In vivo and post-mortem detection of amyloid plaques with a non-specific gadolinium contrast agent

A. E. Petiet[1,2], A. Bertrand[2], C. J. Wiggins[2], D. Houitte[3], T. Debeir[1], T. Rooney[1], and M. Dhenain[2,3]

[1]CNS, Sanofi-Aventis, Vitry-sur-Seine, France, [2]DSV, I2BM, NeuroSpin, CEA, Gif-sur-Yvette, France, [3]Mircen, CNRS URA 2210, Orsay, France

INTRODUCTION

Alzheimer's disease (AD) is the most common type of neurodegenerative disease, but no cure has yet been found. Amyloid deposits, one of the characteristic lesions, constitute the main target for diagnostics and therapeutics and have previously been identified in mouse models using magnetic resonance (MR) microscopy[1,2]. These plaques typically appear as hypointense spots on T2-weighted MR images and their size ranges from 50 µm to 200 µm. One of the challenges in imaging plaques is to achieve high-enough resolution and contrast to detect 50-µm large lesions. While most high-field MR systems can reach high resolution, the lack of contrast between the plaques and the parenchyma often impedes their detection. Some investigators have used targeted contrast agents that selectively bind to amyloid plaques[3], but they are not readily available and cannot be used clinically. We have hypothesized that the use of a non-specific Gd contrast agent coupled with a very high resolution would allow us to identify amyloid deposits in AD transgenic (Tg) mouse brains. We first tested our hypothesis in fixed and stained brains[4] at 23 x 23 x 90 µm^3 and the method was then extended to live anesthetized mice, using intracerebroventricular (ICV) injections of a contrast agent for *in vivo* imaging at 50 x 50 x 200 µm^3 resolution.

METHODS

We used 6 APP Tg mice (24 months old) for the *ex vivo* study and 3 APP/PS1 Tg mice (8 months old) and 7 wild-type (WT) mice for the *in vivo* study. All MR images were acquired on a 7T clinical magnet (Siemens, Syngo MR VB15) with gradients' strength of 80 mT/m and a slew rate of 333 mT/m/s. A preliminary study was run to determine the best contrast agent (CA) titration needed for amyloid plaque detection in fixed brain tissue (passive staining[4], data not shown). The CA concentration in the brain that resulted in the highest SNR (~ 40:1) was 2.5 mM gadoterate meglumine (Gd-DOTA) in PFA. This concentration was then used in all further studies.

For the *ex vivo* study, the brains were imaged before and after staining at very high (23 x 23 x 90 µm^3) and intermediate (65 x 65 x 200 µm^3) resolutions with a 3D gradient-echo-based sequence (FLASH, TR/TE = 100/19.4 ms, scan time = 12 h and 1 h). Histology sections were obtained from all brains and stained with congo red and cresyl violet for MR/histology plaque colocalization.

For the *in vivo* study, the anesthetized animals were bilaterally injected with 1 µl of gadopentetate dimeglumine (Gd-DTPA, 0.5 M) via an ICV route and imaged before and after injection at a resolution comparable to the *ex vivo* study (50 x 50 x 200 µm^3). We used a 3D turbo spin-echo sequence (TR/TE = 4000/54 ms, RARE = 7, scan time = 2 h 28 min) to avoid susceptibility artifacts found on gradient-echo sequences.

Signal and contrast-to-noise ratios (SNR and CNR) were calculated for all brains.

RESULTS

Without CA, the *ex vivo* images showed low parenchymal SNR (11:1) and no detectable plaques (Fig.1a) even at very high resolution. After addition of CA, the SNR increased by a factor of 2. Although some plaques were visible at the intermediate resolution (Fig.1b) with a high CNR of ~ 18, most of them were not resolvable enough to be quantified. Only the very high resolution images distinctly showed a large number of plaques (CNR ~ 11) and could be visually counted (Fig.1c-e). The *in vivo* study on WT animals showed an initial significant 3-fold increase in the parenchymal signal just after injection of CA which then remained elevated for at least 5 h (graph Fig.2), indicating good penetration of Gd into the brain tissue distant from the injection sites. The images of Tg animals showed that no plaques were detectable in the cortex or hippocampus before ICV injection, whereas some plaques were visible after injection (Fig.2). The CNR between the plaques and the parenchyma was about 2-fold lower than in fixed brains (table1), but was sufficient to detect plaques at the resolution used.

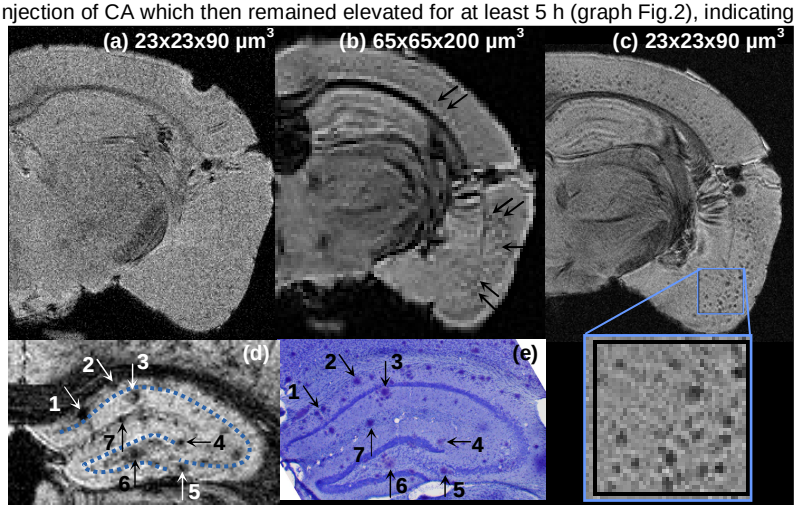

Figure 1: the use of a non-specific CA increased parenchymal SNR by 2X (a vs. c). At a medium resolution (b) some plaques were visible, but only at the very high resolution (c), a large number of plaques were resolved and could be quantified: at least 7 in the hippocampal region of interest (arrows, d) (and (e) histological colocalization) and ~ 20 in the cortical region of interest (bottom right).

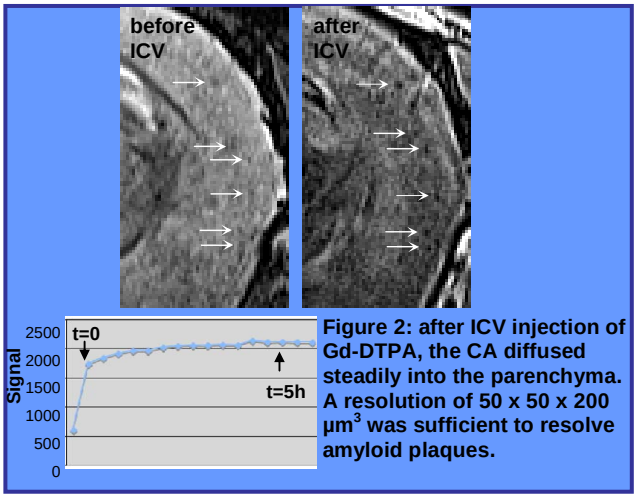

Figure 2: after ICV injection of Gd-DTPA, the CA diffused steadily into the parenchyma. A resolution of 50 x 50 x 200 µm^3 was sufficient to resolve amyloid plaques.

Table1: CNR measurements between plaques and parenchyma.
*WM: white matter; GM: gray matter

	23x23x90 µm^3 *(no CA)*	65x65x200 µm^3	50x50x200 µm^3	23x23x90 µm^3
Ex vivo	NA	18.7 ± 4.1	NA	11.3 ± 0.4
ICV vivo	NA	NA	6.6 ± 2.9	NA

CONCLUSION

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

ACKNOWLEDGEMENTS We thank Fanny Petit (CEA) for her help in histology. This work was supported by Medicen (Pôle de compétitivité Ile-de-France, TransAl program), the France-Alzheimer association and the NIH (R01-AG020197).

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