^{19}F/^{1}H MRI of Brain Inflammation in Experimental Autoimmune Encephalomyelitis

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Introduction: Inflammatory diseases of the central nervous system (CNS) such as multiple sclerosis (MS) involve a recruitment of immune cells during the early stages of pathogenesis, prior to the onset of clinical symptoms [1]. During the development of disease, the blood-brain barrier (BBB) becomes altered and immune cells gain access to CNS parenchyma via a complex, multi-step process that involves crossing both the vascular endothelium and the glia limitans [2]. Using an animal model of MS, the Experimental Autoimmune Encephalomyelitis (EAE), we explored the *in vivo* uptake of fluorine (^{19}F) nanoparticles by inflammatory cells during encephalomyleitis.

Methods: We designed and constructed a 32-leg dual-tunable ^{19}F/^{1}H MRI birdcage-coil [3] dedicated for mouse head imaging. Electromagnetic field (EMF) simulations with CST MWS (CST AG, Darmstadt, Germany) and phantom measurements were performed to assess B_1^+ field strength and homogeneity of the excitation pattern (Fig 1b+c). SJL/J mice were immunized with proteolipid protein, weighed daily and assessed for neurological symptoms as previously described [4]. ^{19}F nanoparticles (C=1200 mM, Z-Average Diam. =160 nm) were prepared from perfluoro-15-crown-5-ether (PFCE, Fluorochem, Derbyshire, UK) as previously described [5], diluted to 100 mM and administrated intravenously (400µl) to EAE mice. Anesthetized mice were placed in a holder designed for the dual-tunable ^{19}F/^{1}H head coil on a Bruker Biospec 9.4T system. Single-^{19}F voxel ^{19}F MRS was performed using point-resolved spectroscopy (PRESS) (TR/TE 1500/11ms, 3x3x3mm voxel, 512 averages, 13min). ^{19}F/^{1}H MRI was performed using a gradient echo sequence (2D FLASH) with 22 sagittal slices for ^{1}H (TR/TE 473/13ms 73x73x400µm, 16 averages, 25min) and one sagittal slice for ^{19}F (TR/TE 15/3.3ms, 440x440x3000µm, 2048 averages, 15min). After terminal anesthesia, mice were transcardially perfused with 4 % formaldehyde and 0.5 % gluteraldehyde prior to brain extraction. A 3 mm³ cube (enclosing the PRESS-voxel used for spectroscopy) was dissected from cerebellum and post-fixed (24 h 2 % gluteraldehyde, 2 h 1 % osmium tetroxide). Following dehydration, tissue was embedded in Poly/Bed 812 (Polysciences, Eppelheim, Germany). Semithin sections were stained with toluidine blue and ultrathin sections were stained with uranyl acetate/lead citrate. Sections were imaged using a FEI Morgagni electron microscope (FEI, Eindhoven, NL) and iTEM software.

Results: The results of the EMF simulations (Fig. 1a-c) as well as phantom studies (data not shown) show that the ^{19}F/^{1}H MRI coil offers optimal B_1^+ field strength and homogeneity. An i.v. administration ^{19}F nanoparticles (containing 40 µmol PFCE) was well tolerated in EAE mice. Fig. 2 illustrates the ^{19}F MRS signal in a 3 mm³ voxel within the cerebellum corresponding to c. 60nmol of PFCE. When we performed ^{19}F/^{1}H MRI, we detected the ^{19}F nanoparticles close to areas of hyperintense lesions within the cerebellum but also in the brain stem of EAE mice (Fig. 3). Upon histological analysis and using differential interference contrast (DIC) microscopy, we observed the ^{19}F nanoparticles in macrophages surrounding the EAE lesions (Fig. 4a). Electron microscopy (EM) revealed the ^{19}F particles as bright smooth spheroids (Fig. 4 b,c) clustered within phagosomes in the cytoplasm of macrophages.

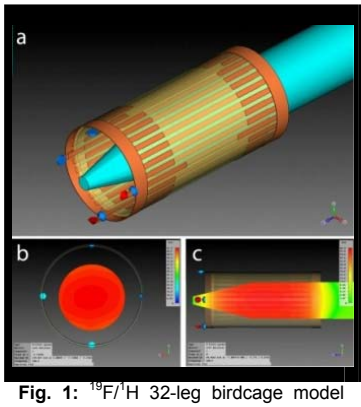

Fig. 1: ^{19}F/^{1}H 32-leg birdcage model simulated in CST a) Geometry of coil b) B_1^+-field distribution on an axial center slice of the coil c) B_1^+-field distribution on a sagittal center slice of the coil

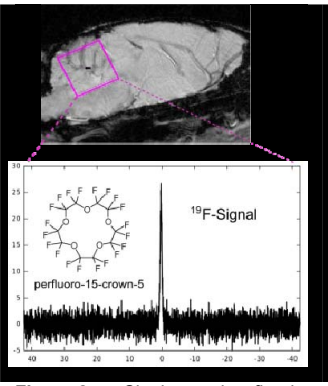

Fig. 2: Single-voxel fluorine PRESS. ^{19}F-PRESS TR/TE 1500/11ms, voxel of 3x3x3mm³, NEX512, 13min; signal corresponds to c. 60nmol PFCE

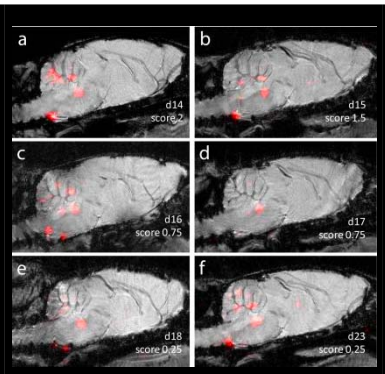

Fig. 3: Sagittal brain anatomical ^{1}H (grey scale) and ^{19}F nanoparticles (red) images during EAE disease (a-f).
^{1}H: 2DFLASH: TR/TE 473/13ms, 22 slices 400x73x73µm³, NEX16, 25min;
^{19}F: 2DFLASH: TR/TE 15/3.3ms, 1 slice 3000x440x440µm³, NEX2048, 15min

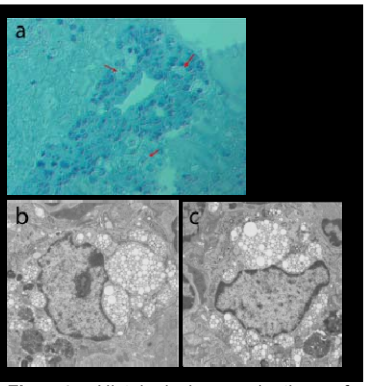

Fig. 4: Histological examination of inflammatory lesions in cerebellum a) semithin sections and DIC microscopy show nanoparticles in macrophages around lesion; b-c) ultrathin sections imaged by EM reveal nanoparticles engulfed in phagosomes

Discussion and Conclusions: ^{19}F MRI is becoming increasingly important for cell tracking and detection of inflammation in small animal imaging [6]. In this study we detected ^{19}F nanoparticle uptake in areas of hyperintense lesions within the brain – predominantly cerebellum – of EAE mice. Since ^{19}F molecules are scarce in the human body, the uptake of ^{19}F nanoparticles by inflammatory cells gives a background free signal in ^{19}F MRI. This is one major advantage of ^{19}F/^{1}H MRI over T_2* imaging of iron oxide nanoparticles; with the latter technology there are sometimes difficulties to distinguish nanoparticles engulfed by inflammatory cells from other susceptibility-related T_2* effects such as paramagnetic deoxygenated blood [7]. Therefore the application of ^{19}F nanoparticles to image immune cells in conditions such as encephalomyelitis is an emerging field to study the kinetics of immune cell localization during the development of inflammation.

References: [1] Stadelmann C (2011) Curr Opin Neurol 24: 224-229 [2] Engelhardt B, Coisne C (2011) Fluids Barriers CNS 8: 4 [3] Waiczies H, Hoffmann W et al. (2011) Abstracts of the 28th ESMRMB, MAGMA 24 Suppl 1: 4-559 [4] Aktas O, Waiczies S et al. (2003) J Exp Med 197: 725-733 [5] Waiczies H, Lepore S et al. (2011) PLoS ONE 6: e21981 [6] Ruiz-Cabello J, Barnett BP et al. (2011) NMR Biomed 24: 114-129 [7] Brooks RA, Brunetti A et al. (1989) Magn Reson Med 12: 241-248