IMAGING MACROPHAGE INFILTRATION OF ISCHEMIC TISSUE IS NOT POSSIBLE FOLLOWING TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION.

T. D. Farr[1], J. Seehafer[1], and M. Hoehn[1]

[1]In-vivo-NMR-Laboratory, Max-Plack Institute for Neurological Research, Cologne, North Rhine-Westphalia, Germany

Background: Stroke produces an inflammatory response partially characterized by blood borne macrophage infiltration of the injured tissue. Magnetic resonance imaging (MRI) of this phenomenon has been attempted using systemic administration of iron oxide contrast agents for phagocytosis by macrophages. This technique has been used primarily in models of photothrombosis (1, 2) and permanent middle cerebral artery occlusion (MCAO) (3, 4). The nature of both models makes interpretation of the results difficult, and a similar experiment using transient MCAO was less promising (5). The aim of the present study was to establish optimal conditions to image inflammation following transient MCAO.

Methods:
Animal model: Male Wistar rats (330-430g) received 60 minutes of transient MCAO using the intraluminal filament technique in which a nylon filament is inserted into the origin of the MCA through an opening in the external carotid artery. Animals were allowed to recover and imaging was performed at 2 (n=3), or 3 (n=3) days post-MCAO. At the conclusion of the imaging session animals were intravenously infused with 300µmol Fe/kg of contrast agent. The 2 day group received superparamagnetic iron oxide (SPIO, Endorem®) and the 3 day group received ultra small SPIO (USPIO, Sinerem®). Endorem® is composed of particles with diameters between 120-180nm, and Sinerem® between 15-35nm. Sinerem® exhibits a blood half life of 5-6 hours in rats at this dose. Animals were imaged again 24 hours after contrast administration.
MRI: The MRI experiments were conducted on a 4.7T Biospec (Bruker BioSpin) equipped with actively shielded gradient coils (100mT/m). Radio frequency transmission was achieved with a Helmholtz coil (12cm diameter) and the signal was detected with a 22mm diameter surface coil. The scan package was composed of a spin echo T_2-weighted scan (TR/TE: 3000/14msec, 16 echoes, FOV: 30 x 30mm, matrix: 128 x 128, 9 contiguous slices 1mm thick), a gradient echo T_2*-weighted scan with similar geometry (TR/TE: 3000/4msec, 32 echoes), and a T_1-weighted scan (TR/TE: 500/9msec, FOV: 30 x 30mm, matrix: 128 x 128, 9 slices 1mm thick, slice gap 0.25mm). The total acquisition time was approximately 30minutes.
Histology: At the conclusion of the experiments animals were perfusion fixed and tissue processed for immunohistochemistry with the macrophage marker ED-1. Adjacent sections were stained with Prussian blue for iron positive macrophages, and counterstained with nuclear red.

Results: The ischemic tissue was clearly visible in the T_2-weighted images and quantitative maps. However, there was no area of observable signal change that could be attributed to the accumulation of iron laden macrophages in the T_2*-weighted images, or maps, in any of the animals. Signal intensity in the vessels decreased in the T_2* images in Sinerem® treated animals (Figure 1).

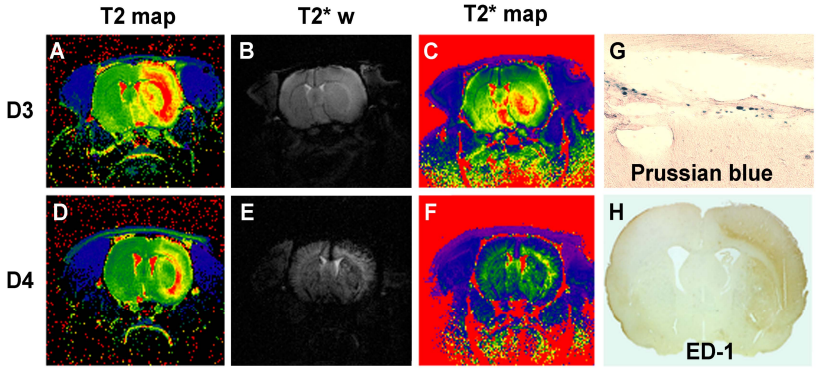

Figure 1. A-C: T_2 maps, T_2*-weighted images (29msec), and T_2* maps from a coronal slice (-0.3mm from bregma) of an animal at 3 days post-MCAO. D-E: Corresponding slices from the same animal at 4 days post-MCAO (24 hours following Sinerem® administration). G: Evidence of Prussian blue positive macrophages next to the ventricle in the peri-infarct zone. H: A coronal tissue section (-0.8mm from bregma) stained with ED-1. Note the increased stain intensity in the ischemic tissue.

In contrast to previous observations (5) no change in signal intensity was detected in the peri-infarct zone of the T_1-weighted images following administration of the contrast agent. ED-1 immunohistochemistry revealed extensive macrophage accumulation in the infarcted tissue. However, Prussian blue positive macrophages were sparse and typically located in areas where small hemorrhagic transformations were thought to have occurred.

Conclusions: The results indicate that imaging the inflammatory response following transient MCAO is not straightforward. Two different contrast agents were used, and administration occurred during a time when activated blood borne macrophages are numerous. There is rising concern that non-specific accumulation of contrast occurs when it is administered near to the time of the insult (3, 4, and 5), or when blood brain barrier breakdown and erythrocyte accumulation is present (1, 2). Therefore, caution should be taken when evaluating experiments that rely on systemic administration of contrast agents to observe the inflammatory response. Future approaches could include *in vitro* macrophage labeling or targeting specific receptors involved in the inflammatory response.

Acknowledgements: The authors wish to acknowledge Drs. Philippe Robert and Claire Corot (Guerbet) for supply of contrast agent. This work was supported by a grant from the Stemstroke EU-FP6 program (LSHB-CT-2006-037526).

References: **1)** Kleinschnitz, *et al*., 2003. JCBFM. 23: 1548-55, **2)** Saleh, *et al*., 2004. NMR in Biomed. 17: 163-9, **3)** Wiart, *et al*., 2007. Stroke. 38: 131, **4)** Rausch, *et al*., 2001. MRM. 46: 1018-22, **5)** Rausch, *et al*., 2002. NMR in Biomed. 15: 278-83