The Direct Contribution of Astrocytes and Microglia to the Pathogenesis of Hepatic Encephalopathy

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Abstract

Hepatic encephalopathy is a neurological complication resulting from loss of hepatic function and is associated with poor clinical outcomes. During acute liver failure over 20% of mortality can be associated with the development of hepatic encephalopathy. In patients with liver cirrhosis, 1-year survival for those that develop overt hepatic encephalopathy is under 50%. The pathogenesis of hepatic encephalopathy is complicated due to the multiple disruptions in homeostasis that occur following a reduction in liver function. Of these, elevations of ammonia and neuroinflammation have been shown to play a significant contributing role to the development of hepatic encephalopathy. Disruption of the urea cycle following liver dysfunction leads to elevations of circulating ammonia, which enter the brain and disrupt the functioning of astrocytes. This results in dysregulation of metabolic pathways in astrocytes, oxidative stress and cerebral edema. Besides ammonia, circulating chemokines and cytokines are increased following liver injury, leading to activation of microglia and a subsequent neuroinflammatory response. The combination of astrocyte dysfunction and microglia activation are significant contributing factors to the pathogenesis of hepatic encephalopathy.


Introduction

Liver disease is a significant cause of morbidity and mortality worldwide. One of the most severe consequences of acute liver failure and decompensated chronic liver disease is the development of neurological complications, a state referred to as hepatic encephalopathy (HE). HE manifests in several different ways and while the severity of symptoms is variable, patients often present with altered mental status, disturbances in sleep-wake cycles and functional impairment.¹ ² Hospitalizations due to HE in the USA increased over the period of 2010 to 2014, from 25,039 to 31,182, though in-hospital mortality decreased from 13.4% to 12.3%.³ In addition to this, HE in-patient charges increased from 8.15 billion USD to 11.9 billion USD over the same time period.³ Therefore, it is evident that HE is a significant financial burden on health care systems and the development of this syndrome is associated with poor patient prognosis and mortality.

The current understanding of HE pathogenesis is that disruption of hepatic function leads to increased ammonia concentrations and impaired urea metabolism, which allows ammonia to accumulate in the blood and the brain. The downstream effects of ammonia accumulation in the brain are astrocyte swelling and dysregulated neurotransmission, which lead to the cognitive deficits present during HE. In addition to this, systemic inflammation, elevation of pro-inflammatory cytokines and microglia activation have been observed during HE in both patients and rodent models and are associated with worse outcomes. Current treatments are primarily focused on ammonia-lowering agents, optimization of nutrition and treatment of comorbid disease processes. While these have been shown to have some efficacy, determining all of the pathophysiological mechanisms that contribute to HE progression is important if novel therapeutic approaches are to be developed.

In this review, we will discuss the clinical definitions and description of HE, further examine the available research regarding the pathophysiology of HE within the central nervous system (CNS), and more specifically discuss the role of astrocytes and microglia within the context of HE pathology.

Clinical epidemiology and description of HE

The neurological and cognitive manifestations that present in patients with liver disease or portosystemic shunting in the absence of liver disease is termed HE.⁴ Two categories of HE exist: covert HE (also known as minimal HE) and overt HE; these are distinguished by the severity of the patient presentation.⁵ Patients with minimal HE have abnormal results with psychometric testing and subtle personality changes that may be evident to caregivers or by those familiar with the
patient. Individuals with minimal HE can have a reduced ability to drive, impairment at work, and inability or difficulty performing other complex tasks.6–10 Diagnosing minimal HE is important clinically because it best predicts future diagnosis of overt HE and is associated with higher rates of hospitalization and lower survival rates.6,9 Overt HE is more evident upon clinical presentation, with increased severity of symptoms, but is diagnosed only after exclusion of other causes of altered mental status.

The West Haven Criteria (WHC) have traditionally been used for grading the severity of overt HE based upon clinical findings, with higher WHC grades being associated with more severe HE. The mortality rate is high for overt HE patients regardless of grade. During acute liver failure, 20–25% of mortality results from increased intracranial pressure and the development of HE.10 In cirrhotic patients, survival rates are estimated to be 42% at 1 year and 23% at 3 years.11 The incidence of overt HE is 8% annually in decompensated cirrhotic patients12 and is estimated to be attributed to 0.33% of hospitalizations in the USA.9

The American Association for the Study of Liver Diseases (commonly known as AASLD) together with the European Association for the Study of the Liver (commonly known as EASL) established guidelines in 2014 consisting of four axes for better categorization and management of HE in the setting of chronic liver disease.6 These guidelines are focused on etiology, severity, time course, and inciting factors. Axis I is categorized via etiology as Type A (acute liver failure), Type B (portosystemic shunting), or Type C (cirrhosis). Axis II is determined by clinical severity of symptoms and is categorized as unimpaired (normal mentation), WHC Grade I (abnormal psychometric testing without apparent mental status changes), WHC Grade II (evidence of disorientation or asterixis), WHC Grade III (gross disorientation, somnolence), and WHC Grade IV (coma).5,6 Axis III is based on time of the clinical course and is categorized as episodic (more than 6 months between episodes), recurrent (episodes occurring within 6 months), or persistent (always present).5,9 Axis IV is categorized as spontaneous or precipitated. Documentation of a patient with HE should include all four axis components and can aid clinicians in consistent categorization of this patient population.

Pathogenesis of HE

The pathogenesis of HE is complex and the exact disease mechanisms have yet to be fully delineated. The most common theory is that the manifestations of HE are due to alteration of the urea cycle and the resulting increase of systemic ammonia levels.1 Glutamine is used by the gut as a primary energy source and the resulting by-products of its metabolism by glutaminase are glutamate and ammonia. In addition, bacteria in the gastrointestinal tract use urease to produce ammonia from urea. Ammonia from the gut diffuses through the intestinal mucosa, enters the mesenteric circulation and is transported to the liver. In the liver, perportal hepatocytes detoxify ammonia via the ornithine cycle, resulting in the production of urea. Urea passes into the systemic circulation via the hepatic veins, where it enters the kidney and is ultimately excreted in urine. Ammonia that does not enter the urea cycle is metabolized by glutamine synthetase in the liver, kidney, muscle and brain to produce glutamine from glutamate.11

When hepatocytes are damaged as a result of liver disease, detoxification of ammonia into urea is impaired and portosystemic shunting leads to ammonia accumulation in the blood. In addition, dysbiosis of the microbiome has been reported during cirrhosis, leading to increased urease activity and even greater production of ammonia in the gut.15 These combined effects lead to a significant increase of circulating ammonia concentrations, which are then metabolized by muscle and other extrahepatic organs. Ammonia also can cross the blood-brain barrier, generating a state of hyperammonemia in the brain. It is not clear whether the increased concentrations of ammonia found in the brain and cerebrospinal fluid are due to active or passive transport but increased systemic levels of ammonia are associated with increased ammonia concentrations in the brain.15 The highest elevations of serum ammonia following liver dysfunction are observed in Type A and Type B HE.16,17 Arterial and venous ammonia have been reported to be elevated during Type C HE compared to controls, though the values observed are lower than the other types of HE, including in patients with acute-on-chronic liver failure.16

Besides ammonia, acute liver injury or chronic liver disease is associated with inflammation that contributes to this syndrome, with neuroinflammation being a pathological contributor to overt HE during both acute liver failure and chronic liver disease. Elevation of circulating pro-inflammatory cytokines has been observed during acute liver failure, cirrhosis, and acute-on-chronic liver failure.18–20 Associated with increased systemic inflammation, is the development of neuroinflammation and HE. In the brain, neuroinflammation is regulated by microglia, the resident macrophage-like cells of the brain. While normally present in a quiescent state, in response to pro-inflammatory signals, they can become activated, producing cytokines generating oxidative stress and contributing to aspects of pathology, and activation of microglia is observed in patients with both Type A and Type C HE.21

In addition to increased ammonia and inflammation are a variety of other dysregulated metabolites that can contribute to this syndrome. The concentrations of various amino acids have been shown to be outside the normal range when assessed in plasma, cerebrospinal fluid and frontal cortex of patients with Grade 3 or 4 HE.22 Similar findings have been observed for bile acids, where concentrations are increased in serum, cerebrospinal fluid and tissue from patients with fulminant hepatic failure.23 Manganese is normally excreted from the body through the hepatobiliary system but during cirrhosis has been shown to accumulate in both the circulation and brain, and can inhibit dopaminergic neurotransmission in the basal ganglia.24–26

In addition to this, a variety of comorbidities, including infections, gastrointestinal bleeding, diarrhea, hyponatremia, hypokalemia, hyperkalemia, benzodiazepine and diuretic use, and others contribute to this syndrome.27 These comorbidities are most often present in patients with liver cirrhosis. In acute-on-chronic liver failure patients, elevated creatinine, as well as altered Jugular venous oxygen saturation ranges, were associated with higher grades of HE.28 This gives support that multiorgan complications during cirrhosis and acute-on-chronic liver failure can exacerbate the development of HE. A diagram of the complex interactions that are involved in the pathophysiology of HE is provided in Fig. 1.
Astrocyte dysfunction

Astrocytes are the most abundant glial cell in the CNS and are responsible for trophic and metabolic support of neurons and neuronal-glial communication, and are essential for blood-brain barrier function. In response to increased ammonia concentrations during HE, astrocytes undergo a change in morphology, developing large pale nuclei known as Alzheimer Type II astrocytosis. A large number of Alzheimer Type II astrocytic nuclei have been detected by autoradiography in hyperammonemic rats induced by methionine sulfoxamine. Hyperammonemia also leads to increased production of glutamine in astrocytes, resulting in increased osmotic pressure via astrocyte swelling. Swelling of astrocytes can lead to cerebral edema and a worsening of HE pathology. Research to date involving astrocytes has primarily focused on the effects of HE on regulation of oxidative stress, glutamine and glutamine synthase, brain water balance, and lactate metabolism.

Glial fibrillary acidic protein (GFAP) and oxidative stress

GFAP is an intermediate filament found in astrocytes that is used for both motility and maintenance of structural stability. Multiple studies have shown that GFAP is decreased during acute liver failure, thereby influencing the morphology and function of astrocytes. Investigating individual brain regions found that GFAP expression was greatly decreased in the corpus callosum and in the hippocampus during HE.

GFAP has been shown to be important for cell volume regulation and therefore could contribute to the development of cerebral edema during HE. This has been demonstrated by the presence of increased brain water content when there was reduced GFAP protein levels during HE. A proposed mechanism for the down-regulation of GFAP during HE is that ammonia can interfere with metabolism, leading to decreased ATP levels and thus reducing GFAP production. In support of ammonia inhibiting GFAP expression, cultured astrocytes treated with ammonium chloride showed an 85% reduction in GFAP mRNA. GFAP expression has also been shown to be reduced during chronic liver disease and HE. Loss of GFAP in chronic hepatitis was found to be related to astrocyte swelling, with subsequent loss of their stellate shape. Similarly, in bile duct-ligated (BDL) rats, altered astrocytes have been found but with no changes in the total number of astrocytes themselves.

Studies have shown a direct relationship between astrocyte swelling and oxidative stress with p47phox-dependent activation of NADPH oxidase, contributing to reactive oxygen species generation from ammonia, in cultured rat astrocytes and cortical mouse brain slices. Oxidative and nitrosative stress can lead to an induction of astrocyte swelling. However, recent studies have shown the reverse is also true, as astrocyte swelling induces oxidative and nitrosative stress—creating a self-amplifying cycle. Hyperammonemia can lead to both oxidative and nitrosative stress, causing protein modifications such as nitration of tyrosine residues along with oxidation of RNA. That being said, oxidative stress...
stress, astrocyte swelling and down-regulation of GFAP likely contribute together to induce HE symptomology. Treatments aimed at reducing oxidative stress in astrocytes, such as treating cells with curcumin, reduce mitochondrial dysfunction and lead to increased GFAP levels. Therefore, many of the studies that are tying oxidative stress to changes in astrocyte swelling in the context of HE could be a result of the down-regulation of GFAP due to oxidative stress.

Glutamine & glutamine synthetase

Currently, astrocytes are considered the primary target of ammonia toxicity because they are responsible for ammonia metabolism in the CNS via glutamine synthetase. Ammonia can freely cross the blood-brain barrier as perivascular processes of astrocytes rapidly metabolize ammonia from the circulation to prevent neurotoxicity using glutamine synthetase. Currently, few reports describe investigations into the role of glutamine synthetase on progression of HE due to acute liver failure. One study using the acute liver failure model of lipopolysaccharide (LPS) and D-galactosamine injection determined that administration of methionine sulfoxime, a glutamine synthetase inhibitor, led to improved survival and reduced plasma tumor necrosis factor alpha (TNF-α) and interferon gamma levels. However, the effect of chronic HE on glutamine synthetase has been more extensively studied and the results are inconsistent with each other. After 4-week portacaval anastomosis, glutamine synthetase activity was significantly decreased in the hippocampus, cerebellum, and cerebral cortex but unchanged in other brain regions. Consistent with these findings, portacaval anastomosis rats have a 15% decrease in glutamine synthetase activity in the cerebral cortex. In addition, portacaval anastomosis rats at 6 months had regional variation of glutamine synthetase activity as well as a strong increase in glutamine synthetase in astrocyte end-feet processes. This regional variation was also shown in BDL rats 30 days after surgery, where glutamine synthetase activity was significantly decreased in the liver but there was no change in the frontal cortex. In addition, the administration of ornithine phenylacetate to reduce ammonia concentrations significantly reduced glutamine synthetase activity in the frontal cortex in BDL rats, indicating that treatments aimed at reducing ammonia may influence glutamine or glutamine synthetase activity. In post-mortem cortical brain tissue from cirrhotic patients with and without HE, glutamine synthase activity was significantly decreased whereas glutamine synthetase protein expression was not affected, which the authors surmise is a consequence of tyrosine nitrination of the enzyme. This is supported, as cultured astrocytes treated with ammonium chloride have reduced glutamine synthetase activity and increased tyrosine nitration of glutamine synthetase.

The elevation of astrocyte glutamine due to increased ammonia concentrations can lead to changes in brain water content. Glutamine is osmotically active and is a cause of brain edema and increased intracranial pressure. Elevated glutamine inside of astrocytes creates a hypertonic state leading to increased water accumulation in astrocytes and cytotoxic astrocyte swelling. In addition, glutamine enters the mitochondria of astrocytes where it is metabolized, yielding glutamate and ammonia. The mitochondrial compartment is small, and the high levels of ammonia that accumulate can lead to mitochondrial permeability transition pore opening, subsequent oxidative stress, and further astrocyte swelling.

Aquaporin-4 (AQP-4)

AQP-4 is a bidirectional transmembrane water channel protein found on astrocyte end-foot and it plays a role in maintaining brain water homeostasis. Direct evidence for a causal role of AQP-4 in brain edema has been demonstrated in AQP-4 knockout mice. Studies have also shown the time course of cerebral edema development differs among brain regions, reflecting differences in AQP-4 distribution. Similarly, silencing AQP-4 gene expression in cultured astrocytes was shown to reduce water permeability under hypoosmotic conditions.

AQP-4 is dysregulated during acute liver failure and HE. Acute liver failure is associated with up-regulation of AQP-4, as evidenced in thioacetamide-treated rats as well as during galactosamine and LPS-induced liver failure. This phenomenon is also present in patients, as there was an up-regulation of AQP-4 expression in the cerebral cortex from 8 patients with acute liver failure. In thioacetamide-treated rats, AQP-4 expression was positively correlated with brain water content. Exposure of rat cortical astrocytes cultures to interleukin (IL)-1β but not ammonia resulted in up-regulation of AQP-4, showing that brain edema may be worsened by inflammation.

During chronic liver disease it was thought that cerebral edema was not a pathological characteristic of HE, as increased intracranial pressure is rare in this patient population. In addition, there is a decrease in grey matter and an increase in white matter in patients with cirrhosis, and low grade cerebral edema has been observed in patients with cirrhosis and minimal HE. In 4-week BDL rats, there is an increase of AQP-4 expression in the cortex, hippocampus, striatum and cerebellum that was associated with increased water content in all brain regions. This was validated in 4-week BDL rats, that also had elevations of AQP-4 in the cortex. Interestingly, these researchers also used a galactosamine and high ammonia diet model in rats where AQP-4 was not changed following LPS injection but brain water percentage was significantly increased. Therefore, there may be other factors outside AQP-4 that need to be better characterized to understand the control of brain water content during chronic liver disease.

Lactate

Lactate is made in every cell from pyruvate, following a reaction catalyzed by lactate dehydrogenase. The liver will metabolize lactate into glucose, where it can serve as an energy source for all organs. In the brain, neurons use lactate as their preferred oxidative energy source and lactate is primarily synthesized by astrocytes, where it can be transported to neurons via the astrocyte-neuron lactate shuttle. In HE, there is possible derangement of this relationship leading to hyperlactatemia. Increased concentrations of lactate, considered a marker of energy failure, are a mechanism of generating brain edema and neuronal dysfunction during HE. Lactate can induce swelling of cultured and primary astrocytes, as determined through in vitro studies. However, lactate has been associated with increased brain edema during acute liver failure in patients and rodents and during chronic liver disease in BDL rats.
Hyperlactatemia has been suggested as a prognostic marker of acetaminophen-induced acute liver failure, as increased arterial lactate correlated with the severity of HE and was present at significantly higher concentrations in non-survivors.76 The use of 1H and 13C NMR spectroscopy on the frontal cortex of rats with acute liver failure secondary to hepatic devascularization determined that lactate was increased 169.2% compared to controls.77 Likewise, use of hepatic devascularization to model acute liver failure determined that there were significant increases in lactate levels, with a 166% increase at 6 h and an increase in 3293% at coma.78 Also, nuclear magnetic resonance spectroscopy was used to examine lactate usage by cells and determined that increased brain lactate synthesis along with impaired glucose oxidation were the major contributing factors to brain edema rather than accumulation of intracellular glutamine.79 It should be mentioned that not every study investigating lactate has found increases, as the use of 1H and 13C magnetic resonance spectroscopy found essentially no change of brain lactate in BDL rats at 4 weeks or 8 weeks following surgery.79

**Microglia activation**

Microglia are cells of myeloid origin, whose main function is to control the immune response of the CNS.80 Additionally, activated microglia are known to induce the inflammatory response in the brain by releasing proinflammatory cytokines, such as IL-1β, IL-6 and TNFα.81 Evidence of neuroinflammation has been shown in HE patients with acute liver failure and chronic liver disease. In patients with acute liver failure, microglia activation occurs as shown by increased immunostaining for human leukocyte antigen DR (CR3/43) when compared to controls.21 In post-mortem cortical brain tissue from patients with liver cirrhosis and overt HE, there is up-regulation of the microglia marker ionized calcium binding adaptor molecule 1 (known as IBA1) when compared to cirrhotic patients without HE.82

Multiple reports have shown microglia activation in the BDL model of chronic HE.34,38,84 Interestingly, one study found that BDL triggered alternative activation of microglia.34 Instead of the classical microglial markers OX6, ED1 and IBA1 along with pro-inflammatory markers IL-1β and inducible nitric oxide synthase were not elevated but transforming growth factor beta 1 (known as TGFβ1) was found to be increased.34 Another study using post-mortem tissue from cirrhotic patients with HE observed activated microglia with hypertrophied cell bodies and thickened processes along with higher levels of IL-6.85 Outside of cytokines, microglia activation can be assessed by 11C-PK11195, which is a positive emission tomography ligand for translocator protein.86 In the context of acute HE, 11C-PK1195 and 18F-DPA-714 have been used and found to detect neuroinflammation in thioacetamide-treated rats by binding to translocator protein.87 Interestingly, translocator protein has been deleted from astrocytes, demonstrating an increase in mitochondria permeability transition and cell volume in response to ammonia, indicating that this protein is involved in more processes than just neuroinflammation.88

That being said, not all evidence shows induction of a pro-inflammatory phenotype during HE as microglia polarization occurs in cirrhotic patients, with both pro-inflammatory M1 and anti-inflammatory M2 phenotypes being present.89 Research involving microglia has primarily focused on signals leading to their activation, chemokine and cytokine regulation, and oxidative stress.

**Ammonia and microglia**

Studies have investigated if hyperammonemia causes microglia activation in both acute and chronic HE. The exposure of primary cell cultures of microglia to ammonia led to an increase in both synthesis and release of IL-6 and TNFα compared to basal microglia.90 Likewise, in the azoxymethane model of acute liver failure, the investigators found microglia to be activated; however, they found microglia to not be activated in mice injected with ammonium chloride.91 In rats fed an ammonia-containing diet for 4 weeks to induce a state of hyperammonemia, microglia activation was observed in the hippocampus, that could be reversed by removing the ammonia-containing diet for 2 or 4 weeks.92 Interestingly, in a co-culture of rat astrocytes and microglia that were treated with ammonium chloride and LPS, it was found that ammonia treatment did not up-regulate the gene expression of IL-1α, IL-1β, IL-6 or TNFα in microglia or cocultured astrocytes and microglia.93 The investigators also found that astrocytes reduced the up-regulation of microglia activation markers induced by LPS.93 As elevated brain ammonia concentrations and neuroinflammation are pathological characteristics of HE, it is evident that gaining greater understanding into the exact influences of ammonia on microglia activation in the different contexts of HE are necessary.

**Chemokines**

Outside of ammonia, chemokines are the primary contributors to activating microglia during states of neuroinflammation. Chemokines are involved with cell-cell communication and regulate neuroinflammation by influencing migration and activation of immune cells.94 Most studies have focused on investigating the pro-inflammatory chemokine ligand 2 (CCL2) and anti-inflammatory C-X-C ligand 1 (CX3CL1). In mice that had undergone bile duct ligation, the release of CCL2 in the brain triggered recruitment of infiltrating monocytes, leading to neurological decline.93 The investigators also found that microglia have increased levels of CCL2 and intraperitoneal injection of anti-TNFα serum led to reduced CCL2 expression in microglia.83 In a model of portal hypertension using triple calibrated portal vein ligation for 1 month in rats, the investigators found that the CX3CL1 expression was not changed in the hippocampus or cerebellum, but its receptor CX3CR1 was significantly up-regulated in both regions while stromal cell-derived factor 1 alpha and C-C chemokine receptor type 4 were up-regulated in only the hippocampus.95 Specific targeting of CCL2 activity through intraperitoneal injection of chemokine receptor 2 and chemokine receptor 4 inhibitors during acute liver failure in azoxymethane- or thioacetamide-treated mice was found to reduce microglia activation and improve neurological function.96,97 In the azoxymethane model of acute liver failure, injection of soluble CX3CL1 was found to reduce microglia activation and improve time taken to reach coma, indicating that an imbalance of CCL2 and CX3CL1 expression may be driving the activation of microglia during HE.98
Cytokines

In patients and animal models of HE, systemic inflammation causes worsening of neurological function and it has been proposed that pro-inflammatory signals act in concert with ammonia to generate the neurological complications of acute liver failure and chronic liver disease. TNFα is a potent pro-inflammatory cytokine that has been shown to activate microglia in a number of experimental models of neuroinflammation. Circulating levels of TNFα are increased as a function of the severity of HE in both patients and experimental animals with liver failure. Moreover, the presence of TNFα gene polymorphisms is known to influence the outcomes of patients with acute liver failure. During HE, systemic levels of TNFα are increased in the azoxymethane model of acute liver failure. Inhibition of TNFα signaling by systemic treatment with etanercept reduced systemic inflammation, attenuated the neurological decline, and prevented microglial activation in the cerebral cortex. These data support the hypothesis that peripherally-derived TNFα at least in part, contributes to the microglial activation and subsequent neurological decline of liver failure. In further support of this concept, neurological complications occurring in BDL mice were shown to be the consequence of monocyte recruitment in response to TNFα signaling and to occur via microglial activation. Specifically, peripheral TNFα signaling stimulates microglia to produce CCL2, which subsequently mediates monocyte recruitment into the brain. Pro-inflammatory mediators other than TNFα can contribute to HE pathogenesis as well. IL-1β and microglia activation are increased in rats after portacaval shunt and administering sildenafil was found to reduce neuroinflammation and microglia activation. In azoxymethane-treated mice, cortex IL-1β, IL-6, TNFα and CCL2 protein expression were increased. The use of anti-TGFβ1 antibodies or genetic ablation of transforming growth factor beta receptor 2 (known as TGFβR2) in neurons of azoxymethane-treated mice led to reduced microglia activation and normalized levels of IL-1β, IL-6, TNFα and CCL2, giving support that TGFβR2-mediated signaling contributes to neuroinflammation during HE. Interestingly, the use of the gamma aminobutyric acid antagonist bicuculine was found to reduce the expression of IL-1β, but not effect microglia activation in ammonium chloride-fed rats. This gives support that the pro-inflammatory state during elevated levels of ammonia in the brain may not be directly linked to microglia activation, though more studies are need in this area to better characterize this.

Clinical management of HE

The most efficacious treatment for patients with HE is liver transplantation, with cognitive measures and metabolites in the brain becoming normalized after transplantation. That being said, in comparison to cirrhotic patients without HE that undergo liver transplantation, those with HE that undergo liver transplantation have impaired cognitive function, with one study showing that 13% of the cohort of patients maintained mild cognitive impairment 6–12 months after liver transplantation. Besides liver transplantation, there are therapeutics commonly used, including lactulose or nonabsorbable antibiotics such as rifaximin. Lactulose is a nondigestible disaccharide that is metabolized by bacteria in the colon, decreasing pH, reducing bacterial ammonia production.
and producing more ammonium from ammonia, which cannot be absorbed into the intestine.\textsuperscript{112} Rifaximin is used to eliminate bacteria with a specific efficacy for anaerobic bacteria and can reduce ammonia production, endotoxin generation, and inflammation.\textsuperscript{113} There are a variety of other treatments that have been investigated, including L-ornithine-L-aspartate, ornithine phenylacetate, glycerol phenylbutyrate, various probiotics, AST-120 and others that are generally aimed at reducing ammonia levels in HE patients that have had limited improvement when compared to lactulose or rifaximin.\textsuperscript{5,114}

Other than these direct treatments of HE, clinical management strategies for HE patients include alleviating precipitating factors that contribute to the syndrome and management of comorbidities. In HE patients that develop hepatorenal syndrome there is a complete disruption of the urea cycle, leading to even greater concentrations of ammonia in the circulation due to both loss of hepatic function and kidney failure. Other conditions can lead to inhibition of ammonia metabolism, including sarcopenia in cirrhotic patients, as these have reduced ability to metabolize ammonia into glutamine due to muscle loss. The presence of sarcopenia is associated with development of both minimal and overt HE.\textsuperscript{115} Infections are also a risk factor for HE with bacterial overgrowth, spontaneous bacterial peritonitis, and other infections contributing to morbidity and HE progression.\textsuperscript{116} Controlling these systemic complications that contribute to HE while lowering circulating ammonia concentrations are the most effective strategies currently used for management of this syndrome.

Summary and future perspectives

HE is a devastating consequence of acute liver failure and chronic liver disease that is associated with increased morbidity and mortality and has few effective treatments besides liver transplantation. The elevation of circulating ammonia levels that occurs following the disruption of the urea cycle leads to accumulation of ammonia in astrocytes. This generates a myriad of complications, including dysregulation of glutamine concentrations, elevation of lactate, generation of oxidative and nitrosative stress, and cerebral edema. In addition, hepatic injury and loss of liver function leads to systemic inflammation, which results in activation of microglia in the brain due to both increases of ammonia and dysregulation of chemokine concentrations. This activation of microglia leads to the production of pro-inflammatory cytokines that ultimately work in concert with astrocyte dysfunction to further increase oxidative stress and cerebral edema. Collectively, this results in increased cerebral edema, intracranial pressure, and worse HE pathology. A working model of these aspects of HE pathology are presented in Fig. 2. Ultimately, if we are to develop new treatments for the management of HE, gaining a greater understanding into the specific cellular contributions to neurological dysfunction associated with HE is necessary to identify new targets that allow for better management of this syndrome.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

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