In vivo magnetic resonance imaging and spectroscopy of Alzheimer__s disease in transgenic mice

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6 General discussion and future outlook*

In this thesis the potential applications of high resolution MR microimaging and spectroscopy to study Alzheimer's disease pathology in transgenic mouse models were explored. The focus was both on the μMRI and the 2D MRS method optimization, as well as their application to visualize and understand the mechanism of AD pathology *in vivo*.

6.1 Visualization of AD hallmarks: amyloid plaques and beyond

The visualization of Aβ plaque deposition in brain, a key feature of Alzheimer's disease, is important for the evaluation of disease progression and the efficacy of therapeutic interventions. In Chapter 3, it was shown that application of the RARE sequence at 9.4T allowed plaques visualization without contrast agents in a scan time as short as 25 min with an in-plane resolution of 78 μm. The straightforward implementation of a T_2-weighted RARE sequence combined with high magnetic field strength provided sufficient resolution for clearly visualizing plaques in living mouse brain.

The basis for intrinsic MRI contrast between individual plaques and normal background tissue is not fully understood. It is presumed that the hydrophobic nature of amyloid deposits, or age related accumulation of iron within the plaques, can have an effect on the T_2 and T_2* relaxation rates in and adjacent to plaques (1,2). Very recently, El Tannir El Tayara *et al.* (2) have shown that T_2 relaxation can be affected by plaque deposition in the absence of histochemically detectable iron. House *et al.* (3) found no correlation between AD plaque density and iron concentration. The distribution of iron in the histological sections of the Tg2576 mouse brain was examined in Chapter 3. Iron was found to be associated with many dense-cored senile plaques, though not all contained iron. This suggests that the intrinsic MR contrast from Aβ plaques may be partly caused by iron, although signal hypointensities arising from the reduced water content in Aβ plaques compared to the surrounding tissue and from other unknown factors cannot be ruled out. Amyloid plaques in AD brains can be circumscript plaques with a dense core or more diffuse plaques. Dense-core plaques are fibrillar deposits of Aβ, showing all the classical properties of amyloid including β-sheet secondary structure, while diffuse plaques are amorphous deposits of Aβ that lack a core (4). It is still an open question if iron is

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associated with dense cored plaques, diffuse core plaques, or both. In addition, it is not yet clear if MRI techniques for the visualization of amyloid plaques are more specifically detecting dense core plaques or diffuse plaques. This aspect warrants further research efforts. It will require the improvement of resolution and specificity of available MRI techniques to distinguish between dense-core and diffuse plaques.

In Chapter 3 it was shown that μMRI can be successfully used for longitudinal studies monitoring the development of Aβ plaques with age in the same animals. The same Tg2576 mice were imaged at regular intervals, which showed a marked age-dependent increase in amyloid deposition in the hippocampus and cortex. This longitudinal study has shown that, using μMRI, a genuine map of plaque deposition can be made as the disease progresses in a living subject. Such a map may subsequently be used to assess the efficacy of putative AD treatment strategies. In addition, using this approach interesting biological issues could be non-invasively addressed; for example, does a particular therapeutic approach arrest plaque growth, shrink plaques, or prevent development of new plaques? Is the effect of a therapeutic a uniform response of all plaques, or are there significant plaque to plaque variations?

Further improvements will be required in the field of MR based amyloid imaging, such as higher specificity of plaque detection, improved resolution of MR images, and a reduction of the required measurement time. Specificity may be increased by the development of safe MR contrast agents that cross the blood-brain barrier and selectively bind Aβ plaques. Improved resolution and reduced measurement times may be achieved by exploiting the improved SNR granted by high magnetic fields. For studies in mice, moving from 9.4T to 17.6T will be beneficial. Very recently Faber *et al.* published the first ultra-high field (17.6T) study of *in vivo* plaque visualization in transgenic AD mice (5). Due to the increased field strength, and the corresponding increase in SNR, it was possible to visualize plaques *in vivo* in the thalamus region with an isotropic resolution of 94×94×94 μm in 34 minutes. The high magnetic field strength also permitted *in vivo* detection of plaques with a lower resolution of 94×234×234 μm in a scan time of only 82 seconds using a gradient echo sequence. At high magnetic field strength, the dipole field of iron in the plaques causes strong signal dephasing in a region larger than the actual size of a plaque, which facilitates detection. Short scan times provide new opportunities for MR in Alzheimer research. For example, rapid scan protocols could be used for high throughput screening of a large number of AD transgenic mice treated with new drugs. However, *in vivo* visualization of plaques in the cortex and hippocampus, which are the most affected areas in human AD patients, has not yet been successful at high field.

For increased specificity in amyloid detection, ultimately molecular imaging will be the method of choice. This approach requires the administering of a suitable contrast agent. Thus far, contrast agent based MR approaches for *in vivo* plaque visualization in transgenic mice have been used in only two studies. The first reported study was by Wadghiri *et al.* in 2003 (6). Relying on the observation that the Aβ peptide avidly binds to amyloid plaques, they developed an effective MRI sensitive plaque ligand by attaching either gadolinium or iron nanoparticles to Aβ. Although the results of this study were convincing, the ligand does not spontaneously pass through the blood-brain barrier, and co-administration of mannitol was required for the ligand to gain access to the brain. Due to the neurotoxicity of Aβ and the requirement for mannitol injection, these methods may have limited utility in longitudinal studies in mice. A subsequent study was reported by Higuchi *et al.* in 2005 (7). They used Congo Red-based amyloidophilic compounds that were labeled with ^{19}F. The authors showed that when this ligand is injected intravenously into living transgenic mice, it successfully crosses the blood-brain barrier and binds amyloid plaques, which can subsequently be detected with ^{19}F-MRI. They proposed that this approach can be used for longitudinal studies in mouse models of AD (7). It is interesting to note that ^{19}F-containing ligands are not specific to only amyloid plaques, as they can bind neurofibrillary tangles and perhaps even Lewy bodies or other β-sheet inclusions as well (7). Sensitivity of ligands to both amyloid plaques and neurofibrillary tangles may be diagnostically relevant, since both are histological hallmarks of AD. Prospective contrast agents for visualizing and quantifying the pathologic burden of β-amyloid plaque should be highly stable *in vivo*, cross the blood-brain barrier non-destructively following intravenous injection, bind specifically to plaques with high affinity, produce local changes in tissue contrast detectable by MRI, and require lower dosages than current contrast agents (8,9).

6.2 2D MRS applications in AD

In Chapter 4, it was shown that the application of localized 2D MRS in mouse brain is possible, and that a large number of metabolites can be uniquely and reproducibly detected using 2D methods. Subsequently, in Chapter 5, the L-COSY method was applied to study the effects of AD on the neurochemical profile of aging Tg2576 mice. Specifically, it was found that the membrane phospholipid breakdown product GPC increases concurrently with the increasing plaque-load in aging Tg2576 mice. Unlike 1D MRS techniques, localized 2D MRS had not been reported in mouse brain until now, and resolving strongly overlapping signals from different metabolites required the use of

spectral editing techniques (10,11) or the application of spectral analysis software such as LCmodel (12). The drawback of spectral editing is that only a single metabolite of interest is targeted per measurement, and for current analysis software, only metabolites in the analysis database are measured. As seen in Chapters 4 and 5, 2D MRS provides a method whereby metabolites overlapping in 1D may be distinguished and uniquely assigned. As described in Chapter 5, glycerophosphocholine appears to be a potential biomarker for the onset and progression of AD. The *in vivo* changes in GPC levels in mice should be monitored in other AD mouse models as well. In addition, changes in GPC levels in human brain may be determined by implementation of 2D MRS on high field (≥ 7T) clinical scanners.

As with MR imaging, several advantages are expected in MR spectroscopy when moving to high magnetic field (13,14). Spectroscopy at high field strengths is enhanced by the increase in SNR and by improved spatial resolution. Finally, increased spectral dispersion will provide more reliable quantification and additional sensitivity gains (13). I made some initial efforts to implement MRS on a Bruker 17.6T (750MHz) vertical bore system fitted with 1T/m gradients, and a 20mm birdcage volume transmit/receive coil. Measurements in a brain phantom yielded a linewidth of ~7 Hz, or 0.0175 ppm, at 9.4T. At 17.6T, the linewidth obtained from the same sample, with the same scan settings, was ~10 Hz, or 0.0133 ppm, which is an improvement of approximately 30%. With further optimization of the scan parameters a greater improvement may be obtained. These preliminary findings are hopeful for future 2D MRS experiments at 17.6T, although further optimization is still required. Thus far MRS at 17.6T has only been tested successfully in phantom solutions. It is expected that the NMR lines in brain or other inhomogeneous samples will be broader than in the phantom. In addition, tissue-inhomogeneity induced susceptibility effects due to the increasing field strength may cause an additional increase in linewidth (15). As reported by Fleysher *et al.* (15), first and second order shimming may not be sufficient to compensate for this effect. Reducing the voxel size can help to limit the linebroadening due to tissue-inhomogeneity (15). It is expected that these limitations. may be less relevant for small animal scanners than for clinical scanners; shimming and gradient systems for small-animal scanners are considerably stronger than systems available on clinical scanners, and allow manual shimming of first, second, and higher order shims, in addition to automatic shimming of the first and second order shims. Efforts to further optimize one- and two-dimensional MRS at 17.6T may thus prove beneficial to the future study of AD.

6.3 Potential challenges for the translation to humans

As the field strengths of clinical scanners are approaching those of small-animal scanners, while Aβ plaques in mice and humans are quite similar in size, from 20 up to 200 μm (16), the exciting question is obvious: Can amyloid plaques be detected *in vivo* in man using MRI sequences that can be realistically applied in a clinical setting? In principle, the *ex vivo* MRI studies in post-mortem human tissue (1,17) and *in vivo* studies in mice (5-7,18-22) have set the stage for further technical innovation to extend amyloid imaging to living human subjects. However, several practical hurdles remain before this can be achieved. Despite the long imaging times and the use of very high field MR systems for plaque visualization in mice, MRI is a relatively insensitive technique. This suggests that MRI hardware and software need further improvement in terms of sensitivity to obtain sufficient contrast-to-noise ratios and resolution in humans in a much shorter imaging time. The optimization of MRI protocols for plaque detection will be difficult due to the uncertainty of the presence of amyloid plaques in suspected Alzheimer patients. Movement artifacts are likely to be a problem; in animal studies, the subjects are anaesthetized during measurement. This cannot be done in human studies. Consequently, patients remain conscious and are required to remain immobile. This may not be a problem for early detection of plaques in patients that do not yet have disease symptoms; however, it may be difficult for patients with advanced Alzheimer's disease to keep still even with a scan duration of only a few minutes. In addition, the specificity of the technique needs to be improved to differentiate Aβ plaques from other structures with a similar appearance on MRI, such as blood vessels and small hemorrhages. Specificity may be increased by the development of safe MR contrast agents that cross the blood-brain barrier and selectively bind and enhance Aβ plaques in MRI. The required doses of contrast agents must be decreased. Currently, PET-based ligands can be administered in the picomolar range or less, whereas MRI-based ligands need to be administered in the micromolar range (8).

With the advent of ultra-high field (≥ 7T) MR in clinical settings, high resolution one- and two-dimensional MRS in humans is becoming feasible as well. As demonstrated previously by Thomas *et al.* (23), an increase in field strength from 1.5 to 3T is beneficial for the reliable detection of metabolites using 2D MRS. A further increase in field strength, from 3T to 7T, is expected to yield similar improvements in spectral dispersion. Keeping in mind the limitations set forth by Fleysher *et al.* (15), who noted that as B_0 rises, the consequent increase in linewidths counteracts the chemical shift dispersion and leads to the overlap of the components of *J*-coupled multiplets (24), the voxel size may

need to be reduced to prevent an increased linewidth due to macroscopic inhomogeneity (15). While localized two-dimensional MRS has yet to be applied in high field clinical scanners, several groups have already successfully applied conventional 1D MRS using 7T clinical scanners, and reported a significant improvement in results compared to studies at lower fields (25,26).

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