A Novel Assay for the *in vivo* Detection of Reactive Oxygen Species Using MRI

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Background: Under normal physiological conditions there is a fine regulation between reactive oxygen species (ROS) and antioxidants within the body. However, in multiple disease states, increases in ROS or a decrease in endogenous antioxidants can cause a perturbation in redox homeostasis. When this balance is disrupted damage can occur to major cellular components such as the nucleus, mitochondrial DNA, membranes, and cytoplasmic proteins. Because of the profound role increases in ROS plays in multiple diseases, including liver, diabetes and neurodegenerative diseases, technologies that enable the ability to detect ROS levels in live animals would undoubtedly provide unprecedented insights into multiple preclinical models of human disease. Recently, our collaborators have designed nano-antioxidant PEG-HCCs, which not only rapidly quench superoxide and hydroxyl radicals but also have been shown to enter cells. Furthermore, the PEG-HCCs accumulate in the liver where they are eventually broken down.

An additional important feature of superoxide and hydroxyl radicals are they have an unpaired electron and thus are paramagnetic, resulting in a shortening of the spin-lattice relaxation time constant, T_1, rendering them MRI detectable. Therefore, in tissues where there are significant increases in superoxide and hydroxyl radicals, there should be a concomitant decrease in T_1. We therefore **hypothesize** that it is possible to use MRI in conjunction with the PEG-HCCs to measure *in vivo* ROS levels. That is, based upon the inherent paramagnetic properties of superoxide and hydroxyl radicals, we should be able to perform a quench assay by measuring the T_1 values before and after the addition of the PEG-HCCs.

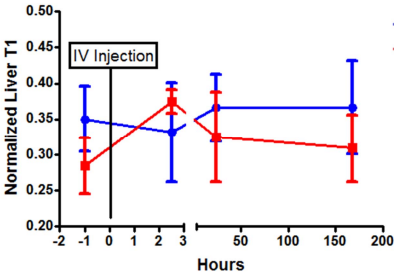

Fig 1: Timeline of normalized liver T_1 before and after PEG-HCC administration in WT (blue) and KO (red) mice. n=2

Methods: ***Animal Model:*** Experiments were conducted with NADH:ubiquinone oxidoreductase iron-sulfur protein 4 (NDUFS4) homozygous KO mice. NDUFS4 is a non-enzymatic, nuclear encoded subunit of complex I. Defects in NDUFS4 KO animals show reduced levels of NDUFS4 protein in various tissues, partial complex I deficiency, increased oxidative stress in brain, liver and heart, and impaired cellular and mitochondrial respiration. WT and KO NDUFS4 mice received a baseline T_1 measurement for the region of interest (ROI). Next, 2mg/kg of PEG-HCCs was administered intravenously and T_1 values were obtained at several time points post injection. 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: Mice were anesthetized using 4% isoflurane, placed into the animal holder with a water phantom, and sustained in an anesthetic plane with 2% isoflurane during imaging. Body temperature (37.0°C) and breathing rate (40bpm) were maintained using a small animal monitoring and gating system (SA Instruments, Stony Brook, NY). All 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 protocol with Variable Acquisition Time (RAREVTR) protocol to measure T_1-times. Imaging parameters used for RAREVTR: TE=6.57ms, TR=30.984 - 15000ms (5 images), Rare Factor =4, FOV=3x3cm matrix size=128x128, taking 11m, 48s and 399ms using Paravision 5.1 software (Bruker BioSpin, Billerica, MA).

Data Analysis: Images were analyzed using Paravision software. Regions of interest (ROI) within the liver and the water phantom were selected. T_1 values of the liver and water phantom ROIs were measured. T_1 values obtained for liver were then normalized to those from the water phantom. Graphs and statistics from MRI data were generated using Prism (GraphPad Software, San Diego, CA).

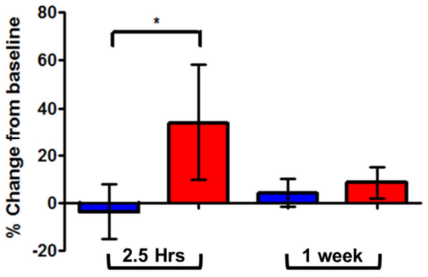

Fig 2: Percent change of normalized liver T_1 from baseline 2.5 hrs and 1 week after PEG-HCC administration in WT (blue) and KO (red) mice. n=2 *$p < 0.05$

Results: Baseline T_1 values in the liver of KO mice (n=2) were found to be lower than those of WT littermates (n=2). After PEG-HCC administration T_1 values in WT mice stayed relatively unchanged while the T_1 in the liver of the KO mice increased after 2.5hrs and returned to baseline levels after 1 week (Fig 1). Two way ANOVA analysis of the percent change in normalized liver T_1 shows that the percent change curves for WT and KO mice pre, 2.5, hours, 24 hours, and 1 week after Peg-HCC injection are statistically different. Further analysis demonstrates that the percent change in normalized liver T_1 is significantly larger for the KO mice than the WT mice (Fig 2).

Discussion: Preliminary data demonstrates our ability to measure differences in T_1 relaxation due to superoxide and hydroxyl radicals. This is further validated after administration of nano-antioxidant PEG-HCCs when decreased T_1 levels in mice exhibiting increased oxidative stress are returned to WT levels.