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The role of Inflammation in the mechanisms of bile acid-induced liver damage

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Abstract

Background: The mechanism by which bile acids induce liver injury in cholestasis remains controversial. Although high levels of bile acids are toxic when applied to liver cells, the level of toxic bile acids in the liver of most cholestatic animals and patients is <10 μM, indicating there must be alternative mechanisms. Recent studies suggest that the inflammatory response may play an important role in bile acid-induced liver injury, as pro-inflammatory cytokine expression is stimulated by bile acids in mouse hepatocyte cultures. To elucidate the mechanisms of bile acid-induced liver injury, we assessed signs of liver damage and gene expression in *Abcb4*$^{-/-}$ mice, a well-known model for cholestasis.

Key Messages: Elevated plasma levels of bile acids were detected as early as 10 days after birth and at all later ages in *Abcb4*$^{-/-}$ mice compared to their wild-type littermate controls. Parallel increases in expression of Tnfα, Ccl2, Cxcl1, and Cxcl2 mRNA occurred at these early time points and throughout 12 weeks in *Abcb4*$^{-/-}$ livers. Marked hepatic neutrophil infiltration was first detected in 3-week mice, whereas histological evidence of liver injury was not detected until 6-weeks of age. Subsequent in vitro studies demonstrated that normal hepatocytes but not other non-parenchymal liver cells responded to bile acids with inflammatory cytokine induction.

Conclusion: Bile acids induce the expression of pro-inflammatory cytokines in hepatocytes in *Abcb4*$^{-/-}$ mice that initiates an inflammatory response. This inflammatory response plays an important role in the development of cholestatic liver injury in this and other cholestatic conditions. Furthermore, understanding of these inflammatory mechanisms should lead to new therapeutic approaches for cholestatic liver diseases.

Keywords

Bile acid; cholestasis; inflammation; Abcb4$^{-/-}$

In the last decade, a number of studies have suggested that bile acids may injure liver cells in cholestatic liver disease, not by a direct toxic effects as a detergent, but by initiating a cytokine-mediated inflammatory response [1,2]. While there is little doubt that bile acids can injure mitochondria, produce oxidative stress and initiate Fas-dependent apoptosis, these observations have been derived primarily from in-vitro studies in isolated cell systems where the concentrations have exceeded those normally found in the serum or liver of cholestatic animal models or patients with cholestatic liver diseases [3,4,5]. As discussed by Woolbright

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and Jaeschke [2] and observed by Trottier et al. [6] serum concentrations of bile acids in cholestatic patients rarely exceed 150–200 μM and the highest levels are non-toxic bile acids like taurocholic and glycocholic acid, while levels of the principle toxic bile acid, glycochenodeoxycholic acid reach concentrations of no more than 30 μM. In contrast, when isolated human hepatocytes are exposed to bile acids in-vitro, little or no injury occurs until concentrations of >50 μM GCDCA are used for 24 h [7]. Similar findings are also seen after bile duct ligation in mouse models of cholestasis [7] where the most toxic bile acid, TCDCA + GCDCA only reach levels ~25 nM during the initial 3 days of injury, whereas concentrations up to 3,000 fold are required to initiate toxicity in hepatocyte cultures from mice [7]. Thus, alternative explanations for bile acid-induced liver injury seem necessary.

A number of studies have demonstrated that bile duct ligation results in the accumulation of neutrophils within the liver parenchyma in areas of cell injury and that adhesion molecules such as ICAM-1 play an important role since ICAM-1 null mice or mice deficient in NHERF-1, an anchoring protein required for ICAM-1 expression markedly reduce liver injury and neutrophil accumulation [8,9]. Allen et al. [1] have shown that the exposure of hepatocytes in culture to levels of bile acids (both toxic and non-toxic) that are seen in-vivo, results in significant increases in mRNA expression of pro-inflammatory cytokines, particularly CXCL2 (MIP-2) and CXCL1 (KC). Importantly, toll-like receptor 4 was not involved in the initiation of this acute inflammatory response [1].

Figure 1 illustrates similar findings from our laboratory in the *Abcb4*$^{-/-}$ mouse model of cholestatic liver injury as it develops over time from birth. Note that the initial abnormalities consist of a rise in serum bile acids at 10 days of birth. This is associated with an increase in hepatic cytokine expression (mRNA), particularly Ccl2 (MCP-1) and Cxcl2 prior to any evidence of liver injury as reflected in the absence of significant elevation of aminotransferases until sometime between day 20 and 40 (fig. 2, 3). Neutrophil infiltration then follows at day 20 when liver injury appears to be initiated at that point of time (fig. 4). These findings strongly suggest that the initial injury is mediated by an increase in serum bile acids at a time that presumably reflects elevated liver bile acid levels.

Further detailed in vitro studies demonstrated that endogenous conjugated bile acids at pathophysiological levels cause ER stress and mitochondria damage, and stimulated cytokine expression in primary hepatocytes from mice and humans but not non-parenchymal liver cells. This cell type-specific event depends on bile acid entry to hepatocytes through its membrane transporters. Disruption of pro-inflammatory chemokines production in vivo in mice reduced liver injury after bile duct ligation [10]. This was seen in Ccl2 null mice and toll-like receptor 9 (Tlr9) null mice as well, as tlr9 can sensor damaged mitochondrial DNA and stimulated chemokine expression [11].

These series of experiments provide new insights into the mechanism by which bile acids injure the liver. First, bile acids at pathophysiologic levels seen in cholestatic liver disease accumulate in the liver where they induce ER stress and mitochondrial injury. As part of this injury response, mitochondrial DNA activates the innate immune system in part by activating TLR9, which then leads to the increase in expression of cytokines including Cxcl2, resulting in the influx of neutrophils and the onset of liver injury.

These findings suggest several novel targets for future therapeutic intervention including: (1) the inhibition of basolateral bile acid transporter, Ntcp, to prevent bile acid accumulation in the liver; (2) the application of drugs that reduce ER stress and mitochondrial injury; (3) inhibitors of cytokine production or release and finally (4) inhibitors of cytokine receptors in order to block subsequent inflammatory cell recruitment. Future studies will be needed to assess these potential targets.

Citations:

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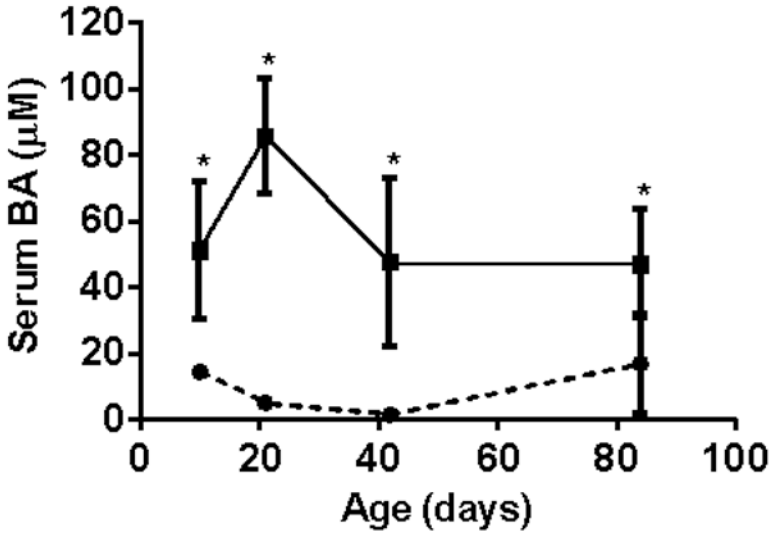

Fig 1.

Serum bile acid levels from days of birth in Abcb4 null mice (■) vs wild type controls (●)

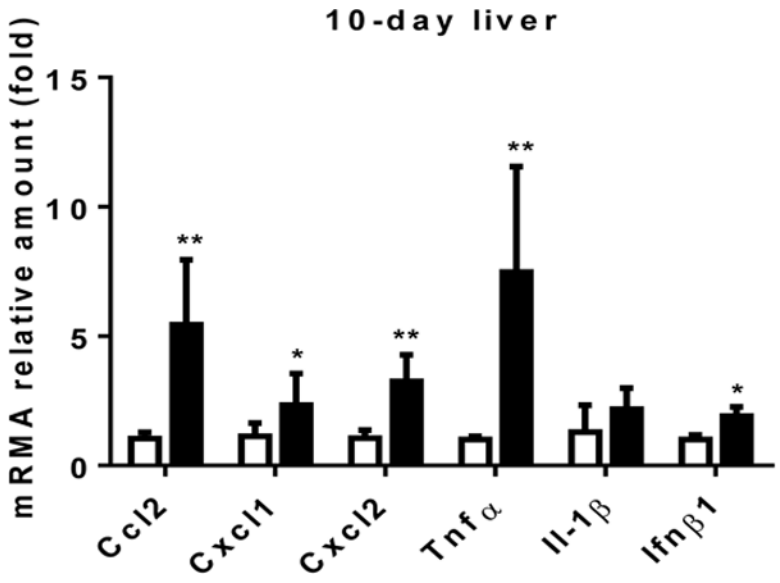

Fig 2.

Cyctokine expression levels in liver in the ten day old Abcb4 null mice (black bars) vs wild type controls (open bars)

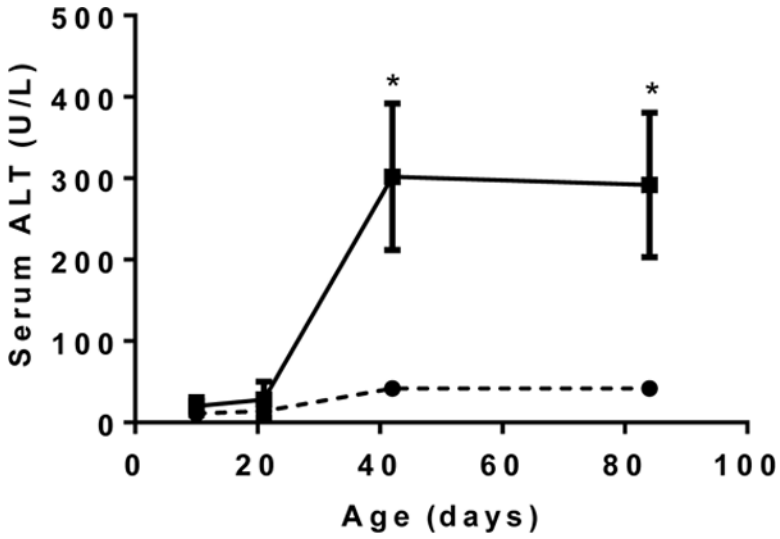

Fig 3.

Serum Alanine aminotransferase levels as a function of age in Abcb4 null mice (■) vs wild type controls (●)

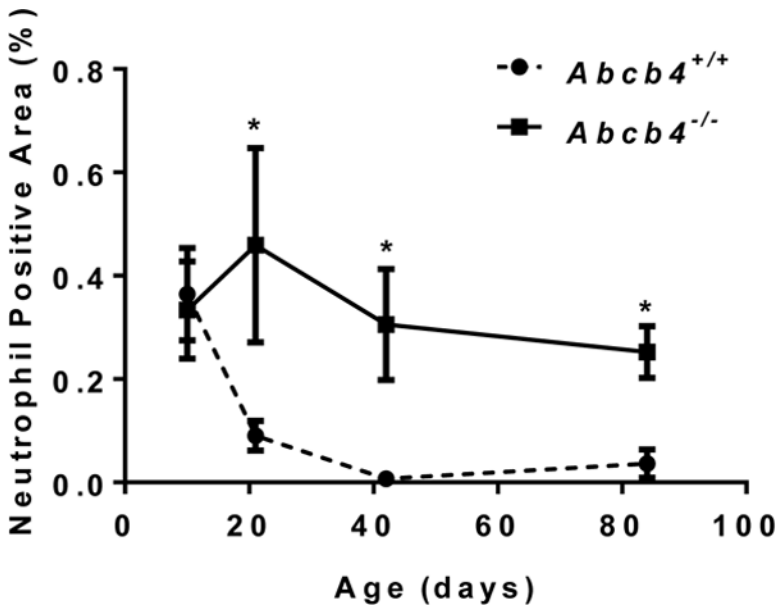

Fig 4.

Liver neutrophil staining as a function of age in Abcb4 null mice vs wild type controls