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Histamine is correlated with liver fibrosis in biliary atresia

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ABSTRACT

Background and aims: Biliary atresia (BA) is a severe neonatal cholestasis disease that is caused by obstruction of extra bile ducts. Liver fibrosis progresses dramatically in BA, and the underlying molecular mechanism is largely unknown.
Methods: Amino acids and biogenic amines were quantified by targeted metabolomic methods in livers of 52 infants with BA and 16 infants with neonatal hepatitis syndrome (NHS). Normal adjacent nontumor liver tissues from 5 hepatoblastoma infants were used as controls. Orthogonal partial least-squares discriminant analysis was used to identify the differences between BA, NHS, and control tissues. Histamine metabolism enzymes and receptors were analyzed by immunohistochemistry and Western blot.
Results: The orthogonal partial least-squares discriminant analysis clearly separated BA from NHS and the controls using amino acid and biogenic amine profiles. Histamine was significantly increased in the livers of BA infants and was positively correlated with the severity of fibrosis. This finding was supported by the elevated L-histidine decarboxylase and reduced monoamine oxidase type B expressions in the BA infants with severe fibrosis. Furthermore, histamine receptor H1 was observed in the cholangiocytes of BA livers.
Conclusions: Histamine was positively correlated with fibrosis and may be a potential target to prevent liver fibrosis in BA.
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1. Introduction

Biliary atresia (BA) is the most life-threatening cholestasis disorder in children that results from a destructive inflammatory process of the extrahepatic bile ducts [1,2]. It is the leading cause of liver transplantations in children [3]. Liver fibrosis progresses dramatically in BA. If untreated, BA will progressively advance to liver cirrhosis, leading to death within approximately 2 years [4].

As a prominent feature of BA, liver fibrosis is an important predictor of the outcome following a Kasai operation [5,6]. However, underlying mechanisms for the rapid progression of liver fibrosis have not been explained. A simultaneous activation of multiple fibrogenic pathways in the background of ongoing cholestasis may result in the pathological consequence of BA. Oxidative stress, epithelial–mesenchymal transformation, and perpetual activation of the tissue repair pathways are believed to play important roles in the liver fibrosis of BA.

The link between amino acids and liver fibrosis and cirrhosis has long been described. In carbon-tetrachloride-induced liver cirrhosis, increased levels of aromatic amino acids (tyrosine and phenylalanine), aspartate, asparagine, methionine, ornithine, and histidine and decreased levels of branched-chain amino acids (BCAA) (such as valine, leucine, and isoleucine) were observed [7]. Serum BCAA are also markedly reduced in patients with stage C alcoholic liver cirrhosis [8]. In addition, L-glutamic acid was reported as a protective regulator for deoxynivalenol-induced damage manifested as oxidative stress and signalling inhibition [9]. Oxidative stress was also found to be significantly increased in serotonin-deficient mice [10]. Some other amino acids or biogenic amine, such as histidine [11] and taurine [12], also show antioxidant potential.

Our former research has identified significant differences of amino acid metabolism between BA and neonatal hepatitis

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syndrome (NHS), both present in severe cholestasis [13]. In this study, we quantitatively evaluated 40 amino acids and biogenic amines using targeted metabolomics in the livers of infants with BA and NHS and infants used as controls. We found that histamine was significantly elevated in the BA infants compared to the NHS infants, and it was positively correlated with liver fibrosis in the BA infants.

2. Materials and methods

2.1. Liver samples

Liver samples were taken by wedge biopsy from 52 BA and 16 NHS infants undergoing operative cholangiography at Xin Hua Hospital, Shanghai, China, from July 2012 to January 2014. Five normal adjacent nontumor liver tissues, taken from hepatoblastoma infants, were used as normal controls. All infants with BA were correctly identified based on operative cholangiography and liver pathology. Cholestasis induced by citrin deficiency, α1-antitrypsin deficiency, Alagille syndrome, progressive familial intrahepatic cholestasis, parenteral nutrition, and choledochal cyst were excluded in this study. All of the parents/guardians of the infants provided written informed consent prior to the start of the study. The Faculty of Medicine Ethics Committee of Xin Hua Hospital approved all aspects of this study.

2.2. Histopathological evaluation

Small pieces of liver tissue were collected and fixed in 10% buffered formalin. The samples were embedded in paraffin and sliced into 5-μm thick sections. Masson's trichrome staining was then applied according to the manufacturer's protocol (GeneMed Scientists, Inc.). Liver fibrosis was evaluated by experienced pathologists, according to the following criteria by Tomita et al. [6]: no portal fibrosis (F0), portal fibrosis without septa (F1), portal fibrosis with rare septa (F2), numerous septa or lobular distortion without cirrhosis (F3), and cirrhosis (F4). Mast cells were stained histochemically using toluidine blue according to the protocols described by Kennedy et al. [14].

2.3. Sample preparation

Approximately 10 mg of liver tissue was weighed, homogenized in 50 μL of ice-cold 40% methanol, and then centrifuged at 20,000 × *g* for 10 min. The supernatant was transferred to a clean tube. Then, 80 μL of ice-cold methanol:chloroform (3:1, v/v) was added to the remaining pellet and was rehomogenized. After centrifugation, the two supernatants were combined, and 10 μL supernatants were used for amino acids and biogenic acids measurement with a P180 absolute IDQ kit.

2.4. Amino acids and biogenic acid quantification

Forty amino acids and biogenic amines were quantitatively measured according to the manufacturer's instructions with minor modifications using an ACQUITY ultra-performance liquid chromatography (BEH C18 1.7 μm 2.1 mm × 100 mm column) coupled with Waters Xevo TQ-S triple quadrupole mass spectrometry. In brief, 10 μL of the internal standard solutions was added to all of the plate wells. Then, 10 μL of liver extractives or calibration standards was pipetted onto the centre of each spot, and samples were dried at room temperature under a stream of nitrogen. Next, 50 μL of freshly prepared 5% v/v phenylisothiocyanate solution was added to each dried well. After 20 min of derivatization, the plate was dried in the nitrogen evaporator for 60 min, and 300 μL of extraction solvent (5 mM of ammonium acetate in methanol) was added to each well and incubated for 30 min. An aliquot of 5 μL of the derivatized amino acids and biogenic amines was injected for analysis.

2.5. Immunohistochemistry (IHC) and Western blot

IHC was introduced to measure the expression of HDC, MAOB, H1HR, H2HR, H3HR, and H4HR. IHC was performed as previously described [15]. Rabbit anti-HDC polyclonal antibody (ab37291), rabbit anti-MAOB monoclonal antibody (ab125010), goat anti-H1HR polyclonal antibody (sc-33970), rabbit anti-H2HR polyclonal antibody (sc-50314), goat anti-H3HR polyclonal antibody (sc-17920), and rabbit anti-H4HR polyclonal antibody (ab97487) were used as primary antibodies. Meanwhile, Western blotting was introduced to measure the expression of HDC and MAOB. The appropriate amount of total liver proteins was loaded and separated on 4–12% SDS-PAGE, and gels were electroblotted to nitrocellulose membranes. Western blotting was performed as previously described [16]. Primary antibodies against HDC and MAOB, mentioned above, were used. As a reference, β-actin was used.

2.6. Statistics

A multivariate statistical model of orthogonal partial least square discriminate analysis (OPLS-DA) was constructed with the software SIMCA-P+ (version 13.0, Umetrics, Umea, Sweden). On the basis of a variable importance in the projection (VIP) threshold of 1, a number of metabolites responsible for the difference in the metabolic profiles of different groups could be obtained. In parallel, the metabolites identified by the OPLS-DA model were validated at a univariate level using the non-parametric Mann–Whitney *U* test (SPSS 21) with the critical *p*-value set to 0.05.

3. Results

3.1. Demographic information and clinical manifestations

Demographic and clinical parameters, including age, gender, total bilirubin (TB), direct bilirubin (DB), total bile acids (TBA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT), are listed in Table 1. Age showed no significant difference between the BA and NHS infants, while the BA infants with severe fibrosis (F4) were significantly older than those infants with mild fibrosis (F1-2) ($p < 0.01$). In this study, there were many more male infants with NHS than with BA ($p < 0.001$). As reported previously, GGT was significantly higher in the BA infants than those infants with NHS ($p < 0.001$). There were no significant differences regarding TB, DB, TBA, ALT, AST, and GGT among the different stages of fibrosis in the BA infants.

3.2. Amino acid and biogenic amine profiles in livers of BA and NHS

UPLC–MS/MS was performed on liver samples from 52 infants with BA, 16 infants with NHS, and 5 infant controls for targeted quantification of amino acids and biogenic amines. A total of 35 amino acids and biogenic amines were quantified, including L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-citrulline, L-glutamine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, asymmetric dimethylarginine, symmetric dimethylarginine, aminoadipic acid, carnosine, creatinine, histamine, kynurenine, methionine sulfoxide, 3-nitrotyrosine, 4-hydroxy-L-proline, petroscine, sarcosine, sarotonin, and taurine. After data normalization with liver weights, the OPLS-DA analysis revealed clear separation among the BA, NHS, and control infants

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