The Role of SOD2 in a Mouse Model of Multiple Sclerosis

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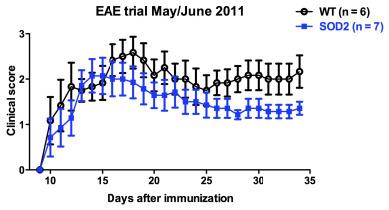
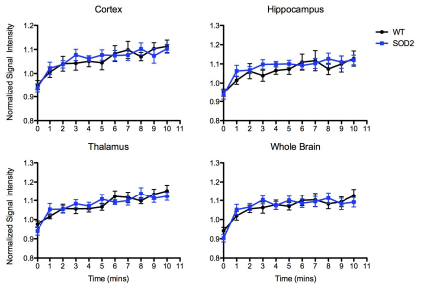
Background: Multiple Sclerosis (MS) is a demyelinating disease of the Central Nervous System (CNS) with demyelination occurring in the brain, spinal cord and the optic nerve. It is slow progressing and ultimately results in debilitation of afflicted individuals. It is characterized by periods of relapse and remission of neurological symptoms that include decreased vision, muscle weakness and loss of coordination. The etiology of this disease is currently unknown, however, it understood that autoimmune mechanisms play a key role in the development of this disease. In order to study this debilitating disease, an animal model of MS has been developed and is called the experimental autoimmune encephalomyelitis (EAE) model. These animals exhibit MS-like symptoms by immunizing them against myelin. Using this model, elevations in reactive oxygen species (ROS) have been implicated in the development of lesions in this disease. Some of the major sources of ROS in MS are activated microglia and macrophages, as well as mitochondrial dysfunction. As a result, *we hypothesized that lowering of ROS by overexpressing an antioxidant, superoxide dismutase 2 (SOD2) in an EAE model of MS would result in decreased EAE phenotype.*

Methods: Experiments were carried out using superoxide dismutase overexpressing (SOD-2^{OV}) mice and their WT controls starting at 10weeks of age. EAE was induced in mice by injecting an emulsion consisting of *mycobacterium tuberculosis*, Freund's adjuvant and mouse myelin oligodendrodrocyte glycoprotein (mMOG) into the flank along with pertussis toxin i.p. The disease severity was measured daily using a clinical scale from 0-5, where 0: no symptoms and 5: moribund. After 35 days of disease, blood-brain barrier (BBB) permeability was assessed by measuring T_1-times and T_1-weighting in conjunction with i.v. injected Magnevist® before and after the administration of contrast agent. Finally, spinal cord tissues taken from these animals were stained with H&E, for CD3, and myelin basic peptide (MBP). All animals were handled in compliance with institutional and national regulations and policies.



Graph 1: Disease severity is reduced in SOD-2OV mice compared to wild type after 35 days of EAE

Imaging Protocol: All images were obtained using a 9.4T, Bruker Avance BioSpec Spectrometer with a 21cm horizontal bore (Bruker BioSpin, Billerica, MA) and a 35mm resonator. Mice were anesthetized using 5% isoflurane with oxygen and placed into the animal holder, where they were kept at 2% isoflurane for the rest of the imaging time. Mice were imaged using a Rapid Acquisition with Refocused Echoes (RARE) protocol to obtain T_1-weighted images and RARE with Variable Acquisition Time (RAREVTR) protocol to measure T_1-times. Imaging parameters used for RARE: TE=11.721ms, TR=590.940 ms, FOV=25mm, matrix size=256x256, taking 2mins, 31s and 280ms and for RAREVTR: TE=10ms, TR=200 - 6000ms, FOV=20mm, matrix size=128x128, taking 4mins, 3s and 200ms using Paravision 4.0 software (Bruker BioSpin, Billerica, MA). During imaging, body temperature was maintained at 37.0°C using an animal heating system (SA Instruments, Stony Brook, NY).



Graph 2: BBB permeability was not detected across genotypes

Data Analysis: Obtained images were analyzed using Paravision 4.0 software. Regions of interest (ROI) within the cortex, hippocampus, thalamus and whole brain were selected. T_1-signal intensity (SI) and times within these ROIs were measured and normalized to SI measured from a water phantom. Graphs and statistics from MRI data, disease severity and immunohistochemistry were generated using Prism (GraphPad Software, San Diego, CA).

Results: After inducing EAE in SOD-2^{OV} and WT mice, we found improved disease severity in SOD-2^{OV} compared to WT mice. There was no difference in to blood brain barrier permeability between genotypes, however, there was a significant decrease in levels of immune infiltrates within the spinal cord of SOD-2^{OV} mice compared to WT. There was also less demyelination seen in the SOD-2^{OV} compared to WT.

Discussion: SOD-2^{OV} mice show an overall improved phenotype compared to their WT counterparts in both disease severity and immune infiltration and demyelination. This indicates that lowering ROS levels may play a neuroprotective role in this mouse model of MS. BBB permeability was not detected possibly due to the short disease course. It is feasible, however, that permeability of the blood-spinal cord barrier is compromised after 35 days due to the ascending nature of EAE. As a result, current and future studies will continue to look at blood barrier permeability within the spinal cord of both genotypes of EAE mice.

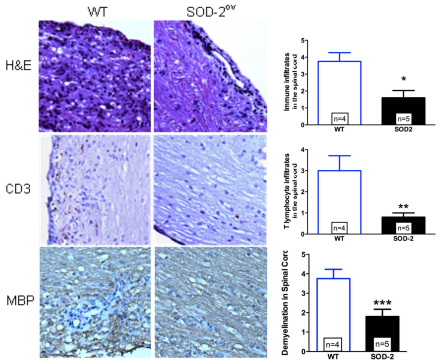


Figure 1: Immunohistochemistry show lower levels of immune infiltrates, T-lymphocyte presences and demyelination in SOD-2OV mice compared to WT

References:

(1) Van Horssen et al. *Biochimica et Biophysica Acta* 2011. 1812(2):141-150, **(2)** Wuerfel et al. *EJ Neuroscienc* 2007. 26(1): 190-198 **(3)** Schellenber et al. *MRM* 2007. 58(2): 298-305 **(4)** Ho et al. *Am J Respir Cel Mol Biol* 1998. 18(4): 538-547