Superoxide dismutase overexpression improves FA and ADC in the brains of a mouse model of Alzheimer's Disease

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Introduction: Alzheimer's disease (AD) is the most common form of age-related dementia with 5.3 million people affected in the United States alone (2010 AD report). Oxidative stress has been implicated in the pathogenesis of AD. Oxidative stress involves an imbalance in pro-oxidant and antioxidant molecules in favor of pro-oxidants such as superoxide anion. Previous studies have shown that the overexpression of the mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2) in the Tg2576 AD mouse model (SOD/AD) can reduce oxidative stress and pathological findings in the model, including reduced levels of amyloid beta, recovered cerebral blood flow and axonal transport rates, and improved performance in learning and memory tests (Massaad *et al.* 2009, Massaad *et al.* 2010). Age-related white matter damage has also been reported in Tg3576 mice using diffusion tensor imaging (DTI) (Sun *et al.*). To determine whether SOD overexpression in AD mice (SOD/AD) can recover white matter damage, we imaged aged SOD/AD mice and their littermates (AD, SOD, and wildtype) using DTI and assessed structural changes in the brains of the different transgenic mice.

Materials and Methods: *Mice:* Adult C57BL6 mice (WT), C57BL6 SOD2 overexpressing (SOD) mice (Ho *et al.*), APP Swedish mutationTg2576 mice (AD), and Tg2576 mice crossed to SOD2 overexpressing mice (SOD/AD) age 15-22 months were anesthetized and perfused. Tissues were processed as described by Tyszka *et al.* Briefly, the heads were fixed overnight in 4% PFA and then transferred to 0.01% sodium azide in PBS at 4°C. After one week, the heads were put in 5mM gadopentetate dimeglumine in 0.01% sodium azide at 4°C for 2 weeks. The samples were equilibrated to room temperature and then imaged. *Imaging:* Mice were imaged on a 9.4 Bruker Avance Biospec Spectrometer, 21-cm bore horizontal scanner with 35 mm volume resonator (Bruker BioSpin, Billerica, MA). A pilot scan was run to orient the scans. A reference scan was used to determine 3D volume placement so that the entire head was covered. The imaging parameters for the diffusion tensor scan were as follows: TR=500 ms; TE=15.4 ms; FOV=1.50x1x2 cm; matrix=256x96x164; NEX=1; diffusion directions=6. DTI scan took approximately 17 hours to acquire using Paravision software (Bruker BioSpin, Billerica, MA). Brains were aligned using (kindly provided by the Henkelman lab). DTI Studio was then used to calculate fractional anisotropy (FA) and apparent diffusion coefficients (ADC) in several brain regions. *Statistics:* One-way ANOVA with Bonferroni post hoc test. A p-value <0.05 was considered significant.

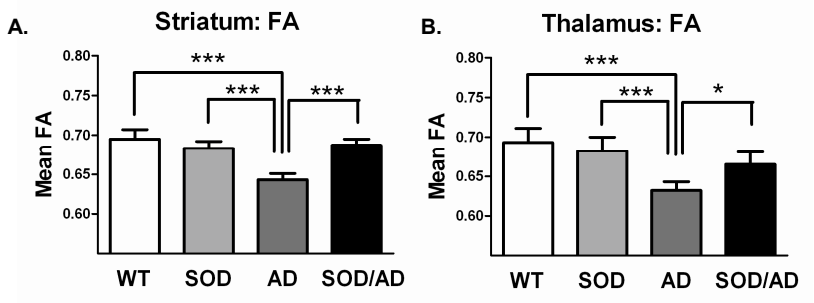

Figure 1. Mean FA values for WT, SOD, AD, and SOD/AD mice. **A**. FA values for the striatum. SOD/AD mice have significant recovery of FA. **B**. FA values for the thalamus. SOD/AD mice have a significant recovery of FA. * p-value<0.05; *** p-value<0.001.

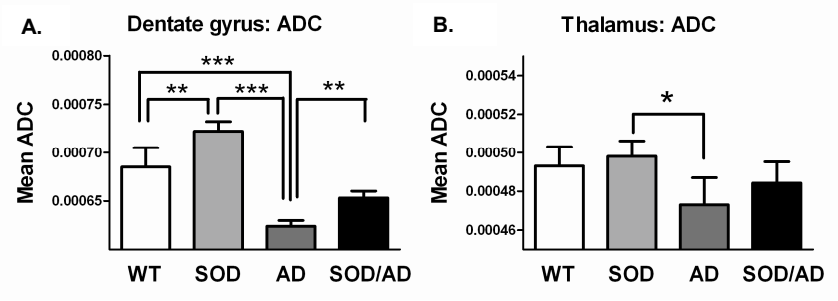

Figure 2. Mean ADC values for WT, SOD, AD, and SOD/AD mice. **A**. ADC values for the dentate gyrus. SOD/AD mice have significant recovery of ADC. **B**. ADC values in the thalamus. AD mice have a significant reduction in ADC from WT with a trend toward recovery in SOD/AD. * p-value<0.05; ** p-value<0.01; *** p-value<0.001.

Results: Significant decreases in FA and ADC were seen in AD mice compared to WT in several brain regions (Figure 1 and 2). In the striatum and thalamus was there a significant recovery of FA levels in AD mice overexpressing SOD2 (SOD/AD) (Figure 1a-b). The AD mice had significantly lower ADC in the dentate gyrus and thalamus (Figure 2a-b) compared to SOD mice. The dentate gyrus showed significant recovery in SOD/AD mice (Figure 2a).

Conclusion: Alzheimer's disease is a progressive form of neurodegeneration and is often associated with oxidative stress. Antioxidants can lower oxidative stress and may have a role in AD treatment. In our study, AD mice had reductions in their FA and ADC values in several brain regions including the thalamus. SOD2 overexpression in the AD mice recovered FA values in the striatum and thalamus and improved ADC values in the dentate gyrus. Additional brain regions and fiber tracking are being analyzed in DTI scans with 20 directions to determine other diffusion alterations in the brain induced by AD and recovered by SOD2 overexpression. These data could help to identify structures in the brains that are readily damaged by oxidative stress during AD and that could be targeted by future antioxidant therapies.

References:

Massaad, C.A. *et al.* Overexpression of SOD-2 reduces hippocampal superoxide and prevents memory deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA*. 106(32): 13576-13581 (2009).

Massaad, C.A. *et al.* Mitochondrial superoxide contributes to blood flow and axonal transport deficits in the Tg2576 mouse model of Alzheimer's disease. *PLoS One*. 5(5): e10561 (2010).

Sun, S.W. *et al.* Detection of age-dependent brain injury in a mouse model of brain amyloidosis associated with Alzheimer's disease using magnetic resonance diffusion tensor imaging. Exp Neurol. 191(1): 77-85 (2005).

Ho, Y.S. *et al.* Transgenic models for the study of lung antioxidant defense: enhanced manganese-containing superoxide dismutase activity gives partial protection to B6C3 hybrid mice exposed to hyperoxia. *Am J Respir Cell Mol Biol*. 18(4): 538-547 (1998).

Tyszka, J.M. *et al.* Statistical diffusion tensor histology reveals regional dysmyelination effects in the shiverer mouse mutant. *Neuroimage*. 29: 1058-1065 (2006).