Targeting Alzheimer's plaques with Dysprosium based probes

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Introduction

Alzheimer's disease is a neurodegenerative process with a long incubation period that precedes the appearance of clinical symptoms. At present, definitive diagnosis requires a postmortem brain examination for the presence of amyloid-ß (Aß) plaques and neurofibrillary tangles. Transgenic mice that develop Aß plaques similar to those seen in AD patients provide an animal model to study plaque deposition and to test new therapeutic strategies aimed at reducing amyloid burden. A noninvasive method that accurately identifies Aß plaques in these mouse models would be invaluable for testing and monitoring these new therapies. While Aß plaques are sometimes visible on non-contrast enhanced MRI due to accumulation of iron [1, 2], sensitivity is low. Therefore, our group and others have developed Aß-binding peptides conjugated to gadolinium chelates (Gd) as targeted contrast agents [2-4]. Upon co-injecting these agents with hyperosmolar mannitol to open the blood brain barrier (BBB), we were able to demonstrate in vivo detection of Aß plaques in mice for the first time [4]. Surprisingly, efficient detection using Gd was obtained when using susceptibility-weighted MRI. Although this technique has proven reliable, comparison of MRI with histological sections indicates that many plaques are not detected and suggests that amyloid burden may be underestimated. To further increase sensitivity we also labeled our amyloid probes with monocrystalline iron oxide nanoparticles (MION) [4]. Although individual plaques appeared better delineated, the overall sensitivity of MION-based constructs led to greater individual variability to detect Aß plaques. This resulted in a poor overall estimate of amyloid burden , likely due to the larger size of the MION-based construct and the reduced corresponding BBB permeability. In the current study, we demonstrate that dysprosium (Dy) labeling of our amyloid probes is a great alternative to increase the effect on transverse relaxation rate without compromising the other physicochemical attributes such as BBB permeability and binding affinity critical for efficient plaque visualization.

Material and Methods

Imaging experiments were performed on transgenic mice both APP (Tg2576) and double mutation APP/PS1 with ages chosen to ensure a reasonable Aß plaque density in each mouse model. 4-6 hours prior to MRI, all transgenic mice as well as their age matched wild-type control were subjected to intra-carotid co-injection of Dy-labeled peptide and mannitol to permeabilize the construct to the blood brain barrier. All µMRI experiments were performed with a Bruker Biospec console (Bruker Biospin, Ettlingen Germany) interfaced to a 7T horizontal bore magnet (Magnex Scientific, Abingdon, UK) equipped with 750mT/m actively shielded gradients (ID=90mm; Resonance Research Inc., Billerica, MA USA). A Litzcage circularly polarized RF coil (ID=25mm, Length=22mm, Doty Scientific, Columbia SC, USA) was used to image the mouse head. A 3DGE sequence with a 4-echoes train (TR=50ms, TE=4.07ms, ES=6.7, FA=15°, BW=50Khz,) was acquired to provide a 100µm isotropic resolution T2* map datasets in less than 2-hours. The isotropic voxels obtained facilitated comparison between brain datasets and co-registration with histology. The brain realignments were assessed with Analyze (v7.0 AnalyzeDirect, Lenexa KS) and compared to histology sections stained for amyloid plaques.

Results and Discussion

Due to their low concentration in Aß plaques, even Gd based contrast agents has been shown to work best as T2 and T2* agents, producing hypo-intensities in T2 and T2* weighted images [2-4]. The lanthanide substitution of Gd by Dy on our DTPA-peptides using the same metallization protocol resulted in an expected significantly stronger T2 and T2* effect on our peptide (see Fig1). Compared to our previous iron oxide nano-particle labeling strategy [4], the gain in sensitivity obtained by Dy-labeling was achieved without altering the overall binding affinity of the resulting construct towards the amyloid ß plaques. Similarly the ability to cross the blood-brain-barrier was maintained using co-injection of mannitol as evidenced by the plaque labeling throughout the brain of an APP transgenic mouse (Fig2.A). The specificity of the Dy-labeled construct was also preserved as evidenced by the lack of enhancement in a wild type mouse injected with the same amount of Dy-labeled peptide (Fig2.B).

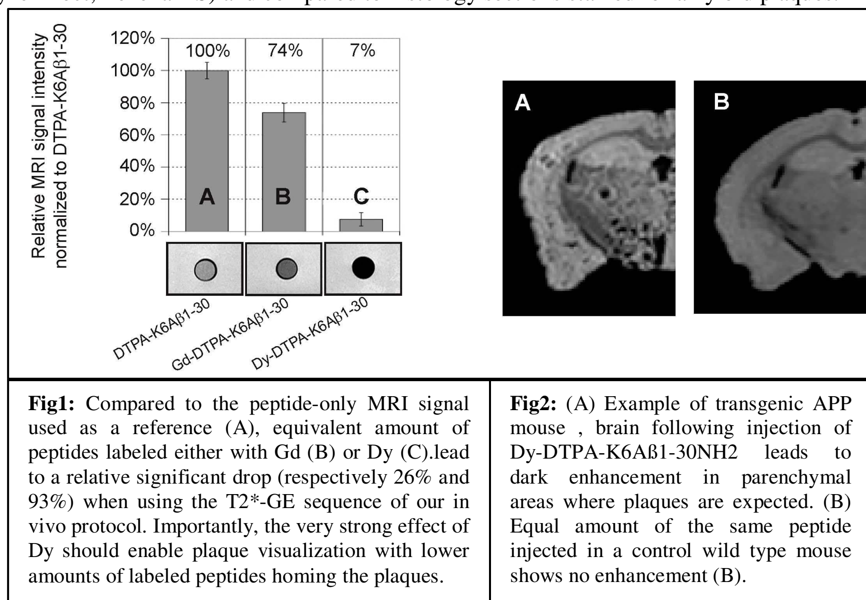

Fig1: Compared to the peptide-only MRI signal used as a reference (A), equivalent amount of peptides labeled either with Gd (B) or Dy (C).lead to a relative significant drop (respectively 26% and 93%) when using the T2*-GE sequence of our in vivo protocol. Importantly, the very strong effect of Dy should enable plaque visualization with lower amounts of labeled peptides homing the plaques.

Fig2: (A) Example of transgenic APP mouse , brain following injection of Dy-DTPA-K6Aß1-30NH2 leads to dark enhancement in parenchymal areas where plaques are expected. (B) Equal amount of the same peptide injected in a control wild type mouse shows no enhancement (B).

Conclusion

Dy labeling appears to be an excellent compromise for increasing sensitivity while maintaining the required physicochemical attributes to efficiently deliver and label the Aß plaques across the brain of AD transgenic mice. A close comparison between Gd- *vs.* Dy-labeling efficiency in each individual mouse and the corresponding correlation with histology is currently being assessed. The ability to label plaques more efficiently with Dy-based contrast agents will demonstrate a higher percentage of Aß plaques than Gd based agents making longitudinal studies more reliable and MRI as a valuable tool to monitor new therapies aimed at clearing the plaques

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