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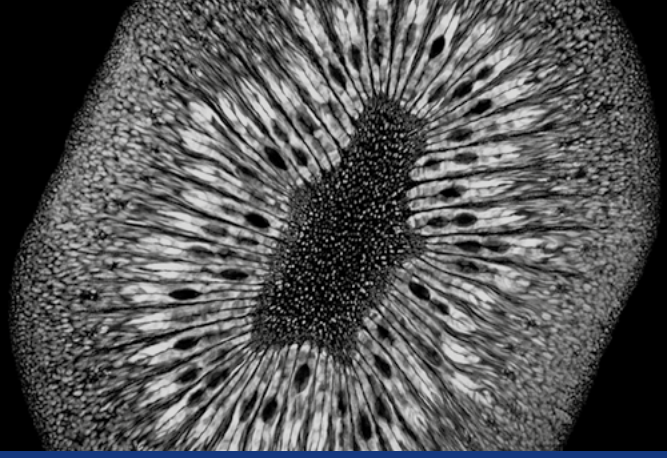
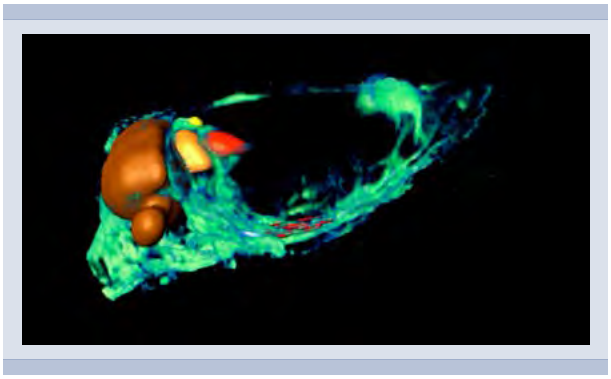


Monitoring clearance of brain waste



Visualization of brain waste clearance pathways using dynamic contrast-enhanced (DCE) MRI and GadoSpin™ P

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Introduction

In the classical model of cerebrospinal fluid (CSF) flow, CSF is actively secreted by the ventricles of the brain and travels through the ventricular system into the subarachnoid space, where it is then reabsorbed into the bloodstream[1, 2]. Contrary to the classical model of CSF flow, our previous studies demonstrated that a large proportion of subarachnoid CSF recirculates through the brain tissue and exchanges with the interstitial fluid (ISF)[3]. This CSF-ISF exchange pathway, which we term the glymphatic system, is believed to facilitate the clearance of wastes from the brain.

In this study we use dynamic contrast-enhanced (DCE) MRI and apply two different imaging agents of varying molecular weight to visualize CSF-ISF exchange in 3D in the live rat brain. The influx and clearance of the clinical MRI agent, Magnevist®, and the innovative Viscover™ MRI agent, GadoSpin™ P, were investigated so as to map the CSF-ISF exchange pathway across the brain, with the aim of identifying key anatomical clearance routes of brain waste.

Materials and methods

Experiments were performed on anaesthetized, healthy female Sprague Dawley rats (n = 14). Non-invasive monitors were used throughout to assure adequate oxygenation, ventilation and normal body temperature. During a brief surgical procedure, an intrathecal catheter, which was used for MRI agent administration, was positioned and fixed into place[4]. Following surgery, the rats were positioned in a custom-made cradle fitted with a stereotaxic head frame (Kopf Instruments, CA, USA). Scanning was performed on a 9.4 T MRI instrument interfaced to a Bruker Avance console and controlled by Paravision 5.0 software. A custom-made 3 cm radio frequency coil was used as receiver, and a 16 cm diameter volume coil was used as transmitter (Bruker BioSpin, Ettlingen, Germany). A 3D T_1-weighted FLASH sequence (T_R = 15 ms, T_E = 3.4 ms, FA = 15°, NA = 1, FOV = 3.0 × 3.0 × 3.2 cm^3, scanning time = 4 min 5 s) was performed. Two different MRI agents having significantly different molecular weights were used: Magnevist® (Gd-DTPA, MW = 938 Da; Bayer HealthCare Pharmaceuticals Inc., New Jersey, USA) and GadoSpin™ P (polymeric Gd-chelate, MW ~ 200,000 Da; Viscover™, *nanoPET Pharma* GmbH, Berlin, Germany). The MRI agents were each diluted to a concentration of 21 mM Gd in 0.9 % NaCl and the rats were intrathecally administered with a total of 80 µL Gd-DTPA (n = 8) or GadoSpin P (n = 6) at an infusion rate of 1.6 µL per min (total infusion time = 50 min). Even after completion of the intrathecal infusion, the MRI acquisitions continued for a total period of ~ 4 h. For all experiments, a 60 mM Gd-DTPA phantom placed in the vicinity of the animal's head was used for image intensity normalization.

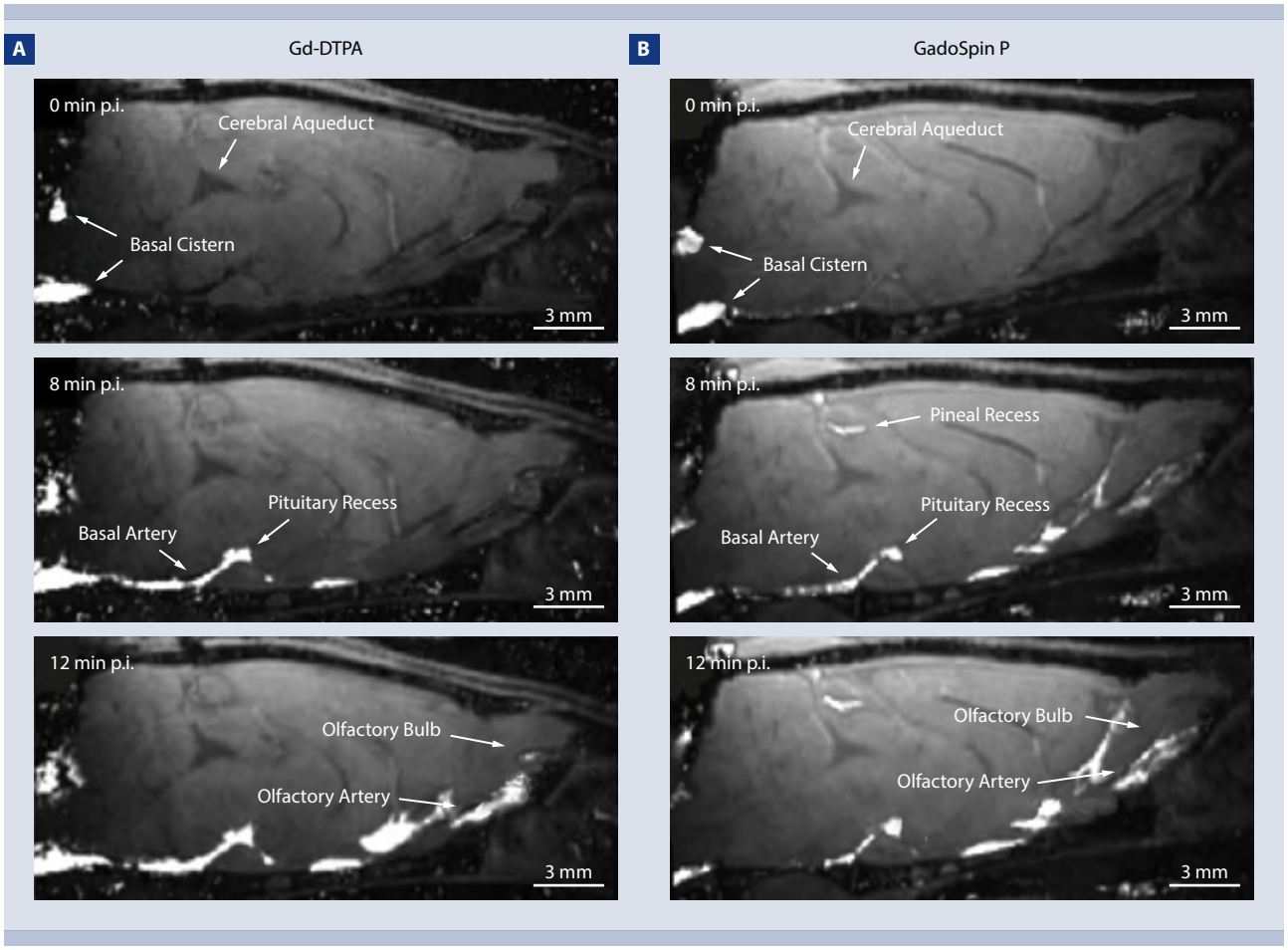

Figure 1: Early influx of CSF within the brain is governed by bulk flow.
Time-dependent MR images during the early infusion period of **A.** Gd-DTPA and **B.** GadoSpin P showing that, despite their differing molecular weight, the contrast agents pass through paravascular pathways at similar rates supporting that CSF bulk flow governs this process.

Results and discussion

To investigate whether the flow of CSF within the brain can be observed by contrast-enhanced MRI, rats were imaged following intrathecal delivery of two different MRI agents. MR images over the early infusion period with Gd-DTPA (Fig. 1A) and GadoSpin P (Fig. 1B) clearly show that both agents first appear in the cisterna magna (Fig. 1 upper row, defined as 0 min p.i.), are transported along the basilar artery to the pituitary recess (Fig. 1 middle row, 8 min p.i.) and then flow along the olfactory artery into the olfactory bulb (Fig. 1 lower row, 12 min p.i.). The obtained images indicate that, even though the two imaging agents differ greatly in molecular weight, they pass through paravascular conduits at similar rates. This supports our initial finding[3] that bulk flow, which is known to be independent of molecular size[5], is the mechanism driving CSF transport along paravascular pathways, facilitating the clearance of wastes from the brain. So as to visualize the CSF-ISF exchange pathway and, thereby, identify key anatomical clearance routes of brain waste, MRI was performed over a prolonged time period of ~ 4 h. The time-dependent signal enhancement at various locations within the brain, corresponding either to areas near paravascular conduits or areas of brain parenchymal tissue, was measured and plotted (data reported elsewhere[4]). The results showed that the transport of GadoSpin P into and through the brain parenchyma was found to be significantly lower to that of Gd-DTPA. This implies not only that CSF from the paravascular pathway moves into and through the brain interstitium, but also that the CSF-ISF exchange mechanism is dependent on molecular size.

In order to directly visualize CSF-ISF exchange in a 3D manner, dynamic time series displays capturing transport through the glymphatic system were acquired over a time period of ~ 4 h. The displays show that initially both Gd-DTPA and GadoSpin P travel similarly along paravascular pathways. However, whereas Gd-DTPA passes readily from the paravascular spaces into the brain parenchyma, GadoSpin P preferentially remains in the paravascular compartment, showing insignificant parenchymal uptake (Fig. 2). This verifies that there is a size-dependence of flow behaviour from the paravascular space into the surrounding brain interstitium. From these observations, we postulate that the exchange of CSF and ISF between paravascular spaces and the interstitium occurs across perivascular astrocytic end feet, which completely ensheathe the cerebral microcirculation and function to restrict the access of larger molecular weight substances into the interstitium.

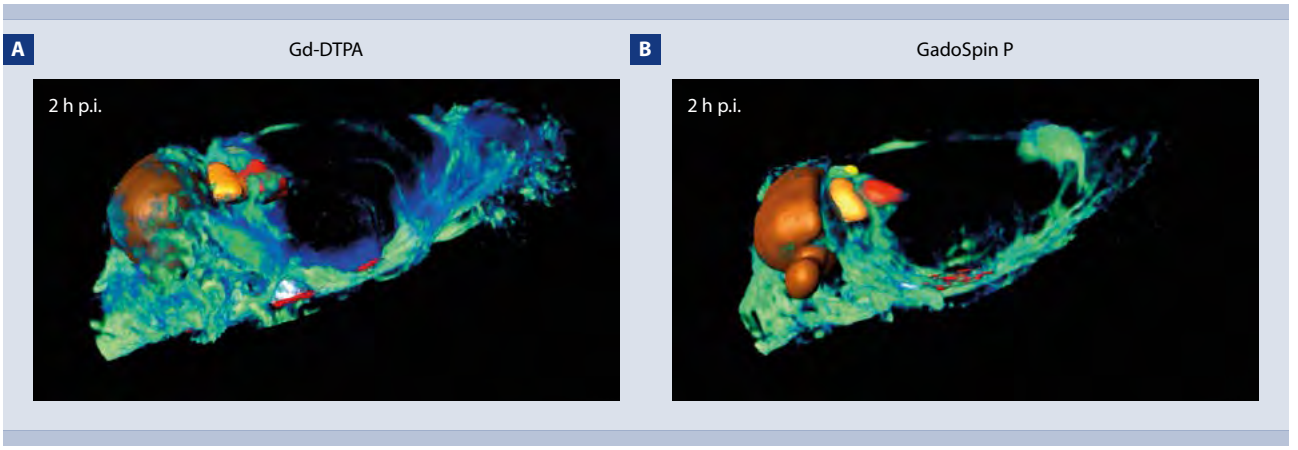

Figure 2: Size-dependence of flow behaviour through the glymphatic system.
MR images 2 h after administration of **A.** Gd-DTPA and **B.** GadoSpin P obtained from dynamic time series displays, showing that the transport of the MRI agents (green/blue color) through the glymphatic pathway is dependent on molecular size. Key anatomical structures are displayed and include the cerebellum (brown), pineal gland (yellow) superior colliculus (orange), inferior colliculus (red), pituitary gland (white) and large basal arteries (red).

Conclusion

In this study we demonstrate that the CSF-ISF exchange pathway, responsible for clearance of wastes from the brain, can be assessed using dynamic contrast-enhanced MRI. Implementation of the polymeric MRI agent, GadoSpin P, in comparison to the small-molecule MRI agent, Magnevist, enabled not only visualization of CSF and ISF flow, but also examination of the size-dependence of flow behaviour. In light of the key role that this pathway plays in the clearance of amyloid β peptides from the brain parenchyma, this MRI approach may provide the basis for a new strategy that allows evaluation of the susceptibility and progression of Alzheimer's disease in humans.

Viscover™ Product	Order No.
GadoSpin™ M, 1 x 5 injections	130-095-134
GadoSpin™ M, 5 x 5 injections	130-095-135
GadoSpin™ P, 1 x 5 injections	130-095-136
GadoSpin™ P, 5 x 5 injections	130-095-137
GadoSpin™ F, 1 x 5 injections	130-095-162
GadoSpin™ F, 5 x 5 injections	130-095-163
GadoSpin™ D, 1 x 5 injections	130-095-164
GadoSpin™ D, 5 x 5 injections	130-095-165

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