Possible role of glymphatic system of the brain in the pathogenesis of high altitude cerebral edema

Marian Simka,[1] Paweł Latacz,[2] and Joanna Czaja[1]

[1]Department of Anatomy, University of Opole, Opole, Poland.
[2]Department of Neurology, Jagiellonian University Collegium Medicum, Krakow, Poland.

Abstract

In this paper we suggest that glymphatic system of the brain can play an important role in the pathogenesis of high altitude cerebral edema (HACE). Water enters the intercellular space of the brain primarily through aquaporin-4 water channels, the main component of glymphatic system, while acetazolamide, pharmacological agent used in the prevention of HACE, is the blocker of the aquaporin-4 molecule. In animal experiments cerebral edema caused by hypobaric hypoxia was associated with an increased expression of aquaporin-4 by astrocytes. Also, glymphatic system is primarily active during sleep, while sleep at high altitude is a well-known risk factor of developing HACE. All these findings support our hypothesis. We suggest that future research on the prevention and treatment of HACE should involve factors that are already known to modify activity of the glymphatic system, such as angiotensin-converting enzyme inhibitors or other pharmaceutical agents affecting noradrenergic system of the brain, body posture during sleep, anatomy of the veins draining the cranial cavity and the influence of physical activity before and during exposure to high altitude, especially in relation to sleep.

Edema of the brain occurring after an exposure to high altitude, the so-called high altitude cerebral edema (HACE), is usually considered to be an extension of acute mountain sickness (AMS), although some experts emphasize that there are significant differences between advanced AMS and HACE (*Hackett and Roach, 2004; Davis and Hackett, 2017*). Nonetheless, while in less severe cases AMS presents with symptoms comprising headache, sleep disturbances, fatigue, dizziness and cognitive impairment, HACE is a life-threatening disease manifesting with nausea, vomiting, ataxia and hallucinations, followed by coma and death. Although it is well known that this type of cerebral edema is primarily caused by hypobaric hypoxia, precise mechanisms responsible for this pathology remain elusive. Consequently, there are no other effective treatments for HACE than administering oxygen and descending to a lower elevation. Dexamethasone is recommended in HACE patients as an adjunctive treatment, especially if rapid evacuation is not possible (*Davis and Hackett, 2017*). In this review we will summarize current knowledge on HACE with focus at recently

discovered glymphatic system of the brain and possible role of this system in the pathogenesis of this type of edema. We will also suggest pathways of future research. Still, we emphasize that research in the field of association of an altered function of glymphatic system with HACE is in its infancy, there is little evidence supporting such a link and all conjectures presented in this paper should be considered as highly hypothetical.

Glymphatic system of the brain

Discovery of the glymphatic system of the brain was inspired by an observation that cerebrospinal fluid fluxes into the brain parenchyma along the perivascular spaces (the so-called Virchow-Robin spaces) surrounding the penetrating cerebral arteries. Further research has revealed that water from the periarterial space is actively transported to the interstitial space through AQP-4 water channels. Then, interstitial fluid is cleared through AQP-4 water channels to the perivascular spaces surrounding cerebral veins. Since AQP-4 molecules are expressed on the astrocytes and these glial cells play the main role in this process, researchers coined the term "glymphatic" to emphasize the importance of glial cells serving purpose of the lymphatics. It seems that most of water found in the cerebral parenchyma enters this space through AQP-4 channels. Also, water molecules primarily leave intercellular space through AQP-4 channels expressed by astrocytes at the venous side of the perivascular space (*Jensen et al., 2015; Plog et al., 2018; Simka, 2015; Thrane et al., 2014*).

Discovery of astroglial-mediated interstitial fluid bulk flow has resolved the enigma how water enters and leaves cerebral parenchyma. Generally speaking, water cannot freely flow into the extracellular cerebral space as it can in other tissues. Endotheliocytes in blood vessels of the brain that make up the blood-brain barrier, which is responsible for maintaining the homeostasis of the central nervous system, differ greatly from those in the periphery. They are characterized by the presence of tight junctions, low expression of adhesion molecules, lack of fenestration and minimal pinocytotic activity. Of note, a passive flow of water from the Virchow-Robin space, because of the distance between perivascular space and majority of neurons, cannot be effective. It has been suggested that an altered cerebral interstitial fluid bulk flow plays an important role in several brain pathologies, including neurodegenerative disorders (such as Alzheimer and Parkinson diseases) (*Jessen et al., 2015; Plog* and *Nedergaard*, *2018*) and also some types of cerebral edema (*Lawley et al., 2016; Tang et al., 2016; Thrane et al., 2014*). Therefore, it is likely that water congestion in the settings of HACE is associated with impairment of the glymphatic system. Consequently, we propose that "glymphatic" model of this disease could provide a useful and potentially rewarding

framework for future research. A role for glymphatic system in the pathogenesis of HACE has already been hypothesized by Lawley and colleagues (*Lawley et al., 2016*). In our paper we describe new findings supporting this hypothesis, suggest what could be investigated in the future and which possible obstacles should be overcome by research in this field.

Findings supporting the role for astrocytic AQP-4 water channels in the pathogenesis of HACE

Acetazolamide, a carbonic anhydrase inhibitor, is the main pharmacological agent used in the prevention of AMS (*Davis and Hackett, 2017; Kayser et al., 2012; Swenson, 2014; Swenson, 2016; Wang et al., 2015*). Yet, contrary to milder forms of AMS, efficacy of acetazolamide in the prevention of HACE has not been unequivocally proven, mostly because of the fact that HACE is a rare pathology and is difficult to replicate in laboratory settings. In addition, at least regarding cerebral component of AMS, it seems unlikely that this disease is simply a consequence of hypoxia of the brain. Characteristics of cerebral symptoms of AMS, which are different of those resulting from normal short-term asphyxia and connection of AMS with sleep at high altitude indicate that more complex mechanisms than neuronal hypoxia play here a role. Importantly, in addition to carbonic anhydrase, aquaporin-4 (AQP-4) is an alternative target for acetazolamide. Crystallographic experiments have revealed that blocking of AQP-4 by this pharmaceutical agent occurs at the extracellular pore entrance of the AQP-4 molecule (*Kamegawa et al., 2016*) - thus, in the brain, at the perivascular side of astrocytic endfeet.
Recent investigations have shown that water molecules cannot passively move into cerebral parenchyma. Majority of water enters intercellular space of the brain through AQP-4 water channels expressed on glial cells, the astrocytes. These water channels not only enable exchange of water between cerebrospinal fluid and cerebral parenchyma, but also facilitate cleansing of the cerebral tissue from waste products (*Jessen et al. 2015; Plog and Nedergaard, 2018*). Taking into account these findings, we suggest that it is possible that HACE does not only result from breakdown of the blood-barrier, as has been already demonstrated (*Lafuente et al., 2016*), but an altered function of astrocytic AQP-4 water channels plays here a significant role. Although disruption of the blood-brain barrier seems to be a prerequisite of cerebral edema, water molecules—irrespective whether they come from the lumen of capillaries or from the Virchow-Robin space—should enter cerebral parenchyma through these water channels. Therefore, expression and activity of AQP-4 should—at least theoretically—influence the formation of cerebral edema in the settings of hypobaric hypoxia. While the glymphatic system has not yet been studied in the context of HACE, there are some

findings that make this hypothesis credible. First, AMS is strongly associated with the sleep at high altitude. A common way to avoid AMS is the "climb high, sleep low" rule. It thus seems that an awake brain is less sensitive to hypoxia at high altitude than an asleep brain, which is counterintuitive, since oxygen consumption by this organ is higher in the state of consciousness. Yet, this enigma could be explained by the fact that the flow of water through AQP-4 water channels occurs primarily during sleep (*Xie et al. 2013*) and therefore an asleep brain would be more prone to develop edema. Second, there are also some recently performed animal experiments that revealed a role of AQP-4 water channels in the pathogenesis of HACE. It has been found that in rodents hypobaric hypoxia was associated with an increased expression of AQP-4 by astrocytes (*Gong et al., 2018; Sun et al., 2018; Wang et al., 2018*). A similarly increased AQP-4 astrocytic expression has been also demonstrated in mice exposed to intermittent hypoxia (*Wang et al., 2018*). However, in another animal experiment an increased expression of AQP-4 by astrocytes was seen only in rodents that received a combination of hypobaric hypoxia and pro-inflammatory stimulus (an injection of liposaccharide). In this experiment researchers have also revealed an increased permeability of the blood-brain barrier and decreased expression of proteins responsible for the integrity of this barrier after combined exposure to hypobaric hypoxia and liposaccharide (*Zhou et al., 2017*). Taking into account these findings, we hypothesize that HACE, in addition to the breakdown of the blood-brain barrier, may be associated with an altered astroglial-mediated interstitial fluid bulk flow, known as the glymphatic system.

Suggested pathways of future research

Inhibition of AQP-4 by acetazolamide in the context of anatomical structure of the glymphatic system (*Jensen et al., 2015; Plog et al., 2018*) may explain low efficacy of this pharmaceutical agent in the prevention of HACE (*Swenson, 2014*). On the one hand inhibition of these water channels in the astrocytes surrounding cerebral arteries would decrease influx of water into interstitial space, but at the same time blocking of AQP-4 at the venous side of the glymphatic system would capture water. Thus, a net effect would be close to nil. Theoretically, a selective inhibition of periarterial AQP-4 molecules should solve this problem, however periarterial and perivenous AQP-4 molecules probably do not exhibit different conformation and therefore such a selective inhibition would not be possible, even by other carbonic anhydrase inhibitors exhibiting blocking capability of the AQP-4 molecule. Therefore, pharmaceutical management of HACE should primarily focus at the prevention of excessive activation of the glymphatic system at high altitude.

It has been suggested that functioning of the glymphatic system may be influenced by venous outflow through the internal jugular veins (*Simka, 2013; Simka, 2015; Zivadinov and Chung, 2013*). It has also been found that some otherwise healthy people present with malformed valves or other obstructing pathologies of these veins, the so called chronic cerebrospinal venous insufficiency (*Zivadinov et al. 2011*). Yet, these venous abnormalities are more often seen in patients with neurodegenerative and neuroinflammatory diseases (Parkinson disease and multiple sclerosis) (*Simka et al., 2011; Simka, 2013; Zamboni et al., 2009; Zamboni and Galeotti, 2010; Zivadinov et al. 2011*). Contrary to intracranial venous pathology (*Wilson and Imray, 2016*), of as yet a role for impaired flow in the extracranial part of the internal jugular veins in the pathogenesis of HACE has not been studied. However, it is tempting to speculate that venous congestion within cerebral venous microvasculature in the settings of chronic cerebrospinal venous insufficiency, caused by a blockage at the level of jugular valve, can result in a higher risk of developing HACE.

If it were the case, then high-altitude trekkers and mountaineers presenting with this venous abnormality (abnormal jugular valves, as well as impaired flow in the internal jugular veins can be quite easy revealed using standard ultrasound scanner) (*Bastianello et al., 2014*), in order to avoid HACE should be advised to ascent slower than it is usually recommended. Of note, a compression of cerebral sinuses and large cerebral veins, as well as small cerebral veins, has already been suggested to play a role in the pathogenesis of HACE (*Lafuente et al., 2016; Sagoo et al., 2016*)

Activation of the glymphatic system during sleep is potentially the most promising target in this field. Currently it is not precisely known which mechanism actually activates AQP-4 water channels in the asleep brain. Some researchers suggested that an increased pulsatility of cerebral blood vessels during sleep activates this system (*Jessen et al., 2015; Lawley et al., 2016; Plog and Nedergaard, 2018*). Even if it actually were the case, what is responsible for this enhanced pulsatility remains elusive. Most likely pulsatility is primarily regulated by the cerebral norepinephine system through specialized neurons located in the locus coeruleus and could be influenced by targeted pharmacotherapy (*Holstein-Rathlou et al., 2018; Russak et al., 2016*). Angiotensin II inhibitors, such as losartan, represent a group of potentially beneficial pharmaceutical agents that affect norepinephine axis of the brain. A preliminary animal study has demonstrated an inhibition of glymphatic flow by losartan injected into the cisterna magna (*Russak et al., 2016*). Of note, it has already been demonstrated that angiotensin-converting enzyme plays a role in the pathogenesis of AMS (*Enhao and Huang, 2016*). Although inhibition of the renin–angiotensin–aldosterone system by losartan had no

observable effect on exercise performance at high altitude (*Myers et al., 2017*), a role for this drug and other angiotensin-converting enzyme inhibitors in the prevention of HACE cannot be ruled out and should be studied, at least in animal experiments.

Activation of the glymphatic system can also be regulated by exercise. Animal studies demonstrated a decreased activity of the glymphatic system in active awake mice (*Xie et al., 2013*) probably due to increased activity of the noradrenergic system of the brain. On the other hand, basic activity of the glymphatic system was higher in mice that ran regularly, still with normal clearance of interstitial space and no congestion within cerebral parenchyma (*Holstein-Rathlou et al., 2018*). It therefore remains unclear which type and timing of physical activity in humans, especially in relation to the sleep, would be beneficial for the prevention of HACE.

The last area of suggested research is the influence of body posture during sleep on the development of HACE. It has been shown that in rodents glymphatic system is differently activated depending on body position during sleep (lateral vs. prone position) (*Lee et al., 2015; Xie et al., 2013*). If this phenomenon occurs also in humans, it would be possible to regulate activity of the glymphatic system by body position during sleep. This may be of particular importance for mountaineers who are often forced to sleep at high altitude, which—especially after incomplete acclimatization—can result in life-threatening HACE.

Finally, it should be emphasized that a treatment and prevention of HACE could probably be achieved by modification of the functioning of the glymphatic system, but not through its complete blockage. A total obstruction of AQP-4 water channels would probably result in cessation of the cleansing of the neurons from waste products, equally life-threatening as HACE, or even in a worsened cerebral edema (*Papadopoulos et al., 2004*).

References:

Bastianello S, Dake MD, Ferral H, Haacke EM, Haskal ZJ, Hubbard D, Liasis N, Mandato K, Sclafani S, Siddiqui AH, Simka M, and Zamboni P (2014) Recommendations for multimodal noninvasive and invasive screening for detection of extracranial venous abnormalities indicative of chronic cerebrospinal venous insufficiency: a position statement of the International Society for Neurovascular Disease. J Vasc Interv Radiol 25: 1785-1794. e17.

Davis C, and Hackett P (2017) Advances in the prevention and treatment of high altitude illness. Emerg Med Clin N Am 35:241-260.

Enhao Z, and Huang L (2016) Association of angiotensin-converting enzyme SNPs and acute mountain sickness. J Am Coll Cardiol 68(suppl.S):C37.

Gong G, Yin L, Yuan L, Sui D, Sun Y, Fu H, Chen L, and Wang X (2018) Ganglioside GM1 protects against high altitude cerebral edema in rats by suppressing the oxidative stress and inflammatory response via the PI3K/AKT-Nrf2 pathway. Mol Immunol 95:91-98.

Hackett PH, and Roach RC (2004) High altitude cerebral edema. High Altitude Med Biol 5:136-146.

Holstein-Rathlou van S, Petersen NC, and Nedergaard M (2018) Voluntary running enhances glymphatic influx in awake behaving young mice. Neurosci Lett 662:253-258.

Jessen NA, Finmann Munk AS, Lundgaard I, and Nedergaard M (2015) The glymphatic system – a beginner's guide. Neurochem Res 40:2583-2599.

Kamegawa A, Hiroaki Y, Tani K, and Fujiyoshi Y (2016) Two-dimensional crystal structure of aquaporin-4 bound to the inhibitor acetazolamide. Microscopy 65:177-184.

Kayser B, Dumont L, Lysakowski C, Combescure C, Haller G, and Tramer MR (2012) Reappraisal of acetazolamide for the prevention of acute mountain sickness: a systematic review and meta-analysis. High Altitude Med Biol 13:82-93.

Lafuente JV, Bermudez G, Camargo-Arce L, and Bulnes S (2016) Blood-brain barrier changes in high altitude. CNS Neurol Disord Drug Targets 15:1188-1197.

Lawley JS, Levine BD, Williams MA, Malm J, Eklund A, Polaner DM, Subudhi AW, Hackett PH, and Roach RC (2016) Cerebral spinal fluid dynamics: effect of hypoxia and implications for high-altitude illness. J Appl Physiol 120:251-262.

Lee H, Xie L, Yu M, Kang H, Feng T, Deane R, Logan J, Nedergaard M, and Benveniste H (2015) The effect of body posture on brain glymphatic transport. J Neurosci 35:11034-11044.

Myers SD, Lucas S, AshdownK, Malein W, Thomas OD, Edsell M, Ladha C, Bradwell A, Wright A, and Gallagher CA (2017) Losartan does not affect maximal exercise performance at high altitude (5000 m): Med Sci Sports Exerc 49(5S):250.

Papadopoulos MC, Manley GT, Krishna S, and Verkman AS (2004) Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. FASEB J 18:1291-1293.

Plog BA, and Nedergaard M (2018) The glymphatic system in the CNS health and disease: past, present and future. Annu Rev Pathol 13:379-394.

Russak A, Plog B, Vates E, and Nedergaard M (2016) Angiotensin II increases glymphatic flow through a norepinephrine-dependent mechanism. Neurology 86(suppl.16): P5.214

Sagoo RS, Hutchinson CE, Wright A, Handford C, Parsons H, Sherwood V, Wayte S, Nagaraja S, Ng'Andwe E, Wilson MH, and Imray CH (2015) Magnetic resonance investigation into the mechanisms involved in the development of high-altitude cerebral edema. J Cereb Blood Flow Metab 37:319-331.

Simka M (2013) What is the relationship between chronic cerebrospinal venous insufficiency and multiple sclerosis? Rev Vasc Med 1:66-70.

Simka M (2015) Recent advances in understanding the lymphatic and glymphatic systems of the brain. Phlebol Rev 23:69-71.

Simka M, Latacz P, Ludyga T, Kazibudzki M, Swierad M, Janas P, and Piegza J (2011) Prevalence of extracranial venous abnormalities: results from a sample of 586 multiple sclerosis patients. Func Neurol 26:197-203.

Sun ZL, Jiang XF, Cheng YC, Liu YF, Yang K, Zhu SL, Kong XB, Tu Y, Bian KF, Liu ZL, and Liu ZL (2018) Exendin-4 inhibits high-altitude cerebral edema by protecting against neurobiological dysfunction. Neural Regen Res 13:653-663.

Swenson ER (2014) Carbonic anhydrase inhibitors and high altitude illnesses. Subcell Biochem 75:361-86.

Swenson ER (2016) Pharmacology of acute mountain sickness: old drugs and newer thinking. J Appl Physiol 120:204-215.

Tang G, and Yang GY (2016) Aquaporin-4: a potential therapeutic target for cerebral edema. Int J Mol Sci 17:1413.

Thrane AS, Thrane VR, and Nedergaard M (2014) Drowning stars: reassessing the role of astrocytes in brain edema. Trends Neurosci 37:620-628.

Wang C, Yan M, Jiang H, Wang Q, He S, Chen J, and Wang C (2018) Mechanism of aquaporin 4 (AQP 4) up-regulation in rat cerebral edema under hypobaric hypoxia and the preventative effect of puerarin. Life Sci 193:270-281.

Wang K, Smith ZM, Buxton RB, Swenson ER, and Dubowitz DJ (2015) Acetazolamide during acute hypoxia improves tissue oxygenation in the human brain. J Appl Physiol 119:1494-1500.

Wilson MH, and Imray CH (2016) The cerebral venous system and hypoxia. J Appl Physiol 120:244-250.

Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, O'Donnell J, Christensen DJ, Nicholson C, Iliff JJ, Takano T, Deane R, and Nedergaard M (2013) Sleep drives metabolite clearance from the adult brain. Science 342:373-377.

Zamboni P, Galeotti R, Menegatti E, Malagoni AM, Tacconi G, Dall'Ara S, Bartolomei I, and Salvi F (2009) Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry 80:392-399.

Zamboni P, and Galeotti R. The chronic cerebrospinal venous insufficiency syndrome. Phlebology 2010;25:269-79.

Zhou Y, Huang X, Zhao T, Qiao M, Zhao X, Zhao M, Xu L, Zhao Y, Wu L, Wu K, Chen R, Fan M, and Zhu L (2017) Hypoxia augments LPS-induced inflammation and triggers high altitude cerebral edema in mice. Brain Behav Immun 64:266-275.

Zivadinov R, Marr K, Cutter G, Ramanathan M, Benedict RH, Kennedy C, (2011). Prevalence, sensitivity, and specificity of chronic cerebrospinal venous insufficiency in MS. Neurology 77:138-44.

Zivadinov R, and Chung CP (2013) Potential involvement of the extracranial venous system in central nervous system disorders and aging. BMC Med 11:260.