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Microbiome therapeutics for hepatic encephalopathy

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Summary

Hepatic encephalopathy (HE) is a complication of cirrhosis characterised by neuropsychiatric and motor dysfunction. Microbiota-host interactions play an important role in HE pathogenesis. Therapies targeting microbial community composition and function have been explored for the treatment of HE. Prebiotics, probiotics and faecal microbiota transplant (FMT) have been used with the aim of increasing the abundance of potentially beneficial taxa, while antibiotics have been used to decrease the abundance of potentially harmful taxa. Other microbiome therapeutics, including postbiotics and absorbents, have been used to target microbial products. Microbiome-targeted therapies for HE have had some success, notably lactulose and rifaximin, with probiotics and FMT also showing promise. However, there remain several challenges to the effective application of microbiome therapeutics in HE, including the resilience of the microbiome to sustainable change and unpredictable clinical outcomes from microbiota alterations. Future work in this space should focus on rigorous trial design, microbiome therapy selection, and a personalised approach to HE.

Keywords

lactulose; cirrhosis; rifaximin; fecal transplant; ammonia; probiotic

Introduction

Hepatic encephalopathy (HE) is a complication of cirrhosis characterised by neuropsychiatric and motor dysfunction. Manifestations can range from subtle (minimal

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PB and AL: conception and design; PB: writing original draft; ET, VY, AL: critical revisions.

Conflict of interest

Patricia P. Bloom serves as a consultant for Synlogic. ASL: no conflict. VBY serves as a consultant to Vedanta, Bio-K+ International and Pantheryx. Elliot Tapper has served as consultant for Axcella, Kaleido, Mallinckrodt, Novo Nordisk, Allergan, and Takeida. He has received unrestricted grants from Valeant and Gilead.

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Supplementary data

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HE) to severe (overt HE) and even coma. HE is associated with considerable patient and caregiver burden, decreased quality of life, and poor survival.¹⁻³ HE therapeutics represent one of the clearest unmet needs in cirrhosis care, where at least 50% of patients on current optimal therapy have breakthrough episodes.

Emerging data closely link HE pathophysiology to the gut microbiome. The role of microbiota in HE therapeutics has undergone a revolution, shaped by several landmark trials and major advances in microbiology.⁴⁻¹² Novel sequencing and analytic methods have enabled the field to move from simply cataloguing the gut ecosystem to understanding the complex interactions between gut microbiota members, and how they react to environmental factors and to therapies. In parallel, we now have results from new investigations on microbiome-targeted therapies for other gastrointestinal and neurological diseases, which influences our understanding of the potential for microbiome therapies in HE.

In this review, we will discuss the limitations of current therapies for HE and the potential for novel microbiome therapies to improve outcomes.

Pathogenesis of hepatic encephalopathy and potential therapeutic targets

In health, the host and microbiota are connected by a shared physical space (the gut), but also by a shared metabolism.¹³⁻¹⁵ There are numerous examples of the host supplying microbiota with life-sustaining nutrients, and separately the microbiota providing key metabolism services to the host. Key elements of protein, lipid, and carbohydrate metabolism are symbiotic between host and microbiota. One important example is that bacteria ferment non-digestible polysaccharides, from the host diet, and produce short-chain fatty acids (SCFAs). In turn, SCFAs are an essential energy source for host colonic epithelium – they increase intestinal epithelial production of tight junction proteins and mucin, both of which contribute to barrier function.¹⁶⁻¹⁸ Intestinal bacteria also play a central role in bile acid metabolism and separately in developing host epithelial immune responses, both of which have been associated with intestinal barrier function. Bacterial products are not universally beneficial to the host, a notable example being lipopolysaccharide (LPS). However, bacterial products are often not absorbed into the systemic circulation due to a robust intestinal barrier. Ammonia, a product of bacterial urea and protein metabolism, is detoxified via the urea cycle in the healthy liver, keeping circulating ammonia levels low.

In cirrhosis and HE, the shared metabolism between the host and microbiota is altered (Fig. 1). SCFA-producing species, *Anaerostipes caccae*, *Bacteroides eggerthii*, and *Clostridial species* are depleted in patients with HE, who appear to have lower intestinal SCFA levels.¹⁹ Intestinal bile acid concentrations are reduced in cirrhosis, as is microbiota-induced bile acid metabolism.²⁰⁻²⁴ Finally, intestinal immune function is altered in advanced cirrhosis.²⁵ These changes to the host-microbiota relationship influence intestinal barrier function and permeability, enhancing the translocation of neurotoxic factors. Investigations at the intersection of neurology and microbiology have identified several pathways that link microbiota to neuropsychiatric disease.²⁶ Altered bile acid signalling may impact blood-brain-barrier permeability and neuroinflammation.²⁷ In HE, ammonia was implicated early on as one such molecule with neurotoxic effects. In cirrhosis, portosystemic shunting

and impaired hepatic ammonia metabolism leads to increased serum ammonia levels, with additional contributions from renal and muscle sources.²⁸ Ammonia is able to cross the blood-brain-barrier and enter astrocytes where it is converted to glutamine which acts as an osmole, causing astrocyte swelling, oxidative stress, cellular dysfunction and ultimately neurological deficits.²⁹ *Streptococcus salivarius* has been identified as a gut bacterial species that produces ammonia and is more abundant in patients with minimal HE than those without.³⁰ Overt HE episodes are characterised by dramatic changes to gut microbiota composition, alongside changes in microbiota-mediated ammonia metabolism.³¹ Systemic inflammation has also been established as a major contributing factor to HE pathogenesis,^{32,33} synergising with ammonia to enhance neurotoxicity via increased blood-brain-barrier permeability and cerebral oxidative stress.³⁴ Despite advances in recent decades, there are likely additional unidentified mediators influencing the relationship between the microbiota, intestinal barrier, and brain in HE.

Key point

Alterations in shared hostmicrobiota metabolism, intestinal permeability, and host immune response play a role in HE pathogenesis.

Given the role of microbiota-host interactions in the pathogenesis of HE, therapies targeting microbial community composition and function have been explored. A variety of approaches have been investigated, including directly targeting the microbiota – either by increasing the abundance of beneficial taxa or decreasing harmful taxa (Table 1). Other approaches have targeted the products of microbiota. Yet unstudied, future therapies may directly target host intestinal barrier function and immune regulation. Precision medicine may be on the horizon for HE, wherein therapies target specific host or microbiota deficits in a patient-specific fashion.

Microbe-targeted therapies: Increase potentially beneficial taxa

Prebiotics

Prebiotics are substrates selectively utilised by host microorganisms that confer a health benefit.³⁵ Prebiotics are most often non-absorbable carbohydrates, able to be fermented by luminal bacteria.¹³ Fermentation of prebiotics leads to increased abundance of beneficial taxa that can utilise these substrates, produce SCFAs, and reduce pH in the intestinal lumen.³⁶ Increased biomass of beneficial taxa reduces available nutrients for invading microbial pathogens.¹³ The reduced pH caused by prebiotics also inhibits pathogen growth. *In vitro* and human studies showed that prebiotics improve intestinal barrier function by stimulating mucus-producing goblet cells, augmenting tight junction assembly, and mitigating inflammation.^{37–40} Lactulose, a non-absorbable disaccharide and a prebiotic, is the primary therapy for HE. While the literature on its actions is not uniform, it appears that lactulose's benefits in HE are mediated through changes in intestinal microbes, likely a result of its pH-lowering effect and positive pressure on beneficial taxa.

Lactulose is an effective treatment for HE. A Cochrane review including 38 randomised controlled trials of non-absorbable disaccharides demonstrated their beneficial effect on HE

(risk ratio 0.58; 95% CI 0.50–0.69).⁷ A more recent network meta-analysis of 25 trials found that when comparing lactulose, rifaximin, probiotics, and L-ornithine L-aspartate (an ammonia-lowering agent) for the treatment of minimal HE, lactulose was the only agent able to meet all 3 endpoints: reverse minimal HE, prevent overt HE, and improve quality of life.⁹ However, many patients experience breakthrough overt HE episodes while on lactulose.⁴¹

Early culture-based studies demonstrated that lactulose promotes the growth of the beneficial taxa *Lactobacillus* and *Bifidobacteria*.^{42–44} Lactulose fermentation by these beneficial taxa requires increased bacterial amino acid synthesis using ammonia as the substrate, leading to reduced luminal ammonia concentrations.^{45–48} Increased amino acid synthesis with lactulose does not occur in germ-free rats, supporting the role of bacteria in this process.⁴⁹ Lactulose transits through the small intestine largely unchanged and is fermented by colonic bacteria,^{28,50} leading to SCFA production and subsequent colonic acidification.⁴⁴ In patients with minimal HE, lactulose decreases bacterial DNA in the serum and improves neurocognitive test scores, presumably through changes to bacterial composition and improved intestinal permeability – the latter of which may be a result of increased SCFA production.⁵¹ In the setting of colonic acidification from SCFAs, ammonia production from gram-negative bacteria decreases, likely reflecting diminished metabolic activity as well as growth inhibition of those bacteria.⁴⁵ A recent multicentre study of lactulose for minimal HE found no significant change in microbial composition (using 16S rRNA sequencing); however, those with a clinical response experienced a significant decrease in certain Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria relative to non-responders.⁵² Lactulose may reduce serum ammonia through additional mechanisms, including trapping ammonium ions in the colon.^{16,25,53} Novel prebiotics such as synthetic glycans, which are in development, appear to be more potent than lactulose in lowering ammonia production; further studies will be required to determine their efficacy in treating HE.⁵⁴

Probiotics

Probiotics are living microorganisms that, when administered in adequate amounts, confer a health benefit on the host.⁵⁵ For decades there has been interest in using probiotics to treat HE, ranging from yogurts to probiotic powders and encapsulated probiotic strains.^{4,56–58} There are 3 evidence-based mechanisms by which probiotics may improve HE: improving intestinal barrier function, immune modulation, and decreasing portal hypertension. First, probiotics in HE may enhance tight junction protein production or integrity, thus improving intestinal barrier function and reducing translocation of bacterial products into the systemic circulation. In a murine model of colitis, VSL#3 (a combination of 8 bacterial strains) and separately *Escherichia coli* (*E. Coli*) Nissle 1917 prevented an increase in intestinal permeability by maintaining tight junction expression and suppressing apoptosis.^{59,60} Probiotics in rodent models of alcohol-related liver disease and non-alcoholic steatohepatitis were also found to reduce serum LPS levels and to increase tight junction expression.^{61–63} Furthermore, a randomised trial of *Lactobacillus GG* in patients with minimal HE reduced serum LPS.⁴ Second, probiotics interact with the host intestinal epithelium, and have an established role in immune modulation.^{13,64} Systemic inflammation enhances the cerebral effect of ammonia and exacerbates HE symptoms.^{32,33} One trial of VSL#3 in patients

with recent overt HE found that the probiotic reduced serum cytokines (tumour necrosis factor [TNF]- α , interleukin (IL)-1 β , and IL-6 levels) in the subgroup (24%) of patients who completed 24 weeks of follow-up.⁸ Part of the immune regulation provided by probiotics may be specific to neutrophil function. Chronic neutrophil activation may lead to neutrophil exhaustion in cirrhosis, leaving patients vulnerable to infection and poor survival. Infection is a very common precipitant of HE. Supplementation with a probiotic mixture of *Bifidobacterium*, *Lactobacillus*, and *Lactococcus* strains in patients with cirrhosis compared to placebo led to increased neutrophil production of reactive oxygen species, thus restoring neutrophil function⁵⁸ – a benefit also seen in studies of *Lactobacillus casei* Shirota as well as with a probiotic mixture of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*.^{10,65} Third, probiotics may reduce portal hypertension. One study found that VSL#3 decreased serum and hepatic vein TNF- α levels in patients with large oesophageal varices, and led to an additional reduction in hepatic venous pressure gradient beyond propranolol monotherapy.⁶⁶ Probiotics additionally produce organic acids, antimicrobial compounds, and bile salt hydrolases – mechanisms that should be explored in future studies of probiotics for HE.¹³

Two recent large meta-analyses found that probiotics improve HE symptoms, reverse minimal HE, lead to fewer episodes of overt HE, and lower ammonia levels, though evidence is low to moderate quality as all but 2 trials are at high risk of bias.^{9,11} In addition, probiotics do not impact patient quality of life or mortality, and were not superior to lactulose for all outcomes. Clinical trial data on probiotics for HE is difficult to compare because each trial uses different probiotic strains.⁶⁷ Furthermore, the low level of colony-forming units in most commercial probiotic formulations limits optimism that probiotics are sufficient to overtake the resident microbial community structure of cirrhosis and HE.¹⁶

Key point

Potential targets for HE microbiome therapeutics include microbiota abundance, microbial products, intestinal barrier function, and host immune responses.

Bacterial genomes can be manipulated by modern genetic tools for therapeutic purposes.⁶⁸ SYN1020 is an *E. coli* Nissle 1917 strain genetically engineered to convert ammonia to L-arginine.⁶⁹ The engineered probiotic successfully reduced ammonia levels in preclinical studies and was well tolerated in a phase I study. However, according to reporting on [ClinicalTrials.gov](https://www.clinicaltrials.gov), SYN1020 did not lower blood ammonia levels in patients with cirrhosis. This should not be taken as a failure of all engineered probiotics for HE; other engineered probiotics could impact different aspects of HE pathogenesis, including immune regulation or intestinal barrier integrity.

Faecal microbiota transplant

Faecal microbiota transplant (FMT) is the transfer of processed stool from a healthy donor to a recipient. Three small trials have investigated FMT for the treatment of HE, with early evidence suggesting a potential clinical benefit.^{5,6,70} All 3 trials enrolled patients with a history of overt HE on lactulose therapy and, in many cases, rifaximin. The first

trial randomised patients to standard of care vs. broad-spectrum antibiotics followed by a single FMT enema, and the second trial by the same study team (Bajaj *et al.*) randomised patients to 1 day of oral FMT capsules vs. placebo capsules.^{5,6} The FMT in both trials was derived from the same donor. The primary outcome of these 2 trials evaluated the safety of FMT and found no safety concerns, aside from a reversible and small increase in model for end-stage liver disease (MELD) score after broad-spectrum antibiotics. The patients who received antibiotics and an FMT enema improved on 2 validated cognitive tests and had fewer episodes of overt HE during long-term follow-up compared to the standard of care arm.^{5,71} Antibiotics were given prior to FMT to decrease host bacterial burden and to enable FMT colonisation,^{72–75} and were not given to the control group, limiting the ability to interpret the relative contributions of antibiotics and FMT to cognitive improvement. Lack of blinding also introduces observer bias. In the second trial by Bajaj *et al.*, the patients who received oral FMT capsules improved in one cognitive test (EncephalApp, a version of the Stroop test) but not in another cognitive test (psychometric HE score).⁶ There was no difference in overt HE episodes during follow-up of 5 months. The third pilot trial is our open-label trial of 5 doses of oral FMT capsules over 3 weeks. Preliminary results suggest improvement in the psychometric HE score but not in EncephalApp 1 week after completing 5 days of oral FMT capsules (administered over 3 weeks).⁷⁰ In this trial, FMT transmitted extended-spectrum beta-lactamase (ESBL)-producing *E. coli* to 1 recipient, despite following FDA-approved donor screening protocols.⁷⁶ Overall, the studies evaluating FMT for HE are small and designed to evaluate safety, not efficacy. Clinical efficacy outcomes were mixed, though there were some promising signals. A single dose of FMT from 1 donor was used in the 2 published trials, and it is possible that benefit may vary by donor and additional doses of FMT may be necessary.⁷⁷ Larger trials powered to detect clinical improvement in HE using different FMT dosing strategies are needed.

There are several potential mechanisms by which FMT may impact the pathogenesis of HE: SCFA production, changes to microbiome community structure, bile acid metabolism, and reduced ammonia production (Fig. 2). Results of the Bajaj *et al.* FMT enema trial are difficult to interpret because of antibiotic use only in the FMT arm: changes in microbial diversity and taxa abundance changed primarily with antibiotic exposure, and returned to pre-antibiotic levels after FMT.⁵ However, there was a clear increase in *Ruminococcaceae* abundance from baseline to post-FMT. *Ruminococcaceae* was heavily enriched in the donor, and is known to produce SCFAs, which in turn impacts intestinal barrier function – a possible mechanism by which these FMT enemas improved clinical outcomes.⁵ However, this increase in *Ruminococcaceae* disappeared over long-term follow-up, despite ongoing improvement in HE clinical outcomes.⁷¹ In an elegant mouse study, the bacteria themselves and not a sterile supernatant led to cognitive improvement after FMT, suggesting that the benefits of FMT in HE are not simply a result of bacterial products but also the influence of the bacteria themselves, perhaps on microbiome community composition and function.⁷⁸ In the second trial by Bajaj *et al.* of FMT for HE, oral FMT capsules did not change alpha diversity in stool samples, but did increase diversity of the duodenal mucosal microbiome, suggesting an impact in the proximal bowel.⁶ There was also an increase in duodenal *Ruminococcaceae* and *Bifidobacteriaceae* (generally beneficial taxa) and a decrease in *Veillonellaceae* after FMT capsules, though duodenal samples were not

studied in the control group so it is unknown if these changes may be due to natural fluctuation. In conjunction with these microbial changes, patients who received FMT had an increase in duodenal antimicrobial protein DefA5, increase in tight junction protein E-cadherin (CDH) expression, and a decrease in IL-6 expression. LPS-binding protein also decreased after FMT, suggesting that oral FMT capsules may change duodenal microbial community structure, influencing several aspects of small intestinal barrier function, and decreasing translocation of bacterial products.⁶ Correlation network analysis showed that certain taxa were linked with improved immunomodulatory milieu and cognitive test performance. Those taxa have also been associated with decreased inflammation and strengthened intestinal barrier function in patients with and without liver disease.⁷⁹ *Post hoc* analysis also revealed an increase in secondary bile acids after FMT for HE.⁷⁹ Patients with cirrhosis have a diminished ability to produce secondary bile acids, likely due to a relative reduction in Clostridial species. Secondary bile acids are associated with protection from pathogenic organisms and impact intestinal barrier function. Therefore, FMT may exert some of its therapeutic effect by influencing bile acid metabolism and, as a result, intestinal barrier function. Finally, the PROFIT trial, a placebo-controlled trial of jejunal FMT delivery in patients with cirrhosis (not all had prior HE) reported that FMT was associated with a reduction in serum ammonia levels through as yet unknown mechanisms.^{80,81} Thus, another potential mechanism by which FMT could help patients with HE is by ameliorating hyperammonaemia.

Synbiotics

Synbiotics are probiotics and prebiotics combined into a single therapy. The hope for such combined products is that prebiotics will enhance the efficacy of probiotics, though evidence of synergism is lacking.³⁷ One small single-centre trial showed cognitive benefit and decreased serum ammonia with a synbiotic (*Bifidobacterium longum* and fructo-oligosaccharide).⁸² In a trial of patients with minimal HE, both a synbiotic and a prebiotic alone reversed minimal HE in half of participants, with the symbiotic demonstrating no clear superiority over the prebiotic alone.⁸³ Patients who received the synbiotic or prebiotic alone developed acidified faecal contents, decreased venous ammonia levels, serum LPS levels, and *E. coli* faecal abundance. The clinical benefits of synbiotics compared to prebiotics and probiotics used alone remains to be confirmed.

Microbe-targeted therapy: Decrease potentially harmful taxa

Antibiotics

Antibiotics have been proposed to treat HE, as a method to deplete intestinal taxa that produce neurotoxins (namely ammonia), increase intestinal permeability, and diminish host systemic immune responses. Certain antibiotics may selectively suppress harmful taxa, while allowing potentially beneficial taxa to survive and even proliferate. Rifaximin is approved in the United States and Europe to reduce the risk of recurrent overt HE. Several randomised controlled trials have found that rifaximin markedly reduces the risk of recurrent overt HE.^{41,84} Rifaximin also improves cognition and quality of life for patients with minimal HE.^{85,86} While the clinical benefits of rifaximin are undisputed, its mechanisms of action in HE are less clear and likely multifactorial.

Rifaximin inhibits bacterial RNA synthesis and has broad-spectrum antimicrobial activity, notably for pathogenic bacteria like enterotoxigenic *E. coli*, *Shigella*, and *Salmonella*.^{87,88} However, several small studies in patients with minimal HE suggest that rifaximin exerts little influence on microbial community composition.^{85,89–91} Since these studies used 16S rRNA amplicon sequencing methods, the effect of rifaximin on changes to subtaxa like species or strains may have been missed. Some effects from microbiome therapies are species and even strain specific.⁹² There are several reasons to suspect that rifaximin's benefit in HE may be a result of relevant microbial composition changes. First, rifaximin changes the ratio of secondary to primary bile acids, which has implications for microbiota composition.⁸⁹ Second, rifaximin has been shown in other populations to change microbial community abundance and structure. In a visceral hyperalgesia rat model, rifaximin decreased the total small bowel bacterial burden, increased *Lactobacillus* species, and decreased small bowel inflammation and permeability.⁹³ In irritable bowel syndrome and Crohn's colitis, rifaximin increased the abundance of *Bifidobacterium* and *Faecalibacterium prausnitzii*, known beneficial taxa, and increased production of SCFAs.^{94,95} While family- and genus-level changes have not been detected with rifaximin treatment for HE, species- and strain-level changes have yet to be explored.

Key point

Lactulose and rifaximin influence intestinal microbiota and have been used with success in HE, with early promise for probiotics and faecal microbiota transplant.

Rifaximin significantly lowers serum LPS levels in humans and animal models, which may be the result of changes in microbiome composition (*i.e.* less LPS produced) or a result of decreased LPS translocation across the intestinal barrier.^{85,89} Rifaximin decreases cytokine expression and intestinal inflammation, and simultaneously increases tight junction protein expression – all of which contribute to barrier function.^{89,96–98} Rifaximin reduces adherence of bacteria to the gut wall and decreases bacterial virulence, both of which are potential mechanisms by which it reduces translocation.^{99–101} Rifaximin also influences bacterial metabolism. Bajaj and colleagues found that 8 weeks of treatment with rifaximin led to an increase in bacterial carbohydrate and lipid metabolism, resulting in an increase in patients' serum long-chain and unsaturated fatty acid levels.⁸⁵ Some of these fatty acids have been shown to increase in the brain with probiotic supplementation and are capable of improving cognitive processes like learning and memory.⁹² Finally, data are mixed with regards to rifaximin's impact on ammonia levels;^{89,101–103} however, one notable study of germ-free mice found a bacteria-independent mechanism by which rifaximin could reduce intestinal ammonia production: via intestinal glutaminase.⁸⁹ Overall, available evidence suggests that rifaximin may enhance intestinal barrier function, ameliorate microbiome-induced inflammatory dysregulation, decrease translocation of bacterial products, and influence gut bacterial metabolism in a way that may improve cognitive function.

To date, no antibiotics have demonstrated superior or even comparable efficacy and safety to rifaximin for HE.^{104–108} Rifaximin's minimal systemic absorption accounts for its excellent tolerability and safety profile.^{109–111} With regards to efficacy, rifaximin may selectively

deplete harmful taxa while allowing beneficial taxa to survive and increase metabolic activities compared to other antibiotics tested for HE.

Bacteriophages

Bacteriophages are viruses which specifically target bacteria. The potential impact of bacteriophages in hepatology was recently highlighted when a bacteriophage was used to target and eliminate cytolysin-producing *Enterococcus faecalis*, a species playing a key pathogenic role in alcohol-associated hepatitis.¹¹² Bajaj and colleagues have recently demonstrated that bacteriophage abundance varies by MELD score, HE status, and HE treatment status, though bacterial composition seemed more relevant to clinical outcomes than bacteriophage composition.¹¹³ In particular, phages for *Streptococcal* species seemed the most influenced by disease severity and rifaximin therapy, which is notable given that many of those species are urease-producing and therefore ammonia-generating.

Therapy targeting microbial products

Postbiotics

Postbiotics is a term used to describe bioactive products of beneficial bacteria. SCFAs are the main postbiotics of interest in HE. SCFAs are produced by bacterial fermentation of non-digestible polysaccharides; they are an essential energy source for the colonic epithelium and contribute to barrier function.¹⁷ They also prime the intestinal epithelium to respond to bacterial products, induce tolerance to commensals, and regulate immune responses.^{25,114–116}

Advanced liver disease and HE are associated with reduced intestinal SCFA levels.¹⁹ In a study of hepatic vein and peripheral blood sampled during transjugular intrahepatic portosystemic shunt placement, a moderate inverse correlation between butyrate and MELD score in both blood sources was observed. Stool SCFA content is also inversely related to MELD score.¹⁹ SCFAs have not been directly tested as a treatment for HE. The most common way to experimentally increase SCFAs in the intestinal lumen is by encouraging growth of bacterial species that produce SCFAs, usually with prebiotics. Not all SCFA types and delivery modalities have the same or even desirable effects.^{17,18,117–121} For example, butyrogenic bacteria are associated with steroid-refractory graft-versus-host disease, possibly through butyrate-induced inhibition of colonic stem cells.¹²² Further research is needed to better understand which SCFAs are beneficial for HE and the best administration strategy.

Absorbents

For the last decade there has been interest in ingestible devices which can absorb undesirable substances in the gut lumen, thus limiting their intestinal absorption.⁵³ AST-120 is one such device: a carbon bead with pores small enough to bind microscopic molecules, including ammonia. AST-120 was able to decrease serum ammonia concentrations and reduce brain oedema in a rat model; however, it did not produce clinical benefit in patients with HE.^{123,124} Yaq-001, another absorbent carbon bead, is able to absorb larger molecules including LPS. It appears that Yaq-001 had a myriad of effects in a rat model: reduced LPS

levels, markers of liver and systemic inflammation, portal hypertension, and HE, and altered the microbiome.⁵³ Unfortunately, the phase I trial of Yaq-001 had to be halted due to the COVID-19 pandemic and results are not yet available.¹²⁵ Despite these challenges, there is still optimism around methods that remove potentially toxic bacterial products in the gut lumen.

Challenges

One major obstacle to the effective use of microbiome-targeted therapies for HE is the resilience of the human adult microbiome. The human microbiome is constantly exposed to external challenges including the diet, medications, and numerous host factors, but it has an incredible ability to restore its equilibrium after perturbation – even if that microbial community structure is associated with disease.¹²⁶ It is possible that the disease state (in this case, cirrhosis) exerts constant pressure on the microbiome, promoting overgrowth of potentially harmful taxa while limiting colonisation of beneficial taxa. In contrast to *Clostridioides difficile* (*C. difficile*) colitis, microbiome therapies for HE will likely need to be administered as recurrent courses or continuously instead of as a single short course.

Another related challenge in using microbiome-based therapeutics is that the gut-liver ecosystem is saturated with connections.¹²⁶ Every functional pathway in the gut and liver involves numerous interconnected components such that if one element changes, there are likely many compensatory mechanisms. Thus, an impact on one microbiome component can have unpredicted ripple effects or no effect at all.

One challenge with antibiotics as a therapy for HE is the potential to promote multi-drug resistance. The prevalence of multi-drug resistant bacteria has grown considerably in patients with cirrhosis in the last decade, from 29% of infections to 38% in a large European cohort.¹²⁷ In a study of 77 patients with cirrhosis starting prophylaxis for spontaneous bacterial peritonitis, nearly 50% carried multi-drug resistant organisms prior to antibiotic initiation, and at 180 days of prophylaxis this prevalence increased to 74%.¹²⁸ Rifaximin, the best validated antibiotic for HE, does not appear to induce bacterial resistance and actually has bactericidal activity against many multi-drug resistant bacteria.^{91,129} However, as we continue to use antibiotics to treat HE, we must increasingly keep in mind the problem of resistance and balance benefit-to-harm.

FMT is associated with its own specific set of challenges (Fig. 3). The central limitation of FMT is an inherent lack of certainty about what is administered. FMT originates from the stool of a healthy individual, and as such there will be changes over time within the same donor and across donors.¹³⁰ Despite extensive testing outlined by FDA-approved protocols, FMT has recently led to infections by ESBL-producing *E. coli*, Shigatoxin-producing *E. coli*, and enteropathogenic *E. coli*.^{76,131,132} In all cases, donor stool was extensively screened for pathogens and in some cases for the ultimate infectious culprit, thus highlighting the challenges in identifying pathogens in FMT material. In at least one of these cases, the antimicrobial resistance patterns differed between the FMT *E. coli* and the recipient's *E. coli*, despite genetic testing confirming they were identical strains.⁷⁶ The presence of divergent antibiograms with clonal bacteria raises the question of whether prior

FMT studies have underestimated the risk of infection related to FMT. While FMT has been reported to be safe in numerous studies, there remains some risk of infection.¹³³ In the COVID-19 era, potentially infectious SARS-CoV-2 is present in the stool of infected individuals¹³⁴ and may be transmitted from asymptomatic carriers. While potential donors could be screened by symptoms and nasopharyngeal swab, those measures do not have perfect sensitivity and no stool-based SARS-CoV-2 test has been approved for clinical use.^{135,136} Furthermore, some studies suggest that virus shedding in stool may outlast virus detection in nasopharyngeal swabs.¹³⁷ As the list of required donor tests appropriately grows in length, the practical limitations of FMT also grow. Prior to COVID-19 and some recent additions to testing, the cost of finding a suitable FMT donor was estimated at \$15,190.¹³⁸ There are other logistical challenges with using FMT to treat HE including identification of appropriate donors, standardisation of the product, and sustainability of supply. There remain significant unanswered scientific questions as well. Each trial of FMT for HE or cirrhosis has used a different route, quantity and timing of FMT administration. At this time, we do not know the best FMT dosing regimen or whether patients will require repeat dosing. In addition, the ideal donor for FMT has not been defined. Studies outside of *C. difficile* infection, in ulcerative colitis for example, have found that only 1 in 6 donors achieve desired clinical endpoints.^{139,140} Given the uncertainty around the mechanism of FMT in treating HE, it is hard to determine criteria for the ideal donor. Optimal donor selection may require analysis of microbiota function and not just composition. Finally, most FMT is aerobically prepared, and thus anaerobes are not administered. Anaerobically prepared FMT was found to induce remission in patients with ulcerative colitis, after several failed trials with aerobically prepared FMT, suggesting a potential therapeutic benefit of the anaerobic components.¹⁴¹ Many probiotics, such as *Faecalibacterium prausnitzii*, are lost with aerobic stool processing and preserved with anaerobic processing.¹⁴² Therefore, there may be benefits to anaerobically prepared FMT in HE, beyond what has been found with aerobic preparations.

Key point

Future research on microbiome-targeted therapies for HE should focus on patient and primary outcome selection, microbiome therapy selection, and a personalised therapeutic approach based on baseline enterotype and other patient factors.

Future directions

Microbiome-targeted therapies for HE have had some success, namely lactulose and rifaximin, with early promise for probiotics and FMT. Despite available therapies, many patients with HE suffer from persistent symptoms and many are unable to tolerate lactulose. Future work in this space should focus on trial design, microbiome therapy selection, and a personalised approach to HE (Table 2).

Future trials should be designed to target the highest-need populations as well as focus on clinically relevant primary outcomes. Trials should be designed with a translational component, to allow for gaps in our mechanistic knowledge to be closed. Practices of

rigorous and reproducible research should be applied, including those which minimise bias and achieve sufficient power to assess clinical efficacy. Microbiome therapy selection is also critical. Microbiome therapies have varying impact throughout the intestines and colon. For example, an FMT enema impacts the distal colon while orally administered FMT capsules have a more dispersed effect. Further studies are needed to identify which segment of the bowel has the most impaired barrier function in cirrhosis and is most responsible for HE, as well as the impact of microbiome therapies on intestinal permeability.¹⁴³ Additional studies on FMT including larger cohorts are needed to determine efficacy, ideal dosing regimen, favourable donor characteristics, benefit of anaerobic preparation, duration of benefit, and predictors of response. Living biotherapeutic products with a larger biomass than traditional commercial probiotics and with known metabolic effects (similar to FMT) are being studied in *C. difficile* infection and inflammatory bowel disease. The therapies that produce metabolites known to be deficient in patients with HE, such as SCFAs and secondary bile acids, should be trialled for HE. Finally, the efficacy of each microbiome therapy may depend on a patient's existing microbiome community, or enterotype. A personalised approach to microbiome therapy, based on baseline community structure and function, may yield the most clinical success.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

| | |
|---------------------|-----------------------------------|
| <i>C. difficile</i> | <i>Clostridioides difficile</i> |
| <i>E. coli</i> | <i>Escherichia coli</i> |
| ESBL | extended-spectrum beta-lactamase |
| FMT | faecal microbiota transplant |
| HE | hepatic encephalopathy |
| LPS | lipopolysaccharide |
| MELD | model for end-stage liver disease |
| SCFA(s) | short-chain fatty acid(s). |

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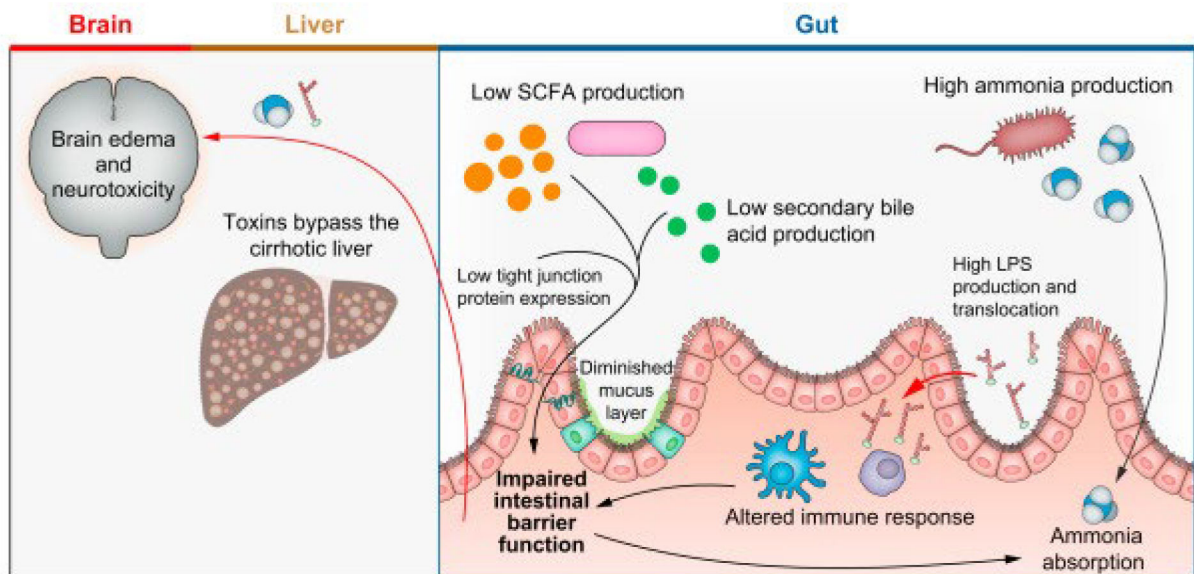


Fig. 1. The pathogenic mechanisms of hepatic encephalopathy.

Changes to SCFAs, secondary bile acid, tight junction protein, and mucus production contribute to increased intestinal permeability. Intestinal bacterial products, including ammonia and LPS, are able to traverse the epithelial membrane and bypass the liver due to hepatic dysfunction and portosystemic shunting. They enter systemic circulation and reach the brain, where ammonia enters astrocytes and leads to neurotoxicity. LPS, lipopolysaccharide; SCFAs, short-chain fatty acids.

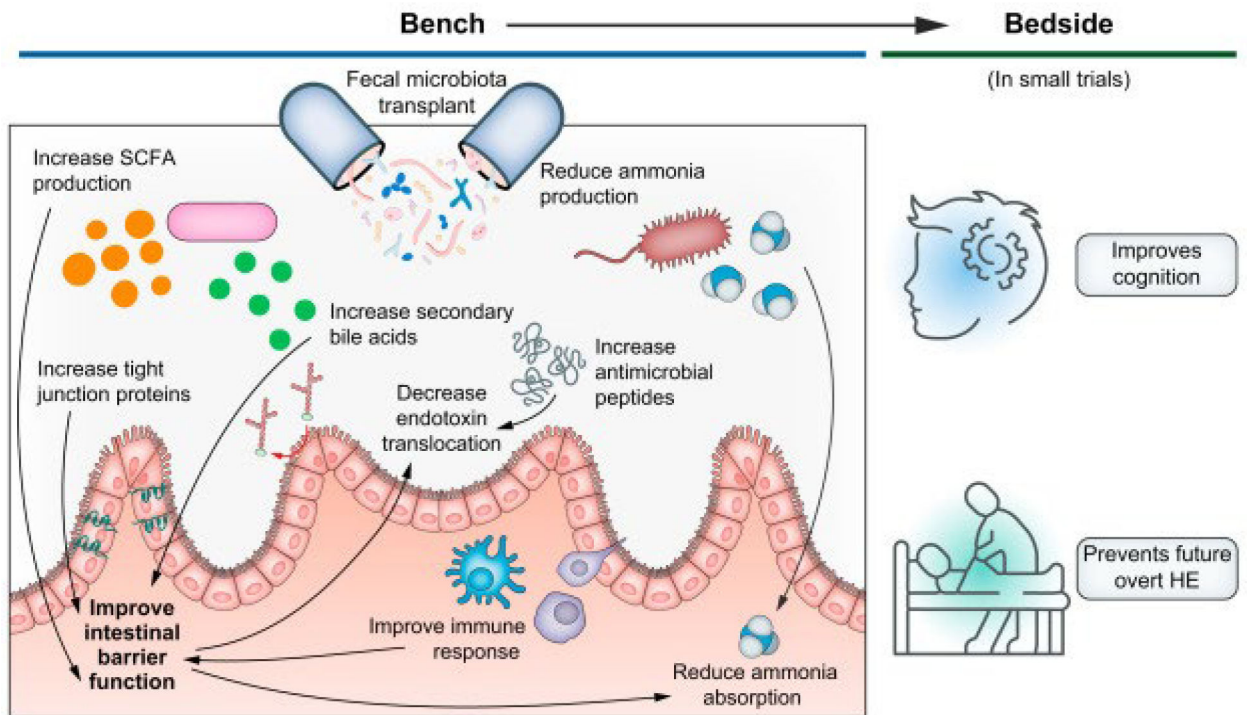


Fig. 2. Faecal microbiota transplant for hepatic encephalopathy: From bench to bedside.

Faecal microbiota transplant has multiple potential therapeutic mechanisms in HE, including several which improve intestinal barrier function: increasing SCFA, secondary bile acid, tight junction protein, and antimicrobial peptide production. By changing microbial community structure, ammonia and endotoxin production and translocation decreases. At the bedside, several small trials have suggested improved cognition and fewer overt HE episodes with faecal microbiota transplant. HE, hepatic encephalopathy; SCFA, short-chain fatty acid.

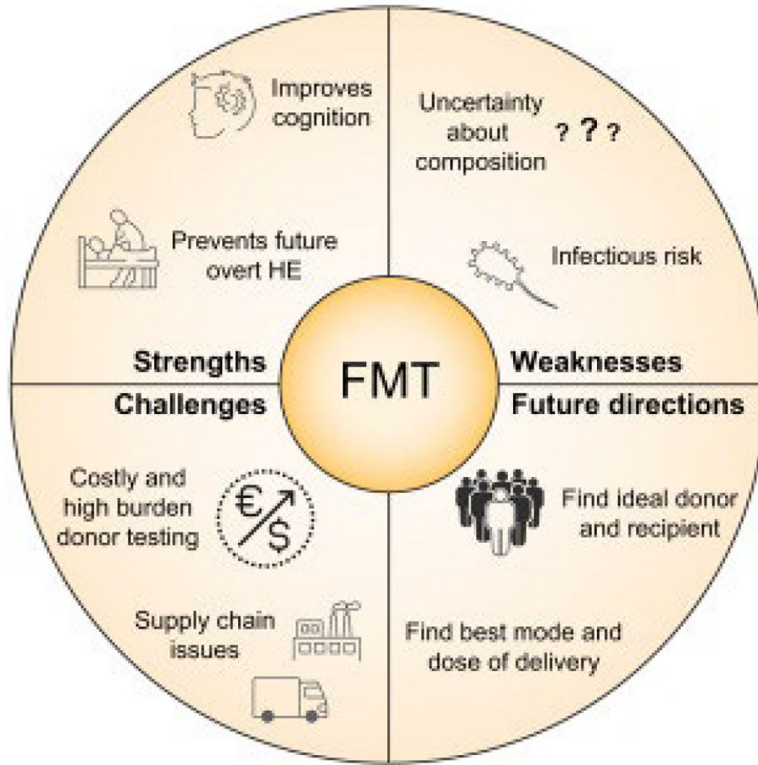


Fig. 3. The strengths, challenges, weaknesses, and future directions of faecal microbiota transplant for hepatic encephalopathy.
FMT, faecal microbiota transplant; HE, hepatic encephalopathy.

Table 1

Potential benefits and limitations of microbiome therapies for hepatic encephalopathy.

| Therapy | Definition | Potential benefits | Clinical efficacy | Clinical limitations |
|--|--|---|---|--|
| Target microbes: Increase potentially beneficial taxa | | | | |
| Prebiotic (e.g. lactulose) | Substrates selectively utilised by host microorganisms, conferring a health benefit | <ul style="list-style-type: none"> Increase beneficial taxa abundance, short-chain fatty acids Inhibit pathogen growth (via lowering luminal pH) Stimulate mucus-producing goblet cells Improve tight junctions | Two large meta-analyses show lactulose reverses minimal HE and prevents overt HE | Lactulose has undesirable side effects and prevents <50% of recurrent overt HE |
| Probiotic | Living microorganisms that, when administered in adequate amounts, confer a health benefit | <ul style="list-style-type: none"> Produce antimicrobial proteins, short-chain fatty acids, secondary bile acids Inhibit pathogen growth (via lowering luminal pH and decreasing available nutrients) Regulate immune response | Low to moderate quality evidence show probiotics improve HE symptoms, reverse minimal HE, and lead to fewer overt HE episodes | Clinical data is low to moderate quality; many trials have high risk of bias; different strains used in each trial |
| Engineered probiotics | Genetically modified bacteria | <ul style="list-style-type: none"> Varies by genetic modification | SYNB1020 reduces blood ammonia in mice | First trial in cirrhosis failed |
| Faecal microbiota transplant | Transfer of processed stool from healthy donor to recipient | <ul style="list-style-type: none"> Decrease ammonia production, intestinal permeability Increase short-chain fatty acids, secondary bile acids, tight junction proteins, antimicrobial proteins | Small single-centre trials suggest FMT improves cognition and reduces overt HE episodes | Limited data; supply and implementation challenges; inherent risk of uncharacterised components |
| Synbiotic | Probiotic and prebiotic in 1 therapy | <ul style="list-style-type: none"> Combined benefits of prebiotics and postbiotics | Limited supportive data | No evidence of synergistic benefit |
| Target microbes: Decrease potentially harmful taxa | | | | |
| Antibiotics (e.g. rifaximin) | Treatment with anti-bacterial effects; target and effects vary by antibiotic | <ul style="list-style-type: none"> Decrease pathogen abundance, gut inflammation, endotoxin Increase long-chain fatty acids, beneficial taxa, tight junction proteins | High quality data that rifaximin reduces risk of overt HE; also improves cognition in MHE | High rate of overt HE recurrence despite rifaximin; other antibiotics have limiting side effects |
| Bacteriophages | Viruses that target bacteria | <ul style="list-style-type: none"> Eliminate specific pathogenic bacteria | No clinical efficacy data | No clinical efficacy data |
| Target products of microbes | | | | |
| Postbiotic | Bioactive products of beneficial taxa | <ul style="list-style-type: none"> Increase tight junction proteins, mucin production Regulate immune response | No clinical efficacy data | No clinical efficacy data |
| Absorbents | Synthetic molecules that absorb undesirable substances | <ul style="list-style-type: none"> Decrease serum ammonia, endotoxin, cytokines | AST-120 and Yaq-001 with positive biological effects in rat model | AST-120 without clinical benefit in humans; Yaq-001 human data not available yet |

HE, hepatic encephalopathy; FMT, faecal microbiota transplant; MHE, minimal hepatic encephalopathy.

Table 2

Future directions of microbiome-targeted therapies for hepatic encephalopathy.

| Future directions | |
|------------------------------|---|
| Trial design | <p>Patient selection: target specific groups in need of more effective and/or better tolerated intervention (<i>e.g. at high risk of developing recurrent overt HE, or those who do not tolerate lactulose</i>)</p> <p>Primary outcome selection: clinically important primary outcomes such as overt HE, cognitive function, quality of life, or other patient-reported outcomes (<i>microbiome changes may be included as secondary outcome</i>)</p> <p>Translational component: explore mechanism within clinical trials</p> <p>High rigour: minimise bias through blinding, randomisation \pm risk stratification; meet enrolment target to achieve adequate power for trial</p> |
| Microbiome therapy selection | <p>Target gut segment: match route of administration to optimal gut segment (upper vs. lower intestine) for that mechanism</p> <p>Living biotherapeutics: select probiotic consortium with biological actions with potential to reverse HE, determine optimal dose and duration, assess need for antibiotic priming to ensure grafting</p> <p>Faecal microbiota transplant: determine characteristics of ideal donor, optimal dose regimen and preparation</p> |
| Personalised approach | <p>Patient enterotype: determine baseline microbiome characteristics to match appropriate microbiome therapy</p> <p>Biomarkers of response: identify other biomarkers predictive of response to microbiome therapies</p> |

HE, hepatic encephalopathy.

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