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Microbiome alterations observed in liver diseases present opportunities for potential fecal transplantation

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ABSTRACT

Many disease processes lead to chronic liver disease, however, progress has been made regarding common findings amongst these disease processes that may suggest a path forward for treatment. In particular, common alterations in the intestinal microflora of patients with different etiologies of liver disease may provide a clue as to the pathogenesis of these disorders as well a potential therapy. Data is still scant at this point, however, what is available suggests a promising opportunity for future studies to expand upon what has been demonstrated.

Keywords: Fecal, stool, transplantation, liver, microbiome

Review

NORMAL HUMAN INTESTINAL MICROBIOME

The human GI tract is colonized by an estimated hundreds of species of bacteria; this microflora has been associated with facilitating digestion and aiding in provision of nutrition, defense against pathogens, and development and maturation of colonic epithelium (1-3). The microflora encountered in a healthy gut appears to vary in composition for each individual, however, some generalizations can be made. Various phyla of Firmicutes appear to make up the majority of the microflora in human guts; estimates vary from ~40-70% (1-5). These bacteria, a collective largely made up of various Clostridial strains along with Fusobaceterial, Faecalbacterial, and other various gena (1,5). Also common, though in lower abundance to the Firmicutes, are the Bacteroides phyla, with various studies estimating their prevalence at ~25% (1-6). Of interest, alterations in the ratio of these two groups appear to be associated with various disease states, most extensively studied in C. difficile colitis (1-3). It appears that there is a 'healthy' or 'normal' balance between these majority phyla, changes in which are associated with pathologic states such as liver disease (1-7). Changes in the microbiome may be induced by factors other than pathology. A 2015 study used pyrosequencing to quantify changes in the microflora in healthy patients receiving clindamycin, ciprofloxacin, as well as a placebo group over 12 months (2). It was demonstrated that overall species diversity decreased in groups receiving either antibiotic, however, not in the placebo group. While it is of note that the microflora appear to remain over time in a healthy patient, it also suggests that changes in microbiome observed in the disease states to be discussed may be influenced by interventions taken by clinicians in those pathologic states.

INTESTINAL MICROBIOME ALTERATIONS IN LIVER DISEASES

Microbiome alterations in cirrhosis

While a variety of disease processes lead to hepatic fibrosis, some generalizations can be made regarding changes in intestinal gut flora exhibited in cirrhotic patients. 95% of the gut microbiota of humans consists of Firmicutes, Bacteroides, and Actinobacteria phyla (4). A 2014 study noted that patients with cirrhosis and hepatic encephalopathy were demonstrated to have relatively reduced abundance of native taxa including Lachnospiraceae, Ruminococcaceae, and a Clostridial strain while having a relatively higher abundance of others such as Enterobacteriaceae and higher proportion of Firmicutes (5). Multiple studies have demonstrated

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this same trend across cirrhotics due to alcohol, HCV, and cryptogenic etiologies (6-9). The ratio of native versus non-native bacterial strains in cirrhotic patients is referred to as cirrhosis dysbiosis ratio (CDR). The CDR can be calculated by quantifying bacterial strain from stools of control patients, patients with compensated cirrhosis, and patients with decompensated cirrhosis. Lower model for end-stage liver disease (MELD) scores were associated in patients in the aforementioned study with higher burden of native microbiome, while increasing morbidity and mortality were seen with increasing dysbiosis. In this same prospective 2014 study, decreased microbial diversity and decreased CDR were associated with increased MELD. A similarly organized 2011 study did not use CDR, however, it did correlate decreased microflora diversity in patients with cirrhosis compared to controls. Diversity was even less in decompensated cirrhotic patients with hepatic encephalopathy (10). The pathology associated with altered CDR is thought to be secondary to reduced production of fatty acids which function to reduce colonic inflammation and reduce intestinal permeability to endotoxins (11). A 2016 study demonstrated this altered CDR as well as increased small intestinal permeability in patients with hepatitis C virus (HCV) cirrhosis, however, a recent study in 2016 demonstrated persistent, stable dysbiosis in patients with HCV cirrhosis treated with peginterferon and ribavirin with sustained virologic response 12-46 months after treatment (8,9). Given the similar profile of dysbiosis across a spectrum of etiologies of cirrhosis, the previously referenced two studies conjointly suggest the etiology of cirrhosis may not be as consequential as the result alteration in microflora. A study examining CDR in alcoholic cirrhosis abstaining from alcohol compared to those continuing to drink alcohol might reinforce this hypothesis. Many current therapies used to alleviate symptoms of chronic liver disease, such as lactulose or rifaximin, indirectly affect the composition of the microbiome (12,13). Fecal transplantation, conversely, would induce its therapeutic effect via direct alterations of the intestinal microbiome.

Microbiome alterations in nonalcoholic fatty liver disease

It also appears that other chronic liver disease states, such as non-alcoholic fatty liver disease (NAFLD), are associated with changes in the intestinal microflora as well. It has been demonstrated that obesity and metabolic syndrome, well known precursors to NAFLD, correlate with significant changes in intestinal diversity (14-16). Generally, it appears that relative levels of Bacteroides and Firmicutes appear to differ amongst lean and obese mice. Obese mice had higher proportions of Firmicutes, similarly observed in human populations. Researchers were able to demonstrate that colonizing germ free mice with the microbiome of obese mice led to increased total body fat despite any changes in diet (16,17). Of interest, a 2012 study demonstrated male patients with metabolic syndrome undergoing fecal transplantation from lean male donors had significantly improved insulin sensitivity over a 6-week period (18). Quantifying fecal microbiota appeared to demonstrate significant increase in ***Roseburia intestinalis***, as well as significantly increased proportions of Firmicute density. To date, no study has been performed assessing the effect of fecal transplantation from a lean donor with "typical" microbiome composition to a recipient with known NAFLD with a "typical" microbiome of this phenotype. Such a study may demonstrate therapeutic effect on liver pathology with 'normalization' of gut microflora.

Microbiome alterations in primary sclerosis

Similarly, while many patients with primary sclerosing cholangitis (PSC) have known associated inflammatory bowel disease (IBD), there is a subset of patients without any known inflammatory colopathy. It has been postulated that chronic cholangitis secondary to endotoxin production or inflammatory response to microbial metabolites may lead to development of PSC (19-21). Generally, alterations in microbial diversity or altered microbial metabolism is thought to produce an aberrant cholangiocyte response, either in terms of inflammation or apoptosis, and/or affecting the cholangiocyte's ability to respond appropriately to injury over time, potentially resulting in chronic liver disease (4,6,21). Biomolecules such as lipopolysaccharide, lipoteichoic acid and bacterial DNA fragments have been detected in bile, cholangiocytes and portal tracts of patients with chronic cholestatic liver disease (4,5,19). Investigations into relative microbiome diversity amongst patients with PSC, both PSC and IBD, and control patients yielded generally complementary results, demonstrating significantly reduced Clostridial strains (22,23). Also noted in PSC patients without markers for IBD demonstrated overall decreased diversity with relatively increased Lactobacillus, Enterococcus, and Fusobacterium (24-26) populations.

FECAL TRANSPLANTATION AND LIVER DISEASES

Fecal transplantation, cirrhosis, and hyperammonemia

There is a known association between hyperammonemia and hepatic encephalopathy associated with worsening prognosis in chronic liver disease. Evidence is scant, however, studies have been done demonstrating that fecal transplantation may be able to replace urease-producing bacteria in the human gut with a more benign population and mitigate the encephalopathic symptoms. As alluded to, mammalian genes do not encode for urease enzymes; ammonia is produced by bacterial urease activity. This ammonia is either reabsorbed or excreted in the feces, suggesting a correlation between fecal and systemic ammonia levels (27). In a 2014 study, mice were inoculated via fecal transplantation with slurry of bacteria with low urease gene activity (28). Significantly reduced fecal ammonia levels were noted in the experimental arm of this study after inoculation. No urease activity was seen in pellets from mice colonized with this low urease-producing colony; this response was also sustained for at least 80 days.

Concomitantly, fecal ammonia levels were lower in mice treated with this low urease activity colony than control mice

treated with low protein diet alone. After these initial measurements were done, hepatic injury was induced using thioacetamide (TAA); the mice having undergone fecal transplantation demonstrated a reduction in fecal ammonia levels and reduced mortality in response to high dose TAA compared with control mice yielding conventional microbiota. Low dose TAA was also introduced at escalating doses over 7 weeks starting 3 weeks after fecal transplantation. Compared with controls, the mice having undergone fecal transplantation demonstrated reduced mortality rates maintained over the 7 week period during which hepatic fibrosis developed in both groups. This research suggests a possible protective effect on cirrhotic patients. Of interest, a 2015 case report appears to reinforce this impression (29). This report notes a 57-year-old patient with cirrhosis secondary to alcohol and HCV with grade 1-2 hepatic encephalopathy with noted response to lactulose and rifaximin, however, experienced issues of inaccessibility to rifaximin and noted subsequent decline in cognitive status. This patient underwent fecal transplantation supplied by universal stool donor with noted improvement in serum ammonia levels, cognition, and function as measured by a variety of clinical exams. Fecal analysis of his microbial diversity demonstrated significant change in the recipient's microbiome to reflect that of the donor, although the changes do not appear to be in line with the aforementioned expected changes in microbiome composition. Regardless, the change in composition correlated with improved degree of encephalopathy. Unfortunately, 10 weeks after his last fecal transplantation (week 14), the patient's cognitive status had reverted to his baseline encephalopathy. There is no data available to determine whether the patient's microbiome had reverted from a population closer to his original composition and further from the donor's. Regardless, such a case presents an opportunity for future research on a greater number of patients and prolonged courses or intervals of fecal transplantation to assess response in terms of both symptomatic control as well as maintenance of microbial diversity.

Fecal transplantation and other disease states with hepatic consequence

Given the aforementioned evidence of altered microbiome in PSC patients, fecal transplantation may have a role in this disease state as well. Evidence of improved ALP levels in PSC patients treated with low dose vancomycin (21), as well as a case of PSC in a patient status post liver transplantation with resolution after administration of vancomycin (30), it suggests that fecal transplantation may have a role in alleviating chronic inflammation in PSC. Probiotics may also have a role in PSC, however, studies demonstrate discordant results, perhaps due to a variety of treatment durations and multiple options of probiotic compositions (31). Fecal transplantation may be a more direct means of studying the effect of intestinal microflora on this disease process. An ongoing clinical trial at Brigham and Women's Hospital is looking at the impact of fecal transplantation on patients with PSC; primary outcome measures include analysis and comparison of the recipient's microbiome pre and post-transplant as well as a comparison of the post transplantation biome to the donor's microbiome. The study will also trend liver chemistry over 3 months, utilizing as therapeutic success a 50% improvement in alkaline phosphatase, total bilirubin, alanine aminotransferase, or aspartate aminotransferase. This study is set to conclude in April 2017 (32).

OPPORTUNITIES FOR FUTURE RESEARCH

Given the above information, it appears there is promising yet scant evidence for the use of fecal transplantation in chronic liver disease by either altering the composition of the microbiome or its metabolism. While it appears well established that dysbiosis is associated with multiple etiologies of chronic liver disease, further studies with larger sample sizes, prolonged duration and treatment, and follow up of microbiome identification and quantification will be needed to adequately assess the impact of dysbiosis on these disease processes.

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