Detection of Alzheimer’s Amyloid in Transgenic Mice Using Magnetic Resonance Microimaging

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The presence of amyloid-β (Aβ) plaques in the brain is a hallmark pathological feature of Alzheimer’s disease (AD). Transgenic mice overexpressing mutant amyloid precursor protein (APP), or both mutant APP and presenilin-1 (APP/PS1), develop Aβ plaques similar to those in AD patients, and have been proposed as animal models in which to test experimental therapeutic approaches for the clearance of Aβ. However, at present there is no in vivo whole-brain imaging method to detect Aβ plaques in mice or men. A novel method is presented to detect Aβ plaques in the brains of transgenic mice by magnetic resonance microimaging (μMRI). This method uses Aβ(1-40 peptide, known for its high binding affinity to Aβ, magnetically labeled with either gadolinium (Gd) or monocrystalline iron oxide nanoparticles (MION). Intravenous injection of magnetically labeled Aβ(1-40, with mannitol to transiently open the blood–brain barrier (BBB), enabled the detection of many Aβ plaques. Furthermore, the numerical density of Aβ plaques detected by μMRI and by immunohistochemistry showed excellent correlation. This approach provides an in vivo method to detect Aβ in AD transgenic mice, and suggests that diagnostic MRI methods to detect Aβ in AD patients may ultimately be feasible. Magn Reson Med 50:293–302, 2003. © 2003 Wiley-Liss, Inc.

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Several transgenic mouse lines have been generated in an attempt to produce genetically relevant animal models of Alzheimer’s disease (AD). In particular, mice overexpressing either mutant amyloid precursor protein (APP), or both mutant APP and presenilin-1 (APP/PS1), develop amyloid-β (Aβ) plaques—one of the hallmark histopathological features of AD (1,2). These transgenic mice are now being used to test experimental approaches for amyloid clearance (3,4). Currently lacking is a sensitive in vivo method to assess amyloid burden in the brains of transgenic mice and AD patients, which makes it impossible to make an early definitive diagnosis of AD or to dynamically monitor the effects of new therapeutic agents on Aβ plaques.

Prior in vitro studies have shown that radiolabeled Aβ binds to Aβ plaques (5). More recent studies have shown that radiolabeled Aβ derivatives (6–8) or other radioactive probes that bind to Aβ (9,10) enable the visualization of Aβ plaques following autoradiography of tissue sections. Systemic administration of a radiolabeled Aβ derivative has allowed the in vivo visualization, using nuclear imaging, of implanted synthetic Aβ deposits in the biceps femoris muscle of rats (10). In addition, both topical and systemic injections of fluorescent Aβ-binding probes have enabled the in vivo detection of amyloid lesions near the surface of exposed transgenic mouse brains, using multiphoton optical microscopy (11,12). These results have generated considerable excitement and led to the expectation that similar probes might be radiolabeled for noninvasive detection of Aβ using high-resolution, whole-brain, in vivo imaging methods, such as positron emission tomography (PET).

Magnetic resonance imaging (MRI) provides a superior whole-brain evaluation compared to optical imaging, and higher spatial resolution than PET, and may therefore be better suited to assess amyloid burden in vivo. Benveniste and colleagues (13) reported that they were able to detect Aβ plaques in postmortem brains from AD patients using T₂*-weighted MR microimaging (μMRI), but the ~20-hr imaging time needed to achieve the required resolution of [40 μm]³ was prohibitive for in vivo studies. In contrast to Benveniste et al.’s (13) results, Dhenain and colleagues (14) recently reported that, using T₂*-weighted μMRI at a very high field strength (11.7 T) and very high spatial resolution of [23 μm]³, they were unable to detect Aβ plaques in tissue samples from the brains of AD patients. The development of a contrast agent that selectively binds to Aβ might enable plaque detection with sufficient temporal, spatial, and contrast resolution using in vivo MRI in AD patients and transgenic mice. We have tagged Aβ1-40, an Aβ peptide with high binding affinity for Aβ plaques.
(15), with two paramagnetic MRI contrast agents: gadolinium-diethylentriaminepentaacetic acid (Gd-DTPA), and monocrystalline iron oxide nanoparticles (MION). Our results demonstrate that these two magnetically labeled ligands, Gd-DTPA-Ab1-40 and MION-Ab1-40, can be used to detect Ab plaques in the brains of both APP and APP/PS1 transgenic mice after intravascular coinjection with manniitol to transiently open the blood–brain barrier (BBB).

**MATERIALS AND METHODS**

**Magnetically Labeled Peptides**

MR contrast agents were linked to Ab1-40 peptide using standard methods. Ab1-40 with and without a metal chelating arm was synthesized on an ABI430A peptide synthesizer using standard protocols for tBOC (tert-butyloxycarbonyl) chemistry. The chelating arm, diethylentriaminepentaacetic acid (DTPA), was attached to the amino terminus of the peptide as the final step of synthesis. The peptides were cleaved from the resins using hydrofluoric acid, and purification was performed by high-pressure liquid chromatography (HPLC) on a Vydac C18 preparative column, 2.5 x 30 cm (Vydac Separations, Hesperia, CA), using linear gradients from 0–70% of acetonitrile in 0.1% trifluoroacetic acid. Gadolinium (Gd) was chelated to the DTPA-Ab1-40 using Gd (III) chloride hexahydrate (Aldrich, Milwaukee, WI) with a 24-hr incubation in water at pH 7.0. The Gd-DTPA-Ab1-40 complex was then purified again, and the peptide was lyophilized in preparation for in vivo injection.

Monocrystalline iron oxide nanoparticles (MION) were obtained from the Center for Molecular Imaging Research (Massachusetts General Hospital, Boston, MA) as a brownish, stock solution containing 12.05 mg Fe/ml. Each MION particle contains an average of 2064 Fe atoms (16). For injection into each mouse, 300 μg of dextran-coated MION particles (15.8 μl of stock solution), were adsorbed with 120 μg of Ab1-40 in a volume of 120 μl of H2O by overnight mixing at 4°C (17). We calculated that approximately 17 Ab1-40 molecules were bound to each nanoparticle of MION-Ab1-40.

**Binding Studies Between Gd-DTPA-Ab1-40 or MION-Ab1-40 and Ab1-42**

Experiments were performed to assess the ability of the magnetically labeled Ab1-40 peptide to bind Ab1-42 peptide. The interaction between Gd-DTPA-Ab1-40 or MION-Ab1-40 and Ab1-42 was evaluated by enzyme-linked immunosorbent solid phase assays, as described in our previously published protocol (18). Ab1-42 was used for these affinity binding studies because this peptide is the major constituent of AD plaque amyloid (19), and therefore the calculated affinity constants approximate the binding of the MR ligands to Ab plaques in the AD-transgenic mice. Briefly, polystyrene microtiter plates were coated overnight at 4°C with freshly dissolved Ab1-42 in 50 mM NaHCO3, and then blocked (Superblock, Pierce). The Ab1-42 coated wells were exposed to increasing concentrations (0–11,000 nM) of Ab1-40, Gd-DPTA-Ab1-40, or MION-Ab1-40 in Tris-buffered saline. All binding studies were performed in triplicate. Following washing, bound Ab1-40, Gd-DPTA-Ab1-40, or MION-Ab1-40 was detected using an anti-Ab1-40 antibody (44-348; Biosource International, Hopkinton, MA) that does not cross-react with Ab1-42. After washing, the plates were incubated for 1 hr with an anti-rabbit horseradish peroxidase-linked antibody (Amersham Life Science), developed for 15 min with a TMB peroxidase kit (Bio-Rad, Hercules, CA), and quantified at 450 nm on a 7520 microplate reader (Cambridge Technology, Watertown, MA). The data were analyzed by a nonlinear regression fit algorithm in Prism (v3.0, GraphPad Software, San Diego, CA). Controls for nonspecific binding included wells without coating Ab1-42 and omission of the anti-Ab1-40 or secondary antibodies. Using Ab1-40, Gd-DPTA-Ab1-40, or MION-Ab1-40 to coat the plates, the anti-Ab1-40 antibody detected Ab1-40 equally well.

**μMRI**

μMRI experiments were performed on an SMIS console interfaced to a 7 Tesla horizontal bore magnet equipped with 250 mT/m actively shielded gradients (Magnex Scientific, Abingdon, UK). Preliminary phantom imaging experiments were performed to qualitatively assess the relaxivity effects of the magnetically labeled peptides.

Ex vivo μMRI of fixed whole brains was used initially to determine the feasibility of the magnetic labeling approach, and to optimize the pulse sequences. Extracted, fixed mouse brains were embedded in 3% aqueous agarose gel to prevent dehydration during μMRI. Fixed brains were imaged because unfixed brains were more likely to become distorted during removal and embedding, and would deteriorate significantly during the imaging procedure, compromising subsequent immunohistochemical analysis. Samples were maintained at 20°C during μMRI by means of a water-circulating system. A custom threeturn solenoid coil (ID = 16 mm) was developed to fit closely around the fixed mouse brain samples. For each brain, 11 coronal image slices, spaced 500 μm slice to slice, were acquired from the olfactory bulb to the cerebellum, with 59 μm x 59 μm in-plane resolution and a slice thickness of 500 or 250 μm. We explored a variety of T1- and T2-weighted spin-echo (SE), and T2*-weighted gradient-echo (GE) pulse sequences for detecting the magnetically labeled peptides in the mouse brain samples. The pulse sequence timing parameters (echo time (TE) and repetition time (TR)) were as follows:

1. T1-SE (2D): TE = 10 ms, TR = 250 ms.
2. T1-SE (3D): TE = 8 ms, TR = 150 ms.
3. T2-SE (2D): TE = 30-40 ms, TR = 2 s.
4. T2*-GE (2D): TE = 15 ms, TR = 1.5 s.

For in vivo μMRI, the mice were initially anesthetized with isoflurane (5%) in air, and anesthesia was maintained with 1.5% isoflurane in air (2 l/min flow rate). The rectal temperature and respiratory rate of each mouse were monitored throughout the scan. A custom saddle coil (ID = 22 mm) was incorporated into the holding device, and a tooth bar was used to fix the head in a reproducible and stationary position during data acquisition. As in the ex vivo imaging protocol, 11 coronal brain image slices were
acquired for each mouse: $T_2^*$-weighted SE (TE = 30 ms; TR = 2 s; in-plane resolution = 78 μm × 78 μm; slice thickness = 500 μm; total imaging time = 120 min); $T_2^*$-weighted GE sequence (TE = 15 ms; TR = 1.5 s; in-plane resolution = 78 μm × 78 μm; slice thickness = 250 μm; total imaging time = 59 min). Following in vivo μMRI, the anesthetized mice were killed by transaortic perfusion, and their brains were removed for further ex vivo μMRI and/or histological analysis.

**Mice and Contrast Agent Injection**

All mice used in these studies were maintained according to protocols approved by the Institutional Animal Care and Use Committee at the New York University School of Medicine. Imaging experiments were performed on 15–16-month-old APP transgenic mice (1), 5–6-month-old APP/PS1 transgenic mice (2), and age-matched nontransgenic controls (Table 1). These ages were chosen to ensure a time of maximum AD plaque labeling following systemic injections, with minimal nonspecific labeling of blood vessels, is between 4 and 6 hr (6,8).

Since neither Gd-DTPA nor MION cross the BBB alone, the magnetically-labeled peptides were coinjected with mannitol. Gd-DTPA-αβ1-40 was prepared before each administration by suspending 400 μg in 100 μl water and dissolving immediately before infusion with a solution of 15% mannitol in PBS (600 μl), in order to temporarily open the BBB (20,21). This preparation was injected directly into the common carotid artery at a rate of 0.25 ml/kg/s. This rate is below that which causes hypertensive opening of the BBB, but produces optimal BBB disruption without neurotoxicity (21,22). The dosage of Gd-DTPA-αβ1-40 used in these studies was limited by the solubility of the peptide (400 μg Gd-DTPA-αβ1-40 per 700 μl total volume). This represents a much smaller dose of Gd-DTPA per body weight than is typically used in human contrast-MRI (~0.003 mmol/kg, or 3% of the recommended human dose).

In other experiments, MION-αβ1-40 was used as the contrast agent. In this case each mouse received 300 μg of MION particles, onto which 120 μg of αβ1-40 was adsorbed (as described above, corresponding to 190 mg Fe/kg). The dosage of MION reported in human studies has ranged between 1.1 and 2.6 mg Fe/kg (23–26); hence, the mice received a substantially higher dose. Immediately before the MION-αβ1-40 was injected into the common carotid artery, it was mixed with 600 μl of 15% mannitol in PBS.

Both Gd-DTPA-αβ1-40 and MION-αβ1-40 were coinjected with mannitol into AD-transgenic mice and age-matched, nontransgenic controls (Table 1). Additional control experiments were performed injecting Gd-DTPA-αβ1-40 or MION-αβ1-40 without mannitol, Gd-DTPA or MION with mannitol but without αβ1-40, or administering no injection (see Table 1). Six hours after intracarotid injection, the mice were either imaged in vivo or anesthetized with sodium pentobarbital (150 mg/kg, intraperitoneal) and perfused transaortically with 0.1 M phosphate-buffered saline (PBS), pH 7.4, followed by 4% paraformaldehyde in PBS. The time point of 6 hr was chosen because our own and other studies using 125I-αβ1-40 have shown that the time of maximum AD plaque labeling following systemic injections, with minimal nonspecific labeling of blood vessels, is between 4 and 6 hr (6,8).

**Histology**

After μMRI, the brains were placed in 2% DMSO/20% glycerol in PBS overnight. Coronal sections (40 μm thick) were cut and analyzed for the presence of αβ and iron (to detect MION). Immunohistochemistry was performed, using an antibody (6E10) that recognizes αβ, as previously described (27). Briefly, sections were incubated in 6E10 (1:1000, Senetek), and antibody staining was revealed with a Vectastain MOM kit (Vector Laboratories) and diaminobenzidine (DAB; Sigma) with or without nickel ammonium sulfate intensification. Mallory iron-staining was performed by putting defatted and hydrated sections for 10 min in a solution containing 2.5% potassium ferrocyanide and 2.5% hydrochloric acid. The slides were then rinsed in distilled water, and the sections were dehydrated, cleared in Hemo-De, and coverslipped.

**Aβ Plaque Density Quantitation**

Immunostained tissue sections were quantified with a Bioquant stereology image analysis system (R&M Biometrics)
Aβ deposits were analyzed under ×165 magnification in regions of cortex and hippocampus, using anatomical landmarks defined by Franklin and Paxinos (28). The area of the grid was 800 μm × 800 μm, and Aβ deposits were analyzed in 18 frames per mouse (640 μm × 400 μm). Intensification with nickel ammonium sulfate resulted in black Aβ with minimal background staining that facilitated threshold detection. Only lesions larger than 200 μm² were counted. This criterion included the vast majority (>90%) of Aβ plaques, and ensured that only extracellular amyloid was included in the analysis. The number and area of Aβ plaques per mm² was measured in each test area.

For quantification of Aβ plaque density with μMRI, digitized image files with a calibrated scale marker were imported into the Bioquant stereology system. The brightness of the images was altered so that the average optical density measurement for each imported image was similar. Regions of interest (ROIs) were manually drawn on the cortex and hippocampus, corresponding to the same anatomical landmarks used for histological Aβ quantification. Dark spots, with intensity below a preset threshold value (equal for all μMR images) were counted as “Aβ plaques.” The numerical density of plaques was calculated, dividing the number of plaques by the ROI area.

Quantitative analysis of Aβ plaque density by histology and μMRI was performed by an individual blinded to the treatment of the mice. The correlation between numerical plaque densities detected by histology and μMRI was analyzed using Pearson’s correlation coefficient in Statistica (Version 6.1, StatSoft Inc., Tulsa, OK).

We recognize the complications involved in comparing plaque density results between μMRI (with slice thickness = 250 or 500 μm) and histology (with slice thickness = 40 μm). We attempted to minimize these differences by averaging plaque density results from several (serial) histology slices matched to each μMRI slice. In the future, it may be useful to investigate “virtual sectioning” approaches using 3D-rendered histology and μMRI data.

RESULTS
Analysis of Magnetically Labeled Aβ1-40
Mass spectroscopy of the HPLC-purified Gd-DTPA-Aβ1-40 gave a mass of 4975.76, in agreement with the expected mass of 4976.6. Analytical HPLC showed a purity of >90–95%, and the molar ratio of Aβ1-40 to Gd was 1:1. Purification and analysis of Aβ1-40 gave similar results for the expected molecular weight and purity.

To determine the effectiveness of the Aβ1-40 adsorption onto MION, the mixture was ultracentrifuged after incubation at 100000 g for 40 min. This produced a clear supernatant and a brownish lower phase. HPLC analysis revealed that >99% of the Aβ1-40 and MION were found in the lower phase. In experiments incubating Aβ1-40 alone for 24 hr, the peptide did not pellet at 100000 g, but remained in solution.

Binding Affinity Studies
We performed experiments to determine the binding affinity between the targeting peptides and Aβ1-42. Serial dilutions of Gd-DTPA-Aβ1-40 with wells coated with Aβ1-42 revealed a dose-dependent binding that saturated at 1000 nM. Assuming 1:1 stoichiometry between Aβ1-42 and Gd-DTPA-Aβ1-40, the KD was calculated to be 577 nM (±166, SEM). This compared to a KD of 266 nM (±50) between Aβ1-40 and Aβ1-42, suggesting that the covalent modification of Aβ1-40, by adding Gd-DTPA, lowered the affinity of labeled Aβ1-40 to Aβ1-42 by a factor of approximately 2. The KD for binding of MION-Aβ1-40 to Aβ1-42 was 202 nM (±40), which was not significantly different from the binding of Aβ1-40 to Aβ1-42.

FIG. 1. Aβ plaques were detected with ex vivo μMRI after injection of Gd-DTPA-Aβ1-40 with mannitol. Ex vivo T₂-weighted (TE = 30 ms; TR = 2 s; in-plane resolution = 59 μm × 59 μm; slice thickness = 500 μm; total imaging time = 120 min) SE coronal μMR images show 6-month-old control (a) and APP/PS1-transgenic (b) mouse brains. Both brains were extracted and prepared for imaging 6 hr after carotid injection of Gd-DTPA-Aβ1-40 with 15% mannitol. Note the obvious matching of many larger plaques (arrowheads) between μMRI (b) and immunohistochemistry (c).
Phantom experiments, imaging the labeled peptide in solution, demonstrated the expected $T_2$ and $T_2^*$ signal loss due to the susceptibility effects of Gd-DTPA-Aβ1-40 and MION-Aβ1-40 alone, while unlabeled Aβ1-40 produced no signal effect (data not shown). $T_1$-weighted images of the Gd-DTPA-Aβ1-40 peptide also showed an expected positive enhancement (data not shown). These results encouraged us to proceed to μMRI experiments in mice using both Gd-DTPA-Aβ1-40 and MION-Aβ1-40.

**Gd-DTPA-Aβ1-40**

In vivo injection of Gd-DTPA-Aβ1-40 into the carotid artery of both 15–16-month-old APP transgenic mice (N = 3) and 6-month-old APP/PS1 transgenic mice (N = 9), along with 15% mannitol to transiently open the BBB, resulted in the detection of numerous dark spots in all transgenic mice imaged with ex vivo (N = 5/5) and in vivo (N = 7/7) $T_2^*$ (Fig. 1) and $T_2^*$-weighted μMRI (Fig. 2). Significantly, the patterns of hypointense spots were very similar to the Aβ distribution seen in matched histological sections, and the larger μMRI spots corresponded exactly to Aβ plaques (see arrowheads in Figs. 1 and 2). Ex vivo positive enhancement of some Gd-labeled plaques was also observed with $T_1$-weighted SE, but there were far fewer $T_1$-hyperintense than $T_2^*$-hypointense lesions, and these were only seen with a short TR (150 ms) and very long imaging time (>16 hr) using the 3D sequence, so this approach was not pursued further (Fig. 3). $T_2^*$-weighted μMRI provided su-
The hypointense spots seen in all AD transgenic mice were quite characteristic and distinct from the uniform image intensity seen in age-matched controls. Indeed, dark spots were rarely observed in wild-type control mice injected and imaged using the same protocols, on both ex vivo (N = 5/5; Fig. 1) and in vivo μMRI (N = 4/4). Hypointense blood vessels were identified in both transgenic and control mice, especially on $T_2^*$-weighted μMRI. Although this is a potential confounding factor in plaque detection, the vessels were usually easily distinguished from plaques since the former were less hypointense and hence were eliminated by thresholding during quantitative analysis. Furthermore, blood vessels generally presented on μMRI as a small number of isolated or symmetrically distributed linear structures, unlike the clustered patterns of spots in AD-transgenic mice (Fig. 2). In any event, there was a marked and obvious difference in both the qualitative and quantitative appearance of the μMRI brain images from AD-transgenic and control mice.

Additional control experiments were performed to determine the necessity of injecting Gd-DTPA-AB1-40 or mannitol to detect Aβ plaques (Table 1, Fig. 4). In noninjected wild-type and transgenic mice, Aβ plaques were not detected; however, they were present in the transgenic mice imaged, as confirmed by histology (Fig. 4a; N = 2/2). In addition, in wild-type and transgenic mice injected with Gd-DTPA (without AB1-40) and mannitol, Aβ plaques were not detected on μMRI, although they were present in all the transgenic mice imaged, as confirmed by histology (N = 3/3; data not shown). Finally, in transgenic mice injected with Gd-DTPA-AB1-40 without mannitol, Aβ plaques were not detected on μMRI, although they were present in the brains of the transgenic mice imaged, as confirmed by histology (Fig. 4b; N = 2/2).

**MION-AB1-40**

In vivo coinjection of MION-AB1-40 with 15% mannitol into the carotid arteries of AD-transgenic mice also resulted in numerous dark spots on ex vivo μMRI, which matched the histologically detected Aβ plaques in the same mice (Fig. 5; N = 6/6). As with Gd-DTPA-AB1-40, the images of transgenic mice were markedly different in appearance from wild-type mice imaged with the same protocol (N = 4). Significantly, colocalization of MION-AB1-40 in Aβ plaques was confirmed histologically by double staining for iron and Aβ (N = 6/6, Fig. 5c, inset). As with Gd-DTPA-AB1-40, Aβ plaques were not detected in transgenic mice injected with MION-AB1-40 without mannitol (Fig. 4c), or with MION alone (without AB1-40) with mannitol (data not shown), although Aβ plaques were present in all the AD-transgenic mice imaged (N = 4/4).

**Quantitative Correlation Between μMRI and Histology**

The amyloid burden, defined as the percent tissue area under examination occupied by Aβ plaques, was assessed...
FIG. 4. Control experiments indicated that both magnetically-labeled Aβ1-40 and mannitol were required to detect Aβ plaques. Ex vivo $T_2$-weighted SE coronal μMRI images (left panels) failed to show Aβ plaques in a number of experiments, in 16-month-old APP-transgenic mice with numerous histologically-confirmed Aβ plaques (right panels): (a) uninjected transgenic mouse; (b) transgenic mouse injected with Gd-DTPA-Aβ1-40 alone, without mannitol; (c) transgenic mouse injected with MION-Aβ1-40 alone, without mannitol. In b and c, the brains were extracted and prepared for imaging 6 hr after carotid injection of magnetically-labeled Aβ1-40.

by quantitative immunohistochemical analysis of cortical sections of the APP and APP/PS1 transgenic mice in this study. The results of this analysis were 1.00% ± 0.68 (SD) in APP transgenics ($N = 17$), and 1.87% ± 0.92 (SD) in APP/PS1 transgenics ($N = 7$). These results are consistent with previously published data from the same transgenic mice ($4,29$).

In AD-transgenic mice coinjected with Gd-DTPA-Aβ1-40 and mannitol, there was a statistically significant correlation between the densities of Aβ plaques detected by μMRI and histology ($r = 0.871, P = 0.011; \text{Fig. 6}$). The ratio of lesion densities (histology : μMRI) was 1.1 ± 0.3 (SD). Furthermore, the line of fit between histology and μMRI was very close to the line of identity, indicating that the majority of Aβ plaques detected by immunohistochemistry were also detected on μMRI. In mice imaged with both $T_2$- and $T_2^*$-weighted μMRI, $T_2$-weighted SE was slightly less sensitive for in vivo detection of lesions compared to $T_2^*$-weighted GE. $T_2$-weighted images revealed 85% ($±13\%$, SD; $N = 2$) of lesions detected by the $T_2^*$-weighted images, an effect that can be attributed to higher sensitivity of the $T_2^*$-weighted sequence to the susceptibility effects of the paramagnetic contrast agent. Of note, in quantitative analysis of control (wild-type) mice lacking Aβ plaques, dark spots in the cortex and hippocampus were rarely detected following thresholding. In all control mice the μMRI-determined “amyloid” burden was $<0.005\%$.

The sensitivity of MION-Aβ1-40 to detect Aβ plaques was slightly lower than with Gd-DTPA-Aβ1-40, likely due to the reduced BBB permeability of MION-Aβ1-40. The ratio of lesion density (histology : μMRI) was 1.3 ± 0.1 (SD) for MION-Aβ1-40. In addition, in AD-transgenic mice injected with MION-Aβ1-40 and mannitol, the correlation between μMRI and histological lesion density did not reach the level of statistical significance, due to greater individual variability.

DISCUSSION

Our results show clearly that systemic injection of Aβ1-40 peptide, chelated to Gd or adsorbed onto MION, with an agent to increase BBB permeability, can be used to detect Aβ plaques by μMRI in the brains of AD-transgenic mice. $T_2$-weighted SE and $T_2^*$-weighted GE μMRI protocols were used in this study because they allow clear identification of hypointense lesions, corresponding to Aβ plaques identified by histology, with relatively short acquisition times (1–2 hr). In contrast, $T_1$-weighted μMRI-detected Aβ plaques, labeled with Gd-DTPA-Aβ1-40, were hyperintense but required very long MR acquisition times (>16 hr) with the 3D SE sequence used, which was not feasible for in vivo imaging. Furthermore, we observed fewer enhancing Aβ plaques in ex vivo $T_1$-weighted images, compared to the number of $T_2$-hypointense lesions in the same brains. We speculate that the lack of strong $T_1$ enhancement in our studies may be related to the difficulty of removing susceptibility effects from $T_1$-weighted images at the high field strength (7T) of our magnet. It is therefore possible that Gd-tagged Aβ ligands may still prove useful in conjunction with $T_1$-weighted imaging at the lower field...
strengths (1.5T) more commonly used for human diagnostic MRI.

We performed a number of experiments to better define the utility and limitations of the magnetically labeled Aβ1-40 peptides used to detect Aβ plaques in transgenic mice. In particular, we demonstrated that Aβ1-40 peptide is essential for the targeting of MION and Gd-DTPA to Aβ plaques, since plaques were not detected when the contrast agents were injected alone in transgenic mice. Some blood vessels also presented as hypointense spots on $T_2^*$- and $T_1^*$-weighted images, but the small number of suspected blood vessels were easily distinguished from Aβ plaques by their size, location, shape, and intensity. Our results were obtained 6 hr following a single injection of magnetically-labeled Aβ1-40 in order to maximize Aβ binding while eliminating nonspecific vessel labeling. This time point was chosen based on previous work using radiolabeled Aβ1-40 (6,8). Given the difficulties in exactly matching results from μMRI (thickness = 250–500 μm) and histology (thickness = 40 μm), there was a remarkable degree of correspondence between the Aβ plaques detected by μMRI and immunohistochemistry. The magnetically labeled peptides used in these studies preferentially led to the detection of larger parenchymal deposits. Indeed, our quantitative analysis was limited to plaques larger than 200-μm$^2$ in area, which included the majority of Aβ plaques and guaranteed that we were examining extracellular amyloid on immunohistochemistry. It is likely that the Aβ ligands used in these studies would also label amyloid associated with cerebral blood vessels. However, the AD-transgenic mice in this study had minimal amyloid angiopathy, so this potential application could not be examined.

The dosage of Gd-DTPA (scaled to the mouse body weight) used in our experiments is significantly lower than the corresponding Gd-DTPA dosage used in human contrast-MRI studies, while the converse was true for MION. In spite of the lower dose, there was a slightly better detection of Aβ plaques using Gd-DTPA-Aβ1-40. These differences likely reflect the lower BBB permeability of MION-Aβ1-40 vs. Gd-DTPA-Aβ1-40, even though mannitol was co-injected with both targeting peptides. Despite the high permeability of Aβ1-40 via receptor-mediated transport (30,31), no lesions were detected when Gd-DTPA-Aβ1-40 or MION-Aβ1-40 were injected without

FIG. 6. There was good correlation between the numerical density of lesions detected by immunohistochemistry and μMRI, after coinjection of Gd-DTPA-Aβ1-40 and mannitol ($r = 0.871$, $P = 0.011$). The dashed line indicates the 95% confidence level.

FIG. 5. Aβ plaques were detected with ex vivo μMRI after injection of MION-Aβ1-40 with mannitol. Ex vivo $T_2^*$-weighted SE coronal μMRI images show 16-month-old control (a) and APP-transgenic (b) mouse brains. Both brains were extracted and prepared for imaging 6 hr after carotid injection of MION-Aβ1-40 with 15% mannitol. Many μMRI lesions matched to Aβ plaques (arrowheads), as revealed by immunohistochemistry (c). High-power microscopic examination of the amyloid plaques, double-stained with an Aβ (6E10) antibody (brown color) and a Mallory stain for iron (azure color, red arrow), demonstrated the colocalization of MION with Aβ plaques (inset).
mannitol, showing that magnetic labeling lowered the BBB permeability of the Aβ1-40. This was as expected, since it has been well documented that Gd-DTPA and MION alone do not cross the BBB. The control experiments described in this work also highlight the important point that with the current approach, Aβ plaques in the AD-transgenic mice were not visualized without the presence of a contrast agent. The fact that mannitol is required to transiently open the BBB does not preclude the use of these amyloid ligands in longitudinal μMRI studies in AD-transgenic mice. However, less traumatic approaches should be considered for routine diagnostic applications of brain-targeting ligands (32)—for example, by coupling the Aβ peptide to a carrier that is normally transported into the brain, such as putrescine (33,34). In addition, intravenous injection of the targeting ligands should be explored as a safer alternative to intraarterial injection for longitudinal studies.

Finally, alternative Aβ ligands should also be explored to minimize potential toxicity, with the ultimate goal of making this method more suitable for human use. Aβ1-40 is the major sequence of Aβ peptides found physiologically in biological fluids (35), with longer peptides, including Aβ1-42, being more closely linked to the initiation of amyloid deposits and AD-related pathology (36). However, at concentrations Aβ1-40 can be toxic in tissue culture (37), and Aβ1-40 is the major form of amyloid in vascular walls (38). Therefore, toxicity is a concern with the use of this peptide, although there was no evidence of toxicity during our experiments. A number of methods could be used to circumvent this problem. We and others have designed Aβ homologous peptides that are nontoxic, but retain a high affinity for native Aβ peptides (4,27), and could be magnetically labeled in the same way as Aβ1-40. Alternatively, anti-Aβ antibodies could be magnetically labeled, as has been previously described for the detection of tumors in vivo (39,40). Overall, the results show that with the use of the MRI approach presented here, early detection of Aβ plaques—one of the hallmarks of AD—may be within reach. Once effective Aβ-clearing therapies are available for AD, the ability to make an early diagnosis, before the onset of marked clinical symptoms, will be critical in preventing the irreversible neuronal damage that eventually leads to dementia and ultimately death.

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