Assessment of the uptake of magnetite labeled nanoparticles in the rat using MRI

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Introduction: Nanoparticles (NP) are expected to facilitate transport of drugs over the blood brain barrier (BBB) thus allowing a more effective and targeted application of therapy [1]. So far, MRI is the most promising method for tracing the uptake and distribution of NP in vivo but suffers from the limitation that the NP per se do not provide intrinsic image contrast.

Aims: The aim of this experimental study was to explore the potential of magnetite labelling for making NP traceable in phantoms and *in vivo*. Phantom studies were performed to assess the relaxivities of different NP configurations. In addition, in vivo experiments in rats were performed to look for distribution patterns of the NP and to test if the relaxivity effect in the brain is high enough to allow in vivo tracking.

Methods: 1.) NP preparation: Human serum albumin (20 mg) was dissolved in 2 ml purified water and the pH was adjusted to 8.0. The solution was filtered through a 0.22 µm cellulose acetate membrane filter. An aliquot (1 ml) of this solution was mixed with 33.3 µl magnetite dispersion with an average particle size of 8 nm (PlasmaChem, Germany) and 10 µl 1 M NaCl solution. The mixture was incubated for 1h at 20°C under constant stirring (600 rpm). To initiate nanoparticle formation 4 ml ethanol were added using a tubing pump. Particles were stabilised by crosslinking using 5.8 µl glutaraldehyde solution. The crosslinking was performed for at least 12 h under constant stirring at room temperature. A similar procedure was repeated with varying particle size of the magnetite. 2.) MRI: All MR studies were performed on a clinical 3.0T scanner (Tim Trio, Siemens Healthcare, Germany) using a 2-element surface coil for signal reception. R1 in the phantom containing different concentrations of NP was determined with an IR sequence (TR=6s, TE= 9.2ms) with multiple inversion times (TI= 50, 100, 200, 400, 800, 1600, 3200 ms). R1 in the rat brain was estimated with driven equilibrium single pulse observation of T_1 (DESPOT, FA= 4° and 22 °; TE_1= 5.6 ms, TE_2=11.1 ms; TR= 19 ms; THK= 1 mm; FOV= 40 mm; matrix= 192 × 192) by averaging the signal from both echoes [2]. As this study focused on the longitudinal changes of T_1, no attempts were made to assess B_1 in the rat brain. A phase sensitive IR sequence (TE= 8.6, 9.20 ms; TR= 5000 ms; section thickness= 1.44 mm; FOV= 50 mm; matrix= 180 × 256) was used to qualitatively assess the uptake of the NP in the body of the rats. No physiological triggering was used. Instead a 2D acquisition mode and flow compensation was selected to minimize artefacts from physiological motion. 3.) In vivo experiments: For the in vivo measurements only the NP were used that showed the strongest R1 relaxivity effect in the phantom measurements. Following anaesthesia, baseline brain scans and body scans were performed and repeated after injection of 150 µl NP (corresponding to 0.9 mg NP per animal and/or 81 µg magnetite per animal) in the leg vein. 4. Image analysis: After calculation of T1 maps of the brain, brain tissue was segmented and analyzed with a histogram technique (Fig 1.) The histogram was fitted with a double Gaussian function to assess gray and white matter simultaneously. The peak positions of the gray and white matter peak was used as the global T1 value for these structures which facilitated robust analysis of NP induced relaxivity changes.

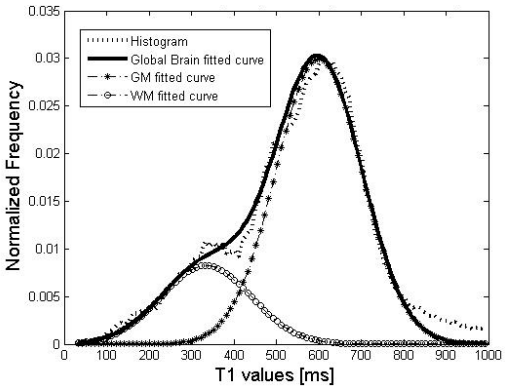

Figure 1: Whole brain histogram (dotted line) of segmented T1 maps of a rat. Double Gaussian fitting allowed for separation of brain white matter (circles) and brain gray matter (asterisks).

Results: Results from the phantom studies are summarized in Figure 2. In line with theoretical considerations T1 shortening was found to be linearly related to NP concentration. The strongest R1 effect was found for the NP with a mean diameter of approx. 200 nm and a 8 nm magnetite loading of 90.9 µg per mg NP. In the body of the rat, most pronounced changes were observed in the liver and spleen. NP could not be visually assessed in the T1 maps (Figure 3) but histogram analysis revealed that there was a NP related R1 increase of approximately 3% in brain.

Conclusion: Our results indicate that even small amounts of NP can be visualized *in vivo* by incorporating magnetite. While the most significant uptake in the rat occurs in the spleen and liver, only a small amount of NP seem to cross the BBB. However, with a robust histogram techniques it is possible to detect even these small variations in R1. Further studies will have to confirm these results with auto-fluorescence imaging. In addition, new strategies will have to include factors increasing the BBB permeability [3].

References: [1] Wohlfart et al, Journal of Controlled Release, 2011; [2] Deoni et al, Magnetic Resonance Medicine, 2005; [3] Zensi et al, Journal of Controlled Release, 2009.

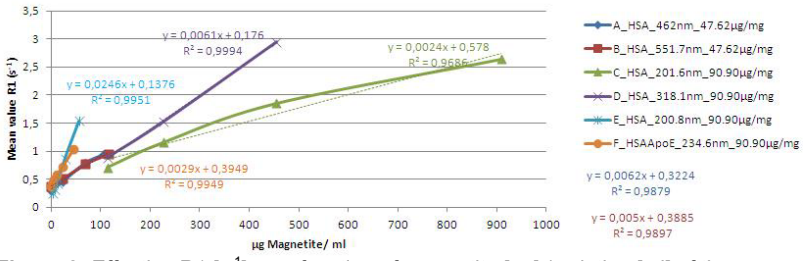

Figure 2: Effective R1 [s^{-1}] as a function of magnetite [µg] / solution [ml] of the different NP formulas.
(LetterSerie_ProteinComposition_Diameter_Magnetite/Nanoparticle).

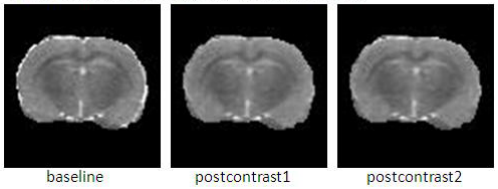

Figure 3: T1 maps from coronal rat forebrain slices before NP injection (baseline) and after NP injection (postcontrast1=20min, postcontrast2=40min).