Indirect sensitive MR detection of Aβ plaques with USPIO in Alzheimer transgenic mice

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Introduction

High field (>7T) and high resolution (<60µm) are needed in MR imaging for direct Aβ plaques visualization in Alzheimer disease [1]. USPIO-enhanced approach allows an increase of sensitivity and was evaluated in animal experiments and clinical studies as MRI markers for the diagnostic of neuroinflammatory disease associated with high macrophage phagocytic activity like multiple sclerosis [2,3]. It is known that activated microglia may play a role in the pathogenesis of AD as they cluster around Aβ plaques [4]. In this context, the aim of this study was to evaluate the potentiality of an USPIO to increase sensitivity to indirectly detect, via microglial phagocytic activity, Aβ plaques, in transgenic mice.

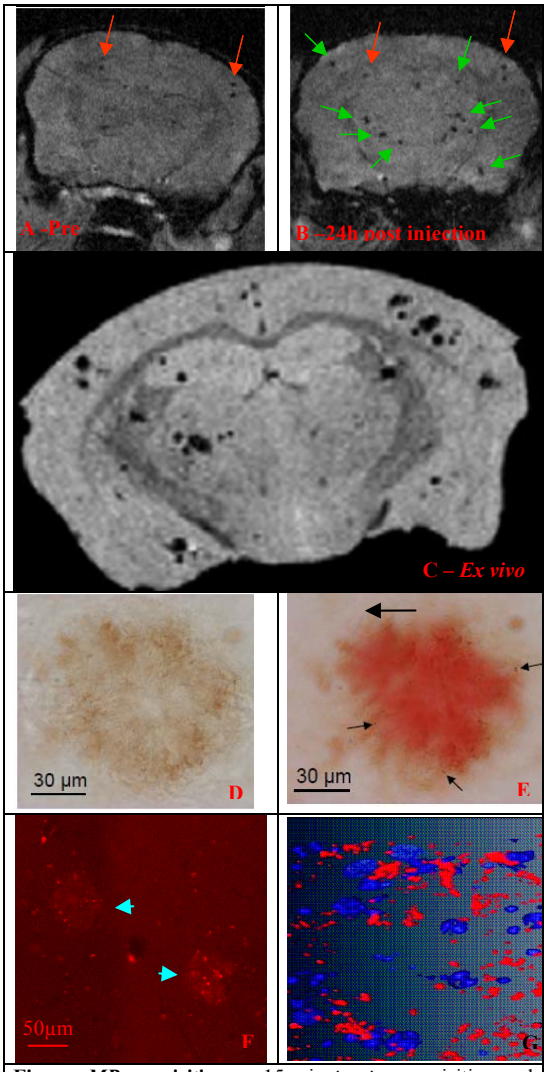

Figure :***MR acquisitions*** : 15 min *in vivo* acquisition and iron accumulation in mice brain, before (A / red arrows) and 24h after P904 injection (B / green arrows); *ex vivo* MRI (C); ***On histological slices*** : iron and activated microglia staining (D); activated microglia (brown), Aβ plaques (red) and iron (arrows) staining (E); P904 fluorescence (F / blue head arrows); fluorescence (red) and nucleus (G / blue)

Material & Methods

Alzheimer model *:* Eight mice, Tg2576 (19-24 month-old, n=2) and APP/PS1dE9 (15-20 month-old, n=6), were realized.
Contrast agent : P904 [Guerbet Research] was iv administrated (1000 µmol. Fe/kg). Rhodamine was added to P904 for fluorescence.
MR acquisitions *:* T2*w GE/SWI sequences were carried out on a 7T Pharmascan (*in vivo*, pre and 24h post injection) and a 2.35T BioSpec (*ex vivo*) scanners [Bruker, Ettlingen, Germany]. Parameters were 10/75ms TE/TR, 17° flip angle, 78*80*220µm, 15min acquisition time for *in vivo* imaging and 10-24/75ms TE/TR, 17° flip angle, 78*80*100µm, 8h acquisition time for *ex vivo* experiment.
Histological slices *:* The histological sections were selected from the MR images most relevant to achieving nearly colocalization of MR images and histological staining of microglia (CD45), iron (Perl's), Aβ plaques (Congo Red), nucleus (DAPI) and Rhodamine fluorescence.

Results

On MR acquisitions :

- Post P904 injection, several susceptibility artefacts were observed as focal spots all over the mouse brain (green arrows, B) whereas only limited ones were noticed before injection probably due the presence of endogenous iron deposit in large amyloid plaques(red arrows, A).
- The sensitivity of USPIO detection is increased on the ex vivo MR acquisitions (C) as hyposignal diameters range from 100 µm (pixel size) to 500 µm (clinical resolution).
- Tg2576 transgenic mice present a higher response compared to APP/PS1dE9.

On histological slices :

- Iron staining was visible as small granules inside or in neighborhood of plaque and CD45 positive staining (D)
- Activated microglia cells were seen in and around plaque-like structures which were always positive also for Congo Red indicating heavy amyloid load in the plaques (E).
- Fluorescence was observed in same areas than microglia and aβ plaques staining, with similar spherical distribution (F).
- P904 (fluorescence) is colocalized to nucleus (DAPI) (G)
- Large variability is observed between mice. APP/PS1dE9 transgenic mice present a higher staining.

Conclusion

Histological analyzes show the localization of both iron nanoparticles and fluorescence of the P904 inside and in the neighborhood of Aβ plaques, in activated microglia cells which match with the low signal areas (cortex, hippocampus, thalamus) in MR images. However, due to slice thickness differences between MR (100-200µm) and histological slices (20µm) and signal exportation form slice to slice, no correlation was observed between numbers of MR hyposignals and degree of target staining.

P904 can penetrate into the central nervous system either via transport mechanism of macrophages and/or microglia or with some other mechanism such as transcytosis and finally ending to the plaques. These results suggest that P904 could be a very sensitive tool for Alzheimer disease diagnostic and prognostic for therapeutic monitoring.

References

[1] Dhenain et al., NMR in Bimed (2007), 15:197-203.
[2] Dousset et al., AJNR (1999), 20:223-7.
[3] Corot et al., Invest Radiol (2004), 39:619-25.
[4] Arends et al., Neurobiol. Aging. (2000), 21:39-47