Prevention of hepatic fibrosis by probiotics in mice

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Hepatic fibrosis is the final destination of all types of chronic liver injuries including chronic viral hepatitis, alcoholic liver diseases, non-alcoholic fatty liver diseases, and autoimmune hepatitis. It may lead to irreversible cirrhosis with portal hypertension, resulting in development of gastroesophageal varices, ascites, and hepatic encephalopathy, unless intervened at early stages.1

Hepatic fibrosis may occur predominantly in portal or periporal areas (e.g., chronic viral hepatitis) or central areas (e.g., alcoholic liver disease) according to their etiologies. Although relative distribution of extracellular matrix (ECM) within the hepatic lobules varies according to the type of the insults, hepatic fibrosis is characterized by increased deposition and altered composition of ECM components such as collagens I, III, and IV. It is well known that hepatic stellate cells (HSCs, also known as lipocytes, fat-storing, or Ito cells) are central to the process of hepatic fibrogenesis as they are the main source of ECM proteins.2 In the normal liver, HSCs produce large quantities of cytokines such as prostanoids (prostaglandin (PG) F2α, PGD2, PGI2, PGE2; leukotriene(LT)-C4, LT-B4), leukocyte mediators (macrophage colony-stimulating factor (M-CSF), monocyte chemoattractant protein-1 (MCP-1), platelet-activating factor (PAF)), acute phase components (α2-macroglobulin, interleukin-6), mitogens (hepatocyte growth factor (HGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), stem cell factor (SCF), insulin-like growth factor (IGF)-I and II, α-fibroblast growth factor (αFGF)), adhesion molecules (I-CAM-1, V-CAM-1, N-CAM), vasoactive mediators (endothelin-1 (ET-1), nitric oxide (NO)), fibrogenic compounds (transforming growth factor (TGF)-β1, 2, 3, and connective tissue growth factor (CTGF). HSCs also have other major functions such as expression of membrane receptors, cell matrix synthesis and degradation, and regulation of hepatic sinusoidal blood flow owing to their contractility. In particular, HSCs play a critical role in hepatic fibrogenesis during chronic liver injury by transforming into proliferating, fibrogenic myofibroblast-like cells. Once activated, they typically express α-smooth muscle actin (α-SMA) and over-produce collagens, leading to hepatic fibrosis.3,4

Hepatic fibrogenesis and activation of hepatic stellate cells (HSCs), the main fibrogenic cell type in the liver, almost exclusively occur in an inflammatory environment. Proinflammatory signaling
pathways contribute to fibrogenesis by activating survival pathways in HSCs and by promoting the recruitment of leukocytes. LPS is one of the strongest known inducers of inflammation and an important contributor to hepatic injury and inflammation. It has recently shown that lipopolysaccharide (LPS) and its receptor toll-like receptor 4 (TLR4) are required for hepatic fibrogenesis and that HSCs are highly responsive to LPS. Hepatic fibrosis is associated with elevated portal and systemic levels of LPS in patients as well as in mouse models. Elevations of LPS are caused by abnormalities in the intestinal mucosal structure, intestinal motility as well as changes in the bacterial flora and subsequent increases in bacterial translocation. A significant proportion of patients with hepatic fibrosis display increased levels of lipopolysaccharide (LPS), a membrane component of Gram-negative bacteria, that is among the strongest inducer for proinflammatory signaling cascades. The increased levels of LPS are due to abnormalities in the coordinated motor function of the small bowel and the resulting decreased intestinal motility, intestinal overgrowth, and the subsequent bacterial translocation. Previous studies demonstrated that gut-derived LPS and its receptor Toll-like receptor 4 (TLR4) are required for hepatic fibrogenesis. Taken together, HSCs, the main fibrogenic cell type of the injured liver, express abundant TLR4 on the cell surface and are, therefore, highly responsive to LPS, leading to liver fibrogenesis.\(^5\)

On the other hand, probiotics have shown the beneficial effects for irritable bowel disease, ulcerative colitis of inflammatory bowel diseases, and even some liver diseases such as non-alcoholic fatty liver diseases, alcoholic liver diseases, and complications of decompensated liver cirrhosis.\(^6\) In addition to the effect of reduction in intraintestinal inflammation through preventing the overgrowth of Gram negative bacteria competitively, probiotic bacteria may not only repress the bacterial translocation of pathogenic ones but also suppress the LPS movement to extraintestinal tissues. Theoretically, liver fibrogenesis can be in part prevented by probiotic treatment. To date, there is no study about the effects of probiotics in the early stage of liver fibrogenesis.

Based on the previous data that showed a crucial role for TLR4 and its signal LPS in early hepatic fibrogenesis, it was hypothesized that probiotics are more effective in preventing hepatic fibrosis than treating complications of established fibrosis. In the present study, it was investigated whether probiotics may prevent hepatic inflammation by preventing bacterial translocation before the onset and at early time points of fibrogenesis. This study also investigated probiotics as a potential treatment option for liver fibrosis. If so, probiotics will be a feasible and safe treatment option in patients.

Male Balb/c mice and C57BL/6 (8 weeks old from the Jackson Laboratory; Bar Harbor, ME, USA.) were housed in a specific pathogen-free, climate-controlled animal facility under a 12-hour light-dark cycle. Ten mice were used for each group. After a week of acclimatization, hepatic fibrogenesis was induced by ligation of the common bile duct (BDL). Control mice underwent sham operation. Briefly, mice were anesthesized with xylazine and ketamine. After midline laparotomy, the common bile duct was ligated twice with 4-0 silk and transected between the two ligations. Sham operations were performed similarly with the exception of ligating and transecting the bile duct. Ten