Manganese-enhanced MRI in a mouse model of de- and remyelination

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Introduction: Due to their calcium-like behavior and shortening of T1 manganese ions (Mn^{2+}) are widely used in experimental animal research to visualize brain structures, neuronal activity and connectivity [1]. However, only little is known about Mn^{2+} accumulation under pathological conditions. Here we took advantage of the well characterized mouse model of cuprizone-induced toxic de- and remyelination [2] and analyzed the Mn^{2+} accumulation in the brain by in vivo MRI at different time points of cuprizone treatment. Selected tissue samples were analyzed by synchroton based X-ray fluorescence microscopy.

Methods: Twelve C57BL/6 mice at the age of 8 weeks were fed with 0.25% cuprizone in ground breeder chow *ad libitum* over 2 weeks (group A, n = 4) and 4 weeks (groups B and C, n = 4 each), respectively. After withdrawal of cuprizone, group C received normal food for additional 10 days. Two additional mice served as controls and were maintained on a normal diet for the duration of the experiment. At the respective end of treatment MRI was performed on isoflurane-anesthetized mice before and 24 h after $MnCl_2$ (40 mg/Kg, i.p.) administration using a 3D spoiled FLASH sequence (TR/TE=17/3.6 ms, flip angle 25°, 100 µm isotropic resolution). In addition, one mouse per group underwent MRI at 2 h and 8 h after Mn^{2+} injection. All measurements were performed at 9.4 T (Bruker Bio Spin, Germany). After MRI, mice were sacrificed for histology including immunohistochemical staining of amyloid precursor protein (APP), anti-glial fibrillary acidic protein (GFAP), CD3+, and MAC3+ cells.

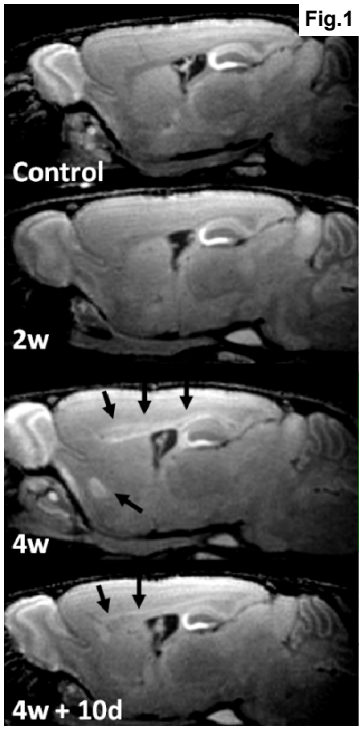

Fig.1

Results and Discussion: Compared to controls (**Fig. 1, control**) no alterations of Mn^{2+} distribution were observed at 2 weeks of cuprizone treatment (**Fig. 1, 2w**).

In contrast, after 4 weeks of treatment, 24 h after Mn^{2+} administration a distinct signal enhancement was observed mainly at the anterior commissure and the outer layers of the corpus callosum (**Fig. 1, 4w**, arrows).

After 10 days of recovery and remyelination this signal enhancement was decreased (**Fig. 1, 4w+10d**, arrows). At that time point distinct accumulations of Mn^{2+} appeared in the striatum as shown in a horizontal section (**Fig 2. 4w+ 10 d**, arrows).

This pattern of signal enhancement after Mn^{2+} administration was correlated with microglia activation (**Fig. 2**, bottom left: MAC3 staining, right: MRI 24 h after Mn^{2+}).

Synchroton based X-ray fluorescence microscopy revealed a point-shaped, cell-body like accumulation of Mn^{2+} (**Fig. 3 A**, arrows, Mn – blue, Fe – red) and a co-localization with Ca^{2+} (**Fig. 3 B**, arrows, Mn – blue, Ca – red). The fact that MRI signal enhancement was first observed at about 8 h after Mn^{2+} injection, indicates an unaffected permeability of the blood brain barrier for Mn^{2+} (**Fig. 4**).

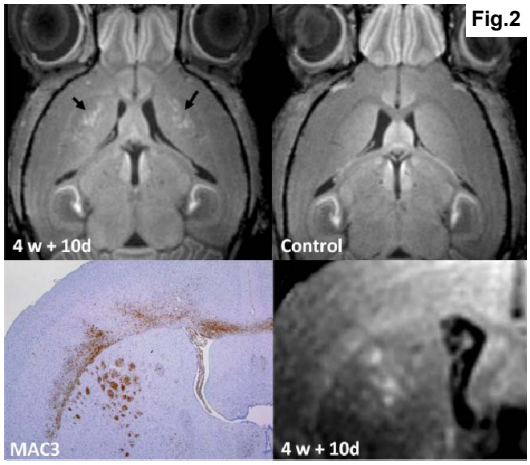

Fig.2

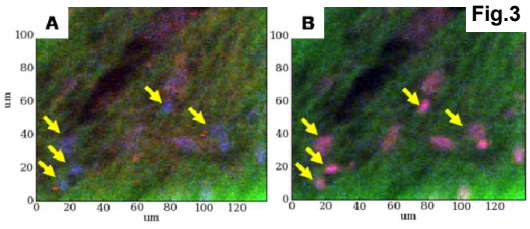

Fig.3

Summary: Mn^{2+}-enhanced MRI revealed a distinct Mn^{2+} accumulation in specific brain regions that refers to activated microglia cells as evidenced by immunohistochemistry. X-ray fluorescence spectroscopy demonstrated a co-localization of manganese with calcium in cell-body like structures that most likely represent microglia cells and no accumulation in neuronal fibers. Although Mn^{2+} is often used to measure neuronal activity, a possible bias in animal models with inflammation has to be taken into account. The specific accumulation of manganese indicates that manganese-enhanced MRI may significantly contribute to the understanding of immune-mediated diseases in the CNS and potentially also in other tissues.

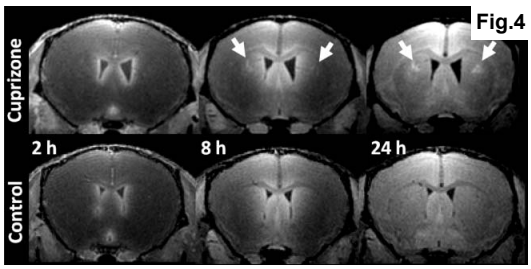

Fig.4

References: [1] Boretius et al, Methods Mol Biol., 2011; [2] Matsushima et al, Brain Pathol., 2001