Mapping glymphatic solute transportation through the perivascular space of hippocampal arterioles with 14 Tesla MRI

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Abstract
The perivascular space (PVS) plays a crucial role in facilitating the clearance of waste products and the exchange of cerebrospinal fluid and interstitial fluid in the central nervous system. While optical imaging methods identify the glymphatic transport of fluorescent tracers through PVS of surface-diving arteries, their limited depth penetration impedes the study of glymphatic dynamics in deep brain regions. In this study, we introduced a novel high-resolution dynamic contrast-enhanced MRI mapping approach based on single-vessel multi-gradient-echo methods. This technique allowed the differentiation of penetrating arterioles and venules from adjacent parenchymal tissue voxels and enabled the detection of Gd-enhanced signals coupled to PVS of penetrating arterioles in the deep cortex and hippocampus. By directly infusing Gd into the lateral ventricle, we eliminated delays in cerebrospinal fluid flow and focused on PVS Gd transport through PVS of hippocampal arterioles. The study revealed significant PVS-specific Gd signal enhancements, shedding light on glymphatic function in deep brain regions. These findings advance our understanding of brain-wide glymphatic dynamics and hold potential implications for neurological conditions characterized by impaired waste clearance, warranting further exploration of their clinical relevance and therapeutic applications.

Introduction

The glymphatic system facilitates the dynamic interchange of cerebrospinal fluid (CSF) and interstitial fluid (ISF), contributing to maintaining regular physiological equilibrium and effectively removing interstitial substances[1-4]. Among the various compartments encompassing the intracranial interstitial extracellular space, the perivascular space (PVS) between the astrocyte endfeet and the parenchymal perforating vessels has been proposed to play a crucial role underlying the glymphatic clearance[5-9]. Two-photon optical imaging studies have well described the glymphatic cerebrospinal fluid circulation through the PVS from the surface diving arteries[10]. And, the latest work has shown the glymphatic flow through PVS of surface diving arteries can be further regulated by neuronal activity of the adjacent cortex[11]. However, given the limited penetrating depth of the optical imaging method, the dynamic changes of glymphatic flow in the deep cortex or subcortical regions remain to be elucidated.

Magnetic resonance imaging (MRI) has been applied to detect the PVS in human brains[12,13], in particular, to detect the enlarged PVS as a potential marker of brain dementia[14]. To measure the potential glymphatic flow through PVS, the Gd-based dynamic contrast-enhanced (DCE) MRI has been applied to map both animal and human brains[12-24]. Through either intracisternal or intraventricular delivery of the Gd agent, the PVS-specific signal enhancement is also mainly detected from the large diving arteries, e.g. the middle cerebral artery (MCA) or through the posterior cerebral artery (PCA) supplying the hippocampus[25]. Most of the rodent DCE MRI studies have applied preclinical high-field MRI scanner at 9.4T or higher to ensure sufficient signal-to-noise ratio (SNR) up to 100um isotropic resolution. However, the measurement of solvent transportation through PVS of micro-vessels penetrating the deep cortex or subcortical brain regions with 20-50μm diameters remains challenging given the limited spatial resolution.

In this study, we developed a novel DCE MRI approach based on the single-vessel multi-gradient-echo (MGE) imaging scheme[26,27] to measure Gd-enhanced PVS in mouse brains using a 14T preclinical MRI scanner. To ensure sufficient SNR with high spatial resolution images (50um isotropic resolution for the whole brain or 50x50x250um for temporal dynamic mapping at 10s TR), we implanted a radiofrequency (RF coil) above the skull with a micro-capillary targeting the lateral ventricle of the mouse brain for real-time DCE-MRI. This high-resolution DCE method enabled the differentiation of vessel voxels from peri-vessel voxels to verify the Gd-enhanced signals in PVS of penetrating arterioles of the cortex and hippocampus of mouse brains.

Materials and Methods

Animal surgical procedures

All animal surgical and experimental procedures were conducted following the Guide for the Care and Use of Laboratory Animals and approved by the Massachusetts General Hospital Subcommittee on Research Animal Care (Protocol No. 2007N000050). We used 3 healthy 6-month-old female C57/BL6 mice (The Jackson Laboratory, Maine, United States), one for perivascular space distribution, and 2 for glymphatic flow dynamics. Craniotomies were made above the right lateral ventricle (LV) under the microscope. 100nm/g Gd-filled capillary tube (Silica, Polyimide Coated Smooth Solid Tubing, 0.24mm, Digikey, MN), pre-connected to a micro-syringe (10 μL, WPI, FL) at one end, was inserted into the LV with the following coordinates: caudal 0.59mm, lateral 1.25mm, ventral 1.85mm from bregma. The mice then

underwent craniotomy surgery to install an MRI coil, and a customed MRI coil was positioned over the cortex and tested for tunning and matching signal with a network analyzer. The coil ring was lifted ~0.5mm above the surface of the skull and held in place while a thin layer of 404 cyanoacrylate glue was applied to connect the skull with the coil. Once dried (~8 mins), 2part dental cement (Dental Cement Kit, Stoeling, IL) was mixed and applied to cover the coil and exposed bone paying special note to the base of the coil to firmly secure it and avoiding drips into the eye. The edges of the skin were then attached with 454 cyanoacrylate glue. After the dental cement had fully hardened (~10 mins), the mouse was released from the stereotaxic stage and received subcutaneous injections of Dexamethasone and Cefazolin. During surgical sessions, mice were anesthetized at the same mixture of medical air (70 mL/min), oxygen (15 mL/min), and isoflurane (2%). A heating pad was used to ensure the mouse's body temperature was maintained near 37° C throughout anesthesia. A PE10 tube was inserted into the lateral tail veins of the mice for Gd intravenous infusion.

Magnetic Resonance Imaging

After the animal was secured in an MRI holder with ear and tooth bars, isoflurane was then reduced to 1.5%. The respiration rate and rectal temperature were continuously monitored (Model 1030, SAII, USA), with the temperature maintained at 36.5-37C by an air heater. The physiology was maintained within normal ranges, with a respiration rate of 80-110 breaths per minute throughout the MRI scans.

MRI was obtained by using a 14T/13 cm horizontal-bore magnet (Magnex Scientific) interfaced through the Bruker AV Neo system (Bruker). The scanner has a 6 cm gradient set with a strength of 100 Gauss per cm (G/cm) and a 150 μs rise time, as well as 6 channels up to $2^{nd}/3^{rd}$ order shimming (Resonance Research Inc.). An implantable RF transceiver surface coil with an outer diameter of 9.5 mm (Mribot Inc.) was used for image acquisition.

To examine whether the perivascular space of deep cortical layers and subcortical regions is involved in the glymphatic flow circulation, we apply the ultra-high resolution MGE sequence (50um isotropic) to map differential Gd-enhanced signals between intravenous and intraventricular Gd administration. A 3D MGE sequence was applied with the following parameters: TR: 50 ms; TE: 2, 4.5, 7, 9.5 ms; flip angle: 85°; matrix: 256 × 256 x 160; in-plane resolution: 50 μm isotropic. Scan time: 1h 30min.We first acquired blood Gd-based flow maps with MGE MRI (tail-vein Gd infusion, 12nl/min for 0.1-0.2ml for 90 min), which highlights all vessel voxels as vascular control maps. Secondly, we acquired another set of MGE images with the intraventricular Gd infusion (20nl/min, for 1-2ul for 90 min).

Besides mapping the accumulative glymphatic transportation with whole brain MGE, we acquired DCE MGE images with ultra-high resolution to cover both the hippocampus of the mice. A 2D MGE sequence was applied with the following parameters: TR: 80 ms; TE: 2.5, 5.5, 8.5, 11.5ms; flip angle: 85°; matrix: 256 × 256; in-plane resolution: 50 × 50 μm^2; slice thickness: 250 μm. Scan cycle:13.68s, repetition:120. Scan time: 27min22s. MGE images were acquired with the intraventricular Gd infusion (100nl/min, 1ul for 10 min) starting at 21st cycle.

Results

We implemented the ultra-high resolution MGE sequence to characterize two aspects of glymphatic solvent transport through PVS.

First, the 3D whole brain Gd-enhanced MR images were acquired with 50µm isotropic resolution using the MGE sequences in two conditions: intravenous infusion of Gd (Gd_{iv}) and intraventricular infusion of Gd (Gd_{ivtr}) consecutively (**Fig 1A**). 3D images with Gd_{iv} detected some penetrating vessels both in the cortex and subcortical regions, e.g., hippocampus. After intraventricular infusion of Gd, there is a clear signal enhancement in the parenchyma regions near the ventricles. To verify the potential Gd-enhanced PVS, three penetrating vessels in the cortex were identified based on the Gd_{iv} condition. Fig 1B showed MGE images at the first and third TE, enabling the differentiation of arterioles from venules [26]. The deoxygenated blood in venules at longer TE, i.e., 3rd TE, led to faster T2* decay to dimmish the venule signal despite the Gd effect. **Fig 1C** showed the line profiles around the three penetrating vessels, demonstrating the three vessels with high signal intensity at $Gd_{iv\text{-}TE1}$ (**Fig 1B**, colored overlap image) but only two arterioles remained with high signal intensity at $Gd_{iv\text{-}TE3.}$ Meanwhile, we also plot the line profile of the Gd_{ivtr}, presenting signal enhancement located at the two arterioles but with a slighter wider distribution (**Fig 1C**). Similarly, multiple penetrating vessels through the hippocampus of the two hemispheres were also identified in MGE images based on the Gd_{iv} condition (**Fig 1D**). Line profile plots of Gd_{ivtr} also showed a wider spatial distribution of signal enhancement near the hippocampal vessels (**Fig 1E, F**). Fig 1G showed the averaged vessel plots centered at the peak signal, showing a slightly wider distribution for the Gd_{ivtr}, indicating the potential PVS-related Gd enhancement. It should be noted that no signal enhancement was detected near venules in the Gd_{ivtr} condition, indicating that glymphatic Gd transport is less affected by the blood Gd.

Second, we acquired slice-selective 2D MGE images with 50x50 um in-plane resolution with a specific orientation to be perpendicular to the penetrating hippocampal vessels (**Fig 2A**) as previously reported [28]. The hippocampal single-vessel image was acquired with a 10s per frame sampling rate with real-time intraventricular Gd infusion. High-resolution MGE images enabled the identification of vessel, peri-vessel and adjacent parenchymal tissue voxels (**Fig 2B**). The Gd-enhanced DCE signals from three different ROIs were plotted as the function of time before and after 10 min Gd infusion (**Fig 2C**), demonstrating a salient early signal increase in the peri-vessel ROIs than the adjacent parenchymal tissue ROIs. Also, the slight signal drop in the vessel ROI could be caused by the potential T2* shortening by blood Gd during 10 min infusion. This result clearly demonstrates the PVS-specific glymphatic Gd transport in hippocampal penetrating vessels.

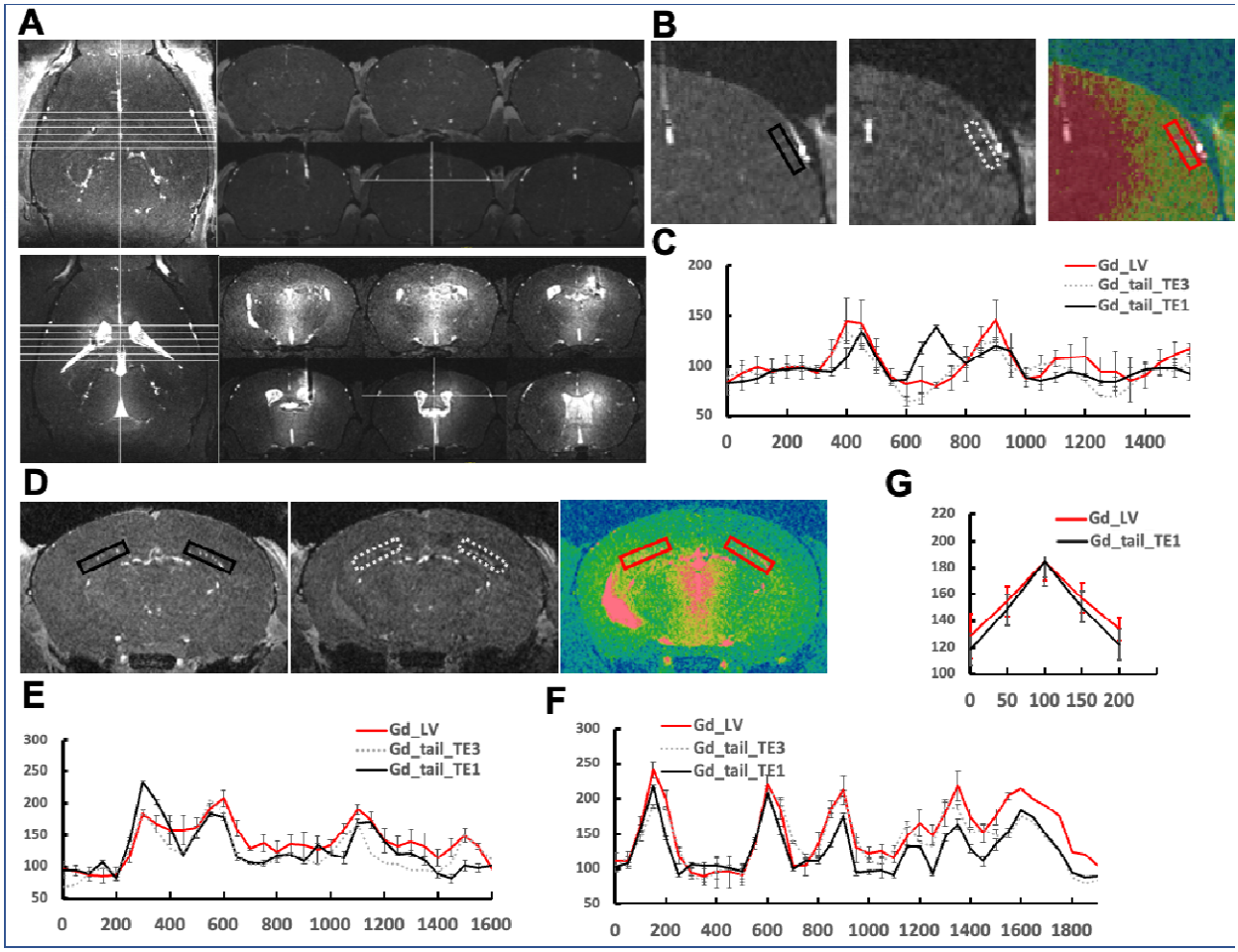

Figure 1. *Perivascular space distribution.* ***A****, Gd-enhanced single-vessel map (upper panel) and Gd-enhanced CSF map (lower panel).* ***B,*** *Representative slices of cortical single-vessel map with short (left, show all vessels) and long TE (middle, diminishing venules due to faster T2* decay, but not in arterioles), and of Gd-enhanced CSF (right) in the same area.* ***C,*** *The line profiles of vessels show wider Gd distribution space in Gd-enhanced CSF map (red) compared with Gd-enhanced single-vessel maps, indicating the perivascular space.* ***D,*** *Representative slices of hippocampal single-vessel map with short (left, show all vessels) and long TE (middle, diminishing venules due to faster T2* decay, but not in arterioles), and of Gd-enhanced CSF (right) in the same area.* ***E&F,*** *The line profiles of vessels (E, left; F, right) show wider Gd distribution space in Gd-enhanced CSF map (red) compared with Gd-enhanced single-vessel maps, indicating the perivascular space.* ***G****, The line profiles of the average of 10 vessels in the ROIs in* ***B&D****.*

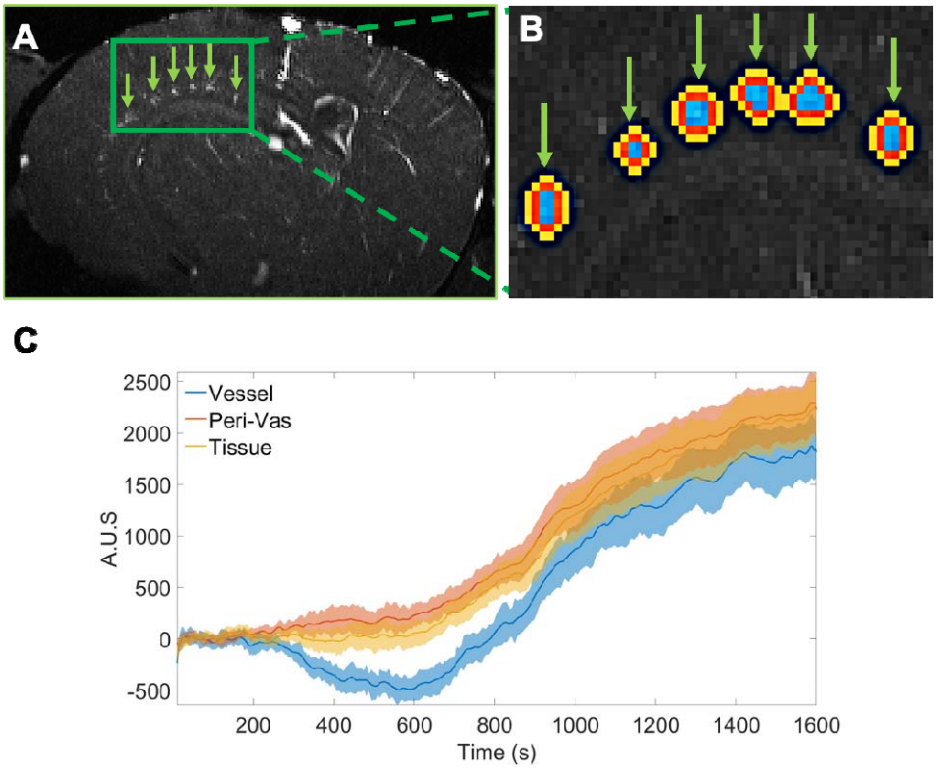

Figure 2. *Hippocampal perivascular influx.* ***A****, Vessel-specific hippocampal map, arrows indicate vessels.* ***B****, ROIs of vessel (blue), perivascular space (red), and tissue (yellow).* ***C****, Time courses of Gd signal change, indicating different dynamics in vessels, perivascular space, and tissue (2 trails/mouse, two mice).*

Discussion

The present study aims to investigate whether glymphatic transport occurs in PVS of penetrating vessels in the deep brain regions. In contrast to the optical imaging methods that provide high spatial resolution to identify microscopic measurements of PVS from diving arteries primarily located at the cortical surface, Gd-based DCE MRI has been used to measure the brain-wide glymphatic flow dynamic changes. There are two ongoing challenges related to this issue with MRI. First is the limited spatial resolution to distinguish vessels from PVS and adjacent parenchymal tissues with conventional MRI methodology. Second is the difficulty in differentiating the diffusion through extracellular matrix versus glymphatic PVS-specific solvent transportation.

We tackled this problem by implementing a novel DCE MRI mapping scheme based on single-vessel MGE methods, originally developed to map hemodynamic responses from penetrating arterioles and venules. The MGE sequence enables image acquisition at different echo times (TE). By setting a large flip angle with a short time of repetition (TR), we are able to highlight

certain penetrating vessels based on their orientation to the MRI slices. Also, due to the different T2* values of the oxygenated and deoxygenated blood in arterioles and venules, it also offers a possibility to distinguish arterioles from venules. When Gd is infused into blood, it can further enhance the vessel detection at shorter TE, and leads to faster T2* decay of venules. Thus, the MGE method offered an ideal image scheme to detect the penetrating micro-vessels with unique orientation with regard to the geometrical alignment of the MRI image field of view. It should be also noted that the hippocampal penetrating vessels align parallel to the cortical surface. This alignment is hard to detect by functional ultrasound based on the Doppler effect with a probe directly attached to the mouse head. Whereas, as a less sensitive method in comparison to the optical or acoustic imaging methods, it is critical to ensure sufficient SNR when mapping the micro-penetrating vessels with high spatial resolution and temporal resolution. We also implanted an RF coil to be attached to the skull, which enables a significant signal increase[29]. Given Gd-driven T1 shortening, it is practical to achieve ultra-high spatial resolution images with 30-50um to measure PVS-related signal changes near arteries and venules in deep brain regions. It should also be noted that the actual PVS near arteries with a diameter of 20-30µm is around 1-2µm[30] The G-enhanced signal in peri-vessel voxels is still mainly due to partial volume effects from PVS. Importantly, Fig 2 showed the opposite sign of signal changes in vessel voxels and peri-vessel voxels indicating the PVS-specific contribution to signal increase in the peri-vessel voxels despite the relatively large voxel size.

In contrast to most of the Gd-based DCE MRI with intracisternal administration, we directly infused Gd into the lateral ventricle and focused on measuring PVS Gd transport in the hippocampal vessels. This administration scheme also avoided the time delay of CSF flow from the cisterna magna to the other ventricles, which can confound with the direct interstitial diffusion through the extracellular matrix. It has been shown that the low-frequency oscillation of arteriole diameter changes (i.e., vasomotion) can alter the glymphatic flow rate, leading to more efficient solvent transport through PVS than molecule dispersion-based diffusion[15,31]. The increased transportation rates are brain state dependent and vary from single-digit to double-digit percentage increase from quiet wakefulness to sleep. The little difference further complicates the measurement of PVS-specific Gd signal enhancement of penetrating vessels from adjacent parenchymal tissues. The 2D MGE slice was positioned to measure the cross-section of the hippocampal penetrating arteries that were around 1mm away from the lateral ventricles. Previous optical imaging studies have estimated a 10-30um/s glymphatic flow rate through peri-arteriole PVS based on 70-2000kDa solutes. And given the obstructive scale in the PVS, the actual transportation rate was further slowed down to be around 40-70s per 100µm distance. In our study, the Gd-DTPA is near 1kDa, which is much smaller than the fluorescent tracers used for optical imaging. The PVS-specific glymphatic transportation showed significant signal enhancement around 140s, enabling the estimate of 7s per 100 µm distance. We will further verify the Gd-DTPA-specific diffusion through the interstitial fluid of the extracellular matrix, which can be compared with the PVS-specific Gd transportation. It should also be noted that the estimated glymphatic flow rate in venule PVS is much slower, which may not be sufficient to be differentiated from the parenchymal diffusion effect.

In conclusion, this study represents a significant step forward in the field of glymphatic research by mapping the perivascular pathway and dynamics through micro-vessels penetrating deep brain regions, e.g., the hippocampus, of the mouse brain. The findings contribute to our

understanding of glymphatic function and have the potential to shed light on neurological conditions where impaired waste clearance is implicated. Further research is warranted to explore the clinical implications and therapeutic potential of these discoveries.

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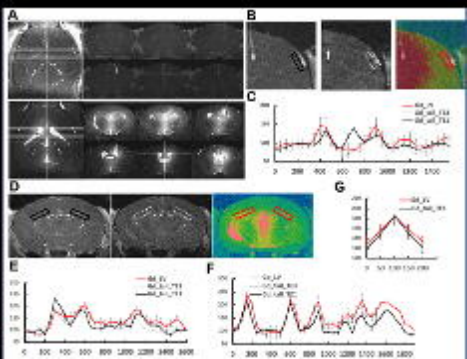
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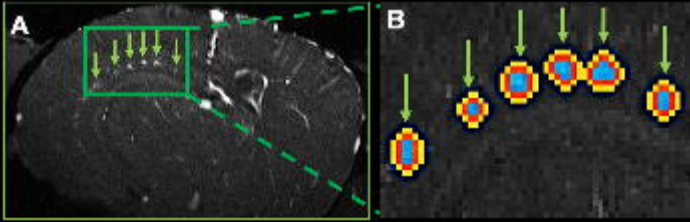
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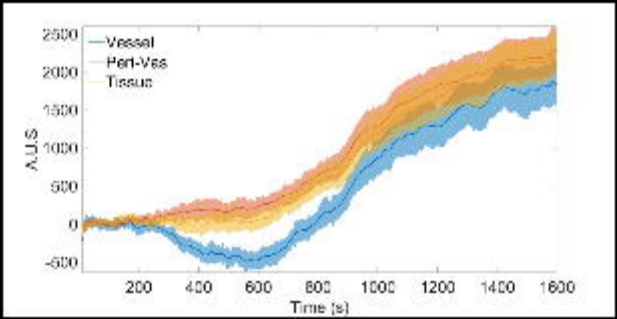
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