EXPERT REVIEWS

Recent insights into the pathogenesis of hepatic encephalopathy and treatments

Expert Rev. Gastroenterol. Hepatol. 8(1), 83–100 (2014)

Jonathan P Sturgeon and Debbie L Shawcross*

Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, Denmark Hill, London, SE5 9RS, UK
**Author for correspondence:*
Tel.: +44 203 299 3713
Fax: +44 203 299 3167
debbie.shawcross@kcl.ac.uk

Hepatic encephalopathy (HE) encompasses a spectrum of neuropsychiatric disorders related to liver failure. The development of HE can have a profound impact on mortality as well as quality of life for patients and carers. Ammonia is central in the disease process contributing to alteration in neurotransmission, oxidative stress, and cerebral edema and astrocyte swelling in acute liver failure. Inflammation in the presence of ammonia coactively worsens HE. Inflammation can result from hyperammonemic responses, endotoxemia, innate immune dysfunction or concurrent infection. This review summarizes the current processes implicated in the pathogenesis of HE, as well as current and potential treatments. Treatments currently focus on reducing inflammation and/or blood ammonia levels and provide varying degrees of success. Optimization of current treatments and initial testing of novel therapies will provide the basis of improvement of care in the near future.

Keywords: acute liver failure • ammonia • astrocyte • cerebral edema • cirrhosis • hepatic encephalopathy • inflammation • treatments

Hepatic encephalopathy (HE) is a complication of liver disease that is difficult to treat and is associated with significant morbidity and mortality.

This review aims to look at:

- Definition and classification of HE;
- The central role of ammonia in the disease and how ammonia is regulated by the body;
- The effect of ammonia on the brain;
- The role of inflammation in HE including neuroinflammation;
- Treatments for HE including novel and potential therapies.

Hepatic encephalopathy

HE is a reversible neuropsychiatric condition, which is usually associated with liver failure. It encompasses a wide spectrum of disorders ranging from mild defects in executive function only detectable with psychometric tests through to coma [1]. HE is associated with similarly diverse liver etiologies and a Working Group in 1998 at the World Congress of Gastroenterology suggested standardizing the HE nomenclature to reflect this [2]. They proposed three classes of HE, shown in Table 1.

These differentiations are important because disease processes differ, especially between acute and chronic HE [3]. Even so, this classification may fail to fully encompass the complex diversity of HE: for example, encephalopathy caused by inherited defects in the urea cycle, which some have said is HE [4] falls outside of these definitions.

Clinical outcomes are significantly affected by the presence of HE. In acute liver failure (ALF), the mortality rate is high, but has improved significantly from 83% in 1973–1978 down to 38% in 2004–2008 [5]. In cirrhotic liver disease, the presence of at least one acute episode of HE is associated with mortality of 58% at 1 year [5]. Minimal HE, detectable using psychometric testing, was associated with a 1-year mortality rate of 39.1% [6] and is predictive of future episodes of overt encephalopathy [7]. Both of these mortality rates are considerably worse than the 1-year mortality rate following orthotopic liver transplantation (OLT) of approximately 10% [8].

 10.1586/17474124.2014.858598 ISSN 1747-4124

Table 1. Different categories of hepatic encephalopathy, as defined by the 1998 Working Group at the World Congress of Gastroenterology.

HE type	Nomenclature	Subcategory	Subdivisions
A	HE associated with acute liver failure		
B	HE associated with portosystemic shunt (bypass) and no hepatocellular disease		
C	HE associated with cirrhosis and portal hypertension and/or portosystemic shunt	Episodic	Precipitated Spontaneous Recurrent
		Persistent	Mild Severe Treatment dependent
		Minimal	

HE: Hepatic encephalopathy.
Data taken from [2].

HE also exerts a significant burden on the quality of life of patients and care givers. In cirrhotics, overt HE occurs in 30–50% [9], whereas minimal HE can affect 30–84% of cirrhotic patients [10]. It can be associated with impaired ability to drive [11,12], impairment in daily functioning, sleep, work and memory [13].

Pathogenesis of hepatic encephalopathy

The pathogenesis of HE is incompletely understood. A rise in blood ammonia levels is central to the disease process [14,15], but ammonia levels correlate poorly with severity of HE. A synergistic action with concurrent inflammation has been suggested [16–19]. Changes in the brain can include cerebral edema, neuroinflammation and deranged neurotransmission.

Ammonia

Ammonia (NH_3) is a nitrogen compound usually produced as a result of nitrogen metabolism in the body. At physiological pH values, ammonia is over 98% dissociated as an ammonium (NH_4^+) ion [20], and for simplicity, this review will refer to NH_3/NH_4^+ as 'ammonia.'

Normal blood ammonia levels are 10–50 μmol/l. Arterial ammonia concentrations in ALF can rise to as much as 100–450 μmol/l, while in chronic liver failure vary from normal to 150 μmol/l. The brain:blood ammonia concentration ratio also increases from around 2 in healthy individuals to 3–4 in chronic liver disease and to 8 in ALF [14].

The biogenesis and regulatory systems in the body for ammonia are shown in Figure 1. The majority of ammonia is generated from the gut. The main energy source for enterocytes in the small bowel is the amino acid glutamine. With the enzyme k-phosphate-activated glutaminase (k-PAG), enterocytes convert glutamine to glutamate. This produces energy, as well as nucleotides and ammonia. When a glutamine meal is given to a patient, a corresponding increase in serum ammonia is seen around 1 h after administration, corresponding to the location in the gut the meal has reached. Although the colon does also produce ammonia from the breakdown of amino acids, the majority of the colon's ammonia production results from the breakdown of urea by intestinal flora-derived urease. Intestinal k-PAG activity is enhanced in cirrhotic patients compared with healthy controls, and the level of enhancement correlates with the severity of MHE [21].

Ammonia-rich blood then flows to the liver for detoxification. Periportal hepatocytes convert ammonia to urea via the urea cycle. Further downstream perivenous glutamine synthase-containing hepatocytes – with a high affinity for ammonia – convert it to glutamine. This second 'scavenger' pathway allows ammonia that has missed the urea-synthesizing cells to be removed [22]. This system ensures that under normal physiological conditions, the liver metabolizes nearly all the gut-derived ammonia [15]. Altering the percentage of ammonia that is removed by each of these two pathways allows the liver to help regulate pH balance: removal of ammonia via the urea cycle irreversibly removes bicarbonate from the system [22,23].

In patients with portosystemic shunts, blood from the gut bypasses the liver and flows directly into the systemic circulation. The extent to which this bypasses the liver depends on the type of shunt. The formation of a portacaval shunt reduces ammonia detoxification capacity by 50% and following a transjugular intrahepatic portosystemic shunt, this can be as high as 93% [24].

The kidneys play an important role in regulating ammonia. They contain both the enzyme glutamine synthetase (GS) and k-PAG so that they are able to generate and use up ammonia [25]. Under normal physiological conditions, they are a net producer of ammonia. From the ammonia produced, 30% of it is excreted by the kidneys into the urine, and the remaining 70% is reabsorbed back in to the circulation by the renal vein. In cases of hyperammonemia [26] and acidosis [27], the kidney becomes a net excretor of ammonia. This has been shown to help modulate the increase in ammonia during the anhepatic phase of OLT [28]. Excretion of ammonia during acidosis also helps to normalize the acid–base balance as bicarbonate is

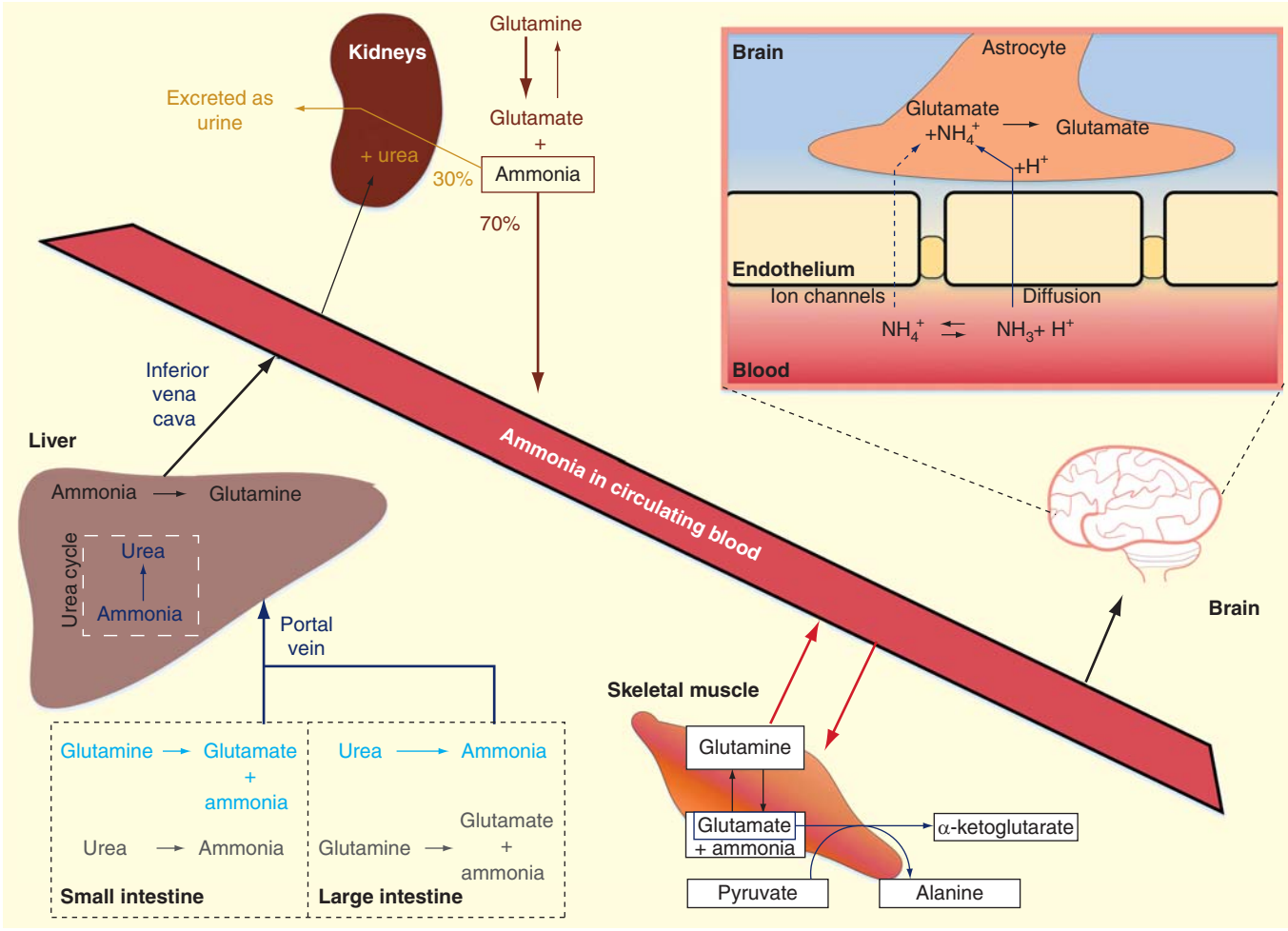

Figure 1. Ammonia generation and metabolism pathways in the body.

generated and protons are excreted [27]. Following a gastrointestinal (GI) bleed in patients with cirrhosis, it is the kidney's ammoniagenesis that results in a significant increase in circulating ammonia. In patients given protein loads to simulate GI bleeds, renal production of ammonia increases sixfold, with no change in ammonia production in portal-drained viscera [29].

Skeletal muscle contains GS, catalyzing the integration of ammonia into glutamine. Such conversion, especially involving skeletal muscle, should only be viewed as a temporary removal of ammonia, as the glutamine is converted back to glutamate and ammonia in the enterocytes and the kidney. Skeletal muscles also possess the enzyme alanine aminotransferase. This converts pyruvate into the amino acid alanine, which contains a nitrogen atom. This can travel in the blood to the liver where it can be converted to urea in an energy intense process. This is therefore a pathway to remove nitrogen from the body.

Astrocytes also contain GS and this will be looked at in more detail by looking at the effect of ammonia on the brain.

Ammonia & the brain in HE

Ammonia in high concentrations is neurotoxic [30]. Ammonia in the bloodstream as NH_3 is able to simply diffuse across the blood–brain barrier, and the ammonium ion NH_4^+ is similar to K^+ so is able to cross via potassium channels or transporters [31], as well as by speculated dedicated ammonium transporters [32]. N^{13}-labeled ammonia is able to readily build up in brain tissue at a rate linearly correlated to plasma concentration of ammonia [33]. Astrocytes, the cells, which biochemically support the blood–brain barrier and synapses, have been shown to protect neurons from the effect of ammonia [30]. They produce GS so that they are able to amidate glutamate to remove free ammonia. Following an ammonia load in cirrhotic patients, brain glutamine increases significantly compared with placebo [34]. Neurons do not normally produce any GS so are unable to use this process to protect themselves from ammonia. Interestingly, in the absence of astrocytes or in Alzheimer's disease with defective astrocytes, neurons are able to adaptively express GS [35].

The clinical picture of altered brain physiology in HE differs between acute and chronic liver failure. In ALF, cerebral edema in the presence of high ammonia plays a main role, along with oxidative stress, altered neurotransmission and inflammation. In chronic liver disease cerebral edema, if present, is unlikely to be significant; neuro/systemic inflammation and altered neurotransmission still contribute.

Cerebral edema & cell swelling

Acute liver failure

Cerebral edema is often seen in patients with ALF and can lead to increased intracranial pressure, brain herniation and death. The exact processes involved are not well defined, but it is a widely held opinion that a cytotoxic process due to the effects of ammonia plays a significant part [36]. At the cellular level, neurons exposed to very high levels of ammonia quickly show extensive degeneration and undergo increased rates of apoptosis and necrosis.

Additionally, astrocytes swell in the presence of high ammonia. This is traditionally ascribed to cell hypertonicity from an increase in cellular glutamine. This swelling may contribute to the intracranial hypertension seen in ALF. Arterial ammonia concentrations in ALF have been shown to correlate with the severity of cerebral herniation and death and with severe grades of HE [37]. Patients with ALF who have not progressed to Grade 3/4 HE are unlikely to progress to develop intracranial hypertension [36,38].

Chronic liver failure

In cirrhosis, there may be chronic low-grade cerebral edema [39]; the extent of this cerebral edema does not correlate with the level of HE [40,41], and it is reversed following liver transplantation [39]. Any cerebral edema in cirrhosis with MHE may not be due to astrocyte swelling, but due to an increase in extracellular interstitial fluid, possibly as a result of migration of macromolecules outside the astrocytes in an attempt at intracellular osmoregulation. With worsening HE, astrocyte swelling is observed. In the brains of cirrhotic patients who died of HE, an Alzheimer's Type 2 astrocytosis is seen: astrocytes have a large swollen nucleus, expansion of cytoplasm and proliferation of organelles [42].

Neurotransmission

N-methyl-D-aspartate *receptor activation*

In HE, the interaction between neurons and neurotransmission is changed. Some of the neurotransmission pathways believed to be implicated in HE are shown in Figure 2. The effect of ammonia on *N*-methyl-D-aspartate (NMDA) neurotransmission depends upon whether there is acute or chronic liver failure.

Acute liver failure

In ALF, the ammonia levels are considerably higher resulting in an increase in NMDA receptor activation that leads ultimately to an increase in cyclic guanosine monophosphate (cGMP) production by the glutamate–nitric oxide–cGMP pathway, shown in Figure 2. Very high levels of ammonia (2 mM) have been shown to depolarize hippocampal neurons by 10 mV [43], but this is not enough to depolarize the cell. By raising the resting membrane potential, this helps partly to remove the voltage-dependent Mg^{2+} block on NMDA receptors, increasing their activity [44]. Intraperitoneal injections of ammonia in rats led to an increase in cGMP in the extracellular space, suggesting increased activation of NMDA receptors [45], but only very high doses of ammonia were able to do this. The NMDA activation may be implicated in the development of coma and death, as ammonia-induced deaths of animals were almost completely prevented by 10 different antagonists of the NMDA receptor [46].

Chronic liver disease

In chronic hyperammonemia, long-term exposure to sublethal levels of hyperammonemia has been shown to be protective against metabolic abnormalities and death in acute hyperammonemia that would otherwise be fatal [47–49]. Compensatory mechanisms mean that there is a reduction in the overall cGMP production. In rats that had been exposed to chronic hyperammonemia, production of cGMP following stimulation of the cerebellum with NMDA was decreased [50]. By providing another source of nitric oxide (NO), and there still being no increase in cGMP, Hermenegildo *et al.* were able to show that the step limiting the production of cGMP in hyperammonemia was soluble guanylate cyclase, and this experiment has been reproduced on cirrhotic patients with the same results [51]. This is significant for HE because the Glutamine–NO–cGMP pathway has been implicated in learning: in rats, inhibition of NO synthase reduces learning of spatial tasks, impairs memory consolidation and learning of passive avoidance [52]. Hyperammonemic rats with impaired learning and cGMP production were able to have their learning ability restored by increasing the brain extracellular cGMP. This worked either through directly injecting cGMP, or through inhibiting the phosphodiesterase that breaks down the cGMP with zaprinast [53] or oral sildenafil [54].

Increased GABAergic tone

γ-aminobutyric acid (GABA) is one of the main inhibitory neurotransmitters, and increased GABAergic tone has long been suggested as an alternative cause of HE due to increased neuroinhibition [55]. This was based on similarities seen between animals with liver failure and those treated with GABA agonists. Additionally, the drug flumazenil, a benzodiazepine antagonist, caused some improvement in patients with HE. This is unlikely to be due to increased GABA in the brain or increased $GABA_A$ receptors, but more likely represents a response to endogenous neurosteroids in the brain some of which are able to activate the $GABA_A$ receptor [56]. Neurosteroids are synthesized by glial cells within the CNS following activation of the 'peripheral-type' benzodiazepine receptor (PTBR) on the outer mitochondrial membrane of astrocytes. Ammonia has been shown to upregulate the PTBR, and they are upregulated in models of ALF [57]. Increased PTBR-binding site densities have been seen in autopsied brains of patients who have died of HE [58], and increased levels of the neurosteroid pregnenolone have also been observed [59]. Additionally, increased central benzodiazepine-binding sites (part of the $GABA_A$ receptor complex) have been seen in chronic HE [60]. These observations may help to explain the sensitivity to benzodiazepines seen in patients with HE and provides a potential target for future therapies.

Cauli *et al.* demonstrated an increased GABAergic tone in the cerebellum of rats exposed to chronic hyperammonemia but decreased GABAergic tone in the cortex [61]. Blocking the $GABA_A$ receptor with intraperitoneal bicuculline restored the function of the glutamate–NO–cGMP pathway in the cerebellum, as well as restoring learning ability in the hyperammonemic rats [61].

Oxidative stress

In ALF, several mechanisms appear to place oxidative stress on cells exposed to elevated ammonia, which will increase metabolic rate and even contribute to inflammation. 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 as shown in Figure 2 [62]. This removal of a substrate in the tricarboxylic acid (TCA) cycle, as well as ammonia being an inhibitor to enzymes required for TCA cycle activity (such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase), go some of the way to explaining the high levels of pyruvate and lactate seen in brains of HE patients [63].

The activation of the glutamate–NO–cGMP pathway and NMDA receptors allow entry of Ca^{2+} to enter the neuron. Sodium ions enter as well, and the removal of these ions from the cell uses up ATP by increased activity of Na^+/K^+-ATPase. Following ammonia intoxication, Na^+/K^+-ATPase activity was increased to 76% [64]. Much of the influx of Ca^{2+} into the cell is sequestered by the mitochondria. This alters membrane potential, reducing respiration and thus ATP production and potentially releasing free radicals [46].

The 'Trojan horse' hypothesis has been recently proposed as an alternative theory by Albrecht and Norenberg to explain the development of astrocyte swelling and brain edema and suggests an important role for both ammonia and glutamine [65]. The excess glutamine synthesized within astrocytes is transported into mitochondria where it is metabolized by k-PAG to ammonia and glutamate [66]. Glutamine, the 'Trojan horse', thereby acts as a carrier of ammonia into the mitochondria, where its accumulation can lead to oxidative stress and ultimately astrocyte swelling.

One critical consequence of oxidative and nitrosative stress is the induction of mitochondrial permeability transition (MPT) [67]. The MPT usually develops in response to an increase in mitochondrial Ca^{2+} levels and results in a sudden opening of the permeability transition pore, a large nonselective permeability pore in the inner mitochondrial membrane. This leads to increased permeability on the inner mitochondrial membrane to protons, ions and other small solutes. As a result, the inner mitochondrial membrane potential dissipates causing mitochondrial dysfunction. The MPT is therefore associated with movement of metabolites across the inner mitochondrial membrane, spilling of the mitochondrial matrix, defective oxidative phosphorylation and adenosine triphosphate (ATP) production and generation of free radicals [68]. Production of free radicals through MPT induction further aggravates the MPT,

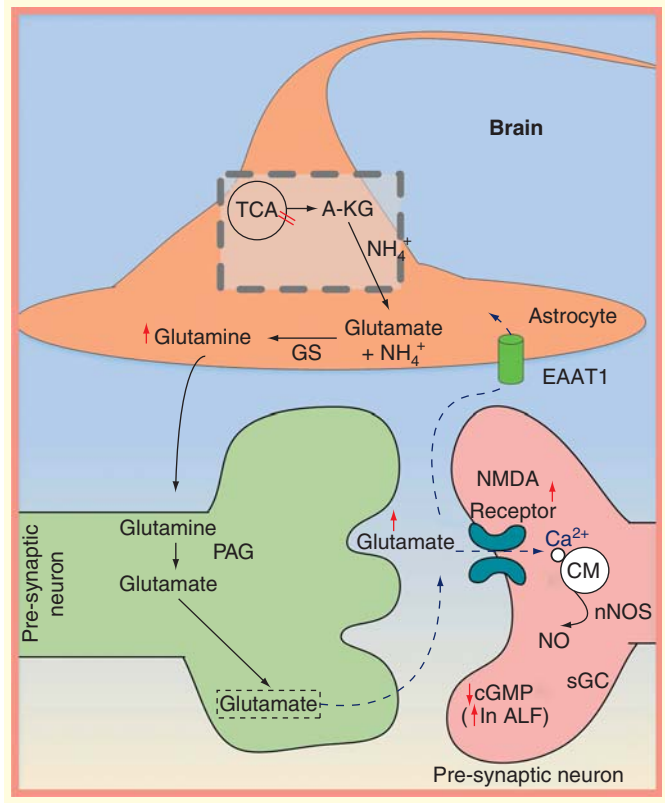

Figure 2. Diagram showing some effects of excess ammonia on glutamatergic neurons.
A-KG: α-Ketoglutarate; GAD: Glutamate decarboxylase; GS: Glutamine synthetase; NMDA: N-methyl-D-aspartate; nNOS: Neuronal nitric oxide synthase; PAG: Phosphate activated glutaminase; sGC: Soluable guanylate cyclase; TCA: Tricarboxylic acid cycle.

resulting in a vicious cycle. Induction of the MPT was described in cultured astrocytes exposed to ammonia [67]. The mechanism underlying MPT induction most likely involves oxidative stress, as antioxidants including superoxide dismutase, catalase and vitamin E were able to inhibit the development of the MPT by ammonia [69].

Inflammation

Ammonia alone does not strongly correlate with the severity of HE [70], particularly in patients with cirrhosis. Although frequently raised in HE, one study showed that 69% of the patients with no HE had a raised ammonia, and several of the patients with Grade III/IV encephalopathy had normal arterial ammonia levels [71]. Despite a positive correlation between arterial ammonia levels and severity of HE ($r = 0.61$), substantial overlap between groups means single ammonia levels have little utility in the diagnosis of HE. Another study concurs with this and notes that even in patients whose HE had resolved, ammonia levels remained high and some cases even elevated [72]. It has been suggested that inflammation plays a synergistic role in the pathophysiology of HE, augmenting the effect of ammonia. Inflammation can be systemic, but there also appears to be a role for autogenously produced proinflammatory cytokines

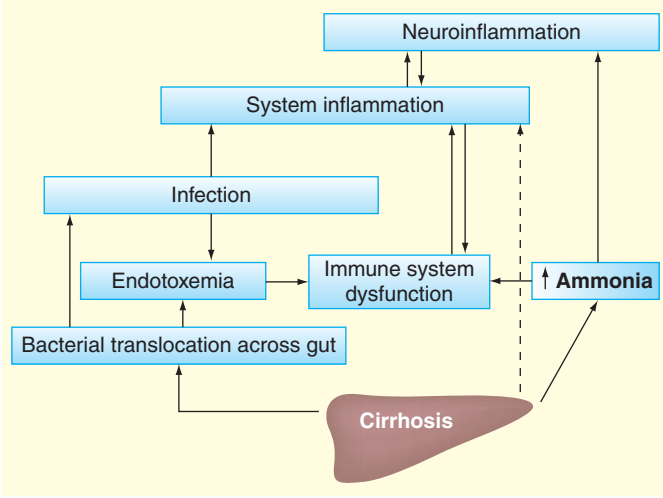

Figure 3. Inflammation in cirrhosis.

from the brain in the presence of ammonia giving rise to neuroinflammation [17].

Systemic inflammation & HE

Acute liver failure

In ALF, inflammation arises from hepatic necrosis and the susceptibility to developing infection – one study showed 80% of ALF patients had bacteriologically proven infection and 32% had fungal infection during their admission [73]. Rolando *et al.* showed that even in the absence of proven infection, a systemic inflammatory response syndrome (SIRS) was seen on admission in 39.8% of ALF patients. At least in part, this is due to inflammation because of ALF *per se* [74]. Importantly, this 'sterile' inflammation was associated with worsening HE and death. Another study showed that acquisition of infection during Grades I and II of encephalopathy was shown to be significantly associated with progression to Grades III and IV encephalopathy in ALF induced by acetaminophen. Again, SIRS at admission increased the probability of progressing to Grade III/IV HE [75]. The systemic inflammation seen in ALF even in the absence of infection is that of a 'cytokine storm,' with very high levels of TNF-α, IL-1β and IL-6 [76]. The necrotic liver plays a significant part in this cytokine release and removal of necrotic liver in ALF results in a reduction in the severity of intracranial hypertension [77].

Chronic liver failure

Inflammation is significant in cirrhosis and minimal hepatic encephalopathy (MHE). In patients with cirrhosis, the presence or absence of HE was not dependent on the severity of liver disease or ammonia level but on inflammation [78]. In another study, induced hyperammonemia in cirrhotics resulted in worse neuropsychiatric test scores only in those with concurrent inflammation [16]. Hyperammonemic mice injected with lipopolysaccharide to induce an immune response produced similar cytokine responses; however, learning impairment was more pronounced and lasted longer in the hyperammonemic mice, again suggesting a central role of inflammation in HE [79]. The presence of inflammation in the presence of hyperammonemia even in the absence of any liver abnormality is sufficient to produce a degree of cognitive impairment. This was noted in one study where 'healthy' controls were found to subsequently have a degree of hyperammonemic encephalopathy with raised ammonia and inflammatory markers, but without any evidence of liver impairment [80]. In healthy rats, which were injected with lipopolysaccharide to induce inflammation, rats that were fed ammonia-rich feed developed significant astrocyte edema confirmed on transmission electron microscopy, but only rats that had their bile duct ligated lapsed into a coma [19]. This reaffirms that cerebral edema is not necessarily related to the severity of HE and that inflammation is able to modulate HE.

Patients with cirrhosis have clinical features consistent with a chronic low-grade inflammation: a hyperdynamic circulation, generalized vasodilation and increased systemic output [81]. The cause of inflammation in cirrhosis is multifactorial, with some of the inflammatory processes shown in Figure 3. In cirrhosis and portal hypertension, the gut wall becomes more permeable to bacteria. This is shown by a study that found gut bacterial flora in mesenteric lymph nodes of 30.8% of patients with Child's Pugh C cirrhosis compared with <10% in noncirrhotic patients [82]. These translocated bacteria can either become a source of infection in themselves [83], or translocated bacterial products – especially endotoxins – can become a source of chronic inflammation by inducing an immune response [84]. One study showed that endotoxemia, without sepsis, was seen in 92.3% of patients with cirrhosis and was completely absent in healthy controls. Higher levels of endotoxemia were seen in patients with HE and a high-level predicted mortality [85]. Increased plasma pro-inflammatory cytokines are observed in patients with MHE [78] and a subsequent study showed levels of IL-6 and IL-18 correlated with the presence of MHE in cirrhosis [86].

Immune responses to this chronically endotoxemic, antigen-rich state leads to a dysfunctional immune system. Neutrophils in cirrhosis have a higher resting oxidative burst, further contributing to systemic inflammation, decreased phagocytic capacity and decreased stimulated oxidative burst [87–89]. Similar neutrophil changes can be shown to be induced by raised ammonia [90,91]. Receptors in neutrophils are altered in liver failure: toll-like receptor (TLR) 4, a receptor for endotoxin and TLR9, a receptor for CpG sequences in bacterial DNA are increased in cirrhotics [92]. TLR9-level expression has been shown to correlate with severity of HE [93]. This altered neutrophil function decreases the immune system's ability to protect against infections and may help to explain a high bacterial infection rate of 34% seen in cirrhotics [94].

Neuroinflammation

Inflammation of the brain itself has been suggested to be present in rats with HE – high levels of IL-6, increased activity of cyclooxygenase and inducible NO synthase in the cerebral cortex suggest inflammation [95]. Treatment with chronic ibuprofen resolves this neuroinflammation, restores cognitive

ability and normalizes the glutamate–NO–cGMP pathway abnormalities seen in HE. Inflammation in the brain can be affected by systemic inflammation. Pro-inflammatory cytokines are able to be transported across the blood–brain barrier from the systemic circulation. There is however good evidence that neuroinflammation in HE is derived from the brain itself. The brain contains microglial cells that are essentially macrophages resident in the CNS. These can be activated to release pro-inflammatory cytokines and cause neuroinflammation. Chronic hyperammonemia is sufficient to induce microglial activation [96], and this activation results in brain-derived pro-inflammatory cytokines [97]. The extent of microglial activation is predictive of the level of HE, as well as presence of cerebral edema in ALF [98].

The exact process of microglial activation in HE is uncertain as the point of activation is unclear. In one mouse model of ALF, microglial activation was seen to occur in the final stages of HE rather than when the ammonia levels started to go up [99]. Ammonia-induced oxidative stress in the brain may contribute to the microglial activation. As previously mentioned, ammonia can inhibit the TCA cycle. Lactate levels reach 10–12 mM in end-stage HE in liver failure, which is sufficient to result in increased pro-inflammatory cytokines in cultured microglial cells [100].

Treatments

Current therapeutic regimens for HE are suboptimal, especially in ALF. Initial management of an acute episode of HE, be it in ALF or an acute episode in a cirrhotic patient, is to treat the precipitant cause, followed by supportive treatment and ammonia/inflammation reduction therapies. In patients with ALF who progress to coma, this invariably will result in death unless the patient undergoes liver transplantation. Treatment aims for chronic and MHE is to reduce the severity of the HE and reduce the number of overt HE episodes. A list of current and potential treatment options for HE are shown in Table 2. An increasing number of treatments are aimed at reducing inflammation, and this will likely continue as new therapeutic targets are identified in the inflammatory response in HE. The applicability of different treatments to different types of HE are shown in Table 3.

Current therapies

Nonabsorbable disaccharides

Nonabsorbable disaccharides such as lactulose/lactitol have been considered as the standard treatment for HE since the late 1970s. It is the first-line treatment, and lactulose frequently serves as control treatment in trials assessing new drugs for HE. Disaccharides pass undigested into the colon, where they are partly digested by bacteria in the gut. This produces carboxylic acids, which help to acidify the gut. This process helps to inhibit bacterial production of ammonia by reducing urease-producing gut flora and also traps ammonia as nondiffusible ammonium in the intestinal lumen [101]. Lactulose has a limited role to play in ALF; its use has been associated with an increase in survival time, but it did not affect severity of encephalopathy or survival [102]. One concern over its use in ALF is that it can cause gaseous bowel distension, which can make subsequent liver transplantation difficult [103]. In acute episodes of HE occurring in the context of chronic liver disease, a meta-analysis by Als-Nielsen *et al.* concluded that there is insufficient evidence to support or refute the use of lactulose in acute episodes of HE based upon there being evidence available from only 57 patients enrolled in high-quality controls [104]. Furthermore, the use of lactulose had no favorable impact on mortality.

Lactulose has been shown to be efficacious in improving neuropsychiatric function and quality of life in MHE [105], as well as in the secondary prevention of HE in cirrhotic patients [101]. A subsequent meta-analysis showed that nonabsorbable disaccharides are effective at reducing HE symptoms and in the prevention of HE episodes compared with no treatment [106]. Given their cost and availability, nonabsorbable disaccharides are likely to remain a well-used treatment in HE.

Nonabsorbable antibiotics

Antibiotics such as neomycin, vancomycin and metronidazole have previously been used to reduce ammonia-producing bacteria in the gut and therefore reduce the occurrence of HE. Furthermore, nonabsorbable antibiotics have been shown to be superior to nonabsorbable disaccharides in the treatment of acute HE [104]. The long-term use of these nonabsorbable antibiotics, however, has largely been discontinued due to their toxicities [107]. Because of its low systemic absorption rates, it is hoped that rifaximin-α avoids such side effects. Rifaximin-α is a synthetic, nonabsorbable antibiotic of the rifamycin class. It inhibits bacterial RNA synthesis by binding to the bacterial DNA-dependent RNA polymerase [108]. Rifaximin is poorly absorbed (<0.04%), but in patients with Childs Pugh C, cirrhosis absorption is increased by 20-times [201]. There are no data on the long-term potential toxicity such absorption could induce.

Compared with lactulose alone, rifaximin-α has been shown to be better at [109–111], or at least as good at [112–114], reducing encephalopathy in cirrhotics and has fewer side effects [115]. Treatment with rifaximin was also associated with fewer deaths, fewer cases of sepsis and correspondingly shorter hospital stay [111]. A placebo-controlled randomized trial showed that rifaximin-α is effective at maintaining remission from HE as well as significantly reducing HE-related hospital admissions [116]. Suggested therapeutic effects of rifaximin-α are shown in Figure 4. Despite rifaximin-α not being absorbed, a reduction in the incidence of sepsis in cirrhosis might result from a reduction in the high level [82] of viable bacteria translocated across the gut wall. This theory is supported by a reduction in endotoxemia seen in rifaximin-treated patients [117].

Rifaximin potentially provides a combination of ammonia and inflammation reduction with few side effects. This provides an exciting avenue in treatment of chronic HE and is looking more likely to be included as a staple in treatment of HE poorly controlled by traditional treatments. It is currently

Table 2. Current and potential treatments for hepatic encephalopathy classified in to ammonia reducing and inflammation reducing.

Ammonia reducing		Inflammation reducing
Decreased production	*Increased removal*	
Nonabsorbable disaccharides	LOLA	(Rifaximin)
Rifaximin	Hemofiltration/hemodialysis	Hypothermia
Probiotics	Branched-chain amino acids	Antioxidants, for example, NAC
AST-120	(Acetyl)-L-carnitine	(Probiotics)
Sodium phenylbutyrate/phenylacetate	Plasmapheresis	Plasmapheresis Sildenafil
Sodium benzoate		Minocycline
L-ornithine phenylacetate		Albumin replacement/dialysis

LOLA: L-ornithine-L-aspartate; NAC: *N*-acetylcisteine.

under consideration by NICE in the UK for maintenance therapy of HE with a committee decision due at the end of October 2013.

L-ornithine-L-aspartate

L-ornithine-L-aspartate (LOLA) stimulates the urea cycle and glutamine synthesis. Both ornithine and aspartate are metabolic substrates of the urea cycle, and its administration in rats shows a reduction in ammonia and slight increase in blood urea, suggesting ammonia reduction may be through production of urea in the liver [118]. They are both able to interact with α-ketoglutarate to produce glutamate, and some LOLA treatments in rats show increased glutamate in CSF and plasma [118], although other studies have shown the opposite [119].

Efficacy of LOLA in chronic HE appears to be more favorable. A meta-analysis of four randomized controlled trials in the use of LOLA versus placebo in HE in cirrhotics demonstrated improvement in neuropsychometry and a reduced serum ammonia level [120]. A more recent randomized controlled trial in patients with MHE comparing LOLA, probiotics or lactulose to placebo showed significant improvement in HE in all three groups. [121] Comparing LOLA to lactulose has shown benefit of LOLA in one study [122]. Evidence for LOLA in ALF is lacking with one placebo controlled study showing no improvement in HE grade although this study was criticized for using subtherapeutic doses of LOLA [123].

Hemofiltration/hemodialysis

Hemofiltration/hemodialysis are renal replacement therapies (RRT) commonly used in patients with renal failure, which can lower blood ammonia levels by removing it and other small solutes from the blood. RRT is the standard of care to help reduce ammonia in patients with ALF [36] and in urea cycle defects when the blood ammonia level does not respond medically [124]. The effect of hemofiltration on ammonia in patients with liver disease has been sparsely studied, but one recent study showed that it resulted in a 22% reduction in arterial ammonia concentration after 24 h, but no comment was made on any change in HE grade [125]. RRTs are invasive, resource intensive and are likely to only be used in an intensive care unit setting; because of this, RRT is likely to be used in the context of end-stage liver failure with multiorgan dysfunction, the latter frequently associated with sepsis.

Hypothermia

Moderate hypothermia (reduction of core body temperature to 32–35°C) is likely to convey protection against deleterious effects of HE in ALF by a decrease in the production of proinflammatory mediators as well as a reduction in the effect and the level of ammonia in the blood. An ammonia reduction is likely to be due to a reduction in ammonia production by intestinal bacteria, decreased proteolysis and decreased renal release of ammonia into the blood [126]. Hypothermia has been demonstrated to be effective at lowering medically resistant high intracranial pressure (ICP) in ALF while waiting for transplantation [76,127–129]. Initial results from a randomized multicenter trial appear to show that the utilization of hypothermia as prophylaxis for raised ICP, rather than as a direct response to it, did not alter overall mortality or the subsequent incidence of raised ICP [130]. There are many different variables in hypothermia treatment such as method of cooling, onset and duration of therapy, extent of hypothermia, rewarming protocols, as well as differences in the underlying causes of ALF. Unless these can be standardized, the comparison and interpretation of results from different trials will be difficult. Given hypothermia is effectively used following brain insults ranging from trauma, myocardial infarction, to hypoxic–ischemic encephalopathy, there may well be an enduring place for hypothermia in future treatment regimens of ALF. Optimization of parameters for hypothermia therapy is vital and the publication of the full breakdown of the multicenter study results as well as targeted further studies are eagerly awaited.

Table 3. Use of hepatic encephalopathy treatments and their suitability for use in acute liver failure and cirrhosis.

Treatment	HE in ALF	Cirrhosis		Notes
		Acute HE episode	*Chronic and minimal HE*	
Currently used therapies				
Nonabsorbable disaccharides	(✓)	✓	✓	Caution in ALF
Non-absorbable antibiotics			✓	For example, rifaximin
Enemas	✓	✓		
L-ornithine L-aspartate			✓	
Hemofiltration/hemodialysis	✓	✓		Lowers ammonia. Only used in cirrhosis where RRT required
Mild/moderate hypothermia	✓			
Antioxidants	✓			For example, NAC
Indomethacin	✓			
Mannitol	✓			
Hypertonic saline	✓			
Experimental therapies				
Probiotics			✓	
AST-120			✓	No further drug development planned
Sodium phenylbutyrate and sodium benzoate	✓	✓	✓	
Branched chain amino acids		✓	✓	
L-ornithine phenylacetate	✓	✓		
L-carnitine	✓	✓	✓	
Albumin replacement/dialysis	✓	✓		
Plasmapheresis	✓			
GABA receptor antagonists	✓	✓		For example, flumazenil
Theoretical therapies: no human trials				
Phosphodiesterase inhibitors			✓	For example, sildenafil
Minocycline	✓	✓		The drug can cause liver failure

ALF: Acute liver failure; HE: Hepatic encephalopathy; NAC: *N*-acetylcisteine; RRT: Renal replacement therapies.

Antioxidants

N-acetylcysteine (NAC) already has a crucial role in ALF caused by acetaminophen overdose. Its antioxidant and anti-inflammatory properties have been suggested to be protective in cases of nonacetaminophen-induced ALF as well. In a randomized controlled trial, NAC improved survival when given during early coma stages [131]. In animal models of nonacetaminophen-induced ALF NAC reduces ammonia, some inflammatory cytokines, as well hepatocyte damage [132]. Recognition of the protective effect of NAC in nonacetaminophen-induced ALF is recognized in a position paper by the American Association for the Study of Liver Diseases, but routine use is currently not one of the recommendations [133]. With further supportive evidence, this may well change in the future.

Protein-restricted diet?

Historically, a protein-restricted diet was believed to be beneficial in the treatment of HE [134,135]. However, recent guidelines have acknowledged that protein restriction has no role in the prevention or management of HE. In a randomized comparison of a protein-restricted diet (0 g protein Days 1–3, increased every 3 days to 1.2 g/kg/day on Days 13 and 14) and a normal diet in 30 patients with acute HE, no difference in outcomes could be detected [136]. Moreover, the need to maintain an

BCAA seem to be mixed. A Cochrane review evaluating BCAA treatment in HE concluded that there was no improvement in HE in studies up to 2002 [156]. Further meta-analyses including trials up to December 2012 seem to suggest that BCAA are effective at reducing manifestations of HE [157].

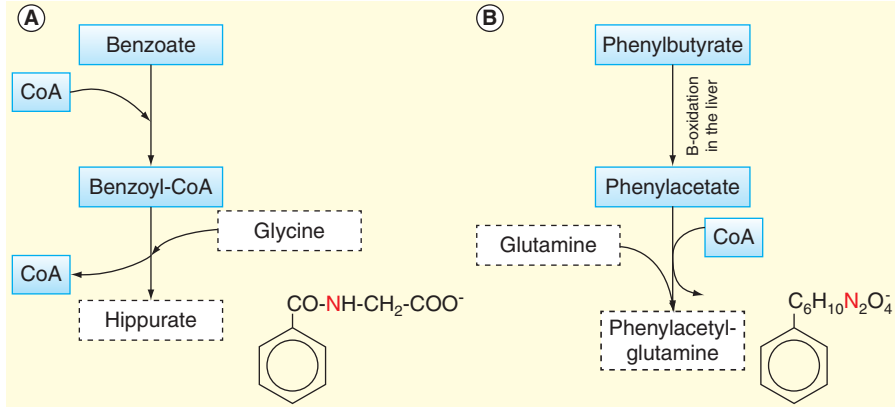

Figure 5. Nitrogen excretion mechanisms. (A) Sodium benzoate. **(B)** Sodium phenylbutyrate. Nitrogen-containing compounds shown in dashed box. CoA also contains nitrogen, but this is unchanged in the reaction.

L-ornithine phenylacetate

This is a novel treatment for HE that aims to combine the effects of L-ornithine observed from treatment with LOLA and the benefits of sodium phenylacetate without the large sodium loading. It aims to have a synergistic effect by both providing more glutamate, and encouraging increased glutamine excretion in the urine, thus reducing the ammonia load [158]. It has been shown to reduce cerebral edema and ammonia concentrations in ALF in pigs [159], and one study in humans has shown that it is well tolerated in decompensated cirrhotics with a reduction in plasma ammonia and glutamine [160].

As with sodium benzoate, the product of sodium or L-ornithine phenylacetate combining with nitrogen products – phenylacetylglutamine – is renally excreted. A functional glomerular filtration rate is therefore required for these nitrogen excretion pathways to be efficacious.

L-carnitine

L-carnitine treatment aims to reduce serum ammonia by inducing ureagenesis. A deficiency of carnitine has frequently been noted in patients who have hyperammonemic encephalopathy as a result of sodium valproate toxicity [161]. Cases of hyperammonemic encephalopathy have been caused simply by a deficiency of carnitine. [162–164] Encephalopathy usually resolved on administration of carnitine. These results have significance to liver failure as there are very close similarities between acute HE and acute hyperammonemic encephalopathy [165]. Rats with HE secondary to portacaval shunts treated with L-carnitine showed a significant reduction in CSF ammonia compared with placebo [166]. Six studies have evaluated carnitine use in HE: two with MHE patients, one with moderate HE and three with severe HE/coma. In MHE, improvement in cognition and reduction in serum ammonia was observed [167,168]. Improvement in moderate HE was observed with better mental fatigue score and reduction in serum ammonia [169]. In severe HE/coma, although there was a reduction in serum ammonia, there was both a reduction [170] and an improvement [171] in Glasgow Coma Score in comatose patients, as well as neuropsychiatric improvement in the severe cases [172].

Albumin replacement/dialysis

Albumin has been shown to have antioxidant properties and is able to scavenge reactive oxygen species [173]. Treatment of dehydration-induced HE with intravenous albumin has been shown to be more effective at reducing HE grade than simple colloid [174]. The detoxification properties of albumin have been utilized in albumin dialysis by passing blood through an albumin-impermeable membrane before going through an albumin cleansing circuit. Because of the invasive nature of this treatment, it would be reserved for those in an intensive care setting. A multicenter trial showed that albumin dialysis was more effective at reducing HE grade in cirrhotics than medical treatment alone [175]. In ALF patients fulfilling transplant criteria, a meta-analysis has shown that the use of albumin dialysis has been shown to reduce HE grade and, although not significant, could act as a bridge to transplantation [176].

Albumin dialysis is usually completed on a circuit that also undertakes hemodialysis/hemofiltration for RRT.

Plasmapheresis

Similar to albumin dialysis, plasmapheresis with plasma exchange of patient plasma for fresh frozen plasma aims to remove albumin-bound toxic substances and large molecular weight toxins, including endotoxin, ammonia and some amino acids [177]. Plasmapheresis often improves the grade of HE, but this is short-lived. In a review of studies on plasmapheresis in HE, there was an improvement in 69 out of 97 patients (71%) treated with plasmapheresis. The review also mentions unpublished data of a randomized controlled multicenter trial on patients with ALF, which showed significant improvement in transplant-free survival and HE [178]. Plasmapheresis is a remarkably simple method to help reduce both ammonia and inflammation. As such, it has great potential in HE treatment, particularly in the context of ALF, and the full results of the trial are eagerly awaited.

Minocycline

Minocycline is a tetracycline antibiotic with anti-inflammatory properties. It has been shown to reduce microglial cell activation in cerebral insults [179]. In rats with induced ALF, minocycline reduced brain edema, and plasma and CSF ammonia

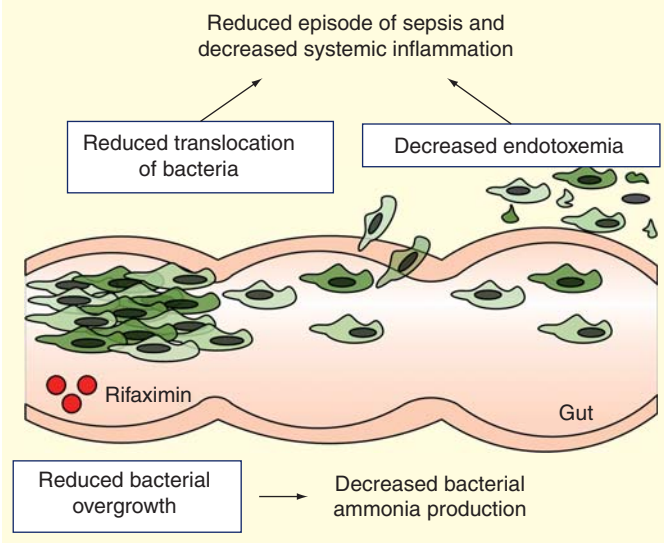

Figure 4. Suggested effects of the nonabsorbable antibiotic rifaximin in the gut in hepatic encephalopathy.

anabolic state in cirrhotic patients, who commonly present with a protein–energy malnutrition as breakdown of bodily protein stores could lead to an increased ammonia load [137], and severe protein–energy malnutrition in itself can cause liver injury [138].

Potential therapies for HE

Probiotics

A probiotic is a microorganism introduced into the body for beneficial reasons. The therapeutic aim is to change the gut flora to reduce the urease-producing species such as *Klebsiella* species (spp) and *Proteus* spp, and replace them with nonurease-producing species such as lactobacilli and bifidobacteria, which are acid-resistant [139]. This would therefore reduce the amount of ammonia produced in the gut. Bifidobacteria have been shown to help maintain equilibrium within the gut flora, and specific species can confer protection against the effect of endotoxins [140]. Treatment of cirrhotics with probiotics has been shown to improve Child's Pugh scores, reduce blood ammonia and endotoxin levels, improve MHE and offer secondary protection against further overt HE episodes [139–142]. However, in a recent meta-analysis, probiotics were shown to have little impact on any clinically significant outcomes [143].

AST-120

AST-120 is a treatment involving spherical oral activated carbon spheres with a selective adsorbent profile for a variety of substances including ammonia. It is not absorbed and considerable data exists with 360,000 'patient exposures' to the product that was developed for the treatment of irritable bowel syndrome. Oral ingestion has been shown to attenuate HE in cirrhotic rats [144], and lower blood ammonia in portacaval shunted dogs [145]. In a Phase II trial comparing AST-120 with lactulose in patients with cirrhosis, there was an improvement in cognitive function in both groups, but no change in venous ammonia levels. There was a significant reduction in side effects such as diarrhea and flatulence in patients who took AST-120 [146]. While this is initially promising data for patients with chronic HE, further development of the drug has not been pursued.

Sodium phenylbutyrate & sodium benzoate

Sodium benzoate and sodium phenylbutyrate have traditionally been used to treat hyperammonemia in patients with urea cycle defects [147]. They provide a pathway other than via urea for nitrogenous waste to be excreted. These pathways are shown in Figure 5. This reduces ammonia production as the nitrogen-containing reactants such as glycine would otherwise produce ammonia if metabolized. Both nitrogen products, hippurate and phenylacetyl glutamine, are readily excreted by the kidneys [124]. The two drugs can be combined together and have been shown to induce substantial decreases in ammonia levels in urea cycle defects, resulting in higher survival rates [148].

In one blinded trial comparing sodium benzoate and lactulose for HE in patients with cirrhosis, sodium benzoate was seen to be as effective as lactulose in reducing HE, as well as arterial ammonia [149]. A very small study of just seven patients looked at benzoate in cirrhotics following an oral glutamine challenge. Patients did not have HE to start with, and there was no change in cognitive function afterward; however, paradoxically a significant increase in plasma ammonia levels was seen in patients following 5 days of sodium benzoate treatment compared with the glutamine challenge before the benzoate treatment [150]. Other studies on HE have shown a significant improvement in neurological function and ammonia levels following treatment with benzoate [151,152]. Further research will be required as it has been suggested that in the absence of ample glycine (which occurs when there is a urea cycle defect), use of CoA by benzoate would use up CoA that is not subsequently released. This in turn could reduce urea cycle activity, which could deleteriously increase levels of ammonia [20]. Further mechanistic studies are therefore warranted.

One potential issue with sodium phenylbutyrate is that in its standard form of sodium phenylbutyrate, the maximum daily dose is 20 g. This equates to 2400 mg of sodium, more than the recommended daily allowance for a healthy adult, let alone a patient with cirrhosis and potential ascites. Glycerol phenylbutyrate has been developed to resolve this, and has been tolerated well in pilot studies in patients with cirrhosis and is able to lower ammonia levels in cirrhotic patients [153,154].

Branched chain amino acids

The branched chain amino acids (BCAA) isoleucine, leucine and valine are nonessential amino acids and are available as nutritional support for patients with cirrhosis. The exact method of how this reduces HE in patients with cirrhosis is not clearly understood. It has been suggested that BCAAs can help with transport of ammonia nitrogen out of the neuron, with brain energy metabolism by providing substrates, and by aiding with metabolites of the TCA cycle [155]. Results for treatment with

levels [180]. Great care will need to be taken if considering introducing this drug as a therapy for HE patients, as minocycline can induce hepatotoxicity that itself has been fatal [181].

GABA receptor antagonists

Flumazenil is a selective benzodiazepine antagonist and is proposed to be beneficial because of the increased GABAergic tone reported in some patients with HE, as previously discussed. A Cochrane review concluded that there is evidence that flumazenil can provide short-term improvement in HE symptoms; it is unclear how long this effect lasts. Additionally, there is no evidence that it has any improvement on recovery or survival [182].

$GABA_A$ antagonists such as bicuculline have been shown to restore learning ability in hyperammonemic rats [61] and transiently reduces HE symptoms in rabbits [183].

Phosphodiesterase inhibitors

Phosphodiesterase-5 inhibitors may potentially be useful in chronic HE. The glutamate–NO–cGMP pathway is dysfunctional, as previously discussed, and results in reduced levels of cGMP. Oral administration of sildenafil or intracerebral administration of zaprinast – both phosphodiesterase inhibitors – result in improved learning in portacaval shunted rats and increased cGMP levels [54]. Sildenafil also exerts a protective effect by reversing oxidative stress probably by its antioxidant properties, which are not fully understood [184]. This could therefore offer a novel therapeutic avenue in HE, and more studies are required.

Expert commentary

It is perhaps unfortunate that we have inherited the term HE. There is no such thing as the disease 'Hepatic encephalopathy.' There is 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 process that has come to be defined as HE. Differences in outcomes, responses to treatments and underlying pathophysiology can be significant between acute HE and chronic HE. The term also fails to articulate quite how systemic HE is; a HE episode might be caused by a change in the gastrointestinal, renal, nervous or immune system without any change in background liver function. The pathogenesis of HE describes a complex disease network involving many interdependent organ systems that is currently not fully rationalized. With the recognition of the synergy of inflammation and oxidative stress in the pathogenesis HE, and the importance of immunoparesis in patients with liver failure, treatment targets for HE are moving further away from the traditional specialty of hepatology.

Five-year view

In the next 5 years, we are likely to benefit from optimization and further trialing of some of the novel treatments outlined in this review, as well as the advent of specific targeted therapies. Further studies of the effect of treatments that have the dual effect of lowering both ammonia level and inflammation, such as rifaximin and plasmapheresis, may well reveal that this combined effect provides better responses. Brand new treatments that look to reduce systemic inflammation, such as TLR antagonists, have the potential to be future therapeutic targets, and their introduction to animal models of HE will play an important part in assessing this.

Financial & competing interests disclosure

D Shawcross has participated in Advisory Boards, consultancy and paid lectures for Norgine, makers of rifaximin alpha. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Hepatic encephalopathy (HE) is a spectrum of neuropsychiatric disorders ranging from subtle cognitive changes through to coma.
- HE is a multisystem disease with dysfunction/compensation seen in the gastrointestinal tract, immune system, central nervous system, kidneys and cardiovascular system.
- High blood ammonia levels, along with systemic or neuroinflammation is the key to the pathogenesis of the disease.
- Circulating ammonia is able to reach the brain. Astrocytes play an important role in protecting the brain from ammonia.
- Ammonia induces changes in neurotransmission, may induce neuroinflammation and induce endogenous production of neurosteroids.
- Disturbances in the immune system in patients with HE leads to a state of immunodeficiency. This makes patients more susceptible to infection, and SIRS is frequently seen in HE patients. This worsens inflammation, HE and prognosis.
- Treatments for HE currently focus on reducing the blood ammonia level, reducing inflammation, or both.
- Current treatments are suboptimal across the spectrum of HE; the only definitive treatment for patients with acute liver failure and severe HE is a liver transplantation. Treatment failure of minimal and chronic HE results in poor quality of life for patients and carers, and worsens prognosis.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Bajaj JS, Wade JB, Sanyal AJ. Spectrum of neurocognitive impairment in cirrhosis: implications for the assessment of hepatic encephalopathy. *Hepatology* 50(6), 2014–2021 (2009).

2 Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy–definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 35(3), 716–721 (2002).

3 Cauli O, Rodrigo R, Llansola M *et al.* Glutamatergic and gabaergic neurotransmission and neuronal circuits in hepatic encephalopathy. *Metab. Brain Dis.* 24(1), 69–80 (2009).

4 Riordan SM, Williams R. Treatment of hepatic encephalopathy. *N. Engl. J. Med.* 337(7), 473–479 (1997).

5 Bernal W, Hyyrylainen A, Gera A *et al.* Lessons from look-back in acute liver failure? A single centre experience of 3300 patients. *J. Hepatol.* 59(1), 74–80 (2013).

6 Dhiman RK, Kurmi R, Thumburu KK *et al.* Diagnosis and prognostic significance of minimal hepatic encephalopathy in patients with cirrhosis of liver. *Dig. Dis. Sci.* 55(8), 2381–2390 (2010).

7 Das A, Dhiman RK, Saraswat VA, Verma M, Naik SR. Prevalence and natural history of subclinical hepatic encephalopathy in cirrhosis. *J. Gastroenterol Hepatol.* 16(5), 531–535 (2001).

8 Alqahtani SA, Larson AM. Adult liver transplantation in the USA. *Curr. Opin. Gastroenterol.* 27(3), 240–247 (2011).

9 Amodio P, Del Piccolo F, Petteno E *et al.* Prevalence and prognostic value of quantified electroencephalogram (EEG) alterations in cirrhotic patients. *J. Hepatol.* 35(1), 37–45 (2001).

10 Quero JC, Schalm SW. Subclinical hepatic encephalopathy. *Semin. Liver Dis.* 16(3), 321–328 (1996).

11 Kircheis G, Knoche A, Hilger N *et al.* Hepatic encephalopathy and fitness to drive. *Gastroenterology* 137(5), 1706–1715, e1701–1709 (2009).

12 Bajaj JS, Saeian K, Schubert CM *et al.* Minimal hepatic encephalopathy is associated with motor vehicle crashes: the reality beyond the driving test. *Hepatology* 50(4), 1175–1183 (2009).

13 Dhiman RK, Chawla YK. Minimal hepatic encephalopathy. *Indian J. Gastroenterol.* 28(1), 5–16 (2009).

14 Butterworth RF. Pathophysiology of hepatic encephalopathy: a new look at ammonia. *Metab. Brain Dis.* 17(4), 221–227 (2002).

•• **A good account of the role of ammonia in hepatic encephalopathy (HE).**

15 Olde Damink SW, Deutz NE, Dejong CH, Soeters PB, Jalan R. Interorgan ammonia metabolism in liver failure. *Neurochem. Int.* 41(2–3), 177–188 (2002).

16 Shawcross DL, Davies NA, Williams R, Jalan R. Systemic inflammatory response exacerbates the neuropsychological effects of induced hyperammonemia in cirrhosis. *J. Hepatol.* 40(2), 247–254 (2004).

• **Significance of inflammation working synergistically with ammonia in HE.**

17 Tranah TH, Vijay GK, Ryan JM, Shawcross DL. Systemic inflammation and ammonia in hepatic encephalopathy. *Metab. Brain Dis.* 28(1), 1–5 (2013).

18 Coltart I, Tranah TH, Shawcross DL. Inflammation and hepatic encephalopathy. *Arch. Biochem. Biophys.* 536(2), 189–196 (2013).

19 Wright G, Davies NA, Shawcross DL *et al.* Endotoxemia produces coma and brain swelling in bile duct ligated rats. *Hepatology* 45(6), 1517–1526 (2007).

20 Rose CF. Ammonia-lowering strategies for the treatment of hepatic encephalopathy. *Clin. Pharmacol. Ther.* 92(3), 321–331 (2012).

21 Romero-Gómez M, Ramos-Guerrero R, Grande L *et al.* Intestinal glutaminase activity is increased in liver cirrhosis and correlates with minimal hepatic encephalopathy. *J. Hepatol.* 41(1), 49–54 (2004).

22 Haussinger D, Lamers WH, Moorman AF. Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* 46 (1–3), 72–93 (1992).

23 Anand U, Anand CV. Teaching the urea cycle: the role of liver in acid-base regulation. *Biochem. Educ.* 25(1), 20–21 (1997).

24 Wright G, Noiret L, Olde Damink SW, Jalan R. Interorgan ammonia metabolism in liver failure: the basis of current and future therapies. *Liver Int.* 31(2), 163–175 (2011).

25 Romero-Gomez M, Jover M, Galan JJ, Ruiz A. Gut ammonia production and its modulation. *Metab. Brain Dis.* 24(1), 147–157 (2009).

26 Dejong CH, Deutz NE, Soeters PB. Metabolic adaptation of the kidney to hyperammonemia during chronic liver insufficiency in the rat. *Hepatology* 18(4), 890–902 (1993).

27 Olde Damink SW, Jalan R, Dejong CH. Interorgan ammonia trafficking in liver disease. *Metab. Brain Dis.* 24(1), 169–181 (2009).

28 Mpabanzi L, van den Broek MA, Visschers RG *et al.* Urinary ammonia excretion increases acutely during living donor liver transplantation. *Liver Int.* 31(8), 1150–1154 (2011).

29 Olde Damink SW, Jalan R, Deutz NE *et al.* The kidney plays a major role in the hyperammonemia seen after simulated or actual GI bleeding in patients with cirrhosis. *Hepatology* 37(6), 1277–1285 (2003).

30 Rao KV, Panickar KS, Jayakumar AR, Norenberg MD. Astrocytes protect neurons from ammonia toxicity. *Neurochem. Res.* 30(10), 1311–1318 (2005).

31 Moser H. Electrophysiological evidence for ammonium as a substitute for potassium in activating the sodium pump in a crayfish sensory neuron. *Can. J. Physiol. Pharmacol.* 65(2), 141–145 (1987).

32 Bakouh N, Benjelloun F, Cherif-Zahar B, Planelles G. The challenge of understanding ammonium homeostasis and the role of the Rh glycoproteins. *Transfus. Clin. Biol.* 13(1–2), 139–146 (2006).

33 Keiding S, Sorensen M, Bender D, Munk OL, Ott P, Vilstrup H. Brain metabolism of 13N-ammonia during acute hepatic encephalopathy in cirrhosis measured by positron emission tomography. *Hepatology* 43(1), 42–50 (2006).

34 Balata S, Olde Damink SW, Ferguson K *et al.* Induced hyperammonemia alters neuropsychology, brain MR spectroscopy and magnetization transfer in cirrhosis. *Hepatology* 37(4), 931–939 (2003).

35 Fernandes SP, Dringen R, Lawen A, Robinson SR. Neurones express glutamine synthetase when deprived of glutamine or interaction with astrocytes. *J. Neurochem.* 114(5), 1527–1536 (2010).

36 Shawcross DL, Wendon JA. The neurological manifestations of acute liver failure. *Neurochem. Int.* 60(7), 662–671 (2012).

37 Clemmesen JO, Larsen FS, Kondrup J, Hansen BA, Ott P. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. *Hepatology* 29(3), 648–653 (1999).

38 Bernal W, Hall C, Karvellas CJ, Auzinger G, Sizer E, Wendon J. Arterial ammonia and clinical risk factors for encephalopathy and intracranial hypertension in acute liver failure. *Hepatology* 46(6), 1844–1852 (2007).

39 Cordoba J, Alonso J, Rovira A *et al*. The development of low-grade cerebral edema in cirrhosis is supported by the evolution of (1)H-magnetic resonance abnormalities after liver transplantation. *J. Hepatol*. 35(5), 598–604 (2001).

40 Cauli O, Llansola M, Agusti A *et al*. Cerebral oedema is not responsible for motor or cognitive deficits in rats with hepatic encephalopathy. *Liver Int*. doi:10.1111/liv.12258 (2013) (Epub ahead of print).

41 Joshi D, O'apos;Grady J, Patel A *et al*. Cerebral oedema is rare in acute-on-chronic liver failure patients presenting with high-grade hepatic encephalopathy. *Liver Int*. doi:10.1111/liv.12257 (2013) (Epub ahead of print).

42 Shawcross DL, Shabbir SS, Taylor NJ, Hughes RD. Ammonia and the neutrophil in the pathogenesis of hepatic encephalopathy in cirrhosis. *Hepatology* 51(3), 1062–1069 (2010).

43 Fan P, Szerb JC. Effects of ammonium ions on synaptic transmission and on responses to quisqualate and N-methyl-D-aspartate in hippocampal CA1 pyramidal neurons *in vitro*. *Brain Res*. 632(1–2), 225–231 (1993).

44 Bosoi CR, Rose CF. Identifying the direct effects of ammonia on the brain. *Metab. Brain Dis*. 24(1), 95–102 (2009).

45 Hermenegildo C, Monfort P, Felipo V. Activation of N-methyl-D-aspartate receptors in rat brain *in vivo* following acute ammonia intoxication: characterization by *in vivo* brain microdialysis. *Hepatology* 31(3), 709–715 (2000).

46 Llansola M, Rodrigo R, Monfort P *et al*. NMDA receptors in hyperammonemia and hepatic encephalopathy. *Metab. Brain Dis*. 22(3–4), 321–335 (2007).

- **Summary of *N*-methyl-D-aspartate changes in HE.**

47 Minana MD, Felipo V, Grisolia S. Protective effect of long term ammonium ingestion against acute ammonium intoxication. *Biochem. Biophys. Res. Commun*. 153(3), 979–983 (1988).

48 Kosenko E, Felipo V, Minana MD, Grau E, Grisolia S. Ammonium ingestion prevents depletion of hepatic energy metabolites induced by acute ammonium intoxication. *Arch. Biochem. Biophys*. 290(2), 484–488 (1991).

49 Kosenko E, Kaminsky YG, Felipo V, Minana MD, Grisolia S. Chronic hyperammonemia prevents changes in brain energy and ammonia metabolites induced by acute ammonium intoxication. *Biochim. Biophys. Acta* 1180(3), 321–326 (1993).

50 Hermenegildo C, Montoliu C, Llansola M *et al*. Chronic hyperammonemia impairs the glutamate-nitric oxide-cyclic GMP pathway in cerebellar neurons in culture and in the rat *in vivo*. *Eur. J. Neurosci*. 10(10), 3201–3209 (1998).

51 Corbalan R, Chatauret N, Behrends S, Butterworth RF, Felipo V. Region selective alterations of soluble guanylate cyclase content and modulation in brain of cirrhotic patients. *Hepatology* 36(5), 1155–1162 (2002).

52 Erceg S, Monfort P, Cauli O *et al*. Role of extracellular cGMP and of hyperammonemia in the impairment of learning in rats with chronic hepatic failure. Therapeutic implications. *Neurochem. Int*. 48(6–7), 441–446 (2006).

53 Erceg S, Monfort P, Hernandez-Viadel M, Llansola M, Montoliu C, Felipo V. Restoration of learning ability in hyperammonemic rats by increasing extracellular cGMP in brain. *Brain Res*. 1036(1–2), 115–121 (2005).

54 Erceg S, Monfort P, Hernandez-Viadel M, Rodrigo R, Montoliu C, Felipo V. Oral administration of sildenafil restores learning ability in rats with hyperammonemia and with portacaval shunts. *Hepatology* 41(2), 299–306 (2005).

55 Schafer DF, Jones EA. Hepatic encephalopathy and the γ-aminobutyric-acid neurotransmitter system. *Lancet* 1(8262), 18–20 (1982).

56 Ahboucha S, Butterworth RF. Pathophysiology of hepatic encephalopathy: a new look at GABA from the molecular standpoint. *Metab. Brain Dis*. 19(3–4), 331–343 (2004).

57 Jones EA. Potential mechanisms of enhanced GABA-mediated inhibitory neurotransmission in liver failure. *Neurochem. Int*. 43(4–5), 509–516 (2003).

58 Lavoie J, Layrargues GP, Butterworth RF. Increased densities of peripheral-type benzodiazepine receptors in brain autopsy samples from cirrhotic patients with hepatic encephalopathy. *Hepatology* 11(5), 874–878 (1990).

59 Ahboucha S, Pomier-Layrargues G, Mamer O, Butterworth RF. Increased levels of pregnenolone and its neuroactive metabolite allopregnanolone in autopsied brain tissue from cirrhotic patients who died in hepatic coma. *Neurochem. Int*. 49(4), 372–378 (2006).

60 Jalan R, Turjanski N, Taylor-Robinson SD *et al*. Increased availability of central benzodiazepine receptors in patients with chronic hepatic encephalopathy and alcohol related cirrhosis. *Gut* 46(4), 546–552 (2000).

61 Cauli O, Mansouri MT, Agusti A, Felipo V. Hyperammonemia Increases GABAergic Tone in the Cerebellum but Decreases It in the Rat Cortex. *Gastroenterology* 136(4), 1359–1367, e1352 (2009).

62 Bjerring PN, Eefsen M, Hansen BA, Larsen FS. The brain in acute liver failure. A tortuous path from hyperammonemia to cerebral edema. *Metab. Brain Dis*. 24(1), 5–14 (2009).

63 Zwingmann C, Chatauret N, Leibfritz D, Butterworth RF. Selective increase of brain lactate synthesis in experimental acute liver failure: Results of a [1H-13C] nuclear magnetic resonance study. *Hepatology* 37(2), 420–428 (2003).

64 Kosenko E, Kaminsky Y, Grau E *et al*. Brain ATP depletion induced by acute ammonia intoxication in rats is mediated by activation of the NMDA receptor and Na+, K(+)-ATPase. *J. Neurochem*. 63(6), 2172–2178 (1994).

65 Albrecht J, Norenberg MD. Glutamine: a Trojan horse in ammonia neurotoxicity. *Hepatology* 44(4), 788–794 (2006).

66 Sonnewald U, Westergaard N, Jones P, Taylor A, Bachelard HS, Schousboe A. Metabolism of [U-13C5] glutamine in cultured astrocytes studied by NMR spectroscopy: first evidence of astrocytic pyruvate recycling. *J. Neurochem*. 67(6), 2566–2572 (1996).

67 Bai G, Rama Rao KV, Murthy CR, Panickar KS, Jayakumar AR, Norenberg MD. Ammonia induces the mitochondrial permeability transition in primary cultures of rat astrocytes. *J. Neurosci. Res*. 66(5), 981–991 (2001).

68 Zoratti M, Szabo I, De Marchi U. Mitochondrial permeability transitions: how many doors to the house? *Biochim. Biophys. Acta* 1706(1–2), 40–52 (2005).

69 Rama Rao KV, Jayakumar AR, Norenberg MD. Role of oxidative stress in the ammonia-induced mitochondrial permeability transition in cultured astrocytes. *Neurochem. Int*. 47(1–2), 31–38 (2005).

70 Shawcross DL, Sharifi Y, Canavan JB *et al*. Infection and systemic inflammation, not

ammonia, are associated with Grade 3/4 hepatic encephalopathy, but not mortality in cirrhosis. *J. Hepatol.* 54(4), 640–649 (2011).

71 Ong JP, Aggarwal A, Krieger D *et al.* Correlation between ammonia levels and the severity of hepatic encephalopathy. *Am. J. Med.* 114(3), 188–193 (2003).

72 Nicolao F, Efrati C, Masini A, Merli M, Attili AF, Riggio O. Role of determination of partial pressure of ammonia in cirrhotic patients with and without hepatic encephalopathy. *J. Hepatol.* 38(4), 441–446 (2003).

73 Rolando N, Philpott-Howard J, Williams R. Bacterial and fungal infection in acute liver failure. *Semin. Liver Dis.* 16(4), 389–402 (1996).

74 Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. *Hepatology* 32(4 Pt 1), 734–739 (2000).

75 Vaquero J, Polson J, Chung C *et al.* Infection and the progression of hepatic encephalopathy in acute liver failure. *Gastroenterology* 125(3), 755–764 (2003).

76 Jalan R, Olde Damink SW, Deutz NE, Hayes PC, Lee A. Moderate hypothermia in patients with acute liver failure and uncontrolled intracranial hypertension. *Gastroenterology* 127(5), 1338–1346 (2004).

77 Jalan R, Pollok A, Shah SH, Madhavan K, Simpson KJ. Liver derived pro-inflammatory cytokines may be important in producing intracranial hypertension in acute liver failure. *J. Hepatol.* 37(4), 536–538 (2002).

78 Shawcross DL, Wright G, Olde Damink SW, Jalan R. Role of ammonia and inflammation in minimal hepatic encephalopathy. *Metab. Brain Dis.* 22(1), 125–138 (2007).

79 Marini JC, Broussard SR. Hyperammonemia increases sensitivity to LPS. *Mol. Genet. Metab.* 88(2), 131–137 (2006).

80 Felipo V, Urios A, Montesinos E *et al.* Contribution of hyperammonemia and inflammatory factors to cognitive impairment in minimal hepatic encephalopathy. *Metab. Brain Dis.* 27(1), 51–58 (2012).

81 Vorobioff J, Bredfeldt JE, Groszmann RJ. Hyperdynamic circulation in portal-hypertensive rat model: a primary factor for maintenance of chronic portal hypertension. *Am. J. Physiol.* 244(1), G52–57 (1983).

82 Cirera I, Bauer TM, Navasa M *et al.* Bacterial translocation of enteric organisms in patients with cirrhosis. *J. Hepatol.* 34(1), 32–37 (2001).

83 Llovet JM, Bartoli R, March F *et al.* Translocated intestinal bacteria cause spontaneous bacterial peritonitis in cirrhotic rats: molecular epidemiologic evidence. *J. Hepatol.* 28(2), 307–313 (1998).

84 Perez del Pulgar S, Pizcueta P, Engel P, Bosch J. Enhanced monocyte activation and hepatotoxicity in response to endotoxin in portal hypertension. *J. Hepatol.* 32(1), 25–31 (2000).

85 Bigatello LM, Broitman SA, Fattori L *et al.* Endotoxemia, encephalopathy, and mortality in cirrhotic patients. *Am. J. Gastroenterol.* 82(1), 11–15 (1987).

86 Montoliu C, Piedrafita B, Serra MA *et al.* IL-6 and IL-18 in blood may discriminate cirrhotic patients with and without minimal hepatic encephalopathy. *J. Clin. Gastroenterol.* 43(3), 272–279 (2009).

- **Hyperammonemia in the presence of raised inflammatory markers can induce a HE in the absence of liver disease.**

87 Panasiuk A, Wysocka J, Maciorkowska E *et al.* Phagocytic and oxidative burst activity of neutrophils in the end stage of liver cirrhosis. *World J. Gastroenterol.* 11(48), 7661–7665 (2005).

88 Bonnel AR, Bunchorntavakul C, Reddy KR. Immune dysfunction and infections in patients with cirrhosis. *Clin. Gastroenterol. Hepatol.* 9(9), 727–738 (2011).

89 Mookerjee RP, Stadlbauer V, Lidder S *et al.* Neutrophil dysfunction in alcoholic hepatitis superimposed on cirrhosis is reversible and predicts the outcome. *Hepatology* 46(3), 831–840 (2007).

90 Shawcross DL, Wright GA, Stadlbauer V *et al.* Ammonia impairs neutrophil phagocytic function in liver disease. *Hepatology* 48(4), 1202–1212 (2008).

91 Taylor NJ, Nishtala A, Manakkat Vijay GK *et al.* Circulating neutrophil dysfunction in acute liver failure. *Hepatology* 57(3), 1142–1152 (2013).

92 Stadlbauer V, Mookerjee RP, Hodges S, Wright GA, Davies NA, Jalan R. Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. *J. Hepatol.* 48(6), 945–951 (2008).

93 Vijay GKM, Abeles RD, Ramage S *et al.* PMO-125 Neutrophil intracellular toll-like receptor (TLR) 9 expression serves as a biomarker that determines presence and severity of encephalopathy in acute liver failure and cirrhosis. *Gut* 61(Suppl. 2), A123–A124 (2012).

94 Borzio M, Salerno F, Piantoni L *et al.* Bacterial infection in patients with advanced cirrhosis: a multicentre prospective study. *Dig. Liver Dis.* 33(1), 41–48 (2001).

95 Cauli O, Rodrigo R, Piedrafita B, Boix J, Felipo V. Inflammation and hepatic encephalopathy: ibuprofen restores learning ability in rats with portacaval shunts. *Hepatology* 46(2), 514–519 (2007).

96 Rodrigo R, Cauli O, Gomez-Pinedo U *et al* . Hyperammonemia induces neuroinflammation that contributes to cognitive impairment in rats with hepatic encephalopathy. *Gastroenterology* 139(2), 675–684 (2010).

97 Jiang W, Desjardins P, Butterworth RF. Direct evidence for central proinflammatory mechanisms in rats with experimental acute liver failure: protective effect of hypothermia. *J. Cereb. Blood Flow Metab.* 29(5), 944–952 (2009).

98 Jiang W, Desjardins P, Butterworth RF. Cerebral inflammation contributes to encephalopathy and brain edema in acute liver failure: protective effect of minocycline. *J. Neurochem.* 109(2), 485–493 (2009).

99 Rangroo Thrane V, Thrane AS, Chang J, Alleluia V, Nagelhus EA, Nedergaard M. Real-time analysis of microglial activation and motility in hepatic and hyperammonemic encephalopathy. *Neuroscience* 220, 247–255 (2012).

100 Butterworth RF. The liver-brain axis in liver failure: neuroinflammation and encephalopathy. *Nat. Rev. Gastroenterol. Hepatol.* 10(9), 522–528 (2013).

101 Sharma BC, Sharma P, Agrawal A, Sarin SK. Secondary prophylaxis of hepatic encephalopathy: an open-label randomized controlled trial of lactulose versus placebo. *Gastroenterology* 137(3), 885–891, 891 e891 (2009).

102 Alba L, Hay JE, Angulo P, Lee WM. Lactulose therapy in acute liver failure. *J. Hepatol.* 36, 33 (2002).

103 Polson J, Lee WM. AASLD position paper: The management of acute liver failure. *Hepatology* 41(5), 1179–1197 (2005).

104 Als-Nielsen B, Gluud LL, Gluud C. Non-absorbable disaccharides for hepatic encephalopathy: systematic review of randomised trials. *BMJ* 328(7447), 1046 (2004).

105 Prasad S, Dhiman RK, Duseja A, Chawla YK, Sharma A, Agarwal R. Lactulose improves cognitive functions and health-related quality of life in patients with

cirrhosis who have minimal hepatic encephalopathy. *Hepatology* 45(3), 549–559 (2007).

106 Gluud LL, Dam G, Borre M *et al.* Lactulose, rifaximin or branched chain amino acids for hepatic encephalopathy: what is the evidence? *Metab. Brain Dis.* 28(2), 221–225 (2013).

107 Riordan SM, Williams R. Gut flora and hepatic encephalopathy in patients with cirrhosis. *N. Engl. J. Med.* 362(12), 1140–1142 (2010).

108 DuPont HL. Biologic properties and clinical uses of rifaximin. *Expert Opin. Pharmacother.* 12(2), 293–302 (2011).

109 Bucci L, Palmieri GC. Double-blind, double-dummy comparison between treatment with rifaximin and lactulose in patients with medium to severe degree hepatic encephalopathy. *Curr. Med. Res. Opin.* 13(2), 109–118 (1993).

110 Loguercio C, Federico A, De Girolamo V, Ferrieri A, Del Vecchio Blanco C. Cyclic treatment of chronic hepatic encephalopathy with rifaximin. Results of a double-blind clinical study. *Minerva Gastroenterol. Dietol.* 49(1), 53–62 (2003).

111 Sharma BC, Sharma P, Lunia MK, Srivastava S, Goyal R, Sarin SK. A randomized, double-blind, controlled trial comparing rifaximin plus lactulose with lactulose alone in treatment of overt hepatic encephalopathy. *Am. J. Gastroenterol.* 108(9), 1458–1463 (2013).

112 Festi D, Mazzella G, Orsini M *et al.* Rifaximin in the treatment of chronic hepatic-encephalopathy – results of a multicenter study of efficacy and safety. *Curr. Ther. Res. Clin. E* 54(5), 598–609 (1993).

113 Paik YH, Lee KS, Han KH *et al.* Comparison of rifaximin and lactulose for the treatment of hepatic encephalopathy: a prospective randomized study. *Yonsei Med. J.* 46(3), 399–407 (2005).

114 Mas A, Rodes J, Sunyer L *et al.* Comparison of rifaximin and lactitol in the treatment of acute hepatic encephalopathy: results of a randomized, double-blind, double-dummy, controlled clinical trial. *J. Hepatol.* 38(1), 51–58 (2003).

115 Jiang Q, Jiang XH, Zheng MH, Jiang LM, Chen YP, Wang L. Rifaximin versus nonabsorbable disaccharides in the management of hepatic encephalopathy: a meta-analysis. *Eur. J. Gastroenterol. Hepatol.* 20(11), 1064–1070 (2008).

116 Bass NM, Mullen KD, Sanyal A *et al.* Rifaximin treatment in hepatic encephalopathy. *N. Engl. J. Med.* 362(12), 1071–1081 (2010).

117 Kalambokis GN, Tsianos EV. Rifaximin reduces endotoxemia and improves liver function and disease severity in patients with decompensated cirrhosis. *Hepatology* 55(2), 655–656 (2012).

118 Rose C, Michalak A, Pannunzio P *et al.* L-ornithine-L-aspartate in experimental portal-systemic encephalopathy: therapeutic efficacy and mechanism of action. *Metab. Brain Dis.* 13(2), 147–157 (1998).

119 Gebhardt R, Beckers G, Gaunitz F *et al.* Treatment of cirrhotic rats with L-ornithine-L-aspartate enhances urea synthesis and lowers serum ammonia levels. *J. Pharmacol. Exp. Ther.* 283(1), 1–6 (1997).

120 Soarez PC, Oliveira AC, Padovan J, Parise ER, Ferraz MB. A critical analysis of studies assessing L-ornithine-L-aspartate (LOLA) in hepatic encephalopathy treatment. *Arq. Gastroenterol.* 46(3), 241–247 (2009).

121 Mittal VV, Sharma BC, Sharma P, Sarin SK. A randomized controlled trial comparing lactulose, probiotics, and L-ornithine L-aspartate in treatment of minimal hepatic encephalopathy. *Eur. J. Gastroenterol. Hepatol.* 23(8), 725–732 (2011).

122 Poo JL, Gongora J, Sanchez-Avila F *et al.* Efficacy of oral L-ornithine-L-aspartate in cirrhotic patients with hyperammonemic hepatic encephalopathy. Results of a randomized, lactulose-controlled study. *Ann. Hepatol.* 5(4), 281–288 (2006).

123 Acharya SK, Bhatia V, Sreenivas V, Khanal S, Panda SK. Efficacy of L-ornithine L-aspartate in acute liver failure: a double-blind, randomized, placebo-controlled study. *Gastroenterology* 136(7), 2159–2168 (2009).

124 Leonard JV, Morris AA. Urea cycle disorders. *Semin. Neonatol.* 7(1), 27–35 (2002).

125 Slack AJ, Auzinger G, Willars C *et al.* Ammonia clearance with haemofiltration in adults with liver disease. *Liver Int.* doi:10.1111/liv.12221 (2013) (Epub ahead of print).

126 Stravitz RT, Larsen FS. Therapeutic hypothermia for acute liver failure. *Crit. Care Med.* 37(7 Suppl.), S258–S264 (2009).

127 Jalan R, O Damink SW, Deutz NE, Lee A, Hayes PC. Moderate hypothermia for uncontrolled intracranial hypertension in acute liver failure. *Lancet* 354(9185), 1164–1168 (1999).

128 Jalan R, Olde Damink SW, Deutz NE *et al.* Moderate hypothermia prevents cerebral hyperemia and increase in intracranial pressure in patients undergoing liver transplantation for acute liver failure. *Transplantation* 75(12), 2034–2039 (2003).

129 Jalan R, Olde Damink SW, Deutz NE, Hayes PC, Lee A. Restoration of cerebral blood flow autoregulation and reactivity to carbon dioxide in acute liver failure by moderate hypothermia. *Hepatology* 34(1), 50–54 (2001).

130 Larsen FS, Murphy N, Bernal W, Bjerring PN, Hauerberg J, Wendon J. 56 the prophylactive effect of mild hypothermia to prevent brain edema in patients with acute liver failure: results of a multicenter randomized, controlled trial. *J. Hepatol.* 54, S26 (2011).

131 Lee WM, Hynan LS, Rossaro L *et al.* Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. *Gastroenterology* 137(3), 856–864, 864, e851 (2009).

132 Bémeur C, Vaquero J, Desjardins P, Butterworth R. N-Acetylcysteine attenuates cerebral complications of non-acetaminophen-induced acute liver failure in mice: antioxidant and anti-inflammatory mechanisms. *Metab. Brain Dis.* 25(2), 241–249 (2010).

133 Lee WM, Stravitz RT, Larson AM. Introduction to the revised American Association for the study of liver diseases position paper on acute liver failure 2011. *Hepatology* 55(3), 965–967 (2012).

134 Sherlock S, Summerskill WH, White LP, Phear EA. Portal-systemic encephalopathy; neurological complications of liver disease. *Lancet* 267(6836), 454–457 (1954).

135 Amodio P, Bemeur C, Butterworth R *et al.* The nutritional management of hepatic encephalopathy in patients with cirrhosis: International Society for Hepatic Encephalopathy and Nitrogen Metabolism Consensus. *Hepatology* 58(1), 325–336 (2013).

136 Cordoba J, Lopez-Hellin J, Planas M *et al.* Normal protein diet for episodic hepatic encephalopathy: results of a randomized study. *J. Hepatol.* 41(1), 38–43 (2004).

137 Plauth M, Merli M, Kondrup J *et al.* ESPEN guidelines for nutrition in liver disease and transplantation. *Clin. Nutr.* 16(2), 43–55 (1997).

138 Rana S, Sodhi CP, Mehta S *et al.* Protein-energy malnutrition and oxidative

injury in growing rats. *Hum. Exp. Toxicol.* 15(10), 810–814 (1996).

139 Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 39(5), 1441–1449 (2004).

140 Malaguarnera M, Greco F, Barone G, Gargante MP, Malaguarnera M, Toscano MA. Bifidobacterium longum with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. *Dig. Dis. Sci.* 52(11), 3259–3265 (2007).

141 Sharma P, Sharma BC, Puri V, Sarin SK. An open-label randomized controlled trial of lactulose and probiotics in the treatment of minimal hepatic encephalopathy. *Eur. J. Gastroenterol. Hepatol.* 20(6), 506–511 (2008).

142 Agrawal A, Sharma BC, Sharma P, Sarin SK. Secondary prophylaxis of hepatic encephalopathy in cirrhosis: an open-label, randomized controlled trial of lactulose, probiotics, and no therapy. *Am. J. Gastroenterol.* 107(7), 1043–1050 (2012).

143 McGee RG, Bakens A, Wiley K, Riordan SM, Webster AC. Probiotics for patients with hepatic encephalopathy. *Cochrane Database Syst. Rev.* (11), CD008716 (2011).

144 Bosoi CR, Parent-Robitaille C, Anderson K, Tremblay M, Rose CF. AST-120 (spherical carbon adsorbent) lowers ammonia levels and attenuates brain edema in bile duct-ligated rats. *Hepatology* 53(6), 1995–2002 (2011).

145 Hiraishi M. The effect of oral adsorbent on surgically induced hepatic failure. *Jpn. J. Surg.* 17(6), 517–527 (1987).

146 Pockros P, Hassanein T, Vierling J *et al.* 105 phase 2, multicenter, randomized study of ast-120 (spherical carbon adsorbent) vs. lactulose in the treatment of low-grade hepatic encephalopathy (he). *J. Hepatol.* 50, S43–S44 (2009).

147 Brusilow SW, Valle DL, Batshaw M. New pathways of nitrogen excretion in inborn errors of urea synthesis. *Lancet* 2(8140), 452–454 (1979).

148 Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW, Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. *N. Engl. J. Med.* 356(22), 2282–2292 (2007).

149 Sushma S, Dasarathy S, Tandon RK, Jain S, Gupta S, Bhist MS. Sodium benzoate in the treatment of acute hepatic encephalopathy: a double-blind randomized trial. *Hepatology* 16(1), 138–144 (1992).

150 Efrati C, Masini A, Merli M, Valeriano V, Riggio O. Effect of sodium benzoate on blood ammonia response to oral glutamine challenge in cirrhotic patients: a note of caution. *Am. J. Gastroenterol.* 95(12), 3574–3578 (2000).

151 Mendenhall CL, Rouster S, Marshall L, Weesner R. A new therapy for portal systemic encephalopathy. *Am. J. Gastroenterol.* 81(7), 540–543 (1986).

152 Uribe M, Bosques F, Poo J *et al.* A double blind randomized trial of sodium benzoate versus lactulose in patients with chronic portal systemic encephalopathy. [Abstract]. *Hepatology* 8(5), 1449 (1988).

153 McGuire BM, Zupanets IA, Lowe ME *et al.* Pharmacology and safety of glycerol phenylbutyrate in healthy adults and adults with cirrhosis. *Hepatology* 51(6), 2077–2085 (2010).

154 Ghabril M, Zupanets IA, Vierling J *et al.* Glycerol phenylbutyrate in patients with cirrhosis and episodic hepatic encephalopathy: a pilot study of safety and effect on venous ammonia concentration. *Clin. Pharmacol. Drug Dev.* 2(3), 278–284 (2013).

155 Bak LK, Waagepetersen HS, Sorensen M *et al.* Role of branched chain amino acids in cerebral ammonia homeostasis related to hepatic encephalopathy. *Metab. Brain Dis.* 28(2), 209–215 (2013).

156 Als-Nielsen B, Koretz RL, Gluud LL, Gluud C. Branched-chain amino acids for hepatic encephalopathy. *Cochrane Database Syst. Rev.* (2), CD001939 (2004).

157 Gluud LL, Dam G, Borre M *et al.* Oral branched-chain amino acids have a beneficial effect on manifestations of hepatic encephalopathy in a systematic review with meta-analyses of randomized controlled trials. *J. Nutr.* 143(8), 1263–1268 (2013).

158 Jalan R, Wright G, Davies NA, Hodges SJ. L-Ornithine phenylacetate (OP): a novel treatment for hyperammonemia and hepatic encephalopathy. *Med. Hypotheses* 69(5), 1064–1069 (2007).

159 Ytrebo LM, Kristiansen RG, Maehre H *et al.* L-ornithine phenylacetate attenuates increased arterial and extracellular brain ammonia and prevents intracranial hypertension in pigs with acute liver failure. *Hepatology* 50(1), 165–174 (2009).

160 Ventura-Cots M, Arranz JA, Simon-Talero M *et al.* Safety of ornithine phenylacetate in cirrhotic decompensated patients: an open-label, dose-escalating, single-cohort study. *J. Clin. Gastroenterol.* 47(10), 881–887 (2013).

161 Lheureux PE, Penaloza A, Zahir S, Gris M. Science review: carnitine in the treatment of valproic acid-induced toxicity – what is the evidence? *Crit. Care* 9(5), 431–440 (2005).

162 Limketkai BN, Zucker SD. Hyperammonemic encephalopathy caused by carnitine deficiency. *J. Gen. Intern. Med.* 23(2), 210–213 (2008).

163 Ling P, Lee DJ, Yoshida EM, Sirrs S. Carnitine deficiency presenting with encephalopathy and hyperammonemia in a patient receiving chronic enteral tube feeding: a case report. *J. Med. case Rep.* 6(1), 227 (2012).

164 Karakoc E, Erdem S, Sokmensuer C, Kansu T. Encephalopathy due to carnitine deficiency in an adult patient with gluten enteropathy. *Clin. Neurol. Neurosurg.* 108(8), 794–797 (2006).

165 McKinney AM, Sarikaya B, Spanbauer J, Lohman BD, Uhlmann E. Acute hepatic (or hyperammonemic) encephalopathy: diffuse cortical injury and the significance of ammonia. *AJNR* 32(7), E142, author reply E143 (2011).

166 Therrien G, Rose C, Butterworth J, Butterworth RF. Protective effect of L-carnitine in ammonia-precipitated encephalopathy in the portacaval shunted rat. *Hepatology* 25(3), 551–556 (1997).

167 Malaguarnera M, Gargante MP, Cristaldi E *et al.* Acetyl-L-carnitine treatment in minimal hepatic encephalopathy. *Dig. Dis. Sci.* 53(11), 3018–3025 (2008).

168 Malaguarnera M, Bella R, Vacante M *et al.* Acetyl-L-carnitine reduces depression and improves quality of life in patients with minimal hepatic encephalopathy. *Scand. J. Gastroenterol.* 46(6), 750–759 (2011).

169 Malaguarnera M, Vacante M, Giordano M *et al.* Oral acetyl-L-carnitine therapy reduces fatigue in overt hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. *Am. J. Clin. Nutr.* 93(4), 799–808 (2011).

170 Malaguarnera M, Pistone G, Astuto M *et al.* Effects of L-acetylcarnitine on cirrhotic patients with hepatic coma: randomized double-blind, placebo-controlled trial. *Dig. Dis. Sci.* 51(12), 2242–2247 (2006).

171 Malaguarnera M, Risino C, Cammalleri L *et al.* Branched chain amino acids supplemented with L-acetylcarnitine versus BCAA treatment in hepatic coma: a randomized and controlled double blind

study. *Eur. J. Gastroenterol. Hepatol.* 21(7), 762–770 (2009).

172 Malaguarnera M, Vacante M, Motta M *et al.* Acetyl-L-carnitine improves cognitive functions in severe hepatic encephalopathy: a randomized and controlled clinical trial. *Metab. Brain Dis.* 26(4), 281–289 (2011).

173 Quinlan GJ, Martin GS, Evans TW. Albumin: biochemical properties and therapeutic potential. *Hepatology* 41(6), 1211–1219 (2005).

174 Jalan R, Kapoor D. Reversal of diuretic-induced hepatic encephalopathy with infusion of albumin but not colloid. *Clin. Sci.* 106(5), 467–474 (2004).

175 Hassanein TI, Tofteng F, Brown RS Jr *et al.* Randomized controlled study of extracorporeal albumin dialysis for hepatic encephalopathy in advanced cirrhosis. *Hepatology* 46(6), 1853–1862 (2007).

176 Kjaergard LL, Liu J, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: a systematic review. *JAMA* 289(2), 217–222 (2003).

177 Nakanishi T, Suzuki N, Kuragano T, Nagasawa Y, Hasuike Y. Current topics in therapeutic plasmapheresis. *Clin. Exp. Nephrol.* 1–9 (2013).

178 Stenbøg P, Busk T, Larsen F. Efficacy of liver assisting in patients with hepatic encephalopathy with special focus on plasma exchange. *Metab. Brain Dis.* 28(2), 333–335 (2013).

179 Stirling DP, Koochesfahani KM, Steeves JD, Tetzlaff W. Minocycline as a neuroprotective agent. *Neuroscientist* 11(4), 308–322 (2005).

180 Jiang W, Desjardins P, Butterworth RF. Minocycline attenuates oxidative/nitrosative stress and cerebral complications of acute liver failure in rats. *Neurochem. Int.* 55(7), 601–605 (2009).

181 Maddrey WC. Drug-induced hepatotoxicity: 2005. *J. Clin. Gastroenterol.* 39(4 Suppl. 2), S83–S89 (2005).

182 Als-Nielsen B, Gluud LL, Gluud C. Benzodiazepine receptor antagonists for hepatic encephalopathy. *Cochrane Database Syst. Rev.* (2), CD002798 (2004).

183 Bassett ML, Mullen KD, Skolnick P, Jones EA. Amelioration of hepatic encephalopathy by pharmacologic antagonism of the GABAA-benzodiazepine receptor complex in a rabbit model of fulminant hepatic failure. *Gastroenterology* 93(5), 1069–1077 (1987).

184 Arafa MH, Atteia HH. Sildenafil citrate attenuates the deleterious effects of elevated ammonia. *Toxicol. Mech. Methods* 23(6), 402–411 (2013).

Website

201 XIFAXAN® (rifaximin) Tablets, Package insert. Salix Pharmaceuticals Inc., Raleigh, NC, USA. www.salix.com/assets/pdf/prescribe_info/xifaxan-pi.pdf (Accessed 20 August 2013)

Recent insights into the pathogenesis of hepatic encephalopathy and treatments

Article *in* Expert review of gastroenterology & hepatology · November 2013

DOI: 10.1586/17474124.2014.858598 · Source: PubMed

2 authors:

Jonathan Peter Sturgeon
Queen Mary, University of London
16 PUBLICATIONS **63** CITATIONS
SEE PROFILE

Debbie L Shawcross
King's College London
157 PUBLICATIONS **3,757** CITATIONS
SEE PROFILE