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Bile Acid Metabolism in Extrahepatic Biliary Atresia: Lithocholic Acid in Stored Dried Blood Collected at Neonatal Screening

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ABSTRACT

Lithocholic acid (LCA) is a potent hepatotoxic compound. Fetal LCA may have a role in the pathogenesis of neonatal cholestasis/extrahepatic biliary atresia (EHBA). Fetal liver efficiently hydroxylates LCA in several positions. This may represent a detox-ification mechanism. In the present study LCA, cholic acid (CA) and chen-odeoxycholic acid (CDCA) were quantitated by gas chromatography-mass spectro-metry using selected ion monitoring in small amounts of stored dried blood from six newborn infants with EHBA and fourteen con-trols. The blood was collected at neonatal metabolic screening. Mean blood levels (±S.E.M.) of LCA were 0.11±0.04 µM in the in-fants with EHBA and 0.08±0.02 µM in the control infants. The corre-spon-ding levels for CA and CDCA were 15.6±3.6 µM and 7.4±2.5 µM in the infants with EHBA and 1.7±0.3 µM and 1.8±0.4 µM in the controls. The increased levels of CA and CDCA in the infants with liver disease can be explained by cholestasis. The low blood levels of LCA indicate a normal fetal metabolism of this bile acid in EHBA.

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INTRODUCTION

Lithocholic acid (LCA) is a potent hepatotoxic compound causing cholestasis in several species (1). It has been suggested that fetal LCA may play a role in the pathogenesis of neonatal choles-tasis/extrahepatic biliary atresia (EHBA) (2). Fetal LCA may have its origin either in primary synthesis in the fetus (3) or it may be a secondary bile acid derived from the mother (4). In the adult liver LCA can be metabolized by sulfation, glucuronidation and hydroxylation (3, 5-8). In addition, the metabolism of lithocholic acid into 3-dehydrolithocholic acid has been shown in human liver microsomes (9). The capacity for sulfation and glucuro-nidation of LCA appears to be low in fetal liver (3, 10), but it has been shown that fetal liver efficiently hydroxylates LCA in several positions (11, 12). Such metabolism may represent a detoxi-fication mechanism. If the pathogenesis of extrahepatic biliary atresia involves increased levels of LCA during fetal life (2, 13), this condition could be associated with impairment in fetal liver hydroxylations of LCA which should lead to increased levels of this particular bile acid at birth. The present report describes analysis of levels of LCA in small amounts of stored dried blood from newborn infants with extrahepa-tic biliary atresia. The blood was collected at the time of routi-ne neonatal metabolic screening during the first days of life.

SUBJECTS

Stored dried blood, obtained at neonatal metabolic screening from six newborn infants, who were later shown to suffer from extrahe-pa-tic biliary atresia, was analyzed as described in Methods. None of the patients was shown to have an underlying cause for their liver disease such as viral infection or inborn error of metabo-lism. As controls, 14 newborn infants, born at the same hospitals as the patients, were used. The control infants had been born immediately before or after the diseased infants. For two of the patients four controls were used, whereas for three of the pati-ents material from two controls, respectively, was av-ailable. For one patient there was no material available from corresponding control infants.

Patient 1 A girl, born after 40 weeks of normal pregnancy. Her birthweight was 3790 g. Laparotomy was performed due to persis-tent hyperbilirubinemia and extrahepatic biliary atresia was diagnosed. At two months of age the patient was operated with a portoenterostomy (Kasai operation) and an external jejunostomy. Microscopical examination of liver tissue supported the diagnosis biliary atresia. The condition of the patient deteriorated gradu-ally and she died at the age of 14 months due to liver failure.

ABBREVIATIONS

LCA, Lithocholic acid (3_-hydroxy-5_-cholanoic acid)
EHBA, Extrahepatic biliary atresia
CA, Cholic acid (3_,7_,12_-trihydroxy-5_-cholanoic acid)
CDCA, Chenodeoxycholic acid (3_,7_-dihydroxy-5_-cholanoic acid)

Patient 2 A boy, born after 36 weeks of normal pregnancy. His birthweight was 2760 g. Extrahepatic biliary atresia was diagnosed when laparotomy was per-formed at two months of age due to persis-tent hyperbiliru-binemia. At 12 months of age the patient was operated with a portoenteros-tomy. Microsco-pical examination of the liver tissue was in agreement with bilia-ry atresia. The liver disease of the patient had a relatively slow progress and at 23 months of age the patient was subjected to orthoptic liver trans-plant.

Patient 3 A boy, born after normal pregnancy with birth weight 3250 g. He developed jaundice from day 5 and later acholic faeces. At 9 weeks, a clinical investigation indicated biliary atresia. Portoenterostomy was performed at the age of 10 weeks. Pre- and intraoperative biopsies showed a liver histology typical for extrahepatic cholestasis. After several episodes of cholangitis and one period of rickets the general condition of the patient improved gradually. At 2 years of age the boy had a mild cirrhosis but an almost normal general condition.

Patient 4 A girl, born small for gestational age after 42 weeks, with a birth weight of 2820 g. Immediately after birth she develo-ped cholestatic jaundice. At 5 weeks extrahepatic biliary atresia was diagnosed at laparotomy and a liver biopsy showed advanced cirrhosis. A Kasai operation, performed at this age, did not influence the course of the disease. The girl died at 10 months of age, while waiting for orthoptic liver transplant.

Patient 5 A boy, born after normal pregnancy, with appropriate birth weight, 3435 g. He developed a major gastrointestinal blee-ding at the age of 1 month due to hypoprothrombinemia caused by anicteric cholestasis. Extrahepatic obstruction was diagnosed. Laparotomy and a Kasai operation were performed at 9 weeks of age. Liver biopsy showed advanced cirrhosis. The patient failed to thrive in spite of vigorous nutritional treatment. He died due to bleeding oesophageal varices at the age of 11 months, while wai-ting for liver transplantation.

Patient 6 A boy, born after normal pregnancy, with birth weight 3820 g. Immediately after birth he developed cholestatic jaundice. An examination indicated extrahepatic biliary obstruction. Laparotomy and a modified Kasai operation with biliary fistula were performed at the age of 1 month. During his first year he had frequent attacks of cholangitis. At one year of age the biliary fistula was removed, whereafter the attacks of cholangitis were less frequent. Although the boy never became anicteric and the initial postoperative development was unfavou-rable, his hepato-cellular func-tions were satisfactory seven years after the opera-tion.

METHODS

[2,2,3,4,4]-^{2}H5 -Cholic acid and [2,2,3,4,4]-^{2}H5 -chenodeoxycholic acid were prepared as described previously (14). [24]-^{14}C-Lithocho-lic acid was obtained from Amersham, Buckinghamshire, England. Filter paper blood samples, collected

according to the Swedish routine neonatal screening program from newborn infants, who were later shown to suffer from biliary atresia, and control infants were used for the analyses (c.f. Subjects). Before analysis, the material was stored for 1-6 years at +4°C (c.f.15). For the analyses, filter paper material corresponding to 25-50 µl of blood were extracted with absolute ethanol-potassium phosphate buffer, 0.1M, pH 7.4 (1:1, vol/vol) (15). Before the extraction deuteriumlabeled CA, CDCA and ^{14}C-labeled LCA were added to the solution. During the work up pro-cedure the material was sonicated for 2 minutes and then heated at 70° C for 15 minutes. After centrifugation, the supernatant was hydrolyzed at 110° C for 10 hours with 2M potassium hydroxide solution. After an initial ether extraction the solution was acidified with hydrochloric acid and the bile acids were extracted twice with ether. Following methyla-tion and trimethylsilylation the bile acids were quantitated by gas chromatography-mass spectrometry. The concentrations of CA and CDCA were obtained from isotope dilution-mass spectrometry as described earlier (14). The concentration of LCA was determined from isotope dilution-mass spectrometry using [24]-^{14}C -lithocholic acid as internal standard by recording the ions at m/z 372 and 374 respectively. The above procedure quantitates conjugated as well as glucuronida-ted LCA and should also include the major part of sulfated LCA present in blood (cf. 16). The very small amounts of material available prevented a detailed analysis of the degree of conjuga-tion and esterification of LCA.

RESULTS

Table 1 shows results of determinations of LCA, CA and CDCA in stored dried blood from newborn infants with extrahepatic biliary atresia and their corresponding controls. CA dominated in blood of the patients. The total concentration of bile acids was higher in the blood of the patients as compared to the controls. Both groups of children had low levels of LCA. There was no evidence for presence of C27-bile acids, neither in the samples of the patients nor in those of the controls.

Table 1. Concentrations of bile acids (µmol/l, mean±S.E.M.) in stored dried blood collected at neonatal metabolic screening from six patients with EHBA and 14 controls

	Lithocholic acid	Cholic acid	Chenodeoxycholic acid
Patients	0.11±0.04	15.6±3.6	7.4±2.5
Controls	0.08±0.02	1.7±0.3	1.8±0.4

DISCUSSION

Dried blood, obtained at neonatal screening, is used to detect several metabolic and endocrine diseases. In addition, saved material can be used to diagnose metabolic abnormalities also when the patient is no longer alive (15). We have earlier used such stored material, collected at neonatal screening, to diagnose Zellweger syndrome, a peroxisomal disease with defects in bile acid synthesis (15). In that study, C_{27}- and C_{29}-bile acids were shown to be present in stored dried blood from newborn infants with Zellweger syndrome (15). In the present study we describe a method for quantitation of LCA, based on isotope dilution mass spectrometry, in small amounts of stored dried blood obtained from newborn infants with EHBA. LCA is a hepatotoxic bile acid, which could have a pathogenetic role in neonatal cholestasis (2, 13). The occurrence of LCA in the newborn may be due placental transfer from the mother (4) or possibly to synthesis in the fetal liver (3). Normally, fetal liver efficiently hydroxylates LCA (11, 12). This may represent a detoxification mechanism (11). If LCA is a pathogenetic factor in EHBA, increased levels of this bile acid should be expected to prevail in newborn infants with this disease. The relative amounts of the different bile acids in the control infants are in accordance with studies performed with radio-immuno assay in serum of a larger number of newborns (17). The results show the presence of low levels of LCA both in newborn infants with EHBA and in corresponding controls. The low levels of LCA found indicate a normal metabolism of this bile acid in fetuses with EHBA, making it unlikely that LCA plays a role in the pathogenesis of this condition. The increased levels of CA and CDCA found in the blood of the patients is probably a result of cholestasis. The predominance of CA in the blood of the patients may be due to the fact that the samples are taken at an relatively early stage in the course of the disease, since this result is in agree-ment with those reported in cases of intrahepatic and extrahepatic cholestasis with normal hepatic parenchyma (2). The finding is in contrast to what has been reported for older infants with EHBA, in which a bile acid pattern with high concentrations of CDCA, possibly reflecting an underlying hepatocellular damage, has been described (18).

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