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Liquid chromatography–mass spectroscopy in the diagnosis of biliary atresia in children with hyperbilirubinemia

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ARTICLE INFO

Article history:
Received 27 September 2017
Received in revised form
3 January 2018
Accepted 14 March 2018
Available online xxx

Keywords:
Biliary atresia
Cholestasis
Liquid chromatography–mass spectroscopy
Bile acids

ABSTRACT

Background: Biliary atresia (BA) is difficult to distinguish from other causes of cholestasis. We evaluated the use of liquid chromatography–mass spectroscopy (LC-MS) and bile acid profiles in the rapid, noninvasive diagnosis of BA.

Materials and methods: Following Institutional Animal Care and Use Committee and Institutional Review Board approval, we used LC-MS to measure 26 bile acids in serum and stool samples from experimental models of BA and in urine, stool, and serum samples from non-cholestatic and cholestatic human infants.

Results: We first evaluated the utility of LC-MS to distinguish bile acid profiles between sham, bile duct ligation, and 3,5-diethoxycarbonyl-1,4-dihydrocollidine mouse models of BA. Serum bile acids were significantly higher and stool bile acids were significantly lower in experimental BA. Next, we evaluated samples from non-cholestatic, cholestatic non-BA, and BA infants. There was no significant difference between cholestatic non-BA and BA stool and urine samples. However, primary bile acids were significantly higher in BA *versus* cholestatic non-BA samples (128.1 ± 14.2 *versus* 61.2 ± 20.5 μM). In addition, the primary, conjugated bile acids glycochenodeoxycholic acid and taurochenodeoxycholic acid were significantly elevated in BA compared with cholestatic non-BA serum samples. Using a receiver operating characteristic curve, we found that a serum glycochenodeoxycholic acid concentration of 30 μM had a sensitivity of 100%, specificity of 83.3%, positive predictive value of 88.9%, and negative predictive value of 100% in the diagnosis of BA.

Conclusions: Our data indicate that bile acid patterns can be used to distinguish experimental and human BA from non-cholestatic and, more importantly, cholestatic disease. This suggests that LC-MS may be useful in the accurate, rapid, and non-invasive diagnosis of BA.

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0022-4804/$ – see front matter
https://doi.org/10.1016/j.jss.2018.03.021

Introduction

Biliary atresia (BA), a rare cause of neonatal jaundice, can be difficult to differentiate from other causes of obstructive cholangiopathy. BA is characterized by a rapidly progressive fibroobliteration of the bile ducts. Although it only affects approximately 1/15,000 infants in the United States, BA is the most common cause of end-stage liver disease and the leading indication for pediatric liver transplantation.[1,2] Current management includes Kasai portoenterostomy to achieve biliary drainage. Without surgical intervention, the disease is uniformly lethal by 2 y. Early diagnosis and management has been shown to improve transplant-free survival.[3] Literature suggests that the diagnosis of BA remains a challenge.[4-8] Current diagnosis requires laboratory evaluation, imaging, and liver biopsy.[1,9,10] While pathologic evidence of periportal fibrosis, ductular reactions, and bile plugging is consistent with BA, it is a nonspecific constellation of findings. Thus, operative evaluation of the extrahepatic biliary tree with a cholangiogram under general anesthesia remains the gold standard for diagnosing BA with a normal cholangiogram disproving BA. We sought to identify a nonoperative technique to rule out BA, avoiding an otherwise unnecessary operation. Currently, no rapid, noninvasive test exists for BA to avoid this circumstance.

Because the excretion of bile acids is disrupted in BA, alterations in the enterohepatic circulation would be expected. Normally, conjugated, primary bile acids are synthesized and secreted into the bile where they can be deconjugated and converted into secondary bile acids by intestinal bacteria. Although most bile acids are reabsorbed, they are also excreted in feces or spilled into the systemic circulation and urine.[11] Obstructive cholangiopathy should prevent the excretion of bile acids into the intestine leading to decreased luminal bile acid concentration and less generation of deconjugated and secondary bile acids. This would significantly disrupt the bile acid pool. Data from our laboratory,[12] as well as others,[13-15] suggest that liquid chromatography–mass spectroscopy (LC-MS) may be able to detect these changes, which may have potential utility as a diagnostic modality.

In this study, we sought to build on previously published data[12] by collecting a new cohort of patients to evaluate LC-MS in the diagnosis of BA. We first confirm the utility of bile acid profiles in animal models of biliary injury and then use human serum, urine, and stool samples to test our hypothesis. We hypothesized that the aberrant biliary drainage seen with BA would lead to higher bile acid levels in blood and urine and lower bile acid levels in stool when compared to cholestatic non-BA patients, allowing bile acid profiles to be used as a noninvasive diagnostic tool for BA.

Materials and methods

Chemicals

To create standard curves, bile acids were purchased from Sigma (St. Louis, MO) except for the following: glycolithocholic acid was purchased from Fisher Scientific (Waltham, MA); glycohyocholic acid, taurohyocholic acid, murocholic acid, alpha-murocholic acid, and omega-murocholic acid were purchased from Steraloids, Inc. (Newport, RI). Deoxymurocholic acid was a generous gift from Dr Alan Hofmann (University of California, San Diego). Deuterated-glycocholic acid and deuterated-glycodeoxycholic acid internal standards were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). Bilirubin assay was purchased from Fisher Scientific.

Liquid chromatography–mass spectroscopy

Bile acid concentrations were measured using high-performance LC-MS (4000 Q Trap System; Applied Biosystems, Waltham, MA). Bile acids were extracted as previously described.[12,16] Briefly, samples were collected and stored at −80°C before processing. After stool samples were lyophilized, 10 mg of dried sample was extracted with NaOH (0.1 mol/L), incubated at 1 h at 60°C, diluted with water, and homogenized by sonication. Resultant samples were centrifuged at 25,000 × *g* for 20 min. The supernatant was extracted using solid-phase extraction cartridges (Waters Corporation, Milford, MA). Urine samples were diluted in 1 mL NaOH (0.1 mol/L) and 2 mL water then extracted using solid-phase extraction cartridges. After extraction, urine and stool samples were dried under a nitrogen stream at 50°C, redissolved in 100% methanol, and centrifuged at 13,500 rpm for 2 min. The resultant supernatant was used for LC-MS injection. Serum samples (50μL) were extracted with 100% methanol, incubated at room temperature for 20 min, centrifuged at 13,000 × g for 12 min, and the supernatant was dried under a nitrogen stream at 50°C. Resultant samples were dissolved in 60% methanol, centrifuged at 13,500 rpm for 2 min, and the supernatant was used for LC-MS injection. Processed samples were separated using LC-MS with a Kinetex C18 column (Phenomenex Inc, Torrance, CA). Quantification was done using bile acid standards and deuterated internal standards to form a standard curve, and data were processed with Analyst Software 1.6.2 (SCIEX, Framingham, MA). Individual bile acids were measured using Analyst and then total, conjugated, unconjugated, primary, and secondary calculations were performed by summation of appropriate corresponding bile acid concentrations.

Animal experiments

Six-week-old male wild-type C57 BL/6 mice (Jackson Laboratories, Bar Harbor, ME) were fed 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) ad libitum for 2 wk to induce cholangiopathy or standard chow.[17] Bile duct ligation (BDL) was performed in 6-wk-old male wild-type C57 BL/6 mice as previously described.[18,19] With either model, mice were euthanized up to 3 wk postinduction for collection of serum and stool samples. All animal procedures were approved by the Children's Hospital Los Angeles/University of Southern California's Institutional Animal Care and Use Committee (IACUC #164-16).