

Gadolinium-Stained Brains Reveal Amyloid Plaques in Live Alzheimer's Transgenic Mice

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INTRODUCTION:

Beta-amyloid (A β) plaques are one of the hallmarks of Alzheimer's disease (AD). Transgenic mouse models of amyloidosis are widely used to test new drugs against AD. Direct in vivo imaging of A β plaques (that measure less than 100 μ m) in such models is very challenging but would be very useful as a screening tool in pharmacological studies. Magnetic resonance microscopy (μ MRI) can be used to detect amyloid plaques in mice^{1,2,3}, but without contrast agents, it mainly detects iron-loaded plaques that are mostly present in old animals. A β -targeted contrast agents are also under development but they are not readily available and most agents do not cross the blood-brain barrier. Here, we developed a protocol based on the use of a non-targeted gadolinium (Gd) contrast agent directly injected in the animals' brains to detect amyloid plaques in vivo.

METHODS:

We used 15 APP/PS1 mice (6-20 months) and 5 control PS1 mice (13-20 months). For the in vivo studies, the animals were bilaterally injected in the lateral ventricles of the brain with 1 μ l of Gd at 0.5 M (gadoterate meglumine, Dotarem®, Guerbet, France) using a stereotaxic frame. In vivo μ MRI exams were run before and after injection at 45 x 45 x 156 μ m³ (original matrix zero-filled to yield a resolution of 22.5 x 22.5 x 78 μ m³) using a 3D gradient echo sequence (FLASH) on a 7T small bore system (Pharmascan, Bruker) with TR/TE = 32/16 ms, FA = 20°, scan time = 1h54min. After sacrifice of the animals by intracardiac perfusion with Formalin, the extracted brains were passively stained⁴ in a Gd solution at 2.5 mM for at least 24 hrs. Ex vivo μ MRI exams were run before and after passive staining at 23 x 23 x 90 μ m³ using a 3D gradient-echo sequence (FLASH) on a 7T wholebody system (Syngo MR VB15, Siemens) with TR/TE = 100/19.4 ms, FA = 25°, scan time = 14h. In vivo and ex vivo relaxation times (T1, T2, T2*) were calculated using Bruker routines. Plaque loads were manually calculated in at least 28 regions in the cortex. After imaging, the brains were sectioned in 40- μ m thick sections and stained with Congo red, anti-A β stain (BAM10) and Perls' for amyloid and iron deposits assessment.

RESULTS:

The relaxation time measurements showed that the Gd contrast agent rapidly diffused into the whole brain after injections (in vivo) and after passive staining (ex vivo): T1s in all brain tissues were shortened (from ~ 1500 ms to ~ 200 ms) and T2/T2* remained high enough to keep the signal in the images high. As a result, the signal was enhanced in the parenchyma (by > 2X) and the contrast was increased between the plaques and the rest of the tissue. This contrast enhancement revealed a large number of hypointense spots in the cortex and hippocampus of APP/PS1 mice at 6-20 months both in vivo (fig.1) and ex vivo (fig.2). We further showed that these hypointense spots correlated with BAM10 immunohistology staining (fig.3) and with Congo red but only very slightly with Perls' iron staining. Plaque load measurements showed a correlation with age (in vivo: $r = 0.87$, $p < 0.001$ and ex vivo: $r = 0.81$, $p < 0.005$) and the MR measurements correlated with the immunohistology measurements (fig.4): $r = 0.77$, $p < 0.01$ (in vivo) and $r = 0.88$, $p < 0.005$ (ex vivo).

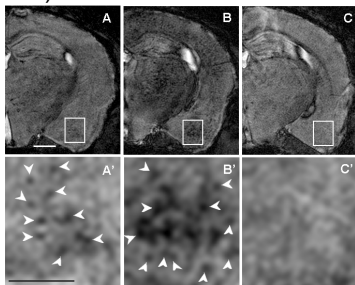


Fig.1. In vivo amyloid plaque detection at 6 months (A, A'), 20 months (B, B') and a control brain (C, C'). Interpolated resolution = 22.5 x 22.5 x 78 μ m. Scale bars = 1 mm.

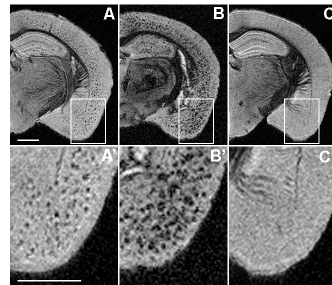


Fig.2. Ex vivo amyloid plaque detection at 6 months (A, A'), 20 months (B, B') and a control brain (C, C'). Resolution = 23 x 23 x 90 μ m. Scale bars = 1 mm.

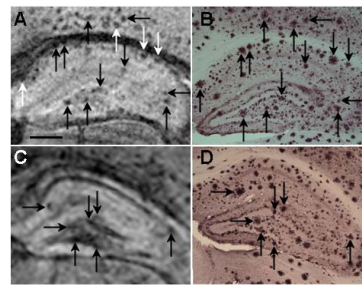


Fig.3. The hypointense spots seen on the MR images (ex vivo: A, in vivo: C) colocalize with A β staining (B, D). Scale bar = 500 μ m.

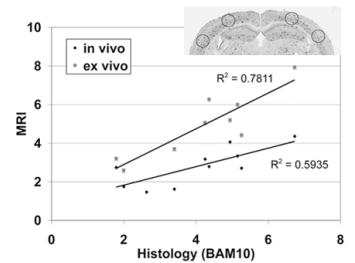


Fig.4. Amyloid plaque count correlation between (in vivo and ex vivo) MRI and immunohistology.

CONCLUSION:

This study demonstrates that amyloid plaques can be detected both ex vivo and in vivo using a non-specific Gd contrast agent delivered through simple passive diffusion in fixed brains, or through intraventricular injections in anesthetized AD Tg mice. This level of detection and quantification should allow us to follow changes in plaque load in pharmacological studies in these animals.

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