Early In vivo characterisation of relapsing experimental auto-immune encephalomyelitis lesions by macrophage cellular imaging (USPIO) predicts late occurence of axonal loss

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Background: Axonal injury and loss are pathological hallmarks of multiple sclerosis (MS) and are probably involved in long term disability. MRI with Ultra-small-super-paramagnetic-iron-oxide (USPIO) is able to disclose *in vivo,* lesions infiltrated by macrophages in experimental auto-immune encephalomyelitis (EAE). MRI with USPIO has been successfully applied to detect active lesions in MS patients.

Objective: To correlate USPIO enhancement by MRI at the first attack of relapsing-EAE with development of acute axonal injury and chronic axonal loss after two attacks.

Methods: Chronic relapsing EAE was induced in Dark Agousti rats. Within 12 to 16 h after appearance of the first clinical signs imaging studies were realized on a 1.5 T magnet. After the first MRI, rats received USPIO infusion (Sinerem®) and a second MRI was performed 20 to 24 hours later allowing the accumulation of USPIO in macrophages and their infiltration to active inflammatory sites in the CNS. Animals were sacrificed either immediately after the MRI at the time of this first attack (n = 9), either at the second attack or 4 days after remission if this second attack did not occur (n = 16). Axonal damage and loss were respectively studied by APP and neurofilament (NF) immunostaining. Extent of demyelination was determined by Luxol Fast Blue (LFB) staining and of inflammatory infiltrates by Hemalun-Eosin (HE) staining.

Results: In 50% of diseased rats, MRI at the first attack revealed signal abnormalities mainly localized in the brainstem, the cerebellum and the cervical part of the spinal cord. Significant differences in tissue alterations were observed between USPIO + and USPIO- rats in MRI.

Demyelination areas (p = 0.0123), areas with acute axonal damage (p = 0.05), inflammatory infiltrates (p = 0.0127) and areas with ED1+ cells recruitment (p = 0,0209) were significantly more important in rats showing USPIO+ enhancement than in USPIO- rats. USPIO+ enhanced MRI alterations at the first attack and the extent of axonal loss detected by NF immunostaining in the rats sacrificed at the second attack were significantly correlated (p = 0.0011).

Conclusion: Macrophage infiltrates as detected *in vivo* by MRI with USPIO is associated with more severe acute axonal injury and are predictive for the late development of axonal loss in relapsing EAE lesions.