In vivo assessment of the number of iron-labeled cells using T2 quantification

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Rationales and objectives:

In recent years, numerous studies have been published on the use of different contrast agents for MRI assessment of macrophage infiltration in inflammatory diseases [1]. Quantitative estimate of iron-labeled cells is highly desirable in such studies, as it could allow to monitor the effects of anti-inflammatory drugs. *In-vitro* studies performed at 7T [2] have shown that there was a linear relationship between iron concentration and 1/T2 for concentrations < 0.36 mM, both for Ferumoxtran-10 (Guerbet, France), a widely used contrast agent, and for AMNP, a new class of anionic nanoparticles (CNRS UMR 7612, France) [3]. In this study, we tested the hypothesis that T2 quantification could be used *in vivo* to estimate the number of iron-labeled cells stereotaxically injected into the brains of mice.

Materials and Methods:

Bone marrow-derived macrophages were incubated in the presence of Ferumoxtran-10 ([Fe]=36-mM) for 36h, or AMNP ([Fe]=1-mM) for 12h [2]. Five healthy C57/Bl6 mice were stereotaxically injected in the striatum with 3-µl saline solution containing either cells (left hemisphere) or free contrast agent (right hemisphere) with the same iron concentration, as detailed in Table 1. MRI was performed on a Bruker Biospec 7T/12-cm magnet, using coronal gradient-echo imaging (TE/TR 3.6/161-ms, flip angle 50°), coronal T2-weighted spin-echo imaging (TE/TR 75/3000-ms), and axial multi-slice multi-echo imaging (MSME) for T2 quantification (TE(interecho delay)/TR 12/4000-ms, 35 echoes). For all exams, the field-of-view was 2x2-cm^2 and the matrix 256x256. Two regions of interest were drawned on axial MSME images: one in the region containing iron ([Fe]) and one in the adjacent region ([Fe] = 0). Iron concentration was estimated as: [Fe] = (1/T2([Fe]) - 1/T2([Fe]=0))/r2, where r2 is the transverse relaxivity previously estimated from *in-vitro* experiments: r2 = 64 $s^{-1}.mM^{-1}$ for Ferumoxtran-10-labeled cells and r2 = 35 $s^{-1}.mM^{-1}$ for AMNP-labeled cells [2]. The number of cells was then estimated using a mean iron load per cell of 0.5 pg for Ferumoxtran-10 and 8 pg for AMNP [2]

Results:

Iron-labeled cells were clearly detected on coronal images, with a typical "arrowhead" artifact on spin-echo images (Fig 1A, dashed arrow) and "clover" artifact on gradient-echo images (Fig 1B, dashed arrow). These effects were due to iron and not to cells, as unlabeled cells did not produce any signal. At the site where free USPIO were injected, coronal and axial T2 images showed a rapidly spreading hypointense signal (Fig 1A, plain arrow), while gradient-echo images showed a faint hyposignal (Fig 1B, plain arrow). Table 1 presents iron concentrations and corresponding number of cells estimated from T2 measurements.

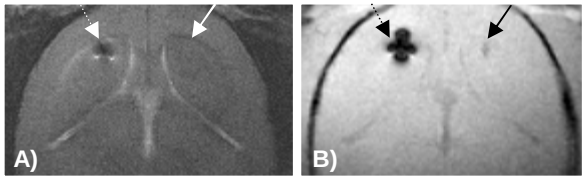

Fig. 1: Coronal T2-weighted spin-echo (A) and gradient-echo (B) images from mouse #3. Note the artifacts in the left hemisphere, where Ferumoxtran-10-labeled cells were injected (dashed arrows), and the spread of hypointense signal along the corpus callosum in the right hemisphere (A, plain arrow), on the side where free USPIOs were injected (B, plain arrow).

Contrast agent	Cell number	Corresponding [Fe] (mM)	Estimated [Fe] (mM)	Estimated cell number
#1-No (control cells)	45,000	0	--	--
#2-Ferumoxtran-10	45,000	0.12	0.16	59,520
#3-Ferumoxtran-10	120,000	0.32	0.29	107,880
#4-AMNP	7,500	0.35	0.52	11,019
#5-AMNP	7,500	0.35	0.41	8,688

Table 1- Injection protocol (gray) and results obtained for each of the 5 stereotaxically-injected mice. "Cell number" corresponds to the number of injected cells, as counted using a Malassez cell, [Fe]: iron concentration.

Discussion and Conclusion:

There was an overall fair agreement between the number of injected cells and the number of cells estimated from T2 measurements. Further studies are needed to assess the potential of this approach to estimate smaller number of cells, as expected in cell tracking studies.

References :

[1] Corot C. et al. Adv Drug Deliv Rev 2006;58(14):1471-1504. [2] Brisset J-C. et al. 16th annual meeting of the ISMRM, Toronto (Canada) 3-9 may 2008 #1687. [3] Wilhelm C. et al. Biomaterials 2003;24(6):1001-1011.