Early Detection of Amyloid β Pathology in Alzheimer's Disease by Molecular MRI*

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Abstract— **Alzheimer's disease (AD) is a degenerative brain disease and the most common cause of dementia. Early stage β-amyloid oligomers (AβOs) and late stage Aβ plaques are the pathological hallmarks of AD brains. AβOs are known to be more neurotoxic and contribute to neuronal damage. Most current approaches are focused on detecting Aβ plaques, which occurs at the late stage of AD, and are limited by poor sensitivity and/or contrast agent toxicity. In previous studies, we developed a new curcumin-conjugated magnetic nanoparticle (Cur-MNPs) to target the Aβ pathologies. In this study, we investigate the *in vivo* feasibility of this novel Cur-MNPs to detect Aβ pathologies at the early and late stages of AD in transgenic AD mice and perform immunohistochemical examinations to validate the specific targeting of various form of Aβ pathologies.**

I. INTRODUCTION

Alzheimer's disease (AD) currently affects more than 20 million people globally, with about 135 million people expected to be affected by 2050 [1-3].

AD is a progressive neurodegenerative disorder with no means yet known for prevention or cure. Although the exact causes of AD have not been clearly identified, brain atrophy, especially around the hippocampal region, is commonly found in AD patients. Soluble amyloid β (Aβ) is normally secreted by brain cells and then cleared from the brain. But in AD brains, Aβ accumulates and aggregates as oligomers and fibrils. They form extracellular Aβ deposits/plaques, which attract reactive astrocytes and microglia. These, insoluble Aβ plaques may contribute to neuronal damage and drive AD progression[4-7].

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Additionally, we have soluble oligomeric forms of Aβ (AβOs) that are more neurotoxic than insoluble Aβ plaques [8-11], occur earlier and also correlate positively with pathological tau proteins [12], which appear downstream during AD progression. Both soluble and insoluble Aβ species coexist in AD brains [5, 7, 13], with predominance gradually shifting from soluble AβOs to insoluble Aβ plaques with progression of the disease.

A potential difficulty in preventing irreversible brain degeneration in AD, even if effective drugs are developed, is the subtlety of the cognitive changes that occur early in the disease. At the time of diagnosis, AD is usually already at a mild to moderate stage. Current treatments cannot stop the progression of dementia, and therefore recent therapeutic approaches are shifting to early intervention aimed at halting neurodegeneration before irreversible damage accumulates [14]. This requires more sensitive diagnostic techniques. Since Aβ plaques and AβOs can be found in the brain before any cognitive changes [15-17], early AD diagnosis and management can be greatly aided by methods of detecting and monitoring Aβ pathology (including both insoluble and soluble Aβ species) *in vivo* [5, 8, 10, 18, 19].

Magnetic resonance imaging (MRI) offers superb spatial resolution and is widely available in clinical settings. In the past decade, MRI methods without exogenous Aβ-specific contrast agents have been developed by exploring high-resolution imaging and changes in tissue proton MR properties (such as T2 and T2*, proton density, magnetic susceptibility, magnetization transfer and T1rho) in transgenic mouse models of AD [18, 20-26]. However, these endogenous contrast-based MRI methods are indirect and generally lack the sensitivity for early Aβ plaque detection [18]. Only large and mature senile Aβ plaques could be visualized in transgenic AD mouse brains, and even those required a high magnetic field and very long scan time. Above all, such approaches lack specificity in the signal changes that may correspond to Aβ deposits [18]. Thus, it is imperative to develop MRI methods to visualize Aβ pathology directly. An exogenous MRI contrast agent that can specifically bind to Aβ species may make MRI a feasible tool for directly detecting Aβ pathology early in AD brains [4, 18].

We recently developed a novel curcumin-conjugated magnetic nanoparticles (Cur-MNPs) that can directly target the Aβ pathologies in AD brain [27]. Curcumin is a natural, safe product extracted from turmeric, and it possesses the ability to bind to Aβ species. Our previous *ex vivo* MRI and

immunohistological analyses indicated that Cur-MNPs could penetrate the BBB and bind to Aβ plaques in aged Tg2576 AD mice, and that MRI can reveal such Cur-MNPs targeted Aβ plaques [27]. In this study, we investigate the *in vivo* feasibility of this novel Cur-MNPs to detect Aβ pathologies at the early and late stages of AD in 5xFAD mice through molecular MRI, and validate the specific targeting of Cur-MNPs on various form of Aβ pathologies via immunohistochemical examinations.

II. METHODS

A. Animal Groups and Preparation

Animal experiments were approved by the Committee on the Use of Live Animal in Teaching and Research of the University of Hong Kong. Four groups of mice were used in this study: 8 old transgenic AD mice (5xFAD, 10-14 months old), 8 old wild type mice (C57BL/6J, 10-14 months old), 4 young transgenic AD mice (5xFAD, 4-5 months old) and 4 young wild type mice (C57BL/6J, 4-5 months old). The strain of 5xFAD was selected due to its quick Aβ pathologies progression. The Aβ pathologies of young and old 5xFAD mice were shown in Figure 1.

Superparamagnetic iron oxide (SPIO) based contrast agent, Cur-MNPs, were used to detect the Aβ pathologies. Cur-MNPs suspension was concentrated to 2.7 mg Fe/ml and immediately injected into the animals (10 μl/g) intravenously through the tail vein. Old mice underwent single injection 4 hours before MRI measurement, and young mice underwent multiple injections (three time points: 4.5, 3 and 1.5 hours before MRI measurement). During the MRI experiments, animals were anesthetized under 1.0-1.5% isoflurane mixed with 99% oxygen, and respiration rate and heart rate were monitored. Warm water was circulated to the MRI animal bed to avoid the occurrence of hypothermia during the scanning.

Figure 1. Immunohistochemically labeled β-amyloid (Aβ) of wild-type (C57BL/6J) mouse (left), young transgenic AD mouse (4-month-old 5xFAD; middle) and old transgenic Alzheimer's disease (AD) mouse (10-month-old 5xFAD; right) demonstrated the progression of Aβ pathologies in AD.

B. MRI Data Acquisition and Analysis

MRI data was acquired on a Bruker 7 T MRI scanner (PharmaScan 70/16, Bruker Biospin GmbH) using volume mouse brain coil. Scout images were first acquired to determine the coronal and sagittal planes of the brain. To detect the magnetic nanoparticles in the brain, T2*-weighted images were acquired by high-resolution 2D gradient recalled echo (GRE) sequence with TR/TE = 3000/12.3 ms, FA = 80.1°, bandwidth = 25 kHz, FOV = 25.6 mm × 25.6 mm, matrix size = 512 × 512, in-plane resolution = 50 μm × 50 μm, slice thickness = 0.38 mm

with 0.02 mm gap, 35 slices, two repetitions, and acquisition time of 51 min.

Two image datasets reconstructed from the two repetitions were realigned before averaging to reduce the effect of animal motion artifacts on high-resolution image quality.

C. Immunohistology

Animals were immediately perfused with saline and stored in Perfluoroalkoxy alkanes (PFA) overnight at 4 ºC. Brain tissues were sectioned at 40 μm thickness. The sections were double-stained with Anti-beta Amyloid 1-42 antibody (Aβ$_{1-42}$ in red) and Prussian blue (Iron in blue) or stained with oligomer A11 Polyclonal antibody (Aβ oligomers, infra-red under fluorescent view). Curcumin was examined under fluorescent view (excitation / emission = 488/510-590 nm).

III. RESULTS

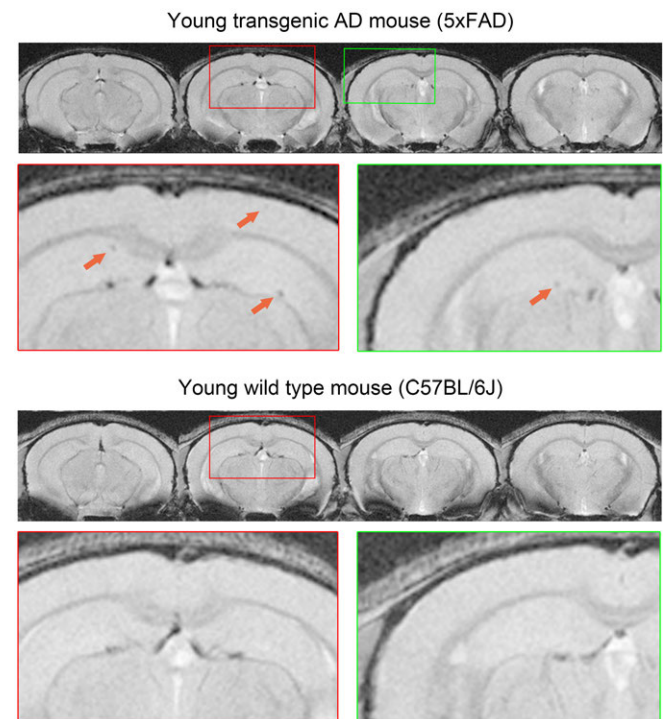### A. Aβ pathologies of AD at early stage visualized by molecular MRI

Figure 2. *In vivo* T2*-weighted images of a representative young 5xFAD mouse 4 hours after injected with Cur-MNPs (top), and the age-matched control (bottom). Iron-induced hypointense spots were observed in young 5xFAD mice brain (orange arrows), but not in the wild type controls.

Figure 2 presents the serial *in vivo* T2*-weighted images of a representative young 5xFAD mouse and age-matched wild type control 4 hours after intravenous Cur-MNPs injection. Hypointense spots were typically observed in the 5xFAD mice but not in the controls. These results directly indicate the capability of Cur-MNPs in detecting Aβ pathologies during the early stage of AD progression. Likely, Cur-MNPs here targeted AβOs rather

than Aβ plaques because the 5xFAD mice are known to predominantly exhibit AβOs at 4 months [28].

B. Aβ pathologies of AD at late stage visualized by molecular MRI

Old transgenic AD mouse (5xFAD)
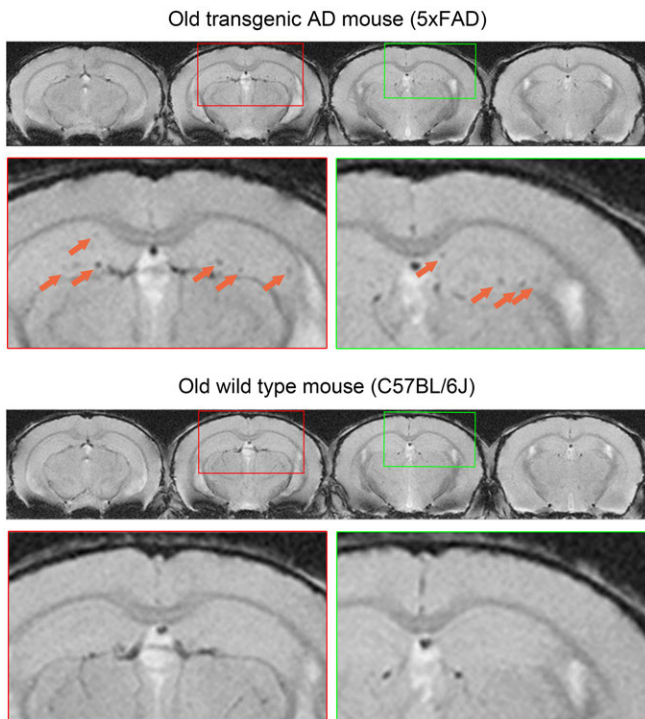

Old wild type mouse (C57BL/6J)

Figure 3. *In vivo* T2*-weighted images of a representative old 5xFAD mouse 4 hours after injected with Cur-MNPs (top), and the age-matched control (bottom). Iron-induced hypointense spots were only observed in old 5xFAD mice brain (orange arrows).

Figure 3 shows the *in vivo* T2*-weighted images of a representative old 5xFAD transgenic mouse and one control 4 hours after intravenous Cur-MNPs injection. Similar with the young mice, hypointense spots were only observed in the 5xFAD mouse but not in the wile type control. However, old 5xFAD mouse showed denser and larger hypointense spots than the young 5xFAD mouse, likely due to the significant increase of Aβ pathologies in old 5xFAD mouse brain, which is shown in Figure 1.

C. Histological validation of Cur-MNPs to target Aβ pathologies
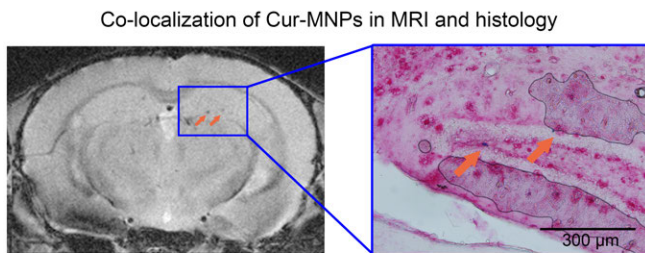
Co-localization of Cur-MNPs in MRI and histology

Figure 4. Co-localization of Cur-MNPs in MRI (left) and histology (right) in old 5xFAD mice. Hypointense spots in T2*-weighted image co-localized with iron, as shown in the corresponding bright-field image of immunohistochemically labeled Aβ pathologies and Prussian blue stained brain section (orange arrows). Iron was presented in blue, and Aβ pathologies were presented in red.
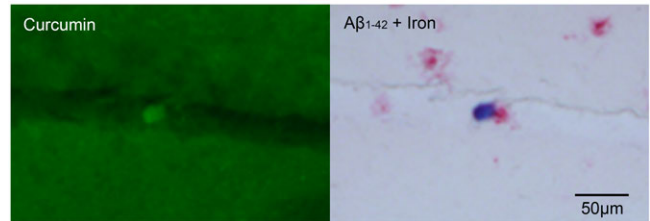
Enlarged view of co-localization of Aβ and Cur-MNPs

Figure 5. Co-localization of curcumin (green under fluorescent view, left) and iron (blue under bright-field view, right), which was bound to the Aβ pathologies (red under bright-field view, right).

To validate the targeting of Cur-MNPs on Aβ pathologies, double staining of Aβ pathologies and iron in old 5xFAD mice was performed. MRI and histology results confirmed that some of the hypointense spots induced by Cur-MNPs in T2*-weighted image (Figure 4, left) were co-localized with iron in corresponding 5xFAD mouse brain sections (Figure 4, right). Furthermore, the co-localization of curcumin (green under fluorescent view) and iron (blue under bright-field view) were shown to bind to Aβ1-42 (red under fluorescent and bright-field view), revealing that Cur-MNPs targeted the Aβ pathologies (Figure 5). These images demonstrate that Cur-MNPs can bind to Aβ pathologies.
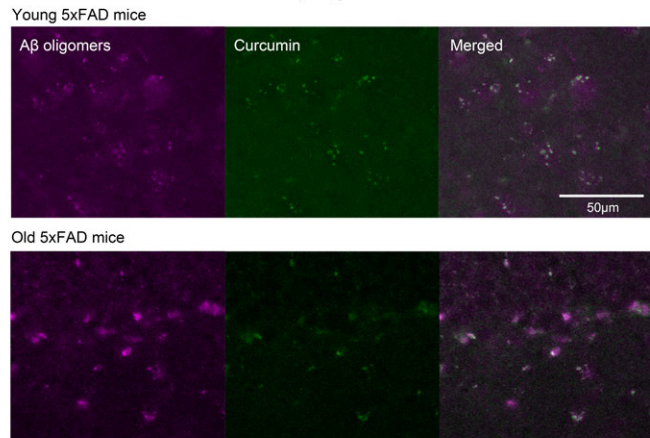
Co-localization of Aβ oligomers and Cur-MNPs

Figure 6. Co-localization of immunohistochemically labeled Aβ oligomers (AβOs) and Cur-MNPs in the hippocampus of young and old 5xFAD mice. This indicates the ability of Cur-MNPs to target the AβOs at the early and late stages of Alzheimer's disease.

To investigate whether the Cur-MNPs could specifically target the Aβ oligomers, we further stained for AβOs in young and old 5xFAD mice. As shown in Figure 6, the co-localization of AβOs (magenta) and curcumin (green) in the hippocampus of young and old 5xFAD mice indicated the ability of Cur-MNPs to target the AβOs at early and late stages of AD.

IV. DISCUSSION

This study demonstrated that our novel MRI contrast agent, Cur-MNPs, could target the Aβ pathologies, including Aβ plaques and AβOs, at both early and late stages of AD by *in vivo* MRI measurements. Four months old 5xFAD mice are known to predominantly exhibit AβOs with little signs of cognitive decline [28]. It is likely that

Cur-MNPs targeted more AβOs than Aβ plaques in young 5xFAD mice. In addition, our *in vivo* findings indicated that old mice exhibit more Aβ plaques and AβOs than young mice, consistent with our histology results (Figure 1) and other AD literature [28]. Moreover, the hypointense spots were mainly concentrated in the hippocampus, no matter young or old 5xFAD mice. This observation could be due to the fact that Aβ is first to aggregate in the hippocampus and the hippocampus has the most Aβ deposition in AD brain [28, 29]. Thus, our MRI and histology findings strongly support that our proposed molecular MRI approach is capable of detecting AD at both early and late stages. This approach will be extremely valuable in preclinical AD research and drug development. More importantly, it holds tremendous potential in MRI detection and monitoring of AD progression in patient diagnosis and/or during therapeutic intervention.

V. CONCLUSION

The present study shows that the curcumin-conjugated magnetic nanoparticles, Cur-MNPs, could target Aβ pathologies at early and late stages of AD progression, and be visualized by our molecular MRI approach. Further, our immunohistological results validate the specific targeting of Aβ pathologies, indicating that Cur-MNPs are able to target not only the Aβ plaques, but also the Aβ oligomers at the early stage of AD progression. Taken together, it presents a powerful imaging approach in our pursuit of AD early diagnosis and drug development.

ACKNOWLEDGMENT

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