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Review

# Modern view on hepatic encephalopathy: basic terms and concepts of pathogenesis

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**Background.** The problem of acute and chronic forms of hepatic encephalopathy (HE) is not clearly identified among modern problems of hepatology and neuroscience in Ukraine. Despite the significant contribution to the development of lethal complications in patients with liver pathology and long history of the study of this issue, there is still no unified opinion on the links of HE pathogenesis.

**The aim** of this review is to conduct a comprehensive analysis of current data on the spreading and mechanisms of development of HE.

HE is a complex of potentially reversible neurocognitive disorders in patients with chronic or acute hepatic failure (ALF). HE is more often a complication of liver cirrhosis and is the second most frequent cause of hospitalization of such patients after ascites. When decompensating liver failure in acute or chronic hepatic pathology in patients severe forms of HE develop, accompanied by a progressive increase in intracranial pressure and the development of coma, which often ends lethal due to poor corrigibility of intracranial hypertension while maintaining hepatogenic neurointoxication. HE is considered as the end result of the accumulation of a number of neurotoxic substances in the brain, among which are ammonia, mercaptans, short chain fatty acids, false neurotransmitters, gamma-aminobutyric acid, manganese. The most popular among the reasons for the development of HE is the neurotoxic theory of ammonia. Ammonia is subjected to detoxification in the liver, turning into urea, a smaller fraction with the participation of glutamine synthetase is used in the synthesis of glutamine in muscles, liver and astrocytes of brain. In case of hepatic dysfunction and/or portosystemic shunting, the concentration of ammonia in blood increases up to 10 times and the main load for its detoxification is shifted to myocytes and astroglia. In ALF glutamine overload of astrocytes occurs with a change in intracellular osmolarity and subsequent edema of astroglia, which is accompanied by the development of cytotoxic edema of the brain. In this case, in astrocytes damaging of mitochondrial respiratory chain occurs and mitochondrial insufficiency develops, as well as processes of nitrosative-oxidative stress and oxidation of astrocytic and neuronal RNA, disruption of gene expression, synthesis of neuro- and gliotransmitters and synaptic plasticity. The increased influx of aromatic amino acids into brain leads to the synthesis of false neurotransmitters, which worsens serotoninergic, GABA-ergic, dopaminergic and glutamatergic neurotransmission. Damage to the components of the blood-brain barrier leads to aggravation of the water imbalance, penetration of hematogenous cytokines, endotoxins and other products of systemic inflammatory reaction into the cerebral parenchyma and development of neuroinflammation, which makes an important contribution to the further progression of cerebral edema.

**Conclusions:** despite a comprehensive study of the problem, many open questions remain in the pathogenesis of HE. Special attention should be paid to more detailed study of the mechanisms of formation and elimination of edematous changes in brain tissue on the background of hepatogenic intoxication and the development of a systemic inflammatory reaction, the role of astroglia and its water channels in these processes, as well as the mechanisms of damage to the blood-brain barrier.

## Сучасний погляд на печінкову енцефалопатію: основні поняття та концепції патогенезу

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**Ключові слова:** печінкова енцефалопатія, патогенез, аміак.

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Проблема гострої та хронічних форм печінкової енцефалопатії (ПЕ) недостатньо чітко позначена серед сучасних проблем гепатології та нейронауки в Україні. Незважаючи на чималий внесок у розвиток летальних ускладнень у хворих із печінковою патологією та тривалу історію вивчення цього питання, і досі не сформовано єдине уявлення щодо механізмів розвитку ПЕ.

**Мета роботи –** всебічний аналіз сучасних даних щодо поширеності та механізмів розвитку печінкової енцефалопатії.

Печінкова енцефалопатія (ПЕ) – комплекс потенційно оборотних нейрокогнітивних розладів у пацієнтів із хронічною або гострою печінковою недостатністю (ГПечН). ПЕ частіше є ускладненням цирозу печінки та другою найчастішою після асциту причиною госпіталізації таких пацієнтів. При декомпенсації печінкової недостатності за наявності гострої або хронічної печінкової патології у хворих розвиваються важкі форми ПЕ, котрі супроводжуються прогресуючим підвищенням внутрішньочерепного тиску та розвитком коми, котра найчастіше закінчується летально через незначні можливості щодо корегування внутрішньочерепної гіпертензії при збереженні гепатогенної нейроінтоксикації. ПЕ розглядається як кінцевий результат накопичення низки нейротоксичних речовин у мозку, серед них розглядають аміак, меркаптани, коротколанцюгові жирні кислоти, псевдонейротрансмітери, гама-аміномасляну кислоту, марганець. Найбільшу популярність серед причин розвитку ПЕ отримала нейротоксична теорія аміаку. Аміак піддається детоксикації в печінці, перетворюючись на сечовину, менша частка за участю глутамінсинтетази використовується в синтезі глутаміну в м'язах, печінці та астроцитах мозку. У разі печінкової дисфункції та/або портосистемного шунтування концентрація аміаку в крові зростає до 10 разів, й основне навантаження з його детоксикації перекладається на міоцити та астроглію. В умовах ГПечН відбувається глутамінове перевантаження астроцитів зі зміною внутрішньоклітинної осмолярності та дальшим набряком астроглії, що супроводжується розвитком цитотоксичного набряку мозку загалом. При цьому в астроцитах відбувається пошкодження дихального ланцюга мітохондрій, розвиваються мітохондріальна недостатність, процеси нітрозативно-оксидативного

стресу й окислення астроцитарної та нейрональної РНК, порушення експресії генів, синтезу нейро- та гліотрансмітерів, синаптопластичності. Підвищене надходження в мозок ароматичних амінокислот призводить до синтезу псевдонейротрансмітерів, що погіршує серотонінергічну, ГАМК-ергічну, дофамінергічну та глутаматергічну нейротрансмісію. Пошкодження компонентів гематоенцефалічного бар'єра призводить до збільшення водного дисбалансу, проникнення в мозкову паренхіму гематогенних цитокінів, ендотоксинів та інших продуктів системної запальної реакції та розвитку в мозку процесів нейрозапалення, що робить свій важливий внесок у дальше прогресування набряку мозкової тканини.

**Висновки.** Незважаючи на всебічне вивчення проблеми, в патогенезі ПЕ залишається багато відкритих питань. На особливу увагу заслуговує детальніше вивчення механізмів формування та усунення набрякових змін мозкової тканини на тлі гепатогенної інтоксикації та розвитку системної запальної реакції, ролі астроглії та її водних каналів у цих процесах, а також механізмів пошкодження гематоенцефалічного бар'єра.

**Ключевые слова:** печеночная энцефалопатия, патогенез, аммиак.

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## Современный взгляд на печеночную энцефалопатию: основные понятия и концепции патогенеза

**Т. В. Шулятникова, В. А. Шаврин**

Проблема острой и хронических форм печеночной энцефалопатии (ПЭ) недостаточно четко обозначена среди современных проблем гепатологии и нейронауки в Украине. Несмотря на значительный вклад в развитие летальных осложнений у больных с печеночной патологией и длительную историю изучения данного вопроса, до сих пор не сформировано единое мнение в отношении звеньев патогенеза ПЭ.

**Цель работы –** проведение всестороннего анализа современных данных касательно распространенности и механизмов развития печеночной энцефалопатии.

ПЭ – комплекс потенциально обратимых нейрокогнитивных расстройств у пациентов с хронической или острой печеночной недостаточностью (ОПечН). ПЭ чаще является осложнением цирроза печени и второй наиболее частой после асцита причиной госпитализации таких пациентов. При декомпенсации печеночной недостаточности при острой или хронической печеночной патологии у больных развиваются тяжелые формы ПЭ, сопровождающиеся прогрессирующим повышением внутричерепного давления и развитием комы, которая чаще всего заканчивается летально ввиду плохой корригируемости внутричерепной гипертензии при сохранении гепатогенной нейроинтоксикации. ПЭ рассматривается как конечный результат накопления ряда нейротоксических веществ в мозге, среди которых рассматривают аммиак, меркаптаны, короткоцепочечные жирные кислоты, ложные нейротрансмиттеры, гамма-аминомасляную кислоту, марганец. Наибольшей популярностью среди причин развития ПЭ пользуется нейротоксическая теория аммиака. Аммиак подвергается детоксикации в печени, превращаясь в мочевину, меньшая доля при участии глутаминсинтетазы используется в синтезе глутамина в мышцах, печени и астроцитах мозга. В случае печеночной дисфункции и/или портосистемного шунтирования концентрация аммиака в крови возрастает до 10 раз, и основная нагрузка по его детоксикации перекладывается на миоциты и астроглию. В условиях ОПечН происходит глутаминовая перегрузка астроцитов с изменением внутриклеточной осмолярности и последующим отеком астроглии, что сопровождается развитием цитотоксического отека мозга в целом. При этом в астроцитах происходит повреждение дыхательной цепи митохондрий, развиваются митохондриальная недостаточность, процессы нитрозативно-оксидативного стресса и окисления астроцитарной и нейрональной РНК, нарушения экспрессии генов, синтеза нейро- и глиотрансмиттеров, синаптопластичности. Повышенное поступление в мозг ароматических аминокислот ведет к синтезу ложных нейротрансмиттеров, что ухудшает серотонинергическую, ГАМК-ергическую, дофаминергическую и глутаматергическую нейротрансмиссию. Повреждение компонентов гематоэнцефалического барьера ведет к усугублению водного дисбаланса, проникновению в мозговую паренхиму гематогенных цитокинов, эндотоксинов и других продуктов системной воспалительной реакции и развитию в мозге процессов нейровоспаления, что вносит свой важный вклад в дальнейшее прогрессирование отека мозговой ткани.

**Выводы.** Несмотря на всестороннее изучение проблемы, в патогенезе ПЭ остается много открытых вопросов. Особого внимания заслуживает более детальное изучение механизмов формирования и нивелирования отечных изменений мозговой ткани на фоне гепатогенной интоксикации и развития системной воспалительной реакции, роли астроглии и ее водных каналов в этих процессах, а также механизмов повреждения гематоэнцефалического барьера.

## Background

The problem of acute and chronic forms of hepatic encephalopathy (HE) is not clearly identified among modern problems of hepatology and neuroscience in Ukraine. Despite the significant contribution to the development of lethal complications in patients with liver pathology and long history of the study of this problem, there is still no unified opinion on the links of the HE pathogenesis.

**The aim** of this review is to conduct a comprehensive analysis of current data on the spreading and mechanisms of development of HE.

Among the causes of endogenous neurointoxication, liver damage occupies one of the leading positions. Liver diseases are accompanied by the development of brain pathology, which corresponds to the term "hepatic encephalopathy" (HE).

HE is a complex of potentially reversible neurocognitive disorders in patients with chronic or acute hepatic insufficiency, manifested by a varying severity neurological deficit, changing in personality and intelligence, and by consciousness disorders from the inversion of "sleep-wake" rhythm to hepatic coma in conditions of functional liver decompensation [1]. Ultimately, the syndrome includes two principal factors: the presence of liver pathology and/or portosystemic shunt, which are sufficient to create and maintain plasma critical concentration of putative neurotoxic substances, and the presence of encephalopathy per se

which developed as a consequence unless another cause of cerebral dysfunction is excluded [2].

HE in most cases is a complication of liver cirrhosis and is the second most frequent cause of hospitalization of patients after ascites [3]. In the US from 2005 to 2009 up to 110000 patients with HE were hospitalized per year [4]. 70–87 % of liver cirrhosis cases are accompanied by latent, or "minimal" HE, while 30–45 % of patients may show symptoms of moderate up to the severe form [5,6], which is accompanied by cerebral edema development, intracranial hypertension (ICH) decompensation and fatal outcome. Approximately 30 % of patients dying in the final stages of liver disease have a signs of severe HE turning into a coma. Thus, the degree of HE severity which is sufficient for patients hospitalization, is associated with a 42 % survival prognosis during 1 year and 23 % within 3 years of monitoring [7,8]. Development of the overt HE in patients, suffering from liver cirrhosis, is a sign of disease decompensation as well as ascites or varicose bleeding, and serves as a marker of unfavorable prognosis and low survival. Besides liver cirrhosis HE can be a complication of surgical portosystemic shunts in 24–53 % of cases [9], and also be one of congenital abnormal intra- and extrahepatic shunting manifestations. To denote the persistent form of HE, the term "chronic hepatic encephalopathy" (CHE) or "portosystemic encephalopathy" can also be used, although these terms are not identical, because CHE can also act as a manifestation of not only a portosystemic dump, but also hereditary urea cycle metabolic disorders, Wilson-Konovalov's disease, porphyria and others [10,11].

HE is the key diagnostic criterion for acute liver failure (ALF). Acute hepatic encephalopathy (AHE) usually occurs in severe form, accompanied by severe ICH, acute cerebral insufficiency and high mortality. The pathogenesis of ICH development on the background of liver failure is not fully understood and is considered as multifactorial. Unfortunately, despite certain successes in intensive care AHE which developed on the background of acute liver failure to date can most effectively be cured only by liver transplantation, which would neutralize the initiating factor of hepatogenic neurointoxication [12].

At the World Congress of Gastroenterology in 1998, 2002 the HE classification was elaborated, according to which it was classified according to 4 characteristics [13,15]:

1. Depending on the underlying disease, the following types are distinguished: type **A** (result of ALF, e. g., in acute hepatitis or decompensated cirrhosis – **ACUTE** Liver Failure); type **B** (predominantly occurs on the background of portosystemic shunting with liver cirrhosis but without hepatocellular insufficiency, or on the background of artificial portosystemic bypass – Portosystemic **BYPASS** or Shunting); a type C (caused by liver cirrhosis and may combine the portosystemic shunting and hepatocellular failure – **CIRROSIS**).

2. According to severity of manifestations degree in accordance with West-Haven criteria HE is divided into:

– Grade 0 – "minimal" or latent HE (previously known as subclinical); Cognitive and psychiatric disorders are diagnosed only when using special psychometric and neurophysiological tests;

– Grade I – the presence of mild neurological and psychiatric symptoms, inversion of the "sleep-wake" cycle;

– Grade II – significant neurocognitive dysfunction, obvious asterixis ("fluttering tremor"), disorientation, significant behavioral abnormalities;

– Grade III – drowsiness, expressed neurocognitive dysfunction, amnesia, disorientation in place and time;

– Grade IV – coma with the presence or absence of response to painful stimuli (precoma-1, precoma-2, coma-1, coma-2 – corresponding to the stages of Glasgow Coma Scale).

HE-0 and the 1st grade referred to as "covert" form of HE, and from the 2nd to 4th grade is considered "overt".

3. Depending on time course of HE: episodic, recurrent and persistent forms.

4. According to the existence of precipitating factors HE can be non-precipitated and precipitated. In the latter case, the following can serve as provoking factors for the emergence of overt HE: infections, excessive protein intake into the body, hyponatremia, hypokalemia, renal failure with azotemia, alkalosis, dehydration, diarrhea, hypotension/hypovolemia, shock, gastrointestinal bleeding, excessive doses of diuretics, constipation, receiving opiates, benzodiazepines, antipsychotics etc. [14].

Despite more than 200 years (since 1765, when Giovanni Battista Morgagni was the first to describe a case of liver cirrhosis with HE) studying of the problem and numerous attempts to formulate a common pathogenetic concept of HE, to date there is no holistic view on the factors of etiopathogenesis and accordingly there is no single approach to managing such patients [15]. Data on the morphogenesis of HE in the current literature are of limited character and are presented in form of single studies of predominantly experimental character. Models using experimental animals reproduce both AHE and CHE. Using nervous tissue cultures (in vitro) in the study of the multifaceted influence of hyperammonemia and other neurotoxic substances on them occupies one of the leading places and is widely reflected in the world literature. Despite such a high popularity, it should be noted that neither in animal models, nor, especially, in tissue cultures, it is possible to reproduce those specific, in many respects unique conditions, in which there is a complex, multilevel system of the brain and the human body as a whole. This fact greatly limits the possibility of studying the factors of neuroaggression, pathogenesis and morphogenesis in conditions close to real ones. On the other hand, modern pathomorphological studies of HE on human material are almost absent in foreign literature and extremely limited in native one. They are based on the study of sectional cases using immunohistochemistry (IHC) methods, as well as general pathohistological techniques. In sectional investigations the subject of research is CHE on the background of the final stages of liver cirrhosis, as AHE on the background of ALF to date is a more rare disease and is due to fulminant forms of viral hepatitis A, B, D, E (which have the highest incidence in Africa and Asia), acute poisoning by hepatotoxic poisons and suicide or accidental poisoning by drugs – mostly acetaminophen (paracetamol, N-acetyl-p-aminophenol [APAP]), – which are responsible for 50 % of all cases of AHE in the US and UK [16]. Reliable data on the incidence of HE in the European Union and Eurasia in the literature are not given in the absence of such studies [12]. In the US, approximately

5 million cases of chronic liver insufficiency are diagnosed each year [17]. These data indirectly reflect the worldwide trend in spreading of chronic liver pathology and indicate high relevance of CHE problem.

There are several theories of the development of HE, however, they all do not exclude, but complement each other. Quite poor described pathomorphological changes in the brain tissue do not allow convincing clinic-morphological comparisons; however, it is fairly clear that in the pathogenesis of HE the main conditions are decrease in the detoxification function of the liver and/or portosystemic discharge of blood with the entry of intestinal and/or liver origin pathogenic factors in the brain tissue. In general, the HE is considered as the final result of the accumulation of a number of neurotoxic substances in the brain, the most actively considering ammonia, mercaptans, short chain fatty acids, false neurotransmitters (tyramine, betaphenylethanolamine, octopamine), gamma-aminobutyric acid (GABA), manganese [15].

The mechanisms of CHE and AHE have significant differences. The main manifestation of HE at ALF is cytotoxic brain edema, furthermore, factors that contribute to a higher risk for its development are identified: childhood, poisoning with paracetamol (acetaminophen), high hyperammonemia, related infectious pathology. Pathogenesis and morphogenesis of edema development of brain in HE is not fully understood, but most researchers come to the conclusion that the prevailing after all is the cytotoxic mechanism.

The most popular among the prospective causes of the development of HE is the neurotoxic theory of ammonia. Since 1890s ammonia is assumed as main factor of neurotoxicity in the development of HE. Over the past century, it was done a large number of experimental studies on animals and tissue cultures, a number of clinical studies during which it was shown the undoubted participation of this metabolite in neurotoxicity, as well as data on the dependence of the severity of HE on the level of hyperammonemia [18]. It is shown that hyperammonemia can be not only of hepatic origin, and the development of encephalopathy on its background has similar mechanisms of development with HE ones [19]. Despite this, in literature there is still no definitive opinion regarding the degree of hyperammonemia level influence on the severity of HE, since in the significant number of clinical studies, particularly in patients with cirrhosis, any such correlation was found [20].

Most of ammonia is synthesized in intestine by the bacterial cleavage of amino acids, amines, purines, urea, during the metabolism of glutamine in enterocytes, and also sources are the liver, muscles and kidneys. Basically, ammonia is detoxified in periportal liver hepatocytes, where it is converted to urea by participating in the ornithine cycle. A smaller portion with the participation of glutamine synthetase is used in glutamine production in muscles, perivenous hepatocytes and brain astrocytes. In case of hepatic dysfunction and/or port-systemic shunting systemic ammonia concentration increases up to 10 times, while the main load for its detoxification is shifted to myocytes and astroglia [21]. In patients with liver cirrhosis and metabolic acidosis renal elimination of ammonium ions reaches 70 % of the total formed volume, and renal failure enhances HE in patients with liver cirrhosis [22]. Ammonia is presented in two forms: a weak base – gaseous form ($NH_3$), and a weak acid – ionized form ($NH_4^+$), has electrolyte conductivity properties and is a substrate and the product of the diversity of enzymatic reactions of brain. Both forms are able to penetrate through the plasma membrane, thereby maintaining acid-base homeostasis in tissue. The gaseous ammonia and ionized form are in dynamic equilibrium, which is defined by the Henderson–Hasselbach equation, so normally, at pH 7.4, the predominant form (approximately 98 %) of ammonia in the body is $NH_4^+$ [23]. Ionized form is capable of dissolving in lipids, thus easily penetrates by diffusion into the CNS. It is known that $NH_4^+$ has very similar ionic properties with $K^+$ ions, which allows them to compete with each other in membrane ion channels, e.g., a voltage-dependent $K^+$ channels and replace $K^+$ ions in $Na^+/K^+$ and $H^+/K^+$ ATP transporters. It has also been shown that $NH4^+$ can be carried in cell by $NH_4^+/Cl^-$ and $Na^+/K^+/Cl^-$ cotransporters and S. M. Saparov et al. showed the possibility of transportation of ammonia through aquaporin channels type 8 [24]. The astrocyte pH depends on the prevalence of a form of ammonia (ionised or gaseous) entry into the cell, which in turn depends on their initial concentration in blood, the rate of entrance into the cell and the quantitative ratio of membrane ammonia transporters. Thus, under physiological conditions, when $NH_3$ is less than 2 %, diffusing, it leads to rapid intracellular contents alkalization. Subsequently, through the membrane channels listed, a smoother intake of $NH4^+$ occurs and an acidification of the intracellular medium develops [23]. Previously it was believed that the electric charge of $NH_4^+$ does not allow it to penetrate the BBB, but now it turned out that it can penetrate through the transcellular barrier through potassium channels and transporters. In conditions of ALF this form of transport may be enhanced, and also joins $NH_3$ paracellular transport path, which increases the total ammonia concentration in the brain tissue [25].

The most common view is that development of AHE is the result of astroglia dysfunction. Evidence of this concept again actively began to appear in last decades' literature. In the 80's Norenberg M. D. proposed to consider the astrocyte dysfunction initiating factor in the launch of a number of pathobiochemical reactions that determine the development of encephalopathy in hyperammonemia and other toxic influences. He suggested calling such a state of astroglia in this application "primary gliopathy" [26]. It has long been known, that at HE on the background of ALF pronounced cytotoxic edema develops in astrocytes, what is believed to be the main cause of edema-swelling of the brain and uncontrolled ICH. In view of the fact that glutamine synthetase (GlnS) enzyme necessary for the metabolism of ammonia in brain is localized exclusively in astrocytes, in conditions of hyperammonemia and developing glutamine transporter SNAT5 dysfunction, there is a rapid saturation of the intracellular pool of glutamine, which leads to hyperosmolarity, osmotic stress in the cytoplasm and its subsequent rapid edema. In animals experiments with the administration of an inhibitor of astrocytic GlnS the severity of edema was significantly decreased [27]. It is also supposed the active participation of glutamine in the mechanisms of oxidative and nitrosative stress, which are of great importance in the pathobiochemical cascade

reactions in brain in conditions of hyperammonemia [15]. On the other hand, in studies carried out on rat model of portocaval anastomoses with additional administration of ammonium acetate, it is shown that when using methionine sulphoximine, an inhibitor of glutamine synthetase, the drug does not completely arrested cerebral edema, indicating that the osmotic properties of glutamine only partially explain the mechanisms of edema development in conditions of hyperammonemia [28].

In 2006, J. Albrecht et al. published a paper on the mechanisms of glutamine participation in ammonia neurotoxicity, calling such model of influence "Trojan horse" scheme. Glutamine, accumulating in astrocytes, enters the mitochondria through histidine-sensitive glutamine transporters, where it is hydrolyzed by phosphate-activated glutaminase on the inner mitochondrial membrane, releasing ammonia and glutamate again. Thus, glutamine as "Trojan horse" is a hidden ammonia carrier. Achieving high concentrations of mitochondrial ammonia leads to changes in mitochondrial membrane potential, damages respiratory chain and leads to the formation of reactive oxygen species and oxidative stress development [15,29]. It is still not known how the ammonia-induced free radicals lead to the development of astrocytic edema. They are expected to damage directly the protein and lipid components of the mitochondrial membrane and cells plasmolemma, leading to violation of membrane permeability through disrupting the ion transport mechanisms. Disturbance of energy production and decreased control of ion transport leads to dysregulation of the cell volume [25]. Described mitochondrial changes are determined by the concept of mitochondrial permeability transition (MPT) – opening large unselective pore (permeability transition pore (PTP)) on the inner mitochondrial membrane, which leads to higher permeability for protons, ions and other small molecules and mitochondrial dysfunction development [15], sharp swelling of the mitochondrial matrix with partial until the total cristolysis.

In addition to disturbing the glutamate-glutamine conversion cycle, ammonia also participates in violation of neurotransmission, weakening of the blood brain barrier (BBB), the synthesis of pro-inflammatory cytokines, decreasing brain tissue energy metabolism, violation of cerebral blood circulation, anomalies of benzodiazepine and GABAergic tone.

An increased level of lactate in the brain tissue can serve the evidence of energy metabolism violation in brain in HE [15]. Between astrocyte swelling and oxidative stress there is a direct positive correlation, where one potentiates the other, forming a vicious circle. It is known that in edematous astrocytes oxidative-nitrosative stress occurs at the level of NADPH-oxidase, nitric synthase and mitochondria. Ammonia-induced oxidative-nitrosative stress leads to nitration of tyrosine protein endings and oxidation of astrocytic and neuronal RNA. Also nitrosative stress mobilizes zinc from intracellular stores, leading to inducing expression of metallothionein mRNA. In rat models of hyperammonemia RNA oxidation is observed predominantly in the perivascular astrocyte endings, as well as in the postsynaptic dendritic spines. This causes disturbance of gene expression, postsynaptic protein synthesis and synaptic plasticity, disturbs intracellular signaling and glia-neuronal, neuro-neuronal interactions and determines neurotransmitter imbalance [30].

In vitro-experiments showed that ammonia, getting into the extracellular space, easily passes through astrocytic plasma membrane and alkalizes cell contents. This facilitates the exit of calcium from intracellular stores and explains the increase of exocytotic $Ca^+$-dependent glutamate release into the extracellular space [31]. Reduced ability of astrocytic glia to reuptake of glutamate from extracellular space under conditions of osmotic stress leads to its accumulation there and development of glutamate excitotoxicity of neurons, enhanced stimulation of astroglial glutamate receptors and aggravation of astrogliopathy [32]. Hyperammonaemia is capable to increase the membrane potential of both neurons and astroglia. Activation of glutamate ionotropic receptor N-methyl-D-aspartate (NMDA) plays an important role in HE development. Opening of NMDA is controlled by extracellular magnesium ions through voltage-dependent block. It is considered that ammonia, increasing membrane potential, removes magnesium blocking, thereby improving the sensitivity of NMDA-receptors. For example, studies in HE animal models indicate increasing of glutamate neurotransmission by NMDA-receptors, at the same time violation of NMDA-dependent glutamate-NO-cGMP pathway can manifest themselves in behavioral abnormalities observed in HE patients [15]. Ammonia induce rapid decrease in astrocytic $K^+$ buffering, what increases its extracellular concentration excessively and activates the $Na^+$-$K^+$-$2Cl^-$ cotransport isoform-1 (NKCC1) in neurons. The subsequent depolarization of neuronal GABA reverse potential ($E_{GABA}$) impairs cortical inhibitory system and can lead to local excitotoxicity that has clinical manifestations in HE [33]. On the other hand, hyperammonaemia causes activation of the astrocytic peripheral-type benzodiazepine receptors [34], which is associated with increase in production of neurosteroids by mitochondria. In turn neurosteroids like benzodiazepines stimulate neuronal GABAergic receptors, which together with astrocytes reduced ability to capture extracellular GABA leads to enhancement of GABAergic tone in brain tissue. Thus, in the brain tissue of patients in hepatic coma pathologically high levels of allopregnanolone and pregnenolone were found [2]. All together it can drive to reduction of neurotransmission, and also provoke encephalopathy symptoms on the background of taking drugs tropic for GABA. Interestingly, in experiments on tissue cultures, allopregnanolone along with tetrahydrodioxycorticosterone showed a significant reduction (up to 60 %) of the permeability of BBB after initial culture treatment with bacterial endotoxin, which initially increased its permeability by 87 % [35], which indicates a simultaneous protective effect of neurosteroids on the BBB during its toxic damage. Hyperammonaemia causes changes in the BBB permeability to various molecules, including branched chain amino acids and aromatic amino acids. The content of the latter in the brain tissue is significantly increased, which is due to the synthesis of glutamine during the detoxification of ammonia. Increased intake of aromatic amino acids leads to violations of catecholamine production (serotonin and dopamine) and instead false neurotransmitters (octopamine and phenylethylamine) synthesis, which can deteriorate the serotoninergic, GABA-ergic and glutamate-ergic

neurotransmission [36]. The total amount of most experimental investigations on animals HE models describe malfunctions of 4 neurotransmitter systems in the brain: GABA-benzodiazepin-ergic, dopaminergic, serotoninergic and glutamate-ergic [1].

Physiologically astrocytes are involved in the regulation of synaptic transmission by $Ca^{2+}$-stimulated release of various "gliotransmitters", including glutamate, ATP and many others. Under normal conditions astrocytic ATP plays an important role in glial calcium fluxes and purinergic modulation of neuronal activity. Thus, increasing level of adenosine in the extracellular space of the brain tissue leads to sleepiness. Astrocytes play a key role in regulating adenosine levels in the extracellular space. Thus, by SNARE (soluble N-ethylmaleimide-sensitive fusion attachment protein receptor)-dependent mechanism they secrete ATP in the form of "dense-core" vesicles contents, and ATP further via ectonucleotidases is converted into adenosine [37]. Violations of this mechanism may underlie the early signs of HE in the form of "sleep-wakefulness" rhythm disturbances. At the same time in prolonged hyperammonemia in vitro studying and in animal HE models with portocaval shunting an inverse relationship was shown between the total level of ATP in the brain with the severity of astroglial changes and the severity of HE in animals.

Water exchange, along with cerebrospinal and neuropial (interstitial) liquid circulation mechanisms lies at the basis of aqueous homeostasis in the brain tissue. Aquaporins (Aqp) – transmembrane channels protein family responsible for water homeostasis in tissues. It is known that among 14 subtypes of aquaporins in the brain Aqp-1, Aqp-9 and Aqp-4 are most expressed, and all of them are involved in edema development. It is believed that from the above Aqp-4 has the greatest value in the mechanisms of edema development in acute and chronic liver failure. Its expression is highest in astrocytic domains responsible for water transport – perivascular astrocytic processes end-feet, submeningeal terminal end-feet – in the border regions between the cerebral parenchyma and the main liquid compartments represented by blood and cerebrospinal fluid. The water movement through astrocytic plasma membrane depends on the permeability of Aqp-4, the heterogeneity of its biochemical structure, the speed and directivity of intracellular transport of vesicles containing Aqp-4 components [37]. To provide the necessary distribution of Aqp-4 in the astrocytic plasma membrane, there are auxiliary intracellular and extracellular proteins. Thus, the intracellular protein α-syntrophin binds Aqp-4 to the dystrophin-associated protein complex, which serves as a link between the transmembrane proteins and the cytoskeleton. Transmembrane protein pair α-β-dystroglycan binds Aqp-4 to the proteins of the vascular basement membrane arginine and laminin, which is necessary for perivascular concentration of water astrocytic channels [38]. During brain tissue damage the expression of these anchor proteins changes, which leads to the movement of Aqp-4 from the astrocytic vascular end-feet toward the rest of the plasmolemma and their wider spread in the soma cell region – a concept called "Aqp-4-dysregulation". Aqp-4-dysregulation phenomenon has been shown in the first three days in animal models of stroke and traumatic injury and coincided in time with the most pronounced degree of cytotoxic edema [39]. On the other hand, this phenomenon can be regarded as an adaptive reaction of astrocytes, which when brain tissue is damaged move their water channels away from the vascular walls to prevent the entry of vasogenic water, as well as processes of astroglia reactivation, which include hypertrophy, migration, production of cytokines and other events [40]. The role of Aqp-4 channels has been shown in various models of cytotoxic and vasogenic edema. Thus, aquaporins are active participants in the development of cytotoxic edema and the processes of excess interstitial fluid elimination when vasogenic edema develops [41]. On the other hand, the role of Aqp-4 in cytotoxic edema in HE is still not clear and is subject to debate, as the data on the neurotoxic effects of ammonia in different experimental models are ambiguous. Rama Rao K. V. et al. when using the astrocytes culture observed pronounced increase in Aqp-4 expression in 10 hours after the treatment by 5 mMol $NH_4Cl$ and further expression growth up to 48 h, which preceded the cytotoxic edema development [42]. G. Bodega et al. in the study of the effect of ammonia on the expression of Aqp-4 in astrocyte culture received conflicting data depending on the method of determining expression levels. However, using a set of techniques, most of the data indicated a decrease in astrocytic expression of Aqp-4, which was combined with the formation of insoluble protein aggregates in the plasma membrane, and apparently was a manifestation of the inactivation of aqueous channels under ammonia influence [43]. G. Wright et al. in experimental rat model of liver insufficiency noted an increase in Aqp-4 expression, however, no correlation was found between the expression levels of Aqp-4 and the level of hyperammonemia, the presence of sepsis, and the development of cerebral edema [44].

Interstitial fluid of the brain tissue is constantly renewed due to the inflow of water and dissolved substances from the bloodstream through the capillary endothelial ion channels and also due to water penetration from the cerebrospinal fluid from the Virchow-Robin periarterial spaces (VR- periarterial S), which are connected with the subarachnoid space. The anatomical and functional features of the VRS have not yet been clearly defined and continue to be the subject of active discussions in modern neuroscience. It is assumed that the exchange between cerebrospinal and interstitial fluid is mainly concentrated on their territory. So, from periarteriolar spaces under the influence of hydrostatic and osmotic pressure, water penetrates through Aqp-channels into the surrounding astrocytic syncytium and further into the parenchyma. In the brain parenchyma interstitial fluid moves transcellular and paracellular and reaching perivenous astrocytic processes, through water channels excreted in the VR-perivenous space. These mechanisms of the formation, exchange and purification of the interstitial fluid of the brain tissue are functional manifestations of the so-called brain "glymphatic system" [45]. This concept was proposed recently and is actively discussed in the current literature. New ideas about the processes of renewal and draining the liquid component of brain tissue, excretion of toxic metabolic

waste have already become a prerequisite for the creation of new theories of the pathogenesis of neurodegenerative diseases, ischemic, traumatic injury and other pathological states of the CNS. Water, as well as small and medium size molecules can penetrate from VR-periarterial spaces into perivascular astrocytic end-feet by paracellular and three transcellular pathways: water can penetrate by diffusion through the cell membrane, through the membrane cotransport through EAAT1 or NKCC1 and finally via Aqp-4 (bidirectional water transport). During glymphatic system functioning in the aqueous transcellular transport mechanisms exactly aquaporin pathway predominates [46].

Given the leading role of astrocytic glia in the functioning of the glymphatic system and the supposed concept of primary astrogliopathy in the pathogenesis of HE, it is to be assumed that the violation of the processes of penetration and drainage of water, as well as the neurotoxic substances dissolved therein, may be of primary importance in the mechanisms of the development of hepatic encephalopathy, and many other toxic damage and may be metabolic disorders of the brain. Number of experimental studies, as well as the study of the autopsy material of patients with ALF demonstrated violation of the expression of several key astrocytic proteins: Aqp-4, GFAP (glial fibrillar acidic protein) [47], glutamine transporter SNAT5, glutamate transporters (GLT-1 (SLC1A2 – solute carrier family 1 member 2; EAAT2 – excitatory amino acid transporter-2)), glycine transporters (GLYT-1), glucose transporters (GLUT-1), peripheral type benzodiazepine receptors [48]. Thus, K. K. Thumburu et al. in the study of the cerebral cortex of 8 patients died in ALF showed an increase in the level of Aqp-4 mRNA and Aqp-4 IHC expression in the region of astrocyte microvascular perivascular end-feet, as well as marked decrease in mRNA level and intermediate filament protein GFAP expression on the background of the brain tissue cytotoxic edema pattern. A study of Aqp-4-knockout animals showed a marked deterioration in astroglia drainage function, which was manifested by a decrease in excretion of substances dissolved in the interstitial fluid [49]. These violations of the astrocytic water canals expression confirm the great importance of disturbances in the functioning of the glymphatic system in the development of HE.

It was previously believed that BBB is impenetrable for ammonia. This opinion was disproved by studies showing an increase extraction of ammonia from the blood by brain tissue in HE but to date this issue is still open [50]. The functions of the endothelium and the BBB tightness can be altered by a variety of substances, including ammonia, interleukins, NO, adenosine, purine nucleotides, steroid hormones, serotonin, bradykinin [36]. The high selectivity of the BBB permeability is due to the presence of tight junctions (TJ) between capillary endothelial cells, weakly expressed pinocytosis transport and thickened basement membrane. The structure of TJ is represented by transmembrane proteins, including junctional adhesion molecules, occludin, claudin, and intracellular protein domains that connect this complex with cytoskeleton elements and ensure the stability of TJ (zona occludin (ZO)-1, -2, and -3). It is known that damage to these protein complexes leads to a significant increase in the permeability of BBB and the development of cerebral edema [51].The role of metalloproteinases (MMPs) is noted among the proposed mechanisms of tight junctions' proteins damage. Being released to the intercellular space they regulate the state of the intercellular matrix, for example, in the process of cell growth, migration, cell and tissue remodeling, synaptic plasticity. It is known that in the adult brain MMPs can be synthesized by microglial cells, astroglia, neurons and in physiological conditions MMPs are either absent or present in undetectable amounts. The pathogenic effect of MMPs is manifested in increasing permeability of BBB, in the processes of demyelination, stimulation of fibrosis of blood vessels, development of gliosis, damage to neurons and other influences [52]. MMP-9 and MMP-2 have the highest value in the development of cerebral edema. In ALF-azoxymethane rat model it was showed that MMP-9 was not detected in the brain tissue during AHE, but in the liver tissue its mRNA level was increased more than 8 times, which could indicate that the effect of MMP-9 on the BBB was performed from the blood side, and the increased plasma level of MMP-9 was due to its release from the damaged liver tissue. In this case, animals in precoma had the highest blood level of MMP-9, and the degree of cerebral edema was the highest in the coma stage [53]. Data regarding the participation of MMPs in the development of edema in HE, especially its chronic forms, are still too insufficient to determine their role in the damage of the BBB and the issue requires further detailed study. Currently, there is an active discussion of whether changes in the permeability of the BBB are the result of the ALF itself, or they are secondary to such complications of ALF, as systemic inflammatory response and sepsis.

Much attention has been paid to the role of the endothelium in edema development in hyperammonemia. Forming the first level of the vascular barrier for incoming neurotoxins, they are the first to undergo their influence. Endothelial cells are able to activate a number of pro-inflammatory factors such as COX-2, iNOS, phospholipase-A2, transcriptional nuclear factor kappa-B (NF-κB), which in turn contribute to the processes of cell swelling through cytokines, reactive oxygen and nitrogen species (ROS/NOS), arachidonic acid, and other factors. Activation of endothelial receptors to IL-1β and TNF-α leads to stimulation and additional cytokines synthesis, exacerbating mechanisms of edema development in AHE [15]. In experiments using the cultures of endotheliocytes, astrocytes and on models of AHE in rats, the formation of oxygen radicals, nitric oxide, signs of oxidative-nitrosative stress, activation of NF-κB in endotheliocytes when addition of ammonia or a mixture of ammonia, endotoxin (lipopolysaccharide, LPS) and pro-inflammatory cytokines were shown. When adding such treated endothelial cells to astrocytic culture, the latter developed a pronounced cytotoxic edema. At the same time the use of ammonia with antioxidants or NF-κB inhibitor BAY 11-7082 the severity of astrocytes swelling decreased significantly. Thus it was shown the important role of endothelial dysfunction in astrocytes swelling and brain edema as a whole in AHE [54].

The results of many experimental studies have shown that HE is characterized by the breakdown of cerebral circulation autoregulation system. Hyperemia and increase rate of cerebral blood flow, which are typical for AHE/hyperammonemia, little depends on glutamine level

in the cerebrospinal fluid (CSF) and follows immediately after reaching the maximum of glutamine in astrocytes and water content in the brain parenchyma, while NOS inhibitors do not prevent cerebral blood flow (CBF) increasing and the occurrence of ICH [55]. This may indicate that the NO-dependent mechanism of hyperemia does not have a proper value in the case of hyperammonemia. A high level of CBF is complicated by the movement of water through the BBB into the osmotically compromised brain tissue and enhances the ICH.

In liver failure, in addition to astroglial changes were detected in the microglial cells, in which there are signs of reactivation and inclusion in neuroinflammatory response. Studies have shown that an increase in the general permeability of BBB in hyperammonemia was followed by reactive changes in microglia [56]. During IHC study of dead patients' brains in HE in 4/9 cases positive expression of PCNA and Ki-67 markers together with such expression of Iba-1 (ionized calcium binding adaptor molecule 1 – a specific marker of microglial cells) in hippocampal subventricular zone were found. Cases with so-called "proliferative HE" were characterized by higher levels of IL-6 in the brain tissue, and a slightly higher density of neurons. While "nonproliferative HE" were characterized by degenerative changes in microglia of the white matter. This suggested an early favorable effect of microglial activation and proliferation in the mechanisms of neuroprotection, which nevertheless proved ineffectiveness under conditions of ongoing hyperammonemia and systemic inflammation [57]. IL-1b, IL-6 and TNF-a play an important role in the development of ICH and HE progression. These proinflammatory cytokines can enter the brain tissue with blood from the damaged liver and can also be synthesized by reactive microglia. Thus, it is known that in extracellular vesicles of microglia IL-1b, IL-6, iNOS and cyclooxygenase-2 [58] are expressed and pro-IL-1b is included in the ectosome together with procaspase-1, the $P2X_7$ receptor and other inflammosomal components described in monocytes [59]. Development of systemic inflammation (systemic inflammatory response syndrome, SIRS) is very typical for ALF. This inflammatory reaction may not be limited to "periphery", but involve the brain and unfold there as a complete neuroinflammatory response [60]. In this context, the synthesis of microglia cytokines and the development of neuroinflammation are expected as a result of the reactive response of microglia to ammonia intoxication [56] and make a significant contribution to the mechanism of nervous tissue damage along with the toxic effects of ammonia.

Over the past few years, the role of systemic inflammation in the development of edema and ICH in HE has actively been discussed, as opposed to the dominant role of hyperammonemia [61]. Infectious factor in HE development plays a significant role, which allows comparing the mechanisms of encephalopathy development in ALF and septic encephalopathy. However, the specific mechanisms for participation of infectious agents and their metabolites in the pathogenesis of HE are not yet fully understood. It is still not known whether the infection itself is the HE exacerbating factor or damaging is the inflammatory response of the body [1]. In the modeling of endotoxemia by administration of LPS in the AHE model, a marked increase in cerebral edema was noted, indicating that systemic inflammation, presence of infection and the concomitant increase in the level of cytokines can significantly aggravate the cytotoxic edema of the brain [60]. Also in blood of patients with minimal HE there is increasing level of pro-inflammatory cytokines TNF-α and IL-6. Also systemic inflammation causes an increase in systemic and intracerebral level of vascular endothelial growth factor (VEGF) and IL-1beta [48]. The pro-inflammatory effect of VEGF is caused by activation of endothelial cells and increase of their permeability. The development of SIRS signs may reflect the presence of subclinical infection, but also means the development of inflammatory response and activation of anti-inflammatory response which ultimately can lead to further activation of infection [62,63]. It was shown that vasogenic edema in AHE is likely caused by infectious complications of ALF in the form of spontaneous peritonitis caused by translocation of bacterial intestinal flora, SIRS or septicemia. Under these conditions, the level of bacterial endotoxins (LPS), that are considered a classic factor of BBB integrity damage [64], will increase in blood.

Recently, interesting results were also obtained in the study of the influence of intestinal microbiota state on the severity of HE. In patients with HE on the background of liver cirrhosis ratio of autochthonous (*Lachnospiraceae*, *Ruminococcaceae* etc.) and other microflora (*Enterobacteriaceae*, *Burkholderiaceae*, *Streptococcaceae*, *Porphyromonadaceae Parabacteroides* etc.) was significantly changed in the intestinal mucosa towards the latter. Such dysbiosis leads to excessive growth of intestinal flora, increased intestinal ammonia level and intestinal barrier permeability, decreased intestinal motility, microflora translocation and its interaction with immune cells in the intestinal wall, increased endotoxinemia and circulating pro-inflammatory cytokines, which in combination supports systemic inflammation and aggravates toxic encephalopathy [65].

## Conclusions

Despite a large number of experimental studies using animal models, tissue cultures and clinical studies of brain tissue in HE in the world literature to date there is no unified view on the mechanisms of HE development in acute and chronic course of the disease on the background of different etiological factors and concomitant pathology. Only discrete links of pathogenesis have been formulated, which still, in view of the postmortal and clinic-anatomical complex studies insufficiency, do not allow creating a single concept of brain damage in HE.

**Prospects for further scientific research.** Given the growing interest in the role of astroglia in neuropathological processes, the latest research on water metabolism in the brain tissue and its drainage system, mechanisms of BBB damage and neuroinflammation processes, there are a number of prerequisites for a deeper and comprehensive study of these aspects in the searching for universal components of HE pathogenesis. These actions could be useful in developing of preventive measures and more targeted therapies aimed at the subtle links of the mechanisms of HE development.

## References


[1] Ferenci, P. (2017). Hepatic encephalopathy. *Gastroenterology Report*, *5*(2), 138–147. doi: http://doi.org/10.1093/gastro/gox013.

[2] Butterworth, R. (2016). Neurosteroids in hepatic encephalopathy: Novel insights and new therapeutic opportunities. *The Journal of Steroid Biochemistry and Molecular Biology*, 160, 94–97. doi: 10.1016/j.jsbmb.2015.11.006.

[3] Volk, M., Tocco, R., Bazick, J., Rakoski, M., & Lok, A. (2012). Hospital Readmissions Among Patients With Decompensated Cirrhosis. *The American Journal of Gastroenterology*, 107(2), 247–252. doi: 10.1038/ajg.2011.314.

[4] Stepanova, M., Mishra, A., Venkatesan, C., & Younossi, Z. M. (2012) In-hospital mortality and economic burden associated with hepatic encephalopathy in the United States from 2005 to 2009. *Clin Gastroenterol Hepatol.,* 10, 1034–1041. doi: 10.1016/j.cgh.2012.05.016.

[5] Hassan, E., Abd El-Rehim, A., Seifeldein, G., & Shehata, G. (2014). Minimal hepatic encephalopathy in patients with liver cirrhosis: Magnetic resonance spectroscopic brain findings versus neuropsychological changes. *Arab Journal of Gastroenterology*, 15(3-4), 108–113. doi: 10.1016/j.ajg.2014.09.003.

[6] Elwir, S., & Rahimi, R. S. (2017). Hepatic Encephalopathy: An Update on the Pathophysiology and Therapeutic Options. *Journal of Clinical and Translational Hepatology*, 5(2), 142–151. doi: 10.14218/JCTH.2016.00069.

[7] Bustamante, J., Rimola, A., Ventura, P., Navasa, M., Cirera, I., Reggiardo, V., & Rodés, J. (1999). Prognostic significance of hepatic encephalopathy in patients with cirrhosis. *Journal of Hepatology*, 30(5), 890–895.

[8] Kaplan, P., & Rossetti, A. (2011). EEG Patterns and Imaging Correlations in Encephalopathy. *Journal of Clinical Neurophysiology*, 28(3), 233–251. doi: 10.1097/WNP.0b013e31821c33a0.

[9] Ding, A., Lee, A., Callender, M., Loughrey, M., Quah, S., & Dinsmore, W. (2010). Hepatic encephalopathy as an unusual late complication of transjugular intrahepatic portosystemic shunt insertion for non-cirrhotic portal hypertension caused by nodular regenerative hyperplasia in an HIV-positive patient on highly active antiretroviral therapy. *International Journal of STD & AIDS*, 21(1), 71–72. doi: 10.1258/ijsa.2009.009038.

[10] Ferenci, P., Litwin, T., Seniow, J., & Czlonkowska, A. ( 2015). Encephalopathy in Wilson Disease: Copper Toxicity or Liver Failure? *Journal of Clinical and Experimental Hepatology*, 5 (Suppl 1), S88-S95. doi: 10.1016/j.jceh.2014.09.002.

[11] Upadhyay, R., Bleck, T., & Busl, K. (2016). Hyperammonemia: What Urea-lly Need to Know: Case Report of Severe Noncirrhotic Hyperammonemic Encephalopathy and Review of the Literature. *Case Reports in Medicine*, 2016, 1–10. doi: http://doi.org/10.1155/2016/8512721.

[12] Wendon, J., Cordoba, J., Dhawan, A., Larsen, F., Manns, M., Nevens, F., et al. (2017). EASL Clinical Practical Guidelines on the management of acute (fulminant) liver failure. *Journal of Hepatology*, 66(5), 1047–1081. doi: 10.1016/j.jhep.2016.12.003.

[13] Vilstrup, H., Amodio, P., Bajaj, J., Cordoba, J., Ferenci, P., Mullen, K., et al. (2014). Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study Of Liver Diseases and the European Association for the Study of the Liver. *Hepatology*, 60(2), 715–735. doi: 10.1002/hep.27210.

[14] Bajaj, J., Cordoba, J., Mullen, K., Amodio, P., Shawcross, D., Butterworth, R., & Morgan, M. (2011). Review article: the design of clinical trials in hepatic encephalopathy – an International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) consensus statement. *Alimentary Pharmacology & Therapeutics*, 33(7), 739–747. doi: 10.1111/j.1365-2036.2011.04590.x.

[15] Perazzo, J., Tallis, S., Delfante, A., Souto, P. A., Lemberg, A., Eizayaga, F. X., & Romay, S. (2012). Hepatic encephalopathy: An approach to its multiple pathophysiological features. *World Journal of Hepatology*, 4(3), 50–65. doi: 10.4254/wjh.v4.i3.50.

[16] Ramachandran, A., & Jaeschke, H. (2017). Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. *Journal of Clinical and Translational Research*, 3 (Suppl 1), 157–169. doi: 10.18053/jctres.03.2017S1.002.

[17] Elwir, S., & Rahimi, R. S. (2017). Hepatic Encephalopathy: An Update on the Pathophysiology and Therapeutic Options. *Journal of Clinical and Translational Hepatology*, 5(2), 142–151. doi: 10.14218/JCTH.2016.00069.

[18] Khan, A., Ayub, M., & Khan, WM (2016). Hyperammonemia Is Associated with Increasing Severity of Both Liver Cirrhosis and Hepatic Encephalopathy. International. *Journal of Hepatology*, 2016, 6741754. doi: 10.1155/2016/6741754.

[19] Acharya, G., Mehra, S., Patel, R., Frunza-Stefan, S., & Kaur, H. (2016). Fatal Nonhepatic Hyperammonemia in ICU Setting: A Rare but Serious Complication following Bariatric Surgery. *Case Reports in Critical Care*, 2016 (2016), doi: 10.1155/2016/8531591.

[20] Dam, G., Keiding, S., Munk, O. L., Ott, P., Vilstrup, H., Bak, L. K., et al. (2013). Hepatic encephalopathy is associated with decreased cerebral oxygen metabolism and blood flow, not increased ammonia uptake. *Hepatology*, 57(1), 258–265. doi: 10.1002/hep.25995.

[21] Walker, V. (2014) Ammonia metabolism and hyperammonemic disorders. *Adv Clin Chem.,* 67, 73–150. doi: 10.1016/bs.acc.2014.09.002.

[22] Wright, G., Noiret, L., Olde Damink, S., & Jalan, R. (2011). Interorgan ammonia metabolism in liver failure: the basis of current and future therapies. *Liver International*, 31(2), 163–175. doi: 10.1111/j.1478-3231.2010.02302.x.

[23] Bosoi, C. R., & Rose, C. (2009). Identifying the direct effects of ammonia on the brain. *Metabolic Brain Disease*, 24, 95–102. doi: 10.1007/s11011-008-9112-7.

[24] Saparov, S., Liu, K., Agre, P., & Pohl, P. (2007). Fast and Selective Ammonia Transport by Aquaporin-8. *Journal of Biological Chemistry*, 282(8), 5296–5301. doi: 10.1074/jbc.M609343200.

[25] Scott, T. R., Kronsten, V. T., Hughes, R. D., & Shawcross, D. L. (2013). Pathophysiology of cerebral oedema in acute liver failure. *Journal of Gastroenterology world: WJG*, 19(48), 9240–9255. doi: 10.3748/wjg.v19.i48.9240.

[26] Norenberg, M. D. (1998) Astroglial dyfunction in hepatic encephalopathy. *Metab Brain Dis.,* 13(4), 319–335.

[27] Desjardins, P., Du, T., Jiang, W., Peng, L., & Butterworth, R. F. (2012). Jiang Pathogenesis of hepatic encephalopathy and brain edema in acute liver failure: role of glutamine redefined. *Neurochem Int.,* 60, 690–696. doi: 10.1016/j.neuint.2012.02.001.

[28] Blei, A. T., Olafsson, S., Therrien, G., & Butterworth, R. F. (1994). Ammonia-induced brain edema and intracranial hypertension in rats after portacaval anastomosis. *Hepatology,* 19(6), 1437–1444. doi: 10.1002/hep.1840190619.

[29] Bustamante, J., Lores-Arnaiz, S., Tallis, S., Roselló, D. M., Lago, N., Lemberg, A., et al. (2011). Mitochondrial dysfunction as a mediator of hippocampal apoptosis in a model of hepatic encephalopathy. *Mol the Cell Biochem,* 354(1-2), 231–240. doi: 10.1007/s11010-011-0822-5.

[30] Görg, B., Schliess, F., & Häussinger, D. (2013). Osmotic and oxidative/nitrosative stress in ammonia toxicity and hepatic encephalopathy. *Arch Biochem Biophys,* 536(2), 158–63. doi: 10.1016/j.abb.2013.03.010.

[31] Rose, C., Kresse, W., & Kettenmann, H. (2005). Acute insult of ammonia leads to calcium-dependent glutamate release from cultured astrocytes, an effect of pH. *J. Biol. Chem.,* 280(22), 20937–20944. doi: 10.1074/jbc.M412448200.

[32] Montana, V., Verkhratsky, A., & Parpura, V. (2014). Pathological Role for Exocytotic Glutamate Release from Astrocytes in Hepatic Encephalopathy. *Neuropharmacology Current*, 12(4), 324–333. doi: 10.2174/1570159X12666140903094700.

[33] Thrane, V. R., Thrane, A. S., Wang, F., Cotrina, M. L., Smith, N. A., Chen, M., et al. (2013). Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. *Nature Medicine*, 19, 1643–1648 doi: 10.1038/nm.3400.

[34] Basile, A., Hughes, R., Harrison, P., Murata, Y., Pannell, L., Jones, E., et al. (1991). Elevated Brain Concentrations of 1,4-Benzodiazepines in Fulminant Hepatic Failure. *New England Journal of Medicine*, 325(7), 473–478. doi: 10.1056/NEJM199108153250705.

[35] Jayakumar, A. R., Ruiz-Cordero, R., Tong, X. Y., & Norenberg, M. D. (2013). Brain Edema in Acute Liver Failure: Role of Neurosteroids. *Archives of Biochemistry and Biophysics*, *536*(2), 171–175. doi: 10.1016/j.abb.2013.03.007.

[36] Skowrońska, M., & Albrecht, J. (2012). Alterations of blood brain barrier function in hyperammonemia: an overview. *Neurotoxicity Research*, 21(2), 236–244. doi: 10.1007/s12640-011-9269-4.

[37] Verkhratsky, A., Matteoli, M., Parpura, V., Mothet, J., & Zorec, R. (2016). Astrocytes as secretory cells of the central nervous system: idiosyncrasies of vesicular secretion. *The EMBO Journal*, *35*(3), 239–257. doi: 10.15252/embj.201592705.

[38] Noell, S., Wolburg-Buchholz, K., Mack, A. F., Beedle, A. M., Satz, J. S., Campbell, K. P., & Fallier-Becker, P. (2011). Evidence for a role of dystroglycan regulating the membrane architecture of astroglial endfeet. *The European Journal of Neuroscience*, *33*(12), 2179–2186. doi: 10.1111/j.1460-9568.2011.07688.x.

[39] Ren, Z., Iliff, J., Yang, L., Yang, J., Chen, X., Chen, M., et al. (2013). 'Hit & Run' Model of Closed-Skull Traumatic Brain Injury (TBI) Reveals Complex Patterns of Post-Traumatic AQP4 Dysregulation. *Journal of Cerebral Blood Flow & Metabolism*, 33(6), 834–845. doi: 10.1038/jcbfm.2013.30.

[40] Li, L., Zhang, H., Varrin-Doyer, M., Zamvil, S. S., & Verkman, A. S. (2011). Proinflammatory role of aquaporin-4 in autoimmune neuroinflammation. *The FASEB Journal*, *25*(5), 1556–1566. doi: 10.1096/fj.10-177279.

[41] Filippidis, A. S., Carozza, R. B., & Rekate, H. L. (2016). Aquaporins in Brain Edema and Neuropathological Conditions. *Journal of Molecular International Sciences*, 18(1), 55. doi: 10.3390/ijms18010055.

[42] Rama Rao, K. V., Chen, M., Simard, J. M., & Norenberg, M. D. (2003) Increased aquaporin-4 expression in ammonia-treated cultured astrocytes. *Neuroreport,* 19, 14(18), 2379–82. doi: 10.1097/01.wnr.0000099610.19426.a6.

[43] Bodega, G., Suárez, I, López-Fernández, L. A., García, M. I., Köofber, M., Penedo, M., et al. (2012) Ammonia induces aquaporin-4 rearrangement in the plasma membrane of cultured astrocytes. *Neurochem. Int,* 61, 1314–1324. doi: 10.1016/j.neuint.2012.09.008.
[44] Wright, G., Soper, R., Brooks, H. F., Stadlbauer, V., Vairappan, B., Davies, N. A., et al. (2010). Role of aquaporin-4 in the development of brain oedema in liver failure. *J Hepatol,* 53, 91–97. doi: 10.1016/j.jhep.2010.02.020.
[45] Iliff, J. J., & Nedergaard, M. (2013). Is there a cerebral lymphatic system? *Stroke; a Journal of Cerebral Circulation*, *44*(601), S93–S95. doi: 10.1161/STROKEAHA.112.678698.
[46] Stokum, J. A., Kurland, D. B., Gerzanich, V., & Simard, J. M. (2015). Mechanisms of Astrocyte-Mediated Cerebral Edema. *Neurochemical Research*, 40(2), 317–328. doi: 10.1007/s11064-014-1374-3.
[47] Thumburu, K. K., Dhiman, R. K., Vasishta, R. K., Chakraborti, A., Butterworth, R. F., Beauchesne, E., et al. (2014). Expression of astrocytic genes coding for proteins implicated in neural excitation and brain edema is altered after acute liver failure. *J Neurochem,* 128(5), 617–627. doi: 10.1111/jnc.12511.
[48] Butterworth, R. F. (2015). Pathogenesis of Hepatic Encephalopathy and Brain Edema in Acute Liver Failure. *Journal of Clinical and Experimental Hepatology*, *5*(Suppl 1), S96–S103. doi: 10.1016/j.jceh.2014.02.004.
[49] Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., et al. (2012). A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β. *Science Translational Medicine*, *4*(147), 147ra111. doi: 10.1126/scitranslmed.3003748.
[50] Goldbecker, A., Buchert, R., Berding, G., Bokemeyer, M., Lichtinghagen, R., Wilke, F., et al. (2010). Blood–brain barrier permeability for ammonia in patients with different grades of liver fibrosis is not different from healthy controls. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *30*(7), 1384–1393. doi: 10.1038/jcbfm.2010.22.
[51] Castejón, O. J. (2012). Ultrastructural pathology of endothelial tight junctions in human brain oedema. *Folia Neuropathol,* 50(2), 118–129.
[52] Brkic, M., Balusu, S., Libert, C., & Vandenbroucke, R. E. (2015). Friends or Foes: Matrix Metalloproteinases and Their Multifaceted Roles in Neurodegenerative Diseases. *Mediators of Inflammation*, *2015*, 620581. doi: 10.1155/2015/620581.
[53] Nguyen, J. H., Yamamoto, S., Steers, J., Sevlever, D., Lin, W., Shimojima, N., et al. (2006). Matrix metalloproteinase-9 contributes to brain extravasation and edema in fulminant hepatic failure mice. *Journal of Hepatology*, *44*(6), 1105–1114. doi: 10.1016/j.jhep.2005.09.019.
[54] Jayakumar, A. R., Tong, X. Y., Ospel, J., & Norenberg, M. D. (2012). Role of Cerebral Endothelial Cells in the Astrocyte Swelling and Brain Edema Associated with Acute Hepatic Encephalopathy. *Neuroscience*, *218*, 305–316. doi: 10.1016/j.neuroscience.2012.05.006.
[55] Larsen, F. S., Gottstein, J., & Blei, A. T. (2001). Cerebral hyperemia and nitric oxide synthase in rats with ammonia-induced brain edema. *Journal Of Hepatology,* 34(4), 548–554. doi: 10.1016/S0168-8278(00)00069-6.
[56] Zemtsova, I., Gorg, B., Keitel, V., Bidmon, H. J., Schror, K., & Haussinger, D. (2011). Microglia activation in hepatic encephalopathy in rats and humans. *Hepatology,* 54(1), 204–215. doi: 10.1002/hep.24326.
[57] Dennis, C. V., Sheahan, P. J., Graeber, M. B., Sheedy, D. L., Kril, J. J., & Sutherland, G. T. (2014). Microglial proliferation in the brain of chronic alcoholics with hepatic encephalopathy. *Metabolic Brain Disease*, 29(4), 1027–1039. doi: 10.1007/s11011-013-9469-0.
[58] Verderio, C., Cagnoli, C., Bergami, M., Francolini, M., Schenk, U., Colombo, A. et al. (2012). TI's – the VAMP/VAMP7 is the SNARE of secretory lysosomes contributing to ATP secretion from astrocytes. *Biol Cell,* 104(4), 213–28. doi: 10.1111/boc.201100070.
[59] Sarkar, A., Mitra, S., Mehta, S., Raices, R., & Wewers, M. D. (2009). Monocyte Derived Microvesicles Deliver a Cell Death Message via Encapsulated Caspase-1. *PLoS ONE*, *4*(9), e7140. doi: 10.1371/journal.pone.0007140.
[60] Butterworth, R. F. (2011). Hepatic encephalopathy: a central neuroinflammatory disorder? *Hepatology,* 53(4), 1372–1376. doi: 10.1002/hep.24228.
[61] Shawcross, D. L., Sharifi, Y., Canavan, J. B., Yeoman, A. D., Abeles, R. D., Taylor, N. J., et al. (2011). Infection and systemic inflammation, not ammonia, are associated with Grade 3/4 hepatic encephalopathy, but not mortality in cirrhosis. *Journal of hepatology*, 54(4), 640–649. doi: 10.1016/j.jhep.2010.07.045.
[62] Wright, G., Davies, N. A., Shawcross, D. L., Hodges, S. J., Zwingmann, C., Brooks, H. F., et al. (2007). Endotoxemia produces coma and brain swelling in bile duct ligated rats. *Hepatology,* 45(6), 1517–1526. doi: 10.1002/hep.21599.
[63] Donnelly, M. C., Hayes, P. C., & Simpson, K. J. (2016). Role of inflammation and infection in the pathogenesis of human acute liver failure: Clinical implications for monitoring and therapy. *World Journal of Gastroenterology*, *22*(26), 5958–5970. doi: 10.3748/wjg.v22.i26.5958.
[64] Aldridge, D. R., Tranah, E. J., & Shawcross, D. L. (2015). Pathogenesis of Hepatic Encephalopathy: Role of Ammonia and Systemic Inflammation. *Journal of Clinical and Experimental Hepatology*, *5*(Suppl 1), S7–S20. doi: 10.1016/j.jceh.2014.06.004.
[65] Bajaj, J. S. (2014). The role of microbiota in hepatic encephalopathy. *Gut Microbes*, *5*(3), 397–403. doi: 10.4161/gmic.28684.

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