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In Vivo Imaging of Molecular Clearance From Human Entorhinal Cortex: A Possible Method for Preclinical Testing of Dementia

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Abstract

Accumulation in the brain of metabolic waste products such as amyloid-β and hyperphosporylated tau (tau) is a hallmark of dementia (e.g., Alzheimer's disease). One possible underlying mechanism is impaired cerebral paravascular (glymphatic) clearance of toxic solutes. Recently, we have provided evidence of glymphatic circulation being present in the human brain, utilizing repeated magnetic resonance imaging (MRI) acquisitions before/after intrathecal injection of an MRI contrast agent, serving as a cerebrospinal fluid (CSF) tracer (glymphatic MRI [gMRI]). In a recent study, we utilized the same methodology to assess glymphatic clearance function within an anatomical region that has a key role in cognitive function—the entorhinal cortex (ERC). gMRI was compared in individuals with the dementia subtype idiopathic normal pressure hydrocephalus (iNPH; $n = 30$) and reference (REF; $n = 8$) subjects. We found delayed clearance of CSF tracer from CSF nearby ERC, the ERC itself, and the white matter adjacent to ERC, which was most evident after 24 hr. The observations were interpreted as indicative of impaired glymphatic circulation and further suggested this being a possible mechanism behind accumulation of amyloid-β and tau in ERC and instrumental for dementia in iNPH. We suggest that gMRI may serve as a tool for assessment of early dementia, or even in the preclinical stage.

Keywords

idiopathic normal pressure hydrocephalus, dementia, glymphatic circulation, entorhinal cortex, cerebrospinal fluid tracer

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Since first described in 2012 (Iliff et al., 2012), the glymphatic system has emerged as a possible important mechanism for clearance of metabolic waste products such as from the brain of amyloid-β and hyperphosporylated tau (tau) (Rasmussen et al., 2018). The glymphatic system was proposed as a brain-wide paravascular pathway for transport of fluid and solutes that is dependent on convective forces for molecular movement along vessels and dependent on the water channel aquaporin-4 (AQP4) that is enriched at perivascular astrocytic endfeet (Jessen et al., 2015). It has been shown that glymphatic function is heavily dependent on brain activity (increased activity during sleep; Xie et al., 2013) and that its function becomes impaired with increasing age (Kress et al., 2014). Moreover, impaired function of the glymphatic system has been proposed to underlie failure of clearance of soluble amyloid-β that in turn may lead to amyloid-β plaque deposition and dementia evolvement (Iliff et al., 2012). When these events affect brain regions involved in memory functions, the cognitive decline characterizing dementia and Alzheimer's disease (AD) evolves.

We introduced glymphatic MRI (gMRI) to examine glymphatic function in vivo in humans (Eide & Ringstad, 2015; Ringstad et al., 2017). This MRI modality utilizes repetitive and standardized T1 acquisitions, before and after intrathecal injection of an MRI contrast agent, serving as a cerebrospinal fluid (CSF) tracer, enabling assessment of clearance of the CSF tracer (Figure 1). We have applied the MRI contrast agent gadobutrol (molecular size 604 Da), which has hydrophilic and nonionic properties, causing it to distribute freely in water. Using this approach, we provided in vivo evidence in humans of a

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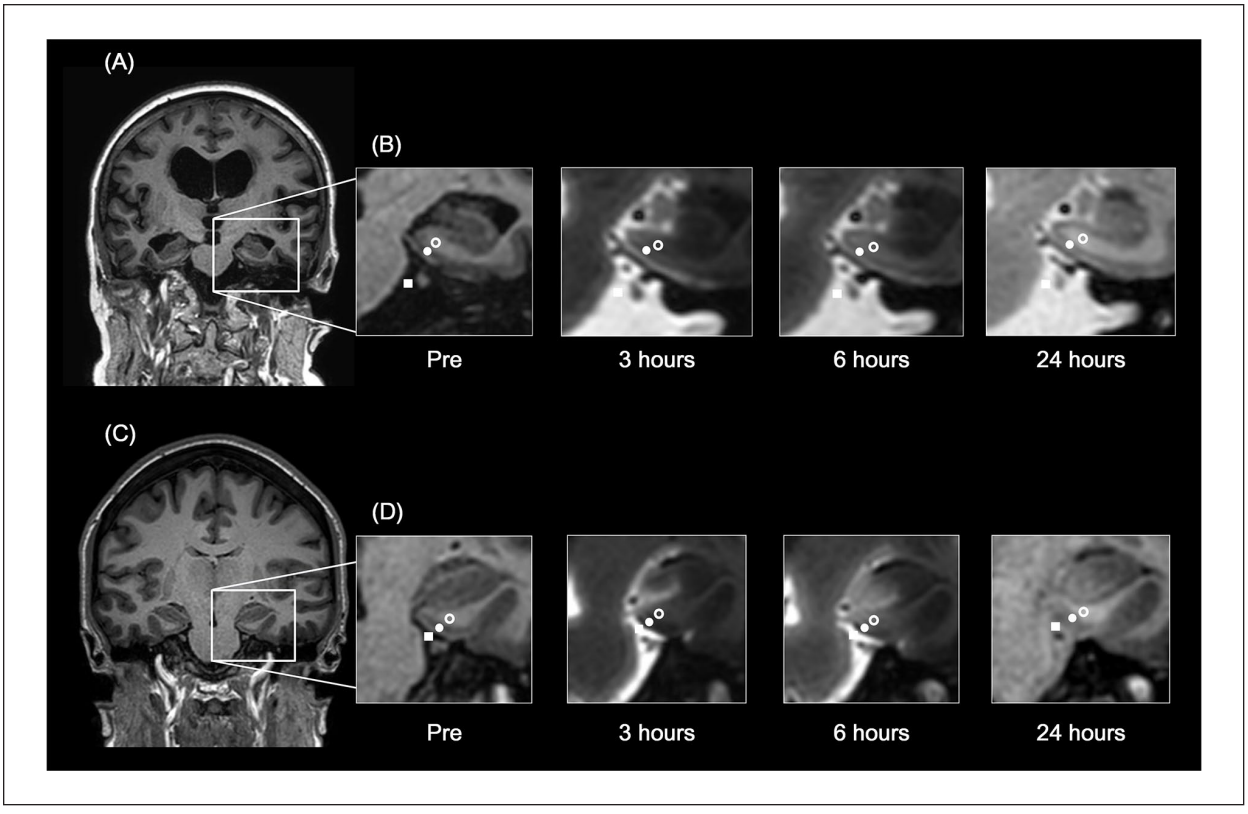

Figure 1. (A) T1-weighted MRI in the coronal plane of an individual with iNPH, where the medial temporal lobe with ERC region in center is squared out. (B) gMRI was obtained before (Pre) and 3, 6, and 24 hr after intrathecal injection of the MRI contrast agent gadobutrol serving as a CSF tracer. The region of interest used for measurement of tracer enrichment in CSF nearby ERC is illustrated by a filled square, in the ERC by a filled circle, and in the white matter beneath the ERC by an open circle. The superior sagittal sinus served as reference region of interest. In this iNPH subject, the signal unit ratio was increased within ERC after 3 (184%), 6 (337%), and 24 (148%) hr, and within the subcortical white matter after 3 (18%), 6 (127%), and 24 (84%) hr. (C) T1 MRI of a reference individual wherein the region of ERC is indicated. (D) gMRI was performed before (Pre) and 3, 6, and 24 hr after intrathecal injection of gadobutrol serving as a CSF tracer. CSF nearby ERC is indicated by a filled square, the ERC by a filled circle, and the subcortical white matter by an open circle. The superior sagittal sinus served as reference region of interest. In this reference individual, the signal unit ratio was increased within ERC after 3 (67%), 6 (200%), and 24 (45%) hr and within the subcortical white matter after 3 (31%), 6 (72%), and 24 (45%) hr. Accordingly, the CSF tracer enrichment within both the ERC and subcortical white matter was more pronounced and with delayed clearance in the iNPH subject, as compared with the reference individual.
Note. MRI = magnetic resonance imaging; iNPH = idiopathic normal pressure hydrocephalus; ERC = entorhinal cortex; CSF = cerebrospinal fluid.

brain-wide glymphatic system and of delayed clearance of CSF tracer in individuals with dementia disease.

In a recent study, we examined in particular whether clearance of the CSF tracer was delayed in the entorhinal cortex (ERC) of patients with a subtype of dementia, that is, normal pressure hydrocephalus (iNPH; Eide & Ringstad, 2019). It is well established that the ERC–hippocampus circuit plays a key role for cognitive function such as learning and orientation (Moser et al., 2015). This anatomical region is affected early in AD, and MRI may reveal thinning of the ERC in the early phase of AD (Pennanen et al., 2004). One mechanism behind degeneration of the ERC is deposition of amyloid-β plaques.

In our studies on dementia, we have examined the dementia subtype iNPH, which is characterized by gait ataxia, urinary incontinence, dementia, and ventriculomegaly, in whom some of the patients may improve clinically following diversion of CSF (Eide & Sorteberg, 2016). This disease has several similarities with AD, such as deposition in brain tissue of amyloid-β and tau (Leinonen et al., 2010), and increased prevalence with increasing age (Jaraj et al., 2014), as well as a high prevalence of vascular co-morbidity (Eide & Pripp, 2014).

The goal of this recent study (Eide & Ringstad, 2019) was to test whether clearance of CSF tracer was altered within ERC of iNPH patients, as compared with reference (REF) subjects. The hypothesis is that delayed clearance is indicative of impaired glymphatic circulation within the ERC and ERC subcortical white matter, which might be one mechanism behind dementia in iNPH.

We included 30 iNPH patients and eight REF subjects. The latter group underwent assessment for tentative idiopathic intracranial hypotension due to suspected CSF leakage. None of the REF subjects had cognitive

impairment, while 28 of 30 iNPH patients had cognitive impairment.

All participants underwent gMRI, which followed a strict and standardized protocol. The three-dimensional (3D) T1-weighted gradient echo volume scans were obtained using a 3 Tesla Philips Ingenia MRI scanner (Philips Medical systems, Best, The Netherlands) with equal imaging protocol settings before and at multiple time points up to after intrathecal injection of gadobutrol (0.5 mL of 1.0 mmol/mL gadobutrol; Gadovist™, Bayer Pharma AG, Berlin, Germany). Gadobutrol served as a CSF tracer, and movement of CSF tracer was assessed by determining change in signal units within circular regions of interest placed inside the ERC and ERC subcortical white matter and CSF nearby ERC on 1-mm-thick, coronally reconstructed T1-weighted images at the level of the hippocampal sulcus. We normalized the mean T1 signal unit for each regions of interest (ROIs) against a reference ROI by dividing any measured T1 signal unit from CSF or brain parenchyma with the value of the reference ROI to correct for any baseline changes of image gray scale due to image scaling (the ratio was denoted the *normalized T1 signal unit*). As described in previous studies, we placed the reference ROI within the posterior part of the superior sagittal sinus in axially reconstructed images from the same T1 volume scan.

As quantifiable biomarkers of neurodegeneration within the ERC, we measured ERC thickness in the coronal plane from reconstructed T1 volume acquisitions with 1 mm slice thickness at level of the hippocampal sulcus and also measured the medial temporal atrophy (MTA) using Schelten's score.

The iNPH patients presented with lower ERC thickness and higher MTA score, providing an anatomical basis for the cognitive decline seen in iNPH subjects. Both higher MTA score and thinning or volume loss of the ERC have previously been associated with cognitive decline and early dementia development. gMRI demonstrated that glymphatic enhancement in ERC precedes enhancement in subcortical white matter and with peak enhancement at 6 to 9 and 24 hr, respectively. The enhancement of CSF tracer within CSF nearby ERC preceded the parenchymal enhancement, indicating that glymphatic enhancement derives from CSF tracer at the surface of the brain and that the tracer propagates through the parenchyma in a centripetal direction. In line with this, we found a strong correlation between enhancement in CSF and adjacent parenchyma. Moreover, the clearance of CSF tracer from CSF nearby ERC was delayed in iNPH. The increased normalized T1 signal units found within all three compartments in iNPH patients at 24 hr was interpreted as reduced clearance of CSF tracer from the respective locations and the intracranial compartment in general.

Considering all 38 patients combined, CSF tracer enrichment after 24 hr in CSF nearby ERC, the ERC itself, and ERC subcortical white matter was higher in iNPH patients. As the destiny of a CSF tracer is to be cleared from the intracranial compartment with a certain rate, the observations can hardly be explained in another way than that clearance of CSF tracer was delayed in iNPH. Therefore, these observations clearly indicated delayed molecular clearance capacity in the ERC, subcortical white matter of ERC, and CSF nearby the ERC in individuals with iNPH. At the same time, ERC thickness was smaller and MTA score higher in iNPH patients compared to REF individuals.

The imaging data of this recent study (Eide & Ringstad, 2019) demonstrate for the first time in patients with cognitive deficits delayed clearance of an in vivo CSF tracer from ERC and subcortical white matter of ERC with its trajectories toward the hippocampus. Our interpretation is that the results support the hypothesis that impaired glymphatic function in these pivotal structures for cognitive function hampers removal of toxic waste solutes, which may lead to neurodegeneration, and eventually dementia in iNPH.

The underlying molecular mechanisms need to be further studied. In iNPH patients, we have found that the expression of the water channel AQP4 and its anchor molecule dystrophin-71 (Dp-71) is reduced at astrocytic perivascular endfeet (Eide & Hansson, 2018; Hasan-Olive et al., 2019). Hypothetically, reduced perivascular AQP4 might hamper transport of fluid and solutes along micro vessels.

It is assumed that arterial pulsations are crucial for movement of molecules along intracranial vessels (Iliff et al., 2013; Mestre et al., 2018). In this regard, we hypothesize that the proximity between ERC in the medial temporal lobe and large artery trunks located at the base of the brain, particularly the circle of Willis, posterior cerebral artery, and superior cerebellar artery, renders for a large impact from pulsations on ERC glymphatic flow. In fact, the parahippocampal region is among the regions with highest CSF tracer enhancement in the entire brain (Ringstad et al., 2018). The arterial pulsations may be restricted in iNPH, thus hampering CSF tracer clearance.

Possibly, glymphatic clearance function might be deteriorated already in the presymptomatic phase of dementia. It could be that the ability of solute clearance from the parenchyma is decoupled from cognitive function level. If impaired glymphatic function has a major role in neurodegeneration and dementia evolvement, glymphatic function might be assessed by means of long term in vivo imaging of CSF tracer metabolism, as done here with gMRI. Based on the results presented in our recent study, gMRI and assessment of the ERC molecular clearance capacity might become a clinical test to measure dementia susceptibility, and AD risk, perhaps even before pathologic Aβ deposition and irreversible neuronal loss occurs.

Declaration of Conflicting Interests

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