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Astrocyte Pathophysiology in Liver Disease

Carlos Pérez-Monter and Aldo Torre-Delgadillo

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Abstract

Liver disease is one of the major chronic disabilities around the world. It is known that global casualties are increasing because of virus C infection, alcohol consumption, or non-alcoholic circumstances. One of the main derived comorbidities of liver disease is the hepatic encephalopathy (HE), a severe neuropsychological syndrome derived from the acute or chronic liver disease. A key feature accounting for HE symptoms in cirrhotic patients is brain edema, which is triggered by hyperammonemia. In basal conditions, ammonia can be metabolized in the central nervous system (CNS) by astrocytes, which synthetize glutamine using ammonia and glutamate as substrates. In hyperammonemic conditions, astrocytes synthetize large amounts of glutamine generating a hyperosmotic condition, inducing these cells to become swollen in shape, invoking the characteristic symptom clinically manifested in patients with HE, as brain edema; this condition is regulated by water channels called aquaporins (AQPs) and by other molecules such as myoinositol. Experimental evidence suggests that some small non-coding RNAs may regulate AQPs expression both *in vivo* an *in vitro* and that some pharmacological interventions improve cognitive impairment in cirrhotic patients. It is undeniable that astrocytes and the different signaling pathways beneath its plasma membrane play a crucial role in liver disease-derived HE and represent some of the novel pharmacological targets to treat comorbidities of the liver disease.

Keywords: liver, hyperammonemia, encephalopathy, astrocytes, astrogliosis

1. Introduction

1.1. General aspects of liver disease and hepatic encephalopathy

Liver disease is one of the leading non-infectious pathologies affecting people around the world. In its report from 2015, WHO indicates that the advanced form of liver disease, meaning cirrhosis, is among the 20 most frequent (2%) causes of death (~1.62×10^6) around the globe (http://www.who.int/). Hepatic encephalopathy (HE) is a pathological condition that

represents the neuropsychiatric disorder derived from liver disease. It is known that about 80% of patients with liver disease (depending on severity), may develop HE, which represents one of the major complications leading to death in about 90% of patients with acute liver failure (ALV) [1]. HE has been classified by the Hepatic Encephalopathy consensus Group, at the World Congress of Gastroenterology in 1998, in type A, associated with ALV; type B related to porto-systemic bypass; and type C related with chronic liver disease, mainly cirrhosis [2]. Further classification includes the severity of symptoms that includes the subcategory called covert (CHE, also known as minimal), persistent (PHE), and overt (OHE). The former is mild in manifestations and is hard to diagnose without specific neurophysiological and neuropsychological tests, such as the visual-based flicker test, the psychometric hepatic encephalopathy score (PHES), the repeatable battery for assessment of neuropsychological status (RBANS), the inhibitory control test (ICT), the cognitive drug research (CDR), and the most recent STROOP App test (*EncephalApp_Stroop*), a practical smartphone App tool for HE screening to be used by the own patients [3, 4]. The PHE and OHE involve more specific clinical symptoms, including seizures, hyperreflexia, rigidity, myoclonus (sudden involuntary jerking of a muscle or a group of muscles), asterixis (hand tremor when wrist is extended), and stupor, which can be detected or reported by the clinician or patient as well [5]. In all of these cases, the main gross causative is the liver failure and the metabolic pathways affected thereafter. In general terms, HE is a debilitating condition in which patients gradually became less psychologically independent and more psychiatrically dependent. The majority of the symptoms in HE are triggered by molecular events within the central nervous system (CNS), and several hypotheses have been proposed in order to understand the pathophysiology. Some of them suggest that metabolites such as ammonia, myoinositol, glutamine, manganese, inflammatory mediators (IL-1, IL-6, and TNFα), and amino acids (Tyr, Phe, Val, Ile, and Leu) [6], regarding its effects over astrocytes and neuronal cells, are responsible of the clinical manifestations of the disease [6–8].

2. Astrocytes as mediators of the pathophysiology of HE

In this and further sections, we will try to introduce to the reader into a review about recent findings that illustrates how the physiological functions of the liver, or to a better extent, a lack of them, may harm at different levels, the metabolic and physiological homeostasis over brain cells and function.

2.1. Ammonium metabolism

The liver is responsible to detoxify the majority of endogenous or exogenous toxic compounds produced by the metabolic or catabolic activity within the organism, including pharmacological metabolites, ammonium, bilirubin, bile salts, etc. Several factors may affect liver functions, such as increased consumption of a fat diet, another one is the chronic alcohol abuse; while the infection by hepatitis C virus (HCV), pharmacological intoxication (mainly paracetamol), complete the pathological scenario. The fatty liver, also known as steatosis (non-alcoholic fatty liver disease or NAFLD), has been called the first hit in liver damage, which when not properly attended, could result in a chronic inflammation or steatohepatitis (NASH). NASH could evolve to fibrosis and eventually to a more critical stage, cirrhosis. Cirrhotic patients suffer a

wide spectrum of comorbidities [9]. Among them is hepatic encephalopathy (HE), a debilitating psychological condition where the patients present a variety of symptoms including loss of memory, mild or manifested tremors, and most importantly, increased plasma levels of ammonia. In basal conditions, ureotelic organisms like humans, should contend with the ammonia derived from the gastrointestinal bacterial activity and muscle metabolism, by converting it to carbamoyl-phosphate (CP), a substrate of the urea cycle, CP is then converted to citrulline, the precursor of arginosuccinate; arginosuccinate is then transformed to arginine and by the action of arginase, arginine is converted to urea and ornithine; all of these reactions takes place within the mitochondria and cytosol of the hepatocytes, resulting in the release of urea into the bloodstream and then in urine [10]. However, in pathological conditions like cirrhosis, hepatocytes are unable to produce urea; therefore, ammonium in its form of gas (NH_3) easily diffuses to the blood brain barrier (BBB). Once within CNS, ammonium is metabolized primarily by the astrocytes, a group of specialized glial cells capable of modulate inhibitory and excitatory neurotransmission. In its form of ion (NH_4^+), ammonia reaches the astrocytes where a different transporter mediates its translocation throughout the plasma membrane. Among this transporters are the Na^+/K^+-ATPase pump, the Na^+/H^+-ATPase antiporter and $Na^+/K^+/2Cl^-$, K^+/Cl^- symporters [5]. Astrocytes metabolize the ammonium by the so-called glutamate-glutamine cycle (GGC). The GGC produces glutamine (Gln) through the action of glutamine synthetase (GS), delivering it to neighboring glutamatergic neurons (**Figure 1**). In neurons, glutamine is subject to the opposite reaction, mediated by the enzyme glutaminase, whose products are glutamate and ammonium. Glutamate is packaged into synaptic vesicles and released to the synaptic cleft in response to electrochemical stimulus, while ammonium is released to the extracellular space [11]. Once released, glutamate binds to ionotropic (N-methyl-D-aspartate (NMDA) or AMPA) receptors, or metabotropic (mGluR1 and mGluR2) receptors in the postsynaptic neuron and astrocytes [11]. Unbound released glutamate can be
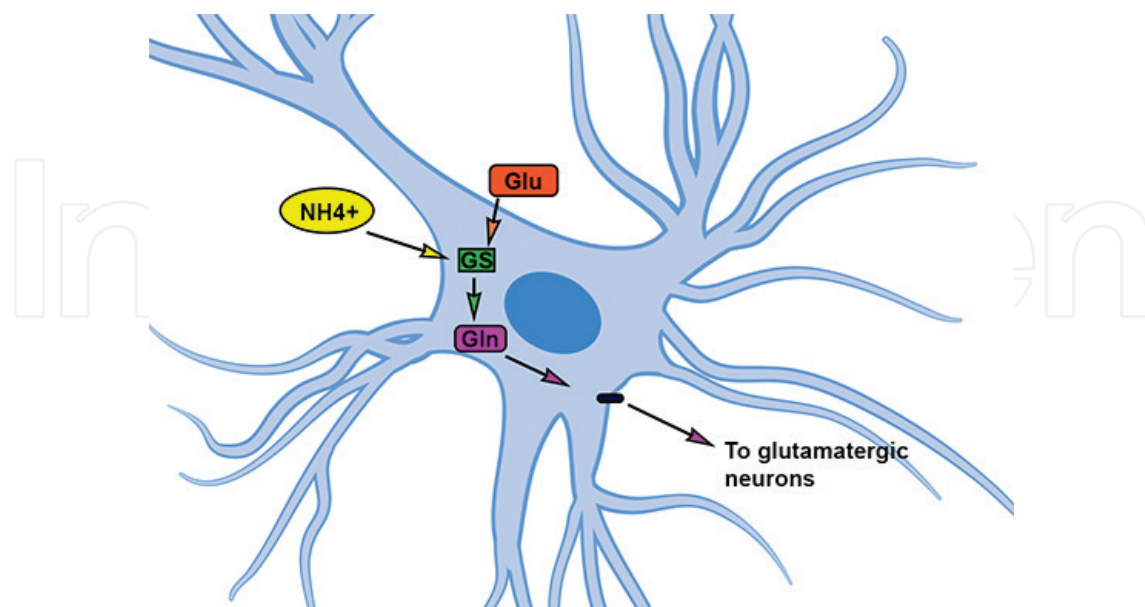
Figure 1. The glutamine (Gln)-glutamate (Glu) cycle within astrocytes. Glutamine synthase (GS) mediates the reaction of ammonia (NH_4^+) and Glu to produce Gln. Gln is then exported by a specific transporter (illustrated here as a small black cylinder) to the glutamatergic neurons near the astrocyte. The cycle completes when Glu is released to the synaptic cleft and then binds to NMDA receptors or is recaptured by astrocytes to began the cycle. See text for details.

recycled when captured by the excitatory amino acid transporters (EAAT) located in the astro-cytes membrane. These events represent the final step in the GGC.

On the other hand, besides GGC, astrocytes may use other systems to titrate ammonium from bloodstream. Those based in the use of the branched chain amino acids (BCAA) on one side, or the use of L-ornithine and L-aspartate in the other. In the first case, BCAA are metabolized by the enzyme branched-chain amino acid transaminase (BCAT) particularly enriched in the cyto-plasm and axons of glutamatergic and GABAergic neurons [12]; cytoplasmic BCAT enzymati-cally mediates the synthesis of branched-chain ketoacids (BCKA), which in turn generates both, a precursor for the tri-carboxylic acid cycle (TCA) in the form of Acetyl-CoA and the excitatory amino acid glutamate; glutamate in turn, can be metabolized by means of the GS to produce glutamine; glutamine is deaminated by the phosphate-activated glutaminase (PAG) which is also present in the cytoplasm of neuronal and glial cells [13]. The second one, the L-Ornithine and L-Aspartate system, operates to generate glutamate by means of the ornithine- or aspartate-aminotransferase (OAT, AAT); both enzymes are enriched in the skeletal muscle, thus contrib-uting to stimulate the glutamine production in this tissue by the GS, diminishing the ammonia derived from circulation [5]. L-ornithine and L-aspartate represent one of the most frequent therapeutic approaches to improve ammonia levels in cirrhotic patients, as we shall see later.

2.2. Astrocytes and the effect of hyperammonemic conditions within CNS

It is known that NH_4^+ and K^+ have comparable physical and chemical properties, so that any change or increase in NH_4^+ concentrations may activate the Na^+ transport by the Na^+/K^+-ATPase pump, leading to abnormal neurotransmission in neurons or even astrocytes [14]. NH_4^+ enters to the astrocytes and induces the increase in the activity of the GS; this event results in the accumulation of glutamine. As intracellular glutamine concentrations rise, the osmotic pressure does it in parallel, provoking the astrocytes to become swelling and to acti-vate two main mechanisms to counteract this morphological change [15]. The first one consists of the release of an osmotic regulator, myoinositol; myoinositol leaves the astrocyte to redress the osmotic balance within the cell to prevent astrocyte and cerebral edema; while the other one is to increase the activity of the water channels called aquaporins (AQPs). One of the main water channel transporters related with regulation of the osmotic response in astrocytes, is the aquaporin 4 (AQP4) [16]. It has been reported that AQP4 increases its activity under hyper-ammonemic conditions, allowing the H_2O molecules to enter into the cell, thus relieving the osmotic pressure induced by the accumulation of glutamine [17]. Until then, it was not clear whether the increased activity of AQP4 was the result of a higher transcriptional activity or an increased rate of mRNA translation [18]. Evidence in favor of both options had been reported. In two separate reports, Jalan et al. and Norenberg et al. demonstrated independently the increased expression of AQP4 protein, in a chronic or acute *in vivo* models of liver failure based on the bile duct ligation (BLD) or the use of thioacetamide (TAA) or acetaminophen (AAP), respectively [19, 20]. Accordingly, *in vitro* evidence indicates that ammonia-treated astrocytes also increase AQP4 protein levels [21]. On the other hand and in favor of tran-scription, additional evidence indicates that ammonium or mannitol, both osmotic stressors, increase the expression of AQP4 mRNA *in vitro* and *in vivo*, probably mediated by the p38-MAPK signaling pathway, which stimulates the tonicity-enhancer binding protein (TonEBP) to interact with the responsive enhancer element (TonE) over the AQP4 gene promoter region

[17, 19, 22]. Further evidence indicates that other AQPs are direct targets of the hypothalamic arginine-vasopressin peptide (AVP) and the angiotensin II (ANGII) hormone; for instance, AVP controls the expression of AQP2 in the renal collecting duct cells under basal conditions, increasing the intracellular levels of cAMP that in turns modifies the activity of one of its targets, PKA; PKA phosphorylates the CREB protein allowing its translocation to the nucleus and its transcriptional activity [23]. It remains to be elucidated if AQP4 gene expression in hepatic encephalopathy conditions is also regulated by similar mechanisms.

As we have seen, hepatic failure is closely related to astrocyte low grade and brain edema mediated by water movement throughout the AQP4 channel. Among the pathogenic factors contributing to aggravate this condition are the free radical production, the MAP-kinase activity, and the induction of the mitochondrial permeability transition (MPT) process. MPT is well known to occur in response to the sudden intra-cytoplasmic increase of molecules around 1.5 kDa in size that enters the mitochondria mediated by the so-called permeability transition pore (PTP), which is located at the inner membrane [24]. These phenomena critically compromises mitochondria homeostasis and the metabolic function of many crucial processes like the TCA and ATP synthesis, promoting the generation of reactive oxygen species (ROS). It seems that HE promotes the generation of ROS, therefore inducing the MPT due to the oxidative stress, because indirect evidence demonstrates that antioxidants are able to reduce MPT. Norenberg et al. demonstrated that MPT is involved in the ammonium-induced astrocyte swelling after their *in vitro* experiments based on the use of cyclosporine A (CsA), a well-known inhibitor of MPT [25]; in this study, the expression of the AQP4 protein was also inhibited by CsA, indicating a plausible crosstalk between different signaling pathways, such as the MAP-kinases or that conformed by the protein kinase A (PKA)/CREB, both of which might be related to the control of cell swelling in hyperammonemic conditions.

2.3. Systemic and local inflammatory pathway effects on astrocyte disturbances

Another important mechanism of regulation of astrocyte physiology in response to hepatic failure is the activation of the pro-inflammatory response. In recent years, an increasing role of the inflammatory-related pathways in response to ammonia-induced brain dysfunction has been reported. The cytokines promoting brain damage are TNFα, interlukin-6 (IL-6), interlukin1-beta (IL-1β), IL-18, and many others. However, there is controversy about the role between these pro-inflammatory cytokines in both acute and chronic hepatic failure. Several groups have provided evidence of a positive correlation in this interaction in human subjects or animal models. In 2007, Wright and colleagues determined the TNFα, IL-6, and IL-1β artery blood levels and found a positive correlation of these cytokines with intracranial pressure in ALF-affected humans [26]. Further evidence indicates that the same cytokines are elevated, at least at the transcriptional level, in the brains of a rat ALF model [27]. A recent interesting proposal about the role of these circulating inflammatory interleukins indicates that they might interact with certain blood-brain barrier (BBB) endothelial cells to reach the CNS, acting synergistically with ammonia. This synergistic model has been expanded through the identification of additional factors such as GABA produced by certain bacterial families within the gut, contributing to modify the GABAergic tone that reach the CNS via the vagal afferent pathway [28]. In view of these facts, systemic inflammation is in part responsible for the astrocyte and neuronal network alterations in both acute and chronic liver disease. A major player in

the systemic inflammation effects over CNS in these pathologies is a member of the danger-associated molecular patterns (DAMPS) called high mobility group box protein 1 (HMGB1), which in basal conditions regulates transcriptional activity [29]; however, in pathological conditions, it can be released passively from damaged cells into the extracellular space in response to pro-inflammatory stimuli, such as LPS; HMGB1 can also be actively released by a JAK/STAT acetylation-mediated process, from immunocompetent cells [30]. In a recent study, Ohnishi et al. showed that HMGB1 significantly decrease the expression of AQP4 mRNA and protein in cultured astrocytes; conversely, the intra-cerebroventricular injection of HMGB1 in adult rats, slightly increase AQP4 protein expression and as expected, the brain edema; further, exposure of microglia to a HMGB had a significant effect over IL-1β mRNA expression and protein release, which apparently regulates AQP4 increased expression; the authors suggest that the pathway IL-1β-NF-κB-AQP4 in microglia might regulate the brain edema in response to HMGB1-mediated systemic inflammation [31]. Whether this pathway might occur with other pro-inflammatory cytokines such as TNFα, IL-6, or IFNγ, remains to be investigated.

The unusual extracellular histone proteins represent another piece in the puzzle of the systemic inflammation response. It has been showed that in ALF or HBV infected patients, increased concentrations of circulating histones correlates with an immunostimulatory effect, leading to multiple organ injuries [32–34]. *In vitro* experiments with hepatic or monocyte cell lines stimulated with sera obtained from ALF patients, elicit cell death or release of inflammatory cytokines, respectively; interestingly, these effects were abolished with heparin—a histone-binding anticoagulant—suggesting that histone proteins are key players in the cellular injury and systemic inflammation observed in ALF-affected patients [32]. In addition to previously described consequences of the liver disease, including hyponatremia, sepsis, variceal bleeding, and renal failure; is the activation of microglial cells, the resident macrophages of the brain. Microglia increase the synthesis and release of inflammatory cytokines in a process called microgliosis [35]. Microgliosis is part of the most general response gliosis, which involves the astrocytes, the microglia, and oligodendrocyte cells; microgliosis is the local response to brain insults and typically represents the scar-promoting mechanism within the CNS.

Along with systemic and local brain inflammation, the increased ammonia and manganese levels in the brain also alter the TCA cycle, promoting lactate accumulation and the promotion of dopaminergic cell death, as well as the generation of reactive oxygen species (ROS) in basal ganglia, and contributing in this way to the parkinsonism-like behavior and cognitive impairment observed in cirrhotic patients [36]. In this line of evidence, oxidative stress is thought to be of relevance for ammonia toxicity in HE. Molecular studies indicate that acute ammonia loading, mechanical or drugs stressors like hypo-osmolarity, diazepam, and TNFα, *in vitro* and *in vivo*, increases ribonucleic acid (RNA) oxidation. Among the oxidized RNA species, the 18 s rRNA and the glutamate/aspartate transporter (GLAST/SLC1A3) mRNA have been described; strikingly, the cerebral RNA oxidation in liver-injured rats predominates in the transport RNA granules located in close vicinity with postsynaptic spines, where learning and memory-associated protein synthesis occurs [37]. These data strongly suggest that ammonia-induced inflammation and RNA oxidation might impair both cognitive events in cirrhotic patients. In addition, it has been demonstrated that ammonia inhibits astrocyte proliferation promoting senescence both *in vitro* and *in vivo*, by means of the multidrug resistance-associated protein (Mrp) 4 and by a p38/MAPK-dependent activation of the cell cycle inhibitor genes GADD45α and p21 [38, 39].

2.4. The role of the intestinal microbiota in the gut-liver-brain axis

The intestinal microbiota has evolved as a new and relevant player in the pathogenesis of several intestinal and non-intestinal diseases. As the liver is the organ in closest contact with the intestinal tract, it is potentially exposed to bacterial components and metabolites. In physiological conditions, nutrients and bacterial compounds translocate to the liver via the portal circulation and contribute to the host homeostasis. As the epithelial wall and mucus layer act as physical barrier to impede that the most of the bacterial components or even bacteria reach the blood flow, it is usually accepted that a small quantity of these compounds enter the portal venous flow, and a tight balance on the immune response is achieved in order to fight against potential exogenous insults. However, in pathological conditions, such a physical barrier can become more permeable to bacterial components, specially lipopolysaccharide (LPS), flagellin, peptidoglycan, and microbial nucleic acids [40]. Evidence about the effect of gut microbiota over hepatic physiology has been demonstrated. In 2010, Gupta and co-workers found that small intestine bacterial overgrowth (SIBO) was a hallmark in cirrhotic patients suffering of HE [41]; in the same year, Jun et al. published evidence about the direct association of SIBO with the peripheral founding of bacterial DNA in cirrhotic patients [42]; later on, in 2012, Henao-Mejia et al. demonstrated that genetic deficiency of inflammasome, a protein complex considered the sensor of endogenous and exogenous pathogen-associated molecular patterns (PAMPS), induced the accumulation of bacterial components in the portal circulation, with the consequent liver damage effect [43]. One important fact about the bacterial overgrowth in cirrhotic patients is that the translocation of bacteria or any of its components promotes and aggravates the liver illness. There are several reports indicating the differences in the fecal microbiota of various populations around the globe, specifically in patients with liver cirrhosis in comparison with healthy subjects. In general, these studies coincides in founding that the *phyla Proteobacteria* and *Fusobacteria*, significantly increases in cirrhotic patients, compared with its healthy counterparts [44–46]; while at the genus level, the pathogenic *Enterobacteriaceae*, *Alcaligenaceae*, *Porphyromonadaceae*, *Veillonellaceae*, and *Streptococcaceae* families prevail and the beneficial taxa such as *Lachnospiraceae* and *Bifidobacteriaceae*, diminished in cirrhotic samples [45, 47]. In our laboratory, we conducted a pilot study in order to explore the changes in the microbiota of cirrhotic patients. Preliminary results indicate that some of these taxa previously reported are also present in the cirrhotic group of VHC-infected patients based in Mexico city; but we have also found that some other taxa have not been reported in other population (unpublished data); our findings are in concordance with the idea that additional variables, such as ethnicity or geographical location and even food ingestion habits, should be taken into account in order to interpret microbiome-derived studies, which eventually can be used in future clinical trials.

Another significant consequence of SIBO is the higher tendency of the microbial community to increase the synthesis and release of organic compounds with a metabolic effect over a wide number of host cells. For example, it has been demonstrated that ingestion of *L. rhamnosus*, diminishes the expression of gamma-amino-butyric acid (GABA) receptor (GABA$_B$) in the cingulate and prelimbic cortical regions of mice brain; this GABA$_B$ regulation implies a direct effect over neuronal physiology, which reflects in less anxiety and more antidepressant-like behavior in mice; moreover, vagotomised mice were unable to respond to *L. rhamnosus*, indicating that vagus nerve acts as an interface between gut bacteria and the brain [28]. Whether these bacteria produce a GABA-like neurotransmitter or directly modify the fire-threshold from vagus

nerve remains a matter of study. Nowadays, molecular studies contributing to explain partially the mechanics behind the role of GABA and the intestinal microbiota have been published. Butterworth and colleagues in 1990 and 2008, found that brain samples from cirrhotic patients who died from HE, had unusual elevated levels of the peripheral-type benzodiazepine receptors (PTBZR) and of neurosteroids such as allopregnanolone (ALLO) or tetrahydrodeoxycorticosterone (THDOC) [48, 49]; interestingly, these neurosteroids are synthesized in the brain following activation of the translocator protein (TSPO), whose main activity is the cholesterol transport through the mitochondria, where stimulates the steroid biosynthesis [50]. Further evidence from Noremberg and co-workers indeed, indicates that astrocyte exposure to ammonia and manganese, significantly increase the TSPO activity, at least *in vitro* [51], indicating that neurosteroids are one of the main metabolites synthetized in response to hyperammonemic conditions as a result of dysbiosis and a lack of a functional urea cycle. However, there is some evidence indicating that some other metabolites produced by gut microbiota, such as the bile acids, tryptophan precursors, serotonin and catecholamine, might be able to signal through membrane receptors located in local cells of the gut epithelium or by a neurocrine or endocrine pathways; among these metabolites are the carbohydrate derivatives butyrate, acetate and propionate, which might be converted to short chain fatty acids (SCFAs); these SCFA in turn can reduce food intake and modulate the immune system response in the host [52, 53]. Different reports indicate that SCFAs are present in the enteroendocrine cells and on neurons of the submucosal and myenteric ganglia [54, 55]. Besides the SCFAs, it cannot be ruled out the possibility that some Gram-positive cell wall components are also capable of inducing neuronal or glial cell damage within the CNS of cirrhotic patients. In 2005, a report indicated that lipoteichoic acid (LTA) and muramyl dipeptide from *Staphylococcus aureus* induce a strong inflammatory response that ultimately lead to neuronal death by increasing nitric oxide (NO), superoxide (O_2-), peroxynitrite (ONOO-), TNFα, IL-1β and IL-6, within astrocytes and microglia [56].

Here, we can say that the net result of the so-called "*dysbiosis*" in cirrhotic patients is that a plethora of metabolic compounds like urea, methanol, short-chain fatty acids (SCFAs), and other volatile compounds are delivered or restricted to the host via the gastrointestinal tract. Altogether, these data indicate that intestinal bacteria play key roles in development and pathophysiology of hepatic liver over the CNS.

2.5. Genetic and epigenetic effects induced by HE in astrocytes

Epigenetic modifications are heritable and reversible stable marks that do not modify gene sequences *per se*, but have tremendous impact over the gene expression process. These marks allow the genome to adapt its transcriptional repertoire to different cellular and molecular conditions in response to environmental cues for fine-tuning of gene expression, and encompass a myriad of processes including histone, DNA and RNA modifications. One of the best-studied DNA modifications is the methylation (5-mC) and 5-hidroxy-methylation (5-hmC) of cytosine bases at the CpG dinucleotide sequence, mediated by the DNA methyltransferase (DNMT) or by the translocated in liposarcoma, *E*wing's sarcoma and *T*ATA-binding protein-associated factor 15 (TET)-family of proteins, respectively [57]. It is widely accepted that the first mark, 5-mC, is a negative signal for gene expression to occur, while the 5-hydroxy-mC indicates the opposite. The role of DNA methylation dynamics within astrocytes is not yet known, at least in the context of HE derived from liver disease; however, specific data indicate

that the NMDA receptor NR2B gene is negatively regulated by the neuron restrictive silencer factor (NRSF) in ethanol-exposed cortical neurons *in vitro* [58]; although specific analysis in this study indicates that NRSF directly interacts with the NR2B gene promoter region, there is no evidence of how ethanol regulates NRSF gene expression. Further evidence about the role of methylation or acetylation of glutamatergic or gabaergic-mediated signaling pathways affected by hyperammonemia remains to be elucidated.

On the other hand, we know that non-coding RNAs (ncRNA) are important players in epigenetic mechanisms regulating cell fate and metabolism. Among the ncRNAs with relevant physiological roles are small RNAs and the long non-coding-RNAs (lncRNAs). A variant of small RNAs includes the microRNAS (miRNAs), which are beginning to acquire significant roles in coordinating astrocyte gene expression. miRNAs are small RNAs (~22 nt in length) that controls gene expression through their binding to the 3'-untranslated region (UTR) in mRNAs, leading transcripts to degradation or translational repression [59]. Long noncoding RNAs (lncRNA) on the other side are sequences of about 200 nt in length, which regulate the expression of neighbor protein-coding-genes in a phenomenon called "transvection" (for a systematic review see [60]). In the last decade, there has been an increase in the number of reports demonstrating the role of both miRNAs and lncRNAs in the physiopathology of HE. Two of the first reports regarding the role of two specific miRNA in the regulation of astrocyte cell swelling came from Singapore; in 2010 and 2012, Jeyaseelan and colleagues published in separate papers that miR-130a and miR-320a directly affect the AQP4 expression in the CNS. In the first case, the role of miR-130a is a non-canonical regulation, because the repression mechanism occurs at the transcriptional level over the AQP4 M1 gene promoter [61], while the mir320a was described as a modulator of AQP1 and AQP4 in a model of cerebral ischemia [62]. In a more wide recent study, Häussinger and co-workers explored the global miRNA profile using NH4Cl stimulated rat brain astrocytes *in vitro*. They were interested in the ammonia-dependent senescence observed in astrocytes and its relationship with miRNAs. By means of miRNA array experiments, they found that 43 of 336 miRNAs were significantly downregulated in hyperammonemic conditions, six of which (miR-31a-5p, miR-221-3p miR-221-5p, miR-222-3p, miR-326-3p, and miR-365-3p) seem to bind and regulate the mRNA encoding the heme oxygenase 1 (HO-1) protein; four of these miRNAs (miR-31a-5p, miR-221-3p, miR-222-3p, and miR-326-3p) were prevented to be downregulated by NH4Cl treatment, when astrocytes were exposed to the glutamine synthase inhibitor, methionine sulfoximine (MSO); moreover, the NADPH oxidase inhibitor, apocynin, fully prevented NH4Cl-mediated downregulation of the four miRNAs predicted to target HO-1, indicating that senescence is regulated by miRNA expression in cultured astrocytes and partly regulated by glutamine synthesis and NADPH-oxidase activity [63]. Data recently obtained in our laboratory indicate that three miRNAs (miR-29a-5p, miR-29b-3p, and miR-148b-3p) are repressed in brain tissue of a mouse model of liver cirrhosis. Interestingly, we found that the same miRNAs were downregulated in *in vitro* experiments using primary astrocytes incubated in the presence of NH4Cl (**Figure 2**); thus indicating that these miRNAs are strongly correlated with the physiological regulation of AQP4, both in basal and pathological conditions. Future experiments will allow us to demonstrate whether overexpression of these miRNAs by means of systemic or direct injection in hyperammonemic conditions counteracts the astrocyte cytotoxic edema.

The lncRNAs, on the other hand, had been more less studied in the context of liver-associated CNS pathologies; however, in a recent study in which 35,923 lncRNAs were screened using
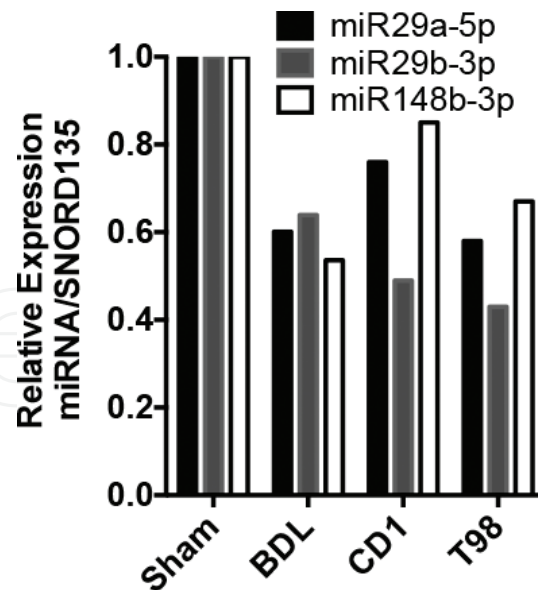
Figure 2. The expression of miR-29 and miR-148 diminished in response to hyperammonemia in vivo and in vitro. Total RNA extracted from the whole brain of bile duct ligated (BDL) mice or control mice (Sham), as well as primary astrocytes (CD1) or the astrocytoma cell line T-98 in the presence or absence of NH_4Cl, was used to evaluate the expression by means of qPCR. The data represent the mean expression of at least three replicates in each case (unpublished data).

microarrays, Silva and colleagues found that 380 and 486 transcripts were upregulated or down-regulated in a mouse model of acute liver failure. The authors found that some of these lncRNAs might be related to the cytokine-receptor interaction, MAPK, insulin, NFκB, and TNFα signaling pathways, all of them related to the inflammatory response; the authors also found that the lncRNA uc007pjf.1 associated with a guanine nucleotide exchange factor (NET1), which regulates RhoA, is involved in the cytoskeleton dynamics, suggesting a relationship of this factor with the astrocyte cell swelling observed in HE [64]; these data are of relevance because contributes to corroborate the role of some genetic pathways already related with ALF and certainly established the roots to new discoveries; however, these findings are the result of a combination of cellular phenotypes within CNS and are hardly extrapolated solely to astrocytes, neurons, microglia or even to non-differentiated precursor cells residing in the frontal cortex; complementary *in vitro* or FACS-sorted cells experiments, should be carried out in order to acutely assign the contribution of each of these cell phenotypes to the physiopathology of liver disease.

2.6. Clinical data in osmoregulation and systemic infections in cirrhotic patients with HE

The maintenance of a constant cell volume is a critical problem of all cells. Cell swelling or shrinkage is undesirable for normal cellular function. Changes in the cell volume mostly occur due to changes in the extra or intracellular osmolarity. As most cell membranes are freely permeable to water and do not possess water pumps in their membranes, cells will shrink or swell in response to changes in the tonicity of the extracellular fluid (ECF) [65]. Cells will shrink in a hypertonic ECF, while they swell in a hypotonic ECF. Similarly, when the tonicity of the intracellular space of the cells increases, the cells try to compensate it and increase the osmolarity by the uptake of water from the ECF—and consequently swell. Extra- and intracellular osmolarity is determined by the concentrations of several compounds, such

as ions, amino acids, etc. Consistent with the adaption against the rise of intracellular osmolarity in patients affected by CLF, it has been proposed that reductions in brain concentrations of myoinositol are implicated in the pathogenesis of ammonia-induced brain edema [66].

In the same line of evidence, it is difficult to elucidate whether the release of taurine (an atypical aminoacid with osmoregulatory properties) from the astrocytes to the ECF is an osmoregulatory response to increased intracellular glutamine and/or cell swelling, since other mechanisms that increased intracellular osmolarity may lead to the cellular release of taurine and taurine may also be released from neurons. Taurine has been related with reversal of hepatic hypertension in a rat model of liver cirrhosis; in a study from 2009, Liang and colleagues found that natural taurine significantly decreased the portal venous pressure, resistance and flow, and markedly decrease the nitric oxide (NO) and cyclic guanine-monophosphate (cGMP), with a concomitant reduction in the pathological status of liver tissue damage and the expression of collagen 1 (COL-1), COL-III, and transforming growth factor-$\beta1$ (TGF-$\beta1$) [67]. Taurine also have clinical uses in the form of a hydrophilic bile acid called tauroursodeoxycholate (TUDCA) for treatment of primary biliary cirrhosis (PBC). In a randomized cross-over study with 12 female patients suffering from PBC, the use of TUDCA showed to improve the enrichment of biliary ursodeoxycholate (UDCA) and was better absorbed than ursodeoxycholate and undergoes less biotransformation than UDCA, thus suggesting that TUDCA is clinically relevant for the treatment of cholestatic liver diseases [68]. As taurine is synthetized in both liver and brain it is plausible that HE might impact on taurine metabolism and biological action. Several reports have indeed described the pathological disturbances in patients with chronic liver disease as well as in liver failure murine models. In both cases, the taurine concentrations were significantly diminished [69, 70]. Astrocytes synthetize and stored taurine, which is involved in ion movement across CNS, mainly K^+ and Ca^{2+}; therefore, is not rare to consider that neural excitability modifications in cirrhotic patients occurs. Evidence in favor of this hypothesis was reported in an experimental model of ALF, in which CSF taurine concentrations significantly increased in a positive correlation with early progression of HE [71]. As taurine has osmoregulatory actions any increase or decrease in its extracellular concentrations could have an impact in the pathogenesis of acute liver failure as has been demonstrated [72]. In contrast, taurine concentrations in chronic liver failure are not changed, but the release of taurine into the extracellular space could represent a control mechanism for volume regulation of astrocytes [73, 74].

Another important metabolite that has been related with HE is the acidic calcium binding protein S100β, produced by astrocytes in the CNS and apparently secreted in response to oxidative stress, exerting its paracrine or autocrine effects on glia, neurons, and microglia [75–77]. S100β is a multifunctional protein that can be measured in basal conditions in CSF and is barely detected in circulating blood flow; however, in pathological conditions, its serum levels might increase, indicating neuronal or astrocytic damage. Our group and others have begun to investigate the diagnostic efficacy of S100β as a biomarker to detect low grade HE in cirrhotic patients. Recent findings from our laboratory indicate that cirrhotic subjects with OHE have higher serum levels of S100β when compared with non-HE cirrhotic or cirrhotic patients with MHE or control subjects, and that 0.13 ng/mL of S100β is the best cut-off for the diagnosis of HE (83 and 64% of sensitivity and specificity, respectively) [78]. These results are in accordance with previous data in which S100β and neuron-specific enolase (NSE) were evaluated in a small Egyptian cohort of 52 subjects, where 62 and 38% of 29 cirrhotic patients had HE grade 1 or 2, respectively; these

groups were compared with non-HE cirrhotic or healthy subjects. Here, NSE showed non-significant differences among the groups, but S100β was increased in serum samples from grade 1 and 2 HE, compared with cirrhotic or healthy subjects; in addition, a significant positive correlation was found between S100β levels and plasma ammonia, in all patients; the main conclusion in this report was that S100β serum levels could be a useful surrogate marker with more than 90 and 50% of specificity and sensitivity, respectively, for detection of mild cognitive impairment in cirrhotic patients, before they progress to more advanced stages of HE [79]. In a similar study, Wiltfang et al. reported that a cut-off value of 112 pg./mL or 0.11 ng/mL of serum S100β, practically the same value found in our group of patients, is able to predict subclinical porto-systemic encephalopathy with a 100 and 57% of specificity and sensitivity, respectively [80].

Osmotic abnormalities account for the cognitive impairment in cirrhotic patients and some clinicians are using the magnetic resonance (MR) in order to clearly demonstrate a relationship among water, manganese, glutamate, glutamine, and myoinositol levels and the degree of brain damage in patients with liver disease. MR might be useful to diagnose brain abnormalities elicited by hyperammonemia, especially in specific regions like the *globus pallidum* and the temporal region. It has been documented that N-acetylaspartate (NAA) is a good biomarker for neuronal loss and phosphocholine (Cho) concentrations reflects phospholipid metabolism and osmotic regulation due to its role in membrane synthesis and myelin destruction. Low levels of Cho have been associated with osmotic changes in the brain of cirrhotic patients. The majority of these metabolites have different echo times in MR spectroscopy with glutamate (Glu), glutamine (Gln), and phosphocreatine (PPC) being some of most constant correlation findings between patients with or without HE. However, in patients with OHE, the MR is difficult to perform because many confounding factors might co-exist, such as infection, renal failure, anemia, alcohol consumption, and other factors [81]. Our group recently conducted a pilot study in which non-infected compensated, infected cirrhotic, and healthy patients were evaluated to assess for cerebral changes in myoinositol and NAA by means of proton magnetic resonance spectroscopy (^{1}H–MRs). Here, we have found that 40 and 30% of the infected patients were by spontaneous bacterial peritonitis or urinary tract infection, respectively; we also registered a significant decrease of myoinositol levels in the temporal region of non-infected cirrhotic and infected cirrhotic patients when compared with the healthy control group; in addition, we found a similar pattern whit NAA, which significantly diminished in the former groups (**Figure 3**) (unpublished data). When we sought over basal ganglia, Creatine, Cho as well as myoinositol levels did also significantly diminished in infected cirrhotic patients; altogether these results strongly suggests that acute infection in cirrhotic patients contributes to regulate brain metabolites and may be a factor related with development of HE (unpublished data).

Besides those data indicating that brain metabolites are also implicated in the pathophysiology of HE, another important issue in end-stage liver disease is the local blood flow. Cirrhotic patients with portal hypertension suffering ascites, hepatorenal syndrome and HE, usually also had distorted cerebral blood flow (CBF), which severely impact their skeletal muscle, brain, and kidney irrigation as a result of vascular resistance. It has been a long way since the 1960s decade, when one of the first studies about cerebral hemodynamics in cirrhosis came into light [82]. Since then, a plenty of studies have contributed to understand the role of vascular pressure over the pathology associated with HE. In 1969, Bianchi-Porro and co-workers reported that after portacaval shunt surgery, cirrhotic patients, had a significance increase in
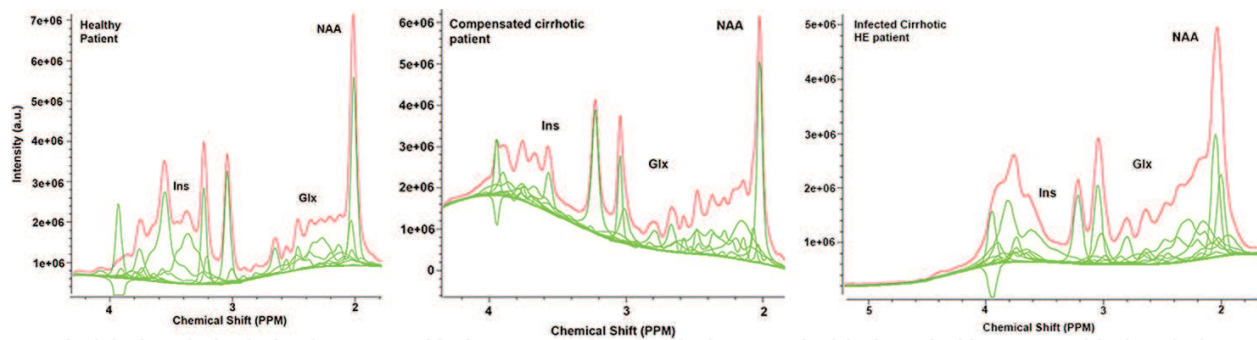
Figure 3. Cirrhotic infected patients have significant abnormalities in the glutamine/glutamate (Glx) ratio and NAA brain levels. See text for details.

CBF and a significant decrease in cerebral vascular resistance (a measure of CBF and metabolism) and the metabolic rate of glucose and the glucose:oxygen quotient did also increase in these patients; the authors suggested that the increased CBF was related to metabolic problems, and that this increase accelerates the removal of toxic substances and allows the disposal of brain metabolites capable of neutralize toxic compounds [83]. It is now well accepted that cirrhotic patients have decreased CBF, portal hypertension, and splanchnic vasodilatation, associated with a hyperdynamic circulatory state, all of which can affect CBF. In a more recent study, we described a maneuver in which we used the transcranial Doppler (TCD) technique (transmission of ultrasound beam through the skull using a pulsed Doppler sectorial probe with a 2 MHz transducer), to measure in an non-invasive and reliable way, the cerebral autoregulation, meaning the physiological mechanisms that maintain CBF at an appropriate levels in response to vasoconstrictors or vasodilators and the middle cerebral artery (MCA) velocities. From here, two functional cerebral hemodynamic indexes were evaluated, (a) the pulsatility index (PI) which assesses the arteriolar vascular integrity and (b) the breath holding index (BHI), which measures cerebrovascular reactivity (CVR). We compared the TCD measures of cirrhotic non HE (n = 30), cirrhotic HE (n = 30) and healthy subjects (n = 30). Here, we found that major basal vessel integrity was not compromised because there were no differences among the three groups when the left and right cerebral arteries were evaluated. However, we observed significant differences in PI and BHI between cirrhotic and control subjects and when the compensated status was taken into account, the PI and BHI was significantly increased and decreased, respectively, when decompensated (CTP > 7 and MELD score ≥ 14) patients were compared with compensated and healthy groups. Similarly and more importantly, when HE status was included in the analysis, the results were the same as before, which means that disturbances in CBF is a novel pathophysiological pathway in HE, and opens an new way for treatment and prevention. Patients with decompensated cirrhosis or HE have a higher risk of cerebral hypoperfusion related with microvascular damage and the ability for autoregulation, which is and important feature for systemic blood pressure sudden changes, particularly hypotension, which can promotes HE [84]. These findings were similar to those reported by two separated groups, one located in Turkey, were 50 decompensated group of cirrhotic patients was compared with 50 healthy volunteers using the same TCD approach [85]; while other in China recruited a healthy control group (n = 40), a cirrhotic without HE group (n = 52), a cirrhotic with MHE group (n = 21) and a cirrhotic with OHE group (n = 19) [86]. Here, in the first case, the authors analyzed the spectra signal derived from the systolic velocity, diastolic

velocity, mean flow velocity (MFV in cm/s), pulsatility, and the resistive indexes (PI = Peak systolic velocity (V_p) – end-diastolic velocity (V_d)/MVF; and RI = V_p – V_d/V_d) of intracranial arteries. Patients with cirrhosis had a lower MFV compared to control healthy group, while cirrhotic patients had a higher PI and RI values and a positive correlation exists with the model for end-stage liver disease (MELD) score and the RI values of patients with ascites, which were higher than those without ascites [85]. In the second case, authors found that mean velocity (V_m), V_d, PI, and RI, as well as the serum ammonia levels, were decreased in the group of cirrhotic patients with MHE subject to lactulose treatment, when compared with the placebo group, while a positive correlation was found between ammonia and PI, RI, cognitive test results and V_d; authors propose that cerebral hemodynamics is related with the severity of HE and that lactulose treatment is able to significantly improve this parameter in cirrhotic patients [86].

3. Novel pharmacological findings over HE treatment

Despite being one of the most frequent and best studied associated pathologies of liver dysfunction, HE still represents a bigger challenge to clinicians due to its devastating effects over cognitive functions. Still, there is no clear consensus about its appropriate treatment because of its complex physiology involving inflammatory responses, neurosteroid-like compounds, reactive oxygen species, etc. In addition, there is another layer of complexity represented by the co-infections and organ failure that many patients develop in the course of the pathology. Nowadays, there had been a lot of attempts to circumvent the signs and symptoms of HE, both experimentally and clinically. One example of this is the use of one of the most popular anti-inflammatory drugs used to treat cirrhotic patients, infliximab, which reduces peripheral inflammation, directly impacts over neuroinflammation, and restores the altered neurotransmission and cognitive impairment, as well as the reversal of activation of microglia and astrocyte GABA transporters (GAT1, GAT3). Infliximab also reduces the synthesis and release of pro-inflammatory cytokines, such as TNFα, IL-1β, etc., as demonstrated by Dadsetan et al. in a murine model of HE [87, 88]. These data are still scarce in human subjects, although some advance have been reported. In an initial trial, Sharma and co-workers reported that patients with severe alcohol-associated hepatitis, who received a single dose of infliximab (5 mg/kg IV), have an improvement of the Maddrey's discriminant factor (DF)—useful in the prediction of short-term prognosis—serum TNFα, C reactive protein (CRP), MELD score and total neutrophil count compared with the before-treatment parameters; interestingly, among the patients who survived only 8% had HE at admission, while among those who died, 67% suffer from HE; the authors concluded that HE at admission of the trial, Lille score and delta bilirubin, predicted 2-month mortality and that infliximab should be carefully used to treat alcoholic hepatitis [89]. Besides the use of infliximab, other therapies aimed to reduce the production of ammonia by the intestinal microbiota, especially the coliforms, have been widely reported. Treatment with lactulose (a non-absorbable disaccharide) or non-absorbable antibiotic rifaximin, are two of the most frequent therapies for cirrhotic patients suffering from HE. The metabolic activity of colonic bacteria which produce short-chain organic acids when metabolize lactulose lowering the pH of the gastrointestinal tract, is the main effect of the disaccharide for the removal of nitrogen; the acidic environment also results in the change of ammonia to ammonium (NH_4^+) a non-absorbable form of nitrogen which diminishes the circulating

ammonia. Further mechanisms include the laxative effect for removal of nitrogen-containing compounds from the gut. In a concise Cochrane review from last year, 38 clinical trials were evaluated and their findings indicate that non-absorbable disaccharides have beneficial effects when compared with placebo/no intervention on mortality, HE, liver failure, hepatorenal syndrome and variceal bleeding; secondary outcomes such as quality of life, also were favored when non-absorbable disaccharides were administered [90]. Additionally, experimental data indicates that lactulose promotes neuro- and astrogenesis in the hippocampus of rats with HE as well as the reduction of plasma ammonia, the locomotor activity impairment and neuronal hyperactivity in brain areas related with locomotor activity [91]. These data are in accordance with those data indicating that Lactulose do also improved cognitive function in MHE patients, as reviewed by Luo et al. [92]. The non-absorbable antibiotic, rifaximin, is the most common additive therapy along with lactulose to treat cirrhotic patients with HE. In a recent prospective observational study, 60 HE patients were divided into two groups, one receiving rifaximin alone and the other one received rifaximin plus lactulose for 7–15 days until discharge from hospital or death. In this study, the authors found that both groups were effectively improved in their mental scores, although they conclude that the combination of both substances was effective, but not superior to lactulose alone in the treatment of HE [93]. The resolution of overt HE has also been tested using rifaximin in comparison with other antibiotics. A meta-analysis of these trials indicates that rifaximin treatment was more likely to resolve an episode in patients with OHE besides an improvement in secondary prevention of HE [94]. Other treatment regimes devoted to reduce the serum ammonia levels, such as polyethylene glycol (PEG), sodium benzoate, odium phenylacetate, glycerol-phenylbutyrate (GPB), ornithine-phenylacetate (OP), phenyl-acetyl-glutamine (PAGN), and a carbon microsphere adsorbent (AST-120) among others, had been also tested for nitrogen removal (for a concise review see [95]).

On the other hand, it is more common to see the rise of many biological compounds that previously were used to treat distinct pathologies; an example of this is Artesunate, a water-soluble hemisuccinate derivative of the Chinese herb *Artemisia annua*, that has been recommended for its use as an antimalarial drug, but it seems that also have anti-inflammatory properties because its use in cancerous cells inhibits its replication and interferes with the expression of pro-inflammatory genes [96, 97]. Artesunate do also have anti-profibrotic properties as was demonstrated recently by Wang and colleagues. Here, Wang et al. investigate the role of treatment with artesunate in a lung fibrosis rodent model; the authors found that artesunate treatment successfully reverted the expression of pro-fibrotic genes such as transforming growth factor (TGFβ), Smad3, and α-smooth muscle actin (α-SMA) as well as the heat shock protein 47 (HSP47) at the protein level, when compared with control animals [98]; these results are in accordance with those of Wang et al., whose findings in the RLE-6TN cell line indicates that TGFβ and Smad3 are inhibited after artesunate exposure [99]. The effect of artesunate on the CNS of rats with HE has been tested too. Rats administered with artesunate (50 or 100 mg/kg), significantly improved its spatial learning ability in the Morris water maze test, while *in vitro*, cerebellar granule neurons treated with artesunate (100 μM) significantly reduced its glutamate release, as well as the Na^+K^+-ATPase activity, indicating that artesunate has a neuroprotective effect, although the effect over astrocytes was not evaluated in this work [100]. Additional evidence about the effect of artesunate over glial cells is lacking, but this drug has a relevant role in the field of the HE treatment choices. A synthetic drug called GR3027 has been tested *in vivo* in a rat model of HE. This new compound has antagonist properties over the $GABA_A$ receptors

when subcutaneously administered and effectively reverses the motor coordination and spatial memory impairment in rats with experimental HE, offering a new way to prevent the ammonia-induced neurological GABA-related damage [101]. These findings are similar to those reported by Turkmen et al. using the UC1011 GABA$_A$ receptor antagonist, who also observed a reduced allopregnanolone effect on the learning test [102].

Although the mechanisms of astrocyte cell swelling are still controversial, some authors propose that the plasma membrane depolarization plays a crucial role, while others suggest that the ionic homeostasis is equally important [103, 104]. In addition, glutamine, manganese, pH changes, and the neurosteroids are also implicated in this process. However, both, *in vivo* and *in vitro* experimental evidence strongly suggests that astrocyte swelling is the primary response to hyperammonemia. One additional factor is the oxidative stress derived from the altered mitochondria. It has been shown that astrocytes exposed to ammonia, results in the activation of the mitochondrial permeability transition pore (MPT), a process related to generation of ROS within the cell. The permeability transition is a sudden increase in the inner mitochondrial membrane to solutes >1.5 kDa and it is known that adenine nucleotides inhibit this process. The MPT is a Ca^{2+}-dependent process that usually culminates in necrosis or apoptosis in hepatocytes; in mammals, cyclophilin D (CyPD) (a conserved cis-trans isomerase) acts as the mitochondrial receptor for cyclosporine A (CsA) (a very well-known immunosuppressive agent). The MTP is a multiprotein complex whose formation is ultimately inhibited by the CyPD/CsA complex. Pre-treatment of astrocytes with CsA completely reverts the cell swelling after treatment with ammonia [25]; in a similar manner, sodium pyruvate, minocycline, magnesium sulfate, and trifluoparazine (TFP), decreased the ammonia-induced MTP-dependent cell swelling, in a significant manner [105]. Other antioxidant compounds have been tested in order to revert the cognitive impairment in HE. For example, the multifunctional soy isoflavone, genistein, has an important activity as an oxygen-derived free radicals scavenger, and as a potent inhibitor of pro-inflammatory cytokines such as IL-4, IL-10, IL-1β, TNFα, etc., as well as the expression of GABA$_A$ and GluR2 receptors in the hippocampus of a rat model of HE, restoring the altered neurotransmission, neuroinflammation and the DNA damage observed in the rat's brain [106, 107]. Further evidence indicates that genistein may inhibit astrocyte swelling by inhibiting the protein tyrosine kinase (PTK) activity and repression of NF-κB-mediated inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO) accumulation [108]. Resveratrol, lipoic acid (LA), and N-acetyl-cysteine (NAC) are important anti-oxidants that can, at least *in vitro*, modulate the expression and activity of the glutamate transporters, increase the glutamate release, reduce the activity of GS and GSH content as well as the ammonia-induced pro-inflammatory response in glial cells [109].

A most recent study, conducted *in vivo*, indicates that TNFα and its receptors, TNFR1 and TNFR2, play major roles in acute ammonia intoxication. In TNFα-deficient or double TNFR1/TNFR2 knock-out mice, ammonia challenge is unable to trigger astrocyte cell swelling, an affect that seems to be related to the expression of the Na^+, K^+, $2Cl^-$ (NKCCl) co-transporter, which is closely related to the NH_4^+ hepatic clearance [110].

L-ornithine and L-aspartate (LOLA) are urea cycle substrates that can lower ammonia levels in cirrhotic patients. The first is a substrate of the ornithine-aminotransferase (OAT) enzyme,

which converts ornithine into glutamate or glutamate-semialdehyde, while L-aspartate is converted by the aspartate-aminotransferase into glutamate or oxaloacetate [5]. A complete review about the effect of LOLA in cirrhotic patients has been published and one of the main conclusions was that LOLA is effective in patients with OHE and less beneficial for those having MHE and had no effects on patients with acute liver failure [111, 112].

An intriguing method to treat liver-affected patients is based in Ayurveda. According to Wikipedia, Ayurveda is a system of medicine with roots in India, where medical knowledge is transmitted from gods to sages and then to humans. Ayurveda therapies are based on complex herbal compounds, minerals, and metal substances. In a clinical research paper, Ayurveda was put into practice to treat a case of HE in India. The report indicates that Ayurvedic therapy, consisting on a mix of four substances (*Siddha Makar Dhwaja*, *Brihat Vata Chintamani Rasa*, *Phyllanthus niruri* extract and a syrup of "*hepatoprotective herbs*"), was administered to a grade three HE male patient currently receiving "*modern therapies*." After a 3-day period of treatment, the patient was found more oriented and awake. The hepatic aminotransferases and bilirubin significantly improved after almost 1 month of treatment [113]. This report, according to authors, is interesting enough to begin a new debate in the area of practice of Ayurvedic medicine. The debate is open.

4. Concluding remarks

Hepatic encephalopathy is a very devastating disease associated with liver failure. Besides the detrimental effects over astrocyte and neuron physiology, HE had a direct impact over the quality of life of affected patients. Recent evidence suggests that many pro-inflammatory pathways are related to these pathology and therapeutic interventions are devoted to counteract it. In this chapter, many of the molecular and pathological events related with ammonia and its effects over astrocytes cells had been addressed. Indeed, ammonia is one of the main factors contributing to the pathophysiology of HE, responsible of the astrocyte swelling along with other metabolites such as myoinositol, manganese, etc. (**Figure 4**). Ammonia is a toxic compound mainly produced by the bacteria in the gastrointestinal tract or by metabolism of ammonium-containing substances. In healthy subjects, urea is the final metabolite of ammonium metabolism in the liver, which is then excreted in urine. Hepatic failure is a one of the most common public concerns in western economies and is beginning to become a major problem in the next 10–15 years. Some of the most feasible and easy-to-implement therapies against liver disease is prevention. Public health policies must be strengthened regarding preventive information not only for liver but also to different metabolic-related illness. The stress and inappropriate feeding habits in general population has enormous impact not only in adults, but also in young or even in elementary-school grade childhood. One of the main tasks of public health should be informative and not only the medical practice. If we want to succeed in our struggle against different maladies affecting human beings, we must to begin to turn the sight to the basics of having a proper balanced diet and to make regular physical activity; this actions would improve and prevent any metabolic imbalance in the short to median term. The society has the future in their hands.
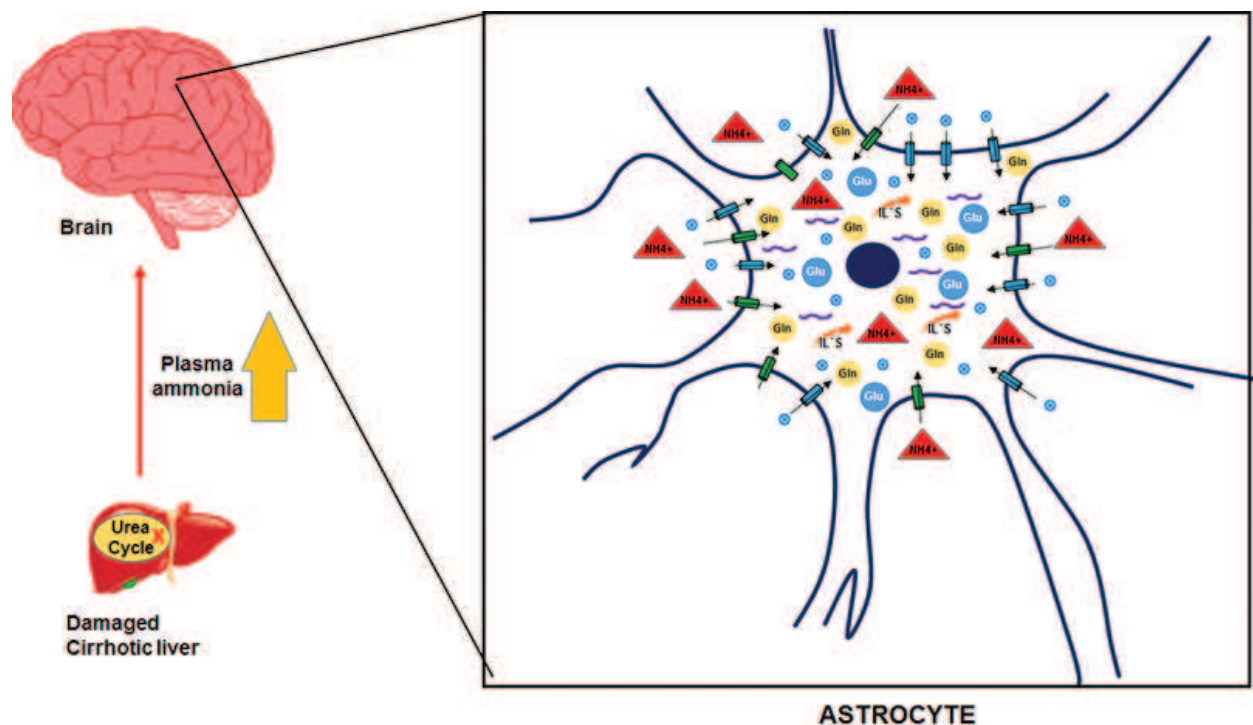
ASTROCYTE

Figure 4. Proposed model of astrocyte dynamic during hyperammonemic conditions. Here, the ammonia (triangle) molecules are increased in the extracellular space and they easily diffuse through the plasma membrane or by means of the NKCC1 channels (dark gray cylinders) once inside ammonia is conjugated with glutamate (**Glu**) by the enzyme glutamine synthase (not shown) to generate glutamine (**Gln**). This is the GGC previously described (see **Figure 1**). In hyperammonemic conditions, such as that resulting from liver cirrhosis, the amount of Gln exceeds the astrocyte capacity to export it. Then, astrocyte increase the expression of the water channel AQP_4 (light gray cylinders) in order to let water (snow flake-like spots) to get inwards. These process is accompanied by the increase of different interleukines like TNFα, IL-1β, IFNγ, and other pro-inflammatory cytokines (**IL´s**), which promotes the mitochondria to become less effective in its metabolic functions, leading the astrocyte to an stressed state. This process generates reactive oxygen species (ROS) and reactive nitrosative species (RNS), as well as oxidized RNAs (**short lines**) that eventually impairs the astrocyte overall function. The whole process generates a condition of low-grade cellular edema and astrocyte cell swelling. See text for additional details: (NH_4^+), (Glu), (Gln), (dark gray cilinders), (light gray cylinders), (snow flake-like spots).

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