Characterization of a Novel MRI-Detectable Nanoantioxidant

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Background: Oxidative stress plays a key role in the pathogenesis and progression of many neurodegenerative diseases such as Alzheimer's disease (AD) and multiple sclerosis (MS). Previous studies in our lab and others, have shown that overexpression of an endogenous antioxidant results in improved outcome, including disease severity, learning and memory, pathology, axonal transport rates, as well as cerebral blood flow in a mouse models of AD. As a result, many antioxidant-based treatments have been tested unsuccessfully due low efficacy, efficiency and poor delivery to needed regions. Recently, we have developed a novel antioxidant, PEGylated-hydrophilic carbon clusters (PEG-HCC) in collaboration with the Tour Laboratory at Rice University that has high efficacy, is targetable, can be utilized as a vector and has been shown to be non-toxic. Modifications have been performed to nanoantioxidants to make them blood-barrier permeable with the addition of adamantane (ADA-PEG-HCC), as well as detectable via MRI with the addition of gadolinium (GDAP). As a result, we hypothesize that our novel nanoantioxidant will be able to address previously unmet needs by earlier antioxidants in the treatment of neurodegenerative disease, namely being able to cross into the central nervous system (CNS), as well as be highly efficacious in scavenging radicals and MRI detectable.

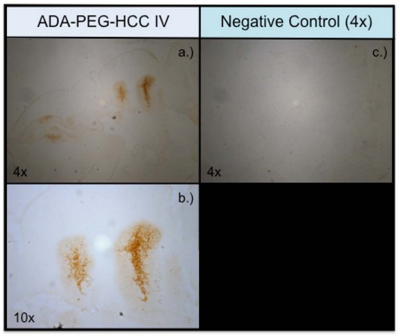

Figure 1: Immunohistochemistry staining for PEG showed positive staining within the cerebellum after i.v. injection with ADA-PEG-HCC

Methods: WT C57 mice were given intravenous (. v.) injections of approximately 100µL of a 200mg/L concentration solution of ADA-PEG-HCCs. Twenty-four hours post-lavage, mice were intracardially perfused with 10% formalin and brain tissue extracted and prepared for cryosectioning. Brain tissue was cryosectioned into horizontal sections and stained with an antibody against PEG. Immunohistochemistry images were taken using a simple light microscope at 4x and 10x magnification. ***Imaging Protocol:*** Images were obtained using a 9.4T, Bruker Avance BioSpec Spectrometer with a 21cm horizontal bore (Bruker BioSpin, Billerica, MA) and a 35mm resonator. Phantoms were imaged using a Rapid Acquisition with Refocused Echoes (RARE) protocol to obtain T_1-weighted images and RARE with Variable Acquisition Time (RAREVTR) protocol to measure T_1-times. Imaging parameters used for RARE: TE=11.721ms, TR=590.940 ms, FOV=25mm, matrix size=256x256, taking 2mins, 31s and 280ms and for RAREVTR: TE=10ms, TR=200 - 6000ms, FOV=20mm, matrix size=128x128, taking 4mins, 3s and 200ms using Paravision 4.0 software (Bruker BioSpin, Billerica, MA). ***Data Analysis:*** Obtained images were analyzed using Paravision 4.0 software. Regions of interest (ROI) within each phantom were selected. T_1-signal intensity (SI) and times within these ROIs were measured. Graphs were generated using Prism (GraphPad Software, San Diego, CA).

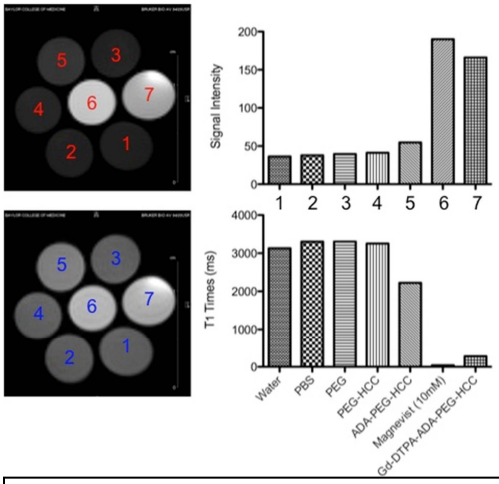

Figure 2: Phantoms of the GDAP and controls were imaged using T1-weighting and measuring T1 times. The T1 signal of GDAP is comparable to a 10mM solution of Magnevist(R) and the T1 times of GDAP also is decreased comparably to the 10mM Magnevist(R) solution. 1-Water, 2-PBS, 3-PEG, 4-PEG-HCC, 5-ADA-PEG-HCC, 6-Magnevist®, 7-Gd-DTPA-ADA-PEG-HCC

Results: Immunohistochemistry of the brain sections showed positive DAB staining in the cerebellum of the mouse given the i.v. injection of ADA-PEG-HCC compared to vehicle injected control sections (Figure 1). MRI T_1-weighted imaging and T_1-time measurement of the Gd-DTPA-ADA-PEG-HCCs and controls showed high degree of signal, intensity as well as lower T_1 times in GDAP phantoms which was comparable to 10mM solution of Magnevist® (Figure 2).

Discussion: Immunohistological staining for ADA-PEG-HCCs in the mouse brain sections indicate that the PEG-HCCs can enter the CNS through the blood-brain barrier with the help of the adamantine group on the PEG-HCCs. The positive data acquired from the MRI analysis of GDAP indicate that the uptake of the molecule can potentially be tracked after administration *in vivo* and also biodistribution of the molecule can also be tracked longitudinally in an *in vivo* system. Overall, these agents show great promise as a therapeutic in that they are highly efficient radical scavengers, they can cross into the CNS, they have also been utilized as vectors, and finally, they can potentially be visualized and tracked in an animal model of human disease.

References: **(1)** Lucente-Schultz et al. *J Am Chem Soc* 2009 131(11): 3934-41, **(2)** Berlin et al. *ACS Nano* 2010 4(8): 4621–4636, **(3)** Massaad et al. *PNAS* 2009 106(32):13576-81, **(4)** Massaad et al. 2010 PloS One 5(5):e10561