The lymphatic drainage systems in the brain: a novel target for ischemic stroke?

Ying-Jie Wang, Yan-Rong Sun, Yan-Hong Pei, Hao-Wen Ma, Ya-Kun Mu, Li-Hua Qin, Jun-Hao Yan

Abstract
Recent studies have proposed three lymphatic drainage systems in the brain, that is, the glymphatic system, the intramural periarterial drainage pathway, and meningeal lymphatic vessels, whose roles in various neurological diseases have been widely explored. The glymphatic system is a fluid drainage and waste clearance pathway that utilizes perivascular space and aquaporin-4 protein located in the astrocyte endfeet to provide a space for exchange of cerebrospinal fluid and interstitial fluid. The intramural periarterial drainage pathway drives the flow of interstitial fluid through the capillary basement membrane and the arterial tunica media. Meningeal lymphatic vessels within the dura mater are involved in the removal of cerebral macromolecules and immune responses. After ischemic stroke, impairment of these systems could lead to cerebral edema, accumulation of toxic factors, and activation of neuroinflammation, while restoration of their normal functions can improve neurological outcomes. In this review, we summarize the basic concepts of these drainage systems, including drainage routes, physiological functions, regulatory mechanisms, and detection technologies. We also focus on the roles of lymphatic drainage systems in brain injury after ischemic stroke, as well as new advances in therapeutic strategies targeting these drainage systems. These findings provide information for potential novel strategies for treatment of stroke.

Key Words: ischemic stroke; brain; edema; glymphatic system; intramural periarterial drainage; ischemic stroke; lymphatic drainage; meningeal lymphatic vessels; neuroinflammation; neurotoxicity

Introduction
Stroke is the leading cause of death and acquired disability in adults and can be broadly classified as ischemic stroke and hemorrhagic stroke (Campbell et al., 2019). Ischemic stroke is responsible for 71% of stroke events worldwide (Campbell et al., 2019) and is a disorder of cerebral blood flow caused by vascular obstruction. The pathophysiology includes excitotoxicity, free radical release, protein misfolding, mitochondrial response, and inflammatory changes, leading to neuronal death and neurological deficits (George and Steinberg, 2015; Zhu et al., 2021; Li et al., 2022; Jiang et al., 2022). Ischemic stroke poses a great threat to human health; however, currently effective treatment methods are limited.

Currently, the theory of intracerebral lymphatic drainage systems includes three parts: the glymphatic system, the intramural periarterial drainage (IPAD) pathway, and meningeal lymphatic vessels (Figure 1). The glymphatic system, first proposed by Iliff et al. (2012), is a new concept. This pathway is highly dependent on aquaporin-4 (AQP4) expressed on the endfeet of astrocytes (Jessen et al., 2015). The glymphatic system provides a fast pathway for the inflow of cerebrospinal fluid (CSF) and the outflow of interstitial fluid (ISF), which facilitates the clearance of metabolic wastes from the brain and maintains the homeostasis of cerebral fluid (Jessen et al., 2015). The IPAD pathway acts as another ISF drainage system, draining solutes through the capillary basement membrane and tunica media of the arteries to the cervical lymph nodes (Carare et al., 2008). Meningeal lymphatic vessels, located in the dura mater, serve as downstream channels to drain ISF, macromolecules, and immune cells out of the cranial cavity, and play a role in the regulation of the immune response in the brain (Aspelund et al., 2015; Louveau et al., 2015a).

In recent years, some studies have focused on the roles of lymphatic drainage systems in the pathology of neurodegenerative diseases, such as Alzheimer’s disease (Reeves et al., 2020) and Parkinson’s disease (Sundaram et al., 2019). In addition, much attention has also been paid to the relationship between lymphatic drainage systems and ischemic stroke, especially its roles in the formation of cerebral edema, activation of neuroinflammation, and modulation mechanism of AQP4 changes after stroke. In this study, we focus on the glymphatic system and cover the latest advances of the IPAD pathway and meningeal lymphatic vessels. The drainage routes, physiological functions, regulatory mechanisms, and detection technologies of the lymphatic drainage systems in the brain are reviewed. Furthermore, their roles in brain injury after ischemic stroke, as well as recent therapeutic advances targeting these systems, especially AQP4, are also discussed.

Search Strategy and Selection Criteria
Studies cited in this review were published from 2000 to 2022 and obtained following a PubMed search using the following keywords: stroke, ischemia, ischemic, glymphatic, intramural periarterial drainage, meningeal lymphatic vessel, edema, inflammation, astrocyte, AQP4. The literature search was completed by YJW on March 22, 2022.
In 2013, dynamic contrast-enhanced MRI was applied to reveal the anatomical routes of perivascular CSF influx (Iliff et al., 2013a). After intracisternal administration of the paramagnetic contrast agent, researchers observed an CSF-ISF exchange process in the rat brain using T1-weighted MRI. Two key influx nodes were identified and they further defined simple kinetic parameters to reflect glymphatic function (Iliff et al., 2013b). To explore the glymphatic system in humans, the effectiveness of lumbar intrathecal infusion has been evaluated. The results showed that intrathecal infusions could improve the glucose metabolism of the brain tissue, although the peak fluorescence intensity was reduced and delayed (Yang et al., 2013). Therefore, clinically established techniques of myelography and cisternography may have the potential to assess glymphatic function. In a case study of Alzheimer’s disease, the IPAD route and the perivenous ISF clearance pathway of the glymphatic system were believed to contribute cooperatively to the clearance of fluids and toxic substances in the brain, although their individual contribution has not been fully determined.

Regulatory mechanisms of the glymphatic system

To date, research on the regulatory mechanisms of the glymphatic system has focused on arterial pulsation and sleep. The arterial pulsation was initially discovered after observing that unilateral internal carotid artery ligation reduced the CSF-ISF exchange capacity of perivascular CSF influx, while increased pulsation with dobutamine could accelerate this process (Iliff et al., 2013b). According to ultrafast magnetic resonance encephalography signal detection, it was observed that although periarterial inflow was driven by the pulsatile movement of the glymphatic system could be regulated by the respiration cycle (Kiviniemi et al., 2016). Inspiration expanded the perivascular space and facilitated glymphatic outflow from the brain, while expiration reversed these effects (Kiviniemi et al., 2016). However, several fluid flow studies challenged this question (Figure 2). The authors proposed that the local movement of the arterial wall acted as a pump to drive the flow of the surrounding CSF (Mestre et al., 2018). In addition, hypertension causes adverse effects on arterial pulsation, increasing flux through the IPAD pathway and thus reducing the drainage rate in the perivascular space (Mestre et al., 2018).

Sleep is an evolutionarily conserved biological behavior, promoting the clearance of harmful metabolites and consolidating memory. Using two-photon imaging, Xie et al. (2013) found that glymphatic function was 90% more active during sleep, while it was significantly suppressed in an awake state. In sleep, the interstitial space increased by 60%, leading to a striking increase in the exchange of CSF-ISF and Aβ clearance. Changes in cell volumes may be related to different noradrenergic signaling derived from the sleep/wake state (Holt et al., 2019). Saito et al. (2019) demonstrated that α2-adrenergic antagonists increases interstitial volume, the power of slow waves and transitional and sleep, and activates γ-aminobutyric acid (GABA)-ergic neurons (Saito et al., 2019). During sleep, increased excitatory neurotransmitters caused by neuronal activity during wakefulness has also been confirmed to interfere with ISF drainage capacity (Li et al., 2020). Furthermore, a recent study found that regulation of glymphatic function was not only dependent on the arousal state, but also exhibited a circadian rhythm that peaked during the day when mice were sleeping and decreased during the night when mice were active (Hablitz et al., 2020). These day-night differences persisted under conditions that eliminated the existence of a normal circadian rhythm, indicating that the circadian rhythm itself contributed to ISF drainage. Maintenance of this circadian rhythm depended on up-regulated AQP4 localization to the perivascular endfeet at midday, since the elimination of AQP4 reduced the day-night difference (Hablitz et al., 2020). Therefore, factors that cause disturbances in the circadian clock may further promote glymphatic dysfunction.

Detection technologies for the glymphatic system

Although the fluorescence imaging method in vivo or in vitro has been confirmed to explore the drainage pathway of the glymphatic system (Iliff et al., 2012), there are inevitable drawbacks to these approaches. Two-photon microscopy has limitations in the shallow field (although intravital diameter), requires invasive operation on the skull and is incapable of probing the deep brain region (less than 250 μm in depth) (Sweeney et al., 2019). For in vitro imaging, the process of perfusion-fixation collapses the perivascular space and significantly reduces the accuracy of the observation (Sun et al., 2010). Magnetic resonance imaging (MRI) can provide a real-time CSF-ISF exchange map of the whole brain in a relatively noninvasive manner, it has become a more favorable detection method for exploring the glymphatic system. In 2013, dynamic contrast-enhanced MRI was applied to reveal the
The lymphatic vessels are considered to be an outflow pathway within the glymphatic system, draining ISF from the central nervous system to the periphery tissue (Louveau et al., 2015b). An MRI study of 35 patients with ischemic stroke confirmed measurable changes in signal intensity in six regions of the brain (Zhou et al., 2020). The results showed that the clearance of the glymphatic system and meningeal lymphatic vessels was associated with aging, and the drainage rate of the glymphatic pathway was significantly faster. These findings further clarified the role of lymphatic vessels in the clearance of interstitial fluid (Mestre et al., 2020). Since meningeal lymphatic vessels can directly drain macromolecules and immune cells from the CSF to the cervical lymph nodes, they are believed to participate in the regulation of immune responses in the brain. Labeled T cell experiments showed that a large proportion of lymphocytes were detected in the cervical lymph nodes, which further drains to the superficial and deep cervical lymph nodes (Louveau et al., 2018). This process relied on the chemokine receptor 7/chemokine C-C motif ligand 21 pathway through which the immune system activated the endothelial and pericyte networks. Phenotyping of meningeal lymphatic vessels blocked the immune cell migration pathway and reduced the activation of ependymal T cells in lymph nodes (Aldea et al., 2019). The severity of meningeal lymphatic dysfunction, such as experimental autoimmune encephalomyelitis (Louveau et al., 2018). Conversely, the integrity of the meningeal lymphatic vessels is essential for anti-tumor immunotherapy. Vascular endothelial growth factor C enhanced meningeal lymphangiogenesis in brain tumors, promoted dendirctic drainage to lymph nodes, and led to the activation of tumor-specific T and B cell populations (Song et al., 2020). With the help of vascular endothelial growth factor-C, immune checkpoint inhibition therapy showed better efficacy in tumor treatment (Hu et al., 2020).

Impairment of the Lymphatic Drainage Systems Following Ischemic Stroke

The dysfunction of lymphatic drainage in ischemic stroke

In ischemic stroke, glucose and oxygen depletion can lead to irreversible neuronal injury, accompanied by the generation of metabolic byproducts (Gabere et al., 2014). Infarct areas are rich in abundant factors related to neurotoxicity and neurodegeneration, such as pro-inflammatory cytokines and chemokines (Zbesko et al., 2016). Therefore, the glymphatic system, as a waste clearance pathway, could be involved in the pathological process of ischemic stroke. Researchers have found that lymphatic drainage function was impaired in ischemic stroke mice. In a study by Gabere et al. (2014), MRI and histological examination showed a blockade of ipsilateral glymphatic perfusion three hours after middle cerebral artery occlusion (MCAO). Twenty-four hours later, the middle cerebral artery was reanastomosed and the lymphatic system function returned to normal. The authors attributed this dysfunction of lymphatic drainage to the fibrinolytic drugs could restore arterial patency and improve lymphatic function (Gabere et al., 2014). A similar phenomenon could be observed in mice with multiple microinfections. The experimental results showed that the flow of fluorescent CSF tracers along the periaxial space was significantly reduced and did not recover even 2 weeks later (Wang et al., 2017). The intensity of the CSF tracer in microinfarct cores increased significantly and CSF protein trapping persisted for up to 14 days. In comparison to young mice, more severe damage, implying that aging brain was more vulnerable to ischemia (Wang et al., 2017). A recent study reported impaired lymphatic function in humans. The DTI-ALPS index in the ischemic hemisphere of stroke patients was significantly lower than that of the contralateral hemisphere and normal controls, which gradually recovered 14 days after stroke (Toh and Siow, 2021).

The capture of toxic solutes within infarcted lesions can serve as persistent foci of chronic neuroinflammation, leading to cognitive dysfunction and progression of dementia (Zbesko et al., 2018). For example, when tau protein clearance by the glymphatic system is interfered with, this may aggravate post-stroke dementia (Bacc et al., 2020). Focal cerebral ischemia can also cause neuronal damage in distal areas with fiber connections to the ischemic area, such as the substantia nigra (Nakane et al., 2001). Lin et al. (2020) found that in a rodent model that in the acute and subacute stages after stroke, the clearance rate of contrast agent was slower in the ipsilateral substantia nigra, indicating a decrease in lymphatic function in this area. Potential mechanisms may include increased intracranial pressure, AQ4P depolarization, and swelling of the astrocytic endfeet (Lin et al., 2020).

In recent years, greater attention has been paid to lymphatic system dysfunction in ischemic stroke. The lymphatic system has been proposed as an adverse effect of ischemic stroke, leading to secondary hypoperfusion, additional tissue loss, increased intracranial pressure, and potential risk of cerebral hernia (Mestre et al., 2020). In Mestre et al.’s study (Mestre et al., 2020), high intensity focused ultrasound treatment was performed to modulate the lymphatic vessels. The results showed that the expression of AQP4 in astrocyte endfeet surrounding blood vessels decreased in a significant manner after US treatment, which improved the drainage of perivascular fluid (Mestre et al., 2020). As a result, most lymphocytes were detected in the cervical lymph nodes, which further drains to the superficial and deep cervical lymph nodes (Louveau et al., 2018). This process relied on the chemokine receptor 7/chemokine C-C motif ligand 21 pathway through which the immune system activated the endothelial and pericyte networks. Phenotyping of meningeal lymphatic vessels blocked the immune cell migration pathway and reduced the activation of ependymal T cells in lymph nodes (Aldea et al., 2019). The severity of meningeal lymphatic dysfunction, such as experimental autoimmune encephalomyelitis (Louveau et al., 2018). Conversely, the integrity of the meningeal lymphatic vessels is essential for anti-tumor immunotherapy. Vascular endothelial growth factor C enhanced meningeal lymphangiogenesis in brain tumors, promoted dendirctic drainage to lymph nodes, and led to the activation of tumor-specific T and B cell populations (Song et al., 2020). With the help of vascular endothelial growth factor-C, immune checkpoint inhibition therapy showed better efficacy in tumor treatment (Hu et al., 2020).

AQP4 changes in ischemic stroke

AQ4P is the most abundant aquaporin in the brain, including two isoforms M1 and M23 (Salman et al., 2022). AQ4P expression is highly concentrated in astrocyte endfeet surrounding blood vessels, while localization in cell bodies is less common. The polarity of AQ4P is related to dystrophic associated complex, which helps anchor AQ4P to the vascular basement membrane (Salman et al., 2022). AQ4P is crucial to the physiological function of the lymphatic system, providing a pathway for the exchange of CSF and ISF driven by an osmotic pressure gradient, therefore the proper expression and distribution of AQ4P ensure the efficiency of the lymphatic system.

Due to the important role of AQ4P, its changes in ischemic stroke have been a central concern. The importance of AQ4P expression has been demonstrated in the transient MCAO model. One hour after stroke onset, the expression of AQ4P in astrocyte endfeet is rapidly up-regulated in the area of the lesion (Ribeiro Mde et al., 2006). At 48 hours, AQ4P expression reaches a second peak and its distribution shifts to the astrocyte soma membrane. Two peaks of brain edema are also observed coinciding with increased AQ4P expression, suggesting that AQ4P might serve as the main route of fluid movement after cerebral ischemia (Ribeiro Mde et al., 2006). In other models of ischemic stroke, such as mice with nicotine and nicotine-induced ischemia, the first peak of AQ4P expression reaches a maximum 24 hours after MCAO, while normalization occurs 28 days later (Badawi et al., 2007; Wang et al., 2012). A recent study revealed the mechanism of altered subcellular localization and increased expression of AQ4P after hypoxia (Kichen et al., 2020). Calcium influx in astrocytes activates a pressure induced membrane protein (AQP4), leading to the phosphorylation of the carboxyl terminus of AQP4, which facilitates the relocalization of AQP4 from intracellular vesicles to the cell membrane. Protein kinase A can also mediate nuclear localization of the transcription factor FoxO3a, which directly activates the AQ4P gene and leads to increased expression of AQ4P (Kichen et al., 2020).
How do these changes affect cerebral edema after stroke? The exact answer may depend on the different time course of the disease. After intracerebral vascular occlusion, cerebral edema progresses from cytotoxic to vasogenic edema over time. Cytotoxic edema is caused by the imbalance of ion gradients and subsequent accumulation of intracellular fluid. While vasogenic edema occurs when leakage of the BBB allows plasma components to flow into the interstitial space. Early studies have confirmed that AQP4 can facilitate both water flow into the brain in cytotoxic edema and excess water removal from the brain in vasogenic edema (Manley et al., 2000; Papadopoulos et al., 2004). Conflicting outcomes in AQP4 knockout mice after ischemic stroke also showed the complexity of AQP4 functions. Research also supports the neuroprotective effect of AQP4 deficiency. In the study by Mestre et al. (2020), AQP4 knockout did not inhibit edema with 15 minutes after MCAO. After 24 hours of permanent ischemia, in AQP4 deletion mice, neurological outcomes showed improvements implied by the reduced mortality rate and deficit score (Papadopoulos et al., 2004). However, other studies held the opposite view and suggested that the lack of AQP4 may hinder the clearance of edema fluid and subsequently exacerbate neurological injury. For example, after 72 hours of reperfusion, the AQP4-/- mice showed increased mortality and neurological deficits (Zeng et al., 2012). Therefore, early up-regulation of AQP4 after ischemic stroke accounts for the development of cytotoxic edema, while a later increase in AQP4 expression may partly serve as a self-protection mechanism to improve water clearance in the phase of vasogenic edema.

Apart from increased expression, loss of AQP4 polarity has also been observed. After an ischemic stroke, the pattern of AQP4 distribution transfers from the perivascular endfeet to the entire astrocytic membrane, which may compromise AQP4 function. Misllocalization of AQP4 is related to enhanced amyloid pathology and neuroinflammation, resulting in vascular cognitive impairment (Back et al., 2017; Lyu et al., 2021). In the ischemic brain hemisphere, the expression of AQP4-M1 increased (Hirt et al., 2009), which could promote astrocyte migration and glial scar formation, but is not conducive to perivascular polarization of AQP4 (Smith et al., 2014). AQP4 also interacts with various ion channels on astrocytes, such as transient receptor potential vanilloid 4 and sulfonylurea receptor 1-transient receptor potential melastatin 4, which also influence water transport through astrocyte membranes and facilitate the edema development (Salman et al., 2022).

**Potential Therapies Aimed at Lymphatic Drainage Systems**

Today, classical strategies such as fibrinolysis and endovascular therapies are still the main treatment methods for ischemic stroke. New treatments are being explored, but their efficacy is not known. Restoring the function of lymphatic drainage systems can improve the prognosis of cerebral ischemia, and some representative drugs are summarized in Table 1. Since AQP4 promotes the formation of cytotoxic edema in early stroke (Mestre et al., 2020), early inhibition of AQP4 function can slow the entry of fluid into the brain parenchyma. There have been multiple attempts to identify AQP4 inhibitors that reduce AQP4-mediated water transport, some of which have shown positive effects. For instance, acetazolamide can inhibit human AQP4-mediated water permeability by 80% (Huber et al., 2007). In a rat model of cerebral infarction caused by bilateral carotid artery ligation, human AQP4-mediated water permeability by 80% (Huber et al., 2007). In a rat model of cerebral infarction caused by bilateral carotid artery ligation, acetazolamide administration reduced brain water content and improved neurological outcomes in MCAO mice and attenuated BBB damage (Igarashi et al., 2011). Moreover, based on a similar mechanism, trifluperazine showed the ability to reduce brain water content during the acute phase of stroke (Sylvain et al., 2021). By inhibiting AQP4 expression through the extracellular signal-regulated kinase 1/2 pathway, methylene blue showed a neuroprotective effect on ischemic stroke and improved cerebral edema and ischemic pathology (Shi et al., 2021). Conversely, restoration of AQP4 polarity is also a potential therapeutic approach to facilitate the clearance of metabolites from infarcted areas; however, few drugs have been reported to have this effect. Experimental studies have demonstrated that sirt protein homologue 2 (He et al., 2020) and caveolin-1 (Flikkenko et al., 2020) play a role in maintaining perivascular AQP4 expression after cerebral ischemia. Dextrin accelerates AQP4 polarization, thus promoting recovery of glymphatic function after hypoperfusion (Cao et al., 2022).

In addition to AQP4, other factors modulate glympathic function. Basement membrane fibrosis induced by transforming growth factor-β leads to impaired CSF-ISF exchange and poor functional recovery in MCAO mice (Howe et al., 2019). Therefore, inhibition of the transforming growth factor-β receptor may be beneficial for cerebral ischemia. Adrenergic receptor antagonism facilitates fresh CSF influx and normalizes extracellular potassium concentration, thus inhibiting cortical SD wave propagation and improving stroke outcome (Monai et al., 2019).

Improving IPAD drainage is also beneficial in preventing the adverse prognosis of stroke, especially vascular cognitive impairment. The treatment strategies targeting IPAD work by regulating the function of SMC and promoting arterial dilation. Cilostazol is an inhibitor of phosphodiesterase-3 in SMC, which can modulate SMC function by upregulating the signaling pathways of cyclic adenosine monophosphate and cyclic guanosine monophosphate (Maki et al., 2014). Cilostazol treatment has been shown to facilitate Aβ drainage along the IPAD pathway and reduce Aβ accumulation in the brain (Maki et al., 2014). Another drug, fasudil hydrochloride acts by stimulating the endothelial nitric oxide synthase pathway and promoting arterial dilation (Nizari et al., 2021). The increase in the number of blood vessels containing Aβ in animals treated with fasudil suggested an improvement in IPAD drainage (Nizari et al., 2021). Taxifolin exerts therapeutic effects on cerebral amyloid angiopathy in many ways. In addition to its antioxidant and anti-inflammatory effects, taxifolin also restores cerebrovascular reactivity and promotes Aβ clearance from the brain (Saito et al., 2021). The application of these drugs after ischemic stroke warrants further exploration in future experiments. The therapeutic targets for lymphatic drainage systems are indicated in Figure 3.

## Conclusions and Perspectives

Three anatomically and functionally related lymphatic drainage systems in the brain, including lymphatic system, IPAD pathway and meningeal lymphatic vessels, were discussed in this review. The lymphatic system involves the perivascular CSF influx and ISF efflux pathway, which promotes metabolite clearance and maintains fluid balance. Arterial pulsation and sleep are the most studied regulatory mechanisms. Meanwhile, the role of the circadian rhythm cannot be ignored. Compared to conventional fluorescence imaging in vivo or in vitro, MRI has more advantages in the detection of the human lymphatic system. DTI, a noninvasive real-time monitoring method, is used to explore the function of the lymphatic system and will contribute to a wider application in daily clinical work in the future, which may help identify patients at high risk of a poor prognosis after ischemic stroke.

### Table 1 | Potential therapies targeting lymphatic drainage systems

| Target | Mechanism | Representative drugs | Effect | Reference |
|--------|-----------|----------------------|--------|-----------|
| **Lymphatic system** | AQP4 | Inhibits AQP4 mediated water transport | Reduces the brain water content and improves pathology caused by infarction changes | Hao et al., 2022 |
| | TGN-020 | Reduces brain edema and infarcted volume, improves motor performance, reduces albumin extravasation, gliosis, and apoptotic cells | Igarashi et al., 2011; Pirici et al., 2017 |
| **Downregulates AQP4 expression** | AER-270 | Controls cerebral edema and improves neurological outcome | Farr et al., 2019 |
| | mir-29b | Reduces infarct volume and edema formation, attenuates blood-brain barrier disruption | Wang et al., 2015 |
| | Methylene blue | Ameliorates brain edema, astrocytic swelling, and pathological changes of ischemia | Shi et al., 2021 |
| | Trifluperazine | Reduces total brain water content during the acute phase of stroke | Sylvain et al., 2021 |
| | Dextrin | Attenuates brain injury and cognitive dysfunction after cerebral hypoperfusion | Cao et al., 2022 |
| **IPAD pathway: SMC** | TGN-020 | Facilitates IPAD drainage along the IPAD pathway, reduces Aβ accumulation in the brain | Maki et al., 2014 |
| | Fasudil hydrochloride | Increases IPAD drainage and the number of Aβ40-containing vessels | Nizari et al., 2021 |
| | Taxifolin | Restores cerebrovascular reactivity and promotes Aβ clearance from brain to blood | Saito et al., 2021 |
Impaired glymphatic system and IPAD in ischemic stroke and therapeutic targets.

The overall lymphatic drainage systems. The meningeal lymphatic vessels act as an important fluid efflux pathway of the glymphatic system, draining CSF and ISF to the deep cervical lymph nodes. AQP4: Aquaporin-4; CSF: cerebrospinal fluid; IPAD: intramural perivascular drainage; ISF: interstitial fluid.

The impairment of the glymphatic system has been proven to be a crucial mechanism in the pathological process of ischemic stroke. According to experimental results, perivascular space enlargement and increased glymphatic flow during early cerebral ischemia could be the potential source of brain edema. In contrast, the decrease of glymphatic inflow and outflow in the later stages aggravates the accumulation of toxic solutes and deteriorates cognitive function. Therefore, it is necessary to intensively explore the entire time course of glymphatic changes. IPAD and meningeal lymphatic vessels are also altered in response to ischemic stroke. Enhancing meningeal lymphatic drainage function is beneficial for improving cerebral edema but is harmful for controlling the extent of the inflammatory injury. Therefore, the long-term effects of meningeal lymphatic drainage on ischemic damage need to be further evaluated.

Restoring the normal functions of the lymphatic drainage systems can improve neurological outcomes. Most studies provide evidence that AQP4 knock-out animals exhibit reduced cerebral edema after ischemic stroke compared to wild-type animals. Therefore, AQP4 inhibitors may have neuroprotective effects if administered during the cytotoxic edema phase. Despite many attempts, the development of a safe AQP4 inhibitor for humans is still far from being achieved. Interestingly, the expression and distribution of AQP4 at the subcellular level may be a promising new target for drug development in the future; however, this pathway also requires more exploration of the potential molecular mechanism involved. Today, few studies targeting IPAD and the meningeal lymphatic system have been reported for the treatment after ischemic stroke, and further exploration of key regulatory sites and modulation mechanisms may offer some improvement.

There are some limitations in this review. First, other pathways of CSF clearance from the brain, such as arachnoid granulations and perineural routes through the cribriform plate, were not fully discussed. Second, less attention was paid to the role of intracerebral lymphatic drainage systems in brain injury after hemorrhagic stroke. Third, the glymphatic influx may also serve as a thrilling route for drug delivery to the brain parenchyma, which has not been thoroughly addressed in this review.

In summary, despite the existence of unresolved questions, the discovery of lymphatic drainage pathways still opens a new avenue for the treatment of ischemic stroke.
Arbel-Ornath M, Hudry E, Eikermann-Haerter K, Hou S, Gregory JL, Zhao L, Betensky RA, Frosch MP, Greenberg SM, Backai Bi (2013) Intravascular fluid drainage is impaired in ischemic stroke and Alzheimer’s disease mouse models. Acta Neuropathol 126:353-364.

Asgari M, de Zélicourt D, Kurtcuoglu V (2016) Glymphatic solute transport does not require bulk flow. Sci Rep 6:38635.

Aspelyn A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, Wiig H, Alitalo K (2008) Solutes, but not cells, drain from the brain parenchyma along basement membrane of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroinflammation. Neuropathol Appl Neuropathol 34:131-144.

Chen J, Li X, Ni R, Yang Q, Zhang Y, Luo L (2019) Cerebrovascular injuries induce lymphatic invasion into brain parenchyma to guide vascular regeneration in zebrafish. Dev Cell 49:697-710.e5.

Chen J, Li X, Ni R, Chen Q, Yang Q, He J, Luo L (2021) Acute brain vascular regeneration occurs via lymphatic transdifferentiation. Dev Cell 56:3115-3127.e6.

Christensen J, Yamakawa GR, Shultz SR, Mychayliuk R (2021) Is the lymphatic system the missing link between sleep impairments and neurological disorders? Examining the implications and uncertainties. Prog Neurobiol 198:101917.

Diem AK, Carare RO, Weller RO, Bressloff NW (2018) A control mechanism for intramural peri-arterial drainage via astrocytes: How neuronal activity could improve waste drainage from the brain. PLoS One 13:e0205276.

Diem AK, MacGregor Sharp M, Gatherer M, Bressloff NW, Carare RO, Richardson G (2017) Arterial pulsations cannot drive intramural periaxial drainage: significance for Aβ drainage. Front Neurosci 11:475.

Du T, Mestre H, Kress BT, Lu G, Sweeney AM, Samson AJ, Rasmussen MK, Mortensen KN, Bork PAR, Peng W, Oleveda GE, Bashford L, Toror ER, Tift JD, Kelley DH, Thomas JH, Hjorth PG, Martens EA, Mehta RI, Hirase H, et al. (2022) Cerebrospinal fluid is a significant fluid source for amniotic fluid. Brain 145:787-797.

Eide PK, Ringstad G (2015) MRI with intrathecal MRI gadolinium contrast medium: a possible method to assess glymphatic function in human brain. Acta Radiol Open 4:205846115606635.

Esposito E, Ahn BJ, Shi J, Nakamura Y, Park JH, Mandeville ET, Yu Z, Chan SJ, Desai R, Eide PK, Ringstad G (2016) Phenylbenzamides inhibit aquaporin-4 reducing cerebral edema and improving neuronal excitotoxicity, accelerates glymphatic clearance, and improves cognition in a multiple microinfarcts model. Mol Brain 13:135.

Hawkes CA, Jaykloyd N, Johnston DA, Bechmann I, Carare RO (2014) Failure of perivascular drainage of β-amylloid in cerebral amyloid angiopathy. Brain Pathol 24:396-403.

He Xi, Li G, Li LL, Li MF, Liang FY, Chen X, Hu XQ (2020) Overexpression of SH2 decreases neuronal excitotoxicity, accelerates glymphatic clearance, and improves cognition in a multiple microininfarcts model. Mol Brain 13:135.

Holtk J, Fritschi SK, Wang C, Pedersen NP, Cirillo JR, Mahan TE, Finn MB, Manis M, Geerling JC, Fuller PM, Lucey BP, Holtzman DM (2019) The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans. Science 363:880-884.

Hofman JW, Furr MJ, Munsly Y, Roy-O’Reilly MA, Mandeski, Moelling J, Frankel EC, D’Agle J, Sensing LH, McLennan LD, Urayama A (2019) Transforming growth factor-β promotes basement membrane fibrosis, alters perivascular cerebrospinal fluid distribution, and worsens neurological recovery in the aged brain after stroke. Geroscience 41:543-559.

Hu X, Deng Q, Ma L, Li Q, Chen Y, Liu Y, Zou F, Zhang C, Shao L, Feng L, He T, Ning W, Kong Y, Huo Y, He A, Liu B, Zhang J, Adams R, He Y, Tang F, et al. (2020) Meningeal lymphatic vessels regulate brain tumor drainage and immunity. Cell Res 30:229-243.

Huber VJ, Tsujiya M, Yamazaki M, Sakimura K, Nakada T (2007) Identification of α-synuclein oligomers as Aquaporin 4 inhibitors. Bioorg Med Chem Lett 17:1270-1273.

Igarashi H, Huber VJ, Tsujiya M, Nakada T (2011) Pretreatment with a novel aquaporin 4 inhibitor, TGN-020, significantly reduces ischemic cerebral edema. Neurol Sci 32:113-116.

Ijji FF, Wang M, Liao Y, Plogga BA, Peng W, Gundersen GA, Benveniste H, Yates GE, Deane R, Goldman SA, Nagelhus EA, Nigedegaard M (2022) A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med 4:147ra111.

Ijji FF, Nigedegaard M (2013) Is there a cerebral lymphatic system? Stroke 44:593-95.

Ijji FF, Le H, Yu M, Feng T, Logan J, Nigedegaard M, Benveniste H (2013a) Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. J Clin Invest 123:1299-1309.

Ijji FF, Wang M, Zeppenfeld DM, Venkatarasan A, Plog BA, Liao Y, Deane R, Nigedegaard M (2013b) Cerebral arterial pulsation drives paravascular CSF-interstitial fluid exchange in the murine brain. J Neurosci 33:18190-18199.

Ijji FF, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, Yang L, Singh I, Deane R, Nigedegaard M (2014) Impairment of paravascular pathway function promotes tau pathology through traumatic brain injury. J Neurosci 34:16180-16193.

Ijji FF, Goldman SA, Nigedegaard M (2015) Implications of the discovery of brain lymphatic pathways. Lancet Neurofl 14:977-97.

Jenssen NA, Munk AS, Lundgaard I, Nigedegaard M (2015) The lymphatic system: A Beginner’s guide. Neurochem Res 40:2583-2599.

Jiang C, Wang ZN, Kang YC, Chen Y, Lu WX, Ren HJ, Hou BR (2022) N2O227 aggravates apoptosis, inflammatory response, and oxidative stress after focal cerebral ischemia injury. Neurog Regen Rep 17:133-149.

Kitchen J, Salaman MM, Halsey AM, Clarke-Bland C, MacDonald JA, Ishida H, Vogel K, Altmann S, Logan A, Kreida S, Al-Jubair T, Winkel Minsel J, Gourdon P, Törnroth-Horrestedt S, Conner MT, Ahmed Z, Conner AG, Bill RM (2020) Targeting aquaporin-4 subcellular localization to treat central nervous system edema. Cell 181:784-799.e19.

Kiviniemi V, Wang X, Korhonen V, Keinänen T, Tuovinen T, Autio J, LeVan P, Keilholz S, Zang RD, Boutelle MG, Brennan KC, Alam AP, Drenchak H, Dohmen C, Fabricius M, Farkas E, Feuerstein D, Graf R, Helbok R, Laruitzen M, Major S, et al. (2017) The continuum of spreading depolarizations in acute cortical lesion development: Examining Leão’s legacy. J Cereb Blood Flow Metab 37:1571-1594.

Lee H, Lee DA, Shin KI, Park JM (2022) Glymphatic system dysfunction in obstructive sleep apnea evidenced by DTS-ALPS. Sleep Med 89:176-181.

Li TT, Wang Q, Zhang X, Xiao Y, Sun LY, Zhang YR, Liu XN, Yang WC (2022) Stellate ganglion block reduces inflammation and improves neurological function in diabetic rats during ischemic stroke. Neuronal Regen Res 17:1991-1997.

Li Y, Han H, Shi K, Cui D, Yang J, Alberts H, Yuan L, Zhao G, Wang R, Cai X, Teng Z (2020) The mechanism of downregulated interstitial fluid drainage following neuronal excitation. Aging Dis 11:1407-1422.

Review
Lin L, Hao X, Li C, Sun C, Wang X, Yin L, Zhang X, Tian J, Yang Y (2020) Impaired glymphatic system in secondary degeneration areas after ischemic stroke in rats. J Stroke Cerebrovasc Dis 29:104828.

Louveau A, Harris TH, Kipnis J (2015a) Revisiting the mechanisms of CNS immune privilege. Trends Immunol 36:569-577.

Louveau A, Smirnov I, Keyes TI, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J (2015b) Structural and functional features of central nervous system lymphatic vessels. Nature 523:337-341.

Louveau A, Herz J, Alme MN, Salvador AF, Dong MQ, Vier KE, Hero G, Knopp J, Setlik JC, Lupi AD, Da Mesquita S, Frost EL, Gaulther A, Harris TH, Cao R, Hu S, Lukens JR, Smirnov I, Overall CC, Oliver G, et al. (2018) CNS lymphatic drainage and neuroinflammation are regulated by meningeal lymphatic vasculature. Nat Neurosci 21:1380-1391.

Lundgaard J, Lu ML, Yang E, Peng W, Mestre M, Hitimoni E, Deane R, Nedergaard M (2017) Glymphatic clearance controls state-dependent changes in brain lactate concentration. J Cereb Blood Flow Metab 37:2112-2124.

Lyu Z, Chan Y, Li Q, Zhang Q, Liu K, Xiang J, Li X, Cai D, Li Y, Wang B, Yu Z (2021) Destructive effects of pyroptosis on homeostasis of neuron survival associated with the dysfunctional BBB-glymphatic system and amyloid-beta accumulation after cerebral ischemia/reperfusion in rats. Neural Plast 2021:4504363.

Ma X, Li S, Li C, Wang R, Chen M, Chen H, Su W (2021) Diffusion tensor imaging along the perivascular space in different stages of Parkinson’s disease. Front Aging Neurosci 13:773951.

Mak T, Okamoto Y, Carare RO, Hase Y, Hattori Y, Hawkes CA, Saito S, Yamamoto Y, Terasaki Y, Ishibashi-Ueda H, Taguchi A, Takashiki R, Miyokawa T, Kalaria RN, Lo EH, Arai K, Ibara M, Cheng P (2014) Phosphodiesterase III inhibitor promotes drainage of cerebrovascular β-amyloid. Ann Clin Transl Neurol 1:519-533.

Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, Chan P, Verkman AS (2000) Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Med Neurol 6:159-163.

Mestre H, Tithof J, Du T, Song W, Peng W, Sweeney AM, Olveda G, Thomas JH, Nedergaard M, Kelley DH (2018) Flow of cerebrospinal fluid is driven by arterial pulsations and is reduced in hypertension. Nat Commun 9:4878.

Mestre H, Du T, Sweeney AM, Liu G, Samson AJ, Peng W, Mortensen KN, Staeger FF, Bok PAR, Bashford L, Toro ER, Tithof J, Kelley DH, Thomas JH, Jhorth PG, Martens EA, Mehta R, Solis O, Bender R, Kleinfeld D, et al. (2020) Cerebrospinal fluid influx drives acute ischemic tissue swelling. Science 367:eaaw7171.

Monai H, Wang X, Yagahai K, Lou N, Mestre H, Xu Q, Abe Y, Yasui M, Iwade Y, Nedergaard M, Hirase H (2019) Adrenergic receptor antagonism induces neuroprotection and facilitates recovery from acute ischemic stroke. Proc Natl Acad Sci U S A 116:10100-11019.

Morris AW, Sharp MM, Albaro Hy, Fernandes R, Hawkes CA, Mestre H, Weller R (2015a) Revisiting the mechanisms of CNS immune privilege. Trends Mol Med 26:285-295.

Maki T, Okami TO, Kanai Y, Alme MN, Salvador AF, Dong MQ, Zhou R, Verma A, Weller RO, Monai H, Wang X, Yahagi K, Lou N, Mestre H, Wang XX, Wang YJ, Yuan J, Lou N, Mestre H, Xu Q, Abe Y, Yasui M, Iwade Y, Nedergaard M, Hirase H (2019) B-amyloid accumulation in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 116:4483-4488.

Smith AJ, Jin BJ, Ratelade J, Verkman AS (2014) Assembly state determines the localization and function of M1- and M23-aquaporin-4 in astrocytes. J Cell Biol 204:559-573.

Song E, Mao T, Dong H, Boisserand LS, Antilla S, Bosenberg M, Altai A, Thomas JL, Iwakasi A (2020) VEGF-C-driven lymphatic drainage enables immunosurveillance of brain tumours. Nature 577:689-694.

Sundaram S, Hughes RL, Peterson E, Müller-Oehring EM, Bronté-Stewart HM, Poston KL, Faerman A, Bhowick C, Schulte T (2019) Establishing a framework for neurovascular physiology: correlates and synaptic function in Parkinson’s disease. Neurosci Biobehav Rev 103:305-315.

Sweeney AM, Plä V, Du T, Liu G, Sun Q, Peng S, Plag BA, Kress BT, Wang X, Mestre H, Nedergaard M (2019) In vivo imaging of cerebrospinal fluid transport through the intact mouse skull using fluorescence microscopy. J Vis Exp e59974.

Syván NC, Salesal MJ, Pushie MJ, Hou H, Meher V, Herlo R, Peeling L, Kelly MF (2021) The effects of trifluoperazine on brain edema, aquaporin-4 expression and metabolic markers during the acute phase of stroke using photoluminescent mouse model. Biochim Biophys Acta Biomembr 1863:183573.

Taoka T, Masatumi Y, Kawai H, Nakane T, Matsuoka S, Yasuno F, Kishimoto T, Naganawa S (2017) Evaluation of glymphatic system activity with the diffusion MR technique: diffusion tensor image analysis along the perivascular space (DTI-ALPS) in Alzheimer’s disease cases. Jpn J Radiol 35:172-178.

Taoka T, Ito R, Nakamichi R, Kamagata K, Sakai M, Kawai H, Nakane T, Abe T, Ichikawa K, Kikutaka J, Aoki S, Naganawa S (2022) Reproducibility of diffusion tensor image analysis along the perivascular space (DTI-ALPS) for evaluating interstitial fluid diffusion and glymphatic function. Change of the ALS index on multiple condition acquisition experiment (CHAMOINDEX) study. Jpn J Radiol 40:147-158.

Toh CH, Siow TY (2021) Glymphatic dysfunction in patients with ischemic stroke. Front Aging Neurosci 13:756249.

van Veluwe SJ, Hou SS, Calvo-Rodriguez M, Arbel-Ornath M, Snyder AC, Frosch MP, Greenberg SM, Baczaki BI (2020) Vasomotion as a driving force for paravascular clearance in the awake mouse brain. Neurobiol 105:5469-561.e5.

Wang M, Ding F, Deng S, Guo X, Wang W, Iliff JJ, Nedergaard M (2017) Focal solute trapping and global glymphatic pathway impairment in a murine model of multiple microinfarcts. J Neurosci 37:2870-2877.

Wang M, Iliff JJ, Liao Y, Chen M, Shinetski MS, Venkataraman A, Cheung J, Wang W, Wiers CE, Demiral SB, Guo M, Kim SW, Lindgren E, Ramirez V, Zehra A, Freeman C, Miller G, Manza P, Srivastava T, De Santis T, Tomàs D, Benveniste H, Holm TK (2018) β-Amyloid immunization in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 115:4483-4488.

Wiers CE, Demiral SB, Guo M, Kim SW, Lindgren E, Ramirez V, Zehra A, Freeman C, Miller G, Manza P, Srivastava T, De Santis T, Tomàs D, Benveniste H, Holm TK (2018) β-Amyloid immunization in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 115:4483-4488.

Wiers CE, Demiral SB, Guo M, Kim SW, Lindgren E, Ramirez V, Zehra A, Freeman C, Miller G, Manza P, Srivastava T, De Santis T, Tomàs D, Benveniste H, Holm TK (2018) β-Amyloid immunization in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 115:4483-4488.

Wiers CE, Demiral SB, Guo M, Kim SW, Lindgren E, Ramirez V, Zehra A, Freeman C, Miller G, Manza P, Srivastava T, De Santis T, Tomàs D, Benveniste H, Holm TK (2018) β-Amyloid immunization in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 115:4483-4488.

Wiers CE, Demiral SB, Guo M, Kim SW, Lindgren E, Ramirez V, Zehra A, Freeman C, Miller G, Manza P, Srivastava T, De Santis T, Tomàs D, Benveniste H, Holm TK (2018) β-Amyloid immunization in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 115:4483-4488.

Wiers CE, Demiral SB, Guo M, Kim SW, Lindgren E, Ramirez V, Zehra A, Freeman C, Miller G, Manza P, Srivastava T, De Santis T, Tomàs D, Benveniste H, Holm TK (2018) β-Amyloid immunization in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 115:4483-4488.