Measuring the accumulation of magnetite labeled nanoparticles in the rat brain

Naira P. Martinez Vera1, Klaus Langer2, Iavor Zlatev3, Robert Wronski1, Manfred Windsch1, Ewald Auer1, Hagen von Brixen1, Sylvia Wagner1, Motti Deutsch5, Claus Pietrzik3, Franz Fazekas4, Reinhold Schmidt1, and Stefan Ropele1

1Department of Neurology, Medical University of Graz, Graz, Styria, Austria, 2Institut für Pharmazeutische Technologie und Biopharmazie, Münster, North Rhine-Westphalia, Germany, 3JSW Life Sciences GmbH, Grambach, Styria, Austria, 4Department of Cell Biology & Applied Virology, Fraunhofer-Institute for Biomedical Engineering, St. Ingbert, Saarland, Germany, 5Physics Department, Schottchenzenter for the Research and Technology of the Cellome, Bar Ilan University, Ramat Gun, Israel, 6Institute of Pathobiochemistry, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Rhineland-Palatinate, Germany

Target audience: Researchers interested in the field of molecular imaging and iron oxide labelled nanoparticles.

Purpose: The blood brain barrier (BBB) maintains the CNS homeostasis and protects the brain from harmful substances. However, specific therapeutic intervention needs to overcome this barrier restriction and this is why nanoparticles (NP) show potential as drug transporters into the brain. This experimental study was performed to explore the feasibility of tracing iron label NP in the rat brain using MRI. The findings were validated using autofluorescence imaging following brain extraction.

Methods: 1) In vivo studies: MRI measurements were performed on a clinical 3.0 T scanner (Trio TIM, Siemens Healthcare, Germany). The magnetite labelled NP used in this study were based on human serum albumin and had incorporated 90.9 µg/NP magnetite (diameter of the magnetite = 8 nm). To study the accumulation of NP in the rat brain, in vivo experiments were performed in 24, 12-30 weeks old female Wistar rats. Before MRI, rats were anesthetized with a mixture of ketamine and Xylazine (2.3:1) where 1.66 µl were injected intraperitoneally. NP induced changes of T1 in the brain were estimated with a 3D mode acquisition and driven equilibrium single pulse observation of T1 (DESPOT, FA = 6° and 30°, TR = 30 ms, THK = 1 mm, FOV = 40 mm, matrix = 192 x 192). To increase the SNR, the sequence was performed with two echoes (TE1 = 4.82 ms, TE2 = 9.96 ms) and with three averages. After baseline T1 mapping, 3.2 µl NP per gram body weight were injected intravenously into the vena femoralis and T1 mapping was repeated twice (total scan time = 65 minutes). In 7 control rats, a saline solution was injected instead of the NP. As this study focused on longitudinal changes of T1, no attempts were made to correct for B1 induced T1 errors. Immediately after MRI, the rats were sacrificed and the brains then were extracted for histological processing. Autofluorescence was assessed in the hippocampus (HC) and in the corpus callosum (CC). 2) Image analysis: Following generation of T1 maps, brain tissue was segmented and analyzed globally with a histogram technique. The T1 distribution in the entire brain was modelled by fitting a triple Gaussian function with a least-square technique. The three Gaussians represented gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) (Figure 1). The peak positions represented the mean T1 values of each component which facilitated robust analysis of NP induced relaxivity changes. The rationale behind this approach was that we expected a global and diffuse distribution of NP that had crossed the BBB. 3) Statistical analysis: Student’s t-test was used to assess NP related autofluorescence and T1 changes following administration of NP. For independent validation, a linear regression was used to study the relationship between NP induced T1 changes and relative levels of autofluorescence.

Results and discussion: Histology revealed that the autofluorescence signal in the hippocampus (HC). P-value associated with the correlation was smaller than 0.001 and the Pearson correlation was 0.683. Number of cases N = 24 rats.

Conclusions: This study demonstrated the feasibility of tracking magnetite labelled NP in vivo. Using a sensitive histogram technique, it was possible to detect even a very small amount of NP. Future work will have to focus on incorporation of drugs into the NP and on ApoE linking, which is expected to facilitate BBB crossing.

References:

