MR Molecular Imaging of Cerebrovascular Amyloid Deposits

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Introduction

Cerebral amyloid angiopathy (CAA) is the most common form of dementia associated with the deposition of amyloid-β peptides (Aβ) after Alzheimer's disease (AD). Both are characterized by the pathological accumulation of Aβ with AD involving the deposition of Aβ peptides in the brain parenchyma and CAA involving the deposition of Aβ peptides in the media and adventitia of small and mid-sized arteries and capillaries of the cerebral cortex and the leptomeninges. The development of a targeted nanoparticle contrast agent and subsequent MR imaging to distinguish these diseases would aid in their diagnosis and treatment by potentially distinguishing between amyloid in the vessels versus parenchymal plaques. Immunotargeted iron oxide nanoparticles (MIONs) have been shown to have the ability to target specific biomarkers in quantities detectable by MRI [1]. Previously, our group successfully targeted the polyamine modified F(ab)´$_2$ fragment of a novel monoclonal antibody IgG4.1 (pF(ab)´$_2$ 4.1), raised against human fibrillar Aβ42, to amyloid plaques in AD transgenic mouse brain [2]. Here we report the development of pF(ab)´$_2$ 4.1 conjugated to MIONs for use as a MRI probe to identify cerebrovascular amyloid *in vivo* using a vessel enrichment technique and to detect these nanoparticles in *ex vivo* mouse brains with MRI.

Methods

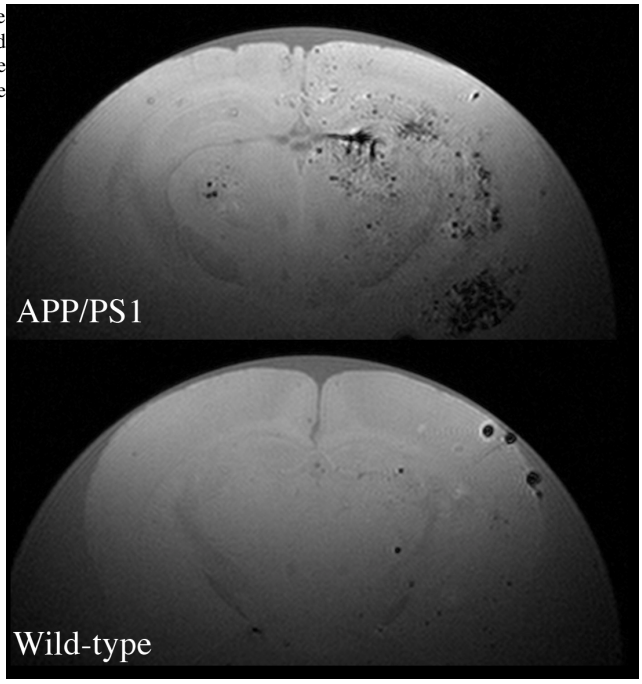
Monodisperse γ-Fe_2O_3 nanoparticles (MIONs) approximately 8 nm in diameter were synthesized via the Hyeon method [3] and coated with a mixture of mPEG 2000, carboxylic acid functionalized mPEG 2000, and lissamine rhodamine tagged phosphatidylethanolamine phospholipids [4]. IgG4.1 antibodies were digested into the F(ab)´$_2$ fragment to decrease the size and weight of the antibody and then polyamine modified prior to conjugation to improve the blood brain barrier permeability of the conjugate [5]. The F(ab)´$_2$ 4.1 antibodies were then conjugated to the carboxylic acid functionalized phospholipids coating the nanoparticles using a carbodiimide reaction with the amine groups on the antibodies, producing F(ab)´$_2$ 4.1-MIONs. Experiments were performed on both APP/PS1 transgenic and B6SJL wild-type mice. The mice were anesthetized using 1.0-1.5% isofluorane, and 150 µL of F(ab)´$_2$ 4.1-MIONs (1 mg) were infused through a catheter into the carotid artery at a rate of 7.5 µL per minute for 20 minutes. For *ex vivo* MRI experiments, the mice were sacrificed and perfused with PBS and 10% formalin 20 minutes post-injection. The brains were removed and fixed in formalin overnight before being embedded in 2% agar for scanning. MR images of the *ex vivo* samples were taken using T_1- and T_2-weighted Spin Echo sequences on a 9.4 T Varian MR scanner. For the vessel enrichment experiments, the mice were perfused with PBS 20 minutes post-injection, and then the brains were flash frozen with dry ice and homogenized in a Tris EDTA sucrose buffer. The vessel fraction was separated from the homogenate using a 1.25 M and 1.5 M sucrose gradient and centrifuged at 58,000 ×g for 1 hour. The collected fraction was incubated with AlexaFluor 647 tagged PECAM overnight to identify blood vessels, rinsed with buffer and then incubated for 5 minutes with thioflavin S to identify cerebrovascular amyloid deposits.

Results and Discussion

Through the use of vascular enrichment and a triple stain, we were able to demonstrate that the pF(ab)´$_2$ 4.1-MION conjugate bound to cerebrovascular amyloid in isolated blood vessels from APP/PS1 mouse brain (Fig. 1a-d) but not wild-type mouse brain (Fig. 1e-h). Blood vessels were labeled with AlexaFluor 647 tagged PECAM antibody and are shown in blue (Fig. 1c,d,g,h, false color). The presence of pF(ab)´$_2$ 4.1-MIONs was indicated by the red fluorescence from the lissamine rhodamine phospholipids in the nanoparticle coating (Fig 1a,d,e,h). Cerebrovascular amyloid deposits in the vessels were detected with thioflavin S and appear green (Fig. 1b,d,f,h). The pF(ab)´$_2$ 4.1-MIONs demonstrate co-localization with the cerebrovascular amyloid deposits in the APP/PS1 mouse brain, as shown by the yellow areas in the composite image, indicating that the targeted nanoparticles bound specifically to the amyloid in the vessels (Fig. 1d). Neither pF(ab)´$_2$ 4.1-MIONs nor vascular amyloid were detected in the wild-type mouse brain vascular enriched fraction (Fig. 1h).

In the T_1-weighted images of the *ex vivo* APP/PS1 mouse brain, dark contrast spots were seen and suggest pF(ab)´$_2$ 4.1-MIONs binding to cerebrovascular amyloid (Fig. 2). In locations with a high concentration of pF(ab)´$_2$ 4.1-MIONs, the resulting contrast change was negative due to the strong T_2 effect of the nanoparticles. The targeting of the MIONs appeared strongest on the injection side of the brain, with a much lower level of targeting on the contralateral side of the brain. The number of spots was significantly lower in the wild type brain due to the absence of vascular amyloid deposits.

Figure 2



References

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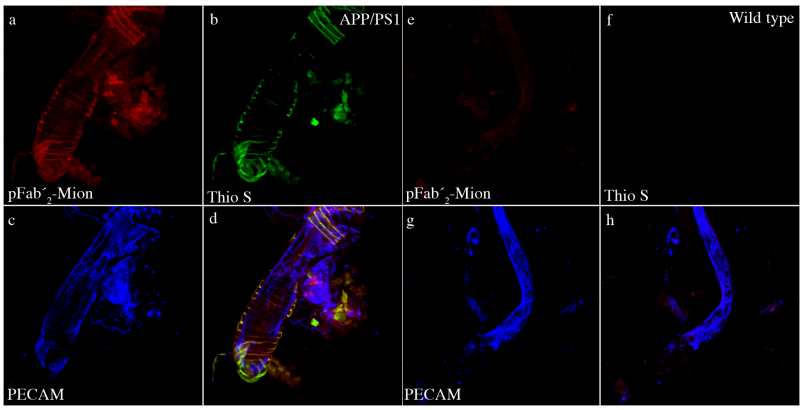
Figure 1



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