentrations are normal when measure by conventional laboratory methods and seemingly incompatible with the extent of cholestasis. In many of the patients, the early presentation of the disease was unremarkable with normal liver function tests, save degree of fat malabsorption. We recently have published a mutational analysis on a cohort of subjects with this defect (14)

Δ^4-3-Oxosteroid 5 beta-reductase deficiency

Soon after the identification of the 3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase deficiency, we described a second inborn error in bile acid synthesis involving nuclear modification, also manifest as progressive neonatal liver disease [7]. This defect involved a deficiency of the cytosolic Δ^4-3-oxosteroid 5β-reductase enzyme responsible for the catalytic conversion of 7α-hydroxy- and 7α,12α-dihydroxy-4-cholesten-3-one into the corresponding 3-oxo-5β(H) analogues [15]. We first reported the defect in monochorionic twin boys born from our GCRC with a marked and progressive cholestasis [7]. A previous sibling born with neonatal hepatitis had died of liver failure following a similar clinical course and the parents subsequently gave birth to a fourth affected boy and later a healthy girl. Thus far, 20 patients have been identified with this disorder, including four infants in which a prior diagnosis of neonatal hemochromatosis was made [16]. In all of these cases, liver function tests revealed an elevation in serum transaminases, a marked hyperbilirubinemia and coagulopathy. Unlike the 3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase deficiency, this defect is not commonly associated with a normal serum GGT concentration.

Liver biopsies from these patients showed marked lobular disarray as a result of giant-cell and pseudoacinar transformation of hepatocytes, hepatocellular and canalicular bile stasis and extrahepatic medullary hematopoiesis. Electron micrographs showed small, slit-like bile canaliculi, lacking the usual microvilli and containing variable amounts

of electron-dense material [17,18, 19]. Since primary bile acids are involved in the canalicular transport of iron [20,21] it is uncertain whether the iron storage defect is secondary to the bile acid inborn error, or vice-versa. In contrast to the previously described defect, the Δ^4-3-oxosteroid 5β-reductase deficiency presents early in life and in most of the cases identified, the diagnosis has been made too late to permit initiation of bile acid therapy.

2- Methylacyl-CoA Racemase Deficiency

We described a new defect in bile acid synthesis in a 3-week old infant who presented with fat-soluble vitamin deficiency and a mild elevation in serum transaminases. The patient, who appeared normal, was investigated because of the death of a sibling a year earlier from an intracranial hemorrhage due to massive vitamin K deficiency. Analysis of the urine by mass spectrometry revealed a virtual lack of primary bile acids, and in their place the liver was synthesizing large quantities of cholestanoic acids. These unusual C_{27} bile acids are typical of bile acids found in the alligator. While the urine and bile of this patient showed striking similarities in bile acid synthesis to that found in patients with the cerebrohepatorenal syndrome of Zellweger, however, clinically this patient had none of the features or symptoms of peroxisomopathies, and liver biopsy indicated the presence of normal peroxisomes.

Remarkable in this case was the fact that the liver from the earlier deceased sibling, presumed to have the same defect, was transplanted and the recipient experienced problems absorbing cyclosporin; this was corrected with bile acid therapy. Analysis of urine collected from the recipient confirmed the same genetic defect in the transplanted liver and this was consequently a unique example of transplantation of a metabolic defect. In collaboration with investigators at Kennedy Kreiger Institute we characterized this child and his sibling's defect to be a deficiency of 2-methylacyl-CoA racemase. Because of reports of adults with this defect with neurologic disease, we are watching her closely and treating her with cholic acid and a phytate reduced diet (22).

Bile Acid Synthesis in Peroxisomal Disorders

Elevated levels of DHCA, THCA and a C_{29}-dicarboxylic bile acid in biological fluids are a consistent feature of Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum's disease and pseudo-Zellweger syndrome [23-26]. Although bile acid synthetic rates are low in these patients [27,28], normal or increased serum concentrations of primary bile acids are frequently found which are a consequence of impaired hepatic function. Additional metabolism of THCA by microsomal hydroxylation in the side-chain (to produce C-24 hydroxylated varanic acid isomers) and the nucleus (to produce C-1 and C-6 tetrahydroxy-cholestanoic acids) gives rise to many tetrahydroxylated cholestanoic acids that are excreted in urine and these metabolites are of diagnostic value [29-31]. Differential diagnosis of these peroxisomopathies is challenging and requires a battery of tests to examine all steps in the β-oxidation of bile acids and fatty acids, complemented by immunoblotting techniques to identify the enzymes.

Severe liver disease is a common manifestation of patients with peroxisomal dysfunction and is almost universal in the Zellweger patient and mild in neonatal adrenoleukodystrophy and infantile Refsum's disease [9]. Infantile Refsum's disease is

a very mild form of the Zellweger syndrome, and the severity of the symptoms and biochemical abnormalities can be used to differentially diagnose these syndromes. The progressive liver disease that develops in Zellweger syndrome may be a result of the increased production and accumulation of cholestanoic (C_{27}) bile acids combined with reduced concentrations of the primary bile acids, cholic and chenodeoxycholic acids. Publication of the results of the studies in these patients is planned for 2005.

Bile acid Conjugation Defect

Under another protocol approved by the IRB (CHMC #90-10-10) we studied a boy from Saudi Arabia who appears to be only the second child to be identified with a defect of bile acid conjugation. As a neonate, he had cholestasis that led to surgery for presumed biliary atresia and the performance of a portoenterostomy. At age 5, he had mild liver disease with pruritus. In addition, this Saudi boy has a 9 year-old sibling with a history or rickets in infancy. After FAB-MS analysis of the urine and mutation analysis of the genes encoding bile acid conjugation both siblings we found to be have the same defect in bile acid conjugation. A previous child had been identified in collaboration with an investigator at UCSD. Unfortunately, both the Saudi and the UCSD patients have subsequently been lost to follow-up. A publication regarding the mutational analysis and biochemical description of these patients is now in preparation. Three further infants have recently been found with this amidation defect and are candidates for bile acid therapy with conjugated cholic acid.

Treatment of inborn errors in bile acid synthesis

Untreated, the progressive cholestasis characteristic of these genetic defects eventually leads to death due to cirrhosis and liver failure. The choice of therapeutic dose (ranging 50-750 mg/day) has been based upon the estimated normal bile acid pool-size for age and titration against the disappearance of the atypical endogenous bile acids as measured by mass spectrometry. Bile acid therapy has proven effective in suppressing the urinary excretion of the atypical bile acids although our experience thus far suggests that it is not possible to completely shut down endogenous bile acid synthesis, but significant down-regulation occurs rapidly after initiating primary bile acid therapy. Concomitant with this response is a marked clinical improvement with concentrations of serum liver enzymes and bilirubin consistently showing a gradual return to normal values. The clinical and biochemical response to therapy is rapid with early therapeutic intervention.

Liver biopsies performed before and 6 months after oral bile acid therapy in several of the patients have revealed a significant reduction in the extent of inflammation and cholestasis, and EM studies have shown a normalization of bile canalicular structure in several patients. We speculate that oral bile acids may have led to maturation in canalicular structure and secretory function. The value of oral bile acid therapy for patients with these inborn errors in bile acid synthesis is apparent from the fact that only those patients who have been treated have survived and have gone on to lead essentially normal lives, while three patients have avoided unnecessary liver transplantation.

Although the cerebrohepatorenal syndrome (Zellweger) and neonatal adrenoleukodystrophy are strictly defects in peroxisome dysfunction, bile acid synthesis

is significantly affected. The biochemical defect involves failure to adequately synthesize the primary bile acids, cholic and chenodeoxycholic acid, although synthesis is usually not completely impaired. Because of enzyme blocks related to peroxisomal dysfunction, levels of dihydroxycholestanoic and trihyroxycholestanoic acids and a C_{29}- dicarboxylic bile acid are increased in biologic fluids including urine, bile and serum. Based upon our hypothesis that atypical, hydrophobic bile acids may be hepatotoxic particularly when present in contracted primary bile acid pools, we developed an orphan drug grant application (FD-R-000995, Treatment of Peroxisomal Disorders with Bile Acids) which was funded beginning 9/1/94. To date, 18 subjects have been evaluated with a protocol which includes, identification and enrollment of subjects, collection of bile to assess pool-sizes of the primary bile acids utilizing stable isotope dilution techniques, percutaneous liver biopsy, collection of urine and serum for assessment bile acid profiles and treatment with 15 mg/kg/day of cholic acid. Enrollment is proceeding with variable results to date. At least in 10 subjects, bile acid therapy appears to have stabilized the liver disease both by histologic and biochemical measures. In the remaining 10 subjects, liver and neurologic disease has progressed despite therapy with 9-10 dying during the course of these studies and 2 lost to follow-up.

IV. Experimental Design and Methods

Study Subjects:

These will consist of infants, children and adolescents of any race or gender who have had samples sent to the Mass Spectrometry Laboratory for assessment for defects in bile acid metabolism because of the presence of cholestasis, or unexplained fat and fat-soluble vitamin malabsorption. They will have been identified by urine bile acid analysis by fast atom bombardment ionization mass spectrometry as having defects in bile acid conjugation based upon characteristic mass spectra. Prior to our planned studies, patients will have undergone a thorough evaluation to define the etiology of their cholestasis or fat-soluble vitamin deficiency. Conventional screening studies (urine culture and clinitest, α1-antitrypsin phenotype, STORCH titers, thyroid function tests, liver ultrasound, liver "function" tests and percutaneous liver biopsy) will have been performed. Patients with other organ dysfunction will not be excluded. Only patients identified with inborn errors of bile acid synthesis caused by defective bile acid conjugation will be candidates for entry to this study. *The possible effect of radiation exposure during this study on an unborn baby is unknown. Because of this possibility, all females that may be able to become pregnant will have a urine pregnancy test done before starting the study, and before any x-ray exposure.* It is anticipated that such defects are rare; however, there are 3 candidates for study at this time and we anticipate identification of one or more new patients each year that would be candidates for study on this protocol.

Study Protocol:

Once a patient is identified by KDRS as having a potential defect in bile acid conjugation, the attending physician will be contacted by JEH to discuss enrollment of the patient in the study. He will explain the study to the parents/patients, and if they are willing, the subject will be brought to the Children's Hospital Clinical Research Center.

On admission, the patient will have studies to assess liver function (bilirubin, ALT, AST, GGT, alkaline phosphatase, cholesterol, albumin, prothrombin time), partial thromboplastin time, serum tocopherol, serum retinol, serum 25-OH vitamin D and PIVKA-II. A full nutritional evaluation will be performed including anthropometrics (height, weight. weight/height and OFC). Serum will be obtained to perform a complete bile acid analysis by gas-liquid chromatography and urine will be obtained for LSIMS and GC-MS analysis (total blood for study purposes <10 cc).

Primary bile acid pool-sizes will be measured to determine whether the hypothesized contraction of pools might play a role in the pathogenesis of cholestasis in these subjects. [2,2,4,4-$^{2}H_4$]Cholic acid (15 mg) and [11,12-$^{2}H_2$]chenodeoxycholic acid (15 mg) will be given by mouth at or soon after 2100 h on the evening of Day 1 on the GCRC. Nothing will be taken by mouth after midnight but a glass of water may be taken up to 6 hours before the procedure to collect bile on day 2. After an overnight fast, a nasoduodenal tube will be passed with the aid of fluoroscopy. Bile will be collected after gallbladder contraction with the octapeptide CCK. After bile collection, the tube will be removed. A blood sample will be taken for DNA sequencing and detection of mutations in the two genes encoding the bile acid conjugation enzymes. On Day 3, after four hour fast 100 IU/kg liquid emulsified dl-alpha-tocopherol will be given orally with collection of blood (1.0 cc/sample) at 0, +4, +8, +12, +18, +24, +48 hours after ingestion (32,33). On Day 4, after a 4 hour fast 1,000 IU/kg Vitamin D_3 will be ingested in 3-5 oz of formula and blood (1.0 cc/sample) collected at 0, 6, 12, and 24 hours as previously described (34,35). Formula may be resumed 1 hour after the test dose of vitamin D. After completion of the last serum sample, the patient will begin therapy with 15 mg/kg glycocholic acid (IND #50,046) by mouth and discharged from the GCRC the morning of Day 5 after receiving at least 2 doses of the bile acid treatment. Liver function tests (as described above), and serum and urine for bile acid analysis by GC-MS and LSIMS. Three to twelve months after initiation of therapy, the patient will return to the GCRC for evaluation and measurement of liver function tests, nutritional assessment, measurement of the fasting primary bile acid pool-sizes, measurement of vitamin E and 25-OH vitamin D absorption. If bile acid therapy is considered to be successful based upon clinical findings, bile acids will be continued indefinitely with periodic follow-up by our group, or by the referring physician. Samples of urine will be collected every 3-12 months to assess compliance and response to therapy. Liver function tests will be performed at the discretion of the attending physician as the standard of care. It is anticipated that they will be collected at least once/year. This follow-up would be considered standard care for patients affected with cholestatic disorders of this nature.

Study Drug:

The study drug, glycocholic acid is administered under an IND approved by the FDA, and is not considered a drug used as standard care for this condition. Glycocholic acid powder is stored in the CCHMC Investigational Pharmacy Department and will be formulated and dispensed by the Investigational Pharmacy personnel and then shipped by overnight courier directly to the patient. We will dispense 3 months supply of the drug at a time, for as long as the drug is available.

Analytical Methods:

Preliminary analysis of urine obtained prior to enrollment and follow up samples will be performed using LSIMS. Samples identified with abnormal patterns of bile acid excretion will be subjected to GC-MS analysis to afford characterization of a specific metabolic defect. Serum bile acids will be measured by GC-MS. All methods are well validated and currently being performed in the Mass Spectrometry Center of the Children's Hospital [36, 37]. Bile acid pools will be measured by the technique of Duane et al. [38]. Serum 25-OH vitamin D and tocopherol levels will be performed using established methods in the GCRC Core Laboratory. Both tests are performed for research as well as clinical testing and are CLIA certified tests. Serum vitamin D levels will be measured by HPLC in the laboratories of Bruce Hollis at the Medical University of South Carolina (39). DNA sequencing will be performed by routine techniques in the Laboratory of Dr. David Russell, Dallas, TX. This sequence analysis is for research purposes only, and will not be used in any clinical decision making.

Data Analysis:

There is no sample-size for this study as the sample-size is based upon the number of subjects identified with this rare metabolic defect. Pool sizes for the conjugates of the primary bile acids (chenodeoxycholic and cholic acids) will be compared before and after treatment using the paired Student's T-test. Assessment of the relative contribution of unconjugated bile acids to the total pool size will be made before and after therapy by the paired Student's T-test. Serial changes in liver function tests (total and direct bilirubin, AST, ALT, GGT, alkaline phosphatase, cholesterol and serum bile acids) will be evaluated by ANOVA using methods for repeated measures. Comparisons of the serum vitamin D and tocopherol absorption tests will be compared by paired T-tests with comparisons of the maximal rise of 25-OHD before and after therapy and the area under the curve (ng/ml*hr) for vitamin D or area under the curve (mg/ml*hr) for tocopherol. Comparisons will be made to previously published results from our laboratories (32,34,35). It is not anticipated that the results of the present study will provide enough information to prove our hypothesis; however, the results will provide a good working model to test in a rodent model of this disorder which is being developed currently.

Special Considerations

1. Radiation Exposure: Fluoroscopy will be performed on 2 occasions. Fluoroscopy time for will be kept to the least possible to complete the intubations. It is anticipated that about 90 seconds of fluoroscopy time will be required for each intubation that will lead to an average exposure of 225 mrems with a total exposure of 450 mrem/year for the entire study. Each exposure is approximately equivalent to that received from 2 abdominal x-rays and is approximately the same as one year's natural radiation exposure in the Cincinnati, Ohio region, estimated at 320 mrem/year. Stable isotopes produce no radiation exposure and are considered safe.

2. Participation in Other Approved Protocols
   None

3. Investigational Drugs.
   Glycocholic acid (IND 50,046)

4. CCHMC facilities to be utilized

All studies will be performed on the Clinical Research Center and in outpatient laboratories of choice of the study subjects for follow-up samples.

V/VI. Potential Risks and Discomforts

Venipuncture may be accompanied by pain and bruising at the site. Cumulative blood losses at each of the two hospitalizations on the GCRC will be 21 ccs which is approximately 5% of the blood volume of even the smallest enrolled subject (6 kg x 70 x 0.05 = 21 cc). The passage of a nasoduodenal tube may result in mild-to-moderate discomfort. The nasopharynx will be anesthetized with a topical anesthetic to minimize discomfort. Fluoroscopy required for passage of the tube results in modest radiation exposure. Fluoroscopy will be performed on 2 occasions. This will produce an average exposure of 225 mrem for each intubation and 450 mrem for the two exposures. The scientific value of the information obtained from this study as it relates to our basic understanding of cholestasis and specifically as it related to the pathogenesis of disease in the study subjects makes this radiation exposure warranted.

There are no recognized hazards associated with the use of small quantities of stable isotopes as proposed in the current protocol. There may be some nausea, abdominal cramping with the use of CCK-octapeptide for collection of bile. Although diarrhea might be a potential complication of bile acid therapy, no significant problem to date has been found in the infants and children treated with UDCA or cholic acid. Treatment of more than 100 cholestatic children with ursodeoxycholic acid has not led to any measurable deterioration in hepatic function. In fact, administration of ursodeoxycholic acid uniformly leads to reductions in serum concentrations of bilirubin, transaminases, and cholesterol in most cholestatic conditions, while primary bile acid therapy has led to normalization of liver function in all patients studied with inborn errors in bile acid synthesis. Similarly, although diarrhea might be a potential complication of bile acid therapy, no significant problem has been found in the infants and children treated with cholic or ursodeoxycholic acids. It is not anticipated that there will be a different safety profile when conjugates of cholic acid are used for treatment.

Risk/Benefit Assessment. The above risks are considered to be more than minimal to these patients; however, the subjects may directly benefit from the proposed therapy with conjugated bile acids. In addition, it is possible that patients with these conditions as a class may benefit by our improved understanding of the pathogenesis of this disease process and the optimization of the therapeutic approach.

Data and Safety Monitoring Plan

This study represents more than minimal risk to subjects with potential benefit. This is a treatment intervention similar to one that has not been associated with any significant adverse events associated with the administration of the drug since the initiation of treatment studies in 1994. Currently, we review any adverse events for causality and they are kept in tabular form in the investigator's password protected database. For any serious adverse events or unanticipated adverse events, the investigator would report

deaths to the IRB, GCRC and FDA within 2 days and other SAEs and unanticipated AEs would be reported to the IRB, GCRC and FDA within 10 working days of the investigator's knowledge of their occurrence. Any SAEs that are considered related or probably related to the use of glycocholic acid will result in halting of enrollment of subjects and review by an Independent Medical Monitor. John Bucuvalas, M.D. will serve as Lead Independent Medical Monitor. He will be responsible for the timely evaluation of any AE's, SAE's and unanticipated AE's for causality and relationship to the study drug at least every six months.

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VII. Estimated Time to Complete Study.

More than 5-10 years.

VIII. Funding

Funding is provided from the NIH supported Cholestatic Liver Consortium (CLiC) Grant # 5U54DK078377-04

IX. Payment for Studies

All costs related to visit 2 will be assigned to the grant from CLiC. There will be no incentives provided for participation in this study. There are no funds available to cover transportation costs to the study site for study related visits.

X. Methods to be used in Procuring Informed Consent

A verbal explanation will be given to the parent/guardian by phone prior to coming to CCHMC for Visit 1. Written consent will be obtained at screening before enrollment in the study at CCHMC. The informed consent form is attached.

XI. Attending Physician Approval

Not applicable

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