Biochimica et Biophysica Acta 1852 (2015) 1646–1657

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Sensitization to autoimmune hepatitis in group VIA calcium-independent phospholipase A2-null mice led to duodenal villous atrophy with apoptosis, goblet cell hyperplasia and leaked bile acids

Li Jiao [a,b], Hongying Gan-Schreier [a], Sabine Tuma-Kellner [a], Wolfgang Stremmel [a], Walee Chamulitrat [a,*]

[a] Department of Internal Medicine IV, University of Heidelberg Hospital, Heidelberg, Germany
[b] Department of Toxicology, School of Public Health, Jilin University, Changchun, China

ARTICLE INFO

Article history:
Received 9 January 2015
Received in revised form 27 April 2015
Accepted 28 April 2015
Available online 7 May 2015

Keywords:
Enterohepatic cycle
Pla2G6
Inflammation
Enteropathy
Hepatic necrosis
Bile acids

ABSTRACT

Chronic bowel disease can co-exist with severe autoimmune hepatitis (AIH) in an absence of primary sclerosing cholangitis. Genetic background may contribute to this overlap syndrome. We previously have shown that the deficiency of iPLA$_2$β causes an accumulation of hepatocyte apoptosis, and renders susceptibility for acute liver injury. We here tested whether AIH induction in iPLA$_2$β-null mice could result in intestinal injury, and whether bile acid metabolism was altered. Control wild-type (WT) and female iPLA$_2$β-null (iPLA$_2$β$^{-/-}$) mice were intravenously injected with 10 mg/kg concanavalinA (ConA) or saline for 24 h. ConA treatment of iPLA$_2$β$^{-/-}$ mice caused massive liver injury with increased liver enzymes, fibrosis, and necrosis. While not affecting WT mice, ConA treatment of iPLA$_2$β$^{-/-}$ mice caused severe duodenal villous atrophy concomitant with increased apoptosis, cell proliferation, globlet cell hyperplasia, and endotoxin leakage into portal vein indicating a disruption of intestinal barrier. With the greater extent than in WT mice, ConA treatment of iPLA$_2$β$^{-/-}$ mice increased jejunal expression of innate response cytokines CD14, TNF-α, IL-6, and SOCS3 as well as chemokines CCL2 and the CCL3 receptor CCR5. iPLA$_2$β deficiency in response to ConA-induced AIH caused a significant decrease in hepatic and biliary bile acids, and this was associated with suppression of hepatic Cyp7A1, Ntcp and ABCB11/Bsep and upregulation of intestinal FXR/FGF15 mRNA expression. The suppression of hepatic Ntcp expression together with the loss of intestinal barrier could account for the observed bile acid leakage into peripheral blood. Thus, enteropathy may result from acute AIH in a susceptible host such as iPLA$_2$β deficiency.

1. Introduction

The liver plays a major role in the defense against pathological insults and disease, such as, viral infection, metabolic syndrome, and autoimmune diseases, and thus this organ is prone to injury triggered by accumulated fat deposition and inflammatory cells. The injury to liver and bile ducts causes hepatobiliary autoimmune disease including primary biliary cirrhosis, primary sclerosing cholangitis (PSC), and autoimmune hepatitis (AIH), and this disease has an inherited element and clusters among families [1,2]. It is known that PSC is the most common hepatobiliary disease seen in association with inflammatory bowel disease (IBD), with 5% of all patients with IBD having PSC, and most patients with PSC ultimately develop IBD, usually chronic ulcerative colitis (UC) [2]. An overlap syndrome of PSC and AIH has also been seen in patients with UC, and severe AIH in an absence of PSC can lead to the development of UC with up to 16% of patients with AIH also having UC [3]. As patients with well-established AIH can develop PSC [4], and that those AIH patients with PSC in the background can develop progressive liver failure from AIH [5], this suggests that the common

Abbreviations: AB-PAS, alcian blue-periodic acid schiff; AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; Bcl-2, B-cell lymphoma 2; CA, cholic acid; CCL, chemokine ligand; CCR, chemokine receptor; CDCA, chenodeoxycholic acid; CK19, cytokeratin 19; ConA, concanavalinA; CXCL12, C-X-C motif chemokine 12; DCA, deoxycholic acid; FXR, Farnesoid X-activated receptor; FGF15, fibroblast growth factor 15; H&E, hematoxylin-eosin; IHC, immunohistochemistry; iPLA$_2$β, group VIA calcium-independent iPLA$_2$; Klf4, Kruppel-like factor 4; LCA, lithocholic acid; LC-MS/MS, liquid-chromatography mass spectrometry; LDH, lactate dehydrogenase; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPS, lipopolysaccharides; MCA, muricholic acid; MCP1, monocyte chemotactic protein1; PC, phosphatidylcholine; PPARγ, peroxisome proliferator activated receptorγ; PSC, primary sclerosing cholangitis; q-RT-PCR, quantitative real-time polymerase chain reaction; SHP, small heterodimer partner; SOCS3, suppressor of cytokine signaling 3; TNFα, tumor necrosis factorα; UDCA, ursodeoxycholic acid; VCAM1, vascular cell adhesion molecule 1; WT, wild-type; α-SMA, α-smooth muscle actin

* Corresponding author at: Internal Medicine IV, Im Neuenheimer Feld 345 EG, 69120 Heidelberg, Germany. Tel.: +49 6221 5638731; fax: +49 6221 565398.
E-mail address: Walee.Chamulitrat@med.uni-heidelberg.de (W. Chamulitrat).

http://dx.doi.org/10.1016/j.bbadis.2015.04.025
0925-4439/

mechanism of AIH and PSC overlap syndrome for UC and IBD may involve a cholestatic phenotype with cholangiopathy and histologic changes of bile duct injury [3,6] as well as alteration in bile acids within the enterohepatic system [7]. Intestinal inflammation and injury has been reported in experimental cirrhosis [8], as well as in patients with decompensated [9] and alcoholic [10] cirrhosis, and the latter has been shown to be associated with alteration in bile acid metabolism. No experimental data have ever been presented to report the involvement of AIH in IBD to confirm patient studies [3], and whether the mechanism is genetically dependent.

In defining a pathogenic mechanism for a contribution of AIH to bowel disease, we designed a mouse AIH model by using a combination of concanavalin A (ConA) [11], and a genetically susceptible transgenic mouse model [12]. ConA induces hepatitis in mice is by an activation of T cells and macrophages with massive granulocyte and CD4 T cell infiltration which leads to apoptosis and necrosis in the hepatocytes and biliary epithelial cells. The latter corresponds to the cholestatic phenotype which is regulated by bile acids [13,14]. Hence, AIH pathogenesis may involve alteration in bile acid metabolism in the enterohepatic loop [10,14] that may play a pivotal role in intestinal homeostasis leading to bowel injury and disease [8–10].

In evaluating a genetically susceptible mouse model, we chose group VIA calcium-independent phospholipase A2 (iPLA$_2$) with alterative names of Pla2G6 or iPLA$_2\beta$ [15], because it has multiple physiologically important functions in numerous cell types and tissues [16]. iPLA$_2\beta$ catalyzes phospholipids to generate 1). lysophosphatidylcholine (LPC) which acts as a 'find-me' signal for removal of apoptotic cells by monocytes [17,18], and 2). lysophosphatidic acid (LPA) plays a role in the migration of monocytes [19,20], and of ovarian cancer cells [21]. Despite of the anticipated dysfunction with iPLA$_2\beta$ deficiency, iPLA$_2\beta$-null mice with the deletion of the lipase-containing exon 9 live normally, but they have defects in glucose homeostasis [15], gain lesser body weight [22], and develop neuroaxonal dystrophy [23] as they age to 1–2 years old. In line with a decrease of LPC leading to a defect in the removal of apoptotic hepatocytes, results from our laboratory have shown that hepatic apoptosis was indeed increased in the livers of iPLA$_2\beta$-null mice together with increased inflammation and susceptibility for endotoxin injury [24] (manuscript under preparation). Because apoptosis is the predominant mechanism of liver cell death during AIH [25], we hypothesize that the deficiency of iPLA$_2\beta$ would represent a genetic susceptible preconditioning for AIH that could lead to bowel injury.

Here we compared the extent of AIH liver injury induced by ConA between wild-type (WT) and iPLA$_2\beta$-null (iPLA$_2\beta^{-/-}$) mice, and determined whether AIH observed in liver was associated with abnormalities in the intestine and colon in regards to epithelial damage, proliferation and bile acid contents. We demonstrated that iPLA$_2\beta$ deficiency sensitized the effects of ConA in promoting liver injury as well as duodenal apoptosis, villous atrophy, intestinal inflammation, goblet cell hyperplasia, and leakage of endotoxin into portal vein. Alteration of bile acids was observed in liver, bile, intestine, feces, and peripheral blood during ConA and iPLA$_2\beta$-deficiency sensitization. Gene expression analysis revealed a feedback regulation of hepatic bile acid synthesis by intestinal FXR/FGF15 axis. Thus, ConA-induced AIH in a susceptible host with iPLA$_2\beta$ deficiency led to enteropathy which was associated with alteration in bile acid metabolism. Our data support the notion that the liver-to-gut axis injury due to AIH in susceptible individuals may lead to bowel disease.

2. Materials and methods

2.1. Animals and treatment

iPLA$_2\beta^{-/-}$ mice were kind gifts from Dr. John Turk (Washington University School of Medicine, St. Louis, Missouri, USA). The breeding and genotyping was performed according to the published work [15]. Control WT mice were mice with iPLA$_2\beta^{+/+}$ phenotype. All mice were housed at the animal facility of the University Heidelberg. Female mice at 13–16 months old were used. For an AIH induction, mice were intravenously injected via tail vein with 10 mg/kg Con A (Sigma Aldrich, Taufkirchen, Germany) or saline. Blood, liver, bile, intestine, colon, and feces were harvested 24 h after ConA administration. All animal experiments were approved by the Animal Care and Use Committee of the University of Heidelberg.

2.2. Biochemical assays

Serum was obtained after centrifugation at 5000 *g* for 15 min. Serum activities of alanine aminotransferase (AST), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (AP) were measured spectrophotometrically by using diagnostic kits from Randox (Krefeld, Germany).

2.3. Histology and immunohistochemistry (IHC)

Liver, intestine and colon specimens were fixed in 10% formalin at room temperature for 18 h, and embedded in paraffin, and paraffin blocks were cut into 5 μm sections. Sections were stained with hematoxylin and eosin (H&E) for histology. For Sirius-red staining of the liver, sections were stained with 0.1% Direct Red 80 Solution (Sigma Aldrich) in a saturated solution of picric acid. For mucin staining of intestine, slides were stained with alcian blue-periodic acid schiff (AB-PAS) reagents (Sigma Aldrich) according to standard procedure.

For IHC, after deparaffinizing and hydration, sections were treated with 10 mM citrate buffer (pH 6.0) and heated to 98 °C for 20 min. Endogenous peroxidase was blocked by treating sections with 3% H_2O_2 for 10 min at room temperature prior to an exposure to a primary antibody overnight at 4 °C. Rabbit primary antibodies used for IHC were cleaved caspase-3 (1:800; cat# 9664, Cell Signaling), α-SMA (1:250; E184, Epitomics), CK19 (1:100; cat# ab133496, Abcam), Ki67 (1:100; cat# ab16667, Abcam), CD3 (1:300; cat# ab5690, Abcam), and CD45R (1:200; cat# ab64100, Abcam). Sections for cleaved caspase-3 staining were stained by using an Abcam Avidin-Biotin Complex kit (cat# ab64261), and the rest of antibodies sections were incubated with a goat anti-rabbit secondary Abcam antibody (cat# ab6721) for 1 h at room temperature. Positive staining was detected by diaminobenzidine. Slides were counterstained with hematoxylin prior to mounting.

Light microscopy was used to visualize stained cells with an Olympus IX 50 microscope. The quantification of Sirius-red- and α-SMA-positive areas in liver was performed by using Image J. For computerized image analyses of intestine, the number of stained cells on a surface area of epithelial cells were determined by using the Olympus Cell^F software. A picture of one field on a slide taken with a 20X objective corresponded to 0.6 mm^2 on the tissue. The number of stained cells on each slide was counted from ten randomly selected fields. Data were presented as the number of stained cells per mm^2 of intestinal epithelium. Evaluation of IHC sections was performed in a blind manner.

2.4. Bile acid profiling using LC-MS/MS

Samples were subjected to extraction using a published method [26], and bile acid concentrations were determined by using liquid-chromatography mass spectrometry (LC-MS/MS) according to a published method [27]. Briefly, the separation of bile acids was achieved by using a Phenomenex Luna C18 column (100 mm × 2.0 mm, 3 μm) fitted on a separation module of a Waters 2695. Binary solvents were 80% H_2O/MeOH with 8 mM ammonium acetate, pH 8.0 (solvent A) and 95% MeOH/H_2O with 8 mM ammonium acetate, pH 8.0 (solvent B). The flow rate was maintained at 0.2 mL/min, and the gradient was started with 100% solvent A for 2.5 min, changed to 100% solvent B in 1 min, held for 16.5 min, and returned to the initial condition in 3 min. Separated fractions were detected on-line by an electrospray