Chapter

Ammonia

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Abstract

Ammonia is a compound that is thought to be central to the pathogenesis of hepatic encephalopathy. It is an important biomarker and may also serve as a prognostic indicator in acute liver disease where ammonia levels may be predictive of cerebral edema and herniation. In this chapter, we aim to review and discuss its role in hepatic encephalopathy to include: the cycle within the human body, appropriate measurement and collection, confounding factors and differential diagnosis, the correlation between levels and development of encephalopathy, the physiopathology and increased morbidity-mortality with the incremental rise, clinical utility of sequential measurement, and lastly, an overview of novel treatments and the tight interconnections with ammonia.

Keywords: ammonia, hepatic encephalopathy, role, pathogenesis, novel treatment

1. Introduction

Ammonia, a colorless gas with a unique odor is thought to be central to the pathogenesis of hepatic encephalopathy (HE). It is an important biomarker and may also serve as a prognostic indicator in acute liver disease where ammonia levels may be predictive of cerebral edema and herniation. In this chapter, we aim to review and discuss its role in HE understanding its rise and fall as part of the urea cycle, appropriate measurement and collection, and examine the paradigms differentiating acute liver failure with chronic liver disease. We want to recognize other diseases that may elevate ammonia levels and discuss how different treatments target its reduction.

2. The ammonia cycle within the human body

The homeostasis of ammonia is a multi-organ process involving the brain, gastrointestinal tract, muscles, adipose tissue, kidneys, and mainly the liver. A study involving patients with end-stage liver disease, revealed that branched-chain amino acids (BCAAs) (**Figure 1**) are not metabolized in the liver but rather by muscle, kidney, adipose, and brain tissue. This is in contrast to the aromatic amino acids (tyrosine, phenylalanine, methionine), which are metabolized and deaminated solely by the liver. BCAA supplementation leads to reductions in hyperammonemia as a result of the metabolism of BCAAs by skeletal muscle. The metabolism of BCAAs supplied carbon skeletons for the formation of α-ketoglutarate which combined with two ammonia molecules to form glutamine [1]. In a 1-year double-blind study of 174 patients with advanced cirrhosis who were randomized to receive BCAAs or equicaloric amounts of lactoalbumin, the group given BCAAs had a significantly decreased incidence of the combined endpoint of death and liver decompensation, as well as hospital admissions, compared with lactoalbumin [2]. In addition, a multi-center randomized study of 646

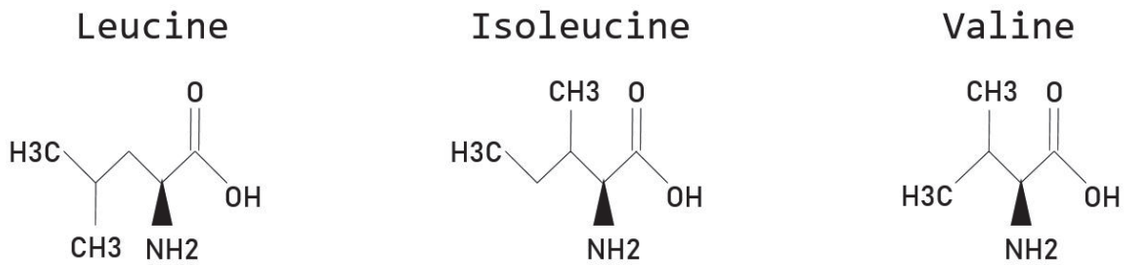

Figure 1.
Branched-chain amino acids.

patients with cirrhosis who were given 12 g of BCAAs per day for 2 years, compared with diet therapy and a defined food intake, found a significant decrease in HE and refractory ascites in the treatment group [3]. Because of their poor palatability and high cost, BCAAs are not routinely recommended, but they were important tools in the proof of concept of liver's importance in ammonia homeostasis.

The mechanism of how the liver processes the ammonia has been described and involves the following steps: ammonia is produced by the enterocytes from glutamine and by colonic bacterial catabolism of nitrogenous sources, such as ingested protein and secreted urea. It then enters the circulation through the portal vein where the liver metabolizes the majority of the ammonia converting it into urea or glutamine and preventing entry into the systemic circulation. These were demonstrated through careful studies of the urea cycle and its disorders [4].

The increase in blood ammonia levels in advanced liver disease is a consequence of impaired liver function and of shunting of blood around/away from the liver. Muscle wasting, a common occurrence in these patients, also may contribute since muscle is also an important site for extrahepatic ammonia removal.

3. Appropriate measurement and collection

The measurement serum ammonia concentration in patients suspected of having HE remains controversial. While it is well known that venous ammonia levels vary immensely and are not useful for screening or following HE [5], arterial ammonia concentrations more accurately correlate with HE as it is further discussed in this chapter. Furthermore, the grade of HE seems to be more closely related to the partial pressure of gaseous ammonia (pNH3) than the total arterial ammonia concentration, since gaseous ammonia readily enters the brain [6]. This can be easily calculated with ammonia levels when correlating with pH.

The accuracy of ammonia determination is influenced by many factors, such as fist clenching, use of a tourniquet, and whether the sample was placed on ice [7]. It is largely recommended that it is tested within an hour of collection, though some agents (sodium borate/l-serine) could potentially stabilize for up to 12 h [8].

Thus, ammonia should be collected in an extremity without trauma with arterial blood, collected in chilled tubes with ammonia-free sodium heparin (green top) or ethylenediaminetetraacetic acid (EDTA; purple top), placed on ice, and delivered rapidly to the laboratory (within an hour). Some chemicals could stabilize for posterior measurement, but more studies are needed to confirm that these agents will not influence in other reactions and measurements.

4. Correlation of levels and development encephalopathy

Normal values for ammonia concentration may differ depending on age groups. It can be often higher in newborns, with the upper limit of normal of ammonia

concentration of healthy term infants at birth of 80 to 90 μmol/L, while normal values in children older than 1 month and adults are less than 50 and 30 μmol/L, respectively [9].

Early studies had shown a correlation of levels of ammonia and worsening HE up to two times the upper limit of normality [6]. Further studies have not only cemented this correlation but have shown a more intricate relationship [10, 11]. It can predict the risk and frequency of HE episodes [12].

5. The physiopathology and increased morbidity-mortality with the incremental rise of ammonia

Proof of the role of ammonia in pathogenesis of HE has come from the efficacy of therapies aimed to lower plasma ammonia in improving its symptoms. The mechanisms causing brain dysfunction in liver disease are still not known to the full extent. In coma of models of acute liver failure, the effects of ammonia are present in brain swelling, impaired cerebral perfusion, and reversible impairment of neurotransmitter systems [13].

Stemming from this proof of concept, several studies have tried to elucidate the effects of hyperammonemia. First, ammonia is believed to be a direct neurotoxin potentialized by other toxins, such as mercaptans and short-chain fatty acids [14]. Second, it impairs the blood–brain barrier by changing the protein transport [15]. Third, it increases the intracellular osmolality of astrocytes leading to edema and extreme cases, herniation [13, 16]. Lastly, it increases oxidative stress. In one study, oxidative stress markers in the brain of patients with cirrhosis with severe hepatic encephalopathy included elevated levels of protein tyrosine-nitrated proteins, heat shock protein-27, and 8-hydroxyguanosine as a marker for RNA oxidation [17].

In a recent study of patients with cirrhosis, there was significant evidence that ammonia levels correlate with not only the severity of hepatic encephalopathy but also the failure of other organs in cirrhosis and is an independent risk factor for 28-day mortality. This data provided evidence that the ammonia level has a clinically relevant utility in providing important prognostic information, signifying its potential role as a biomarker in identifying patients at high risk of mortality. A reduction in ammonia level was associated with improved survival, confirming it as a potential therapeutic target. Classically in urea cycle disorders ammonia levels above 200 μmol/L were considered a poor prognostic factor [18], but in this study in cirrhotics even ≥79.5 μmol/L was associated with increased mortality, indicating an additional role of ammonia in dictating clinical outcomes [11].

Classically, there was a clear distinction of the harmful effects of the ammonia in acute liver failure due to the osmotic component [13] and in lesser degree in chronic liver disease, stating that ammonia in cirrhosis increased morbidity and not mortality. But newer studies and prospective analysis shows that it can be harmful in similar way, increasing mortality [11]. Further studies are needed to corroborate both the utility and prognostic value of ammonia in the setting of chronic liver disease.

6. Confounding factors and differential diagnosis

Ammonia levels may rise due to reasons other than acute or chronic liver disease. This may include increased urea absorption/production, decreased extrahepatic removal, and reduced participation of liver (**Table 1**).

Processes that increase urea absorption/production are the main conditions that make up the differential diagnosis. These conditions include gastrointestinal bleeding, renal disease, urinary tract infection with a urease-producing organism (e.g.,

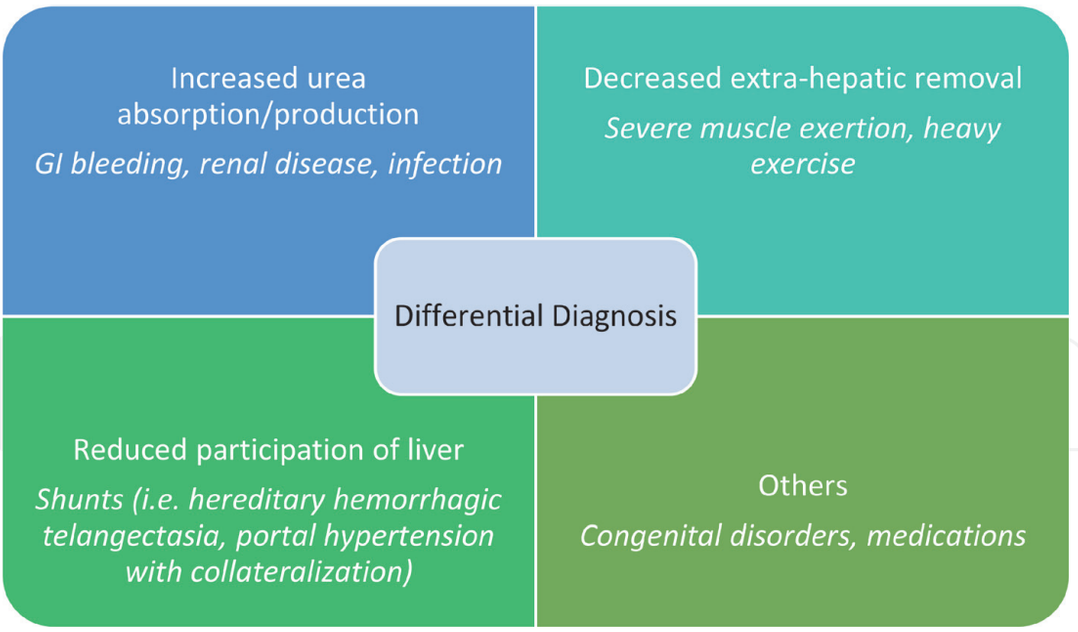

Table 1.
Differential diagnosis for elevated ammonia levels.

Proteus mirabilis), ureterosigmoidostomy, parenteral nutrition, high-dose chemotherapy, and systemic *Mycoplasma hominis* or *Ureasplasma* spp. infection in lung transplant recipients.

Within the conditions that decrease extrahepatic removal of ammonia, diseases affecting the muscles such as severe muscle exertion/heavy exercise are worth noting.

Reduced participation of liver in the removal of ammonia may occur in any cause of portosystemic shunting of blood, such as in hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) and portal hypertension with collateral formation.

Two other groups of conditions are considered controversial in their role in the development of hyperammonemia: congenital disorders (certain inborn errors of metabolism such as urea cycle defects and organic acidemia) and medication induced (valproic acid, barbiturates, narcotics, diuretics, alcohol, and salicylate-Reye syndrome). Some authors classify both as a cause for hyperammonemia while others would englobe in subgroups of liver diseases as they are believed to have similar pathophysiology [19, 20].

7. Overview on treatments

The treatment HE resonates around decreasing ammonia. It can be achieved through three major mechanisms: decreasing ammoniagenic substrates, inhibiting ammonia production, and metabolic removal of ammonia (**Table 2**).

7.1 Decreasing ammoniagenic substrates: enemas

Enemas are the main treatment of this category. These are administered to patients at increased risk of aspiration. Different agents have been used, including

Decreasing ammoniagenic substrates	• Enemas
Inhibiting ammonia production	• Antibiotics • Laxatives • Modification of flora
Metabolic removal of ammonia	• Ornithine-aspartate/Zinc • Sodium Benzoate • Dialysis

Table 2.
Mechanisms used in treatment of hyperammonemia.

tap water, milk/molasses, and lactulose. The efficacy of enema administration has not been evaluated [19].

7.2 Inhibiting ammonia production: antibiotics (neomycin, paromomycin, metronidazole, rifaximin, and vancomycin), laxatives (disaccharides-lactulose/lactitol, polyethylene glycol), and modification of flora (Lactobacillus SF68, acarbose)

The use of laxatives, especially non-absorbable disaccharides, has been the cornerstone of the treatment HE. Oral lactulose or lactitol (the latter is not available in the United States) are thought to have an *in vitro* benefit over other laxatives. This is due their multi-mechanistic properties. Not only do they cause catharsis but they convert ammonia to ammonium and also reduce intestinal pH, thereby reducing ammonia absorption. These agents improve symptoms in patients with acute and chronic encephalopathy when compared with placebo but do not improve psychometric test performance or mortality. Side effects are common and include abdominal cramping, bloating, flatulence, and electrolyte imbalance.

Oral antibiotics have been used with the aim of modifying the intestinal flora and lowering stool pH to enhance the excretion of ammonia. Antibiotics are generally used as second-line agents after lactulose or in patients who are intolerant of non-absorbable disaccharides. Rifaximin given orally in a dose of 550 mg twice daily was approved in 2010 for the treatment of chronic hepatic encephalopathy and reduction in the risk of recurrence of overt encephalopathy in patients with advanced liver disease. The tolerability and side-effect profile of rifaximin are superior to those of lactulose, albeit at greater financial cost. Other antibiotics, including neomycin, paromomycin, metronidazole, and vancomycin, have been studied in small trials and case series, but some may have an increased side effect profile and the effectiveness of others are not well established.

Agents that may modify intestinal flora and modulate the generation or intestinal absorption of ammonia have been evaluated as potential treatments. Acarbose, an intestinal α-glucosidase inhibitor used to treat type 2 diabetes mellitus, inhibits

the intestinal absorption of carbohydrates and glucose and results in their enhanced delivery to the colon. As a result, the ratio of saccharolytic to proteolytic bacterial flora is increased and blood ammonia levels are decreased. A randomized controlled double-blind crossover trial has demonstrated that acarbose improves mild hepatic encephalopathy in patients with cirrhosis and adult-onset diabetes mellitus. Similarly, probiotic regimens (such as *Lactobacillus* SF68) have been used to modify intestinal flora and diminish ammonia generation. Several studies have suggested that these agents may be beneficial in humans with mild encephalopathy. A Cochrane Database review in 2011 was unable to conclude that probiotics improve clinically relevant outcomes [19].

7.3 Metabolic removal of ammonia: ornithine-aspartate (ornithine-transcarbamylase/zinc), sodium benzoate (phenylbutyrate, phenylacetate), and dialysis

Sodium benzoate, sodium phenylbutyrate, and sodium phenylacetate, all of which increase ammonia excretion in urine, are approved by the FDA for the treatment of hyperammonemia resulting from urea cycle enzyme defects and may improve HE in patients with cirrhosis. Administration of sodium benzoate, however, results in a high sodium load, and the efficacy of this agent is not clearly established [21].

Administration of zinc, which has been used because zinc deficiency is common in patients with cirrhosis. Furthermore, because it increases the activity of ornithine transcarbamylase, an enzyme in the urea cycle, it may also improve HE; however, clear efficacy has not been established. L-ornithine–l-aspartate (LOLA), a salt of the amino acids ornithine and aspartic acid that activates the urea cycle and enhances ammonia clearance, has been shown in several randomized controlled studies to improve HE compared with lactulose; however, this agent is not available in the United States.

Extracorporeal albumin dialysis using the molecular adsorbent recirculating system (MARS) has resulted in a reduction in blood ammonia levels and improvement in severe encephalopathy in patients with acute-on-chronic liver failure. Further studies are needed to clarify whether albumin dialysis has a role in treatment of HE [19].

7.4 Treatments on the horizon

Fecal microbiota transplant is being studied prospectively in a few centers in North America. As an established treatment in *C. difficile* colitis, this treatment aims to modify the intestinal flora, as it happens with use of antibiotics, such as rifaximin.

Studies are currently underway comparing different formulations of rifaximin, evaluating the difference between the immediate release against the sustained extended release.

Other antibiotics, cheaper and with safer profiles are being studied prospectively to compare with the current gold standard, rifaximin. One such antibiotic notably is nitazoxanide.

Data regarding dialysis as a treatment modality has not been satisfactory in order to justify its regular use in the setting of HE. There are prospective studies evaluating other exchange therapies such as plasmapheresis as viable alternative treatment options especially in the setting of refractory HE.

AST-120, an oral spherical carbonaceous adsorbent approved and used in chronic kidney disease to decrease uremia by decreasing intestinal indole

absorption and consequently indoxyl sulfate production [23] has been extrapolated to HE with promising results, but still in initial phases and further studies are needed to better characterize its role in the treatment of HE.

8. Conclusions

Our understanding of the interactive physiology between ammonia and HE has greatly increased since its first proposition by Hippocrates of Kos B.C. and its first description in 1860 by von Frerichs [22]. There are multiple effective treatments available and yet others in the horizon. However, there is still much more to be understood about the role of ammonia in HE and other factors may still be involved in the pathophysiology of portosystemic encephalopathy. The future of HE appears bright and future treatment options will hopefully improve the quality of life of patients with this potentially debilitating disease.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

BCAAs	branched-chain amino acids
BC	before Christ
EDTA	ethylenediaminetetraacetic acid
FMT	fecal microbiota transplant
HE	hepatic encephalopathy
HHT	hereditary hemorrhagic telangiectasia
LOLA	l-ornithine–l-aspartate
MARS	molecular adsorbent recirculating system
pNH3	partial pressure of gaseous ammonia
PSE	portosystemic encephalopathy

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