T1 and T2 quantification of free USPIO and USPIO-labeled macrophages at 4.7T and 7T.

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Rationales and objectives: Non-invasive monitoring of activated macrophages using ultrasmall superparamagnetic particles of iron oxide (USPIO)-enhanced MRI is a promising tool for the study of inflammation [1]. Most experimental works using this approach have been performed in rats and mice at high field in order to improve spatial resolution. However, T1 relaxivity of clinically-approved USPIO such as Ferumoxtran-10 is known to vanish with increasing field strength [1], while the impact of macrophage internalization of USPIO on T1 and T2 effects still need to be quantified for this contrast agent at high field. In this context, our aim was to quantify the effects of compartmentalization of the USPIO Ferumoxtran-10 in bone-marrow derived macrophages on T1 and T2 relaxivities at 4.7T and 7T.

Materials and Methods: Four phantoms of free USPIO were prepared in agarose gel at 0.75% w/w with concentrations of [0 5 10 15] mg $Fe.l^{-1}$, in order to obtain the same range of T2 changes as encountered *in vivo* [2]. Phagocytic progenitor cells were derived from cell culture of murine bone marrow during 7 days. These cells were incubated with USPIO (Ferumoxtran-10, Guerbet, France) at [Fe]medium= 0.5 g $Fe.l^{-1}$ for 36 hours. Four phantoms of USPIO-labeled cells and 4 control phantoms of non-labeled cells were prepared in agarose gel at 0.75% w/w with increasing number of cells: [$3.6x10^6$ $7x10^6$ $12x10^6$ $16x10^6$]. T1 quantification was achieved using a spin echo sequence with the following parameters TE/TR(ms): 15/[190 470 875 1500 5000] at 4.7T and 15/[175 445 775 1200 1800 2800 7500] at 7T. T2 quantification was achieved using a multi-echoes sequence with the following parameters TE(interecho delay)/TR(ms): 15/4000, 35 echoes, at 4.7T and 12/4000, 35 echoes, at 7T. T1, respectively T2, were calculated in ROIs using a non-linear least-square curve fitting, with the following functions: SI(TR)=So.(1-exp(-TR/T1), respectively SI(TE)=So.exp (-TE/T2). T1 and T2 relaxivities (r1 and r2) were evaluated from linear regression, assuming $\Delta Ri = ri \times concentration$, where $\Delta Ri = 1/Ti(iron) - 1/Ti(no\ iron)$ and i = 1 or 2. After completion of the last MRI experiment, the phantoms iron concentrations were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Results: The average iron load per cell deduced from ICP-AES measurements was 0.52 pg, resulting in iron concentrations of [3 6 9 13.5] mg Fe/l in phantoms of USPIO-labeled cells. Phantoms of free USPIO showed a linear relationship between R2 and iron concentration on the one hand and between R1 and iron concentration on the other hand, with relaxivity values in agreement with the literature [1]: r1=2.9 $s^{-1}.mM^{-1}$, r2=85 $s^{-1}.mM^{-1}$, at 4.7T and r1=1.6 $s^{-1}.mM^{-1}$, r2=88 $s^{-1}.mM^{-1}$,at 7T (Figure 1). Phantoms of USPIO-labeled cells also showed a linear relationship between iron concentration and R1 and between iron concentration and R2, with lower r1 and r2 compared to free USPIO: r1=1.1 $s^{-1}.mM^{-1}$, r2=57 $s^{-1}.mM^{-1}$ at 4.7T and r1=0.5 $s^{-1}.mM^{-1}$and r2=40 $s^{-1}.mM^{-1}$ at 7T (Figure 1).

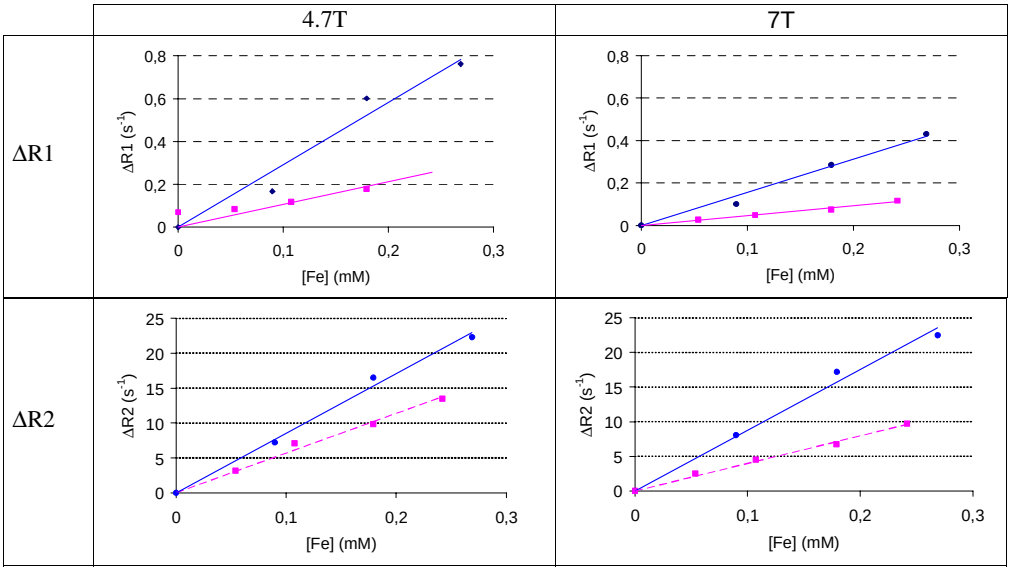

Figure 1- *ΔR1 and ΔR2 of free USPIO (●) and Labeled phagocytic cells (■) at 4.7T and 7T*

Conclusion: There was little difference in T2 relaxivities of free USPIO between 4.7T and 7T, while T1 relaxivity was strongly attenuated at 7T. Internalization of USPIO by macrophages resulted in a decrease of T1 and T2 effects of the iron particles, in accordance with results reported at clinical fields [3]. T1 and T2 relaxivities of USPIO-labeled cells were lower at 7T than at 4.7T. Taken together, these results suggest that working at 4.7T may be a good compromise between sufficient spatial resolution achievable to image small animal brains and USPIO relaxivity properties. In the future, we are planning to develop MR sequences allowing the non-ambiguous discrimination of free USPIO versus USPIO-labeled cells at 4.7T, and to evaluate the relevance of using these calibration curves for iron quantification *post-mortem* and *in vivo* .

References :

[1]Adv Drug Deliv Rev 2006;58(14):1471-1504. [2] Stroke 2007;38(1):131-137. [3]Eur Radiol 2006;16(3):738-745.