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Mechanisms of bile acid mediated inflammation in the liver

Man Li, Shi-Ying Cai, James L. Boyer*

The Liver Center, Yale University School of Medicine, New Haven, CT 06510, USA

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ABSTRACT

Bile acids are synthesized in the liver and are the major component in bile. Impaired bile flow leads to cholestasis that is characterized by elevated levels of bile acid in the liver and serum, followed by hepatocyte and biliary injury. Although the causes of cholestasis have been extensively studied, the molecular mechanisms as to how bile acids initiate liver injury remain controversial. In this chapter, we summarize recent advances in the pathogenesis of bile acid induced liver injury. These include bile acid signaling pathways in hepatocytes as well as the response of cholangiocytes and innate immune cells in the liver in both patients with cholestasis and cholestatic animal models. We focus on how bile acids trigger the production of molecular mediators of neutrophil recruitment and the role of the inflammatory response in this pathological process. These advances point to a number of novel targets where drugs might be judged to be effective therapies for cholestatic liver injury.

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1. Introduction

Bile acids are amphipathic molecules synthesized from cholesterol in the liver and are the major component in bile. Secretion of bile acids and other choleretic compounds by hepatocytes generates bile flow and facilitates elimination of endogenous compounds and metabolites such as bilirubin and hormones, as well as xenobiotics including drugs (Trauner and Boyer, 2003). In humans, most primary bile acids are conjugated with glycine or taurine and form mixed micelles with phospholipids and cholesterol in the bile before they reach the small intestine, where they facilitate digestion and absorption of lipophilic nutrients such as cholesterol, fat and fat-soluble vitamins. At the terminal ileum, approximately 95% of bile acids are reclaimed and transported back to the liver via the portal circulation. The remaining fraction are transformed into secondary bile acids by gut microbiota where they either passively diffuse across the colon or are excreted in the feces.

Bile acid excretion is impaired in cholestatic liver injury either by direct inhibition or genetic deficiencies of canalicular bile acid transporters in hepatocytes or by mechanical or immune mediated obstruction of the biliary ducts. Whatever the cause, bile acid levels increase in the liver and serum, followed by hepatocyte injury and bile duct proliferation. If left untreated, cholestatic liver injury often progresses to liver fibrosis, cirrhosis and eventually liver failure. While the causes of cholestasis have been extensively studied, the molecular mechanisms as to how bile acids initiate liver injury are not well understood. Part of the reason may be due to the diverse physical properties of bile acids. Although all bile acids are metabolites of cholesterol, their structural variation determines their physical properties and physiological function (Hofmann and Hagey, 2008). For example: 1) The hydrophilicity or water solubility of bile acids increases with the number of hydroxylation sites located either in the nuclear ring or side chain, and is also affected by the carboxyl group conjugation; 2) Bile acids with lower water solubility are more cytotoxic; 3) While unconjugated bile acids freely diffuse across cell membranes, conjugated bile acids (which make up the majority in the bile acid pool) require specific protein transporters; 4) Different cell types may respond quite differently to the same bile acid.

Early work suggested that bile acids injured the liver directly through their detergent cytolytic effects, as submillimolar levels of toxic bile acids directly killed hepatocytes when added to these cells in vitro (Scholmerich et al., 1984; Attili et al., 1986; Galle et al., 1990). However, the serum and tissue levels of toxic bile acids rarely reach these submillimolar levels in pathophysiological conditions, suggesting that their cytolytic properties may not be the cause of liver cell death. Subsequently, it was proposed that bile acids induced apoptosis in hepatocytes. This hypothesis is supported by the observation that apoptosis was detected in rat

* Corresponding author. Ensign Professor of Medicine, Department of Medicine, Yale University School of Medicine, 333 Cedar Street, 1080 LMP, P.O. Box 208019, New Haven, CT 06510, USA.
E-mail address: james.boyer@yale.edu (J.L. Boyer).

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hepatocyte cultures when they were treated with >50 μM glycochenodeoxycholic acid (GCDCA) (Patel et al., 1994; Webster and Anwer, 1998), as reviewed by Malhi et al. (2010)). However, 1) GCDCA is not a major bile acid in rats as the serum concentration of total chenodeoxycholic acid is only ~5 μM even in rats with complete bile duct obstruction (Kinugasa et al., 1981); 2) In contrast, taurocholic acid (TCA), the major endogenous bile acid in rats does not induce apoptosis in rat hepatocytes (Webster and Anwer, 1998). Also, taurine conjugation is the major form of conjugates in rodents in contrast to glycine which is dominant in humans; 3) Normally, apoptotic cell death does not elicit an immune response; 4) Most importantly, apoptosis of hepatocytes has not been detected in vivo in the liver of bile duct ligated (BDL) mice or in vitro in bile acid treated human hepatocytes (Allen et al., 2011; Zhang et al., 2012; Woolbright et al., 2013, 2015; Cai et al., 2017); 5) Finally, depletion of macrophages in mice did not reduce liver injury after BDL (Gehring et al., 2006; Osawa et al., 2010), indicating that inflammatory mediators from macrophages do not play a significant role, at least in the initiating stages. Rather, it is the infiltration of neutrophils that best correlates with liver injury in cholestasis (Gujral et al., 2003, 2004; Cai et al., 2017). Altogether, these concerns suggest that under pathophysiological conditions, bile acids must injure the liver by alternative mechanisms rather than by their intrinsic toxicity. This review summarizes recent advances in the molecular mechanism of bile acid induced liver injury focusing on early events and the role of the inflammatory response in this pathological process (Table 1).

2. Cholestatic hepatocytes initiate inflammatory response by releasing cytokines

More recently, Allen et al. proposed that bile acids may induce liver injury via a hepatocyte initiated inflammatory response (Allen et al., 2011; Zhang et al., 2012). In these studies, exposure of cultured mouse hepatocytes to 200 μM of TCA, a major endogenous bile acid in this species, significantly stimulated the expression (mRNA) of a series of cytokines and adhesion molecules, including MCP-1 (Ccl2), MIP-2 (Cxcl2) and ICAM-1. Remarkably, bile acid treatment did not increase caspase 3 activity in these mouse hepatocytes or release alanine transaminase (ALT) activity in the culture medium, suggesting that neither apoptosis nor necrosis had occurred in these cells (Allen et al., 2011; Zhang et al., 2012). Interestingly, increased expression (mRNA) of the early growth response protein 1 (Egr1) was also detected in these bile acid treated cells. Egr1 is a transcription factor that plays an important role in regulating the expression of many genes, including inflammatory cytokines. Furthermore, the authors demonstrated that bile acid induced up-regulation of these inflammatory cytokines in mouse hepatocytes was partially Egr1-dependent but independent of farnesoid X receptor (Fxr/Nr1h4), the bile acid activated nuclear receptor, because these inflammatory genes were not reduced in bile duct ligated *Fxr* knockout mice. In contrast, Egr1-deficiency reduced bile acid induction of some of these cytokines and adhesion molecules in vitro in mouse hepatocyte cultures and in vivo in the liver of BDL mice, where reduced liver injury was also detected (Allen et al., 2011; Kim et al., 2006). To explain how elevated levels of bile acid cause Egr1 activation, the authors proposed that MAP kinases may mediate this transactivation, speculating that there are cell surface receptors involved in this signaling pathway.

To further elucidate the role of inflammatory cytokines in cholestatic liver injury and to gain insights into the mechanism of bile acid induction of cytokine expression, we recently examined this hypothesis in vitro using isolated mouse liver cells and human hepatocytes, and in vivo in cholestatic murine models. First, we confirmed that a 24-h exposure to TCA stimulated inflammatory cytokine expression in a collagen sandwich culture of mouse hepatocytes, a system closely resembling cholestatic liver conditions. In addition, we found that at pathophysiological concentrations (25–200 μM) only hepatocytes, but not isolated liver nonparenchymal cells or cholangiocytes, responded to bile acid stimulation with increased cytokine expression. Furthermore, the cytokines released into the medium of the bile acid treated hepatocyte cultures significantly enhanced neutrophil chemotaxis in a transwell experiment, emphasizing the functional importance of these hepatocyte specific cytokines in initiating the inflammatory response. Knockout of chemokine *Ccl2* significantly reduced hepatic neutrophil infiltration in two cholestatic mouse models, i.e. 1% cholic acid feeding and 7-day BDL, where less liver injury was also detected (Cai et al., 2017).

To understand why hepatocytes are uniquely susceptible to bile acids, we demonstrated that the hepatocyte-specific basolateral bile acid transporter NTCP/SLC10A1 is required for this event because knocking down NTCP or inhibiting the bile acid uptake transporters reduced bile acid induction of chemokines. This is consistent with recent reports in an NTCP-deficient patient and in *Ntcp* knockout mice. Both the patient and the mice were completely protected from cholestatic liver injury, despite extremely high levels of bile acid in the blood (Vaz et al., 2015; Slijepcevic et al., 2015). These findings also indicate that bile acids must first enter and accumulate in hepatocytes to stimulate cytokine expression, rather than the effect being mediated by a specific cell membrane receptor as proposed by others (Allen et al., 2010, 2011). Once accumulated in the cell, bile acid caused ER stress and mitochondrial damage, as previously reported (Botla et al., 1995; Yerushalmi et al., 2001; Iizaka et al., 2007; Bochkis et al., 2008; Tamaki et al., 2008). However, there was no evidence of caspase 3 cleavage in these cells, in agreement with the findings of others that apoptosis is not playing a role (Allen et al., 2011; Zhang et al., 2012; Woolbright et al., 2013; Cai et al., 2017).

Because mitochondria damage was detected in bile acid treated hepatocytes, we hypothesized that the injured mitochondria may release “damage-associated molecular patterns” (DAMPs) that could activate toll-like receptors (Tlr). Tlr9 is one of them and is an intracellular DNA sensor. Previous studies have demonstrated that mitochondrial DNA can activate Tlr9 and stimulate inflammatory cytokines expression (Zhang et al., 2010). To examine whether Tlr9 plays a role in bile acid induced liver injury, we treated *Tlr9* $^{-/-}$ mouse hepatocytes with bile acid and found reduced induction of Cxcl2 in these cells. The involvement of Tlr9 is further supported by the observations that Cxcl2 induction was also significantly reduced in *MyD88/Trif* double knock out mouse hepatocytes. Of note, MyD88 and Trif are downstream molecules in Tlr9 signaling pathway. Reduced liver injury was also found in *Tlr9*$^{-/-}$ mouse liver after BDL (Cai et al., 2017; Gabele et al., 2008). However, Tlr9 normally resides on the endoplasmic reticulum and endosomes, so the mechanism of this activation remains unclear. One possibility is that injured mitochondria undergo autophagy/mitophagy and present mitochondrial DNA to Tlr9 (Carchman et al., 2013). Bile acid induction of cytokines are reduced in mouse hepatocytes when mitochondria are protected by cyclosporine A or norursodeoxycholic acid (Cai et al., 2017). Norursodeoxycholic acid also improves liver function in several cholestatic rodent models and in patients with sclerosing cholanagitis in a recent phase II trial (Fickert et al., 2006; Moustafa et al., 2012; Halilbasic et al., 2017). Together, these findings support a role for mitochondrial damage in the pathogenesis of the cholestatic liver and suggests that bile acids activate the innate immune system.

Similarly, the major human bile acid, GCDCA, also stimulated cytokine expression when applied to human hepatocyte cultures at pathophysiological levels (≥50 μM), including CCL2, CCL15, CCL20,

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