Gd-staining reveals the efficacy of an anti-Aβ antibody to decrease amyloid plaque load *in vivo* in a transgenic mouse model of Alzheimer's disease

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Purpose

Alzheimer's disease (AD) is characterized by two major microscopic brain lesions: amyloid plaques and neurofibrillary tangles. Amyloid plaques measure from 20 to 100 µm and in humans they can occur up to 20 years before the first clinical signs of the disease [1]. Detection of amyloid plaques is therefore key to enable monitoring of the efficacy of anti-amyloid therapies and for an early diagnostics of AD. To date, PET imaging has been the most studied non-invasive imaging technique to detect plaques in AD patients but there are also several efforts aimed at developing new imaging methods for amyloid plaque detection using high-resolution magnetic resonance imaging (MRI). We have recently developed a new MR imaging method for *in vivo* amyloid plaque detection based on the use of a non-targeted Gadolinium (Gd) contrast agent [2]. The aim of the present study was to further validate this imaging protocol (ICV-Gd-staining) by evaluating its ability to detect changes in amyloid load and the efficacy of an anti-Aβ antibody specifically recognizing protofibrillar forms of Aβ, in a longitudinal *in vivo* pharmacology study in a mouse model of AD.

Materials & Methods

Twenty six APP/PS1 transgenic mice and 20 control mice (littermate, PS1, plaque-free) were used in the study. Fourteen of the APP/PS1 mice received a weekly injection of the anti-Aβ antibody (10mg/kg) from 3.5 to 8.5 months whereas 12 others were injected with a control antibody (DM4, not targeting Aβ, 10mg/kg). The control mice (PS1) also received weekly injections of the anti-Aβ (10 animals) or the control antibody (10 animals). Amyloid plaque detection was performed after intracerebroventricular injection of a Gadolinium-based contrast agent (Dotarem, Guerbet; ICV-Gd-staining), as described previously [2]. Gradient-echo MR images (3D, TR/TE=50/25ms, resolution: 29x29x117µm^3, scan time: 1h50 min; 7T-Agilent Tech.) were recorded on each APP/PS1 mice and on two PS1 mice from each group at 5.5 and 8.5 months. After imaging, the mice were sacrificed and the brain removed for histology analysis. Quantification of amyloid plaque load was performed using the MR images of 8 evenly spaced brain slices, from each mouse at 5.5 and 8.5 months. The third slice containing the anterior commissure was used as a reference region. Four regions of interest (ROI) were drawn on each slice and amyloid plaques were manually counted and measured inside these ROIs [3]. The mean amyloid plaque load was derived using the measured size of individual plaques and the size of the ROIs. The same procedure was used to quantify amyloid plaque load on histological sections.

Results

Gd-staining significantly increased the signal to noise ratio in the brain of mice and allowed the detection of amyloid plaques (Fig. 1, 2). The longitudinal study revealed that brain amyloid load increased from 3.4% to 7.5% in the control antibody group and from 3.3% to 5.7% in the anti-Aβ antibody group between the ages of 5.5 and 8.5 months. The amyloid plaque load of the mice treated with the anti-Aβ antibody was significantly lower (~41%) than in the control antibody group (two sample t-test, p=0.004; Fig. 2) at 8.5 months. No plaques were detected in the PS1 mouse treated with the anti-Aβ antibody or with the control antibody (Fig. 1) and no hypointense spots, which could be falsely identified as plaques, were detected in the brain of PS1 littermate mice at 5.5 and 8.5 months (Fig. 2). The reduced amyloid load induced by the anti-Aβ antibody in 8.5 month-old APP/PS1 was also observed by histological analysis (data not shown).

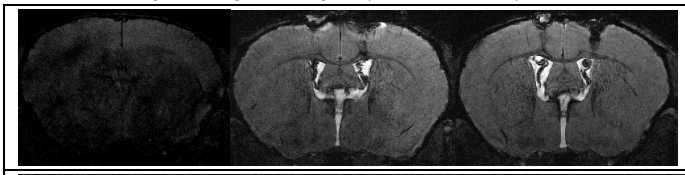

Fig. 1. Increased signal to noise ratio and amyloid plaque detection in the MR images (left) and after Gd staining (center). MR images of PS1 mouse with ICV Gd-Staining at 8.5 month-old (right).

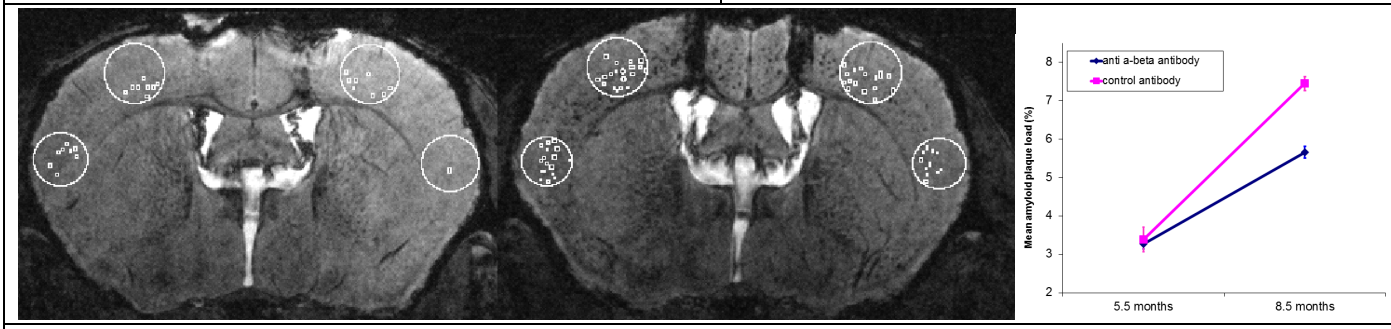

Fig. 2. Longitudinal detection of amyloid plaque load in APP/PS1 mice. Left – *In vivo* ICV-Gd-staining MRI of an APP/PS1 mouse at 5.5 months. White circles represent the ROIs where amyloid load was quantified. Spots identified as plaques are visible within the cortex and drawn in white into the ROIs. Center – MRI of the same APP/PS1 mouse at 8.5 months. Right - Comparison of amyloid plaque load measured in APP/PS1 mice treated with the anti-Aβ antibody or with the control antibody. Error bars stand for standard error of the mean.

Conclusion

In vivo amyloid plaque detection is critical to enable the monitoring of the efficacy of anti-amyloid treatments for Alzheimer's disease in both preclinical and clinical studies. Here, we show that Gd-staining allows to record *in vivo* MR images with a very high resolution (29µm) and to detect amyloid plaques in an animal model of Alzheimer's Disease. In addition, this method makes it possible to follow the age-associated increase of amyloid plaques in transgenic mice and to demonstrate the efficacy of an anti-amyloid therapy.

References

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