2022

Interrelationships of Systemic Changes in Hepatic Encephalopathy

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Abstract: Hepatic encephalopathy is a temporary decline in mental function that is often associated with liver disease and/or portal-systemic disease. Hepatic encephalopathy (HE) symptoms can range from minor cognitive declines to coma and are known to result from excess ammonia accumulations in the blood stream subsequent to liver failure. While HE is known to result from hepatobiliary disorder, many of the physiological process underlying its development and progression remain to be elucidated. Recent studies have identified neurological, metabolic, and microbiome changes implicated in the disease state of HE. In this review, the roles of traditional pharmaceutical interventions and newly developing understandings of the molecular biology underlying hepatic encephalopathy will be explored.

One Sentence Summary: Liver injuries resulting in high blood ammonia concentrations can cause molecular changes in the brain that lead to the development of HE.

Abbreviations: HE=hepatic encephalopathy; OHE=overt hepatic encephalopathy; CHE=covert hepatic encephalopathy; WHC=West Haven Criteria; ISHEN=International Society for Hepatic Encephalopathy and Nitrogen Metabolism, MELD=Model for End-Stage Liver Disease; HRQOL=health-related quality of life; ALF=acute liver failure; GDH=glutamate dehydrogenase; GS=glutamine synthetase; MRI=magnetic resonance imaging; NMDA=N-metil-D-Aspartate; AMPA= α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; cGMP=cyclic guanosine monophosphate

Introduction

Hepatic encephalopathy (herein abbreviated as HE) is a neurological condition resulting from liver failure. Frequently appearing in individuals with cirrhotic liver disease, termed type C HE, its onset typically starts gradually and can lead to severe impairment, including coma. Type A HE is resultant of acute liver injury or failure, as in the case of individuals who suddenly experience chemotoxicity of the liver or physical injury. Type B HE is experienced in the absence of a liver injury, and rather as a result of portal system shunting. This review will expand primarily upon type C HE, though the other types of hepatic encephalopathy will also be implicated. Overt hepatic encephalopathy is estimated to occur in between 30 to 40% of individuals who have cirrhotic liver disease *(17)*. Presence of HE in the cirrhotic patient is related to poor prognosis and may reduce survival when liver transplantation is not performed *(18)*.

Diagnosis

Due to the nature of hepatic encephalopathy as a spectrum disorder, a variety of diagnostic techniques and measurements are implicated in its diagnosis and treatment. The first diagnostic criterion is the presence of liver disease. This may be confirmed by imaging, liver function testing, or biopsy. Certain physical indicators may also align with the diagnosis of HE, namely asterixis of the arms upon outward stretching with the hands bent upward at the wrist, rigidity, tremors, hyperreflexia, or a positive Babinski's sign in adults *(11)*. Though the causes of HE vary, the covert and over symptoms are largely uniform between HE types. Overt symptoms of HE include changes in consciousness, personality, disorientation, and coma. HE can also present much more subtly, at the onset of chronic disease. This phenomenon is called covert HE

and may include minimally noticeable symptoms. Diagnosis of minimal covert HE requires psychometric testing, whereas over HE does not usually require testing to diagnose *(2)*.

Type	Association	Example(s)
A	Acute liver failure without underlying liver disease	Acute liver failure due to NSAID overdose
B	Portal-systemic bypass without underlying liver disease	Adverse effects resulting from transjugular intrahepatic portosystemic shunt procedure (TIPS)
C	Chronic liver disease and/or cirrhosis	Alcoholic liver cirrhosis, untreated hepatitis, non-alcoholic fatty liver disease (NAFLD)

Table 1

Classification of the presence and severity of HE is determined using the West Haven Criteria (WHC), whereby HE can be diagnosed on a grading scale from 0 to IV regarding severity. Of note, the West Haven classification system does not assign a numerical grade to subclinical (minimal) HE, but does recognize it as existing between grade 0, or absence of HE, and grade I. Grade I HE is indicated by altered mood, such as feelings of euphoria or anxiety, decreased attention span, inability to concentrate, altered sleep rhythms, and impaired cognitive functioning, such as decreased ability to perform addition or subtraction. Patients with Grade I HE do not experience changes in orientation. Cognitive impairment and behavioral changes are largely subjective and are noticed by the patient's caregivers and/or healthcare provider when compared to the patient's baseline mental function. WHC Grade II HE is indicated by decreased energy or motivation, disorientation for time, noticeable personality changes, and inappropriate behavior, as well as physical manifestations including dyspraxia and asterixis. Grade III is indicated by drowsiness, decreased responsiveness to stimulation, confusion, memory loss, disorientation of time and space, and inappropriate behavior. Physical symptoms of Grade III HE include all of the previously mentioned physical symptoms of Grades I and II HE, in addition

to a positive Babinski's sign and muscle rigidity. Grade IV HE is the most advanced stage of HE. Patients with Grade IV HE are comatose and do not respond to any stimuli.

The stages of HE as defined by the West Haven Criteria can be split into two categories: overt hepatic encephalopathy, and covert hepatic encephalopathy. Covert HE (CHE) includes subclinical HE and WHC Grade I and are labeled as such due to their less clinically obvious symptoms. Overt HE (OHE) encompasses the group of HE grades that present with physical symptoms, such as asterixis or tremor. OHE includes WHC Grades II through IV and is thought to be more easily diagnosed due to the overt nature of symptom presentation in these grades. In addition to the psychomotor and physical symptoms described above, patients experiencing WHC Grades I through III have also been found to experience abnormalities in the brain's electrical activity as evidenced by electroencephalography. Findings from electroencephalographic studies indicate a triphasic wave form in Grades I, II, and III, and a delta activity in WHC Grade IV patients *(11)*.

Diagnosis of subclinical, or minimal, HE presents a unique challenge for the practitioner, as patients with this stage of HE do not show clinical signs of HE *(13)*. Minimal hepatic encephalopathy (MHE) can be viewed as a precursor to clinical HE, as associated cognitive dysfunction often precipitates further HE symptom development and disease progression *(13)*. Cognitive declines associated with MHE can include decreased ability to learn maps or spatial relationships, inability to perform complex math problems, decreased reaction time, decreased attention span. In addition to a decreased health-related quality of life (HRQOL), MHE is associated with an increased incidence of falls in hospitalized patients *(14)*. Falls present both a burden to the healthcare system, and a significant risk to patients with liver disease, as injuries associated with falls may be complicated by impaired coagulation resulting from the liver

disease itself. Due to their decreased reaction times and spatial awareness, patients with MHE are also four times more likely to be involved in motor vehicle accidents than drivers without MHE *(15)*. Because of these known harms associated with MHE, accurate diagnosis is incredibly important. Healthcare providers may benefit from speaking with family members or other members of a patient's household to identify any behavior changes which may not be otherwise obvious, as well as to obtain information regarding the patient's baseline cognitive and psychomotor functioning. These interviews should accompany psychometric testing to create a diagnosis of MHE and an individualized treatment plan which considers the patient's baseline. Practitioners may also consider a patient's driving history as part of the diagnostic process, as this history may include concrete and quantifiable examples of MHE symptom manifestation *(16)*.

Disease Pathogenesis

The complexity of HE results in part from the various physiological stressors caused by liver failure. As a result of the decrease of blood filtration and detoxification by the liver, metabolic wastes build up in the blood *(1)*. Damage to the liver's mitochondria and surrounding vasculature impairs the conversion of ammonia to urea via the urea cycle. In a healthy person, this process allows for excess ammonia to be excreted via the kidneys as urea; in a patient with liver disease, this excess ammonia may not be removed, and instead builds up in the bloodstream. Ammonia, which is produced by intestinal bacteria, is the most significantly implicated neurotoxin in HE. Ammonia has been implicated in hepatic encephalopathy since the 1890's and has since been a target for medication *(3)*.

While ammonia is a known key player in HE, the exact processes involved in its overproduction, retention, and neuroactivity are still only partially known. Arterial ammonia concentrations are not a reliable diagnostic measure due to each patient's differing sensitivities after prolonged exposure, but arterial ammonia concentrations greater than 300 μM are considered to reliably predict cerebral herniation in patients with acute liver failure *(19)*. A relationship between arterial ammonia concentration has also been discovered, in which increasing ammonia concentrations correlating to increased intracranial pressures in patients with acetaminophen-induced acute liver failure (ALF) *(20)*. Ammonia in the brain increases intracellular osmolarity, furthering disease progression by causing changes in cerebral mitochondrial function and neurotransmitter synthesis and activity *(21)*.

While there exist several known ways in which ammonia itself directly impacts the brain, there are several indirect ways in which ammonia contributes to HE, most notably via its metabolites. Glutamate dehydrogenase (GDH) and glutamine synthetase (GS) are two known enzymes that are active in the brain and can remove ammonia. GDH catalyzes the production of glutamate from alpha-ketoglutarate in both neurons and astrocytes *(22)*. In this reductive amination reaction, GDH fixates ammonia to create glutamate, and this has been postulated to be crucial for neuronal survival *(22)*.

Glutamate produced by GDH is converted to glutamine by GS in a reaction that it thought to occur almost exclusively in astrocytes *(23)*. The conversion of glutamate to glutamine is integral in mediating excess ammonia in the brain, with higher extracellular glutamine concentrations in the brain causing increased cerebral vascularity and corresponding with higher intracranial pressure *(20)*. Glutamine created by GS exits the cell via the SNAT5 transporter *(24)*. Once in the extracellular space, glutamine is taken up by SNAT1 and SNAT2 receptors

into other neurons, where glutaminase converts it back into glutamate *(25)*. This cycle of metabolic activity is responsible both for the uptake and neutralization of ammonia in the brain, as well as indirect effects of hyperammonemia including cerebral edema and neuroinflammation *(26)*.

Intestinal microbiome changes linked to HE

The ammonia that causes many of the symptoms of HE is predominantly created by gram-negative anaerobic bacteria in the lumen of the intestine. Cirrhotic liver disease disrupts normal digestive activity in the lower intestinal tract, resulting in an abnormal gut microbiome that is significantly dysregulated *(4)*. This unbalanced microbiome contains fewer lactobacilli than optimal, and is overrun with *Enterobacteriacaea*, *Archaea, Firmicutes*, and *Prevotella.* These species were found in 2021 to be specifically implicated in increasing ammonia production in the gut in patients with HE *(4*). The presence of ammonia-producing species in the gut relates to several other processes related to HE, namely inflammation and barrier dysfunction, which allow for greater ammonia diffusion across the intestinal epithelia and into the bloodstream. *Enterobacteriaceae* in the gut are closely associated with the cognitive and inflammatory aspects of HE *(5)*. These bacteria have a unique ability to form biofilms in the intestinal tract which impair the mucus layer *(6).* Mucins that form the barrier between intestinal bacteria and the epithelia compose a critically important defense against microbial movement from the lumen into the bloodstream *(7).* The composition of mucins functions both as a physical barrier to the overgrowth of pathogenic species and as a food source for commensal species. In a healthy individual, these commensal species strengthen mucosal layers in the gut

(8). In the liver disease patient, overpopulation of ammonia-producing bacteria combines with the disruption of the intestinal mucus barrier to increase blood ammonia concentrations.

While patients with liver disease tend to experience an increase in intestinal bacterial species which produce ammonia and mucolytic enzymes, these patients also experience drastic decreases in bacteria that may mediate these effects. Decreases in *Bacteroidetes* species have been found to accompany decreases in liver function *(27)*, a change which has also been implicated in the development of obesity and intestinal inflammation in clinical studies *(28)*. Some evidence suggests that patients with liver cirrhosis also experience a delay in gastrointestinal mobility, which increases risk of bacterial overgrowth and increases the digestive time during which ammonia may be absorbed across the gut mucosa *(29)*.

Along with the effects of mucolytic enzymes and delayed small bowel emptying, ammonia may also enter the bloodstream as a result of small bowel inflammation. In patients with significant histories of alcohol consumption this presents a particular concern, as alcohol promotes the growth of Gram-negative bacteria in the small intestine, resulting in a production of endotoxin and acetaldehyde, which increases permeability of the gut mucosa *(30)*.

Neuronal changes in response to ammonia and its metabolites

The symptoms of hepatic encephalopathy all result from insult to the brain on the cellular, molecular, and large-scale anatomical levels. High-resolution magnetic resonance imaging (MRI) studies have shown that patients diagnosed with MHE tend to have structural abnormalities of the cerebral cortex *(31)*. MHE patients with cirrhosis have been further shown to suffer from decreased gray matter volume, and thinning of the superior temporal cortex and precuneus cortex when compared with healthy brains *(32)*. These anatomical changes in the

brain have been likened to changes experienced in Alzheimer disease and schizophrenia. The potential link between HE and Alzheimer disease goes both ways, as changes in liver function have also been postulated to be a precipitating factor for the development of Alzheimer disease *(33)*. While uncommon, severe cases of HE have been documented in which hyperammonemia caused cortical laminar necrosis, the death of cells of the of the cerebral cortex. This phenomenon is usually associated with strokes but has been implicated in several HE case studies *(34)*. Damage to this layer of the cerebral cortex is particularly causal to the development of seizures *(35)*.

The development of cerebral edema in HE has long been attributed to the osmotic effects of ammonia *(21)*, but several additional mechanisms by which ammonia can cause increased intracranial pressure have been discovered more recently *(46)*. Microglia, the brain's immune cells, are heavily implicated in the terminal process involved in Grade IV HE. Activation of microglia does not start until the later stages of disease progression. In fact, microglial activation is found to happen concurrently with opening of the blood-brain barrier and may thereby be associated with cerebral edema *(46)*. This sudden microglial activation constitutes part of an aggressive immune response in the brain, which is believed to exacerbate disease progression to coma and subsequently increase mortality *(46)*. In microglial cell cultures, microglial activation and proliferation can be induced by simulating the microglial membrane NMDA receptor, which provides a potential molecular mechanism by which hyperactivity of microglia can worsen neurodegeneration in correlation with elevated brain glutamate levels *(47).*

By triggering neuroinflammation through microglial activation, ammonia changes the activity of GABA-ergic receptors. The neurotransmitter GABA functions to block impulses between neurons, serving as the brain's way to decrease stimulation and combat anxiety and

hyperactivity *(48)*. GABA membrane receptors in the brain are responsible for maintaining cognition and motor function. Upon exposure to neuroinflammation, GABA-ergic receptors increase expression of the membrane protein GAT-3, which impairs cognition and motor skills *(10*). In rat models, this change was reversible with the administration of sulforaphane. It's believed that this works by reducing altogether inflammation in the brain, and thereby reducing microglial activation.

The effects of hyperammonemia on the brain also impact several of the cellular mechanisms regulating the glutamate-glutamine cycle. As previously mentioned, the production of glutamine from glutamate is largely localized to astrocytes. In cases of acute hyperammonemia, glutamate transporters 1 and 2 (EATT1, EATTT2) are downregulated, causing high levels of extracellular glutamate which cannot enter the astrocyte *(36)*. While high ammonia concentrations in the brain decreases EATT signaling activity, they increase signaling through the N-metil-D-Aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) pathways *(37)*.

Stimulation of NMDA receptors in the brain activates the glutamate-nitric oxide-cGMP pathway, which produces cyclic guanosine monophosphate (cGMP) in response to calcium *(39)*. cGMP plays and important regulatory role in signal transduction and inflammation and has shown direct impact on neuroplasticity and learning ability *(40)*. The healthy brain experiences regulated NMDA signaling activity, but this balance is disturbed by conditions of hyperammonemia, which can cause both excessive and insufficient glutamate-nitric oxide-cGMP activity depending on the duration of ammonia exposure. Overstimulation of the NMDA receptors has been linked to acute hyperammonemia, which may result from acute liver failure

(41). A relationship has been demonstrated between increased extracellular cGMP concentrations and encephalopathic state, with cGMP levels markedly increasing at WHC grades II and III *(38)*. NMDA receptor activation can occur in response to high ammonia levels in the brain and blocking these receptors increases survival in rats with liver failure *(38)*. In contrast to acute hyperammonemia, chronic hyperammonemia is marked by an adaptive response in the brain that attempts to mitigate NMDA receptor hyperactivity. While the NMDA receptors themselves are still hyperactive in chronic hyperammonemia, their tonic activation ultimately reduces glutamate-NO-cGMP pathway activity in rat models *(42)*. These differing neuronal signaling effects resulting from acute and chronic hyperammonemia may provide insight into the molecular mechanisms which cause distinct symptoms in patients with acute onset HE seen in Types A and B, compared to those with Type C HE.

Both AMPA receptors and NMDA receptors are glutamate receptors that participate in excitatory neurotransmission. While AMPA receptors and NMDA receptors share similarities, AMPA receptors are distinct in many of their molecular mechanisms. AMPA receptors are calcium-permeable, and their activation results in an influx of sodium and potassium ions. Studies using rat models have shown that chronic hyperammonemia results in increased membrane expression of the GluR1 subunit of AMPA receptor, but decreased expression of the GluR2 subunit *(43)*. This effect has been experimentally reversed by the administration of extracellular cGMP, which restored appropriate membrane expression of AMPA receptors *(44)*. AMPA receptors are implicated in spatial learning and memory, and these functions are found to decline as a result of AMPA receptor abnormalities in HE *(43,44)* which are also seen in the development of Alzheimer disease *(45)*.

The molecular impacts of ammonia on the brain's structure are incredibly widespread and range from transient, to temporary, to permanent. The brain's natural ability to change, called neuroplasticity, is one of the most heavily contended research areas as of the time of the publication of this review. The American Psychological Association defines neuroplasticity as the brain's ability to respond to its surroundings and stimuli to change to benefit the host. While neuroplasticity remains a poorly understood phenomenon, the effects of lactulose treatment on improving neuroplasticity in patients with minimal HE has been researched *(9)*. Rats with minimal hepatic encephalopathy receiving lactulose have been found to have increased reproduction of brain cells, called neurogenesis *(9)*. The researchers involved with this study concluded that lactulose treatment not only reduced ammonia levels in the bloodstream, but also increased the brain's neuroplasticity. When combined with the molecular effects of NMDA and AMPA receptors in patients with HE *(40; 43-45)*, this study provides a basis for further exploration of neuroplasticity as it relates to HE disease state.

Hepatic Encephalopathy treatment methods

Lactulose

While several pharmacological treatments for HE exist, by far the most common therapies are lactulose and rifaximin. The gold-standard treatment for hepatic encephalopathy, lactulose (1,4 beta galactoside-fructose) is a synthetic disaccharide made of galactose and fructose which cannot be absorbed by the human body. Lactulose was first used to treat HE symptoms in 1966 and received FDA approval for the treatment of HE in 1977 *(49)*. Since its inception, the osmotic effects of lactulose in the small intestine have proven significant in reducing HE symptoms by decreasing the amount of ammonia that enters the bloodstream in the

gut *(50)*. In its syrup form, lactulose can be administered orally or rectally. Because the human body cannot digest this disaccharide, lactulose passes through much of the digestive tract unchanged until it reaches the lumen of the colon, where it causes a host of effects that prevent ammonia absorption across the intestinal lining and into the bloodstream *(51)*.

The intestinal bacteria that produce much of the body's ammonia are targeted both directly and indirectly by lactulose's mechanisms of action. In the large intestine, lactulose is consumed by these bacteria, which causes an increase in osmolality that decreases the luminal pH *(52)*. This increased acid content in the colon causes a laxative effect, which both limits the amount of time that colonic ammonia can diffuse across the intestinal membrane, as well as expels excess ammonia from the body. Lactulose also promotes the conversion of ammonia (NH3) to ammonium (NH4+) by gut bacteria as a response to decreased pH *(51)*. Unlike ammonia, ammonium cannot readily diffuse across the gut membrane, and is thereby trapped in the colon until it's removed via bowel movement *(50)*. Furthermore, lactulose-induced acidic conditions in the intestinal lumen inhibit the survival of urease-producing bacteria, causing changes in the gut microbiome that reduce ammonia production *(53)*.

Rifaximin

The non-absorbable antibiotic rifaximin has been approved by the FDA since 2010 for use in patients with HE as it limits ammonia-producing bacteria in the small intestine. Rifaximin inhibits bacterial reproduction by preventing chain formation in RNA synthesis, a reproductive requirement for many bacterial species, which attributes to its broad-spectrum of activity *(54)*. When administered orally, rifaximin has shown efficacy in treating overgrowths intestinal overgrowths of gram-positive, gram-negative, aerobic, and anaerobic bacterial species (54).

Rifaximin has been found to be well tolerated *(55)* and theoretically less likely result in antibiotic resistance compared to other oral antibiotics *(54)*, which lend to its utility in treating HE long-term. Developments in our understanding of the mucolytic enzymes associated with certain gut microbiota may provide a secondary mechanism of action, whereby rifaximin can prevent diffusion of ammonia across the gut membranes by inhibiting enteric bacteria whose enzymatic activities compromise intestinal mucosa.

Considerations in the development of future HE therapies

While the use of rifaximin and lactulose have shown considerable value in reducing the symptoms of HE by reducing blood ammonia concentrations, these medications fail to treat many of the other molecular mechanisms confounding HE development and progression. Recent developments in our understanding of HE pathogenesis have created numerous potential avenues for treatment, but the development of new therapies may be ethically complex as the already-existing treatments cannot be withheld, which may limit placebo trials of other potential drugs. This particularly affects our ability to study the efficacy of lactulose, and may prevent us from developing alternatives, as the role of lactulose in the treatment of HE has been established for almost fifty years. The efficacy of lactulose compared to other laxatives has also been called into question as many of the therapeutic effects of lactulose result from its laxative properties. This has raised concerns about the efficacy of lactulose compared to other laxatives and caused speculation that other laxatives may have similar or better therapeutic effects *(56)*.

In addition to concerns about the efficacy of lactulose, studies have elucidated potential issues regarding its implementation. The current standard for lactulose treatment emphasizes a titration of dose resulting in 3 daily bowel movements *(57).* but the reasoning for this titration is

contested as patients may experience more side effects of lactulose at this dose *(58)*. Furthermore, this titration of dose is not reflective of efficacy as the frequency of bowel movements is not indicative of cognitive function *(58)*. These questions leave room for further investigation into lactulose treatment and potential adjuncts or alternatives.

Conclusion

The management and treatment of hepatic encephalopathy present unique challenges to our healthcare systems due to the incredible complexity of mechanisms underlying disease development and progression alongside liver injury. While WHC grades II-IV HE present with clinically evident symptoms, the covert nature of symptoms in minimal HE and WHC grade I may convolute diagnosis. Especially in these earlier cases of HE, the formation of a diagnosis should take into consideration the patient's self-reported symptoms as well as observations from the patient's support persons, if possible, and any supportive documents, such as traffic infractions, if applicable. Treatment of covert HE has shown promise in preventing progression to overt HE *(59)*.

As the disease state of HE progresses, patients experience significant changes in intestinal microbiome composition and neuronal signaling, which can lead to structural changes in the brain. Though hyperammonemia resulting from liver failure is known to cause some of these changes directly, there exist a multitude of other molecular mechanisms which have been implicated in HE, many of which interact and with one-another independently of ammonia. Some of these pathways and their alterations have been discovered in other neurodegenerative diseases, such as Alzheimer disease and Parkinson's diseases *(60)*, in which they have been attributed to causing symptoms similar to those of HE. Most notably, changes in the NMDR and

AMPA pathways, as well as microglial hyperactivity, have been related to manifestations including cerebral edema, impaired spatial learning, cognitive decline, and decreased neuroplasticity. Though much remains to be done in the field of HE, further understanding of these processes allows for the development of novel therapies and may even provide insight into other neurodegenerative conditions that share these characteristics.

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