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X-ray phase contrast tomography reveals early vascular alterations and neuronal loss in neurological disorders

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Techniques previously used to investigate damage to vascular and neuronal networks in neurological disorders suffer from several limitations. In particular, 2D imaging restricts spatial coverage, entails destructive sample preparation, and may lead to data misinterpretation due to lack of information on the third dimension. In contrast, recent ex-vivo study in mice demonstrated that imaging by X-ray phase-contrast tomography (XPCT) enables the study of the 3D distribution of both vasculature and neuronal networks, without sample sectioning or specific preparation. We have generated and quantified multiscale XPCT to evaluate alterations in vascular and neuronal networks at relevant disease phases of the animal model for multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), in affected mice and to understand how treatment with mesenchymal stem cells (MSC) modifies them. A direct 3D morphological description of EAE lesions is provided at both vascular and neuronal levels at two different length scales, from the whole spinal cord up to capillaries and single cell. Such a multi-scale direct analysis has never been performed to understand EAE pathology and address the effect of an innovative therapeutic strategy. The results strongly indicate i) a trend in alteration of the micron vessels and occlusions in the capillaries, an observation never obtained in tissue without the use of a contrast agent; ii) neuronal alterations with massive loss of lower motor neurons. Such vascular and neuronal alterations were considerably reduced in MSC-treated mice. We have also applied XPCT to the investigation of other neurodegenerative disorders, i.e. Alzheimer and amyotrophic lateral sclerosis (ALS) and the results will be presented.

The degenerative effects of multiple sclerosis at the level of the vascular and neuronal networks in the central nervous system are currently the object of intensive investigation. Preclinical studies have demonstrated the efficacy of mesenchymal stem cell (MSC) therapy in experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis, but the neuropathology of specific lesions in EAE and the effects of MSC treatment are under debate. Because conventional imaging techniques entail protocols that alter the tissues, limiting the reliability of the results, we have used non-invasive X-ray phase-contrast tomography to obtain an unprecedented direct 3D characterization of EAE lesions at micro-to-nano scales, with simultaneous imaging of the vascular and neuronal networks. We reveal EAE-mediated alterations down to the capillary network. Our findings shed light on how the disease and MSC treatment affect the tissues, and promote X-ray phase-contrast tomography as a powerful tool for studying neurovascular diseases and monitoring advanced therapies.

X-ray Phase-Contrast multiscale-Tomography allows for the 3D simultaneous investigation of the neuronal and vascular microanatomy without any invasive sample preparation or sectioning, which characterize the conventional techniques and limit the reliability of the results.

In this study, 3-D analysis of vascular networks in EAE by micro-XPCT showed a decrease in vessel density at disease onset as compared to healthy mice, which was not observed in EAE-affected mice treated with MSC. While the effect of MSC on vascular remodeling in EAE has not been investigated, vascular alterations have been studied in MS and EAE by 2-D immunohistochemical analysis of tissue slices and/or vascular casting.In contrast to the results obtained with 3-D XPCT analysis, these techniques demonstrated features consistent with angiogenesis and endothelial cell proliferation in both MS and EAE. Such vascular remodeling appeared to be an early process in EAE induced with MOG, as increased vessel areas and endothelial cell proliferation were detected as early as 4 days post disease induction, that is in the pre-symptomatic phase5. However, in a different EAE model induced in rats with guinea pig spinal cord homogenate, an increased density in small blood vessels was observed at a relapsing, later stage of the disease. It has been suggested that this abnormal vascular remodeling response in EAE leads to the formation of leaky angiogenic blood vessels in the CNS concordant with the well-known loss of BBB integrity from early EAE stages.
Alteration of the capillary network in EAE has not been described before, nor has the alteration of the 10–24 micron vessels ever been evidenced without contrast agent. Our results strongly indicate a trend in alteration of the 10–24 micron vessels at the early stage of the disease and our 3D technique applied for the first time to the study of a neurodegenerative disease, has allowed us to image possible occlusions in the capillaries.

Thus, the XPCT has made it possible to investigate the vascular and neuronal alterations simultaneously during EAE. It corroborates findings by 2D techniques showing that MSC administration reduces vascular alteration of vessels as well as the damage to myelin and neurons in EAE, and provides a more informative 3D vision of the situation. In particular, our data support the findings of Vogt et al. who demonstrated, through electrophysiological and morphological analyses, a massive loss of lower motor neurons in MS patients and in mouse MOG-EAE from early disease stages, with evidence of apoptotic neuronal death. Several other studies have also reported neuronal apoptosis and/or abnormalities in the same and other models of EAE as also observed.

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