Gut Microbiota: Its Role in Hepatic Encephalopathy

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Ammonia, a key factor in the pathogenesis of hepatic encephalopathy (HE), is predominantly derived from urea breakdown by urease producing large intestinal bacteria and from small intestine and kidneys, where the enzyme glutaminases releases ammonia from circulating glutamine. Non-culture techniques like pyrosequencing of bacterial 16S ribosomal ribonucleic acid are used to characterize fecal microbiota. Fecal microbiota in patients with cirrhosis have been shown to alter with increasing Child-Turcotte-Pugh (CTP) and Model for End stage Liver Disease (MELD) scores, and with development of covert or overt HE. Cirrhosis dysbiosis ratio (CDR), the ratio of autochthonous/good bacteria (e.g. *Lachnospiraceae, Ruminococcaceae* and *Clostridiales*) to non-autochthonous/pathogenic bacteria (e.g. *Enterobacteriaceae* and *Streptococcaceae*), is significantly higher in controls and patients with compensated cirrhosis than patients with decompensated cirrhosis. Although their stool microbiota do not differ, sigmoid colonic mucosal microbiota in liver cirrhosis patients with and without HE, are different. Linkage of pathogenic colonic mucosal bacteria with poor cognition and inflammation suggests that important processes at the mucosal interface, such as bacterial translocation and immune dysfunction, are involved in the pathogenesis of HE. Fecal microbiome composition does not change significantly when HE is treated with lactulose or when HE recurs after lactulose withdrawal. Despite improving cognition and endotoxemia as well as shifting positive correlation of pathogenic bacteria with metabolites, linked to ammonia, aromatic amino acids and oxidative stress, to a negative correlation, rifaximin changes gut microbiome composition only modestly. These observations suggest that the beneficial effects of lactulose and rifaximin could be associated with a change in microbial metabolic function as well as an improvement in dysbiosis. (J Clin Exp Hepatol 2015;5:S29–S36)

Pathogenesis

Hepatic encephalopathy (HE) is brain dysfunction caused by liver insufficiency and/or porto-systemic shunts. It manifests as a wide spectrum of neurological or psychiatric abnormalities ranging from subclinical alterations to coma.[1] For decades the pathogenesis of HE has been believed to be related to high levels of ammonia resulting from decreased detoxification in liver as a result of liver failure and/or due to the presence of porto-systemic shunts in patients with cirrhosis.[2] Recently, it has been shown that inflammation and oxidative and nitrosative stress play a role in the development of HE. There is evidence that HE is linked to alterations in gut microbiota and their by-products such as amino acid metabolites (indoles, oxindoles), endotoxins, etc. These factors, superimposed on a background of leaky intestinal barrier and immune dysfunction, are involved in the pathogenesis of HE.[3] Thus, modulation of gut microbiota using lactulose, probiotics and nonabsorbable antibiotics is likely to play a role in the management of HE. Insight into the gut–liver axis as well as influence of gut microbiota on liver and *vice versa* is of utmost importance to understand the role of gut microbiota in pathogenesis of HE.

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Abbreviations: CDR: cirrhosis dysbiosis ratio; fMRI: functional MRI; HE: hepatic encephalopathy; IL: interleukin; LGG: Lactobacillus GG strain; LPO: left parietal operculum; MELD: model for end stage liver disease; MHE: minimal hepatic encephalopathy; MRS: magnetic resonance spectroscopy; PAMPs: pathogen-associated molecular patterns; PCR: polymerase chain reaction; RCT: randomized controlled trial; RNA: ribonucleic acid; SBP: spontaneous bacterial peritonitis; SIBO: small intestinal bacterial overgrowth; SIRS: systemic inflammatory response syndrome; TNF: tumor necrosis factor
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GUT–LIVER AXIS

Gut and liver share a close relationship. The liver, which receives 70% of its blood supply from the gut through the portal venous system, is significantly affected by the gut and its contents. The liver also influences intestinal functions through several mechanisms in physiological and pathological conditions. The gut and the liver have a pivotal role in absorption and metabolism of various nutrients and drugs. Abnormal bile acid homeostasis may lead to diarrhea and bacterial overgrowth. Cirrhosis contributes to bacterial overgrowth by decreasing gastrointestinal motility which leads to an increased risk of infections including spontaneous bacterial peritonitis (SBP).

GUT MICROBIOTA

The term microbiota is used to describe the complete population of microorganisms that inhabit the body in various locations including the gut. It numbers approximately 10^{14} microbial cells, and include bacteria, viruses, protozoa, etc.[4–6] It performs vital functions related to nutrition and metabolism, including food processing, digestion of complex carbohydrates and vitamin synthesis.[7,8] It also secretes a number of biologically active compounds which perform various functions like inhibition of pathogens and metabolism of toxic compounds including ammonia.[8]

CHANGES IN GUT MICROBIOTA IN HEALTH

Earlier culture and biochemical typing were the gold standards for the identification of bacterial species; however for last two decades culture-independent techniques have revolutionized knowledge of the gut microbiota. These techniques are based on sequence divergences of the small subunit ribosomal ribonucleic acid (16S rRNA) and are able to demonstrate the microbial diversity of the gut microbiota, providing qualitative as well as quantitative information on bacterial species and changes in the gut microbiota in health and disease.[9,10] Changes have been described in composition of the human gut microbiota from early infancy to old age as well as due to the effect of environmental influences. Gastrointestinal tract is sterile at birth, when colonization of the gut takes place with the mother's vaginal canal flora and thereafter from the surrounding environment. The diversity of microbiota depends upon factors like mode of delivery (vaginal birth versus assisted delivery), gestational age, diet (breast versus formula milk), antibiotics, hygiene and sanitation.[11,12] The gut microbiota of newborn babies has relative dominance of the phyla *Proteobacteria* and *Actinobacteria*; later, the microbiota becomes more diverse with relative dominance of *Firmicutes* and *Bacteroides*, which are typical of adult microbiota.[7,13] Recently, three different genera 'enterotypes', enterotype 1 (*Bacteroides*), enterotype 2 (*Prevotella*) and enterotype 3 (*Ruminococcus*), have been described in the adult gut microbiome from different continents. Their dominance is likely to be independent of factors like sex, age, race and body mass index.[14] Once maturity is reached, the microbiota remains stable until old age when changes can be seen, likely due to changes in diet and digestive metabolism.[15,16]

CHANGES IN GUT FUNCTION AND MICROBIOTA IN CIRRHOSIS OF LIVER

There are multiple mechanisms which are involved in defective gut functions and altered microbiota in patients with cirrhosis. These include impaired small intestinal motility, increased intestinal permeability, impaired antimicrobial defense and small intestine bacterial overgrowth (SIBO). Additionally, decreased bile acids, due to decreased synthesis and defective enterohepatic circulation, contribute to altered gut microbiota.[17,18]

Gut Motility

Evidence suggests that gastrointestinal motility is delayed in patients with liver cirrhosis.[19–22] Several mechanisms have been proposed, including bowel wall edema, autonomic dysfunction, altered concentration of intestinal active peptides and neurotransmitters, and altered intestinal myoelectrical activity.[21,23,24] The abnormal antroduodenojejunal pressure wave leads to increased risk of SIBO in patients with portal hypertension.[25] In addition, HE itself may influence small bowel transit, as transit time has been shown to improve following treatment of HE.[26]

Intestinal Permeability and Impaired Antimicrobial Defense

In cirrhosis, changes in intestinal tight junctional proteins have been described; though the pathophysiology is not clear, alcohol metabolites and proinflammatory cytokines have been postulated to result in leaky intestines.[4] In addition, impaired antimicrobial defense mechanisms, involving intestinal Paneth cells, contribute to the development of bacterial translocation in cirrhosis.[27]

Small Intestinal Bacterial Overgrowth

There is a very high prevalence of SIBO (35–61%) in patients with cirrhosis.[21,22,28,29] The pathogenesis of SIBO in these patients is postulated to include impaired intestinal motility, reduced gastric acid secretion, luminal IgA deficiency and malnutrition.[30] SIBO correlates with the severity of chronic liver disease, is shown to be linked to minimal and overt HE and confers increased risk for development of SBP through bacterial translocation across the gut.[21,22,31,32]

Gut Microbiota in Cirrhosis

In prior studies, culture-based techniques were used for the characterization of gut flora in cirrhosis. Chen et al[33] were the first to use pyrosequencing of the 16S rRNA V3 region and real-time quantitative polymerase chain reaction (PCR) to characterize the fecal microbiota of patients with cirrhosis. They demonstrated that, when compared with controls, in patients with cirrhosis the proportion of phylum *Bacteroidetes* was significantly reduced, whereas *Proteobacteria* and *Fusobacteria* were abundant. At the family level *Enterobacteriaceae, Veillonellaceae,* and *Streptococcaceae* were increased and *Lachnospiraceae* was less prevalent. They observed a positive correlation of Child-Turcotte-Pugh (CTP) score with *Streptococcaceae* and a negative

