Progress in pathogenesis and treatment of type A hepatic encephalopathy in acute liver failure: a comprehensive review

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Author contributions
The study was conceived by Yan Liang and Guang-Ji Wang, while the review was designed by Ye-Xin Xu, He Wang, Kang-Rui Hu, Guang-Ji Wang, and Yan Liang. Literature collection was conducted by Ye-Xin Xu, He Wang, and Kang-Rui Hu. The manuscript was drafted by Ye-Xin Xu, He Wang, and Kang-Rui Hu, with figures drawn by He Wang. Language polishing was performed by Yan Liang, Guang-Ji Wang, Kang-Rui Hu, Bo-Yu Shen, and Lin Xie.

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Abbreviations
AHE, type A hepatic encephalopathy; ALF, acute liver failure; CNS, central nervous system; ICP, intracranial pressure; ROS, reactive oxygen species; TJ, tight junction; BBB, blood-brain barrier; MPT, mitochondrial permeability transition; DON, 6-diazo-5-oxo-L-norleucine; SIRS, systemic inflammatory response syndrome; RHCG, rhesus C glycoprotein; AQ14, Aquaporin 4; GABA, gamma-aminobutyric acid; KA, kainic acid; GS, glutamine synthetase; LDH, lactate dehydrogenase; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; NF-κB, nuclear factor-kappa B; MMP-9, matrix metalloproteinases-9.

Citation

Abstract
Hepatic encephalopathy is a serious neuropsychiatric complication caused by liver failure, which is characterized by the development of cognitive and motor disorders into coma. Typically, hepatic encephalopathy can be divided into three types (A, B, and C) according to the etiology. Type A hepatic encephalopathy (AHE) caused by acute liver failure seriously affects the prognosis of patients, ranging from mild neuropsychological changes to coma, brain edema, and even death. So far, the research on the pathogenesis of AHE has focused on the toxic effects of ammonia on the central nervous system, metabolic disorders (glutamine and lactate accumulation), neurotransmission alteration, systemic inflammation, especially neuro-inflammation. All these mechanisms are not independent, but mutually have synergistic effects. In clinic, treatment of AHE based on only one mechanism is often ineffective. To clarify the pathogenesis and the interaction among the mechanisms will be beneficial to the effective treatment of AHE and reduce the mortality. The aim of this review is to provide comprehensive scientific evidence for the clinical treatment of AHE via collecting and analyzing the latest mechanism of AHE, and clarifying the relationship among these mechanisms combing the investigation of the latest research progress of drug treatment of acute liver failure. Consequently, we find that the pathogenesis of AHE is a complex neurocognitive disorder shaped by interactions among hyperammonemia, inflammation, and changes in neurotransmission, the signaling pathways thereby integrating the inflammatory and neurological inputs to impact pathophysiological or neurobehavioral outcomes.

Keywords: type A hepatic encephalopathy; ammonia; glutamine; lactate, inflammation, blood-brain barrier, neurotransmission
Background

Type A hepatic encephalopathy (AHE) is a serious disease caused by acute liver failure (ALF), with a mortality rate of 80% [1]. Clinically, ALF is usually characterized by central nervous system (CNS) dysfunction including encephalopathy, epilepsy, and brain edema [2, 3]. Brain edema is the main cause of intracranial hypertension (ICP) and herniation, and also the cause of death in patients with ALF [4]. Most of the patients in AHE are progressing very rapidly and liver transplantation might not be a promising treatment [5, 6]. Therefore, Rational and effective approaches for the prevention and treatment of AHE are urgently required. With a great quantity of evidence and animal data being accumulated, ammonia has been confirmed to play an important role in the occurrence and development of AHE [3, 7, 8]. Studies on the history of ALF have shown that elevated arterial and brain ammonia concentration are two positive predictors of the severity of CNS complications in patients with ALF syndrome. Therefore, reducing ammonia has always been the main strategy for the treatment of AHE, but the clinical effect is not ideal [9]. To seek a better therapeutic mode, more and more studies support the view that patients with AHE are in an inflammatory state, and determining the severity of the inflammatory response is a key issue in this field [6, 10]. Moreover, the destruction of blood-brain barrier (BBB), metabolic disorder, and altered neurotransmission have been reported to be involved in the occurrence and deterioration [9]. Some researchers put forward that these changes act independently but it is worth noting that new research shows that it is almost impossible to ignore the interaction of events occurring in the process of AHE occurrence and development [10]. Despite decades of research, our understanding of the pathogenesis of brain edema and AHE in ALF patients is still incomplete and deficient to achieve effective treatment. The review collects the latest and most authoritative articles from different databases like PubMed, ScienceDirect, Web of Science and ResearchGate using relevant keywords in search engines, and analyzes the updated mechanisms and the interaction of various factors that lead to AHE. Novel therapeutic opportunities provided by current research in this area are also presented to further provide comprehensive scientific evidence for the clinical treatment of AHE.

Results

Toxic effects of ammonia on CNS

Direct brain damage caused by hyperammonemia. Ammonia is the intermediate product of normal physiological metabolism, which mainly comes from gastrointestinal tract and skeletal muscle. The liver is the main organ to metabolize ammonia by converting ammonia into urea in hepatocytes around portal vein and glutamine in hepatocytes near the central vein.

In addition, the kidney can also degrade the circulating ammonia in the body, and cooperate with the liver to maintain low concentration of ammonia in human blood. Therefore, ALF patients often have renal failure, which leads to ammonia metabolism disorder. During acute kidney injury, the brain and kidney might interact through the amplification of cytokine-induced damage, extravasation of leukocytes, oxidative stress, and dysregulation of sodium, potassium, and water channels, leading to neurological disorders such as cerebrovascular disease, cognitive impairment, and neuropathy [11].

More than 90% of ALF patients suffer from hyperammonemia [12, 13]. It is suggested that the impaired ability of detoxifying circulating ammonia is the main cause of hyperammonemia [14]. High ammonia in the body can enter the brain through the BBB [13]. Recently, new scholars have proposed a new ammonia transport theory. In addition to simple diffusion, there are also passive patterns of ammonia related to the Rhesus glycoprotein family. Rhesus C glycoprotein (RhCG) is the main protein involved in brain ammonia transport and its expression in astrocytes increases most. When the disease gets worse, RhCG is significantly up-regulated, indicating that more ammonia enters the brain, and RhCG may be a potential therapeutic target of AHE treatment [3]. Ammonia promotes glycosylation by stimulating phosphofructokinase, a critical glycolytic enzyme [16]. Furthermore, ammonia has been demonstrated to inhibit alpha-ketoglutarate dehydrogenase which acts as a rate-limiting enzyme involved in the tricarboxylic acid cycle [17, 18]. Recently, a new pathophysiological hypothesis has been proposed, emphasizing the inhibition of ammonia consumption with α-KG to form glutamate (the first step of ammonia detoxification) in the self-defense system. It should be added that ammonia can also affect the transamination of other amino acids rather than from free ammonia in this detoxification process. The next step is to produce glutamine under the catalysis of glutamine synthetase (GS) to detoxify ammonia, which mainly occurs in astrocytes. In patients with ALF, some amino acids are accumulated in the plasma, but the concentration of isoleucine is low, which is enough to show the general depletion of isoleucine [19]. In this situation, the consumption of isoleucine, which is involved in transamination, can promote glutamate production from α-KG [20]. It has been well established that ALF and following hyperammonemia promote the transamination of branched-chain amino acids and the energy metabolism of isoleucine, valine, and leucine [18, 21].

There are several mechanisms related to CNS dysfunction caused by hyperammonemia in patients with ALF. First of all, hyperammonemia has adverse effects on the function of astrocyte. It has been reported that the increase of intracellular ammonia concentration will lead to the change of protein expression in key astrocyte, including glial fibrillary acidic proteins, transporters of glutamate and glycine, and peripheral benzodiazepine receptors [22]. These events not only affect the volume of astrocytes, but also stimulate or inhibit the levels of extracellular substances. Furthermore, acute ammonia exposure can activate NMDA receptors and related signal transduction pathways. Acute ammonia toxicity also leads to the increase of Ca²⁺ levels in mitochondria, which may cause serious damage on the function of vital enzymes and induce the production of superoxide radicals [23]. Furthermore, ROS is followed by the imbalance of oxidation-reduction, which leads to the increase of reactive oxygen species (ROS) generation and the decrease of several kinds of antioxidant enzymes, including thioredoxin peroxidase, an enzyme that helps to eliminate excessive free radicals [24, 25]. All these findings indeed indicate the poor neurological condition in patients with AHE. In the late stage of AHE, cerebral blood flow was also generally increased due to the accumulation of ammonia, especially the accumulation of ammonia metabolites.

The effect of hyperammonemia on the brain and its mechanism depends on several factors, including the ammonia concentration, the rate of increase in ammonia content, the duration of hyperammonemia and the presence of inflammation [26]. Therefore, a single determination of blood ammonia may be of no significance. In individual patients, the fluctuation of plasma ammonia is usually closely related to the change of neuropathological state, so continuous measurement of blood ammonia is necessary in some cases [27].

Metabolite of ammonia-glutamine. It is generally acknowledged that the most obvious characteristics of ALF are the increased ammonia concentration and the increased detoxification product, glutamine, of ammonia. Under normal physiological conditions, peripheral blood ammonia is converted into urea in the liver. But after liver damage, ammonia metabolism is associated with GS, which is mainly distributed in astrocytes and a few in skeletal muscle [26]. Thus, it is reasonable to take both astrocytes and skeletal muscle into consideration when studying individuals’ ability of detoxifying ammonia. On one hand, it is generally believed that the transformation of ammonia into glutamine in astrocytes can effectively protect the brain from blood-derived ammonia. On the other hand, it has been questioned whether the accumulation of glutamine, as a permeable substance that can produce osmotic gradient, is harmful to astrocytes [29, 30]. In addition, some studies have shown that glutamine is a kind of strong penetrant, and the glutamine concentration will lead to the increase of osmotic pressure, which will lead to the swelling of astrocytes in patients with ALF, and then lead to brain edema [14]. The intracranial hypertension caused by severe...
cerebral edema is also a worrisome complication and the leading cause of death in patients worldwide with ALF. In contrast, Zwingman found that there was no close correlation between glutamine levels and astrocyte swelling in ALF; Chatauret and Zielinska found that several drugs reduced ammonia-induced swelling in brain slices, but barely changed glutamine levels [31–34]. Consequently, whether astrocyte edema of AHE is caused by the osmotic pressure of glutamine is still in question [29].

In a pioneering study conducted in the early 1960s, Warren and Schenker effectively protected mice from acute ammonia toxicity, lowered seizure threshold, and prevented cerebral edema by using GS inhibitor methionine sulfide [35]. It is apparent to conclude that the over-synthesis of glutamine is indeed a fatal factor in mice with AHE. Subsequently, Albrecht proposed the “Trojan horse” hypothesis to explain the toxic effect of glutamine on astrocytes (Figure 1) [29]. This hypothesis suggests that the excess glutamine produced by astrocytes in hyperammonemia enters the mitochondrial matrix and decomposes into ammonia by phosphate-activated glutaminase, which then induces the generation of reactive oxygen species (ROS) in the mitochondrial and causes cellular dysfunction. In addition, ROS is also involved in the nitrification of tyrosine residues of intracellular proteins. The free 3-nitrotyrosine generated after nitrification of proteins results in the decrease of membrane potential of mitochondrial in vascular endothelial cells, the decomposition of tight junction (TJ) proteins which further leads to BBB dysfunction, and ultimately the swelling of astrocytes and brain edema. It seems that there is a vicious circle that oxidative stress promotes the swelling of astrocytes, which in turn promotes the pre-existing oxidative stress. In addition to oxidative stress, mitochondrial permeability transition (MPT) is an indicator of mitochondrial membrane integrity and a critical factor in astrocyte swelling. This process is characterized by the opening of permeability transition pores in the inner-mitochondrial membrane, usually in response to increased levels of mitochondrial calcium ions. It further leads to mitochondrial dilating, mitochondrial dysfunction and energy failure [36,37].

The role of glutamine during MPT was originally recorded in isolated mitochondria, indicating significant morphological changes in the existence of glutamine [38]. In the co-culture of ammonia and astrocytes, 6-diazo-5-oxo-L-norleucine (DON), an inhibitor of phosphate-activated glutaminase, can prevent the transport of glutamine across cell membrane and significantly inhibit the cell swelling induced by ammonia. Rao et al. also observed the inhibition of DON on oxidative stress and MPT by culturing astrocytes pretreated with glutamine at a certain concentration [39]. These findings indicate a hypothesis that DON can inhibit the production of free radicals and prevent the mitochondrial permeability from becoming poor by hindering the hydrolysis of glutamine and reducing the production of mitochondrial ammonia. It is generally believed that glutamine enters the mitochondria through carriers, which can be enhanced by ammonia and are sensitive to histidine. Histidine inhibits the transport of glutamine into mitochondria, markedly suppresses the development of MPT and protects mitochondria from excessive glutamine [40,41]. After the administration of histidine, similar findings of attenuation of MPT and less production of ROS have been reported in rats with thioacetamide-induced ALF [42]. The possible explanation for these findings may be that glutamine does not act as a penetrating agent, but a series of downstream reactions leading to cell edema.

In an in vivo study using microdialysis, extracellular glutamine concentration in the cortex of comatose ALF rats in coma increased almost 5-fold. A recent study found that the expression and activity of GS in the skeletal muscle increased while that in the cerebral cortex decreased [43]. Bosman et al. found that GS activity continued to increase in patients with AHE during coma, while glutamine synthesis in the brain did decrease [44]. One possible explanation for the suppressed synthase activity is the occurrence of protein tyrosine nitrification, which inactivates the enzyme. GS in the brain is uniquely located in astrocytes. When the cultured astrocytes were exposed to the toxic concentration of ammonia, the tyrosine residue on GS was nitrated obviously, which suggests a loss of GS activity. These findings emphasize again that the mechanism of brain protection remains to be further studied.

More recently, another mechanism to explain the high concentration of glutamine has been proposed: the accumulation of glutamine in the brain is significantly due to the decrease of glutamine secretion rather than the increase of synthesis [45]. Thus, the “glutamate-glutamine cycle” is involved in a new mechanism: glutamine produced by GS is released from astrocytes into extracellular space through glutamine transporters and some of the free glutamine is taken up by peripheral neurons via glutamate transporters SNAT1 and SNAT2 (Figure 2). Glutamine in neurons is then converted into glutamate, the major excitatory neurotransmitter. Glutamate is then released into the synaptic space to stimulate glutamate binding to postsynaptic receptors. EAAT-2, a high-affinity glutamate transporter, removes excess glutamate from the synapse to astrocytes. Subsequently, glutamate functions as a substrate of GS and

![Diagram](https://www.tmrjournals.com/pd)

**Figure 1** The “Trojan horse” hypothesis in the astrocyte. The excessive glutamine produced in astrocytes when circulating ammonia accumulates is transported into the mitochondrial matrix and then decomposed into ammonia by PAG, inducing the generation of ROS in the mitochondrial and causing cellular dysfunction. ROS, reactive oxygen species; PAG, Pregnancy-associated glycoproteins; MPT, mitochondrial permeability transition; GS, glutamine synthetase.
continues to circulate. The studies using molecular techniques have shown a reduced expression of glutamate transporter EAAT-2 in astrocytes of animals with hepatic devascularization. Preliminary evidence suggests that selective deletion of SNAT5 expression in the brain is found in the animals with ALF. Due to the loss of SNAT5 expression, glutamine is trapped inside astrocytes, which reduces excretion rather than increases synthesis, leading to glutamine accumulation and subsequent astrocyte swelling. Thus, it is necessary to keep in mind that glutamine not only functions as an osmotic substance in the brain but also closely participates in cellular interactions. It is the key to neurotransmitter regulation.

**Lactate accumulation in the brain.** Ammonia is undoubtedly considered as the culprit of AHE. However, other toxic substances such as lactate and neurosteroids, whose concentration has been detected to be elevated in ALF patients or animals, have also been put forward as agents with the harmfulness of triggering the transformation of microglia from a resting state to an active state, leading to CNS disorder in liver damage [46, 47]. It has been observed that concentrations of cerebral lactate increased in multiple animal models, such as hepatic devascularization, hepatectomy, and acetaminophen-induced liver injury, etc. The increase of lactate concentration in the brain is a common event occurring in ALF, which has been confirmed to be correlated with the mechanism of cerebral edema [48]. The evidence comes from reports about astrocytes swelling when exposed to a pathophysiological concentration of lactate [49]. The cultured glial cells exposed to lactate in the range of 10–20 mmol, which was equivalent to actual concentration of lactate in the brain of animals with ALF, and were stimulated to release inflammatory factors including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) [10]. In other studies, there was a significant correlation between the accumulation of cerebral lactate and the severity of AHE [50, 51]. Moreover, the increased synthesis of cerebral lactate detected by 13C nuclear magnetic resonance (NMR) could predict the grade of AHE and the existence of brain edema caused by ALF [32]. Chavarría et al. used magnetic resonance technology to demonstrate that in rats with AHE, with the significantly elevated concentrations of cerebral lactate concentration, the rats entered coma stage [52]. Therefore, hyperlactacidemia and levels of serum lactate observed in ALF patients have been identified as prognostic indexes for both acetaminophen-induced and non-acetaminophen-induced ALF [53–55].

In the normal state, circulating lactate is metabolized into glucose in the liver. Impaired liver function will reduce the elimination of lactic acid and prolong its half-life. As a result, the level of serum lactic acid increases with the excretion of intracellular substances including lactate from dead hepatocytes [56]. It is uncertain whether the alteration of cerebral lactate is the cause or the result to date. There are several possible mechanisms related to the increase of lactate: 1) Lactate derived from circulating blood crosses the BBB; 2) Energy failure in the brain results in more glycolysis, and glucose produces lactate; 3) Increased release or decreased uptake of lactate. It is generally suggested that the increase of lactate means an energy deficit caused by an imbalance between energy supply and demand. As mentioned above, ammonia stimulates the demand for ATP, reduces the supply of ATP by affecting the production of lactate, and breaks the balance in cerebral energy metabolism.

As a sign of energy impairment, lactate is also an energy source for neurons [57]. Lactate is metabolized specifically by lactate dehydrogenase (LDH), which is called LDH1 in neurons and LDH5 in astrocytes. Previous studies have shown that LDH1 has a higher affinity for lactate than LDH5 [58]. Therefore, after being activated by glutamate, glucose transportation and utilization in astrocytes increased, while in neurons decreased, although it increased with the increase of energy demand [59]. One possible mechanism of the increase in lactate may be the coupling between synaptic activity of neurons and energy metabolism in astrocytes [60]. Glutamate released by neurons stimulates astrocytes, which are responsible for the synthesis and release of lactate. Then, neurons absorb lactate through transporters and convert it to pyruvate, which enters into the tricarboxylic acid cycle, via lactate dehydrogenase. Therefore, in ALF animals with grade III or IV AHE, an extensive number of glutamates are released into extracellular space, which is sensed by astrocytes and thus leads to an increase in lactate formation.

**The liver-brain axis in liver failure**

**Systemic inflammation in ALF.** The disorder of ammonia metabolism is not enough to represent the root cause of all neurological abnormalities in AHE. Emerging evidence suggests that elevated arterial blood ammonia levels are synergistic with systemic inflammatory response syndrome (SIRS) commonly in AHE patients [61]. Meanwhile, there is also evidence to suggest that hyperammonemia can induce the occurrence of peripheral inflammation and is sufficient to induce microglial cell activation and neuroinflammation, thereby mediating many of its effects on the brain, including altering neurotransmission and leading to cognitive impairment [46]. According to reports, the mortality of AHE is only 23%, whereas 63% of clinical cases have SIRS markers. Additionally, two or more SIRS criteria are usually characterized by higher ICP and

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Figure 2 The new mechanism: “glutamate-glutamine cycle”. Glutamine produced by GS is released from astrocytes into extracellular space via glutamine transporters SNAT-5 and some of the free glutamine is taken up by surrounding neurons via glutamine transporters SNAT1. Glutamine is then converted into glutamate inside the neurons. Release of glutamate into the synaptic space ensues, stimulating combination with postsynaptic receptors and excess glutamate is removed from the synapse into the astrocytes by the glutamate transporter EAAT-1 and EAAT-2. Then glutamate functions as a substrate for GS and the cycle continues. GS, glutamine synthetase.
higher mortality. It has traditionally been considered that acute liver injury is followed by an inflammatory response and raised concentrations of several pro-inflammatory cytokines, such as TNF-α, IL-6, cxcl2, and interleukin-1 beta (IL-1β), especially in patients with elevated ICP [9, 62-64]. The accumulation of these pro-inflammatory cytokines in the peripheral circulation may be caused by the secretion of the liver [65].

Neuroinflammation in acute liver failure. The two-hit hypothesis has been proposed to explain the mechanism of AHE, i.e. the initial event of AHE is the increase of ammonia level in the artery, which results in astrocyte swelling, and lays a foundation for the deterioration of ICP by increasing cerebral blood flow in the concurrent inflammatory state. In the presence of inflammation, hyperammonemia can aggravate the induction of AHE, and it can also enhance the sensitivity of the body to the immune response [46].

There is a causal relationship between induction of neuroinflammation and impaired cognitive and motor function when AHE occurs. Despite hyperammonemia, measure targeting inhibition of neuroinflammatory response can correct cognitive impairment and motor deficit in patients with ALF, even though with hyperammonemia. There are several conjectures about how peripheral inflammation evolves into neuroinflammation: 1) Although cytokines cannot directly pass through the BBB, they can prompt the release of various inflammatory mediators in the brain by activating endothelial cells, further stimulate microglia and astrocytes to express an extensive number of precursor inflammatory factor, leading to neuroinflammation response and impairment; 2) Infiltration of monocytes or lymphocytes in the brain; 3) Cytokines can act directly on brain parenchyma through active transport or entry into brain regions due to lack of an intact BBB [66]. A traditional view suggests that systemic inflammatory signals often lead to increased permeability of BBB to cytokines in patients with liver disease [67].

A major feature of neuroinflammation is the activation of glial cells, including microglia and astrocytes [68]. The activation of microglia occurred in the early stage of ALF and further increased with the onset of encephalopathy and cerebral edema. In particular, there is evidence that the degree of microglial activation could predict the severity of brain edema and AHE in patients with ALF [69]. In different ALF models, the mediators involved in neuroinflammation are primarily released by microglia. Therefore, the morphology of microglia plays an important role in the development of AHE and cerebral edema [70]. Recently, in an animal model of ALF induced by azoxymethane, McMillin M found that the increased concentrations of TGFB-β1 in circulation after fulminant hepatic failure causes microglial activation [71]. As mentioned earlier, inflammatory mediators trigger or aggravate encephalopathy by changing the permeability of endothelial cells to neurotoxin in the brain. Cytokines TNF-α, IL-6, IL-1β could not only enhance the permeability of cerebral vascular endothelial cells to ammonia but also the permeability of BBB, which exactly explains the disproportionate increase of cerebral ammonia in experimental animals with ALF [70]. Regardless of etiology, levels of TNF-α in peripheral blood in patients with ALF are associated with the severity of AHE. Earlier studies have shown that the expression of TNF-α in the brain leads to cognitive dysfunction, while non-steroidal anti-inflammatory drugs (NSAID) such as ibuprofen have been proved to restore the motor and cognitive function of rats with AHE. Interestingly, less brain edema is observed in transgenic mice lacking TNF-α, IL-1α, IL-1β, and IL-6 receptors when compared with wild-type mice with ALF. Some studies have proved a possible direction in the animal model of ALF by using a new TNF-α targeting aptamer which might prevent cerebral injury based on the treatment of TNF-α [63]. It is suggested that the increased expression of proinflammatory cytokines gene encoding TNF-α and IL-1β found in experimental animals with ALF is due to ischemic or toxic liver damage and the influence of TNF-α and IL-1β gene deletion in cerebral edema [72]. The important role of neuroinflammation in the pathogenesis of ALF syndrome cannot be overemphasized. A study of cultured astrocytes has shown that pathophysiological concentration of ammonia leads to the activation of the transcription factor nuclear factor-kappa B (NF-kB), and the inhibition of this activation leads to an apparent morphological change of astrocytes. It is well known that NF-kB can induce the expression of certain enzymes, including inducible nitric oxide synthase (iNOS) and NADPH oxidase (NOX), whose products are known to cause astrocyte swelling. In addition, NF-kB has been shown to activate the phospholipase A2 (pla2) and COX-2 genes, and its products arachidonic acid and prostaglandin E2 can both cause astrocyte swelling.

BBB permeability. The BBB is composed of cerebral capillary endothelial cells, TJ proteins, astrocytes, outer membrane cells, perivascular microglia, and basal membrane. The endothelial cells of cerebral capillaries are the basic skeleton of the BBB, and the TJ proteins play the most important role in the BBB. Once BBB function is destroyed, it will lead to brain edema and herniation.

In the past 40 years, the notion of BBB destruction in ALF has been the subject of debate. In vitro and in vivo studies have been shown that exposure to pro-inflammatory cytokines can increase BBB permeability [70, 73]. These results suggest that the proinflammatory status characterized by ALF results in the destruction of BBB permeability and provides a potential liver-brain signaling pathway. Earlier studies have provided evidence of BBB damage in animals with hepatectomy or galactosamine-induced toxic liver failure. However, other findings of subsequent electron microscopy studies in the brain from experimental animals or patients with ALF were not consistent with earlier studies. These studies suggest that the brain edema of AHE is characterized by cytotoxicity caused by cell swelling rather than vascular damage caused by loss of BBB integrity [73, 74]. Nitta et al. found that a slight alteration in BBB permeability in the state of AHE, which allowed small molecular substances, such as water and ammonia, to pass through the membrane without causing abnormal structural destruction to BBB [75]. Moreover, Nguyen et al. also found that the angiogenesis mechanism involved in brain edema in ALF patients was different from the regulatory mechanism of encephalameda in cerebral ischemia and traumatic brain injury [76]. In other words, brain edema is usually associated with increased permeability of the BBB, and subtle changes in the function of BBB play an important role in AHE.

The issue of BBB integrity in ALF was taken to another angle following a report in 2011: brain injury in ALF includes the initial disruption of BBB permeability caused by vasogenic edema in certain areas, such as the cerebellum, rather than the frontal cortex [60]. In the early stage of ALF, edema associated with increased ICP is primarily vascular, suggesting that cell swelling is not the initial trigger of edema and ICP. Due to the gradual increase of cerebral ammonia and glutamine levels, astrocyte swelling may cause cytotoxic edema in many areas, which continuously affects intracranial pressure. In the late stages, NMDA receptors are eventually activated and lactate accumulates to a toxic concentration, further increasing ICP and leading to death. In summary, there are different manifestations of edema in different periods of ALF.

A significant change in BBB permeability was once reported to be the event of terminal AHE in AOM-induced ALF mice [60, 77]. On the contrary, researchers did not find an alteration of BBB permeability in mice administered lipopolysaccharide, an agent used to cause microglial activation and increased expression of inflammatory factors. It is suggested that the inflammatory response and other AHE-related factors have a synergistic effect on the unequivocal physical rupture of the BBB [78]. In addition, the proteins related to BBB are also widely concerned. Tavelin et al. observed the extravasation of Evans blue dye across the BBB, which indicated that the leakage of BBB may be the result of slight changes in TJ [79]. Subsequent studies have proven that matrix metalloproteinases-9 (MMP-9) has regulatory effects on the structure of TJ proteins in ALF mice [78]. The specific monoclonal antibody or inhibitor of MMP-9 prevents brain edema caused by ALF and normalizes the brain levels of MMP-9. Thus, these studies demonstrate the role of MMP-9-TJ-BBB pathway in brain edema of ALF.

Other researchers are also in agreement that aquaporin 4 (AQP4) is mainly expressed in astrocytes and ependymal cells, which is closely
related to the occurrence of cerebral edema caused by ALF. Obviously, the increase of AQP4 production is parallel to the swelling process of astrocytes [80]. At the same time, the activity of AQP4 can be decreased due to phosphorylation, which is mediated by protein kinase C and promoted by the activator of protein kinase C. Targeting this pathway may be an effective strategy for preventing deterioration of brain edema.

**Neurotransmission**

**GABAergic neurotransmission.** The neurological alterations in ALF patients include attention deficits, mild cognitive impairment, mental retardation, hand, and visual motor coordination disorders. Neurological and psychiatric alterations in ALF are the consequence of altered neurotransmission, not caused by a single pathway in the body, but multiple neurotransmission systems that coordinate the cognitive and motor functions, in which glutamatergic and GABAergic neurotransmission play a major role in different brain regions and neural circuits [81]. Gamma-aminobutyric acid (GABA), as the main cerebral inhibitory neurotransmitter, is responsible for hepatic coma in ALF patients. Its neuronal activity pattern is similar to that induced by benzodiazepines, barbiturates, and other-receptor agonists [82].

In recent years, increased levels of GABA have been found in animal models of ALF, which are associated with the severity of AHE. Increased GABAergic tone in the cerebellum leads to cognitive dysfunctions in hyperammonemia rats [61]. The possible mechanisms of GABAergic upregulation are: 1) the increased expression of GABA in the brain; 2) the increase of GABA receptors; 3) the elevated levels of endogenous benzodiazepine-like compounds with the potential of activating GABAa receptors in the brain; 4) the increased concentration of neurosteroids, which may also induce GABAa receptor activation in the brain and 5) the enhancement of GABA receptor activation directly by ammonia [83].

Neurogenic edema is often observed in ALF patients. It is postulated that the intestinal GABA would pass through the BBB with abnormally high permeability, thus inducing its special receptor in the brain. Binding to these receptors promotes the increase of the number and sensitivity of postsynaptic GABA receptors, which further leads to a significant inhibitory effect. It is followed by increased concentrations of neurosteroids, such as pregnenolone and isopregnenolone, which enhanced the activation of GABAa receptors. Neurosteroids are known to modulate GABAa receptors, for example, isopregnenolone tends to reduce the activation of GABAa receptors, while progesterone has the opposite regulatory effect. However, the net effect of different neurosteroids on GABA receptor activation has not been thoroughly studied. The concentrations of both pregnenolone and isopregnenolone are found to be elevated in ALF mice induced by thioacetamide and mice injected with ammonium salt [36, 84]. In cultured astrocytes, ammonia also upregulates the synthesis of pregnenolone. These results suggest that hyperammonemia is responsible for the increase of neurosteroids in patients with AHE. Ammonia can directly promote the combination of GABAa receptors and its ligands. In cortical neurons, ammonia enhances GABA induced Cl current through GABAa receptors [85]. Moreover, a low concentration of ammonia is reported to facilitate the process of binding of agonists and GABAa receptors [86]. Potentiated GABAergic tone involves activation of GABAa receptors and related processes include the glutamate-NO-cGMP pathway, whose inhibition is the main result of alterations of GABAergic tone. In cortical neurons, ammonia enhances GABA-induced Cl current through GABAa receptor.

Although the level of extracellular GABA in most brain area of ALF patients has not changed [38, 87], changes in some regions may result in significant alteration of cerebral functions. A study in portacaval shunt rats revealed that the concentration of extracellular GABA was not influenced in the motor cortex or substantia nigra, but more susceptible in the ventromedial thalamus [88]. It was observed that the increased GABA had a marked effect on motor function in PCS rats, and the normalization of GABA level could restore deterioration of motor function. Another study reported the changes of GABAergic tone in several brain regions of rats with hyperammonemia. It was worth mentioning that GABAergic tone in the cerebellum was enhanced and that of GABAergic in the cortex is decreased. This opposite effect might be explained by the different subtypes and expression of GABA receptors in different regions. Considering the widely accepted view that the effects of GABAa receptors depend on their specific subunits, they will also have a different binding with the same neurosteroids when different GABAa receptors are distributed in disparate brain regions. The composition of GABAa receptors in the cerebellum is mainly characterized by the high level of α-6 subunit, which is expressed exclusively in the cerebellum, and α-1 subunit is rare in the cortex [89]. The increase of α-6 subunits and α-1 subunits induced by ammonia provides a cogent explanation for the opposite effect.

**Glutamatergic neurotransmission.** Net absorption of ammonia has been detected in the brain of ALF patients, and both glutamine and glutamate are produced by the brain. It is well known that glutamine from astrocytes circulates to presynaptic neurons, converts to glutamate, then releases into the synaptic gap, and is re-uptaken into astrocytes. This profound dynamic circulation has been demonstrated to be the basis for the formation of brain edema [45]. Recent studies support the idea that ammonia inhibits the activity of glutaminase in neurons and leads to decreased hydrolysis of glutamine and release of glutamate. Thus, this finding provides compelling evidence about the reduced glutamate detected in the body and also in the brain of patients suffering from ALF [44, 90]. However, glutamate is distributed in different brain regions and the metabolism of glutamate in the mammalian brain is highly compartmentalized. In general, it is distributed between the metabolic and neurotransmitter pools, as well as intra- and extracellular compartments [91]. The decreased total tissue glutamate concentrations do not necessarily mean a defect in glutamate-mediated neurotransmission. Therefore, it seems inappropriate to conclude the relationship between the change of glutamate concentration and the function of neurotransmitter system. Since then, many studies using cerebral microdialysis technology have proved the increase of extracellular glutamate concentration in patients with ALF [38, 92, 93]. According to these studies, the increase in extracellular glutamate concentration is considered to be a crucial factor in the deterioration of neurological function. On the other hand, evidence for glutamatergic synaptic dysregulation in the brain of patients with ALF includes decreased expression of the glutamate transporter GLT-1 in astrocytes, loss of binding sites of AMPA and kainate (KA) receptor ligands [92]. In cultured astrocytes, the expression of glutamate transporter is lost after exposure to ammonia or proinflammatory cytokines, resulting in reduced uptake of glutamate. The NMDA, AMPA, and KA subclases of glutamate receptors are all part of ligand-gated ion channels.

**ASD2** shows the key processes of glutamate metabolism in neurons and astrocytes, including the activation of glutamate receptors on neuronal and astrocytic membranes, which are ligand-gated ion channels (NMDA, AMPA, and KA) and metabolic receptors. A subsequent study provides evidence for the loss of the expression of AMPA/KA receptors in the brain of ALF animals, which indicates a relative priority in NMDA receptor-mediated transmission. The hyperactivation of NMDA receptors provides clues for the activation of glutamate-NO-cGMP pathway, which is associated with excessive formation of NO and cGMP, both of which lead to animal death through excitotoxic mechanism [26]. In vivo data from ALF patients support the effect of increased cGMP due to acute ammonia exposure, which is deemed as an inducement of some more severe neurological symptoms [22]. The activation of NMDA receptors has been proven to increase in animals suffering from acute hyperammonemia, while administration of NMDA receptor antagonist, MK801, to rats with acute ammonia intoxication was found to have a significant protective effect [82, 94-96].

Thus, the question arises: should the elevated level of extracellular glutamate in the brain be deemed as the primary cause of AHE, or just a side effect? After the release of glutamate into the synaptic cleft, the...
joint of glutamate and its receptors may have different effects: compensatory down-regulation or direct toxicity. It seems reasoned that glutamate receptors are down-regulated due to the increased exposure to an abnormal level of glutamate [97]. Some researches have shown a decrease in the number of glutamate receptors in the AHE state, while others have found no obvious change [98, 99]. On the other hand, exposure to more glutamate has adverse effects and there is evidence that the long-term exposure to glutamate is harmful to the brain [100]. Studies in vitro have established that elevation of glutamate concentration is poisonous to both neurons and glial cells [101]. The toxic effect of glutamate is mainly due to the long-term depolarization of glutamate at postsynaptic receptor, which will have a significant effect on the cell membrane permeability and lead to the destruction of ion homeostasis.

Present situation of the treatment for AHE
Due to the rapid development and life-threatening complications of AHE, how to effectively treat it is a challenging problem. Liver transplantation, the most effective treatment at present, is difficult to achieve because of the rapid development of disease. Stated thus, many factors are involved in the development of the disease, but most treatment measures mainly aimed at reducing the accumulation of ammonia and aggravation of inflammation. These measures are conducive to reducing the risk of brain edema and timely treatment will greatly improve the survival rate of patients suffering from AHE [65]. However, the fact is that the therapeutic effect often turns out to be unsatisfactory. Nonspecific drug, such as hypertonic saline and mannitol, can only temporarily relieve brain edema, because they have the effect on the osmotic pressure gradient of astrocytes, rather than hyperammonemia, which mainly stimulates the osmotic pressure gradient [30].

There still remain difficulties to overcome. For instance, animal models can not completely replicate human ALF disease. Although there exist several commonly used animal models of ALF, cases of ALF in patients are sometimes more complex and multifactorial. At the same time, the biochemical criteria used to indicate the presence of AHF in animal models often have little in common with those used in clinical practice. These might lead to the situation that efficacy in animal models cannot be fully realized in the human body. Studies on animals with ALF have shown that cooling can prevent or limit the development of brain swelling. However, in a randomized controlled trial for patients with severe ALF, researchers compare the effects of different temperatures and find that lowering temperature does not help improve survival rate or prevent brain swelling [102]. A large amount of literature has pointed out that L-ornithine-L-aspartate can effectively reduce the level of ammonia and delay the occurrence of hepatic encephalopathy in rats with ALF [103]. However, in a placebo-controlled and blinded clinical study, it is found that L-ornithine-L-aspartate infusion could not improve survival rate or reduce complications and the ammonia level of ALF patients, which may be related to the severely damaged hepatocyte function in ALF [104].

With more combination of animal experiments and clinical practice, there are also some effective measures that have been proven to have a therapeutic effect on AHE. ALF studies in rats and pigs have confirmed the decrease of blood ammonia during L-ornithine phenylacetate infusion, which proves the ability of this compound as an effective ammonia scavenger [105]. In patients with ALF, the significant effect of L-ornithine phenylacetate on lowering ammonia is also determined [30]. In this process, glutamic acid is produced by the transamination of ornithine, and glutamine is produced by the detoxification of ammonia by GS. Excess glutamine combines with phenylacetic acid to form phenylacetylglutamine, which can be excreted with urine.

With the occurrence of ALF, acute renal injury often occurs. The amount of ammonia excretion in the kidney of ALF patients is reduced, which further aggravates the hyperammonemia. Recently, there are clinical studies using renal replacement therapy, as an auxiliary treatment, to improve the hyperammonemia and the survival rate of patients [13]. In conclusion, more translations of work in animal models into clinical practice are needed to ensure that some emerging therapeutic approaches are truly effective for AHE patients. At the same time, it is important to choose species with similar metabolic and physiological characteristics of humans to establish a more practical model.

Discussion
The transition from ALF to intracranial hypertension can be triggered by several events. In particular, the fundamental role for elevated blood ammonia, neuroinflammation, BBB permeability, and metabolic disturbance during AHF development have been identified (Figure 3). Although great progress has been made in revealing the mechanisms of AHE development and progression, there is no effective treatment that can completely reverse the progression of AHE entirely. As discussed in this review, various mechanisms promote the deterioration not only in the liver but also in the brain. Since there are so many triggers that interact with each other, it is necessary to clarify both their interactions and individual effects. Because of the complexity of AHE, a deep understanding of the key factors for disease development is helpful to improve the survival rate and life quality of ALF patients. There are many challenges in the future, for instance, the treatment of patients with different etiologies may need to be classified. What's more, the lack of treatment for other triggers involved in the development of AHE, such as inflammation or metabolic disorder, is a major limiting step in this field. ALF may cause other organ failures at the same time, so it is necessary to prevent complications during treatment. Researchers should fully understand every aspect of AHE, so as to control the occurrence and development of AHE as soon as possible.

Figure 3 Summary of all factors known to contribute to the pathogenesis of hepatic encephalopathy

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Considering the critical role of the interaction between inflammation and neural homeostasis, the potential therapeutic targets including oxidative defense, endoplasmic reticulum stress, and balance of neurotransmitter levels should warrant attention. For instance, some endogenous hydrophilic antioxidants, like glutathione, has been reported to effectively protect cells from various reactive oxygen and nitrogen species [106]. Besides, glycine, an inhibitory neurotransmitter, has been reported to attenuate brain injury-induced neuronal death by regulating microglia polarization and inhibiting NF-κB p65/Hex-1α signal [107]. Thus, these endogenous small molecular metabolites targeting neural inflammation and homeostasis can be the potential therapeutic agents for further research.

References


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