Measuring the accumulation of magnetite labeled nanoparticles in the rat brain

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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 (Tim Trio, 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)[1]. 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[2] (DESPOT, FA = 6° and 30°, TR = 30 ms, THK = 1 mm, FOV = 40 mm, matrix = 192 × 192). To increase the SNR, the sequence was performed with two echoes (TE_1 = 4.82 ms, TE_2 = 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.

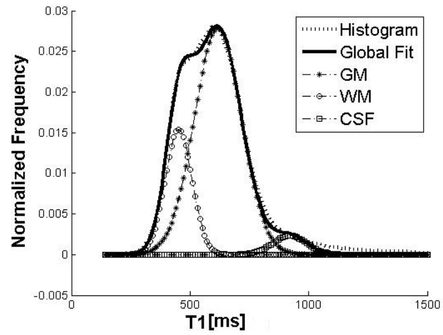

Figure 1: Whole brain histogram (dotted line) of segmented T1 maps of a rat brain. Triple Gaussian fitting allowed for separation of white matter (circles), gray matter (asterisks), and cerebrospinal fluid (squares).

Results and discussion: Histology revealed that the autofluorescence signal in the HC and CC was significantly increased after NP administration indicating that some NP could cross the BBB (Figure 2). Significant effects were also seen in the T1 peak position of GM and WM. These changes scaled linearly with the intensity in the autofluorescence image (Figure 3) and were found for gray matter ($p < 0.001$, R = 0.683) and white matter ($p < 0.001$, R = 0.732).

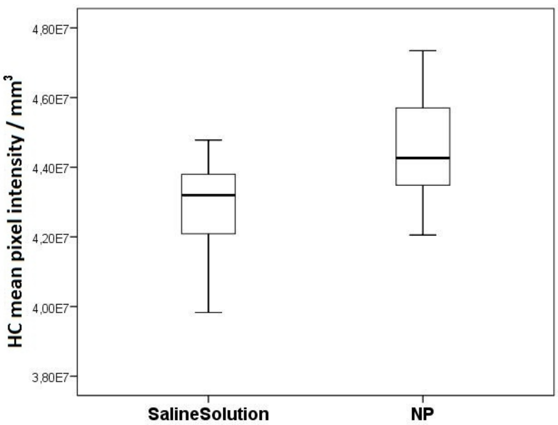

Figure 2: After NP administration, the autofluorescence signal in the hippocampus (HC) was significantly increased when compared to control rats.

Conclusion: 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[3] linking, which is expected to facilitate BBB crossing.

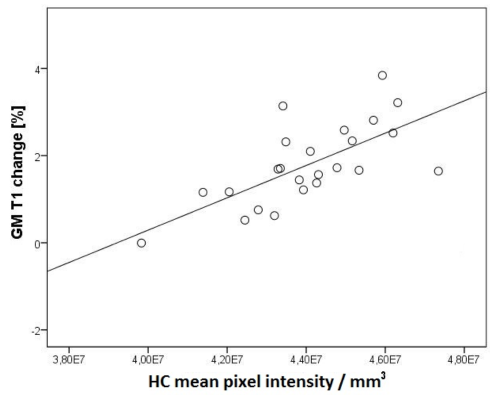

Figure 3: T1 changes in gray matter (GM) versus autofluorescence signal in the hippocampus (HC). P-value associated with the correlation was smaller than 0.001 and the Pearson correlation was 0.683. Number of cases N = 24 rats.

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