Future Therapies of Hepatic Encephalopathy

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KEYWORDS

• Hepatic encephalopathy • Portal hypertension • Experimental therapeutics
• Disease management

KEY POINTS

- Hepatic Encephalopathy remains a common and highly morbid complication of chronic liver disease.
- There are few current therapies, which are limited especially by patient tolerance and cost.
- As the pathophysiology of encephalopathy becomes better understood, there are multiple promising potential targets for therapeutics.
- This review provides an overview of the emerging therapeutics for hepatic encephalopathy and their relation to pathways of disease.

INTRODUCTION

Hepatic Encephalopathy (HE) is a spectrum of neuropsychiatric disturbances that is a common and highly morbid complication of chronic liver disease. It confers significant reduction in health-related quality of life[1] and is associated with increased mortality overall.[2] In addition, although HE is classically thought to be reversible, emerging evidence suggests that HE has lasting effects on cognition and well-being even after correction of an acute episode.

The classification and varying presentation of HE are beyond the scope of this review; however, it should be noted that HE can arise from various diseases, including acute and chronic liver disease and portosystemic shunting. Likewise, HE can be episodic or persistent and can exist in a spectrum of severities from subtle changes (termed covert encephalopathy) to coma (overt encephalopathy), graded according to the West Haven Criteria.[3] HE is most commonly seen in chronic liver disease, where up to 40% will eventually develop overt disease, and the prevalence of covert HE is greater than 50%.[4] A significant portion of HE is precipitated by an acute destabilizing event, especially in those with cirrhosis; such events include infection, bleeding,

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electrolyte abnormality, constipation, renal failure, sedating medication, or treatment nonadherence.

HE is now thought to have persistent cognitive damage, potentially owing to astrocyte senescence, with research demonstrating that those with HE have worse 1- and 5-year outcomes even after transplant.[5] Early detection and appropriate therapy are therefore crucial, but treatment options remain limited for clinicians. This review briefly discusses the few approved options, before expanding on emerging therapeutics and how they relate to HE pathophysiology.

The pathophysiology of HE is complex and incompletely understood, but the broad overview offered here contextualizes current and proposed therapeutics for HE. Ammonia has long been understood to be a critically important neurotoxic agent in the development of HE.[6] A common inorganic nitrogenous waste, it is produced both by human tissue such as muscle and intestine, and especially, by urease-expressing bacteria in the gut.[7] Reduced hepatic metabolism and shunting owing to portal hypertension lead to drastically increased systemic exposure to ammonia. This ammonia crosses the blood-brain border where it is metabolized by a combination with glutamate-to-glutamine in astrocytes,[8] creating an osmotic gradient with subsequent astrocyte swelling and neuroinflammation.[9] Ammonia and other toxins also potentiate neuroinhibitory cascades and have a direct neurotoxic effect on synaptic transmission.[10,11] In cirrhosis, progressive frailty and reduced muscle mass also deprive patients of an alternative route for ammonia detoxification, and reduced branch chain amino acids in cirrhosis reduce capacity for peripheral glutamine synthesis from ammonia.[12] In addition, systemic inflammation and oxidative stress present in cirrhosis are recognized as significant factors in the pathogenesis of HE. This review focuses on current and emerging treatment options for HE, with a focus on how disease pathophysiology is being addressed by experimental therapeutics (**Fig. 1**).

CURRENT THERAPEUTICS

The nonabsorbable disaccharides lactulose and lactitol have long formed the backbone of HE management.[13] They are metabolized into a short-chain acidic form by gut bacteria and exert their protective effects in multiple ways. First, they increase osmolality of colonic contents, with a laxative effect that decreases colonic transit time and reduces burden of ammonia-genic bacteria. In addition, they reduce the pH of the colonic lumen, promoting conversion of ammonia into the nonabsorbed positively charged ammonium state. The lowering of colonic pH also helps to reduce proliferation of pathogenic bacteria. Usage is most often limited by side effects, including flatulence, abdominal bloating, and diarrhea; nonadherence to lactulose is a key factor in recurrent HE.[14]

The nonabsorbable antibiotic rifaximin inhibits bacterial RNA synthesis[15] and likely has multimodal action in HE improvement. It selectively modulates ammonia production by acting on pathogenic colonic bacteria to reduce efficacy of the RNA polymerase without altering microbiome diversity.[16] It also may help improve gut barrier function, reducing endotoxemia and systemic inflammation.[17] A poorly absorbed antibiotic, it has fewer systemic side effects than other antibiotics that have been previously used in HE, making it a useful agent, especially in recurrent HE.[18] However, its high cost sometimes limits availability.

Finally, standard-of-care management in acute HE entails identifying and correcting underlying exogenous factors, such as infection, bleeding, or electrolyte imbalance. Identifying and correcting such factors may result in up to 90% resolution rate in an

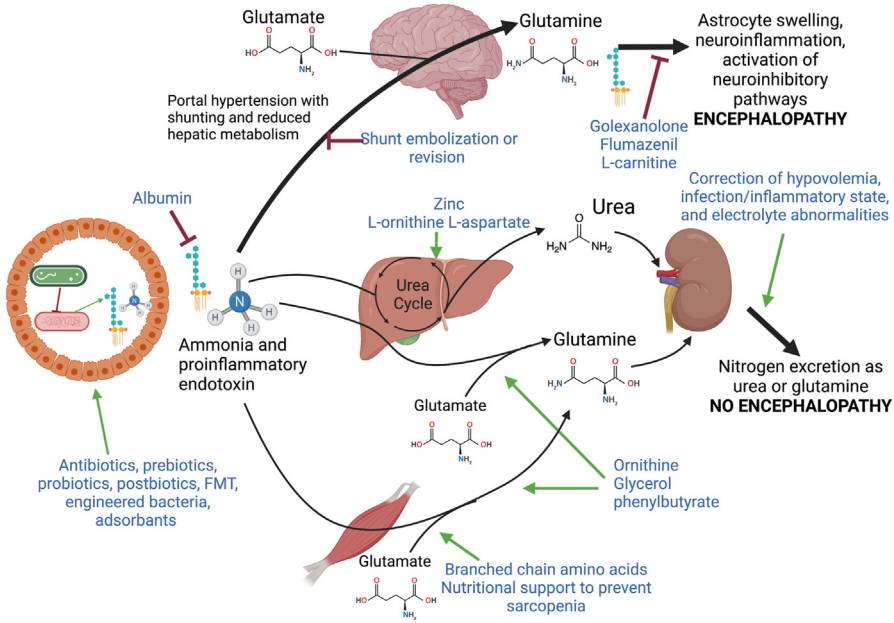

Fig. 1. Nitrogen metabolism in the form of ammonia is a multiorgan process, and increased ammonia exposure in the brain in the setting of cirrhosis and portal hypertension is a major factor in the development of HE. This simplified schematic demonstrates how many experimental therapeutics favor reduced cerebral hyperammonemia and may help treat HE. Created with BioRender.com.

acute episode.[19] Similarly, patients with large portosystemic shunts (either spontaneous[20] or surgical[21]) may benefit from modification, closure, or obliteration.

DISCUSSION ON EMERGING AND EXPERIMENTAL THERAPEUTICS

Albumin

Albumin has established utility in the management of renal insufficiency, overdiuresis, and infection in cirrhosis, all of which can be precipitants of HE. However, because of its known anti-inflammatory properties, as well as studies suggesting that albumin may lower serum ammonia, colloid therapy with albumin has been investigated as a possible treatment or prevention tool in HE. The RELIEF trial demonstrated no improvement in overall outcomes with albumin dialysis, but a trend toward improved HE.[22] When added to lactulose, treatment with albumin improved serum ammonia, inflammatory markers, and proportion of patients with complete HE reversal in a small randomized study by Sharma and colleagues.[23] Although another study by Simón-Talero and colleagues[24] failed to find a clear benefit, a meta-analysis of 12 pooled studies suggested that albumin was associated with decreased risk of HE among those both without prior HE (OR, 0.53; 95% CI, 0.32, 0.86) or prior HE (OR, 0.43; 95% CI, 0.27, 0.68).[25] Finally, a recent randomized double-blind placebo-controlled study of weekly infusions of albumin in outpatients with a history of HE showed reduced cognitive impairment and inflammatory markers with albumin treatment.[26] Despite this, well-controlled studies are lacking at this time, especially to help adjudicate whether the benefit in HE is in fact simply by reversing predisposing factors.

Urea Cycle Modulators

In liver failure and with shunting of portal flow into systemic circulation, the hepatic urea cycle is underused. The urea cycle converts ammonia to urea, primarily in the liver, for excretion in the kidneys, and is the primary source of ammonia disposal in the healthy state.[27] The reduced utilization of the urea cycle owing to hepatic dysfunction and portosystemic shunting results in higher levels of systemic ammonia exposure.

L-ornithine and L-aspartate (LOLA) has been proposed as a method to augment the urea cycle. A mixture of 2 amino acids, LOLA stimulates urea synthesis in periportal hepatocytes and glutamine synthesis in skeletal muscle. A randomized blinded study of 193 patients with acute overt hepatic encephalopathy (OHE) suggested that LOLA reduced recovery time (1.92 vs 2.50 days; $P = .002$) and ammonia levels relative to lactulose and ceftriaxone.[28] In one large Cochrane review and meta-analysis of 36 clinical trials with 2377 participants, LOLA was associated with reduced mortality (RR, 0.42; 95% CI, 0.24, 0.72), but this beneficial effect was not statistically significant when excluding trials with a high risk of bias.[29] This meta-analysis did, however, find that LOLA appeared safe relative to standard-of-care management. The investigators concluded that although it appears LOLA may have a mortality benefit and improve outcomes, higher-quality randomized data are needed before supporting its use, and it remains unavailable in the United States.

Zinc is an important cofactor in the urea cycle, and zinc metabolism impairment and subsequent deficiency are common in cirrhosis.[30] For this reason, and its widespread availability and low expense, supplementation has long been of interest in the management of HE. Unfortunately, small trials, such as a crossover study of 15 participants[31] and a subsequent meta-analysis, have failed to find clear clinical benefit.[32] For this reason, it is not currently considered useful in HE management.

Urinary Ammonia Excretion

The kidneys have net positive ammonia production, with regulation of ammonia excretion a key factor in acid-base homeostasis.[33] In settings of increased acidemia or high ammonia load, healthy kidneys can rapidly upregulate ammonia excretion,[34] and this method of nonurea urinary ammonia excretion takes outsized importance in chronic liver disease. In conditions of hypokalemia and acidosis in chronic liver disease, the kidney also uses glutamine preferentially for potassium recovery in the renal tubules and proton disposal, respectively.[35]

Ornithine phenylacetate is a proposed ammonia scavenger that increases urinary excretion of glutamine in the form of phenylacetylglutamine, preventing it from being used by the kidneys for formation of new ammonia.[36] Although preliminary data suggested that ornithine was effective in lowering serum ammonia,[37] a recent randomized trial failed to demonstrate a statistically significant improvement in time to clinical response, meaning further studies are needed before clinical utilization.[38]

Glycerol phenylbutyrate acts similarly to ornithine phenylacetate, by trapping glutamine for urinary excretion. Already used in genetic urea cycle disorders, it has been shown to reduce serum ammonia levels in small studies.[39] A double-blinded randomized controlled trial of 178 patients with cirrhosis found a reduction in proportion of first-time HE events (21 vs 36%; $P = .02$) and total events (35 vs 57; $P = .04$). As a phase II trial, it was somewhat underpowered but shows promise if studied further.[40]

Antibiotics

Nitazoxanide is an antiprotozoal agent Food and Drug Administration approved for management of diarrheal illnesses. A small head-to-head study comparing twice-

daily nitazoxanide with twice-daily rifaximin demonstrated prolonged remission (137 vs 67 days on average; $P<.01$).[41] Despite the promising results, this study conducted in Egypt must be followed by larger controlled studies, with one study currently recruiting (NCT04161053).

Rifaximin soluble solid dispersion (SSD) tablets are a novel preparation to improve delivery and pharmacokinetics of rifaximin therapy. Because of reduced bile acid concentrations in cirrhosis, the largely water-insoluble standard rifaximin formulation may have reduced efficacy compared with a water-soluble formulation (SSD).[42] A recent phase II study of rifaximin SSD in various formulations did not meet its primary endpoint relative to placebo for increased time to hospitalization or all-cause mortality, but in a second trial an immediate release formulation reduced time to OHE recovery.[43] A phase III study is currently planned (NCT05071716).

Microbiome Modulation with 'Biotics (Prebiotics, Probiotics, and Postbiotics)

Endotoxemia and systemic inflammation are integral to development and progression of HE. It is increasingly clear that altered microbiome in those with worsening liver disease contributes to a proinflammatory state.[44] It is widely accepted that the gut microbiome is altered in those with cirrhosis. Subsequent gut translocation potentiates systemic inflammation and is also thought to be a driver of HE development.[45] A lower intestinal pH is unfavorable to the survival of several bacteria known to produce urease, thus increasing ammonia production, including *Klebsiella* and *Proteus*.

Severity of dysbiosis is directly related to severity of liver dysfunction.[46] For this reason, further refinement of microbiome-targeted therapies is a key development opportunity for HE management (**Fig. 2**). Such therapies could target either reduced

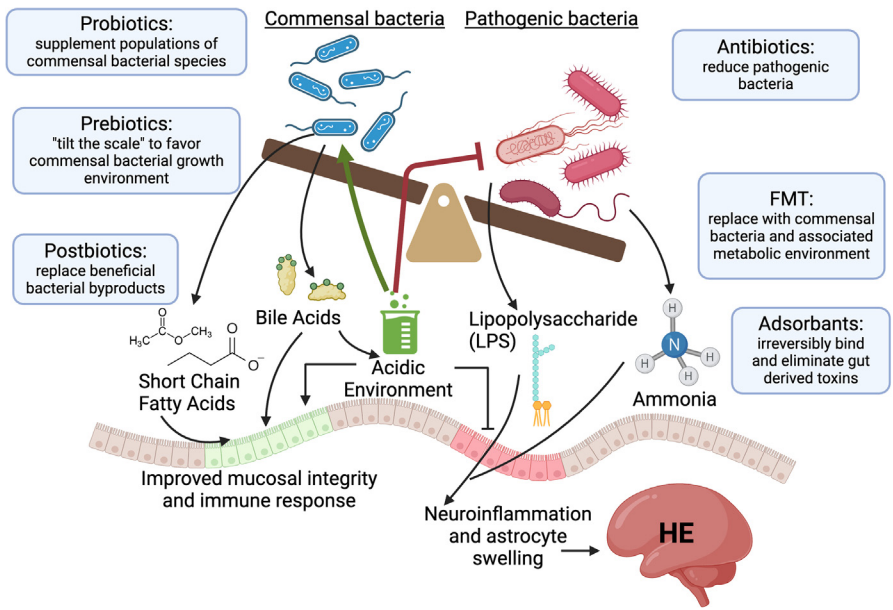

Fig. 2. Progressive dysbiosis and gut membrane dysfunction are now clearly recognized in the pathophysiology of HE. Several experimental therapies in HE address either gut microbial composition or the gut metabolic environment to favor reduced systemic ammonia absorption and gut-induced inflammation. Created with BioRender.com.

inflammation or reduced ammonia load produced by gut bacteria by modulation of relative gut microbial composition. A key study previously demonstrated that reduction of relative *Escherichia coli* and *Staphylococcal* populations in favor of non–urease-producing *Lactobacillus* using probiotics and a prebiotic improved systemic inflammation and covert hepatic encephalopathy (CHE) symptoms.[47]

Prebiotics are nondigestible food products intended to favorably stimulate fermentation and growth of beneficial microorganisms. Lactulose itself is a prebiotic, and its ability to safely modulate ammonia production in the gut has been attractive for HE research. One prebiotic, *Gelsectan*, is derived of mucosal protective agents, such as xyloglucan, with polysaccharides that are thought to promote commensal bacteria and may increase mucosal integrity.[48] It has demonstrated some promise in irritable bowel syndrome, and a clinical trial has been registered to evaluate it in HE (NCT05189834).

Probiotics, which are live micro-organisms that are generally ingested, are a commonly used way to manipulate the microbial composition of the gut. Several strains have been noted in controlled studies to reduce ammonia levels and incidence of overt disease in those with HE. Lactobacillus supplementation, specifically, has been repeatedly demonstrated to be effective at reducing ammonia levels and improving cognitive function, either as an isolated strain or in combination with other bacterial formulations, such as in the *VSL #3*/Visbiome (VSL Pharmaceuticals, Inc/ExeGI Pharma). In one unblinded randomized study, use of VSL #3 reduced the incidence of overt HE episodes in those with cirrhosis.[49] Similar results have been found in other studies and using different strains.

Research thus far has been relatively limited, and a legal dispute between the developer of VSL #3 and the subsequent ownership over a unilateral change in strain composition speaks to the somewhat ephemeral and variable nature of the various brands and strains. A Cochrane review published in 2017 asserted that although probiotics (either in combination with or instead of lactulose) may improve recovery and prevent disease progression, there is no clear impact on mortality.[50] In addition, extant research in this field was thought to be possibly affected by bias and random error. Another limitation to the current use of probiotics is that the exact target population is unclear. In one small study, VSL #3 was found to have more profound protective inflammatory changes in patients with alcohol or metabolic liver disease than in those with cirrhosis owing to hepatitis C.[51] There also may be resistance to long-term colonization and durable uptake of strains not already adapted to the gut environment, limiting use.[52]

One additional limitation of probiotic therapy is that it does not change the host environment (metabolites, gut milieu) that perpetuates dysbiosis in those with cirrhosis. For example, bile acids are an important modulator of the gut microbiome and have decreased concentration in those with advanced liver disease.[53] Potentially by influencing primary and secondary bile acid concentration in the gut, indirect influence on microbial populations could lead to reduced inflammatory markers and improved outcomes in HE.[54]

Other such microbial derived metabolites, often called "*postbiotics*," may eventually have clinical utility either independent of or in combination with classical HE treatment options. These include tryptophan derivatives, short-chain fatty acids, and choline compounds.[55]

Microbial-based therapy not only is confined to the hindgut but also as periodontal treatment has been demonstrated to reduce salivary dysbiosis and improve cognition in those with HE by decreasing endotoxemia and salivary inflammation.[56]

One future avenue for probiotic therapy in HE is the use of *engineered bacteria*, potentially to consume a toxic metabolite, such as ammonia, converting it to a

nontoxic byproduct, such as L-arginine.[57] However, the first in-human randomized trial failed to show effective lowering of serum ammonia, making any clinical application some distance away.[58]

Stool-derived Therapies

Fecal microbiota transplantation (FMT) has been demonstrated to improve outcomes in other conditions of significant gut dysbiosis, such as recurrent *Clostridium difficile* infection.[7] FMT involves the transfer of a complete community of gut microorganisms from a donor, with significantly increased diversity over the limited number of strains in a probiotic. This potentially leads to a more comprehensive restoration of gut microbial balance. They also incorporate enzymes and metabolites from the donor stool, above and beyond the transplanted organisms, which may also affect the urea/ammonia cycle. In a seminal study of those with recurrent HE, FMT using a donor specifically selected for high levels of short-chain fatty acid–producing bacteria, such as *Lachnospiraceae*, resulted in improved cognition and reduced hospitalization at 30 days.[59] Interestingly, follow-up in the same group of patients suggested that the population of *Lachnospiraceae* returned to pre-FMT levels, but cognition remained better in the FMT group, and HE hospitalizations remained lower.[60] One limitation to FMT therapy at this juncture is that donor stool characteristics have been shown to influence outcomes, making standardization difficult.[61] Future research will need to better elucidate the appropriate donor profile, as well as the recipients most likely to benefit. An FMT study by Bloom and colleagues[62] in HE demonstrated that the relative concentration of short-chain fatty acid–producing bacteria in donor stool influenced recipient outcome. Finally, the promise of FMT must be balanced with the understood risk of inducing an infection, potentially with a drug-resistant organism, in patients with known immune dysfunction.[63]

This risk of infection and the variability of donor profiles may limit broad uptake of FMT in clinical practice. One ongoing clinical trial (NCT04899115) is evaluating the use of *VE303*, a group of 5 strains of commensal, nonpathogenic *Clostridia* species. These strains are derived from donor stool and manufactured from clonal cell banks to increase standardization and reduce risk of resistant strain infection.[64] A phase II study in VE303 recently demonstrated efficacy in prevention of recurrent *C difficile*.[65]

Similarly, *RBX7455* is a standardized donor-derived live therapeutic bacterial product that has been lyophilized ("freeze-dried") after being obtained in large aliquots from a single donor and tested for viable bacterial content. It has the added benefit of room temperature stability for storage and being available in a pH-resistant capsule. Early studies have been promising in *C difficile* infection,[66] with an early-stage trial enrolling for HE (NCT04155099).

Gut Adsorbents

AST-120 and *Yaq-001* are synthetic carbon-based ingestible microspheres that have been developed to adsorb gut toxins, such as ammonia, and, because they are not absorbed systemically, reduce systemic ammonia levels and inflammation. Although promising data were found in rat models,[67] the largest randomized study of AST-120 found no clinical benefit.[68] A clinical trial of Yaq-001 was terminated because of COVID-19,[69] suggesting it will be some time before this potential therapeutic is ready for routine use in HE.

Neurotransmitter Modulation

The influx of toxins, especially ammonia, through the blood-brain barrier leads to increased neuroinflammation mediated by reactive oxidative species, a critical step

in the development of HE. For this reason, modulation at the level of the brain is an attractive target for treatment. Neuroinflammation leads to neurosteroid-induced potentiation of the GABA-A system, which is neuroinhibitory and has negative effects on memory, cognition, vigilance, and sleep.[70] A recently developed GABA-A receptor antagonist, *golexanolone* (GR3027), is under investigation for possible cognitive benefits in HE. In a small, randomized trial of 45 patients with cirrhosis, golexanolone improved vigilance and some cognitive markers, although further studies will be needed to evaluate its possible use to improve outcomes.[71]

L-carnitine crosses the blood-brain barrier, where it facilitates mitochondrial uptake of acetyl Co-A and stimulates phospholipid synthesis while serving as substrate for cerebral energy production.[72] It also has been postulated to be protective against neuroinflammation by reducing free radical production.[73] In a small randomized double-blinded placebo-controlled trial, L-carnitine supplementation improved quality of life and reduced serum ammonia concentration.[74] Unfortunately, a subsequent Cochrane systematic review found that all 5 trials with 398 patients evaluating L-carnitine use were conducted by the same research group with high potential for bias, with no clear clinical benefit.[75] No clinical trials appear to be currently recruiting.

Flumazenil is a synthetic benzodiazepine antagonist hypothesized to have utility in HE through its modulation of inhibitory GABA-A complex receptors, given the posited increased GABAnergic "tone" in those with HE as a result of ammonia and manganese upregulation.[76] In a randomized, double-blinded placebo-controlled trial of flumazenil versus placebo in patients hospitalized with severe HE, flumazenil improved neurologic score in more patients (14.7% vs 3.8%; $P<.01$).[77] A follow-up meta-analysis, however, found that most of the 14 studies evaluated had significant risk of bias but overall suggested a possible short-term benefit for flumazenil in severe HE without a mortality benefit.[78] Its short-half life and need for intravenous administration limit it to use only in hospitalized patients.

Skeletal Muscle Metabolism

Muscle is an important alternate source of ammonia metabolism, especially in the presence of hepatic dysfunction. In portal hypertension, muscular glutamine synthetase conversion of glutamate and ammonia into glutamine helps compensates for reduced hepatic metabolism.

Branched chain amino acids (BCAAs), typically derived from dietary protein, are important metabolic precursors of glutamate, which as above is crucial for subsequent ammonia metabolism.[79] Transamination in the liver of BCAAs results in glutamate, but a combination of portal hypertension and malnutrition results in relative depletion.[80] Reduced relative BCAA concentration (compared with aromatic amino acids) is also thought to negatively affect neurotransmission in the brain. Accordingly, supplementation with either oral[81] or intravenous[82] BCAAs may be beneficial in HE, especially in those with sarcopenia or nutrition deficiency. Across 16 clinical trials, a Cochrane systematic review and meta-analysis concluded that insufficient evidence was present for mortality benefit, but an overall beneficial effect on HE (RR, 0.76; 95% CI, 0.63–0.92) when combining oral and parenteral studies.[83] Unfortunately, they have not been extensively studied in comparison, or as an adjunct therapy, with standard of care in controlled settings. Several early-phase studies are currently evaluating BCAAs in acute-on-chronic liver failure with HE (eg, NCT05700695), but a published abstract suggested mixed results with early but not sustained improvement.[84] The amino acid mixture *AXA1655*, a combination of BCAAs and LOLA, was studied, demonstrating increased relative BCAA concentration and reduced ammonia in a small trial (n = 40)[85]; however, a follow-up phase II clinical trial was recently

terminated by the sponsoring company (NCT04816916). An additional ongoing study (NCT04096014) is evaluating the use of timed protein supplementation with Ensure in the evenings and in the mornings and its effect on muscle mass and HE.

SUMMARY

As the pathophysiology, predisposing factors, and various clinical presentations of HE become better understood, the need for better and more precise therapeutic options grows. As previously posited,[86] an ideal treatment regimen would take into account individual patient characteristics and directly address the multifactorial nature of HE. In general, the current armamentarium of lactulose and rifaximin addresses only a limited scope of the problem, is not uniformly effective, and may not be right for every situation especially when considering toxicity and cost. Given the vast array of potential alternatives that have been tested or are being studied, the future of HE management is potentially bright. It is critically important that well-controlled prospective studies be funded in these areas in order to improve outcomes, while considering additional factors, such as muscle, renal, and neurologic drivers of disease.

CLINICS CARE POINTS

- Lactulose and rifaximin remain the backbone of HE therapy but emerging therapeutics may help close the care gaps that remain.
- In particular, microbiota derived therapies including FMT have shown promise but still require standardization and have limitations including risk of accidental infection.
- While urea cycle modulators such as Zinc and L-ornithine and L-aspartate are fairly low risk, they have repeatedly failed to demonstrate significant advances over current standard therapies, limiting their usefullness.

DISCLOSURE

The authors have nothing to disclose.

REFERENCES

1. Montagnese S, Bajaj JS. Impact of hepatic encephalopathy in cirrhosis on quality-of-life issues. Drugs 2019;79(Suppl 1):11.
2. Bohra A, Worland T, Hui S, et al. Prognostic significance of hepatic encephalopathy in patients with cirrhosis treated with current standards of care. World J Gastroenterol 2020;26(18):2221–31.
3. Weissenborn K. Hepatic encephalopathy: definition, clinical grading and diagnostic principles. Drugs 2019;79(1):5–9.
4. Das A, Dhiman RK, Saraswat VA, et al. Prevalence and natural history of subclinical hepatic encephalopathy in cirrhosis. J Gastroenterol Hepatol 2001;16(5):531–5.
5. Wong RJ, Aguilar M, Gish RG, et al. The impact of pretransplant hepatic encephalopathy on survival following liver transplantation. Liver Transpl 2015;21(7):873–80.
6. Shawcross DL, Olde Damink SWM, Butterworth RF, et al. Ammonia and hepatic encephalopathy: the more things change, the more they remain the same. Metab Brain Dis 2005;20(3):169–79.

7. Butterworth RF, Giguère JF, Michaud J, et al. Ammonia: key factor in the pathogenesis of hepatic encephalopathy. Neurochem Pathol 1987;6(1–2):1–12.
8. Norenberg MD. Astroglial dysfunction in hepatic encephalopathy. Metab Brain Dis 1998;13(4):319–35.
9. Jayakumar AR, Rao KVR, Schousboe A, et al. Glutamine-induced free radical production in cultured astrocytes. Glia 2004;46(3):296–301.
10. Felipo V, Butterworth RF. Neurobiology of ammonia. Prog Neurobiol 2002;67(4):259–79.
11. Szerb JC, Butterworth RF. Effect of ammonium ions on synaptic transmission in the mammalian central nervous system. Prog Neurobiol 1992;39(2):135–53.
12. Lattanzi B, D'Ambrosio D, Merli M. Hepatic encephalopathy and sarcopenia: two faces of the same metabolic alteration. J Clin Exp Hepatol 2019;9(1):125–30.
13. Bircher J, Müller J, Guggenheim P, et al. Treatment of chronic portal-systemic encephalopathy with lactulose. Lancet 1966;1(7443):890–2.
14. Chow KW, Ibrahim BM, Yum JJ, et al. Barriers to lactulose adherence in patients with cirrhosis and hepatic encephalopathy. Dig Dis Sci 2023;68(6). https://doi.org/10.1007/S10620-023-07935-Z.
15. Gerard L, Garey KW, DuPont HL. Rifaximin: a nonabsorbable rifamycin antibiotic for use in nonsystemic gastrointestinal infections. Expert Rev Anti Infect Ther 2014;3(2):201–11.
16. Yu X, Jin Y, Zhou W, et al. Rifaximin modulates the gut microbiota to prevent hepatic encephalopathy in liver cirrhosis without impacting the resistome. Front Cell Infect Microbiol 2022;11:1427.
17. Patel VC, Lee S, McPhail MJW, et al. Rifaximin-α reduces gut-derived inflammation and mucin degradation in cirrhosis and encephalopathy: RIFSYS randomised controlled trial. J Hepatol 2022;76(2):332–42.
18. Eltawil KM, Laryea M, Peltekian K, et al. Rifaximin vs conventional oral therapy for hepatic encephalopathy: a meta-analysis. World J Gastroenterol : WJG 2012;18(8):767.
19. Strauss E, Tramote R, Silva EPS, et al. Double-blind randomized clinical trial comparing neomycin and placebo in the treatment of exogenous hepatic encephalopathy. Hepato-Gastroenterology 1992;39(6):542–5. https://europepmc.org/article/med/1483668.
20. Laleman W, Simon-Talero M, Maleux G, et al. Embolization of large spontaneous portosystemic shunts for refractory hepatic encephalopathy: a multicenter survey on safety and efficacy. Hepatology 2013;57(6):2448–57.
21. Kochar N, Tripathi D, Ireland H, et al. Transjugular intrahepatic portosystemic stent shunt (TIPSS) modification in the management of post-TIPSS refractory hepatic encephalopathy. Gut 2006;55(11):1617.
22. Bañares R, Nevens F, Larsen FS, et al. Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: the RELIEF trial. Hepatology 2013;57(3):1153–62.
23. Sharma BC, Singh J, Srivastava S, et al. Randomized controlled trial comparing lactulose plus albumin versus lactulose alone for treatment of hepatic encephalopathy. J Gastroenterol Hepatol 2017;32(6):1234–9.
24. Simón-Talero M, García-Martínez R, Torrens M, et al. Effects of intravenous albumin in patients with cirrhosis and episodic hepatic encephalopathy: a randomized double-blind study. J Hepatol 2013;59(6):1184–92.
25. Teh KB, Loo JH, Tam YC, et al. Efficacy and safety of albumin infusion for overt hepatic encephalopathy: a systematic review and meta-analysis. Dig Liver Dis 2021;53(7):817–23.

26. Fagan A, Gavis EA, Gallagher ML, et al. A double-blind randomized placebo-controlled trial of albumin in outpatients with hepatic encephalopathy: HEAL study. J Hepatol 2023;78(2):312–21.
27. Adeva MM, Souto G, Blanco N, et al. Ammonium metabolism in humans. Metabolism 2012;61(11):1495–511.
28. Sidhu SS, Sharma BC, Goyal O, et al. L-ornithine L-aspartate in bouts of overt hepatic encephalopathy. Hepatology 2018;67(2):700–10.
29. Goh ET, Stokes CS, Sidhu SS, et al. L-ornithine L-aspartate for prevention and treatment of hepatic encephalopathy in people with cirrhosis. Cochrane Database Syst Rev 2018;2018(5):CD012410.
30. Himoto T, Masaki T. Associations between zinc deficiency and metabolic abnormalities in patients with chronic liver disease. Nutrients 2018;10(1). https://doi.org/10.3390/NU10010088.
31. Riggio O, Ariosto F, Merli M, et al. Short-term oral zinc supplementation does not improve chronic hepatic encephalopathy. Results of a double-blind crossover trial. Dig Dis Sci 1991;36(9):1204–8.
32. Shen YC, Chang YH, Fang CJ, et al. Zinc supplementation in patients with cirrhosis and hepatic encephalopathy: a systematic review and meta-analysis. Nutr J 2019;18(1). https://doi.org/10.1186/S12937-019-0461-3.
33. Weiner ID, Verlander JW. Renal ammonia metabolism and transport. Compr Physiol 2013;3(1):201.
34. Dejong CHC, Deutz NEP, Soeters PB. Renal ammonia and glutamine metabolism during liver insufficiency-induced hyperammonemia in the rat. J Clin Invest 1993;92(6):2834–40.
35. Gabuzda GJ, Hall PW. Relation of potassium depletion to renal ammonium metabolism and hepatic coma. Medicine 1966;45(6):481–90.
36. Ventura-Cots M, Arranz JA, Simón-Talero M, et al. Safety of ornithine phenylacetate in cirrhotic decompensated patients: an open-label, dose-escalating, single-cohort study. J Clin Gastroenterol 2013;47(10):881–7.
37. Ventura-Cots M, Concepción M, Arranz JA, et al. Impact of ornithine phenylacetate (OCR-002) in lowering plasma ammonia after upper gastrointestinal bleeding in cirrhotic patients. Therap Adv Gastroenterol 2016;9(6):823–35.
38. Rahimi RS, Safadi R, Thabut D, et al. Efficacy and safety of ornithine phenylacetate for treating overt hepatic encephalopathy in a randomized trial. Clin Gastroenterol Hepatol 2021;19(12):2626–35.e7.
39. Kortbeek S, Gilkes C, Khan A, et al. Secondary ammonia scavenge with glycerol phenylbutyrate improves hyperammonemia following portosystemic shunting. JPGN Rep 2022;3(3):e210.
40. Rockey DC, Vierling JM, Mantry P, et al. Randomized, double-blind, controlled study of glycerol phenylbutyrate in hepatic encephalopathy. Hepatology 2014;59(3):1073–83.
41. Glal KAM, Abd-Elsalam SM, Mostafa TM. Nitazoxanide versus rifaximin in preventing the recurrence of hepatic encephalopathy: a randomized double-blind controlled trial. J Hepatobiliary Pancreat Sci 2021;28(10):812–24.
42. Darkoh C, Lichtenberger LM, Ajami N, et al. Bile acids improve the antimicrobial effect of rifaximin. Antimicrob Agents Chemother 2010;54(9):3618–24.
43. Bajaj JS, Hassanein TI, Pyrsopoulos NT, et al. Dosing of rifaximin soluble solid dispersion tablets in adults with cirrhosis: 2 randomized, placebo-controlled trials. Clin Gastroenterol Hepatol 2022;21(3):723–31.e9.
44. Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature 2014;513(7516):59–64.

45. Fukui H. Role of gut dysbiosis in liver diseases: what have we learned so far? Diseases 2019;7(4):58.
46. Wang J, Wang Y, Zhang X, et al. Gut microbial dysbiosis is associated with altered hepatic functions and serum metabolites in chronic hepatitis B patients. Front Microbiol 2017;8(NOV):2222.
47. Liu Q, Duan ZP, Ha DK, et al. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. Hepatology 2004;39(5):1441–9.
48. Trifan A, Burta O, Tiuca N, et al. Efficacy and safety of Gelsectan for diarrhoea-predominant irritable bowel syndrome: a randomised, crossover clinical trial. United European Gastroenterol J 2019;7(8):1093.
49. Lunia MK, Sharma BC, Sharma P, et al. Probiotics prevent hepatic encephalopathy in patients with cirrhosis: a randomized controlled trial. Clin Gastroenterol Hepatol 2014;12(6). https://doi.org/10.1016/J.CGH.2013.11.006.
50. Dalal R, Mcgee RG, Riordan SM, et al. Probiotics for people with hepatic encephalopathy. Cochrane Database Syst Rev 2017;2017(2). https://doi.org/10.1002/14651858.CD008716.PUB3.
51. Loguercio C, Federico A, Tuccillo C, et al. Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. J Clin Gastroenterol 2005;39(6):540–3.
52. Fehervari Z. Mechanisms of colonization resistance. Nature Research 2021;. https://www.nature.com/articles/d42859-019-00018-y. [Accessed 8 June 2023].
53. Kakiyama G, Pandak WM, Gillevet PM, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. J Hepatol 2013;58(5):949.
54. Schwabl P, Hambruch E, Seeland BA, et al. The FXR agonist PX20606 ameliorates portal hypertension by targeting vascular remodelling and sinusoidal dysfunction. J Hepatol 2017;66(4):724–33.
55. Bajaj JS, Ng SC, Schnabl B. Promises of microbiome-based therapies. J Hepatol 2022;76(6):1379.
56. Bajaj JS, Matin P, White MB, et al. Periodontal therapy favorably modulates the oral-gut-hepatic axis in cirrhosis. Am J Physiol Gastrointest Liver Physiol 2018;315(5):G824–37.
57. Kurtz CB, Millet YA, Puurunen MK, et al. An engineered E. coli Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. Sci Transl Med 2019;11(475). https://doi.org/10.1126/SCITRANSLMED.AAU7975.
58. Safety, tolerability and pharmacodynamics of SYNB1020 - study results - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/results/NCT03447730?term=SYNB1020&rank=2. [Accessed 3 June 2023].
59. Bajaj JS, Kassam Z, Fagan A, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. Hepatology 2017;66(6):1727.
60. Bajaj JS, Fagan A, Gavis EA, et al. Long-term outcomes after fecal microbiota transplant in cirrhosis. Gastroenterology 2019;156(6):1921.
61. Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. Gastroenterology 2015;149(1):102–9.e6.
62. Bloom PP, Donlan J, Torres Soto M, et al. Fecal microbiota transplant improves cognition in hepatic encephalopathy and its effect varies by donor and recipient. Hepatol Commun 2022;6(8):2079.

63. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant E. coli bacteremia transmitted by fecal microbiota transplant. N Engl J Med 2019;381(21):2043–50.
64. Dsouza M, Menon R, Crossette E, et al. Colonization of the live biotherapeutic product VE303 and modulation of the microbiota and metabolites in healthy volunteers. Cell Host Microbe 2022;30(4):583–98.e8.
65. Louie T, Golan Y, Khanna S, et al. VE303, a defined bacterial consortium, for prevention of recurrent clostridioides difficile infection: a randomized clinical trial. JAMA 2023;329(16):1356–66.
66. Khanna S, Pardi DS, Jones C, et al. RBX7455, a non-frozen, orally administered investigational live biotherapeutic, is safe, effective, and shifts patients' microbiomes in a phase 1 study for recurrent clostridioides difficile infections. Clin Infect Dis 2021;73(7):e1613–20.
67. Bosoi CR, Parent-Robitaille C, Anderson K, et al. AST-120 (spherical carbon adsorbent) lowers ammonia levels and attenuates brain edema in bile duct–ligated rats. Hepatology 2011;53(6):1995–2002.
68. Bajaj JS, Sheikh MY, Chojkier M, et al. 190 AST-120 (spherical carbon adsorbent) in covert hepatic encephalopathy: results of the astute trial. J Hepatol 2013; 58:S84.
69. Safety and tolerability of yaq-001 in patients with cirrhosis - full text view - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03202498. [Accessed 11 June 2023].
70. Agusti A, Llansola M, Hernández-Rabaza V, et al. Modulation of GABAA receptors by neurosteroids. A new concept to improve cognitive and motor alterations in hepatic encephalopathy. J Steroid Biochem Mol Biol 2016;160:88–93.
71. Montagnese S, Lauridsen M, Vilstrup H, et al. A pilot study of golexanolone, a new GABA-A receptor-modulating steroid antagonist, in patients with covert hepatic encephalopathy. J Hepatol 2021;75(1):98–107.
72. Malaguarnera M. Acetyl-L-carnitine in hepatic encephalopathy. Metab Brain Dis 2013;28(2):193–9.
73. Rose C, Felipo V. Limited capacity for ammonia removal by brain in chronic liver failure: potential role of nitric oxide. Metab Brain Dis 2005;20(4):275–83.
74. Malaguarnera M, Bella R, Vacante M, et al. Acetyl-L-carnitine reduces depression and improves quality of life in patients with minimal hepatic encephalopathy. Scand J Gastroenterol 2011;46(6):750–9.
75. Martí-Carvajal AJ, Gluud C, Arevalo-Rodriguez I, et al. Acetyl-L-carnitine for patients with hepatic encephalopathy. Cochrane Database Syst Rev 2019;2019(1). https://doi.org/10.1002/14651858.CD011451.PUB2.
76. Ahboucha S, Butterworth RF. The neurosteroid system: implication in the pathophysiology of hepatic encephalopathy. Neurochem Int 2008;52(4–5):575–87.
77. Barbaro G, Di Lorenzo G, Soldini M, et al. Flumazenil for hepatic encephalopathy grade III and IVa in patients with cirrhosis: an Italian multicenter double-blind, placebo-controlled, cross-over study. Hepatology 1998;28(2):374–8.
78. Goh ET, Andersen ML, Morgan MY, et al. Flumazenil versus placebo or no intervention for people with cirrhosis and hepatic encephalopathy. Cochrane Database Syst Rev 2017;2017(7). https://doi.org/10.1002/14651858.CD002798.PUB3.
79. Kawaguchi T, Izumi N, Charlton MR, et al. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. Hepatology 2011;54(3):1063–70.
80. Yamato M, Muto Y, Yoshida T, et al. Clearance rate of plasma branched-chain amino acids correlates significantly with blood ammonia level in patients with liver cirrhosis. Int Hepatol Commun 1995;3(2):91–6.

81. Marchesini G, Dioguardi FS, Bianchi GP, et al. Long-term oral branched-chain amino acid treatment in chronic hepatic encephalopathy. A randomized double-blind casein-controlled trial. The Italian Multicenter Study Group. J Hepatol 1990;11(1):92–101.
82. Naylor CD, O'Rourke K, Detsky AS, et al. Parenteral nutrition with branched-chain amino acids in hepatic encephalopathy. A meta-analysis. Gastroenterology 1989; 97(4):1033–42.
83. Gluud LL, Dam G, Les I, et al. Branched-chain amino acids for people with hepatic encephalopathy. Cochrane Database Syst Rev 2017;5(5). https://doi.org/10.1002/14651858.CD001939.PUB4.
84. Mehtani R, Premkumar M, Garg S, et al. Intravenous branched chain amino acid infusion is associated with early but ill-sustained recovery of overt hepatic encephalopathy in acute-on-chronic liver failure: a pilot protocol. J Clin Exp Hepatol 2022;12:S30.
85. Chakravarthy MV, Neutel J, Confer S, et al. Safety, tolerability, and physiological effects of AXA1665, a novel composition of amino acids, in subjects with Child-Pugh A and B cirrhosis. Clin Transl Gastroenterol 2020;11(8):e00222.
86. Tapper EB, Jiang ZG, Patwardhan VR. Refining the ammonia hypothesis: a physiology-driven approach to the treatment of hepatic encephalopathy. Mayo Clin Proc 2015;90(5):646–58.