REVIEW

Amyloid Imaging Using High-Field Magnetic Resonance

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(Received March 8, 2010; Accepted March 24, 2010)

The formation of senile plaques followed by deposition of amyloid β peptides ($A\beta$) are the earliest pathological changes of Alzheimer's disease (AD); thus, detection of the plaques remains the most important early diagnostic indicator of AD. Amyloid imaging is a noninvasive technique for visualizing senile plaques in the brains of patients with Alzheimer's using positron emission tomography (PET) or magnetic resonance (MR) imaging. Several types of probes have been developed for PET, but few ligands have been developed specifically for MR imaging detection of amyloid plaques. This review presents recent advances in amyloid imaging using MR imaging and includes our studies.

Keywords: *Alzheimer's disease, amyloid imaging, cell tracking, contrast agents, ¹⁹F-MRI*

Introduction

Alzheimer's disease (AD) is a cognitive impairment disorder characterized by cerebral atrophy, deposits of amyloid β peptide ($A\beta$), neurofibrillary tangles, and neuronal cell loss. According to the amyloid cascade hypothesis for AD, formation of senile plaques followed by the deposition of $A\beta$ are the earliest pathological changes of AD.^{1,2} Detection of senile plaques is therefore the most important indicator for early diagnosis of AD.

Amyloid imaging is a noninvasive technique that uses positron emission tomography (PET) or magnetic resonance (MR) imaging to visualize $A\beta$ plaques in the brain. With the increasing incidence of AD as the average age of many populations increases, amyloid imaging has attracted increasing attention for the early diagnosis of AD. Representative ligands for PET, such as N-methyl-[¹¹C]2-(4-methylaminophenyl)-6-hydroxybenzothiazole (PIB)³ and 2-[2-(2-dimethylaminothiazol-5-yl) ethenyl]-6-[2-(fluoro)ethoxy] benzoxazole (BF-227),⁴ have been used in clinical research, but no clinical studies of amyloid imaging using MR imaging have been conducted. The likely bottleneck is the lower sensitivity of MR imaging than PET. Many re-

searchers are thus trying to develop novel contrast agents (Table) and pulse sequences⁵ to increase the sensitivity of amyloid detection. We review the trend in amyloid imaging using MR imaging and include results from our studies.

Amyloid Imaging without Contrast Agents

Senile plaques in the brains of patients with AD contain iron and iron-binding proteins, such as lactotransferrin and melanotransferrin,^{6,7} and so appear as areas of low density by MR imaging using T₂*-weighted gradient echo⁸ and T₂-weighted spin echo sequences^{9,10} at high magnetic fields. However, the many hours to obtain a complete image of the senile plaques preclude such studies for *in vivo* imaging. Amyloid imaging without contrast agents is the ideal alternative, although additional research is needed before applying this approach in human patients. Recently, Chamberlain and colleagues⁵ reported on amyloid plaque contrast generated by a novel imaging method called susceptibility-weighted imaging (SWI). Their *ex vivo* study presented important observations for the future of *in vivo* MR imaging of amyloid.

Amyloid Imaging with Contrast Agents

The essential characteristics of contrast agents

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for amyloid imaging are binding affinity, capacity to pass through the blood-brain barrier (BBB), nontoxicity, and strong signal intensity in the brain. In general, amyloid-binding compound de-

rivatives and paramagnetic-labeled compounds are used.

1. Gadolinium-labeled contrast agents

Gadolinium (Gd) is a typical contrast agent used for MR imaging. Gd-labeled A β peptide^{11–13} and A β antibody¹⁴ have been injected into transgenic mice with AD to detect amyloid plaques in the mouse brain. Using high magnetic field MR systems, senile plaques in the mouse brain are visualized with T₁-weighted and T₂-weighted MR images as the high and low signal intensities, respectively. The above results suggested that amyloid plaque might also be detected using other magnetically labeled A β peptide and A β antibody, such as monocrySTALLINE iron oxide nanoparticle (MION)-A β 1-40.¹²

2. ¹⁹F-labeled contrast agents

The advantage of ¹⁹F-MR imaging is the high sensitivity of the ¹⁹F to MR ratio (¹H: ¹⁹F = 1: 0.83), high natural abundance ratio (100%), and absence of endogenous ¹⁹F. However, disadvantages to amyloid imaging by ¹⁹F-MR imaging include the low concentration accumulated in the body and the lack of clarity regarding chemical properties of probes that improve amyloid detection.

(E,E)-1-fluoro-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (FSB) (Fig. 1a),¹⁵ bis-styrylbenzenes (Fig. 1b, c),¹⁶ and trifluoromethoxy-benzylated (TFMB) compounds (Fig. 1d, e)¹⁷ have been reported as amyloid imaging ligands for ¹⁹F-MR imaging. FSB allowed the detection of A β

Table. Reports on amyloid imaging with and without contrast agents using magnetic resonance (MR) imaging

Contrast agent	Magnetic flux density	Reference
<i>Without</i>	9.4 tesla (T)	5, 8–10
<i>Gadolinium (Gd)-label</i>		
Gd-DTPA-K6A β 1-30	7T	11
Gd-DTPA-A β 1-40	7T	12, 13
PUT-Gd-A β 1-40	7T	13
Gd-A β 1-40	7T	13
Gd-DOTA-pF(ab') ₂ 4.1	9.4T	14
<i>¹⁹F-labels</i>		
FSB	9.4T	15
Polyfluorinated bis-styrylbenzene	—	16
TFMB	7T	17
<i>Other labels</i>		
MION-A β 1-40	7T	12
Resovist-labeled microglia	7T	18–20

Abbreviations and acronyms

FSB: (E,E)-1-fluoro-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene, Gd-DOTA: gadolinium-tetraazacyclododecanetetraacetic acid, Gd-DTPA: gadopentetate dimeglumine, MION: monocrySTALLINE iron oxide nanoparticles PUT: putrescine, TFMB: trifluoromethoxy-benzylate.

Fig. 1. Structure of amyloid imaging ligands for ¹⁹F-magnetic resonance imaging

plaques in transgenic mice with amyloid precursor protein (APP) using 9.4-tesla MR imaging,¹⁵ but we could not detect the ^{19}F -signal of injected FSB (200 $\mu\text{g}/\text{head}$) in mouse brain at 7T. We therefore investigated developing novel ^{19}F -labeled amyloid-imaging ligands for MR imaging and improved instrumentation.

First, we synthesized 2-(4'-dimethylaminostyryl)-6-{2-[(3'-trifluoromethoxy)benzylamino]ethoxyethoxy}benzoxazole (TFMB-2Et) and 2-(4'-dimethylaminostyryl)-6-{2-[(3'-trifluoromethoxy)benzylamino]ethoxyethoxyethoxy}benzoxazole (TFMB-3Et), both of which have stronger ^{19}F -signals than FSB. We attempted to localize TFMB-2Et and TFMB-3Et in mouse muscle and brain using 7T MR imaging, but detecting ^{19}F -signal in mouse brain was difficult.¹⁷ *Ex vivo* MR imaging and *in vitro* nuclear magnetic resonance (NMR) analyses showed the NMR signals of ^{19}F to be very sensitive to the tissue environment and that brain tissues inhibit amyloid detection in ^{19}F -MR imaging by affecting the ^{19}F -NMR signals.¹⁷ Comparing the log P values and the intensities of ^{19}F -NMR signals in brain homogenates using several compounds with a trifluoro-base revealed that hydrophilic chemicals (low log P) have relatively strong ^{19}F -NMR signals. Therefore, effective MR imaging of amyloid in the brain requires optimizing probe hydrophilicity while retaining good brain permeability of the probe.

Second, we optimized the radiofrequency (RF) coil because we detected an unexpectedly high ^{19}F signal with our home-built, solenoid-type volume coil designed to detect the ^{19}F signal of TFMB-2Et (Fig. 2A). The ^{19}F chemical shift image of a TFMB-2Et tube in 3 orthogonal planes with a larger field of view showed ^{19}F -signal of the TFMB-2Et (Fig. 3D-I, arrow) and additional high signal from the capacitors (Fig. 3D-I, arrowhead). We successfully removed the noise by replacing the existing capacitor with glass parts (Fig. 2B). Attention should also be paid to instrument noise in detecting weak ^{19}F

signals.

Amyloid Imaging by Cell Tracking

Because microglia accumulate around senile plaques and remove $\text{A}\beta$ deposits in the brain,¹⁸ we could track microglia around senile plaques by MR imaging. Because native cells are not detected by MR imaging, cells must be labeled with a contrast agent for MR tracking. The most popular such agents are superparamagnetic iron oxides (SPIOs). We¹⁹ and others²⁰ have developed a highly efficient labeling method using a nonviral vector system, the hemagglutinating virus of Japan (HVJ; Sendai virus) envelope (HVJ-E) vector. The high effectiveness of HVJ-E vector labeling allowed us to detect a few cells at each spot in the mouse brain using MR imaging.²¹

We then injected β -amyloid peptide $\text{A}\beta_{1-42}$ ($\text{A}\beta_{42}$) into the left hippocampus and saline into the right hippocampus of rats and labeled microglia with a SPIO (Resovist, Bayer, Osaka, Japan) administered via the carotid artery or lateral ventricle. MR imaging revealed clear signal changes attributable to Resovist-containing microglia accumulated in the areas injected with $\text{A}\beta_{42}$.^{22,23} Because microglia accelerate the removal of $\text{A}\beta$, this technique might also be applicable to amyloid imaging and cell therapy for AD.

Conclusion

Although many researchers have addressed the development of novel contrast agents and pulse sequence to increase the sensitivity of amyloid detection, many issues still exist in using amyloid imaging for MR imaging in humans. However, such application of MR imaging is very useful in terms of safety, cost, and apparatus penetration rate and is expected to progress in the future.

Fig. 2. ^{19}F -single pulse spectra of 2-(4'-dimethylaminostyryl)-6-{2-[(3'-trifluoromethoxy)benzylamino]ethoxyethoxy}benzoxazole (TFMB-2Et) corrected by home-made coil (A) and new home-made coil with the glass parts of the capacitors replaced (B).

Fig. 3. ^{19}F -chemical shift imaging of 2-(4'-dimethylaminostyryl)-6-{2-[(3'-trifluoromethoxy) benzylamino]ethoxyethoxy}benzoxazole (TFMB-2Et) (arrow) and noise (arrowhead) in the 3 orthogonal planes with bigger field of view **A, B:** View showing 3 orthogonal planes of a home-built, solenoid-type volume coil. **C-E:** ^{19}F -nuclear magnetic resonance (NMR) signals obtained by chemical shift imaging. **F-H:** Merged images.

Acknowledgments

This study was supported by the Japan Science and Technology Agency (JST) Practical Application Research Program and Grants-in Aid for Scientific Research (No. 1930058).

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