

HISTOLOGICAL CORRELATION OF MANGANESE ENHANCED MRI IN THE DEMYELINATING DISEASE MODEL BRAIN.

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Introduction

Multiple sclerosis (MS) is one of the most common autoimmune diseases of the central nervous system (CNS), and that is associated with progressive oligodendrocyte loss, neuronal loss and demyelination. Cuprizone is a copper chelating mitochondrial toxin that induces apoptosis of oligodendrocytes and demyelination in the CNS, and is used for animal model of de- and remyelination in MS [1,2]. Manganese ion is one of T1 shortening contrast agents of MRI, that enhances visualization of brain microstructures. Recently, manganese enhanced MRI (MEMRI) is utilized to reveal the mechanisms of neuroinflammatory and neurodegenerative disorders, especially it enhances image contrast of the glial (astrocytes and microglia) activations in the pathological conditions. In this study, we investigated manganese contrast enhancement in cuprizone induced demyelinated mouse brain using 11.7T ultra high field MRI, and analyzed with histology and glial marker expressions.

Materials and Methods

The demyelination mouse model (C57BL/6) were created by feeding 0.2% cuprizone contained breeder chows *ad libitum* over 5 weeks. One week prior to start cuprizone feed, 0.1M MnCl₂ were started to administer with drinking water and continued up to 5th week. MRI investigations were performed at 11.7T vertical scanner with micro imaging system (AVANCEII 500WB, 1.5T/m high power gradient: Bruker Biospin, and 15mm inner diameter linear volume coil: m2m). T1w (TR/TE= 600/7.0ms, ETL=2, 0.0625mm², 0.3mm thickness, 25nex, 15min), T2w (TR/TE= 5000/31.5ms, ETL=8, 0.0625mm², 0.3mm thickness, 12nex, 24min) and saturation recovery T1 (TR/TE=[6, 3, 2, 1, 0.75, 0.5, 0.25, 0.15s]/8.8ms, ETL=2, 128x128matrix, 0.3mm thickness, 2nex, 15min) RARE were scanned every week. Immuno-histochemical staining (KB [Kluver-Barrera] for myelin density, GFAP for astrocyte distribution) and electron microscopic observation were performed for evaluating demyelination level and distribution of glial cells. In addition, RT-PCR (GFAP for astrocyte activity, Iba-1 for microglia activity) was performed for evaluating the expression level of astrocyte and microglia markers.

Results and Discussion

In MEMRI, positive signal enhancement in corpus callosum was observed at 3rd week, the signal strength had a tendency to peaking at 4th week and reducing to 5th week (**Fig.1 a**, arrows). Volume reduction of external capsule was observed at 3rd week and strong positive enhancement was also observed at 4th week (**Fig.1 b**, arrows). A shortening of quantitative T1 value of corpus callosum was detected at 3rd week and peaked at 4th week then reducing to 5th week, however no significant changes were observed in hippocampus and cortex (**Fig.2**). In spite of MnCl₂ administration, conventional T2 weighted images show hyperintense in corpus callosum at 3rd week, and its size and enhancement were increased up to 5th week (**Fig.1 c**, arrows). In histology, demyelination of entire white matter was started to observe in KB (myelin) staining at 1st week and increased up to 5th week (**Fig.3**). In electron microscope, minor degradation of myelin sheath was observed at 1st week and strong depletion of myelin was detected at 3rd week and was continued up to 5th week (**Fig.4**). In contrast, GFAP (astrocyte) staining was depicted activated astrocytes in corpus callosum at 5th week but in controls were not detected. In RT-PCR, GFAP (astrocytes) expression was monotonically increased to 5th week, however Iba-1 (microglia) expression showed peak at 3rd week and diminished at 5th week (**Fig.5**), that was similar as MEMRI enhancement. In conclusion, MEMRI may reflect phenomenon accompanying microglia activation on the cuprizone induced demyelination.

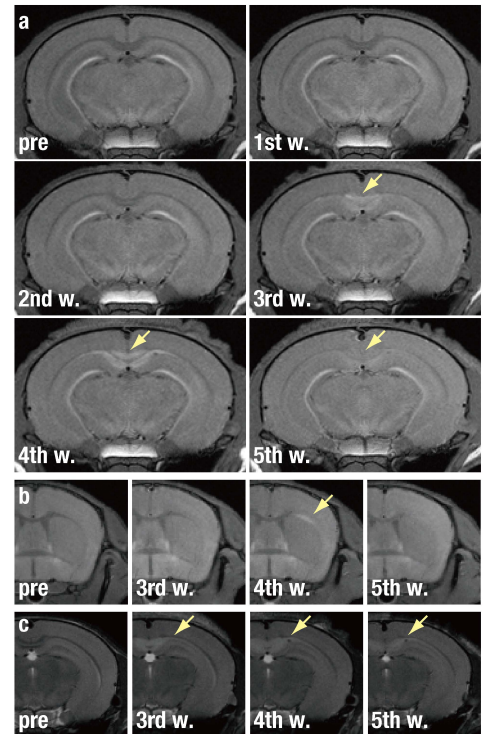


Fig. 1

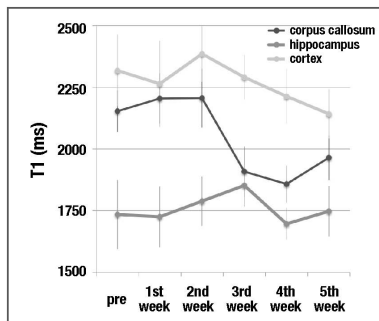


Fig. 2

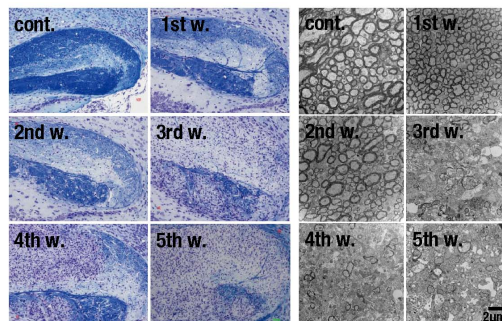


Fig. 3

Fig. 4

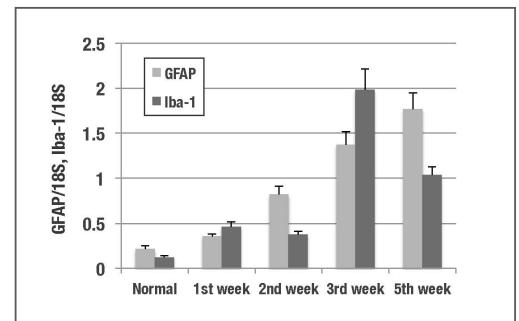


Fig. 5

References: [1] Brain Pathol. 11:107-16, 2001, [2] Acta Neuropathol. 118:723-36, 2009. This work was supported in part by Grand-in-Aid for Scientific Research (C) of JSPS.