Editorial

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Hepatic encephalopathy: Another brick in the wall☆,☆☆

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Introduction

Hepatic encephalopathy (HE) is defined as a neurological and neuropsychological complication caused by liver disease and/or portosystemic shunting. The implication of hyperammonemia has been proposed more than a century ago in a dog animal model, *i.e.*, Eck's fistula,[1] and more than a half century ago in humans with porto-systemic encephalopathy.[2] The implication of systemic inflammation has been shown more recently.[3] Yet, hyperammonemia and systemic inflammation do not explain all the abnormalities that characterize HE.[4] The cerebral accumulation of several substances has been described over the years: aromatic amino acids, benzodiazepine-like substances, bile acids, manganese, indols, mercaptans and even xenobiotics,[5] providing evidence for an altered permeability of the blood-brain barrier (BBB) in the context of HE.

The wall

Located at the interface between the blood and the central nervous system, the BBB controls exchanges between blood and brain.[6] The BBB is formed by cerebral endothelial cells that are mainly characterized by the expression of intercellular tight junctions and the expression of polarized transport systems. Different cell types are involved in the BBB architecture: cerebral endothelial cells lying on a basal lamina, pericytes, astrocytes and neurons. While cerebral endothelial cells express intercellular adherent junctions formed by the homophilic interaction of vascular-endothelial (VE)-cadherins like other endothelial cells in the body, they are unique in that they express apical tight junctions formed by the homophilic interaction of occludin, claudin-3 and -5, zonula occludens (ZO)-1 and other molecules such as junctional adhesion molecules molecules, cingulin, AF-6 and 7H6. Cerebral endothelial cells lying on the basal lamina are surrounded by astrocytic protrusions, the astrocytic endfeets, that cover microvessels and form the glial limitans. This unique organization is responsible for an extremely low permeability to solutes, proteins, plasmatic nutrients, xenobiotics and cells, *i.e.*, leukocytes. This provides the brain a protection against plasmatic xenobiotics and contributes to brain homeostasis. Nevertheless, specific transport systems, *i.e.*, receptor-mediated transporters, solute-carrier (SLC) and ATP-binding cassette transporters, are expressed by cerebral endothelial cells to ensure the supply of nutrients to the brain and the elimination of waste products.

A leaky wall in HE

The alteration of BBB permeability in HE has been reported both in patients and in animal models.[7–10] Patients with HE display increased permeability to solutes and several substances. Cerebral edema is a hallmark of HE: vasogenic edema is present in acute and in acute-on-chronic liver failures and to a lesser extent in cirrhosis. Cytotoxic edema is present in both acute and chronic liver diseases. The increase of BBB permeability in HE has also been called into question.[10,11] The different techniques used to assess BBB's permeability could largely explain discrepancies. For example, even though the classical cut-off value of BBB passage is 500 Daltons (Da), Evans Blue, a dye of 70 kDa molecular weight has been used by investigators who concluded that BBB permeability was not increased.[10] Studies, in which dyes of lower molecular weights, *i.e.,* 10 or 40 kDa, were used, demonstrated increased BBB permeability in HE.[8,9] Therefore, the range of increased permeability between 0.5 and 70 kDa have been frequently overlooked. Animal studies could also show a decrease in the expression of tight junction proteins and an increase in the activity of metalloproteases, MMP-2 and -9, classically associated with impaired BBB permeability.[8] Clinical studies in humans with HE showed the accumulation of several substances in the brain parenchyma or the cerebrospinal fluid (CSF): aromatic amino acids, bile acids and even xenobiotics.[5,7] Whereas BBB abnormalities readily explain cerebral edema and the accumulation of aromatic amino acids,

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they poorly explain the accumulation of some others, *i.e.* bile acids or small molecules.

Digging under the wall

Recently, a paravascular fluid pathway has been described in the brain and has been suggested to account for the clearance of small molecules encompassing waste products, outside the brain.[12,13] This pathway termed glial-lymphatic (glymphatic) system (Fig. 1), is dependent on glial astrocytic endfeets that surround the cerebral vessels and it is functionally close to the lymphatic system.[13] Thus, CSF of the subarachnoid space enters the brain parenchyma by following paravascular spaces that surround penetrating arteries, *i.e.*, Virchow-Robin spaces, located between the basal membrane of smooth muscle cells and astrocytic endfeets expressing aquaporin-4 (AQP-4) water channels. Similar paravascular spaces are present on large caliber cerebral veins and contribute to the clearance of small molecules and waste products, from interstitial fluid to the CSF which will then reach cervical lymphatic vessels and lymph nodes or the newly described meningeal lymphatic vessels.[14] It has been proposed that the AQP-4 water channel was the main driving force of the glymphatic system and an impairment of glymphatic clearance associated with a decrease in the expression of AQP-4, has been described in animal models of Alzheimer's disease or traumatic brain injury.[12,15] The glymphatic system is implicated in the pathophysiology of other diseases: Parkinson's disease, small vessel diseases, subarachnoid hemorrhage or high altitude cerebral edema.[16] Presumably, its function is modulated by sleep, acetazolamide, dobutamine or deep cervical lymph node ligation in animal models. This system could also account for the cerebral accumulation of amyloid-beta (A-beta) proteins after sleep deprivation,[17] as the inhibition of glymphatic clearance is associated with the cerebral accumulation of A-beta proteins.[12] The glymphatic system is still a subject of debate,[16,18] notably with respect to the existence of paravascular spaces, or the role of AQP-4 water channels. Yet, the glymphatic system has emerged as a pathway for the cerebral supply and clearance of small molecules in parallel to the transvascular route, depending on the BBB.

Another brick in the wall

In this issue of the *Journal*, Hadjihambi *et al.*[19] address the possible impairment of the glymphatic clearance system in cirrhosis and HE. They took advantage of the bile duct ligation (BDL) animal model of chronic liver disease with HE, and used mass-spectroscopy techniques and dynamic contrast-enhanced MRI to study the glymphatic system. The authors could show that BDL animals compared to Sham presented both a reduced

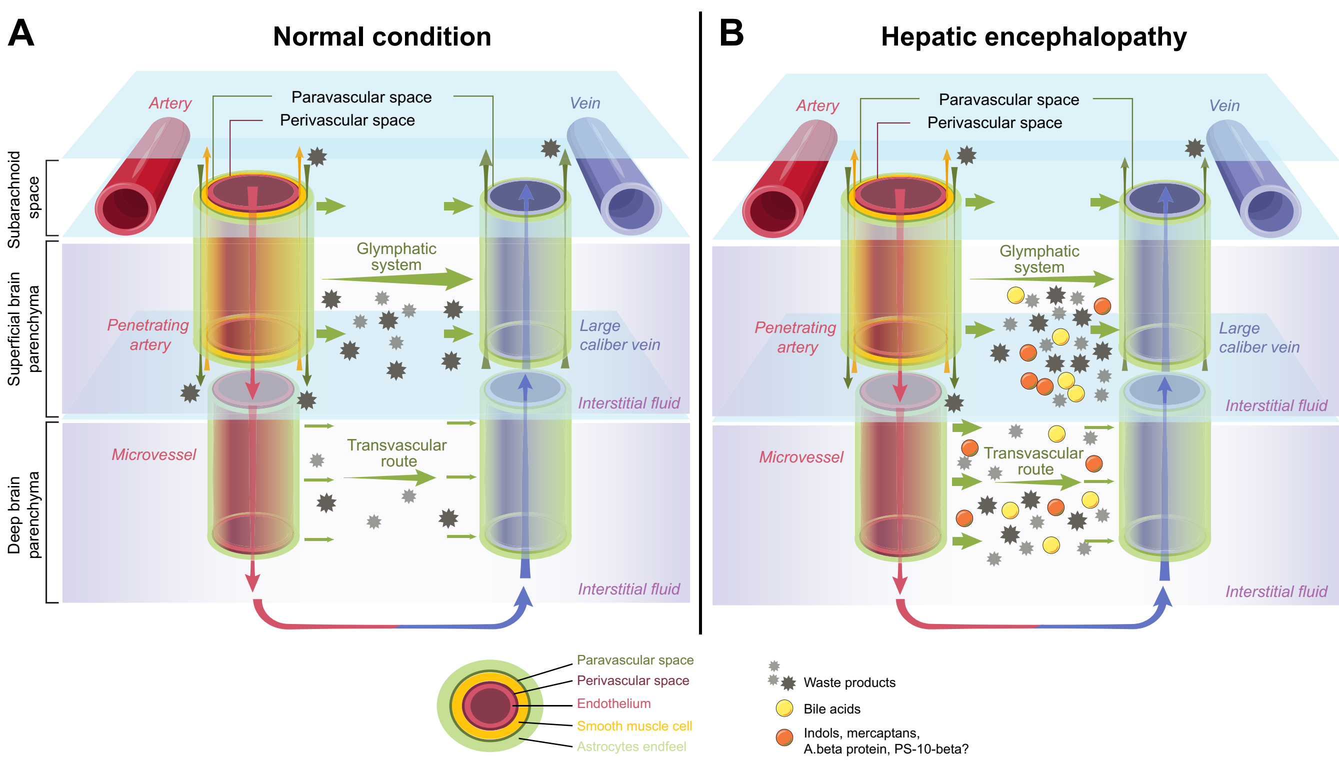

Fig. 1. The glymphatic and the transvascular route. (A) In normal conditions, the cerebrospinal fluid of the subarachnoid space enters inside the brain parenchyma by following paravascular spaces (dark green vertical arrows) surrounding penetrating arteries (red), *i.e.* Virchow-Robin spaces. Those are located between the basal membrane of smooth muscle cells (orange) and astrocytic endfeets (green) expressing aquaporin-4 (AQP-4) water channels. An exchange with cerebral interstitial fluid is possible (green horizontal arrows). Similar paravascular spaces are present on large caliber cerebral veins (blue) and contribute to the clearance from interstitial fluid of small molecules (dark grey stars), waste products, to the CSF. Some data questioned the reality of this clearance through the veins and suggested an alternative pathway for waste product elimination through perivascular spaces located between the basal membrane of smooth muscle and the endothelium of penetrating arteries. This perivascular flux (orange vertical arrows) would be in the opposite direction of the paravascular flux (dark green vertical arrows). This system is coined the glial-lymphatic (glymphatic) system. In the deep brain parenchyma, microvessels have a peculiar organization, the blood-brain barrier that dramatically restrict exchanges. However, specific transporters are expressed by cerebral endothelial cells and exchanges with the interstitial fluid is possible there (green horizontal arrows). This is the transvascular route. (B) In hepatic encephalopathy, the glymphatic system could be impaired. According to recent data, venous paravascular flux and/or perivascular flux could be decreased and could be responsible for the accumulation of waste products (grey stars), bile acids (yellow circles) and maybe other small molecules (orange circles).

CSF brain influx and a reduced CSF brain efflux. They confirmed as we did that bile acids accumulate in the CSF of patients with HE.[5] By immunostaining, they confirmed that AQP-4 water channel expression was decreased as previously shown in other models in which the glymphatic system was impaired. They also showed that the neurocognitive abnormalities displayed by BDL animals corresponded to the brain regions where the glymphatic system was the most profoundly impaired. All these data bring new insight into HE pathophysiology and provide strong evidence to indicate that clearance in the glymphatic system is impaired in HE.

Breaking the wall

This work is remarkable in many respects. First, it indicates for the first time that the glymphatic pathway may be implicated in HE pathophysiology. Second, it provides a possible explanation for the cerebral accumulation of bile acids in HE. Third, as the glymphatic system mediates the accumulation of small molecules such A-beta or alpha-synuclein proteins in neurodegenerative disorders, it provides a possible explanation for fixed lesions in HE. Finally, this study is the result of a collaboration between neuroscience and hepatology. Such interdisciplinary research should be promoted to better understand unresolved questions in HE pathophysiology and to suggest new therapeutic strategies.

The next step will be to confirm glymphatic system impairment in patients with HE. Different MRI techniques based on contrast enhancement have enabled the study of the glymphatic system in patients,[20,21] but these techniques are time-consuming. The measurement of Virchow-Robin spaces on MRI without contrast enhancement could also be used to evaluate the glymphatic system and would be more usable in cirrhotic patients with HE. The most useful tool would be plasmatic biomarkers. Indeed, plasmatic concentrations of protein S-100-beta or neuron-specific enolase, two major cerebral biomarkers, are commonly used in long-term neurological prognostication of cardiac arrest or traumatic brain injury. Interestingly, the plasmatic concentrations of these biomarkers are poorly predicted, based on the concept of BBB. If the cerebral clearance of these biomarkers goes through the glymphatic pathway, they could be used in human studies? Once these short-comings are resolved, it will be possible to study the impact of treatments. Would the specific treatment of sleep disturbances in cirrhotic patients be able to correct glymphatic system impairment? Indeed, the glymphatic system constitutes an exciting research avenue in HE.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2018.10.016.

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