The Role of Mitochondrial Superoxide in Alzheimer's Pathology

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Introduction:

Alzheimer's disease (AD), the most common form of dementia in the elderly, is a progressive neurodegenerative disease characterized clinically by the impairments of cognitive functions and changes in behavior and personality. It is now widely accepted that accumulation of A, a small peptide with very high propensity for aggregation, is central to the pathogenesis of AD. A is generated through proteolytic cleavage of amyloid precursor protein (APP) by - and -secretases, and it has been shown that mutations in the gene encoding APP can lead to excessive accumulation of A. One such mutation called the Swedish mutation (K670N:M671L) led to the formation of a transgenic mouse (Tg2576) very widely used as an animal model for AD. Although the potential neurotoxic effects of A have been known for over a decade, it is not clear how A participates in the cascade of events that lead to neurodegeneration. Recently, however, several reports have suggested the involvement of mitochondrial abnormalities and oxidative damage in the etiology of AD. A is thought to enter the mitochondria, induce generation of free radicals and lead to oxidative damage of the brain. 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 mitochondrial superoxide dismutase (SOD-2) and catalase. Our work focuses on this aspect of A pathogenesis. Specifically, we determined that SOD-2 overexpression in Tg2576 mice, prior to the onset of oxidative stress, prevents A plaque deposition and ameliorates the axonal transport deficits characteristic of the Tg2576 mice. We also have preliminary behavioral and electrophysiological results showing that SOD-2 improves the learning deficits observed in AD animals. Together, our results suggest an important role for mitochondrial ROS in AD pathogenesis.

Methods:

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 25m sections were mounted on glass slides. Staining for amyloid plaques was done using the thioflavin S stain and then pictures taken on a Zeiss fluorescence microscope.

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 a combination of 7.5 mg/ml of ketamine and 0.5 mg/ml xylazine at a dose of 0.17 ml/10g body weight. Following anesthesia, 0.77g/ml $MnCl_2$ was pipetted into the nasal cavity of the mouse at a total of 4 1 (2l/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 Advance imaging spectrometer with a micro-imaging gradient insert and a 30mm 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=128x128; 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. 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+}.

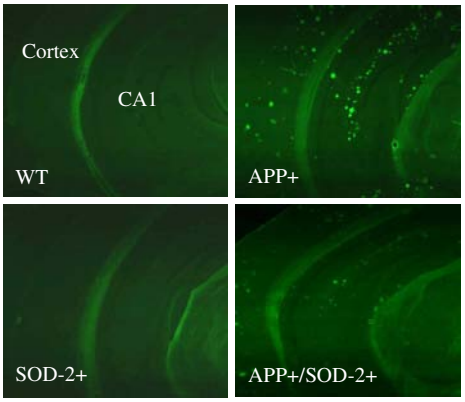

Figure 1: SOD-2 overexpression prevents plaque formation in APP+ mice. Thioflavin S staining of dense amyloid plaques in WT, APP+, SOD-2+ and APP+/SOD-2+ mice.

Results:

Tg2576 mice have already been shown to exhibit abnormal learning behavior and amyloid plaque deposition as early as 6 months of age. 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, leads to an alleviation of all these symptoms, indicating an involvement of mitochondrial ROS in the pathogenesis of AD. Figure 1 shows a large load of plaque deposition in the hippocampus (area CA1) as well as cortex of 24-months-old APP+ mice, as expected, but almost no plaque deposition in the APP+/SOD-2+ mice, indicating that mitochondrial SOD overexpression does alleviate the plaque burden caused by APP overexpression. Both WT and SOD-2+ mice do not have any plaque deposition. Figure 2 shows preliminary MEMRI studies in 24 months-old mice indicating that APP+ mice exhibit a null transport rate, which is consistent with work from the literature showing that APP blocks axonal transport. APP+/SOD-2+ mice appear to have a normal transport rate indicating that overexpression of SOD-2 in APP+ mice seem to have a beneficial effect on the APP-induced blockade of axonal transport.

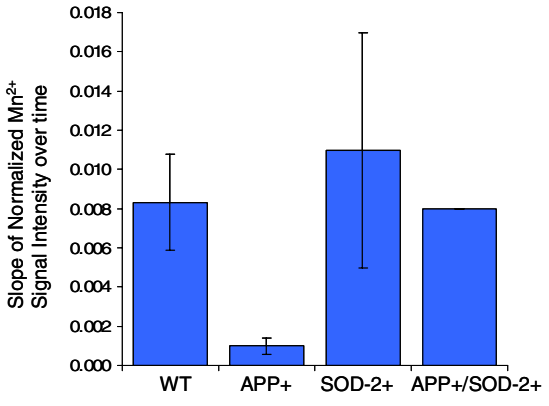

Figure 2: SOD-2 reverses the blockade of axonal transport caused by APP in Tg2576 mice. Normalized Mn^{2+} signal intensity over time in the olfactory bulb of WT, APP+, SOD-2+ and APP+/SOD-2+ mice. Linear regression shows the following slopes: WT (0.008, n=3), APP+ (0.001, n=2), SOD-2+ (0.011, n=3) and APP+/SOD-2+ (0.08, n=1)

Conclusion and future directions:

In addition to the data reported here, we have preliminary behavior and electrophysiology experiments showing a beneficial effect of SOD-2 on the learning deficits exhibited in mice with AD (data not shown). Taken together, these data indicate that mitochondrial ROS possibly play a role in AD pathology and therefore overexpression of mitochondrial SOD will have beneficial effects on learning, synaptic plasticity and axonal transport impairments observed in the animal model for AD. The effect of SOD-2 overexpression in Tg2576 mice will be investigated over time (4,6,12 and 16 months of age) to determine the critical age at which the beneficial effects of SOD-2 take place. The study will include an assessment of learning and memory by behavioral and electrophysiological studies as well as measurements of plaque deposition and axonal transport by MEMRI, both in the olfactory bulb and in the hippocampal formation, following setereotaxic Mn^{2+} injections.

References:

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