Validation of Magnetoliposomes as MR contrast agents for in situ labeling of endogenous neuronal progenitor cells in the mouse brain

R. Vreys[1], C. Peleman[2], M. Geraerts[3], M. De Cuyper[2], Z. Debyser[3], V. Baekelandt[4], A. Van der Linden[1]

[1]Bio-Imaging Lab, University of Antwerp, Antwerp, Belgium, [2]Interdisciplinary Research Centre, KULAK, Kortrijk, Belgium, [3]Molecular Virology and Gene Therapy, KULeuven, Leuven, Belgium, [4]Experimental Neurosurgery and Neuroanatomy, KULeuven, Leuven, Belgium

Introduction: MRI has recently become a powerful tool for in vivo non-invasive monitoring of the migration of superparamagnetic iron oxide (SPIO) labeled stem cells. However, labeling of the stem cells is principally performed in cell culture by incubating the cells with the contrast agent. A protocol for labeling endogenous neuronal stem cells in situ has first been described by Shapiro et.al[1]: subventricular neuronal precursor cells were labeled in situ in the rat brain by injection of micron-sized SPIO particles in the lateral ventricle and their recruitment along the rostral migration stream (RMS) up to the olfactory bulb (OB) was visualized with in vivo MRI. The goal of this study was to test different Magnetoliposomes (ML's) in a related protocol to evaluate there potentials for labeling endogenous neuronal progenitor cells (NPC) in healthy adult mice aiming at future applications in different mouse models for neurodegenerative diseases.

Methods: In this study we used four new developed nano-sized Magnetoliposomes (iron oxide containing liposomes), each type with a different surface coating (neutral, anionic, cationic coating or a coating which included PEGylated phospholipids)[2]. These ML's were stereotactically injected (1.5µl) in the left RMS of C57BL/6J mice (n=8). At four different time points (5, 13, 21 and 60 days post injection), the distribution of the contrast agents in the mice brain was monitored in vivo with T_2* weighted gradient-echo MRI (TR/TE = 500/6 ms, matrix = 256x128, FOV = 20 mm). At the end of the in vivo experiments (60days) mice were sacrificed and perfused with 1 mM Gd-DTPA doped 4% paraformaldehyde. Decapitation was performed for high resolution in-vitro 3D-FLASH MRI (66µm isotropic resolution). The brains with intact olfactory bulbs were removed for histology. Sections (50µm) of the brain were stained with nucleus Red and Prussian blue for detection of iron particles. Every third section was also stained for Dcx (migrating NPC) and NeuN (mature neurons) using immunohistochemistry.

Results: As shown in figure 1 in vivo MRI revealed ML's (hypo-intensity) at the injection site and particle relocation into the olfactory bulb, except for the cationic ML's, 5 days after RMS injection in the adult brain. High resolution in vitro MRI (figure 2) detected movement of all the ML's into the OB. In contrast to the MRI, histology showed ML's near the injection site in the vicinity of the RMS but the presence of iron oxide in the OB could not be revealed. Additional to the movement of ML's into the OB, relocation of the ML's along white matter tracts such as the corpus callosum (cc) and the anterior commissura anterior (aca) could be detected for al types of ML's with in vitro MRI as shown in figure 3.

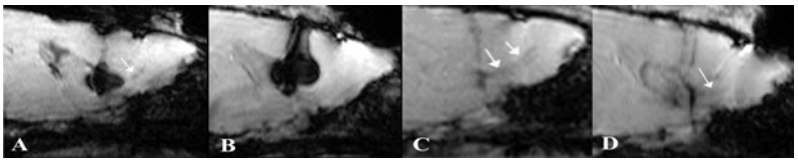

Figure 1: In vivo sagittal sections of the forebrain and olfactory bulb of mice 5 days after injection of A) neutral ML's, B) anionic ML's, C) cationic ML's and D) PEGylated ML's. The white arrows show the ML's relocation into the olfactory bulb.

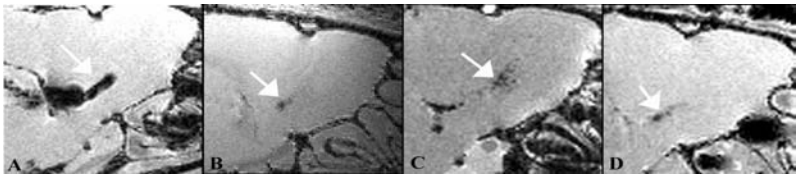

Figure 2: In vitro sagittal MRI of adult mice brains 60 days after injection of A) neutral ML's, B) anionic ML's, C) cationic ML's and D) PEGylated ML's. The white arrows show the ML's relocation into the olfactory bulb.

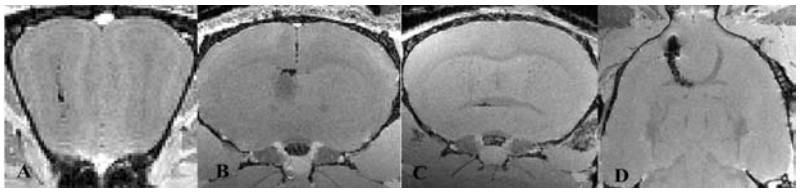

Figure 3: In vitro MRI of adult mice brains 60 days after injection. A and B) Transverse sections where anionic ML's are visible in the anterior commissural interbulbar and the corpus callosum. In vitro transverse section (C) and horizontal section (D) where neutral ML's are located in the anterior commissura anterior.

Discussion and conclusion: The results of this work highlight the possibility of misinterpretation of in vivo MRI data on neuronal recruitment using SPIOs. There was a movement of the ML's into the OB but this particle relocation was probably not due to an uptake of the magnetoliposomes by migrating endogenous NPC as no iron oxide particles could be detected in NPC with histology. A possible explanation for this migration of ML's, visible with MRI, could be the fact that the particles followed a white matter tract, namely the anterior commissura interbulbar (aci). It is known that the RMS runs parallel to this white matter tract. The proposed self-migration of the ML's could be confirmed by their relocation along other white matter tracts as the corpus callosum and the anterior commissura anterior.

References:

1. Shapiro E et.al,*ProcISMRM* 2004,#166
2. De Cuyper M, et.al,*J.Phys.:Condens.Matter* **15,** 1-12 (2003)