19F/1H 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 (19F) nanoparticles by inflammatory cells during encephalomyelitis.

Methods: We designed and constructed a 32-leaf dual-tunable 19F/1H 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 B1 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]. 19F 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 19F/1H head coil on a Bruker Biospec 9.4T system. Single-19F voxel 19F MRS was performed using point-resolved spectroscopy (PRESS) (TR/TE 1500/11ms, 3x3x3mm voxel, 512 averages, 13min). 19F/1H MRI was performed using a gradient echo sequence (2D FLASH) with 22 sagittal slices for 19F (TR/TE 473/13ms, 73x73x400µm, 16 averages, 25min) and one sagittal slice for 1H (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 PolyBed 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 19F/1H MRI coil offers optimal B1 field strength and homogeneity. An i.v. administration of 19F nanoparticles (containing 40 µmol PFCE) was well tolerated in EAE mice. Fig. 2 illustrates the 19F MRS signal in a 3 mm³ voxel within the cerebellum corresponding to c. 60nmol of PFCE. When we performed 19F/1H MRI, we detected the 19F 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 19F nanoparticles in macrophages surrounding the EAE lesions (Fig. 4a). Electron microscopy (EM) revealed the 19F particles as bright smooth spheroids (Fig. 4 b,c) clustered within phagosomes in the cytoplasm of macrophages.

Discussion and Conclusions: 19F MRI is becoming increasingly important for cell tracking and detection of inflammation in small animal imaging [6]. In this study we detected 19F nanoparticle uptake in areas of hyperintense lesions within the brain – predominantly cerebellum – of EAE mice. Since 19F molecules are scarce in the human body, the uptake of 19F nanoparticles by inflammatory cells gives a background free signal in 19F MRI. This is one major advantage of 19F/H MRI over T2* imaging of iron oxide nanoparticles: with the latter technology there are sometimes difficulties to distinguish nanoparticles engulfed by inflammatory cells from other susceptibility-related T2* effects such as paramagnetic deoxygenated blood [7]. Therefore the application of 19F nanoparticles to image immune cells within the conditions such as encephalomyelitis is an emerging field to study the kinetics of immune cell localization during the development of inflammation.