Rapid *in-vivo* Imaging of Amyloid Plaques Using µ-MRI Gd-Staining and Ultrasound-Induced Blood Brain Barrier Opening

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Introduction

Alzheimer's disease (AD) is characterized by two complementary microscopic brain lesions: amyloid plaques and neurofibrillary tangles. Amyloid plaques measure from 20 to 100 µm. They occur up to 20 years before the first clinical signs of the disease [1] and numerous experimental treatments try to suppress these lesions. Imaging amyloid plaques is thus critical to follow-up these treatments and for an early diagnostic of AD. Thus, several efforts aim at developing methods for amyloid plaque detection with high-resolution magnetic resonance imaging (MRI). The approaches using NMR contrast agents need opening of the blood-brain barrier (BBB), a specialized system which prevents certain substances from penetrating the brain. BBB opening can be achieved with agents such as mannitol but this approach leads to large mortality. Recently our group developed methods of *ex vivo* (Passive staining) [2] or *in vivo* [3] amyloid plaque detection based on the use of a non-targeted Gadolinium (Gd) contrast agent. For *in vivo* detection of amyloid plaques, the agent had to be injected within the cerebral ventricles (ICV-Gd-staining) to bypass the BBB. However, being able to detect amyloid plaques after IV injection of contrast agents would be easier. Some studies showed the feasibility of BBB opening with the use of ultrasound (US) and ultrasound contrast agents (encapsulated gas microbubbles) [4-6]. Under the action of an ultrasound beam, microbubbles oscillate and allow the opening of the BBB. The aim of this work was thus to develop a new protocol (US-Gd-staining) to detect amyloid plaques after intra-venous (IV) injection of NMR contrast agent and BBB opening with ultrasound and microbubbles.

Materials & Methods

This study was done using 6 APP/PS1 transgenic mice (8 to 17 months-old) exhibiting cerebral amyloid plaques and 6 control (littermate, PS1) plaque-free mice. BBB opening was obtained by means of a controlled acoustic excitation leaded by an unfocalized ultrasound transducer (Imasonic) and encapsulated gas microbubbles (Sonovue, Bracco) injected IV. NMR contrast agent (Dotarem, Guerbet) was also IV-injected. 3D Gradient-echo NMR images (TR/TE=30/15ms, resolution: 29x29x117µm^3, Nex=1, scan time: 32 min; 7T-Varian) were recorded.

The ability to detect amyloid plaques with this method was compared with detection of amyloid plaques with previously developed methods based on intracerebroventricular injection of Dotarem as described in [3] (same 3D Gradient-echo imaging protocol than for US-Gd-staining) and with *post mortem* detection of amyloid plaques. For this latter experiment, the mice were sacrificed and their brains were removed and soaked in a 1:200 solution of Dotarem two days before to be imaged with a high resolution 3D gradient-echo protocol (TR/TE=40/15ms, resolution: 25x25x100 µm^3, Nex=16, scan time=11h39min).

Results

US-Gd-staining, but also *in vivo* ICV-Gd-staining and *post mortem* passive staining largely increased the signal to noise ratio in the brain of mice (Fig. 1).

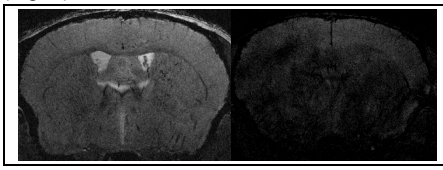

Fig. 1. Left – *In vivo* MRI of an amyloid-free PS1 mouse obtained with US-Gd-staining. No hypointense spots identified as plaques are visible within the cortex. Right – MRI of an APP/PS1 mouse without NMR contrast agent.

No hypointense spots which could be falsely identified as plaques were detected within the brain of amyloid-free littermate mice with the three methods described: US-Gd-staining (Fig. 1, left panel), ICV-Gd-staining and *post mortem* passive staining. No plaques could be *in vivo*-detected without the use of NMR contrast agent (Fig. 1, right panel). In transgenic mice, hypointense spots could be detected in the cortex of all animals imaged with *in vivo* US-Gd-staining (Fig. 2, Left panel, arrows), *in vivo* ICV-Gd-staining (Fig. 2, right panel) and *post mortem* passive staining (data not shown) protocols. The hypointense spots are amyloid plaques [3]. These lesions were identified even in the youngest mice used in this study (8-months old).

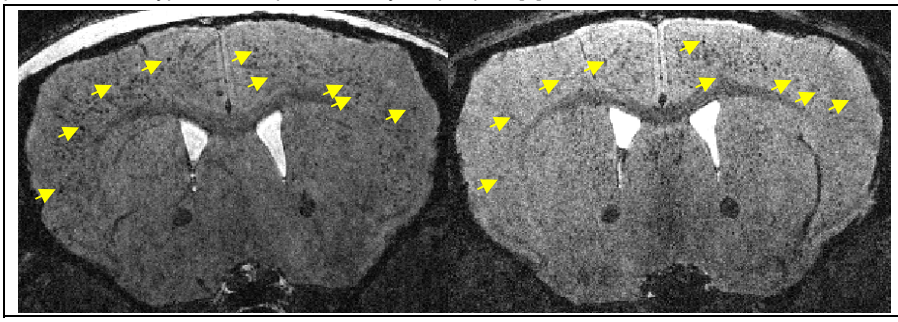

Fig. 2. Left – *In vivo* MRI of 8 months-old APP/PS1 mouse obtained with US-Gd-staining. Dark spots within the cortex are amyloid plaques (arrows). Right – *In vivo* MRI with bilateral *in situ* injections of NMR contrast agent (*in vivo* ICV-Gd-staining), arrows indicate the presence of plaques.

Discussion

To our knowledge this is the first study showing the feasibility of amyloid plaque imaging in mouse with a peripheric injection of NMR contrast agent and the non invasive opening of the BBB by US. This suggests that, in humans, contrast agents that can cross the BBB will allow the detection of amyloid plaques by MRI. *In vivo* US-Gd-staining should also permit a better follow-up of new therapies targeting amyloid in murine models of AD.

References

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Acknowledgements

Medicen (Pôle_de_compétitivité Île-de-France, TransAl program), France Berkeley Fund, France-Alzheimer association, NIH (R01-AG020197).