In vivo detection of Alzheimer's plaques in transgenic mice: Requirement for targeted contrast agents

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Introduction

Noninvasive *in vivo* evaluation of amyloid burden in Alzheimer's disease (AD) patients would be instrumental for the early diagnosis of AD as well as the development and monitoring of new treatment strategies. In MRI, ongoing efforts have been devoted in developing a method that would visualize the amyloid plaques [1-8]. The initial approach proposed was based on the premises that the higher iron content in plaques would serve as an endogenous source of contrast that would be discernable at higher field through the susceptibility effect. The first study to report this effect using MRI was assessed in post-mortem human brain tissues with a high anatomical detail [1]. Subsequently a similar study was unable to confirm these findings although employing even greater spatial resolution [2]. These discrepancies observed in human samples can be partially explained by dependence in the tissue preparation leading to a possible loss of iron or to the difference in nature of the amyloid deposits studied. Similar studies have been assessed in mouse models of amyloidosis leading to a much greater success from several groups [3-6]. Although encouraging, these studies required long imaging hours that are prohibitive for *in vivo* consideration in order to achieve an efficient visualization of the plaques in the brain parenchyma. Alternatives approaches have proposed the injections of MR labeled peptide that would specifically target the amyloid deposits to improve the sensitivity [7-8], leading to the first *in vivo* observation of AD plaques [8]. In this current study we have explored with a high spatial resolution both approaches *in vivo*, endogenous detection as well as the targeted labeling technique using intravenous or intracarotid injection at various stages of the disease.

Material and Methods

All MRI experiments were performed with a SMIS console interfaced to a 7T horizontal bore magnet equipped with 250mT/m actively shielded gradients (ID=120mm) (Magnex Scientific, Abingdon, UK). Both single transgenic APP (Tg2576) and double transgenic APP/PS1 mice, together with age-matched wild-type control mice were imaged under isoflurane anesthesia (1.5% in air, 2 l/min flow rate) using a homemade head coil (ID=22mm). Mice at early and late stages of disease were examined to test the role of amyloid load in MRI detection. Each individual mouse was imaged 3 times with a 2 week interval between scans, allowing the subjects time to recover from anesthesia administered during MRI. The first image session was used as a reference prior to the injection of targeted contrast agents and to determine if some plaques could be detected without contrast-enhancement. Subsequent imaging was used to test the effect of intravenous injection of Gd-DTPA tagged peptides designed to target amyloid plaques [8]. The last imaging session was included to monitor the clearance of the plaque labeling agents. Two targeting constructs, identically MR-tagged were examined: Aβ1-40 that was previously used with success [7-8] and a less toxic form K6Aβ1-30. These two constructs were both covalently linked to putrescine to allow brain uptake when intravenous administration was considered [7]. K6Aβ1-30 was also tested through an intracarotid co-injection with mannitol to permeabilize the blood brain barrier (BBB).

A 3DGE sequence (TR=50ms,TE=15ms) was used to provide a 100μm isotropic resolution in less than 2 hours while facilitating image comparison during longitudinal studies and co-registration with histology. The image sets were realigned (Analyze v6.0, AnalyzeDirect, Lenexa KS) to match MR slices from different imaging times and to compare to histological brain sections acquired after MRI and stained for amyloid plaques.

Results and Discussion

In older transgenic mice with high amyloid burden, large plaques were observed *in vivo* in pre-contrast images (N=4; Fig.1.A). Following intravenous injection of the targeted agents, contrast-enhancement was observed in the previously detected plaques and many additional smaller plaques were observed on MRI (Fig. 1.B), and confirmed by histology (Fig. 1.C). In transgenic mice with low amyloid burden, plaques were only observed following injection of the targeted contrast agents (N=3; Fig. 2.B). Furthermore, clearance of the contrast agents after 2 weeks demonstrated that longitudinal studies to monitor the progression of amyloid deposition are possible (Fig. 2.C). These results confirm previous *ex vivo* results that amyloid plaques can be detected in transgenic mice with high field MRI [4-6], and provide the demonstration that some amyloid plaques can be detected *in vivo*. Detection without contrast agents appears to be limited to large plaques, but likely also reflects a difference in the nature of the amyloid deposits (iron content, old *vs.* young plaques, vascular *vs.* parenchymal). The ability to label plaques efficiently after intravenous injection, combining putrescine to the targeting construct to permeablize the BBB, is a significant improvement over existing MR plaque imaging methods and makes longitudinal studies possible from early stages of plaque formation. This new MRI approach can now be used to monitor experimental therapies aimed at clearing plaques in transgenic mice. The ability to visualize amyloid plaques before and after administration of the targeted agents may be useful in the future for differentiating plaque types which may ultimately improve our understanding of AD disease progression. Finally, the use of contrast agent appears to be required for early plaque detection, suggesting that these agents will be needed to detect plaques in Alzheimer's disease prior to clinical symptoms. Early diagnosis of Alzheimer's should lead to a more effective therapy.

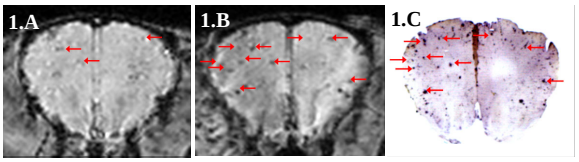

Fig. 1: *In vivo* MRI plaque detection in a 20-month old APP transgenic mouse: A) detection of larger plaques in pre-contrast MRI; B) marked enhancement of numerous plaques using Gd-K6Aβ1-30 peptide; C) matched histology stained for amyloid. Arrows indicate histologically confirmed amyloid plaques.

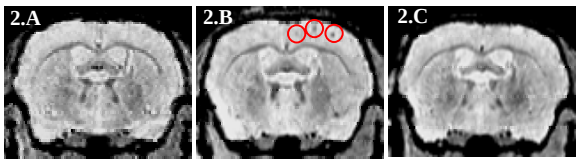

Fig. 2: In a 20-month old APP mouse with low amyloid burden, plaques are only observed following injection of targeted contrast agent. A) normal MRI appearance of the brain in the pre-scan; B) sparse but marked enhancement in the cortex using putrescine labeled Gd-K6Aβ1-30 peptide. C) Follow up 2 weeks after injection demonstrating the clearance of the contrast agent. Circles indicate histologically confirmed amlyoid plaques.

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