About the origins of enhanced visualization of amyloid plaques on APP/PS1 mouse brain using GdDOTA passive staining

B. Marty[1], J. Flament[1], J. Valette[1], F. Boumezbeur[1], M. Dhenain[1], F. Lethimonnier[1], and S. Mériaux[1]

[1]Neurospin, I2BM, Commisariat à l'Energie Atomique, Gif-sur-Yvette, France

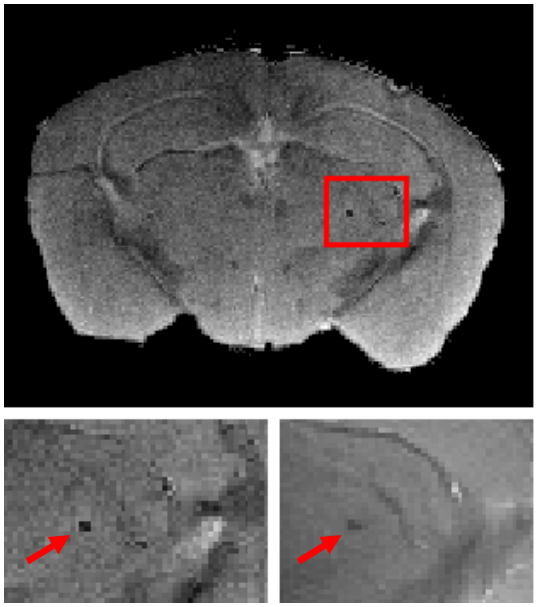

Fig.1 T_2^*-weighted µMRI of APP/PS1 transgenic mouse brain exhibiting amyloid plaques zoomed in (bottom left) along with the corresponding phase image (bottom right).

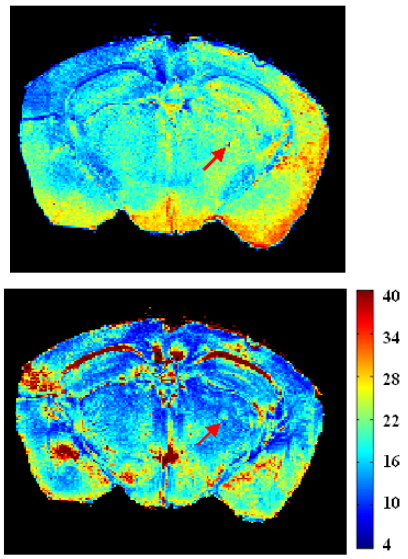

Fig.2. Proton density map after 3 days of passive staining (top, arbitrary unit) and T_2* parametric map (bottom, in ms).

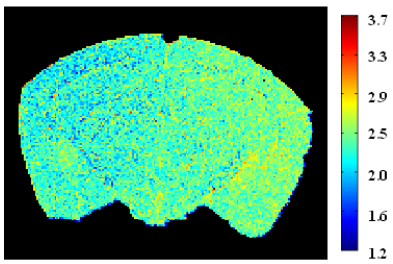

Fig.3. Concentration map of DOTAREM (in mM). The mean concentration over the brain is 2.3±0.4 mM.

Introduction

T_1-relaxating contrast agent (e.g. GdDOTA) "passive staining" is a smart strategy to enhance signal-to-noise ratio (SNR) for MRI microscopy (µMRI) of *ex vivo* tissue samples. The principle of the method is to immerse organs (e.g. brains) in a solution of GdDOTA and let it diffuse for days in order to reduce the T_1 and further reduce the optimum relaxation time (TR) and accordingly the acquisition time. Such strategy has been adopted successfully to detect amyloid plaques in high-resolution T_2^*-weighted images of APP/PS1 transgenic mice brain [1]. Nevertheless, mechanisms of this contrast enhancement of amyloid plaques were not thoughtfully investigated. In the present study, we have carried such investigation and established how GdDOTA is diffusing throuh tissues during passive staining and what the consequences are for the contrast of amyloid plaques vs. healthy brain tissue.

Materials and Methods

Passive staining protocol. A transgenic APP/PS1 mouse was sacrificed and its brain was immersed first in a solution containing 4% buffered formalin (Sigma, St Louis, MO, USA) for control imaging and later in a mixture of 4% buffered formalin and 2.5mM GdDOTA (DOTAREM, Guerbet, Roissy, France) during 4 days according to a protocol previously established [1]. For imaging, the brain was placed in a perfluorinated fluid (Fluorinert FC-40, 3M, St. Paul, MN, USA).

Relaxivity of DOTAREM. r_1 and r_2 relaxivities of GdDOTA were measured *in vitro* in a 6-tubes phantom each containing GdDOTA at various concentrations (0, 0.2, 0.5, 1.25, 2.5 and 5 mM) at T° = 20°C in a low-gelling point 4.5% agarose matrix.

MRI acquisitions. Experiments were realized on a 7T small animal MRI scanner (Bruker PharmaScan, Ettlingen, Germany) using a 2.6-cm-diameter bird-cage ^{1}H coil for acquisition and reception. T_1 maps were acquired with a high resolution IR-SE sequence (TE/TR=8/1000ms, TI=0, 60, 100, 200, 300, 500ms, resolution 60x60x120 µm). T_2 maps were acquired with a MSME sequence (TR=1100ms, TE=8, 16, 24.5, 33, 41, 49, 57, 65ms, resolution 60x60x120 µm). T_2* maps were obtained with a GE sequence (TR=1000ms, TE=4, 8, 12, 16, 20, 24, 28, 32ms, resolution 60x60x120 µm).

Maps generation. Parametric T_1, T_2, T_2^* and proton density maps were generated using a pixel by pixel fitting procedure written in Matlab (The MathWorks Inc, Natick, MA, USA). Concentration maps were calculated from T_1 maps using the measured relaxivity $r_1 = 3.4\ mM^{-1}.s^{-1}$ of GdDOTA under the hypothesis that the relaxivity hardly differ between the agarose matrix and the formalin-fixed brain tissue [2].

Results and Discussion

After 4 days of passive staining, the contrast agent seems to have diffused throughout the whole brain (Fig.3), which is confirmed by the homogeneity of the concentration map and the mean estimated concentration (2.3±0.4 mM) being close to the 2.5 mM of the solution concentration of GdDOTA. On anatomical T_2^*-weighted images, some amyloid plaques can be observed (Fig.1): this confirms the great potential of T_2^*-weighted sequences for amyloid plaques detection because they take advantage of the susceptibility effect of iron frequently trapped in this kind of plaques. As already reported [3], the presence of amyloid plaques is confirmed by an intense phase shift also due to the susceptibility effect (Fig.1 bottom left). On the proton density map (Fig.2, top), the amyloid plaques appear as a hypointense signal (red arrow), which is consistent with their hydrophilic aspect. By placing one region of interest on the detected amyloid plaques and one region of interest in the surrounding healthy tissue, the CNR (Contrast to Noise Ratio) is found to be 16.8% on the proton density map and 26.3% on the corresponding T_2* parametric map (Fig.2, bottom), whereas the CNR reaches 43.8% on the T_2^*-weighted image shown Fig.1. It appears that the contrast observed on the T_2^*-weighted images is a combination of susceptibility effect and poor proton density. Note that no amyloid plaque was detected on the T_1 and T_2 parametric maps (results not shown) probably because of the whole brain GdDOTA diffusion which strongly decreases T_1 and T_2 contrasts.

Conclusion

In this study, we shown that in experiences of passive staining for observing amyloid plaques, T_2* sequences are well adapted. They are more sensitive to susceptibility effects due to iron, but the contrast observed is also due to proton density differences between healthy tissues and amyloid plaques. The following step will be to determine an optimized passive staining protocol (GdDOTA concentration, duration of immersion, sequence parameters) which will lead to greater CNR and an enhanced capability of amyloid plaques detection.

Acknowledgments

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References

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