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Neuroinflammatory Signals during Acute and Chronic Liver Diseases

Matthew McMillin and Sharon DeMorrow

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Abstract

A spectrum of neurological complications can result from acute and chronic liver diseases and is termed hepatic encephalopathy. The precise pathogenic mechanisms by which hepatic encephalopathy occurs is unclear. However, it is commonly accepted that the development of hepatic encephalopathy shares a long-standing relationship with neuro-inflammation. This chapter will outline the evidence for a role of neuroinflammation and proinflammatory cytokines in the pathogenesis of hepatic encephalopathy. Furthermore, we will identify the possible circulating factors, released from the liver after damage, that may contribute to the neurological complications of hepatic encephalopathy, including neuroinflammation. Lastly, we discuss the current and experimental treatment options aimed at reducing neuroinflammation for the management of hepatic encephalopathy.

Keywords: hepatic encephalopathy, microglia, acute liver disease, liver cirrhosis, cytokines

1. Introduction

Hepatic encephalopathy (HE) describes a spectrum of neurological complications that arise during acute liver failure or chronic liver diseases and can be classified depending upon the underlying liver pathology. Specifically, Type A HE is associated with acute liver failure. Acute liver failure, or fulminant hepatic failure, is a rapid deterioration of liver function without any pre-existing liver disease. It can arise due to drug-induced liver injury (e.g., acetaminophen overdose), viral hepatitis or ischemic hepatitis. Type B HE arises from a portal-systemic bypass without underlying liver disease. This occurs when blood bypasses the liver, thereby bypassing the detoxification function of the liver, resulting in an increased buildup of toxic substances in the blood stream and subsequent neurological impairment. Lastly, type C HE is

a result of liver cirrhosis. In late stage chronic liver diseases, when severe fibrosis is evident, the liver decompensates leading to the development of HE. Recently, it was suggested that a 4th type of HE exists that results from acute-on-chronic liver failure. This occurs when an acute liver insult (e.g., an infection) occurs in a patient with an existing chronic liver condition. The HE that may arise has features in common with both Type A and Type C HE and therefore perhaps should be characterized as its own entity.

Regardless of the type of HE, clinical symptoms of HE range from altered cognitive function, mood changes, disorientation, and neuromuscular problems such as asterixis and ataxia, which ultimately culminate in hepatic coma. Associated with these symptoms are cerebral edema, leading to increased intracranial pressure (evident only in HE due to acute liver failure), astrocyte swelling, neuronal dysfunction and neuroinflammation.

The neurological changes during HE are thought to arise due to the buildup of toxic or inflammatory substance in the blood stream as a result of impaired liver function. With the increased permeability of the blood-brain barrier observed in patients and in animal models of HE, these substances are able to cross the blood-brain barrier and alter cognitive function. While the full scope of liver-derived substances may not yet be fully appreciated, some of these include ammonia buildup, increased bile acids and circulating proinflammatory cytokines. The consequences of these agents on the brain, and neuroinflammation in particular, will be discussed in detail below.

2. Microglia activation during HE

Neuroinflammation is a key feature in common with all types of HE and is predominantly modulated by microglia, the resident macrophage-like cell in the brain. Microglia are normally found in their quiescent or ramified form, characterized morphologically by small cell body and long, branching processes. The cell body typically remains stationary, whereas the processes are constantly moving and surveying their microenvironment for proinflammatory signals released by surrounding damaged neurons, infectious agents etc. Upon activation, microglia undergo morphological changes, including a thickening and retraction of the branches and increased cell body volume, and produce increased amounts of proinflammatory cytokines and recruitment molecules (e.g., chemokines). A schematic diagram of these morphological changes can be seen in **Figure 1**. Furthermore, reactive microglia undergo rapid proliferation to increase their number. The increased microglia number at a site of trauma is thought to be a combination of proliferation of resident microglia and recruitment of microglia from neighboring areas.

Microglial activation has been demonstrated to be a key feature in the pathogenesis of HE regardless of the type. Indirect clinical evidence for microglial activation has been demonstrated by an upregulation of the microglial marker Ionized calcium binding adaptor molecule 1 (Iba-1) in postmortem cortical brain tissue from patients with liver cirrhosis and HE, when compared to cirrhotic patients without HE [1]. In addition, data from a comprehensive gene expression profile analysis demonstrated an upregulation of markers for both the
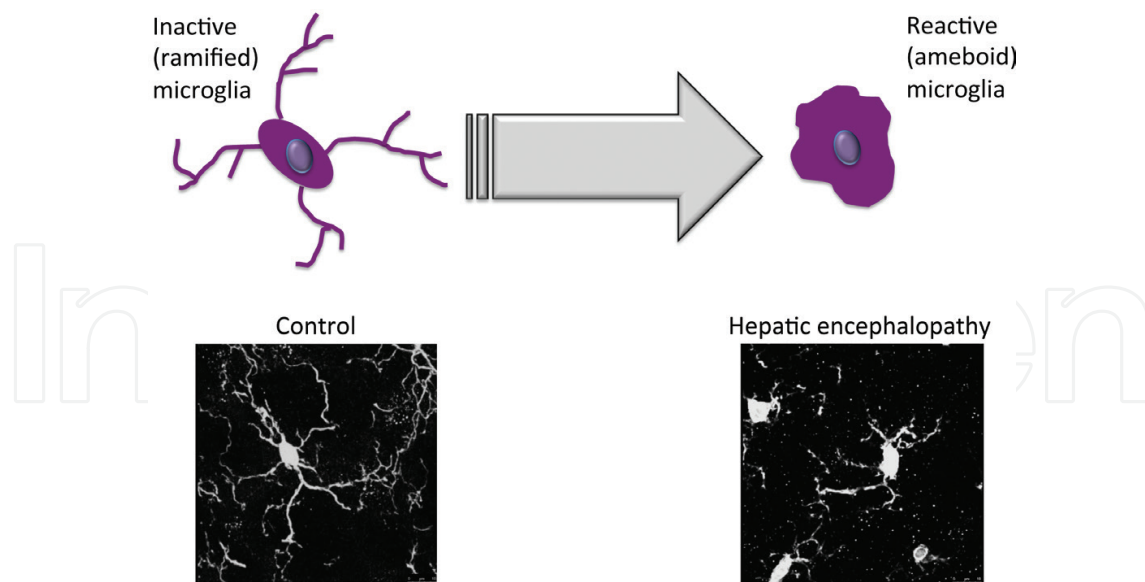
Figure 1. Schematic diagram depicting changes in microglia morphology during neuroinflammation. Lower panels show immunofluorescent staining of microglia using an Iba1-specific antibody in a mouse model of HE.

proinflammatory M1 and anti-inflammatory M2 microglial phenotypes, suggesting that both subpopulations of microglia may be present in patients with HE due to cirrhosis [2]. Taken together, these clinical data indirectly support a role of microglia activation in HE.

In contrast, evidence for a direct role for microglia activation in the neurological consequences of both acute liver failure and liver cirrhosis is more striking in animal models of these diseases. Furthermore, in many of the models used, treatment modalities shown to inhibit microglia activation also alleviated or prevented the cognitive impairment and neurological decline observed during HE. Specific details are described below.

2.1. Toxic liver injury

A range of hepatotoxic agents have been used to uncover basic mechanisms responsible for the CNS complications of liver failure. This topic was reviewed by a panel of experts nominated by The International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) who, after careful deliberation, recommended two toxic models based upon the extent of their characterization. The two models of acute liver failure were the azoxymethane (AOM) mouse model and the thioacetamide (TAA) rat model [3]***. Very elegant and detailed analyses of the morphological changes in microglia and real-time analysis of microglial dysmotility after AOM have been demonstrated [4]. Both microglia activation (as demonstrated by an ameboidal phenotype) and motility (as demonstrated by analysis of the turnover rate) were shown to be altered in the cerebral cortex at late stages of HE when severe neurological symptoms were evident, coinciding with the appearance of brain edema [4]. Furthermore, increased number of microglia [5–7] and increased reactive phenotype [6] has been demonstrated in the cerebral cortex of AOM-injected mice. This HE-associated microgliosis could be attenuated with anti-inflammatory treatment modalities [5–7], which also attenuated or delayed various neurocognitive deficits

observed in this model of Type A HE, indicating that microglia activation may be contributing to the behavioral abnormalities observed in HE rather than as a consequence.

While microglia activation has not been assessed specifically in the ISHEN-recommended TAA rat model of acute liver failure, Faleiros et al. recently assessed this phenomenon using the relatively uncharacterized TAA mouse model of acute liver failure [8]. While mice injected with TAA displayed a significant reduction in locomotor activity, which was accompanied by increased expression of certain proinflammatory cytokines and chemokines, no microglia activation was observed [8]. However, it is conceivable that this observation may be an anomaly of this model rather than evidence of a lack of microglia involvement.

2.2. Ischemic liver failure

Experimental acute liver failure can be induced by the performing an end-to-side portacaval anastomosis followed by hepatic artery ligation and is thought to mimic ischemic liver failure. Rats undergoing this surgery exhibit key clinical features of HE, including cerebral edema and hyperammonemia, which ultimately result in grade 4 HE (hepatic coma). An increase in the number of OX-42/CD11b positive microglia has been demonstrated in the frontal cortex, thalamus, hippocampus and cerebellum starting 6 h after surgery (early stage HE) and worsening at the time of coma/edema [9, 10]. These pathological effects, to include brain edema and HE progression, could be alleviated by either mild hypothermia [9] or treatment with minocycline [10].

2.3. Portal-systemic (bypass) encephalopathy

In a related, more subtle model of HE induced by end-to-side portacaval shunt surgery without subsequent hepatic artery ligation, rats develop mild cognitive impairment over the following 3–4 weeks. Associated with this mild form of HE (or minimal HE) is a change in the microglia morphology to a more ameboid, activated phenotype [11, 12]. Curiously, these changes were restricted to cerebellum. Chronic infusion of a p38 mitogen-activated protein kinase inhibitor [11] or the phosphodiesterase inhibitor Sildenafil [12] reversed the morphological changes observed in microglia and prevented the cognitive impairment.

2.4. Biliary cirrhosis

Obstruction of the common bile duct induces a reproducible model of biliary cirrhosis in rats. Bile duct-ligated (BDL) animals have liver failure, developing jaundice, portal hypertension, portal-systemic shunting, bacterial translocation and immune system dysfunction. BDL rats are hyperammonemic but show only low-grade or minimal encephalopathy (decreased locomotor activities) [3]. Using this model, microglia are activated predominantly in the cerebellum with only traces of activation in the striatum and thalamus [13]. Treatment with ibuprofen reduced microglia activation and reversed the concomitant cognitive impairments observed [13]. Similarly, microglia activation has been shown after BDL in mice, as demonstrated by morphological changes in Iba-1 positive microglia [14]. However, in contrast to

the rat model, the activation of microglia was localized to the cerebral cortex rather than the cerebellum. The cause of these region-and species-selective changes remains unknown. The activation of microglia in BDL mice is thought to subsequently recruit monocytes to the brain that contribute to the cognitive impairment observed [15].

3. Mechanism of microglia activation during HE

As mentioned above, the development of HE appears to be due to the buildup of toxic or inflammatory substances in the blood stream as a result of impaired liver function. While the identity of all of these substances is likely unknown, a summary of the identified factors and their involvement in HE is shown in **Figure 2** and is described in greater detail as follows:
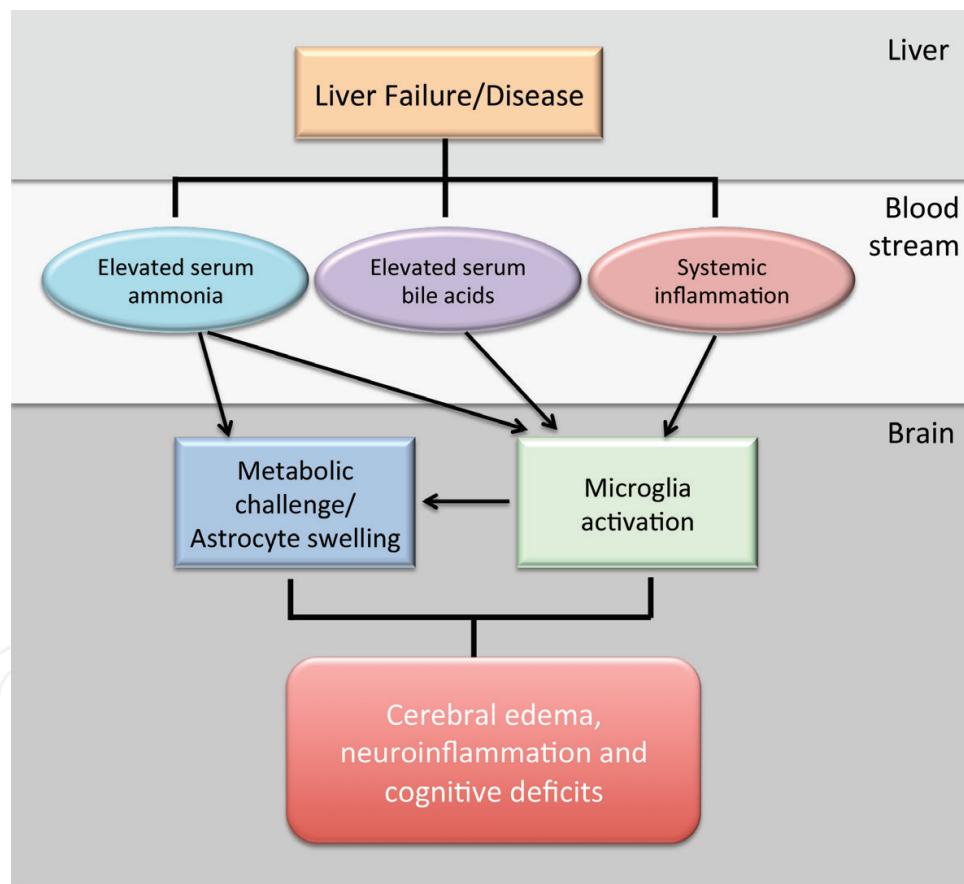

Figure 2. Schematic diagram depicting the current knowledge of the pathogenic mechanisms of HE. Following liver failure or chronic liver disease, there are elevations of serum ammonia, circulating bile acids and systemic inflammation. All of these are able to promote microglia activation as has been shown by numerous studies. In addition, elevated cerebral ammonia leads to an elevation of glutamine in astrocytes causing a metabolic challenge and swelling of astrocytes. There are potential mechanisms at play in which activated microglia promote astrocyte swelling. Both astrocyte swelling and microglia activation promote the development of HE.

3.1. Ammonia

Ammonia is a nitrogenous compound that is the best-characterized neurotoxin that contributes to the development of HE. Ammonia is generated through both gut bacteria and enterocytes and is subsequently metabolized by the liver into urea after its passage through the portal tract [16, 17]. Urea, unlike ammonia, can be excreted from the body via the kidney. However, when the liver is damaged or diseased, this detoxification of ammonia into urea by the liver is impaired leading to significant elevations of ammonia in the bloodstream. Ammonia has the capability to cross the blood-brain barrier during HE and once in the brain, ammonia is taken up by astrocytes [18]. Astrocytes metabolize ammonia into glutamate and subsequently into glutamine via glutamine synthetase. The increased levels of glutamine inside of astrocytes cause an osmotic gradient which results in the swelling of astrocytes and cytotoxic edema [19]. This results in a morphological change in astrocytes, which have been characterized as Alzheimer type II astrocytes, which are a neuropathological marker of this disease state [20].

Ammonia can contribute to other aspects of pathology other than the swelling of astrocytes as this metabolite has been shown to induce oxidative stress and neuroinflammation, which contribute to the pathology of HE. This is evident in cell culture studies where treating rat primary microglia with 5 mM of ammonia was found to induce the expression of reactive nitrogen and oxygen species [21]. In addition, treating rat primary astrocytes with conditioned media from these ammonia-treated microglia induced cell swelling [22]. The idea that ammonia was the primary factor necessary to induce encephalopathy is not be the case in every circumstance. Injection of LPS into sham-operated, ammonia-fed and BDL rats determined that significant neurological deficits and cytotoxic brain edema were observed only in the BDL rats administered LPS [22]. This gives support that inflammation induced by organ injury works in tandem with LPS-induced inflammation to contribute to HE. Therefore, while ammonia does play a significant role in HE, microglia-induced inflammation is a synergistic partner that also contributes to the pathology of this disorder.

3.2. Bile acids

It is well accepted that increased serum bile acids can be an indication of liver damage [23] and have been observed in the cerebrospinal fluid of patients with fulminant hepatic failure [24], and with liver cirrhosis [25], however, their contribution to the pathogenesis of HE has only recently been suggested [26, 27]. Increased serum bile acids have been implicated in the increased blood-brain barrier permeability observed in a rat model of chronic liver disease [28] hereby allowing access of bile acids and other signaling molecules to the brain. Furthermore, increased bile acid content in brain tissue has been demonstrated in rodent models of both acute and chronic liver diseases [26, 27]. In the AOM mouse model of Type A HE, increased total bile acid content was observed in the frontal cortex, and strategies to reduced circulating bile acids (e.g., cholestyramine feeding or the use of a genetically modified mouse with impaired bile acid synthesis) proved neuroprotective [26].

Activation of microglia is a delicate balance between the proinflammatory chemokine ligand 2 (CCL2) and the anti-inflammatory chemokine fractalkine, which in physiological conditions favors the dampening of microglia activation [6]. However, during type A HE, this balance is

dysregulated leading to an increased production of CCL2 [5] and a downregulation of frac-talkine [6] and subsequent microglia activation [5, 6]. This imbalance may, at least in part, be attributable to aberrant bile acid signaling in the frontal cortex during acute liver failure [29].

3.3. Proinflammatory cytokines

It is commonly accepted that systemic inflammation contributes to the progression of HE. Indeed, in patients and in animal models of HE, systemic inflammation causes worsening of the encephalopathy, and it has been proposed that proinflammatory signals act syner-gistically with ammonia toxicity to bring about the neurological complications of acute and chronic liver failure [30–32].

Because the proinflammatory cytokines released from the liver during liver damage are often identical to those released from activated microglia, it is difficult to determine the precise origin and role of each source of cytokine during HE. However, a number of liver-derived proinflammatory cytokines have been definitively demonstrated.

Tumor necrosis factor-alpha (TNFα) is a potent proinflammatory cytokine. Circulating levels of TNFα are increased as a function of the severity of HE in both patients [33] and experi-mental animals [10] with liver failure. Moreover, the presence of TNFα gene polymorphisms is known to influence the clinical outcome in patients with acute liver failure [34]. In experi-mental models of acute liver failure, mice lacking the TNF receptor 1 gene had a delayed onset of encephalopathy and an attenuation of brain edema [35]. TNFα has been shown to activate microglia in a number of experimental models of neuroinflammation [36, 37]. With respect to HE, systemic levels of TNFα are increased in the AOM model of acute liver failure [7]. Inhibition of TNFα signaling by systemic treatment with etanercept reduced systemic inflammation, attenuated the neurological decline, and prevented microglial activation in the cerebral cortex [7]. These data support the hypothesis that peripherally derived TNFα, at least in part, contributes to the microglial activation and subsequent neurological decline in liver failure. In support of this concept, neurological complications occurring in the BDL model of biliary cirrhosis were shown to be the consequence of monocyte recruitment in response to TNFα signaling and occurred via microglial activation. Specifically, peripheral TNFα signal-ing stimulates microglia to produce CCL2, which subsequently mediates monocyte recruit-ment into the brain [14]. These findings were suggested to constitute a novel immune-to-brain communication pathway with the potential to result in altered neuronal excitability and neu-rological complications during cholestatic liver disease.

The role of transforming growth factor β (TGFβ) in the inflammatory response is largely con-text dependent. Specifically, TGFβ has both anti-inflammatory and proinflammatory effects on various immune cells in the body, including microglial activation. Increased levels of TGFβ have been demonstrated in the liver and serum in the AOM model of acute liver failure [38]. The authors demonstrated that peripheral TGFβ has implications on microglial activation [39]. Specifically, systemic treatment of mice with a neutralizing anti-TGFβ antibody, that did not significantly alter the underlying liver damage, but inhibited the actions of circulat-ing TGFβ delayed the neurological decline observed in AOM-induced acute liver failure [38], and attenuated the morphological changes in Iba-1 positive microglia [39]. However, whether

liver-derived TGFβ is acting directly on microglia to regulate the neuroinflammatory response in these models of HE, or whether the changes in microglial activation are an indirect effect of the protective effect of anti-TGFβ neutralizing antibodies remains to be established.

3.4. Neuron and astrocyte crosstalk with microglia

Microglia activation does not occur in an isolated system, and various studies have demonstrated that both neurons and astrocytes have the capability to crosstalk with microglia and promote their activation during neuroinflammatory states. This does occur in conditions other than HE. For example, in a mouse model of Alzheimer's disease, astrocytes have upregulated CCAAT/enhancer-binding protein and proinflammatory cytokines, which are associated with microglia activation and migration [40]. During hyperammonemia in rats, it was found that ammonia induces both astrocyte and microglia activation along with increased production of interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) [41]. In a recent report, LPS-stimulated microglia have increased production of proinflammatory cytokines including IL-1β, IL-6 and TNFα which were reduced when microglia were co-cultured with astrocytes indicating that astrocytes may play an immunomodulatory role [42].

In contrast, neurons have been demonstrated to induce the activation of microglia during HE. In the cortex of mice with acute liver failure, there is an elevation of CCL2 in neurons, which signals through chemokine receptor 2 (CCR2) and chemokine receptor 4 (CCR4) [5]. Antagonism of CCR2 or CCR4 was found to improve HE outcomes and reduce microglia activation compared to controls [5]. In physiological states, neurons are able to inhibit microglia expression by producing fractalkine, which signals through CX3CR1 on microglia [6]. Fractalkine in neurons was found to be suppressed in the cortex during HE in mice with acute liver failure and infusion of soluble fractalkine into the brain led to reduced microglia activation [6].

4. Treatment strategies to reduce neuroinflammation during liver disease

At this time, most therapies used for the treatment and management of HE are not targeted directly at neuroinflammation, per se. As ammonia was the first identified neurotoxin to play a role in HE, current treatment strategies are aimed at reducing circulating ammonia levels during this disease state. Some of these treatments appear to have efficacy in certain conditions, while others do not. Current treatments and future potential therapeutic strategies will be discussed below.

4.1. Current treatments for HE

A majority of current therapies are aimed at reducing the levels of circulating ammonia by targeting the bacteria of the gut. It should be noted that these treatments may indirectly reduce inflammation due to the synergism of ammonia and neuroinflammation during HE described above. The non-absorbable disaccharides lactulose and lactitol are commonly used for HE

treatment. These are metabolized by the gut microbiota, which acidifies the colon, reduces the number of ammonia producing bacteria, and converts ammonia to ammonium, which cannot be absorbed [43]. While studies have reported improved outcomes of HE patients during lactulose treatment [44], a meta-analysis assessing 30 studies determined that lactulose treatment did not significantly reduce mortality in HE patients though it did reduce the risk of no improvement [45]. Lactitol has been shown to be just as efficacious as lactulose and has less severe side effects but is not available in the United States [46].

Non-digestible antibiotics are another therapy targeted at reducing ammonia production of intestinal bacteria and can be used in conjunction with lactulose. Rifaximin has the least number of side effects and is the most well-characterized [47]. Rifaximin is effective against both Gram-positive and Gram-negative bacteria of the gastrointestinal tract. Rifaximin works by disrupting transcription by binding RNA polymerase and has been demonstrated to reduce ammonia concentrations and improve mental status to a greater degree than lactulose or other antibiotics in HE patients [48].

L-Ornithine-L-aspartate (LOLA) is aimed at reducing ammonia concentrations by increasing the generation of urea through the urea cycle. Oral administration of LOLA is not recommended for the management of HE as the studies assessing its efficacy have been conflicting with some stating no benefit compared to placebo [49]. Newer studies have identified that intravenous administration of LOLA is more efficacious at lowering ammonia levels and is recommended for patients that do not respond to lactulose treatment [50].

Probiotics are dietary supplements containing viable bacteria that are designed to deprive pathogenic bacteria of nutrients, while supplying beneficial bacteria with growth-promoting substrates. While there have not been definitive studies during acute liver failure, probiotics have shown efficacy during type C HE. A meta-analysis of probiotics usage in patients with minimal HE described that they are associated with significantly improved outcomes [51]. In another study, it was found that the probiotic VSL#3 improved outcomes in patients with minimal HE and had comparable efficacy to lactulose [52].

Other treatments employed are designed to minimize the complications of HE. Two of these are mannitol and hypertonic saline which aim to reduce cerebral edema and intracranial pressures that are a result of cytotoxic edema and inflammation [50].

4.2. Pre-clinical therapies targeting inflammation

While the current therapies being employed are largely focused on ammonia, there are prospective therapeutic approaches that are targeted at reducing neuroinflammation with many of the studies reporting improved HE outcomes.

Therapeutic hypothermia has been employed in rodent models and in patients. In rats with end-to-side portacaval anastomosis, moderate hypothermia (33°C) was found to reduce cerebral edema and TNFα, IL-1β and IL-6 concentrations in the cortex [9]. A similar finding was observed in HE patients where reducing their core temperature to 32–33°C was able to decrease levels of circulating TNFα, IL-1β and IL-6 as well as reduce cerebral edema and

intracranial pressure [53]. However, a recent report investigating moderate hypothermia (33–34°C) in HE patients from acute liver failure determined that this treatment strategy did little to reduce increased intracranial pressures or mortality [54]. Therefore, more studies are necessary to determine the clinical potential of therapeutic hypothermia in patients with HE.

Chemokines and cytokines may also be a potential target for the management of HE. Systemic antagonism of CCR2 or CCR4 was found to reduce neuroinflammation and neurological decline in AOM-treated mice [5]. In addition, supplementation of CX3CR1-mediated signaling via soluble fractalkine infusion in the brain reduced microglia activation and neuroinflammation in AOM-treated mice [6]. TNFα-mediated signaling seems to play a significant role in neuroinflammation and outcomes during HE as infliximab, etanercept and p38 inhibitors all reduced serum and brain levels of proinflammatory cytokines and improved cognitive and motor functions in rodent models of HE [7, 11, 55].

N-acetylcysteine is a therapeutic agent that is known to be efficacious in the treatment of hepatotoxic acetaminophen overdose by increasing bioavailability of the antioxidant glutathione. In regard to HE, it has been shown that in acute liver failure not due to acetaminophen overdose that N-acetylcysteine is able to reduce IL-17 and improve outcomes but this occurs only in patients with grade 0-II HE [56, 57]. These beneficial effects of N-acetylcysteine were not observed in children with minimal HE due to non-acetaminophen-induced acute liver failure as there was no change in 1-year survival [58]. In fact, it was observed that children younger than 2 years old actually had a significantly reduced 1-year transplant free survival compared to controls [58].

Non-steroidal anti-inflammatory drugs (NSAIDs) also show promise at mitigating neuroinflammation and improving outcomes during HE. Ibuprofen has been shown to reduce microglia activation, cerebellar IL-1β concentrations and improved learning and motor functions in BDL rats [13]. In portacaval shunt rats with HE, ibuprofen treatment reduced inducible nitric oxide synthase expression and improved motor and cognitive function compared to controls [59]. Indomethacin has shown conflicting results with this treatment reducing intracranial pressures in patients with acute liver failure while increasing TNFα and mortality in TAA-induced liver failure in rats [60]. More studies are necessary with NSAIDs in patient populations before these agents should be used for the treatment of HE [61].

The elevation of bile acids in the brain and CSF of HE patients due to acute liver failure has been previously described [24]. Recently, we published a report that bile acids are elevated in the cortex of AOM-treated mice, and the use of cholestyramine (to promote fecal excretion of bile acids) or Cyp7A1-null mice (that have reduced bile acid synthesis) were protected from neurological decline [26]. This is not unique to this model as BDL rats 3 weeks after surgery have significant elevations of lithocholic acid in the brain [62]. Bile acids in other systems have been demonstrated to have the ability to modulate inflammation, giving support that they could contribute to neuroinflammation during HE.

4.3. Clinical trials

At this time, there are 83 open clinical trials assessing aspects of HE. That being said, the numbers directly assessing HE are only 15 and of these 15, 6 involve lactulose or rifaximin that

are already being readily used in the clinic. Of these remaining studies, only 6 involve novel treatments not currently in use.

Due to the lack of efficacy for probiotics during HE due to acute liver failure, fecal microbiota transplants are being proposed to better control the makeup of the gut microbiome. Fecal microbiota transplants are taken from a health donor and are then administered in a diseased individual with the goal of altering their gut bacteria to a healthier population. The first trial is aimed at cirrhotic patients with recurrent HE that do not respond to lactulose or rifaximin (NCT02255617). The second trial is also in cirrhotic patients and is aimed at determining the feasibility and safety of fecal microbiota transplants in HE patients (NCT02862249).

GABA receptor antagonists could also be a potential therapy for HE as this neurotransmitter is modulated by both positive and negative regulators [63]. As GABA activation suppresses neural circuits, this neurotransmission pathway could suppress the CNS and promote hepatic coma. Flumazenil is a GABA receptor antagonist that is being proposed for use in non-alcoholic cirrhotic patients that have HE (NCT02048969). This trial will employ proton magnetic resonance spectroscopy to determine the metabolic and biochemical changes in these patients that are on flumazenil or placebo.

Metformin is one of the primary medications used for the treatment of type 2 diabetes. During HE, it has been shown that metformin inhibits glutaminase activity and was protective against HE in cirrhotic patients with diabetes [64]. In order to determine whether metformin is beneficial in cirrhotic patients with diabetes and minimal HE, a clinical trial is being performed that will assess neurobehavioral outcomes using psychometric tests in these patients (NCT02470546).

Albumin infusion is a method employed to scavenge substances, and proteins in the blood to improve patient outcomes. In cirrhotic patients with HE, albumin infusion has been previously used and was found to not reduce the occurrence of HE during hospitalization, but did improve survival after hospitalization [65]. The new trial is comparing infusion of human albumin into cirrhotic patients with HE and is assessing survival at 90 days and 180 days following initial dose (NCT02401490).

The last clinical trial is the joint administration of nitazoxanide and lactulose in cirrhotic patients with HE (NCT02464124). Nitazoxanide is an oral medication that is used to treat *Giardia lamblia* and *Cryptosporidium parvum* during infectious diarrhea. The outcomes of this trial are to determine the number of patients with total reversal of HE.

5. Conclusions

In conclusion, the evidence for a role of neuroinflammation in HE is unequivocal. However, the precise molecular mechanism by which neuroinflammation, and more specifically microglia, is activated is not completely understood. Targeting the neuroinflammatory aspect of HE may prove to be a useful strategy for the development of experimental therapeutics to manage the neurological complications of HE.

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