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THE BRAIN'S LYMPHATIC SYSTEM?

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Abstract

Despite the great metabolic activity of the brain, there doesn't seem to be an anatomically defined lymphatic system. The search for the way the brain gets rid of its waste products revealed the existence of a system which was called glymphatic due to its similarity to the body's lymphatic system and its dependence on glial cells, specifically water aquaporin 4 (AQP4) channels located on the endfeet of astrocytes which surround blood vessels in the brain. The components of the glymphatic system are cerebrospinal fluid, interstitial fluid, periarterial and perivenous spaces. Cerebrospinal fluid flows from the subarachnoid space to periarteriolar spaces, following pial arteries down through the brain, entering astrocytes through AQP4 and then the parenchyma of the brain. There it interchanges with the interstitial fluid and flows into the perivenous spaces, 'flushing' away waste products. The pathway ends in the cervical lymph nodes. The role of several manipulations which lower glymphatic activity was examined in various experiments. One of the manipulations is sleep deprivation. Dysfunction of this 'cleansing' mechanism could be responsible for the development of diseases characterized by accumulation of metabolites, such as Alzheimer's disease, and possibly glaucoma. The glymphatic system is also considered responsible for the appearance of markers for traumatic brain injury in the peripheral blood. All of these findings point to the importance of the glymphatic system, and its assessment could help monitor and treat patients with neurodegenerative diseases.

KEYWORDS: Alzheimer disease, aquaporin 4, cerebrospinal fluid, glymphatic system, traumatic brain injury

INTRODUCTION

The lymphatic system drains excessive fluid and metabolic products from interstitial space in our tissues. Density of lymphatic vessels is proportional to the metabolic rate of the tissue itself. So one would think that the central nervous system (CNS), which has a high metabolic rate, would also have a dense lymphatic network. Considering that no anatomical structures resembling classical lymphatic vessels had been found so far, alternative drainage pathways were proposed. The first pathway suggests that cerebrospinal fluid (CSF) drains directly into the blood from the subarachnoid space via arachnoid villi of the superior sagittal sinus. The second pathway drains into the lymph nodes via subarachnoid spaces around the olfactory nerves and nasal lymphatics. The third route leads to the cervical lymph nodes following the capillaries and arteries which branch off the internal carotid artery.

THE GLYMPHATIC SYSTEM

So how does the brain 'flush' away all of its waste products? The answer is via the glymphatic system. CSF travels along the arteries of the brain inside of perivascular spaces (Virchow-Robin spaces).[1] As the arteries go deeper into the brain parenchyma the perivascular spaces become continuous with the basal lamina. The basal lamina does not interfere with the flow of CSF due to its permeability. The force which drives the flow of the CFS into the perivascular spaces is a combination of arterial pulsatility, respiration, slow vasomotion and CSF pressure gradients.[1] All blood vessels within the brain parenchyma are surrounded by astrocytic endfeet. Here the CSF interchanges with the interstitial fluid (ISF) with the help of aquaporin-4 (AQP4) channels expressed in a polarized manner in the astrocytic endfeet. CSF travels to the parenchyma via these AQP4 channels, through the astrocytes. ISF is then driven towards perivenous spaces by the movement of the CSF.[1] This theory was put to the test in an experiment where fluorescent tracers were infused into the subarachnoid space of mice. The movement of tracers was assessed *both ex vivo* via fixed vibratome brain slices and *in vivo* using two-photon laser scanning microscopy. Tracers of smaller molecular weight (3 kD) moved through the brain as described above, whereas larger molecules (2000 kD) stayed within the perivascular spaces.[2] The word glymphatic refers to the similarity in function with the lymphatic system while stressing the importance of the astrocytic glial cells and their AQP4. If not for these water channels, CSF would have to rely simply on diffusion which in this case would be too slow and, therefore, ineffective. Different researches showed that certain manipulations de-

creased glymphatic-associated clearance in mice. They were: (1) AQP4 deletion which disabled the flow of CSF from the perivascular spaces to the parenchyma, (2) cisterna magna cisternotomy which reduced the pressure created by the CSF by physically draining it, (3) acetazolamide treatment which reduced CSF production, and (4) sleep deprivation. [1]

HOW SLEEP AFFECTS THE GLYMPHATIC SYSTEM

An experiment was conducted to see how sleep affects glymphatic clearance. It was shown that during sleep the interstitial space is increased by 60%.[3] It allows easier CSF-ISF exchange and makes the brain more permeable to CSF. The change in interstitial space volume is achieved by changing astrocyte cell volume with the help of AQP4. Since the same results were shown both in naturally sleeping and anesthetized mice, circadian rhythms are not responsible for the change in volume. Researchers hypothesized that the state of arousal itself was responsible. In fact, locus coeruleus noradrenergic signaling, which is associated with wakefulness, reduces interstitial volume and decreases clearance. This was proven by giving awake mice a cocktail of adrenergic antagonists and measuring the clearance afterwards, which was increased.[3] It is possible that one of the major roles of sleep is also clearance of toxic biomolecules by altering the function of the glymphatic system.

EFFECTS OF AGING ON THE GLYMPHATIC ACTIVITY

In old mice glymphatic function decreased by ~ 80-90% compared to that in young mice.[1] The reason why this is the case may lie in AQP4, which plays a crucial role in CSF-ISF exchange. Genetic deletion of AQP4 was previously shown to decrease CSF-ISF exchange by~ 65%.[2] Since aging is associated with loss of perivascular AQP4 polarization it is probable that these channels are the main reason for the decrease of glymphatic function with age. AQP4 is depolarized from the vascular endfeet to the parenchymal processes which can be seen after traumatic brain injury. Also, with aging there is a decline in CSF production by 66% and CSF pressure by 27% which together along with the reduction of arterial pulsatility due to stiffening of the arterial wall can lead to a change in glymphatic function. This can be the reason why the risk of neurodegenerative disorders becomes higher with age. Neurodegenerative diseases are characterized by the accumulation of different proteins which were considered to be mainly intracellular. However, it was found that the protein aggregates are present in the CSF and ISF, e.g. misfolded β-amyloid (Aβ) and tau protein in Alzheimer's disease (AD) and misfolded α-synuclein in Parkinson's disease. The flow of CSF and ISF via glymphatic pathways helps clear these proteins, especially Aβ, from the extracellular space.[1] In AD pathology Aβ accumulates in perivascular spaces and the reason may be low activity of the glymphatic system. The second most common category of dementias are vascular dementias which are characterized by deformation of the perivascular space. Abnormalities in the perivascular space may have an impact on the glymphatic system due to a change in pathways of the CSF.[1] The before mentioned mislocalization of AQP4 from astrocytic endfeet to parenchymal processes, following traumatic brain injury, increases the risk of developing premature dementia and AD.

ROLE OF THE GLYMPHATIC SYSTEM IN GLAUCOMA

Glaucoma is a group of eye diseases which lead to optic nerve damage and vision loss. It is the number one cause of blindness worldwide. Most common is the primary open-angle glaucoma (POAG) which is caused by a blockage of the draining system in the anterior eye chamber, causing intraocular pressure (IOP) to increase. Another type of glaucoma which leads to a high IOP is the closed-angle glaucoma. There is also a normal-tension glaucoma where the IOP is not elevated. In all types of glaucoma a degeneration of retinal ganglion cells (RGC) is observed.
Since the eye is a direct extension of the brain is it possible that there is a glymphatic pathway similar to the one in the brain itself? We will start by reviewing the role of Aβ in glaucoma. RGCs exposed to chronic elevation of IOP showed abnormal processing of β-amyloid precursor protein which increased expression of Aβ.[4] Aβ is toxic to RGCs in higher amounts. Since 65% of Aβ in the normal brain is cleared via the glymphatic system, there is a possible link between glaucoma and AD. Several studies have shown that patients with AD have a higher prevalence of glaucoma.[5,6]

TRANSPORT OF TRAUMATIC BRAIN INJURY MARKERS TO THE BLOOD

Traumatic brain injury (TBI) is an injury to the brain caused by a trauma to the head. Since it is a growing problem causing a variety of symptoms, efforts were made to identify peripheral blood markers that would reflect the severity of the brain injury. The most studied marker is S100β which is a calcium-binding protein found in the cytoplasm of astrocytes but also in peripheral cells (Schwann cells, chondrocytes, adipocytes). A more specific marker would be GFAP, a principal intermediate filament making up the astroglial cytoskeleton which is expressed specifically in astrocytes. Also a glycolytic enzyme neuron specific enolase (NSE), specifically its γ-γ isoform, is predominantly found in neurons and is a potential TBI marker.[7]

A group of scientists conducted a series of experiments on female mice to see the effect of glymphatic inhibition on the blood levels of TBI markers after TBI.[7] The formerly mentioned four manipulations (section The Glymphatic System) that decrease glymphatic activity were used in this experiment. Mice whose glymphatic function was altered prior to the induced TBI had lower serum levels of markers in comparison to those in the control group (mice only with TBI). It shows that the glymphatic system transports endogenous molecules from the brain to the peripheral blood. On the other hand, TBI alone will decrease the function of the glymphatic system.[7] Therefore, this method of measuring blood marker levels to evaluate TBI is not objective. It is important to find a clinically appropriate method to measure glymphatic activity.

CLINICAL APPROACH TO EVALUATING THE GLYMPHATIC FUNCTION

Neurodegenerative disorders, such as those mentioned earlier, present a serious and still mysterious problem. The role of the glymphatic pathway may shed a little light on these conditions. That is why it is important to find a way to clinically assess its function.

An experiment was conducted in rats to determine the best way of introducing fluorescent tracers into the glymphatic system without causing damage to the central nervous system.[8] Two options were explored. One was an infusion into the cisterna magna which is contraindicated in humans because it shows high potential for iatrogenic complications, including traumatic tissue injury. Intrathecal lumbar delivery route, however, is routinely used in administering contrast for CT-myelography, spinal anesthetics, post-operative analgesics and chemotherapeutics, and presents a viable option for contrast-imaging in humans.

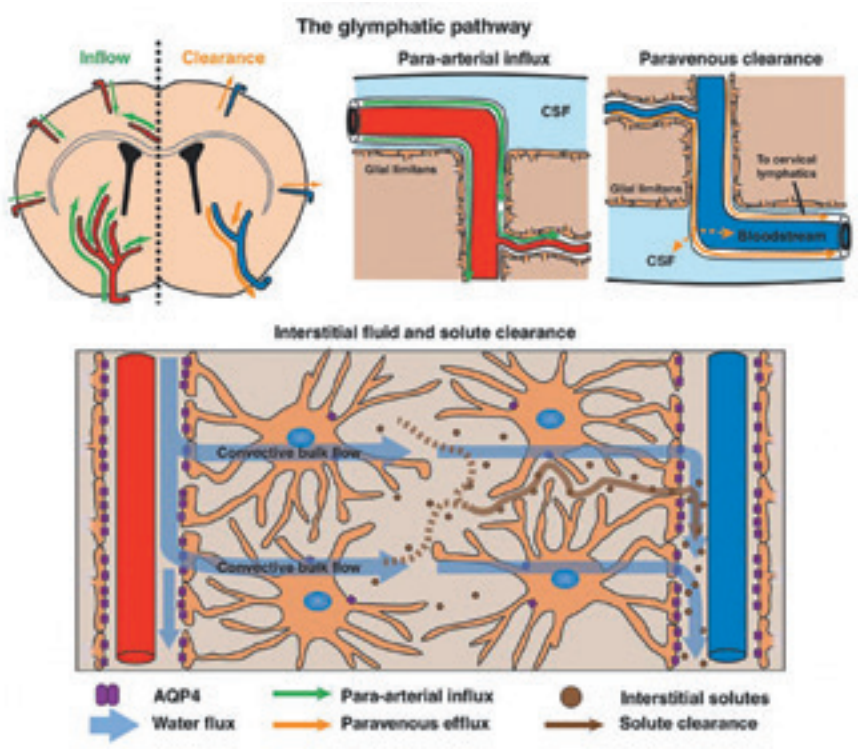

Figure 1. SF enters the brain following arteries in para-arterial spaces. Blood vessels are surrounded by astrocytic endfeet containing water channels, AQP-4, that allow CSF to move through brain parenchyma and interchange with ISF. Interstitial fluid and solutes are then cleared from the brain along paravenous routes
Source: Iliff JJ, Wang M, Liao Y, et al. A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β. Sci Transl Med. 2012;4(147):147ra111. doi:10.1126/scitranslmed.3003748.

CONCLUSION

The glymphatic system serves as a drainage system for waste products of the brain. CSF flows perivascularly around brain's arteries and enters the brain parenchyma with the help of AQP4. It interchanges with ISF which then exits perivenously. Lower activity of the glymphatic system possibly contributes to the development of various diseases and disorders associated with accumulation of different proteins, especially in AD and Parkinson's.

It also affects the presence of TBI markers in the peripheral blood. In a clinical setting, a way to assess glymphatic function could help with early diagnosis and tracking of the progression of diseases such as AD. It is a promising field which offers a lot of space for further research.

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LIMFNI SUSTAV MOZGA

Sažetak

Unatoč velikoj metaboličkoj aktivnosti mozga, anatomski definiran limfni sustav u mozgu ne postoji. Dugo se tragalo za načinom na koji se mozak oslobađa svojih otpadnih produkata. Potraga je nedavno urodila otkrićem takvog sustava, koji je nazvan glimfatičkim, zbog svoje sličnosti s limfnim sustavom u ostatku tijela i ovisnosti o gliji, točnije kanalima za vodu, akvaporinima-4 (AQP4) na nožicama astrocita koji okružuju krvne žile u mozgu. Sastavnice glimfatičkog sustava su cerebrospinalna tekućina, intersticijska tekućina, periarterijalni i perivenozni prostori. Cerebrospinalni likvor iz subarahnoidalnog prostora ulijeva se u periarterijalne prostore slijedeći pijalne arterije koje zaranjaju u dubinu mozga te kroz AQP4 ulazi u astrocite i dalje u moždani parenhim. Tamo se izmjenjuje s intersticijskom tekućinom i ulijeva se u perivenozne prostore, usput odnoseći otpadne produkte. Na kraju se ulijeva u cervikalnu limfnu mrežu. U brojnim eksperimentima dokazana je uloga nekoliko bitnih manipulacija glimfatičkim sustavom koji smanjuju njegovu funkciju, kao što je, primjerice, nedovoljno sna. Disfunkcija ovog mehanizma "čišćenja" mogla bi biti odgovorna za razvoj bolesti karakteriziranih nakupljanjem metabolita, kao sto su Alzheimerova bolest, a moguće i glaukom. Također, funkcija glimfatičkog sustava povezana je s prisutnošću markera traumatske ozljede mozga u perifernoj krvi. Sva ova otkrića upućuju na obećavajuću ulogu glimfatičkog sustava i procjene njegove funkcije u dijagnostici i liječenju pacijenata s neurodegenerativnim bolestima nakupljanja.

KLJUČNE RIJEČI: Alzheimerova bolest, akvaporin-4, cerebrospinalna tekućina, glimfatički sustav, traumatska ozljeda mozga

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