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Dysregulated Bile Transporters and Impaired Tight Junctions During Chronic Liver Injury in Mice

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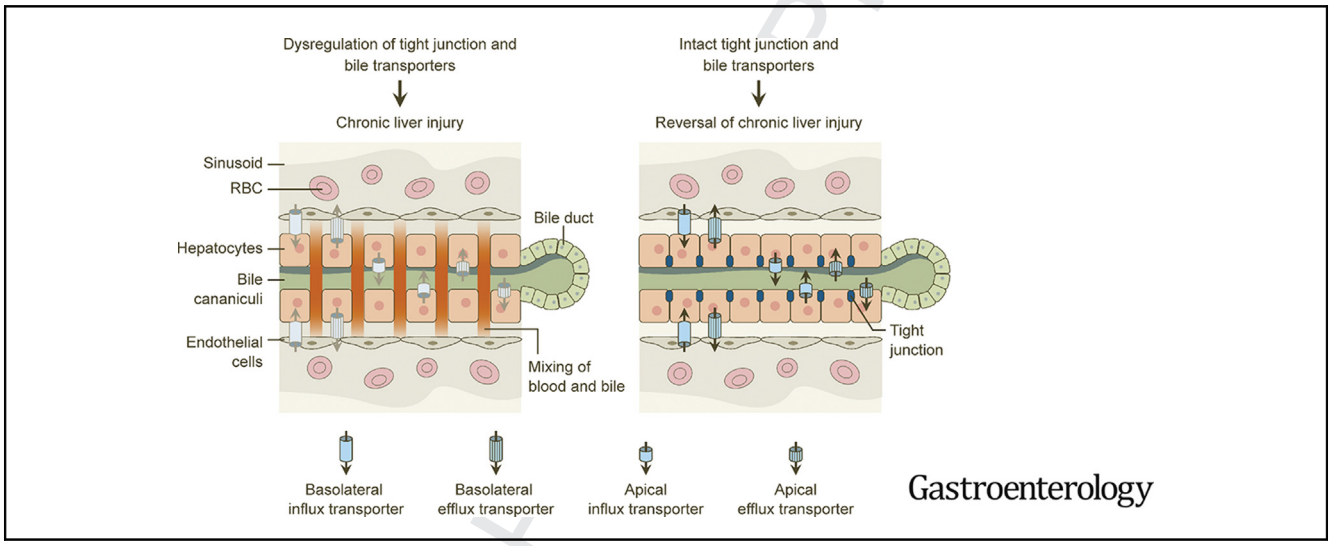

BACKGROUND & AIMS: Liver fibrosis, hepatocellular necrosis, inflammation, and proliferation of liver progenitor cells are features of chronic liver injury. Mouse models have been used to study the end-stage pathophysiology of chronic liver injury. However, little is known about differences in the mechanisms of liver injury among different mouse models because of our inability to visualize the progression of liver injury in vivo in mice. We developed a method to visualize bile transport and blood-bile barrier (BBlB) integrity in live mice. **METHODS:** C57BL/6 mice were fed a choline-deficient, ethionine-supplemented (CDE) diet or a diet containing 0.1% 3,5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC) for up to 4 weeks to induce chronic liver injury. We used quantitative liver intravital microscopy (qLIM) for real-time assessment of bile transport and BBlB integrity in the intact livers of the live mice fed the CDE, DDC, or chow (control) diets. Liver tissues were collected from mice and analyzed by histology, immunohistochemistry, real-time polymerase chain reaction, and immunoblots. **RESULTS:** Mice with liver injury induced by a CDE or a DDC diet had breaches in the BBlB and impaired bile secretion, observed by qLIM compared with control mice. Impaired bile secretion was associated with reduced expression of several tight-junction proteins (claudins 3, 5, and 7) and bile transporters (NTCP, OATP1, BSEP, ABCG5, and ABCG8). A prolonged (2-week) CDE, but not DDC, diet led to re-expression of tight junction proteins and bile transporters, concomitant with the reestablishment of BBlB integrity and bile secretion. **CONCLUSIONS:** We used qLIM to study chronic liver injury, induced by a choline-deficient or DDC diet, in mice. Progression of chronic liver injury was accompanied by loss of bile transporters and tight junction proteins.

Keywords: Blood Bile Barrier; Claudins; Diet-Induced Liver Injury; Hepatocyte Tight Junction.

Abbreviations used in this paper: **ALP, alkaline phosphatase; ALT, alanine aminotransferase; BBlB, blood-bile barrier; CDE, choline-deficient, ethionine-supplemented; CF, carboxyfluorescein; CFDA, carboxyfluorescein diacetate; CLD, chronic liver disease; DDC, 3,5-diethoxycarbonyl 1,4-dihydrocollidine; EpCAM, epithelial cell adhesion molecule; IV, intravenously; PCR, polymerase chain reaction; qLIM, quantitative liver intravital microscopy; TJ, tight junction; TXR, Texas red.**

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

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NEW FINDINGS

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LIMITATIONS

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Chronic liver disease (CLD) is the 12th leading cause of death in the United States.[1,2] Current treatments for CLD are limited, and liver transplantation is the only treatment available to patients diagnosed with liver failure. The onset of CLD is primarily attributed to excessive alcohol consumption, viral hepatitis, nonalcoholic fatty liver disease, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, and genetic conditions such as hereditary hemochromatosis.[2–7] Although chronic liver injury is the hallmark of all types of CLDs, the cellular, molecular, and biophysical mechanisms that contribute to progression of chronic liver injury are incompletely understood. Identifying the common molecular pathways that promote liver injury in diverse experimental models of chronic liver injury would enable the development of improved therapies to prevent or halt the progression of CLDs.

Chronic liver injury is characterized by iterative cycles of insult to the liver parenchyma leading to inflammation, matrix deposition, hyper-bilirubinemia, angiogenesis, and progressive fibrosis.[3] Recently,[8] we have identified that chronic liver injury is associated with loss of blood-bile barrier (BBlB). BBlB is a physical barrier formed by liver epithelial cells or hepatocytes that separates bile from sinusoidal blood. The BBlB function is enabled by the junctional adhesion complexes known as tight junctions (TJs), which are maintained by the homotypic interaction of adhesion molecules such as claudins (1–31), occludin, coxsackievirus and adenovirus receptor, and zonula occludens (ZO-1, -2, and -3).[9] In addition to TJs, BBlB function is also regulated by highly polarized basolateral and apical transporters present on hepatocytes that modulate the collection and release of bile acids from the blood and into the bile-canaliculi, respectively.[10–12]

Here, we have used 2 widely used experimental models of chronic liver injury in mice fed choline-deficient, ethionine-supplemented (CDE)[13–15] or 3,5-diethoxycarbonyl 1,4-dihydrocollidine (DDC)[16–19] diet, respectively. Using our recently developed quantitative liver intravital microscopy (qLIM) approach, we show that chronic liver injury involves physical breach of BBlB, mixing of blood with bile, and loss of bile transport across hepatocytes. Our data suggest that loss of TJ adhesion molecules such as claudins and basolateral or canalicular bile transporters from hepatocytes is the central pathophysiology in chronic liver injury irrespective of the experimental model. Finally, we show that the recovery from liver injury is dictated by the ability of BBlB to regain physical integrity through reappearance of TJ adhesion molecules and bile transporters on hepatocytes.

Methods

Mice

C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and Taconic Biosciences (Hudson, NY). Mice used in this study were ages 4–6 weeks and weighed around 18 kg. All animal experiments and procedures were performed under the guidelines of the National Institutes of Health and under an animal protocol approved by the Institutional Animal Use and Care Committees at the University of Pittsburgh.

Diet

DDC Diet. Mice were fed a special diet containing 0.1% DDC (Bioserve,) for up to 4 weeks.

CDE Diet. Mice were given unlimited access to choline-deficient diet (Envigo, Frederick, MD) and drinking water supplemented with 0.15% DL-ethionine (MP Biomedicals, Santa Ana, CA) for up to 4 weeks. Q9

Surgical Preparation and qLIM Imaging

Mice were anesthetized with an intraperitoneal injection of 100 mg/kg of body weight ketamine HCl (100 mg/mL; Henry Shein Animal Health, Dublin, OH) and 20 mg/kg of body weight xylazine (20 mg/mL; Lloyd Laboratories, Shenandoah, IA). When anesthetized, mice were given a 1-mL intraperitoneal injection of warmed saline and were placed on a heated stage in the supine position. A tracheotomy was performed, and a short length of PE90 tubing was inserted into the incision site and tied to the trachea using a silk suture (Supplementary Figure 1). Next, the right carotid artery was cannulated with heparinized PE10 tubing. Mice were repositioned in the right lateral decubitus position. The right lobe of the liver was exposed through removal of the overlying skin and fat. To gently immobilize the liver, we used a micro-machined thoracic liver window inspired by Looney et al,[20] which provided a light suction through the use of a vacuum pump (Roscoe Medical Inc; Strongsville, OH). The modified thoracic liver device had a larger viewing window (diameter, ~5 mm) that allows more stable imaging over extended durations. A round coverslip (diameter, 12 mm) was placed on top of the window and held in place using vacuum grease. Once the vacuum was applied, the liver window was gently lowered to immobilize a small region of the lower half of the right liver against the coverslip. Next, intravascular fluorescent dyes were injected through the Q10 Q11 Q12