Overexpression of SOD-2 reduces Aβ levels and improves the axonal transport deficits in the Tg2576 Alzheimer model mice

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Introduction: Alzheimer's disease (AD), the most common form of dementia in the elderly, is a progressive neurodegenerative disease characterized by impaired axonal transport and a decline in cognitive functions. It is now widely accepted that accumulation of Aβ, a small peptide with very high propensity for aggregation, is central to the pathology of AD. Although the potential neurotoxic effects of Aβ have been known for over a decade, it is not clear how Aβ participates in the cascade of events that lead to neurodegeneration. Recent evidence points towards the early forming soluble oligomeric Aβ as being the culprit, rather than the late accumulated plaques. Additionally, several reports suggest a link between mitochondrial reactive oxygen species (ROS) and AD. ROS that are formed in the mitochondria as a result of leakage from the electron transport system are usually scavenged by enzymes like the mitochondrial superoxide dismutase (SOD-2). Using the Tg2576 AD mouse model in conjunction with a mouse that overexpresses SOD-2, we showed that SOD-2 overexpression reduces plaque formation in the Tg2576 mice and improves the axonal transport and the learning and memory abilities that are deficient in the old AD mice (>16 months old). Our data in the old AD mice suggested an important role for mitochondrial ROS in AD pathology. In the present study, we measure the *in vivo* axonal transport rates in 8 months old animals, which already exhibit an increase in the soluble Aβ levels but have no plaques yet. We also determine the effects of SOD-2 overexpression on soluble Aβ levels. Our studies show that the axonal transport deficits occur in 8 months old animals prior to plaque formation, and that these deficits are reversed by SOD-2 overexpression. We also show that these deficits correspond to an increase in soluble Aβ levels, which are also reduced by SOD-2 overexpression. Taken together, our present and previous results suggest that mitochondrial ROS come first in the cascade of events leading to AD. The model would be:

ROS → soluble Aβ → Plaques + axonal transport deficits + cognitive impairments.

Methods: ***ELISA:*** Heterozygous SOD-2 transgenic mice were crossed to heterozygous mutant APP mice to obtain wild type mice (WT), SOD-2 overexpressing mice (SOD-2), Tg2576 mice, and mice that overexpress SOD-2 and mutant APP (Tg2576/SOD-2). Their brains were dissected and homogenized with a glass dounce homogenizer in cold Tris buffered saline containing protease inhibitors. Soluble Aβ was extracted from the brain homogenates by 2% SDS in TBS followed by sonication and centrifugation for 1 hour at 4°C at 100,000g. Insoluble (fibrillar) Aβ was extracted from the pellets by formic acid extraction followed by sonication and centrifugation for 1 hour at 4°C at 100,000g. The supernates from both steps, containing the soluble and insoluble Aβ species were then tested for the levels of Aβ1-40 and Aβ 1-42 by sandwich ELISA using Signet Aβ 1-40 and Aβ 1-42 kits with their proprietary reagents.

MEMRI: The same groups of mice were also tested for axonal transport deficits by manganese-enhanced MRI (MEMRI) in the olfactory bulb. Mice were anesthetized with 5% isoflurane in 100% oxygen. Following anesthesia, 0.77g/ml $MnCl_2$ was pipetted into the nasal cavity of the mouse at a total of 4 μl (2μl/naris). Mice were allowed to recover on a warming pad for about 45 minutes, allowing the loading of Mn^{2+} into the olfactory receptor neurons located in the olfactory epithelium. They were then sedated with 2% isoflurane in 100% Oxygen and then imaged for 80 minutes. The zero time point for imaging was at 60 minutes post Mn^{2+} exposure. T_1-weighted, spin-echo 2D data sets were acquired of the mouse brain using a horizontal bore 9.4T Bruker Avance imaging spectrometer with a micro-imaging gradient insert and a 30 mm birdcage RF coil. Mice were anesthetized and maintained on 1 – 2 % isoflurane in a seterotaxic holder for the duration of the imaging experiment. The imaging parameters were as follows: Multi-Slice/Multi Echo 2D imaging protocol, matrix dimensions = 128 x 128; FOV = 3.0 cm x 3.0; slice thickness = 1 mm; repetition time (TR) = 504.1ms; echo time (TE) = 8.2 ms; NA = 2, number of images = 15, time per image = 2 min. The short TR ensures a heavily T_1-weighted image that will provide positive signal enhancement in regions with an accumulation of the paramagnetic Mn^{2+}. Because axonal transport is a temperature dependent process, the body temperature of the mouse was monitored and maintained at 37°C using an air heater. 4 axial slices were selected with the first slice aligned with the leading edge of the olfactory bulb. Each slice spans 1 mm. In all studies, slice 2 of the 4 slices was assayed for axonal transport and the dorsal lateral portion of the olfactory bulb was selected as a region of interest (ROI). Changes in the signal intensity of this ROI were measured using Bruker's Paravision software and plotted using Microsoft Excel and GraphPad Prism. All signal intensities were normalized to non-enhanced muscle outside of the brain. A least squares method was used to determine the change in signal intensity over time, reflective of the rate of transport of Mn^{2+}.

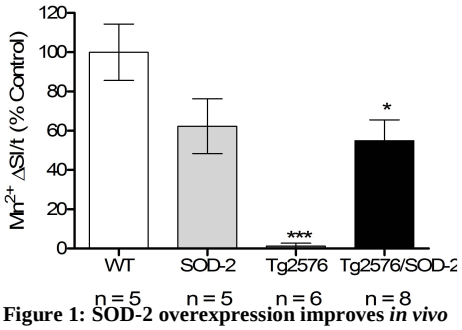

Figure 1: SOD-2 overexpression improves *in vivo* axonal transport deficits in Tg2576 mice. Normalized Mn^{2+} signal intensity over time in the olfactory bulb of 8 months old WT, SOD-2, Tg2576 and Tg2576/SOD-2 mice. Significance, as compared to control, was assessed by a student's t-test with *** p<0.001, * p<0.05

Results: Tg2576 mice have already been shown to exhibit abnormal learning behavior and amyloid β plaque deposition as early as 6 months of age and we confirmed that in our studies. They have also been shown (by our lab) to have blockade of axonal transport. Our current study shows that overexpression of SOD-2 in Tg2576 mice, prior to the onset of oxidative stress and prior to accumulation of plaques, leads to an alleviation of all these symptoms, indicating an involvement of mitochondrial ROS and soluble Aβ in the pathogenesis of AD. Figure 1 shows a significant deficit in axonal transport in Tg2576 mice and a full recovery of this deficit by overexpression of SOD-2. This effect is mirrored at the cellular level by an increase in soluble and insoluble (fibrillar) Aβ 1-40 and Aβ 1-42 in Tg2576 mice (Figure 2). SOD-2 overexpression leads to a reduction of all Aβ species with the exception of soluble Aβ 1-40 (Figure 2). We do however have early indication that Aβ 1-40 is reduced by SOD-2 at later stages (12 months – Data not shown).

Conclusion: In addition to the data reported here, we have behavioral, histological and MEMRI data showing a beneficial effect of SOD-2 on the learning deficits, axonal transport deficits, amyloid level increase and plaque accumulation exhibited in old mice with AD (>16 months). Taken together, these data indicate that mitochondrial ROS play a role in AD pathology, possibly upstream of Aβ events, and therefore overexpression of mitochondrial SOD will have beneficial effects on learning and axonal transport impairments observed in the animal model for AD.

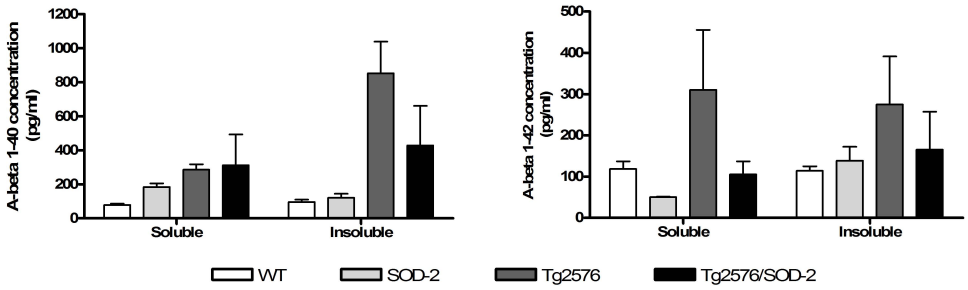

Figure 2: SOD-2 overexpression reduces the levels of soluble Aβ 1-42 and insoluble Aβ 1-40 and Aβ 1-42. Levels of soluble and insoluble Aβ 1-40 and Aβ 1-42 in 8 months old WT (n=3), SOD-2 (n=2), Tg2576 (n=3) and Tg2576/SOD-2 (n=2).

References: **1.** Reddy PH, Beal MF. Brain res Brain Res Rev 2005; 49(3): 618-632; **2.** Serrano F, Klann E. Ageing Res Rev 2004; 3(4): 431-443; **3.** Smith K et al., Neuroimage 2007; 35(4): 1401-1408; **4.** Hsiao K et al., Science 1996; 274 (5284): 99-102; **5.** Massaad CA et al., Proceedings of the ISMRM 2007; Abstract 937