Liver Cirrhosis and Intestinal Bacterial Translocation

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Intestinal barrier dysfunction, facilitating translocation of bacteria and bacterial products, plays an important role in the pathophysiology of liver cirrhosis and its complications. Intestinal defense system including microbial barrier, immunologic barrier, mechanical barrier, chemical barrier, plays an important role in the maintenance of intestinal function. Under normal circumstances, the intestinal barrier can prevent intestinal bacteria through the intestinal wall from spreading to the body. Severe infection, trauma, shock, cirrhosis, malnutrition, immune suppression conditions, intestinal bacteria and endotoxin translocation, can lead to multiple organ dysfunction. The intestinal microflora is not only involved in the digestion of nutrients, but also in local immunity, forming a barrier against pathogenic microorganisms. The derangement of the gut microflora may lead to microbial translocation, defined as the passage of viable microorganisms or bacterial products from the intestinal lumen to the mesenteric lymph nodes and other extraintestinal sites. In patients with cirrhosis, primary and intestinal flora imbalance, intestinal bacterial overgrowth, intestinal mucosal barrier dysfunction, endotoxemia is associated with weakened immunity.

Key words: Cirrhosis; Intestinal barrier; Bacterial translocation; Endotoxemia

Intestinal microflora plays many important functions, among which maintaining the microbial barrier against potential pathogens is the major role. Furthermore, it can influence the motility and perfusion of the intestinal wall, stimulate the intestinal immune system and therefore the so-called common mucosal immune system, reducing bacterial translocation and producing vitamins. Immune homeostasis at mucosal level results from a controlled response to intestinal luminal antigens. Many changes, mostly an increase in bacterial overgrowth and translocation occurred in liver cirrhosis. Among the complex enteric microbial communities, a different bacterial phyla with more than 15 000 species-level bacterial phylotypes harbor in intestinal lumen.¹ Firmicutes and Bacteroidetes could make up the majority of these phylotypes in

mice and humans.²⁻⁴ Microflora maintains a symbiotic relationship for healthy individuals' intestine. Balance of microflora number, distribution and composition is regulated by the innate and adaptive immune system including host antimicrobial proteins secreted from paneth cells and intestinal epithelial cells. Dysbiosis is known as the disruption and enteric disequilibrium between the microbiota and colonized host, and is associated with diseases including inflammatory bowel disease.⁵ Intestinal dysbiosis and bacterial translocation is common in patients with advanced liver disease, and there is strong evidence that the translocation of bacteria and their products across the epithelial barrier drives liver disease progression in experiment. Cell death in the intestinal epithelium should be tightly compared. Excessive or misplaced epithelial cell death may result in barrier dysfunction and uncontrolled translocation of microbial flora components from the lumen into the bowel wall.⁶

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Microbial translocation in cirrhosis

Intestinal microflora plays many important roles, in particular to maintain the microbial barrier against established as well as potential pathogens, and furthermore, it can influence the motility and perfusion of the intestinal wall, also stimulate the intestinal immune system, and therefore the so-called common mucosal immune system, reducing bacterial translocation and producing vitamins. Bacterial translocation is defined as the passage of viable indigenous bacteria or their products, such as lipopolysaccharide and bacterial DNA, from the gut to extraintestinal sites, notably the mesenteric lymph nodes and the systemic circulation. The presence of bacterial DNA in an animal model of cirrhosis has also been established as a surrogate marker of bacterial translocation, associated with marked inflammatory response in the host.⁷ Such migration of bacteria and bacterial products has been implied in spontaneous bacterial peritonitis and sepsis in patients with end-stage liver diseases.⁸ Intestinal bacterial translocation occurs mainly in small intestine, also it was reported that the occurrence of intestinal bacterial translocation was higher in cecum of cirrhotic rats.⁹

Small intestinal bacterial overgrowth (SIBO)

SIBO was reported frequently occur in the setting of chronic liver diseases and related to the degrees of hepatic dysfunction.¹⁰ Bacterial overgrowth and translocation were observed in animal models of end-stage liver diseases.^{11,12} Among cirrhotic rats with bacterial translocation, there is a higher prevalence of bacterial overgrowth. The prevalence of overgrowth in cirrhotic rats was 67%, with 47%-78% animals also exhibiting bacterial translocation.¹³⁻¹⁵ Among experimentally-induced cirrhosis via bile duct ligation, there is an increase on number of Gram-negative bacteria in the cecum of animals, which includes *E. coli*, *Enterococci*, *Klebsiella*, *Pasteurella*, *Proteus*, *Pseudomonas* and *Shigella*. The reasons of bacterial overgrowth for liver disease is still unknown. One hypothesis suggests that impaired bile flow results in bacterial proliferation and mucosal injury in small intestines. As liver dysfunction progresses, less conjugated bile acids are produced and available

in the small intestines, which may contribute to small intestinal bacterial overgrowth. Among patients with cirrhosis of liver bile secretion, intestinal bacteria decomposition increased and intestinal bile concentrations decreased. A result suggested that small intestinal bacterial overgrowth was frequent in patients with progressive familial intrahepatic cholestasis.¹⁶ Dysmotility has also been proposed as a mechanism for stasis and bacterial overgrowth in patients with cirrhosis. Moreover, a higher gastric pH may contribute to bacterial overgrowth and translocation. Less gastric acid secretion with higher transit times from intestinal dysmotility enables bacterial colonization of the upper gastrointestinal tract. With a reduction in protective conjugated bile acids, cirrhotic patients are prone to bacterial overgrowth and translocation.

Impaired immunity

Bacterial infections in patients with cirrhosis are common, which often accounts for significant mortality. Compared with patients with compensated liver disease, those patients with decompensated cirrhosis have more frequent episodes of infection. Spontaneous bacterial peritonitis is the most common infection among these patients. The development of cirrhosis is associated with impairment of immune system. Risk factors associated with development of infections in patients with cirrhosis were severe liver failure, variceal bleeding, low ascitic protein level and prior episodes of SBP.¹⁷ Cirrhosis is accompanied by several abnormalities of both systemic and local immune system. Bureau and the whole body immune function damage is one of important factors for liver cirrhosis patients with intestinal bacterial translocation. High pressure of intestinal mucosal hyperemia portal venous was caused by damage of intestinal mucosa intravenous phagocytic cell migration. Decreased hepatic synthetic ability, immune function and phagocytosis occurred during patients with liver cirrhosis. Due to impaired phagocytosis activity of Kupffer cells and decreased activity of liver cirrhosis reticular endothelial system, bacteria can't be effectively removed and metabolites of portal

vein and peripheral circulation may not be relieved, thus endotoxin, an important reason for liver cirrhosis may cause dysfunction of immune system.

Increased permeability

Cirrhosis associated portal hypertension (PHT) may be related with increased small intestinal permeability (SIP), prone to malnutrition and bacterial translocation, which may cause septicemia, endotoxemia and spontaneous bacterial peritonitis. Thus the structure of intestinal mucosa may change and membrane permeability may increase in patients with cirrhosis. Portal hypertension may cause intestinal mucosal blood filling, intercellular broadening and thickening of mucosal muscularis edema, etc.

Pijls et al aimed to investigate both small and large intestine permeability in patients with stable compensated cirrhosis compared with healthy controls and to evaluate the expression of TJ proteins in mucosal biopsies at duodenal and sigmoid level,¹⁸ which showed that gastroduodenal and small intestine permeability did not alter, whereas large intestine permeability increased in patients with stable compensated cirrhosis. Only limited alterations were found related to the expression of TJ proteins in both small and large intestine. Structural and functional changes in intestinal mucosa may increase its permeability and contribute to the development of MT. Structural abnormalities, the most important determinant appears to be related with portal hypertension, including vascular congestion, thickened muscularis mucosa, fibromuscular proliferation, and a reduced villus/crypt ratio.¹⁹ Tight junctions normally join together at the apicolateral membranes of enterocytes, providing a mucosal barrier against paracellular diffusion of intestinal contents. Damage to these protective barriers may result in structural deficiencies that cause bacterial translocation.²⁰⁻²² Several studies considered oxidative stress on intestinal mucosa as a possible etiology for barrier dysfunction. Acute intake of alcohol has been shown to alter the epithelial barrier in colonic mucosa of rats via ethanol oxidation into acetaldehyde by enteric microflora with subsequent downstream

activation of mast cells.²³ An increase in tissue oxidative stress in jejunal, ileal and colonic mucosa was also found as early as 4 weeks after alcohol administration in rats.²⁴ Furthermore, LPS was known to activate macrophages, resulting in subsequent release of pro-inflammatory cytokines, which in turn induced liver damage.²⁵ Cytokines such as tumor necrosis factor (TNF) α , interleukin 1 β (IL-1 β), iNOS and IL-6, had been shown to be elevated in the distal ileum of mice administered alcohol for 14 ds. And IL-1 β and TNF α were known to cause a disruption of tight junctions.²⁶

Intestinal bacterial translocation and cirrhosis complications

Bacterial infections and sepsis were recognized as a distinct stage in natural progression of chronic liver disease, as they may accelerate organ failure and contributed to the high mortality observed in decompensated cirrhosis.²⁷ Bacterial infection was very common and was considered as one of the most important reasons for progression of liver failure, development of liver-related complications and mortality in patients with cirrhosis. In fact, bacterial infections may be a triggering factor for the occurrence of gastrointestinal bleeding, hypervolemic hyponatremia, hepatic encephalopathy, kidney failure and development of acute-on-chronic liver failure.²⁸ Bacterial infections were associated with increased morbidity and mortality in patients with liver cirrhosis. A wide range of bacterial infections can decompensate liver function and lead to deterioration or death in patients with cirrhosis.²⁹⁻³⁰ Bacterial infection itself, as well as inflammatory mediators, had been confirmed as potential triggering factors in many complications of liver cirrhosis, including variceal bleeding, hepatic encephalopathy, renal failure or impairment in clotting factors.³¹⁻³² Recent studies had shown that intestinal bacterial translocation in patients with liver cirrhosis complications (endotoxin hematic disease, upper gastrointestinal bleeding, hepatic encephalopathy) may play an important role in the development of spontaneous bacterial peritonitis, which was one of the most common complication with bacterial infection during the progress of liver cirrhosis. Also,

bacterial translocation may participate in upper gastrointestinal bleeding, hepatorenal syndrome, hepatic complications such as pulmonary syndrome, hepatic encephalopathy, which mainly mediated by hyperdynamic circulatory state.

Searching for effective therapeutic options: selective intestinal decontamination and probiotics

In recent years, probiotics had become a prospective alternative for the treatment of gastrointestinal and various other diseases. Modulation of the intestinal microflora through the use of selective intestinal decontamination (SID) and probiotics has been proposed as an emerging therapeutic strategy in the management of chronic liver diseases. A symbiotic relationship between the liver and intestinal tract enables the healthy status of both organs. Microflora resident in intestinal lumen plays a significant role in hepatocytes function. Probiotics assisted the maintenance of normal mucosal in gut, which not only help maintain normal function of the gut mucosa, but also protect mucosa from injurious factors such as toxins, allergens and pathogens. The beneficial effect of probiotics is mediated by multiple mechanisms, including cytoprotection, cell proliferation, cell migration, resistance to apoptosis, synthesis of proteins and gene expression. One of the important cytoprotective effects of probiotics in intestinal mucosa was to strengthen the epithelial tight junctions and preservation of mucosal barrier function. Probiotics not only enhances barrier function by inducing synthesis and assembly of tight junction proteins, but also prevents disruption of tight junctions from injurious factors. Bioactive factors released by probiotics may trigger activation of various cell signaling pathways that lead to strengthening of tight junctions and the barrier function.³³ Alterations to the type and amount of microorganisms that live in the intestinal tract can result in serious and harmful liver dysfunctions such as cirrhosis, nonalcoholic fatty liver disease, alcoholic liver disease and hepatic encephalopathy. An increased number of pathogens, especially *Enterobacteriaceae*, *Enterococci* and *Streptococci* species causes the elevation of intestinal permeability

and bacterial translocation. The presence of high levels of lipopolysaccharide (LPS) and bacterial substances in blood resulted in portal hypertension and hepatocyte damage. Several methods including the usage of antibiotics, prebiotics and probiotics can be applied to prevent the overgrowth of pathogens. Compared with prebiotic and antibiotic therapy, probiotics strains are safer and less expensive. Despite the initial reported negative influence on the course of disease, such as acute pancreatitis,³⁴ these medicines were still considered safe, which had been intensively studied and described in hepatology.³⁵ Probiotics had been suggested as a treatment for different types of chronic liver damage due to their abilities to augment intestinal barrier function and prevention of BT.³⁶⁻³⁷ Administration of probiotics was reported to reduce BT in a rat model.³⁸ Both viable and heat-killed yeast cells could prevent BT, which was considered to be the results of an immune modulatory effect and the maintenance of gut barrier integrity.^{39,40} Administration of probiotics, nonpathogenic *Escherichia coli* and gentamicin orally could decrease BT and attenuate liver damage, resulting the decrease of TNF- α , IL-6, IL-10 and IL-12.⁴¹ A diet supplemented with *Chlorella sp. microalgae* had significantly protective effects on intestinal mucosal barrier in a rat model of obstructive jaundice and reduced BT.⁴² Probiotics supplied by dietary supplements of live microbes including microorganisms *Lactobacillus* and *Bifidobacterium* could benefit the health.⁴³ These microbes are considered to enhance the production of anti-inflammatory cytokines, stimulate the secretion of antibacterial proteins and alter the intestinal microflora, ultimately reducing the production and translocation of endotoxin.⁴⁴ A randomized prospective trial demonstrated that probiotic treatment (*Bifidobacterium bifidum* and *Lactobacillus plantarum*) for five days resulted in restoration of colonic bowel flora and improvement of serum indexed in patients with alcoholic liver injury.⁴⁵ Prebiotics are complex carbohydrates that can not be digested by pancreatic or intestinal enzymes and ultimately metabolized by the gut microflora. Commonly administered prebiotics are fructo-oligosaccharides (FOS), galacto-oligosaccharides and

lactulose, which are commonly applied in patients with decompensated cirrhosis for the treatment and prevention of hepatic encephalopathy.

Conclusions

Intestinal microflora is not only involved in the digestion of nutrients, but also in local immunity, forming a barrier against pathogenic microorganisms. Alterations of intestinal microbiota seem to play important roles in the induction and promotion of liver damage and the development and severity of nonalcoholic steatohepatitis (NASH). Bacterial overgrowth, immune dysfunction, alteration of luminal factors and alteration of intestinal permeability are all involved in the pathogenesis of NASH and its complications, including progression to cirrhosis, infections, hepatic encephalopathy, SBP and renal failure. The derangement of gut microflora may lead to microbial translocation, defined as the passage of viable microorganisms or bacterial products (i.e., LPS, lipopeptides) from the intestinal lumen to the mesenteric lymph nodes and other extraintestinal sites. A better understanding of cell-specific recognition and intracellular signaling pathway involved in sensing gut-derived microbes may contribute to achieve an optimal balance in gut-liver axis and amelioration of liver diseases. Therefore, early diagnosis of intestinal barrier dysfunction may reduce the incidence of bacterial translocation, which is significant for treatment and prevention of diseases. Therapeutic strategies aiming at modulation of gut microflora and reduction of MT should focus on antibiotic-based options such as selective intestinal decontamination, and nonantibiotic-based options such as prokinetics and probiotics.

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