BIOMECHANICAL PROPERTIES QUANTIFIED IN VIVO BY MAGNETIC RESONANCE ELASTOGRAPHY CORRELATE WITH MYELINATION AND BRAIN PARENCHYMAL INTEGRITY – A COMBINED 7 TESLA MRE AND HISTOPATHOLOGY STUDY IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

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Background: Magnetic Resonance Elastography (MRE) non-invasively visualizes and quantifies biomechanical tissue properties. Recent preliminary clinical studies, e.g. in multiple sclerosis (MS), revealed a high sensitivity of MRE in detecting occult brain parenchymal damage (1). However, it has remained unclear, how these in vivo measured viscoelastic properties translate into cellular and molecular conditions. In a mouse model of reversible toxic demyelination, we assessed longitudinally the influence of various aspects of neuroinflammation including demyelination, cellular infiltration, astrogliosis and extra-cellular matrix alterations.

Methods: Transient demyelination was induced in two groups comprising 30 female C57BL/6 mice by a diet enriched with 0,2% Cuprizone (CPZ). After nine weeks, one group returned to normal chow allowing for remyelination in these animals, whereas the other group continued with the CPZ diet. Both groups were compared to age- and sex-matched healthy control mice. In each mouse, full 3D-MRE with an isotropic image resolution of 300 µm as well as anatomical imaging was performed every three weeks. For MRE, mechanical waves at 1000 Hz were transmitted into the mouse brain in a setting presented last year (2). Mean values and standard deviation of the complex-valued shear modulus \(G^* = G_d + iG_i\) in regions of interest (ROI) covering the corpus callosum (CC) were calculated and analyzed on reconstructed maps. Additionally, highly resolving T2-weighted anatomical scans within identical slice positioning were acquired. After each scanning session, subsets of 2-3 mice of each cohort were sacrificed for further histological analysis. Conventional H&E and alcian blue stainings, as well as extensive immunohistochemical and immunofluorescent studies were performed.

Results: The complex-valued shear modulus in the CC increased continuously during adolescence, related to brain maturation and progressing myelination. This development was reciprocal to the age-dependent viscoelasticity reduction reported at senescence (3) (Fig. A). However, in the CPZ cohort, this increase was reversed and resulted in a significant decrease at week 12, corresponding with demyelination in fluoromyelin stainings (Fig. E,F). In T2-weighted images, the CC became increasingly isointense in CPZ mice, corresponding to developing demyelination (Fig. C). Astrogliosis and accumulation of activated macrophages/microglia in the CC of CPZ mice started after week 3 of treatment, peaking at week 9 (Fig D and E), and declined again at week 12. In conventional histological stainings (hematoxylin and cosine, alcian blue), we detected a progressive degradation of the extracellular matrix, deliberation of elastic fibres and brain parenchymal spongiosis peaking at week 12 (data not shown). However, when omitting the CPZ diet and returning to normal chow in one subgroup at week 9, CC remyelination occurred, that was accompanied by partial recovery of the viscoelasticity (Fig. A, B).

Interpretation: MRE is sensitive to the degree of myelination under physiological conditions. CPZ treatment resulted in a significant decrease of the complex-valued shear modulus correlating with progressive demyelination and degradation of the extracellular matrix. The activation of macrophages/microglia, but also astrogliosis started early during the CPZ treatment at time points, when a significant impact on viscoelasticity was not yet detectable. However, when a subgroup of animals returned to normal chow, remyelination could be closely monitored in vivo by a recuperation of the viscoelastic properties.

Figure legend: A The complex-valued shear modulus of the CC increased continuously during adolescence, reaching a maximum at week 9. However, CPZ treatment decreased viscoelasticity significantly (see week 9 and 12). Halting the CPZ diet in week 9 resulted in a partial recovery. B Exampley G* maps of a CPZ treated (upper image) and a control mouse at week 12 (lower image). C In T2-weighted images, CPZ diet causes isointensity of the corpus callosum (arrows) with partial recovery due to remyelination when omitting the treatment (week 12, lower right panel). D CPZ diet induced strong astrogliosis in the corpus callosum (arrowheads) peaking at week 9 (GFAP staining). E CPZ diet resulted in demyelination (arrows) and accumulation of activated macrophages/microglia (arrowheads) in the corpus callosum (immunofluorescent stainings: red: fluoromyelin → myelin; green: IBA-1 → macrophages/microglia; blue: Hoechst → cell nuclei). F Remyelination in CPZ-treated mice was initially detectable at week 6 and reached a maximum at week 9 (fluoromyelin staining).