Nano-antioxidants improve axonal transport deficits in a mouse model of Alzheimer's disease

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Background: As the 6th leading cause of death and the leading cause of dementia in the US, Alzheimer's disease (AD) is a neurodegenerative disorder where sufferers undergo progressive cognitive decline, as well as a gradual loss of memory, cognition and behavioral stability. An integral part of normal brain function is fast axonal transport (FAT), which is responsible for the movement of cellular cargoes. Studies have shown in multiple models of AD that deficits in FAT develop before amyloid-β plaque and tau deposition [1, 2], with oxidative stress being implicated in the process. In particular, reactive oxygen species (ROS) have proven to decrease FAT [3], while overexpression of innate anti-oxidants can rescue axonal transport deficits in AD [4,5]. Therefore we **hypothesize** that that PEG-ylated hydrophilic carbon clusters (PEG-HCCs) with anti-oxidant capabilities [6] can reduce ROS related axonal transport deficits in a mouse model of Alzheimer's disease.

Methods: ***Animal Model*:** Experiments were carried out using the APP/PSEN1 mouse model of Alzheimer's disease. Mice, at 3 and 5 months of age, were treated with 2 µl per naris of 0.0685 mg/kg PEG-HCCs or vehicle control. Treatment continued every 3 days for a maximum of 10 doses. 24h after last treatment manganese axonal transport rates were measured using manganese enhanced MRI (MEMRI). Prior to imaging, mice were anesthetized with 5% isoflurane with oxygen and received 0.75g/mL manganese chloride ($MnCl_2$) pipetted into the nasal cavity for a total of 4µl (2µl/naris). All animals were handled in compliance with institutional and national regulations and policies. Animal protocols were approved by Institutional Animal Subjects Committee at Baylor College of Medicine.

Imaging Protocol: Images were obtained using a 9.4T, Bruker Avance BioSpec Spectrometer, with a 21cm horizontal bore and 35mm resonator (Bruker BioSpin, Billerica, MA). 1 hour post-lavage animals were anesthetized as described previously and maintained during imaging with 2% isoflurane. Animals were placed in a prone position within a mouse holder, with a water phantom, and body temperature (37.0°C) and breathing rate (40bpm) were maintained using a small animal monitoring and gating system (SA Instruments, Stony Brook, NY). One hour post-injection, mice were imaged using a multi-spin/multi-echo (MSME) protocol with TE=8.54ms, TR=504ms, FOV=3cm, matrix size=128x128, NEX = 2; number of cycles = 15; with each cycle taking ~2min using Paravision 5.0 (Bruker BioSpin, Billerica, MA).

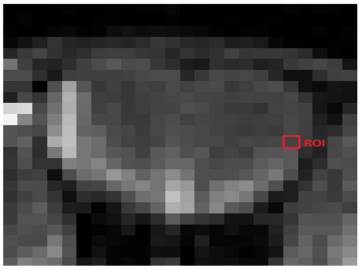

Fig 1: Representative MRI demonstrating the ROI in the olfactory receptor neurons.

Data Analysis: Images were analyzed using Paravision software. A region of interest (ROI) was selected in the dorsal lateral portion of the right olfactory bulb (OB) and the water phantom consisting of one pixel and 9 pixels respectively and copied across each of the 15 cycles. Signal intensities (SI) of the OB and water phantom ROIs were measured (Fig 1). SI values obtained for the OB were then normalized to those from the water phantom. The correlation between normalized signal intensity in the OB and time were assessed using Prism (GraphPad Software, San Diego, CA).

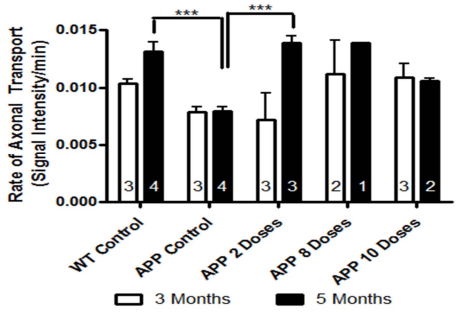

Fig 2: Rate of axonal transport measured *in vivo* via MEMRI in 3 (open bar) and 5 (closed bar) month old WT, and APP mice treated with vehicle or 2, 8 or 10 doses of PEG-HCCs.
*** $p < 0.001$

Results: Using MEMRI, axonal transport rates were measured for 3 and 5 month old WT and APP/PSEN1 mice. Decreases in axonal transport in APP/PSEN1 mice compared to WT littermates are evident at 3 months of age, reaching significance by 5 months (Fig 2). When treated with 2, 8 or 10 doses of PEG-HCCs axonal transport rates increased in 3 month old APP/PSEN1 mice. In 5 month old APP/PSEN1 mice, rates significantly increased in as few as 2 doses of PEG-HCCs.

Discussion: Preliminary data indicates that impairments in axonal transport begin as early as 3 months of age in APP/PSEN1 mice, further decreasing with age. Furthermore, deficits in axonal transport can be restored with the use of anti-oxidant PEG-HCCs, implicating ROS in the degradation of fast axonal transport during AD.

References:

[1] Kim et al. Neuroimage. 2011
[2] Stokin et al. Science. 2005
[3] Fang et al. Mol Neurodegeneration. 2012
[4] Massaad et al. PNAS. 2009
[5] Massaad et al. PlosOne. 2010
[6] Lucente-Schultz et al. J. Am. Chem. Soc. 2009