

# Overexpression of SOD-2 Rescues Reduced Cerebral Blood Flow in the Tg2576 (APP) Mouse Model of Alzheimer's Disease

S. K. Amin<sup>1</sup>, C. Massaad<sup>1</sup>, L. Hu<sup>1</sup>, E. Klann<sup>2</sup>, and R. G. Pautler<sup>1</sup>

<sup>1</sup>Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, United States, <sup>2</sup>New York University, New York City, NY, United States

## Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized clinically by the impairments of cognitive functions and changes in behavior and personality. The accumulation of a small peptide called A $\beta$  is central to the pathogenesis of AD. In the Tg2576 mouse model of AD (designated as APP), soluble A $\beta$  levels start increasing at 6 months of age followed by plaque formation as early as 9 months of age.

Although the potential neurotoxic effects of A $\beta$  have been known for over a decade, it is not clear how A $\beta$  participates in the cascade of events that lead to AD pathology. Several reports have suggested that involvement of mitochondrial abnormalities and oxidative damage in the etiology of AD. A $\beta$  is thought to enter the mitochondria, induce generation of free radicals and lead to oxidative damage of the brain parenchyma<sup>1</sup>. Reactive oxygen species (ROS) that are formed in the mitochondria as a result of leakage from the electron transport system are usually scavenged by enzymes like the superoxide dismutase (SOD-2) and catalase.

Our current data (not shown) indicates that SOD-2 overexpression in APP mice reduces A $\beta$  plaque deposition, ameliorates the learning, memory, and the axonal transport deficits characteristic of the APP+ mice. Few studies, however, have addressed the involvement of A $\beta$  in oxidative impairment of cerebrovascular regulation in AD. Only two studies have provided evidence of vascular oxidative stress in APP mice and prevention of A $\beta$ -induced attenuation in functional hyperemia with free radical scavengers using laser-Doppler flowmetry.<sup>2,3</sup> Our work focuses on this aspect of A $\beta$  pathogenesis using APP/SOD-2 transgenic mice with FAIR-EPI (Flow-sensitive Alternating Inversion-Recovery EPI) arterial spin labeling (ASL). Based upon our studies showing that SOD-2 overexpression leads to a reversal of multiple AD phenotypes at the cellular level (manuscript in preparation), we wanted to determine if SOD-2 overexpression in APP mice is also capable of a similar reversal at a systemic level.

## Methods:

**Perfusion Arterial Spin Labeling (ASL):** 12-13 month old mice were sedated with 2% isoflurane in 100% oxygen. Following anesthesia, the mice were placed in a horizontal bore 9.4T Bruker Avance imaging system with the head positioned in the center of the probe and maintained in 1-2% isoflurane for the remainder of the imaging session. The body temperature of the mice was monitored and maintained at 37°C using an air heater. The imaging parameters were as follows: TR = 7145.973 ms, TE = 24.26 ms, Number of averages = 1. The inversion recovery time (TIR) = 100ms, the number of TIR = 8, the TIR increment = 1000 ms, the global inversion slab thickness = 6 mm, and the slice package margin = 2.50 mm. The effective bandwidth = 200kHz. The field of view (FOV) = 15 x 15 mm, matrix = 64 x 64 and slice thickness = 1 mm. We utilized Bruker Biospin's Paravision 4.0 software to calculate the rCBF. Briefly, the imaging slice was positioned transversely through the center of the cortex for both the slice selective and non-selective slice T1 series. Regions of interests (ROI) were defined for the calculation of T<sub>1</sub> for both selective and global inversion datasets: right cortex, left cortex. The data points from each ROI were fitted to a T<sub>1</sub> inversion regression curve. The values are then to calculate CBF according to: Relative CBF (rCBF) =  $\lambda (1/T_{1\text{selective}} - 1/T_{1\text{nonselective}})$  where  $\lambda$  is the blood-brain partition coefficient, a constant equal to 90ml blood/100g of tissue. The factor 60000 was used to convert ms to minutes and, consequently, the unit for rCBF is ml/(100g\*min). The rCBFs were compared among groups and processed utilizing the software, Prism. P values were determined utilizing a t-test analysis.

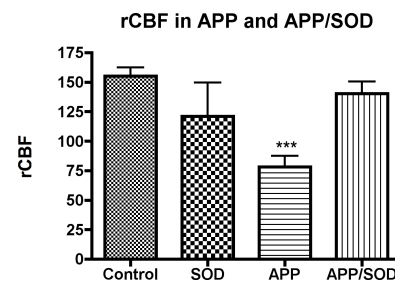
**Histology:** Heterozygous SOD-2 transgenic mice were crossed to heterozygous mutant APP mice to obtain wild type mice (WT), SOD-2 overexpressing mice (SOD-2+), mutant APP mice (APP) and mice that overexpress SOD-2 and mutant APP (APP/SOD-2+). Their brains were dissected and fixed overnight in a 4% paraformaldehyde solution, followed by overnight immersion in a 30% sucrose in PBS solution. After that, it was briefly washed in PBS, then 100% ethanol then frozen in isopentane on dry ice. The frozen brains were sectioned on a cryostat, and the obtained 25 $\mu$ m sections were mounted on glass slides. Staining for amyloid  $\beta$  plaques was performed using thioflavin S stain and then images taken utilizing a Zeiss fluorescence microscope.

## Results:

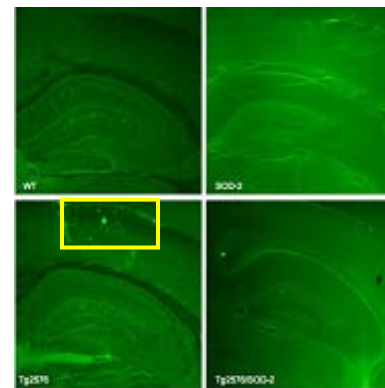
We observed a significant CBF reduction in the Tg2576 mice (APP) compared to both control groups (wild-type and SOD-2 mice) with an insignificant difference in CBF between the two controls (Fig. 1). In addition, when SOD-2 was overexpressed in T2576 mice (APP/SOD-2), the CBF was not only significantly higher than that of the Tg2576 mice (P value < 0.003), it was comparable to the CBFs of both control groups. Furthermore, the Thioflavin S staining of dense amyloid plaques in the cortex of the mice revealed plaque reduction in the APP/SOD-2 mice compared to the APP mice (Fig.2).

**Conclusion and Future Directions:** In conclusion, we provided evidence showing that not only does oxidative damage on cerebrovasculature occur early in the AD pathogenesis, but that it can also be "rescued" with overexpression of the major mitochondrial ROS scavenger (SOD-2). This study corroborates and strengthens what we found in our other studies—evidence that SOD-2 overexpression leads to a reversal of multiple AD phenotypes, starting at the cellular level where there is a significant reduction in amyloid plaques and a significant amelioration of the *in vivo* axonal transport rates, going to the system's level where it ameliorates the cognitive impairments as well as the cerebral blood flow deficits characteristic of AD. Our present findings support the idea that ROS formation precedes plaques in the chain of events of the disease. We are currently investigating the effect of SOD-2 overexpression on soluble A $\beta$  levels, which is thought to be a pivotal in AD. One interesting finding we have is that Tg2576 mice exhibit the same deficits at 8 months of age, prior to plaque deposition, and that SOD-2 overexpression alleviates these deficits (data not shown). Elucidating the mechanisms by which SOD-2 reverses AD symptoms will be critical for the design and possible use of pharmacological antioxidant agents for the treatment of AD.

**References:** 1. Vina, J et al, J Alz Dis, 2007, 11(2):175-181. 2. Park L, et al. Journal of Cerebral Blood Flow & Metabolism 2004; 24:334-342. 3. Niwa K, et al. Proc Natl Acad Sci 2000; 97(17): 9735-974



**Figure 1: SOD-2 reverses reduction in CBF caused by ROS in Tg2576 mice.** Inverse regression curves using ASL shows the following rCBF: WT (155.25, n=2), SOD-2 (121, n=2), APP(78.17, n=5), APP/SOD-2 (140.15, n=5). \*\*\*p < .003



**Figure 2: SOD-2 overexpression prevents plaque formation in Tg2576 (APP) mice.** Thioflavin S staining of dense amyloid plaques in WT (upper left), APP (lower left), SOD-2+ (upper right) and APP/SOD-2+ (lower right) mice. A region of dense plaques in the Tg2576 mouse is boxed in yellow. Note the lack of plaque formation in the APP/SOD-2 mouse.