In-Vivo Mouse Brain Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) Detects Gender and Region Specific Pathology Induced by Cuprizone

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Introduction:

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS), with a large heterogeneity in clinical course, MRI patterns and responses to therapy[1]. It is characterized by extensive loss of myelin in acute or chronic plaques, as well as by axonal injury and inflammation. Clinical observations show that the course of MS is gender dependent, women reach disability milestones at older ages than males and the male sex is associated with a more progressive and severe outcome than female sex[2,3]. The question of "why these gender differences in MS" was mainly addressed by the examination of hormonal influences in animal models but no in vivo longitudinal brain imaging study, meant to characterize the brain microstructure in demyelinating males and females was carried out till now. In mice, the cuprizone diet model[4,5] raised particular interest because it underlines the progression of acute demyelinating pathology to the chronic state. By using brain diffusion tensor magnetic resonance imaging (DT-MRI) in the cuprizone mouse model we aimed to characterize the region specific vulnerability of the brain tissue to cuprizone (especially in the early phase of the disease) and to identify in vivo the pattern of microstructural changes overtime, comparatively, in male and female mice.

Materials and Methods:

Male and female 8-week old C57BL/6 mice were treated with 0.2% cuprizone for 12 weeks, underlining the progression of active demyelinating processes towards the chronic state. In vivo brain DT-MRI exams were performed at different time points (week 0 – before cuprizone treatment, w4, w8 and w12 – 4, 8 and 12 weeks after starting the cuprizone diet) as presented in the Figure-A. Duplicates for each group were kept in the same conditions of housing and treatment and used for the histopathological examination.

MRI: The mice were scanned under isoflurane anesthesia, using a 9.4T small bore animal Scanner (Biospec 94/20, Bruker, Germany). A transmit/receive 1H mouse quadrature birdcage resonator (35 mm inner diameter) was used for data acquisition. A RARE T2-weighted sequence was employed to obtain 31 axial slices ($78 \times 78 \times 500 \mu m^3$) with the following parameters: TR/TE = 4200/35 ms, a RARE factor of 4 and NEX= 4. Mouse brain **DT-MRI** was performed using a 4-shots DT-EPI sequence. Diffusion gradients were applied in 45 non-collinear directions for a b factor of 1000s/mm^{2}. The brain axial slices were acquired with TR/TE = 5000/30 ms, time (Δ) between the application of diffusion gradient pulses of 17ms, diffusion gradient duration (δ) of 7ms. The in-plane image resolution was 156 x 156 μm at a FOV=20 x 20 mm and an acquisition matrix of 128x92. Partial Fourier with an acceleration factor of 1.35 and 31 overscan lines were used. The movement artifacts were avoided by performing respiratory gating. The total acquisition time varied in a range of 1h39min – 2h15min, depending on the respiratory rhythm of the animal. The diffusion tensor was calculated using in house developed DT-MRI software. Different diffusion tensor parametric maps were generated, including fractional anisotropy (FA), mean diffusivity <D>, radial ($D\perp$) and axial diffusivity ($D_\parallel$) as well as directional encoded images. The values of these parameters were assessed in different ROIs of white (WM) and gray matter (GM), comparatively in males and females, and statistical analysis was performed at different scanning points. DT-MRI results were further correlated with the histological evaluation, assessing the myelin and axonal state and the modifications in the glial cell population (oligodendrocytes, astrocytes).

Results and Discussion:

Cuprizone treatment induced marked white matter (WM) pathology: region specific oligodendrocyte depletion, gliosis, demyelination and axonal damage. These pathologic alterations, observed starting with the early phase (week 4) of the disease, caused changes of DT-MRI derived parameter values. DT-MRI evidenced high vulnerability of the rostral external capsule at w4 (red arrows, ec, Fig. B), with subtle differences quantified between male and female brains. In the rostral part of the male brain, FA decreased in the major WM areas (genu of the corpus callosum-gcc, external capsule-ec and anterior commissure-ac), while in females the most affected region was the external capsule (Fig.-B, FA). The early differences between males and females could also be seen in color coded and T2-weighted images (w4, Fig.-B, arrows). At this timepoit, the axial diffusivity values drastically decreased (e.g. Fig-F, w4) from normal (Fig-F, w0) in both male and female corpus callosum (cc), with stronger decline observed in the male's WM (Fig-D). Axonal damage and gliosis observed in histopathological investigation accounted for the abnormally low values of axial diffusion. The assessment of the radial diffusivity overtime showed a faster progression towards abnormally high values in male white matter, when compared with data obtained from females. At w12, $D\perp$ values calculated in the corpus callosum of the male mice were significantly higher than those obtained in females, suggesting a more severe demyelinating phenotype. The increased values of $D\perp$ correlated with the oligodendrocyte apoptosis and the myelin loss. At the chronic phase of the disease (w12), enlarged ventricles and infiltration of the cerebrospinal fluid in the surrounding tissue were observed in both genders. This aspect of the pathology occurred with higher frequency and from earlier phases of the pathology (w4 or w8) in males. The gender specific WM abnormalities were evidenced also in the color coded maps (Fig.-E, white arrows) acquired from more caudal part of the brain and describing the fiber's orientations. Fiber tracking was also performed, helping to assess the impact of demyelinating pathology on fiber's architecture and integrity.

A. Experimental design

B. Comparison between male and female brains at w0 and w4

C. Higher values of $D\perp$ are observed in males cc, in early (w4) and late phase of the disease(w12).

D. Statistical significant differences in $D_\parallel$ values are seen at w4, between male and female WM.

E. Subtle differences are seen between male and female brains in color coded maps at week 4 (w4) – see arrows.

F. The strong decrease of the axial diffusivity values at w4 (see red arrows) was correlated with the axonal damage seen in histology.

Conclusion:

The combination of in vivo DT-MRI and histology allowed the characterization of region specific vulnerability of the WM to cuprizone and provided evidences of subtle differences in the acute and chronic demyelination pattern between male and female mice. When compared with females, the progressing pathology in the male brains had a stronger impact on the values of DT-MRI derived indices (e.g. $D\perp$ and $D_\parallel$), suggesting a faster and more severe course of the disease. The female sex hormones might play an important protective role in early phases of demyelinating disorders and could be exploited for the development of treatments. The existence of a sexual dimorphism in demyelination processes implies also a gender specific response to different therapeutic strategies developed to induce remyelination.

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