

Pathogenesis of Hepatic Encephalopathy: Role of Ammonia and Systemic Inflammation



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The syndrome we refer to as Hepatic Encephalopathy (HE) was first characterized by a team of Nobel Prize winning physiologists led by Pavlov and Nencki at the Imperial Institute of Experimental Medicine in Russia in the 1890's. This focused upon the key observation that performing a portocaval shunt, which bypassed nitrogen-rich blood away from the liver, induced elevated blood and brain ammonia concentrations in association with profound neurobehavioral changes. There exists however a spectrum of metabolic encephalopathies attributable to a variety (or even absence) of liver hepatocellular dysfunctions and it is this spectrum rather than a single disease entity that has come to be defined as HE. Differences in the underlying pathophysiology, treatment responses and outcomes can therefore be highly variable between acute and chronic HE. The term also fails to articulate quite how systemic the syndrome of HE can be and how it can be influenced by the gastrointestinal, renal, nervous, or immune systems without any change in background liver function. The pathogenesis of HE therefore encapsulates a complex network of interdependent organ systems which as yet remain poorly characterized. There is nonetheless a growing recognition that there is a complex but influential synergistic relationship between ammonia, inflammation (sterile and non-sterile) and oxidative stress in the pathogenesis HE which develops in an environment of functional immunoparesis in patients with liver dysfunction. Therapeutic strategies are thus moving further away from the traditional specialty of hepatology and more towards novel immune and inflammatory targets which will be discussed in this review. (J CLIN EXP HEPATOL 2015;5:S7-S20)

Hepatic encephalopathy (HE) is the term used to encapsulate the broad spectrum of neuropsychiatric disturbances associated with both acute and chronic liver failure (ALF and CLF, respectively), as well as porto-systemic bypass in the absence of hepatocellular disease. The clinical manifestations of HE can be extremely heterogeneous in nature, with symptoms presenting anywhere on a continuum spanning from seemingly normal cognitive performance, right the way through to states of confusion, stupor and coma. In be-

tween these extremes, patients with HE may exhibit signs such as inattentiveness, blunted affect, impairment of memory or reversal of the sleep-wake cycle, as well as physical manifestations such as tremor, myoclonus, asterixis and deep tendon hyperreflexia.

ALF is defined by the onset of coagulopathy alongside any degree of encephalopathy in patients with no evidence of pre-existing liver disease.¹ The presence of HE in those with ALF is prognostic, with up to a quarter of cases developing raised intracranial pressure.² Patients presenting with ALF are at risk of developing its cardinal, life-threatening feature, cerebral edema. Left untreated, cerebral edema can rapidly progress to cause herniation of the uncus through the falx cerebri, leading to compression of the brainstem and, ultimately, death. Historically, cerebral edema was believed to develop in up to 80% of patients with ALF and be the most common cause of death.³ However, recent data following a review of 3300 patients presenting to a single tertiary liver center has shown that the proportion of patients with intracranial hypertension (ICH) fell from 76% in 1984-88 to 20% in 2004-08 ($P < 0.0001$). In those who developed ICH, mortality fell from 95% to 55% ($P < 0.0001$). This mirrored a fall in the markers of disease severity on intensive care admission reflecting earlier recognition, improved care, and use of salvage emergency liver transplantation.⁴

Keywords: hepatic encephalopathy, ammonia, inflammation, infection, systemic inflammatory response syndrome

Received: 9.12.2013; **Accepted:** 5.6.2014; **Available online:** 30.6.2014

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Abbreviations: HE: hepatic encephalopathy; ICH: intracranial hypertension; AoCLF: acute-on-chronic liver failure; MHE: minimal hepatic encephalopathy; GS: glutamine synthetase; CNS: central nervous system; BBB: blood-brain barrier; PAG: phosphate-activated glutaminase; CBF: cerebral blood flow; iNOS: inducible nitric oxide synthase; PTP: permeability transition pore; MPT: mitochondrial permeability transition; ATP: adenosine triphosphate; TLR: toll-like receptor

<http://dx.doi.org/10.1016/j.jceh.2014.06.004>

In patients with CLF, the symptoms of HE tend to be far less severe and occur insidiously in keeping with the chronic nature of this disease. Factors such as sepsis, upper gastrointestinal bleeding, constipation or electrolyte disturbances, can precipitate the clinical decompensation of pre-existing cirrhosis and may lead to the development of organ dysfunction; a state sometimes referred to as acute-on-chronic liver failure (AoCLF). Phenotypically, AoCLF may be indistinct from ALF, and in some cases patients may even develop cerebral edema, although this is generally considered to be rare.⁵ Great emphasis is put on actively seeking out and treating any precipitating factors in patients with cirrhosis presenting with overt HE, so as to minimize their risk of developing potentially fatal complications.⁶

Minimal hepatic encephalopathy (MHE) cannot, by definition, be detected by the clinician alone, and its diagnosis therefore hinges on detailed assessment of the patient's history and comprehensive examination of the neurologic system, as well as formal psychometric testing.⁷ It has therefore recently been redefined as covert HE.⁸ The prevalence of MHE in patients with cirrhosis has been estimated to lie between 30% and 84%, with variations in the diagnostic criteria thought to be responsible for this wide range.⁹

THE ROLE OF AMMONIA IN THE SYNDROME OF HEPATIC ENCEPHALOPATHY

Ammonia was first implicated in the pathogenesis of HE by a team of Nobel Prize winning physiologists led by Pavlov and Nencki at the Imperial Institute of Experimental Medicine in Russia in the 1890's. Hahn and colleagues demonstrated the induction of an encephalopathic state in dogs following the formation of a surgical shunt, known as Eck's fistula, which served to divert nitrogen-rich blood from the portal vein directly to the inferior vena cava, therein bypassing the liver. Six weeks post-operatively, the dogs began to exhibit increased levels of aggression, irritability, ataxia, as well as experiencing seizures and eventually lapsing into coma especially following ingestion of an ammonia-rich meal.¹⁰ Two years later, in another canine study with surgical portocaval fistulas, it was discovered that the urinary concentration of ammonia salts was elevated, leading to the logical first suggestion that ammonia may be key in the development of this neurobehavioural syndrome.¹¹ The ingestion of ammonium salts was subsequently shown to exacerbate the neurobehavioural symptoms in these dogs, causing them to become comatose and die. This causally implicated the inability of the bypassed liver to convert the neurotoxic ammonia into urea and its subsequent accumulation in the brain, in the syndrome which was later termed HE.^{12,13}

Some years later, Gabuzda and colleagues¹⁴ performed a therapeutic trial in 12 cirrhotic subjects which aimed to

assess the efficacy of three different cation-exchange resins in the treatment of ascites; this followed reports that cation-exchange resins were effective in treating the fluid overload state associated with congestive cardiac failure. Whilst results from this study indicated that the resins were indeed effective diuretics, significantly reducing ascites and edema, almost all of the cirrhotic subjects receiving the ammonium-containing cation-exchange resins developed marked neurological and behavioral disturbances. Patients became drowsy, apathetic, weak, confused and disorientated to time and place, and exhibited various inappropriate behaviors. These neurocognitive changes presented within a few days of the administration of the ammonium-containing cation-exchange resins and resolved soon after their discontinuation, therein illustrating the generally reversible nature of HE. This prompted further investigation by Phillips and colleagues later on in the same year.¹⁵ In this study, patients with advanced cirrhosis were administered ammonium chloride, urea, protein or di-ammonium citrate, and observed. These substances precipitated the development of a syndrome identical to that of impending hepatic coma and lay the foundations of our understanding that ammonia is central in the pathogenesis of HE.

Beginning in mid-1950s, studies began to focus on whether or not it was possible to establish a quantitative relationship between blood ammonia concentration and the severity of neurocognitive impairment in HE in cirrhosis. Broadly speaking, those patients who were experiencing significant neurological disturbances had elevated blood ammonia levels^{16,17} but whilst blood ammonia levels were generally higher in cirrhotic patients with either past or present neurological disturbances, blood ammonia concentration was not predictive or consistent with severity HE.^{18,19} This finding has been replicated with one such study showing that 69% of individuals with no overt signs of HE had elevated blood ammonia levels, whilst a number of patients with more significant grade 3 or 4 HE had either normal or only slightly elevated levels of ammonia in their blood.²⁰

This is in contrast to ALF whereby the relationship between blood ammonia levels and the clinical severity of HE is more clear-cut. Bernal and colleagues demonstrated ammonia to be an independent risk factor for the development of both HE and ICH, with the latter occurring in 55% of patients with blood ammonia concentrations >200 $\mu\text{mol/L}$.² Blood ammonia levels $\geq 150 \mu\text{mol/L}$ have also been shown to predict a greater likelihood of cerebral herniation and death in patients presenting with ALF.²¹ Persistent arterial hyperammonaemia for 3 days following hospital admission predicts a greater likelihood of complications and death in individuals with ALF.²²

Discrepancies in the direct correlation between ammonia concentration and the severity of HE in patients with cirrhosis, have contributed to the general consensus

that whilst ammonia has an irrefutable, and key role in the pathogenesis of HE, it may not be solely responsible for the neurocognitive sequelae and other factors might be contributing. Over the last decade or so, a fast-growing body of literature has been emerging which supports the role of other factors, such as sterile and non-sterile systemic inflammation and its associated 'cytokine storm', in acting synergistically with the deranged nitrogen metabolism found in patients with liver failure to culminate in, and propagate, the clinical picture of HE.

INTERORGAN AMMONIA METABOLISM AND REGULATION

Ammonia is a by-product of nitrogen metabolism, and its formation in the body is predominantly a consequence of the action of the enzyme glutaminase located within enterocytes of the small intestine and colon, as well as the action of the vast number of urease-producing bacteria located in the gut. Ammonia derived from the gut is absorbed into the hepatic portal circulation and transported to the liver where, under normal physiological conditions, it enters the urea cycle and is metabolized. Ammonia that bypasses this primary fate is subsequently 'picked up' and detoxified by glutamine synthetase (GS), an enzyme found in the hepatocytes surrounding the hepatic vein (as well as in muscle and astroglial cells), which catalyses the conversion of ammonia and glutamate to glutamine.²³ Whilst the liver is critical in the homeostatic control of blood ammonia levels, other organs such as the brain, muscle and kidney are also known to play a role in regulating them. Insult to the liver, whether acute or chronic in nature, reduces its capacity to metabolize ammonia and this exerts an ammonia burden on extrahepatic tissues which can result in hyperammonaemia up to five times that of normal blood ammonia levels. The occurrence of hyperammonaemia is not specific to liver dysfunction, and can also be observed in various other disease states including, but not limited to, inborn errors of the urea cycle, Reye's syndrome and valproate poisoning.²⁴ In the context of liver failure, the brain, and more specifically, astrocytes, act as an alternative metabolic pathway for ammonia; but not without a toll.

AMMONIA AND THE BRAIN: THE SICK ASTROCYTE

Astrocytes are the most abundant cells of the central nervous system (CNS) and are the cells most commonly found to be affected in patients with HE owing to the exclusive localization of GS within the CNS to astrocytes.^{25,26}

Astrocytes are involved in numerous functions in the brain, such as the provision of nutrients and mechanical support to surrounding neurones, the regulation of ion transport and neurotransmitter uptake in the brain, as

well as being key components of the blood-brain barrier (BBB). Whilst astrocytes are sensitive to the effects of ammonia, neurones are almost completely unaffected by exposure to this neurotoxin. The impact of ammonia is significantly greater on pure populations of neurones, as compared to populations of neurones co-cultured with astrocytes, highlighting a neuroprotective role for astrocytes.²⁷

The "strategic" positioning of astrocytes within the neurovascular unit (composed of the cerebral microvascular endothelium, astrocytes, pericytes and extracellular matrix) is thought to facilitate the efficiency with which astroglial cells can detoxify ammonia and trap it in the brain. Whilst the BBB has been shown to remain anatomically intact in HE,²⁸ PET studies utilizing ¹³N-ammonia have demonstrated an increased uptake and trapping of ammonia in the brains of individuals with CLF, with controversy prevailing over the respective roles that alterations in the permeability of the BBB, and blood ammonia levels, may have in this observation.²⁹⁻³²

From the neuropathological standpoint, significant astrocyte swelling and cytotoxic brain edema are cardinal features of human ALF. Using electron microscopy, Kato and colleagues observed marked swelling of astroglial foot processes in samples of cerebral cortex obtained from patients succumbing from ALF.³³ Similar results have been gathered from animal models of ALF,³⁴ as well as from CT studies of the brains of children with ornithine carbamoyl transferase deficiency, a congenital disorder of the urea cycle associated with acute episodes of hyperammonaemia³⁵ and cultured astrocytes exposed to pathophysiologically relevant concentrations of ammonia.³⁶ Recent MRI studies of patients with ALF demonstrate evidence of interstitial brain edema as well as cytotoxic edema, implying there may be a vasogenic component to the cerebral edema in ALF.^{37,38} In an animal model of ALF, astrocyte swelling, extravascular and interstitial edema has been described. However, brain capillary endothelial cells and their tight junctions appeared intact.^{34,39} Nguyen has also described physically intact tight junctions in ALF, but these were lengthened and tortuous in shape.⁴⁰

In CLF, astrocytes typically exhibit morphological features of Alzheimer type II astrocytosis, which include a large swollen nucleus, prominent nucleolus, margination of the chromatin pattern and significant enlargement of the cytoplasm. These alterations to astrocyte morphology can be seen in the brains of patients with chronic hyperammonaemia due to congenital disorders of the urea cycle enzymes, as well as in various experimental animal models of hyperammonaemia,^{41,42} and in astrocyte cultures chronically exposed to hyperammonaemia.⁴³ Experimental models of CLF in rats have consistently shown no evidence of BBB breakdown,^{28,44} however Chavarria and colleagues⁴⁵ have recently provided evidence for the

presence of both cytotoxic and vasogenic edema in cirrhotic patients awaiting liver transplantation. It has been proposed that low-grade astrocyte swelling, as may be seen in CLF,⁴⁶ could have significant functional consequences despite the absence of clinically overt ICH, and impairment of the cross-talk between swollen astrocytes and neurones has also been suggested to alter cerebral function.⁴⁷

Evidence suggests that the neuropsychological effects of induced hyperammonaemia, and the subsequent elevation of astrocyte glutamine levels, are determined by the intrinsic ability of the brain to buffer these changes by losing key osmolytes such as myo-inositol⁴⁸; a process which may in itself be modulated by other factors such as hyponatraemia, a major risk factor for the development of overt HE in patients with CLF.^{49,50}

In states of hyperammonaemia, ammonia detoxification within astrocytes leads to an intracellular accumulation of glutamine which, it is widely postulated, generates an osmotic stress and causes astrocytes to swell in HE; this is known as the osmotic gliopathy theory, and the reader is directed to a review by Brusilow and colleagues for a more detailed account.⁵¹

The 'Trojan horse' hypothesis has recently been proposed as an alternative theory to explain the development of astrocyte swelling and implicates an important role for both ammonia and glutamine.⁵² The excess glutamine synthesized within astrocytes is transported into mitochondria where it is metabolised by phosphate-activated glutaminase (PAG) to ammonia and glutamate. Glutamine, the 'Trojan horse', thereby acts as a carrier of ammonia into mitochondria, where its accumulation can lead to oxidative stress and ultimately, astrocyte swelling.

Ammonia interferes with mitochondrial energy metabolism and studies have reported depletion of ATP *in vitro* and *in vivo* models of ammonia neurotoxicity.⁵³ The implications of energy failure in ALF have largely been disregarded despite the presence of higher lactate levels in patients with ALF, which is a consequence of energy failure.⁵⁴ In an experimental rodent model of ALF,⁵⁵ in the early (pre-coma) stages of HE there was a significant 2–4.5-fold increase in total brain glutamine and lactate but in the severe (coma) stages of HE and brain edema, there was a further significant increase in brain lactate but no such increase in glutamine implying that impaired glucose oxidative pathways rather than intracellular glutamine accumulation *per se* may play a more dominant role.^{56,57}

SYSTEMIC INFLAMMATION

Sepsis and inflammation are terms often used synonymously, however they are not equivalent clinical entities. Systemic inflammation, also commonly referred to as SIRS (systemic inflammatory response syndrome) can present as a consequence of many pathologies in both sterile and non-sterile environments [Figure 1].⁵⁸ It is not contingent on the presence of infection and may occur purely as a consequence of liver inflammation and necrosis. SIRS is defined by the presence of 2 or more criteria as outlined in Table 1.

SIRS is the clinical manifestation of the systemic release of pro-inflammatory cytokines and mediators including, but not limited to TNF- α , IL-1 β , IL-6, IL-8 and IL-12. Particular insults that can induce SIRS include injury direct to hepatocytes, such as acetaminophen-induced toxicity or acute alcoholic hepatitis, or may develop in the periphery in

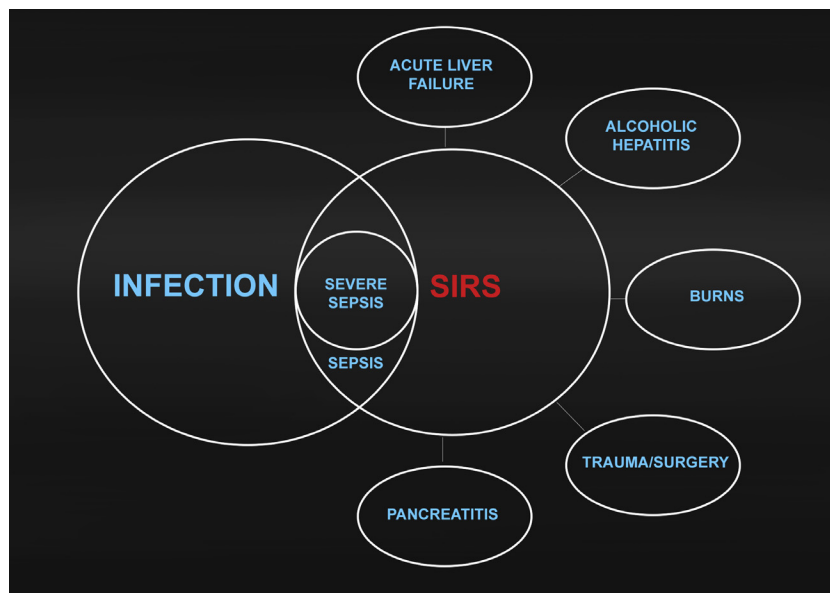


Figure 1 The systemic inflammatory response syndrome.

Table 1 Definition of the Systemic Inflammatory Response Syndrome.⁵⁸

The response is manifested by 2 or more of the following conditions:	
1	Temperature >38 °C or <36 °C
2	Heart rate >90 beats per minute
3	Respiratory rate >20 breaths per minute or PaCO ₂ <32 mmHg
4	White blood cell count >12,000/mm ³ or less than 4000/mm ³ or <10% immature neutrophils.

response to sterile injury such as pancreatitis, burns, surgery or trauma.⁵⁸ SIRS can culminate in the development of tissue injury following activation of neutrophils and microvascular dysfunction which induces vasodilatation, capillary leak, mitochondrial dysfunction and disseminated intravascular coagulation which lead to impaired tissue oxygenation, cell death and multiorgan failure akin to that observed in patients with septic shock or ALF.

Increasingly and perhaps unsurprisingly, sepsis, or a systemic inflammatory state is being recognized as a key player in precipitating and exacerbating HE, possibly by rendering the brain more susceptible to concurrent hyperammonaemia.

Septic encephalopathy or delirium, which will be discussed in an accompanying review in this journal, is well documented and can present similarly to HE with altered consciousness and motor activity, in the absence of cerebral infection. Although the mechanisms are discreet, it is possible that infection can factor into the precipitation of an encephalopathic state, whether the patient has underlying liver disease, or not. Indeed, one study has shown that 66% of cirrhotic patients presenting with advanced HE necessitating airway support had evidence of systemic inflammation (46% had positive cultures and 20% had evidence of sterile SIRS). The severity of the HE did not however correlate with the arterial ammonia level, serum biochemistry or the underlying disease severity as measured by the MELD score.¹⁹

THE ROLE OF SYSTEMIC INFLAMMATION IN ENCEPHALOPATHY IN ACUTE LIVER FAILURE

There are striking similarities between the clinical presentation of septic shock and ALF with them sharing the common cardinal features of encephalopathy, coagulopathy and cardiovascular collapse. It is perhaps therefore not a surprise to observe that in those patients with ALF in whom there is concurrent inflammation (SIRS), have more advanced manifestations of HE and a poorer prognosis. In a pig model of ALF, animals administered lipopolysaccharide and amatoxin developed more pronounced ICH than pigs given amatoxin alone, despite the fact the arterial ammonia concentrations were similar in both groups.⁵⁹ In a seminal study, Rolando and colleagues observed a rapid progression in the severity of HE with inherent poor prognosis in those patients with ALF that had more marked inflammation as measured by the SIRS Score [Figure 2]⁶⁰ and in a study from the US ALF Group, progression of HE from mild to deeper stages was temporally associated with the development of infection.⁶¹ Furthermore, during acetaminophen-induced ALF, it has been shown that the inflammatory cascade is activated within the brain itself; proinflammatory cytokines including IL-1 β , IL-6 and TNF- α are released into the arterial circulation (shown by increased brain cytokine flux) and arterial cytokine levels correlate well with the development of ICH.⁶² In an azoxymethane mouse model of ALF, etanercept, a TNF- α neutralizing molecule, significantly delayed the onset of hepatic coma and significantly reduced peripheral inflammation as evidenced by decreased plasma IL-6 and CD40L levels. Etanercept also decreased IL-6 levels in the brain, attenuated microglial activation as assessed by OX-42 immunoreactivity, and increased brain glutathione concentrations.⁶³ Together, these studies support the role of inflammation, whether caused by infection or as a consequence of ALF itself, as being manifest in determining the severity, progression and outcome of HE in ALF.

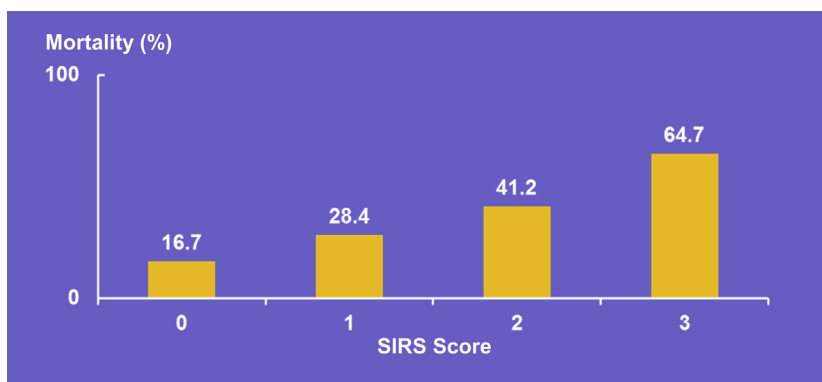


Figure 2 The systemic inflammatory response syndrome in the outcome of hepatic encephalopathy in acute liver failure adapted from Rolando et al.⁶⁰

Studies have also shown that inflammation may exert its effects in part through alterations of cerebral blood flow (CBF). It has been shown that increasing inflammation has a direct correlation with increasing CBF⁶⁴ which in turn is known to raise intracranial pressure.⁶⁵ This has been further demonstrated by studies which have examined therapeutic strategies that reduce systemic inflammation and cerebral hyperemia.

Bémour and colleagues have investigated the impact of proinflammatory gene deletions on the onset of brain edema in an animal model of ALF.⁶⁶ Deletion of the IFN- γ gene had no effect on brain water levels or neurocognitive status. However, IL-1 β and TNF- α gene deletions significantly delayed the onset of HE and brain edema. This may correlate with the parallel observation of early cerebral microglial activation in animal models of ALF which increases as HE and brain edema ensues.^{67,68}

THE ROLE OF SYSTEMIC INFLAMMATION IN ENCEPHALOPATHY IN CHRONIC LIVER FAILURE

As with ALF, there is direct evidence for the role of inflammation in exacerbating the severity of HE in patients with cirrhosis. In MHE, patients have elevated plasma levels of inflammatory markers including IL-6 and IL-18 which correlates with the presence and the severity of HE⁶⁹ but is not determined by the severity of underlying liver disease or ammonia levels *per se*. Furthermore, in stable cirrhotic patients undergoing neuropsychological testing, there was a significant deterioration in scores following induced hyperammonemia in the inflammatory state, but not after its resolution, suggesting inflammation and its mediators may be important in modulating the cerebral effect of ammonia.⁷⁰ This observation applies equally to patients with cirrhosis that develop advanced HE with infection and systemic inflammation, but not ammonia, being implicated in the development of advanced HE.¹⁹

In an animal model of MHE, Cauli and colleagues demonstrated an improved learning ability following the administration of the NSAID, ibuprofen.⁷¹ This may act by reducing the inducible nitric oxide activity within the cerebral cortex. In chronic liver disease experimental models, portal vein ligated animals have not been found to exhibit microglial activation, however, feeding rats an ammonium-containing diet or performing bile duct ligation (BDL) was sufficient to induce microglial activation and neuroinflammation which was reduced by administering ibuprofen.⁷² Zemtsova and colleagues have demonstrated up-regulation of the microglial activation marker ionized calcium-binding adaptor molecule-1 in the cerebral cortex from acutely ammonia-intoxicated rats and in the cerebral cortex from patients with cirrhosis who had HE, but not from patients with cirrhosis who did not have HE. However, ammonia had no impact on microglial glutamate release,

prostaglandin synthesis, and messenger RNA (mRNA) levels of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and the proinflammatory cytokines IL-1 α/β , TNF- α , or IL-6. Unexpectedly however, mRNA and protein expression of iNOS and COX-2 and mRNA expression of proinflammatory cytokines and the chemokine monocyte chemoattractive protein-1 (MCP-1) in the cerebral cortex from deceased patients with liver cirrhosis and HE did not differ compared to patients without HE and non-cirrhotic controls.⁷³ However, D'Mello and colleagues have elegantly shown in a bile duct-ligated resection mouse model that there is significant infiltration of activated monocytes into the brain accompanying microglial activation. They show that in the presence of hepatic inflammation, mice demonstrate elevated cerebral MCP-1 levels, as well as increased numbers of circulating CCR2-expressing monocytes. Cerebral recruitment of monocytes was abolished in MCP-1/CCL2 or CCR2 knockout mice. The activated microglia produce MCP-1/CCL2 prior to cerebral monocyte infiltration in response to peripheral TNF- α signaling and in TNF-R1 deficient mice microglial expression of MCP-1/CCL2 and cerebral monocyte recruitment were both markedly inhibited.⁷⁴

A whole human genome micro-array approach in post mortem brain samples from cirrhotic patients with or without HE and non-cirrhotic controls, has indicated altered expression levels in over one thousand genes related to oxidative stress, microglial activation, receptor signaling, inflammatory pathways, cell proliferation and apoptosis. Despite an up-regulation of genes associated with microglia activation, proinflammatory cytokine mRNA profiles remained unchanged in the brain of patients with liver cirrhosis and HE as compared to controls supporting the previous findings of the Zemtsova study.⁷⁵ Perhaps the most interesting observation of this study however was that many anti-inflammatory genes were up-regulated in the cerebral cortex suggesting that the brain is able to induce appropriate compensatory anti-inflammatory responses in HE.⁷⁶

AMMONIA AND INFLAMMATION: A COMPLEX SYNERGISTIC RELATIONSHIP

As inflammation, infection and ammonia have all been shown to be important interdependent factors in the pathogenesis of HE, the next question that demands to be answered is as to whether infection and inflammation have a synergistic relationship with ammonia. Hyperammonemic mice injected with endotoxin to induce an immune response produce potent proinflammatory cytokine responses, inducing learning impairment that is more pronounced and more enduring than in hyperammonemic control mice suggesting that ammonia sensitizes the brain to the effects of systemic inflammation.⁷⁷ This may occur even in the absence of any underlying liver

dysfunction. This was observed in a recent study where 'healthy' controls with keloid scars were found to subsequently have a degree of hyperammonemic encephalopathy with raised ammonia and inflammatory markers, but without any evidence of liver impairment.⁷⁸

When BDL rats are fed an ammoniagenic diet, cerebral ammonia levels rise with evidence of the development of the characteristic Alzheimer Type II astrocytosis. Both ammonia-fed and control BDL animals have evidence of active inflammation but the rats fed ammonia had a significant rise in brain edema, glutamine, and reduction in myo-inositol and on co-ordination testing, had impaired motor function suggesting either an additive, or possibly synergistic effect of these two factors.⁷⁹ Further supporting evidence can be gleaned when endotoxin is administered to BDL rats exacerbating cytotoxic brain edema with the induction of pre-coma, despite a preserved BBB. Systemic and local brain inflammation is observed in these animals which increases further in those given endotoxin. Nitrosation of proteins in the frontal cortex of BDL and endotoxin-treated animals can be demonstrated. However, ammonia cannot be responsible alone because protein nitrosation was not demonstrated in ammonia-fed sham-operated and ammonia-fed BDL rats in the absence of an inflammatory stimulus. Therefore both ammonia and an additional inflammatory insult need to be present for nitrosation of brain proteins to occur.²⁸ These studies clearly demonstrate that the neurocognitive manifestations of hyperammonaemia can be exacerbated in an inflammatory environment.

SYSTEMIC INFLAMMATION AND THE BLOOD BRAIN BARRIER

An integral role of the neurosupportive astroglial cells is to form the BBB, determining cerebrovascular tone and with the capacity to secrete an array of different neurotrophic factors and cytokines such as IL-1 β , IL-6 and TNF- α . These cytokines are produced in response to inflammation and can affect the BBB with TNF- α being released early and subsequently promoting IL-1 β and IL-6 release. *In vitro* studies have shown that the BBB can become compromised by the presence of IL-1 β via intracellular endothelial cell cyclooxygenase and TNF- α activity, which induces endothelin-1 production promoting cerebral inflammation and disrupting the permeability of brain microvascular endothelial cells.^{80,81} Chastre and colleagues have shown that endotoxin administration in an ALF mouse model led to a rapid precipitation of hepatic coma and BBB permeability to the 25-kDa protein immunoglobulin G (IgG). This extravasation of IgG was accompanied by significant up-regulation of matrix metalloproteinase-9 (MMP-9), an endopeptidase known to modulate opening of the BBB in a wide range of neurological disorders.⁸²

OXIDATIVE/NITROSATIVE STRESS AND NEUROINFLAMMATION

In ALF, astrocytes exposed to ammonia develop signs of oxidative stress. When ammonia combines with glutamate in the astrocytes to form glutamine, there is a reduction in the amount of glutamate in the cell. A shortage of glutamate is partly avoided by amination of α -ketoglutarate to produce glutamate.⁸³ This removal of a substrate in the Tricarboxylic Acid Cycle (TCA), as well as ammonia being an inhibitor to enzymes required for TCA cycle activity (such as pyruvate dehydrogenase and α -ketoglutarate dehydrogenase), is likely to explain the high levels of pyruvate and lactate seen in brains of HE patients.⁵⁵

One critical consequence of oxidative and nitrosative stress is the induction of mitochondrial permeability transition (MPT).⁸⁴ The MPT usually develops in response to an increase in mitochondrial calcium levels and results in a sudden opening of the permeability transition pore (PTP), a large non-selective permeability pore in the inner mitochondrial membrane. This makes the inner mitochondrial membrane more permeable to protons, ions, and other small solutes. As a result, the inner mitochondrial membrane potential dissipates causing mitochondrial dysfunction with spilling of the mitochondrial matrix, defective oxidative phosphorylation and adenosine triphosphate (ATP) production, and generation of ROS.⁸⁵ The mechanism underlying MPT induction most likely involves oxidative stress, as antioxidants including superoxide dismutase, catalase, and vitamin E can inhibit the development of the MPT by ammonia.⁸⁶

The role of oxidative and nitrosative stress will be discussed at length in the supplement accompanying this review.

SYSTEMIC INFLAMMATION AND IMMUNE DYSFUNCTION IN ACUTE AND CHRONIC LIVER FAILURE

In a similar fashion to the septic shock-like syndrome observed in ALF, patients with cirrhosis have clinical features consistent with a chronic low-grade inflammation which include a hyperdynamic circulation, generalized vasodilation, and increased cardiac output.⁸⁷ In patients with cirrhosis and portal hypertension the gut wall becomes more permeable to bacteria. One study demonstrated gut bacterial flora in mesenteric lymph nodes of 30.8% of patients with Child's Pugh C cirrhosis, compared with less than 10% in non-cirrhotic patients.⁸⁸ These translocated bacteria can either become a direct source of infection,⁸⁹ or translocated bacterial products including endotoxins can become a source of chronic inflammation by inducing an immune response.⁹⁰ One study showed endotoxemia, without sepsis, was seen in 92.3% of patients with cirrhosis and was completely absent in healthy

controls with higher levels of endotoxemia seen in those with HE, and a high level predicted mortality.⁹¹ The immune response in a patient with cirrhosis is generated in response to this chronically endotoxemic, antigen-rich, state and promotes a dysfunctional immune system.

Neutrophils forming an integral component of the innate immune response have a higher resting production of ROS, which further contribute to systemic inflammation, decreasing microbiocidal defenses including neutrophil phagocytic capacity and respiratory burst.^{92,93} Experimental data has shown that neutrophils exposed *ex vivo* to ammonia levels typically seen in patients with cirrhosis (50–150 μM) were swollen and spontaneously produced ROS, with impaired phagocytic activity of *E. coli*. This indiscriminate production of ROS would almost certainly induce endothelial dysfunction and ‘bystander’ tissue damage which could contribute to the promotion of systemic inflammation and SIRS.^{94,95} *In vivo* supportive evidence of neutrophil malfunction including spontaneous over-production of ROS and impaired phagocytic activity has also been derived from neutrophils isolated from ammonia-fed rats and also from patients with cirrhosis given an oral ammonia load.⁹⁵ Thus hyperammonaemia is thought to induce dysfunction in one of the key cells of the inflammatory response.

ALF in addition to inducing sterile SIRS and organ dysfunction confers susceptibility to the development of

sepsis and a recent study by Taylor and colleagues has shown that circulating neutrophils in ALF have impaired bacteriocidal function similar to that seen in severe sepsis.⁹⁶

Toll-like receptors (TLR) are a key part of the innate immune system whereby they recognize pathogen-associated molecular patterns. Manakkat Vijay and colleagues showed that intracellular expression of neutrophil TLR-9 which binds to CpG sequences in bacterial DNA correlated with HE severity in patients with ALF and acute variceal bleeding and may be a useful biomarker for assessing patients with suspected HE.⁹⁷

THERAPEUTIC STRATEGIES TARGETING SYSTEMIC INFLAMMATION

The treatment strategies utilized in the management of acute and chronic HE will be discussed separately in this supplement and historically have focused on ammonia, either by reducing its production or promoting its excretion in patients with HE. More recently, hepatologists have become aware that it is important to target the precipitating factors implicated in the pathogenesis of HE and therefore, it goes without saying, that therapies which might impact upon systemic and cerebral inflammation and immune dysfunction may be a far more efficacious path to pursue. These strategies will now be discussed [Figure 3].

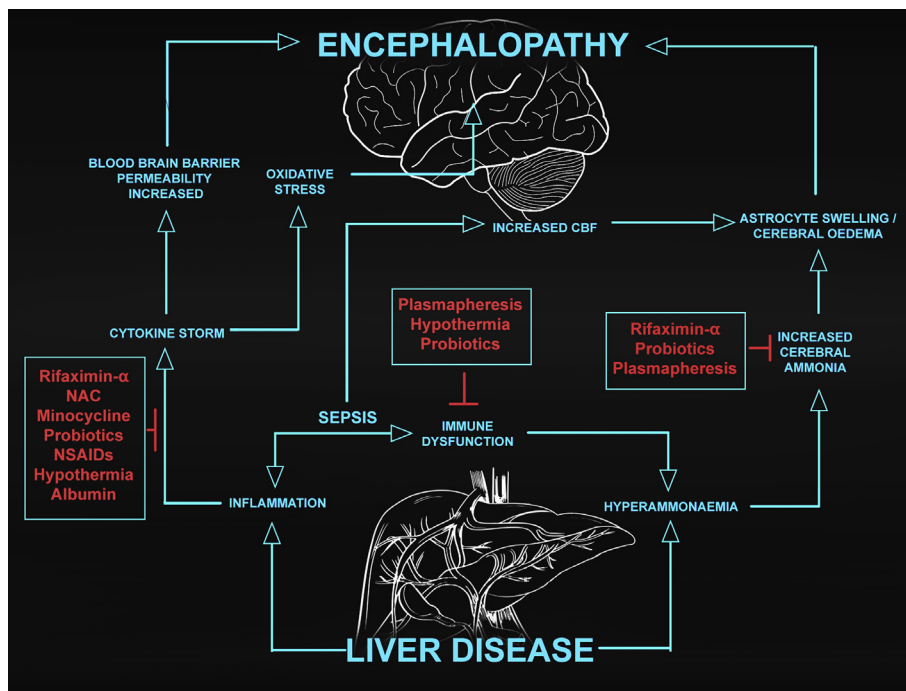


Figure 3 A figure summarizing how ammonia and immune dysfunction contribute to the propensity to develop hepatic encephalopathy and brain edema in the context of acute and chronic liver failure. The sites of the potential action of therapies targeting inflammation are shown. Abbreviations: CBF: cerebral blood flow; NAC: N-acetyl cysteine; NSAID: Non-steroidal anti-inflammatory drug.

N-Acetylcysteine

NAC has a potential therapeutic role as both an antioxidant and anti-inflammatory agent. In acetaminophen-induced ALF, early administration of intravenous NAC can prevent hepatic necrosis by increasing hepatic stores of glutathione.⁹⁸ NAC has been shown to increase oxygen delivery to the tissues and increases oxygen consumption, concurrent with increased arterial blood pressure and cerebral perfusion pressure.⁹⁹ It has been shown that these effects are mediated through increased nitric oxide/guanylate cyclase enzyme activity.¹⁰⁰

In a BDL model of CLF, animals administered NAC for two weeks had improved spatial memory and reduced motor deficits. NAC supplementation decreased lipid peroxidation and restored the activity of antioxidant enzymes as well as structural deficits observed in the cortex and cerebellum. These results suggest the protective effect of NAC is mediated through attenuation of oxidative stress.¹⁰¹

Non-steroidal Anti-inflammatories

A study in patients presenting with ALF first suggested that the NSAID indomethacin, which is a non-specific inhibitor of cyclo-oxygenase, may offer benefit in the treatment of uncontrolled brain edema.¹⁰² Chung and colleagues also demonstrated that indomethacin can prevent the development of ammonia-induced brain edema in rats that have undergone portocaval anastomosis.¹⁰³

In a chronic portocaval-shunted rat model, Cauli and colleagues showed that the NSAID ibuprofen restored the ability of the rats to learn the Y-task maze, in addition to normalization of the function of the glutamate-nitric oxide cyclic guanosine monophosphate enzyme pathway in the cerebral cortex.⁷¹ Unfortunately, NSAID drugs can only have a limited role in the treatment of HE in patients with cirrhosis as they have adverse cardiovascular and renal toxicities which impact upon the protective role of prostaglandins in cellular metabolism.

Minocycline

Minocycline is a broad-spectrum tetracycline antibiotic which has been shown to attenuate endotoxin-induced neuroinflammation.¹⁰⁴ Jiang and colleagues have explored its use in an experimental model of ALF and found that it delayed the progression of HE and brain edema.⁶⁷ It is postulated to exert a potent inhibitory action on microglial activation independently of its antimicrobial properties. There are no data in human ALF but the recognized hepatotoxicity of the drug may well limit clinical trials.

Plasmapheresis

High volume plasmapheresis can alleviate brain edema in ALF and improves systemic hemodynamics despite increasing CBF.¹⁰⁵ Plasmapheresis is likely to have a posi-

tive impact on systemic immune and endothelial dysfunction by reducing the proinflammatory milieu and thus SIRS. The preliminary analysis of a recent trial of high volume plasmapheresis in 120 patients suggests that it may improve survival in patients unsuitable for liver transplantation (verbal communication—Dr Finn Stolze Larsen).

Hypothermia

Moderate hypothermia (33 °C) has been extensively investigated as a therapeutic modality in patients presenting with ALF and uncontrolled ICH.¹⁰⁶⁻¹⁰⁸ It has been postulated to improve outcomes through a variety of mechanisms including reducing CBF, brain ammonia uptake, systemic inflammation, ROS production and oxidative stress which helps to lower ICH.⁶⁴ The use of mild hypothermia (cooling patients to <35 °C) has now become standard of care in many tertiary liver centers¹⁰⁹ but its role in patients unsuitable for liver transplantation remains debatable.

Human Albumin Solution

The protein albumin has antioxidant properties and is an endotoxin scavenger. Recent studies have shown that not only albumin concentration but also albumin function is reduced in liver dysfunction. This observation led to the concept of effective albumin concentration,¹¹⁰ which represents the fact that plasma albumin concentration *per se* does not reflect its functional capacity.¹¹¹ Infusion of albumin has benefits above that of simple volume expansion and can reduce the severity of HE.¹¹² Stadlbauer and colleagues have demonstrated that albumin prevented the deleterious effect of patients' plasma on neutrophil phagocytosis, spontaneous oxidative burst and TLR expression.¹¹³ In a recently published double blind randomized controlled trial, 56 cirrhotic patients with an acute episode of HE (grade II-IV) were randomized to receive albumin (1.5 g/kg on day 1 and 1.0 g/kg on day 3) or isotonic saline. The number of patients without HE at day 4 did not differ between groups. However, significant differences in survival were found at day 90 (albumin 69.2% versus saline 40.0%; $P = 0.02$) suggesting that the development of HE may identify a subgroup of patients with advanced cirrhosis that may benefit from the administration of albumin.¹¹⁴

Albumin dialysis has also been studied in a randomized controlled trial in patients with HE and advanced cirrhosis, and found to be effective for the treatment of HE,¹¹⁵ however the benefits of albumin dialysis appear to be independent of changes in ammonia level or cytokines.¹¹⁶

An albumin replacement system with a novel endotoxin ligation (ARSeNEL) component has been developed and tested in a porcine ALF model. Improvements were demonstrated in survival, endotoxemia and intracranial pressure

index which support exploration in larger clinical human trials.¹¹⁷

Agents Targeted to Key Pro-inflammatory Mediators and the Innate Immune System

There might also be hypothetical benefits to utilizing agents that abrogate the augmented inflammatory state, particularly in ALF as shown for example by Chastre and colleagues in a mouse model of ALF whereby they utilized etanercept, a TNF- α neutralizing antibody, to abrogate the onset of hepatic coma.⁶³ However, the proinflammatory response which develops as a consequence of acute liver injury is vital in initiating liver repair and regeneration and it may be detrimental to use agents targeted to key proinflammatory mediators. Moreover, as functional immunoparesis is a consistent finding in patients with ALF and CLF,^{94,118,119} this could be detrimental rendering patients susceptible to bacterial and fungal infection. However, novel strategies that target ammonia-induced neutrophil dysfunction would be of particular interest to explore such as modulators of p38-Mitogen Activated Phosphokinase⁹⁵ and TLR-9.⁹⁷

Rifaximin- α

Rifaximin- α is a broad-spectrum antibiotic which has minimal systemic absorption. A large double-blinded randomized controlled trial of 299 patients, demonstrated an improvement in maintained remission from HE and a reduction in hospitalizations due to HE over a six month period in patients with cirrhosis who were administered rifaximin versus placebo.¹²⁰ Increased levels of the anti-inflammatory cytokine IL-10 have been found in rifaximin-treated groups which may allude to its mechanism of action being an anti-inflammatory rather than ammonia-lowering in nature.¹²¹ Furthermore, a recent observational study demonstrated that rifaximin- α intriguingly reduced systemic endotoxin levels and disease severity score¹²² and may therefore have a therapeutic role in HE by reducing systemic inflammation rather than lowering blood ammonia levels.

Probiotics

Probiotics may have a role in reducing the bacterial translocation of endotoxin and other bacterial activators of TLRs through modulation of the intestinal microbiota. A reduction in blood ammonia levels, endotoxemia and an improvement in neurocognitive impairment have been reported in patients with cirrhosis and MHE who were administered probiotics.¹²³ Probiotics have also been shown to restore neutrophil phagocytic activity by lowering endogenous levels of IL-10 and TLR-4 expression in patients with cirrhosis.¹²⁴ However, in other studies no changes in the reduction of the plasma proinflammatory milieu¹²⁵ or outcomes have been demonstrated.¹²⁶

SUMMARY

Seminal serendipitous studies first implicated hyperammonaemia as a key driver in the development of HE, however the co-existent systemic inflammatory state is now considered pivotal in both the precipitation and exacerbation of HE. While the full mechanism is yet to be elucidated, there is a large body of evidence implicating ammonia in both direct and indirect toxic effects on the brain culminating in metabolic disarray, astrocyte dysfunction and cerebral edema. This, combined with altered neurotransmission, increased oxidative/nitrosative stress and immune dysfunction are thought to underpin the development of HE.

Accordingly, treatment of HE with pure ammonia lowering strategies is becoming obsolete as novel strategies which target systemic inflammation gather a greater evidence base including rifaximin- α which is quickly becoming the new mainstay in the treatment of HE whilst other anti-inflammatory therapies are undergoing scrutiny.

CONFLICTS OF INTEREST

All authors have none to declare.

ACKNOWLEDGMENT

This review was supported by the Medical Research Council (MRC) Centre for Transplantation, King's College London, UK—MRC grant no. MR/J006742/1 and the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

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