Novel Method for In Vitro Evaluation of Amyloid Plaque Binding Contrast Agents In Alzheimer's Disease

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Introduction

Currently, no in vivo diagnostic method exists for Alzheimer's disease (AD). Molecular imaging may provide a method to not only visualize plaques but also diagnose AD at much earlier stage. One important problem in development of targeted contrast agents is an ability to assess binding properties of the contrast agent to the tissue or cellular target of interest. The technique described here is a novel in vitro assay to evaluate the effectiveness of contrast agent binding to the target of interest. This method involves gentle homogenization of unfixed AD mouse brain, incubation of the homogenate with a contrast agent, removal of unbound contrast agent by centrifugation and washing, and resuspension of the homogenate in gelatin in an MRI compatible glass tube.

Methods

The entire PBS-perfused, unfixed cerebral cortex minus the meninges of a 24-month-old doubly transgenic APP-PS1 (amyloid precursor protein and presenilin 1) mouse was gently homogenized and aliquots were incubated with various concentrations of the contrast agent based on the sequence of the first 30 amino acid residues of Aβ with asparagyl/glutamyl-4-aminobutane residues (N-4ab/Q-4ab) substituted at unique Asp and Glu positions and with Gd-DTPA-aminohexanoic acid covalently attached at the N-terminal Asp[1], centrifuged to remove unbound contrast agent and suspended in 10% gelatin. Each sample (~1 mL) was degassed after it was transferred into a 12-mm outer diameter spherical glass bulb (Wilmad-LabGlass).

Magnetic Resonance Microimaging (MRMI) experiments were performed using a Varian INOVA console which was interfaced to a 9.4-T, 31-cm horizontal bore magnet (Magnex Scientific) equipped with actively shielded gradients capable of reaching 450 mT/m in 300 μs (Resonance Research Inc.). A single-loop 400-MHz ^{1}H surface radiofrequency coil was used to transmit and receive.

The T_2-weighted spin-echo images were obtained using a previously described pulse sequence[2]. The imaging parameters were as follows: T_R = 2 s, T_E = 52 ms, *x*, *y*, *z* matrix = 256 x 96 x 32 at corresponding FOV of 15.36 x 5.76 x 3.84 mm^3 resulting in voxel dimensions of 60 μm x 60 μm x 120 μm, respectively, and a scan time of 1 hr 40 min. The T_1-weighted spin-echo images were obtained using the following parameters: T_R = 0.4 s, T_E = 7 ms, BW = 80 kHz, 2 scans, *x*, *y*, *z* matrix = 256 x 128 x 32 at corresponding FOV of 15.36 x 7.68 x 3.84 mm^3 resulting in voxel dimension of 60 μm x 60 μm x 120 μm, respectively, and a scan time of 54 min.

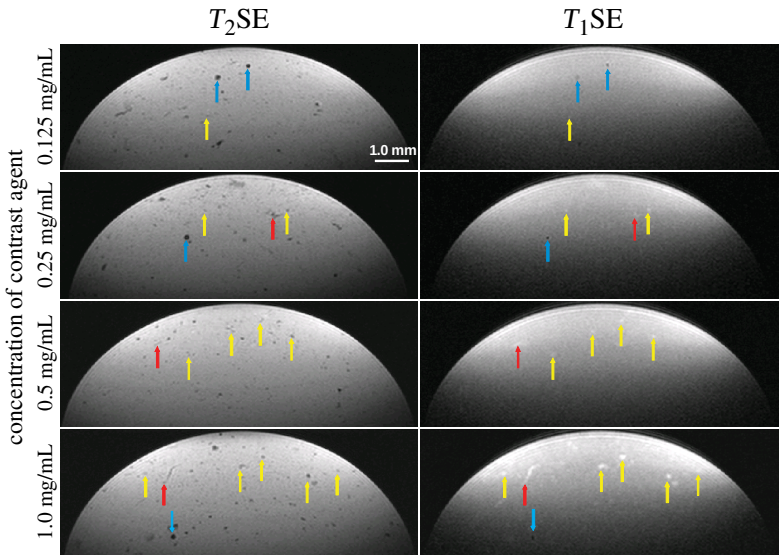

Figure. MRM images obtained using homogenate samples. Yellow arrows indicate amyloid plaques, red arrows indicate fragments of blood vessels, and blue arrows indicate micro-bubbles.

Following imaging, an image analysis tool with a linked cursor system was used to identify spatial position points that were common to the T_2- and T_1-weighted images. Validation of the method was based on the known contrast properties of plaques on T_2-weighted images (dark due to documented intrinsic iron content) and expected properties of plaques on T_1-weighted images (bright when incubated with plaque labeling contrast agen).

Results and Discussion

Figure shows the T_2- and T_1-weighted images from three samples. Individual plaques were resolved on T_2 images in all samples (dark areas). The expected signal of hyperintense foci was observed on the T_1 images and was attributed to incubation with the contrast agent. Moreover, relative plaque brightness demonstrates appropriate dose response behavior. Two-way spatial correspondence revealed excellent correlation between individual plaques visualized with the T_2 sequence and hyperintense foci on the T_1 scans. Additionally, apparent vascular amyloid deposits were also observed and labeled by the contrast agent as indicated by the thread-like structure present in the images. The ability to detect both amyloid plaques and blood vessels indicates that the architecture of both is well preserved in this preparation.

The method we describe seems to appropriately represent the biology of interest (amyloid plaques) in a manner that lends itself to the convenient testing of the binding properties of amyloid-labeling MRI-specific contrast agents. It seems highly unlikely that mechanical homogenization of mouse brain tissue would disrupt the architecture of plaques. Plaques themselves are composed primarily of insoluble crosslinked amyloid beta fibrils. Second, the fact that intact blood vessels were visualized in the homogenate indicates that the much more durable plaque architecture is also likely preserved.

The use of brain homogenates for evaluating plaque binding contrast agents has advantages over the use of intact animals in that uncertainties of variable delivery of the plaque labeling compound and BBB penetration are eliminated. Likewise use of this homogenate model has advantages over incubating intact tissue slices. These include avoiding variable physical access to plaques by contrast agent molecules diffusing through tissue; the need to fix tissue in order to mount it in gelatin; and partial volume effects when imaging the slices. In addition, this homogenate preparation allows a more rigorous evaluation of the kinetics of contrast agent binding to individual plaques which is a necessary prerequisite to *in vivo* studies.

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References: 1. Poduslo, J. F. *et al*., *Biochemistry* **43**, 6064-6075 (2004). **2.** Jack, C. R. *et al*., *Magn. Reson. Med.* **52**, 1263-1271 (2004).