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POINT-COUNTERPOINT

The Benevolent Bile: Bile Acids as Stimulants of Liver Regeneration

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Bile acids are amphiphilic signaling molecules involved in a number of pathophysiological processes including digestion and adsorption of fats, inflammation, cell proliferation, and cancer.[1–3] Whereas an increase in the intracellular levels of bile acid is generally associated with pathogenic outcomes, bile acids are critical for multiple nondigestive physiological processes at normal levels. One such process is liver regeneration, where bile acid plays a critical regulatory role.[4–7] The liver is known for its remarkable capacity to regenerate after surgical resection or injury induced by viruses and chemicals.[6,8] Having a robust hepatic regeneration response is critical for maintaining liver health and overall animal survival. Although the role of bile acid in stimulating liver regeneration has been known since late 1960s, detailed mechanistic studies in the past 3 decades have revealed involvement of various bile acid receptors and the role of gut-liver crosstalk in the regulation of liver regeneration by bile acid. These studies conclusively indicate that bile acids at physiological levels are involved in stimulating liver regeneration.

Bile acids are derived from cholesterol in a CYP7A1-dependent process exclusively in the hepatocytes.[9] They are secreted by hepatocytes into the biliary system in the form of bile, which is stored in the gallbladder. Upon stimulation by cholecystokinin, bile is released and enters the duodenum where it is required for the digestion and absorption of fats and fat-soluble nutrients. Further down the gut in the ileum, bile acids are reabsorbed and recycled to the liver via the portal circulation. Bile acid stimulates farnesoid X receptor (FXR)-dependent fibroblast growth factor (FGF)-15/19 production in the ileum, which plays an important role in regulation of total bile acid pool via FGF receptor 4–mediated suppression of cytochrome P450 family 7 subfamily A member 1 (CYP7A1).[4] Because of this enterohepatic circulation, bile acid and bile acid–stimulated FGF-15/19 form the crux of the gut-liver signaling axis (Figure 1).

One of the most common models to study liver regeneration is partial hepatectomy in rodents, where approximately two-thirds of liver is surgically removed, and the remnant liver is allowed to grow back.[8] This is an excellent model to study liver regeneration because it is highly reproducible, is not complicated by toxicant-induced tissue injury, and is a good surrogate for liver regeneration after partial liver resection performed in humans. Studies using rats show that draining bile before partial hepatectomy surgery inhibits liver regeneration after partial hepatectomy. This is also observed in humans where external biliary drainage in patients undergoing partial hepatic resection results in decreased postoperative regeneration.[10] In addition, moderate bile acid supplementation stimulates liver regeneration and bile acid sequestration, and depletion by using the exchange resin cholestyramine results in attenuated liver regeneration after partial hepatectomy.[11] Finally, deletion or inhibition of transporters and enzymes involved in bile acid uptake and synthesis results in delayed liver regeneration. These data clearly demonstrate that bile acids are critical stimulators of liver regeneration.[4]

Bile acids signal via at least 4 nuclear receptors (FXR, VDR, CAR, and PXR) and 3 membrane receptors (TGR5, S1PR2, and CHRM2). Of these, bile acid signaling via FXR and TGR5 has been conclusively shown to be involved in liver regeneration.[4,5] TGR5 is expressed on nonparenchymal cells in the liver including Kupffer cells, endothelial cells, and cholangiocytes. Deletion of TGR5 in the setting of partial hepatectomy results in increased hepatic necrosis because of the inability to compensate for increased bile acid load and subsequent induction of proinflammatory cytokines such as interleukin 6 in the regenerating liver. Furthermore, the TRG5 null mice show delayed liver regeneration.[12]

FXR is expressed in both the hepatocytes in the liver and enterocytes in the ileum. The role of FXR in liver regeneration after partial hepatectomy has been studied by using both the whole-body and tissue-specific gene deletion mice. The global FXR null mice show severe impairment in liver regeneration leading to significant mortality.[11] Interestingly, hepatocyte-specific deletion of FXR produced only a moderate delay in liver regeneration without any mortality.[13] A similar but smaller delay in liver regeneration was observed in mice with intestine-specific deletion of FXR.[14] Hepatic FXR deletion resulted in decreased expression of the cell cycle transcription factor FoxM1B, whereas intestine-specific deletion resulted in decreased FGF15 expression after partial hepatectomy. Ectopic expression of FGF15 in intestine-specific FXR

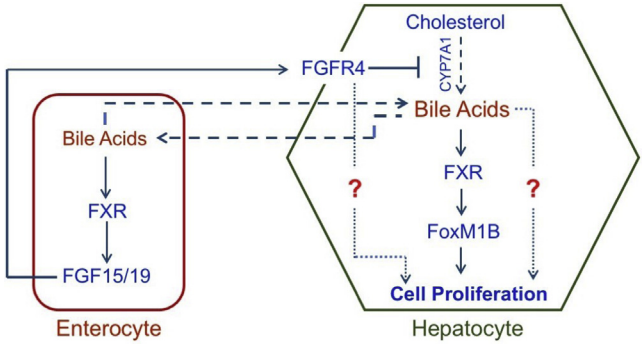

Figure 1. Role of bile acids in stimulation of liver regeneration. Schematic shows enterohepatic circulation of bile acids central to the liver-gut axis of signaling, which plays a critical role in liver regeneration. *Question marks* indicate unknown mechanisms.

null mice restored liver regeneration after partial hepatectomy. These data revealed a role for FGF15 in liver regeneration beyond its role in regulation of bile acids. This was confirmed in further studies using FGF15 knockout mice, which showed extensive liver injury, delayed regeneration, and mortality similar to global FXR knockout mice.[15] Whole body deletion of FXR results in complete disruption of the bile acid regulation system, leading to massive injury, lack of regeneration, and cancer. However, deletion only in either the liver or intestine maintains partial function by maintaining elements of the regulatory loop. These data indicate that bile acid signaling via FXR in the liver and intestine both plays a coordinated role in regulation of liver regeneration.

The role of bile acid in the regulation of liver regeneration and cytoprotection has been also demonstrated in drug-induced liver injury models. The majority of these studies have been performed in conjunction with the partial hepatectomy studies mentioned above using CCl_4 as an injury-inducing toxicant. In addition, a pro-regenerative role of bile acid has been also shown in the more clinically relevant model of acetaminophen overdose, the major cause of acute liver failure in the Western world.[6,7] Bile acids play a dual role in acetaminophen-induced acute liver failure such that they prevent development of injury and also stimulate liver regeneration. Depletion of endogenous bile acid by administration of the bile acid sequestrant cholestyramine markedly aggravates acetaminophen-induced liver injury in mice.[7,16] In addition, supplementation of cholic acid in the diet has a reverse effect by causing delayed development of liver injury after acetaminophen overdose in mice. The protective effects of endogenous bile acids during acetaminophen-induced acute liver injury are possibly due to their role in regulation of hepatic glutathione replenishment.[7,16] Glutathione is an important antioxidant molecule that is depleted during acetaminophen-induced acute liver injury, leading to massive oxidative damage caused by acetaminophen toxic metabolites. Bile acid depletion via cholestyramine feeding in mice impairs hepatic glutathione regenerative capacity, which can be fully prevented by administration of a glutathione replenishing agent, N-acetylcysteine. Bile acids also might govern glutathione homeostasis by inhibiting the cysteine dioxygenase type-1–mediated cysteine catabolic pathway via an FXR–dependent mechanism because cysteine is a rate-limiting precursor in glutathione synthesis.[16]

The protective role of bile acid in acetaminophen-induced acute liver failure may be, at least in part, due to FGF15 signaling. The FGF15 null mice display higher susceptibility to acetaminophen-induced liver injury.[17] Furthermore, treatment with engineered FGF19, which is the human homologue of FGF15, can drastically decrease liver injury after both sub-lethal and lethal overdose of acetaminophen in mice.[18] In fact, engineered FGF19 decreases liver injury even after late administration after acetaminophen overdose in mice, when N-acetylcysteine (the standard therapy for acetaminophen intoxication) is known to be ineffective, which demonstrates its potential therapeutic applicability. FGF15/19 might play a role in bile acid–driven regulation of glutathione homeostasis during acetaminophen-induced liver injury because treatment with engineered FGF19 can accelerate recovery of hepatic glutathione levels after acetaminophen overdose in mice.[18]

Apart from the direct protection from development of acute liver injury, bile acids are also involved in promoting recovery after liver injury by inducing liver regeneration. This is evident from our previous study showing that cholic acid feeding in mice can result in faster and stronger regenerative response to acetaminophen-induced hepatotoxicity, leading to early regression of injury and recovery.[7] The bile acid–driven higher proliferative response in hepatocytes in the acetaminophen model can be attributed to its regulation of cyclin D1, which is a critical regulator that governs entry into cell cycle. Whereas cholic acid feeding causes rapid and strong induction of cyclin D1 after acetaminophen overdose in mice, cholestyramine-driven bile acid depletion causes delayed and inhibited induction of cyclin D1.[7] FGF15 also contributes to bile acid–driven improved liver regeneration after acetaminophen overdose in mice. Treatment with engineered FGF19 not only attenuates liver injury but also boosts liver regeneration in the murine acetaminophen overdose model.[18] In fact, engineered FGF19 treatment improves liver regeneration and survival even after severe acetaminophen overdose that normally results in failed spontaneous regeneration and high mortality. However, it is not clear whether this is due to a direct pro-regenerative effect of FGF19 or secondary to attenuated liver injury because FGF15 null mice do not show

any alteration of liver regeneration compared with wild-type mice after treatment with equitoxic doses of acetaminophen.[17]

In summary, the evidence for pro-regenerative role of bile acid in liver regeneration and recovery is strong and compelling. The data indicate that maintaining normal levels of bile acid and activity of bile acid signaling pathways is critical for proper liver regeneration. Depletion of bile acid below physiological levels seems to remove the protection against development of injury they afford and completely eliminates pro-mitogenic activity. Although the protective role of bile acids in acute drug-induced liver injury and regeneration is clear, the mechanisms by which bile acids regulate liver regeneration needs further investigations. This is of high significance considering strategies involving bile acids have significant therapeutic implications for the treatment of acute liver failure.

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