

Nanoantioxidants in the treatment of diabetic complications

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Background:

Diabetes Mellitus (DM) is a metabolic diseases affecting more than 23.6 million people in the United States alone. If left untreated, diabetes has been shown to cause an elevation in reactive oxygen species (ROS), leading to oxidative stress. Oxidative stress has been shown to modify macromolecules such as DNA, proteins and lipids and activate stress-signaling pathways. It is associated with many of the diabetic complications seen in DM such as high blood pressure, stroke, and other vascular abnormalities, which increase the patient's risk of premature death. One of the current theories is that DM complications can be reduced by lowering oxidative stress. Previous antioxidant-based therapies, however, have shown mixed results in clinical trials, possibly due to low potency and/or low localization in needed areas of the antioxidants used. Recently, the Tour laboratory at Rice University has reported that hydrophyllic carbon clusters (HCC) are potent antioxidants with approximately 5 times the radical scavenging capacity of trolox, a vitamin E analogue. In our current work, we assessed the regional cerebral blood flow (rCBF) in diabetic mice treated with PEGylated-HCCs and our data demonstrates that there is an improvement in rCBF post-treatment.

Methods: Experiments were carried out using WT C57 mice either injected with streptozotocin (STZ) (0.17g/g b.w.) or sodium citrate vehicle. STZ is toxic to pancreatic beta cells and injected mice are a model of type 1 diabetes. After 4 weeks of hyperglycemia (blood glucose level of >250mg/dL) rCBF of mice was measured via MRI at baseline, 1, 2, 3 and 4 hours after injection of 100 μ L of PEG-HCC ([130mg/L]). All animals were handled in compliance with institutional and national regulations and policies.

Imaging Protocol: 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. Mice were anesthetized using 5% isoflurane with oxygen and placed into the animal holder, where they were kept at 2% isoflurane for the rest of the imaging time. Mice were imaged using a flow sensitive alternating inversion recovery (FAIR) arterial spin labeling (ASL) echo planar imaging (EPI) protocol before treatment, at 1, 2, 3 and 4 hours post-treatment with PEG-HCCs. Imaging parameters used: TE=16.73ms, TR=7555.373ms, FOV=15mm, matrix size=64x64, NEX=2

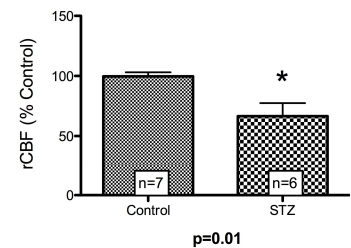


Figure 1: STZ treated mice show a significant decrease in cortical rCBF after 4 weeks

taking approximately 2 mins and 885 ms using Paravision 4.0 software (Bruker BioSpin, Billerica, MA). Selective and nonselective ASL images were acquired for each mouse. During imaging, body temperature was maintained at 37.0°C using an animal heating system (SA Instruments, Stony Brook, NY).

Data Analysis: Obtained images were analyzed using Paravision software. Regions of interest (ROI) within both the left and right cortex were selected. T1 times within these ROIs were measured and rCBF was calculated. Graphs and statistics were generated using Prism (GraphPad Software, San Diego, CA).

Results: Diabetic mice show a significant decrease in cortical rCBF at 4 weeks after injection with STZ (Fig 1). rCBF of diabetic mice treated with PEG-HCC

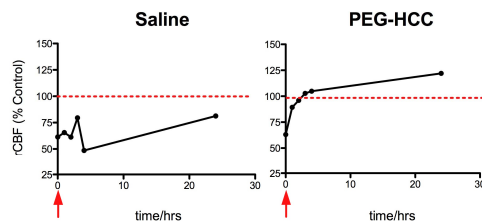


Figure 2: Diabetic mice show an increase in rCBF measured after i.v. treatment with PEG-HCCs.

shows increased blood flow within the cortex within 4 hours of treatment compared to animals treated with vehicle saline (Fig 2). This increased blood flow can be visualized using blood flow maps shown in Fig 3.

Discussion: Our data indicates that there is an increase in rCBF within the cortex of diabetic mice after treatment with PEG-HCC potentially as a result of decreased oxidative stress within the vasculature. These results indicate the potential of PEG-HCCs as nanoantioxidants in the treatment and prevention of complications that result from diabetes, as well as a host of other diseases where oxidative stress plays a major role in disease progression and pathology.

References:

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- (3) Lucente-Schultz RM et al. J. Am. Chem. Soc 2009 Mar;131(11):3934-3941.

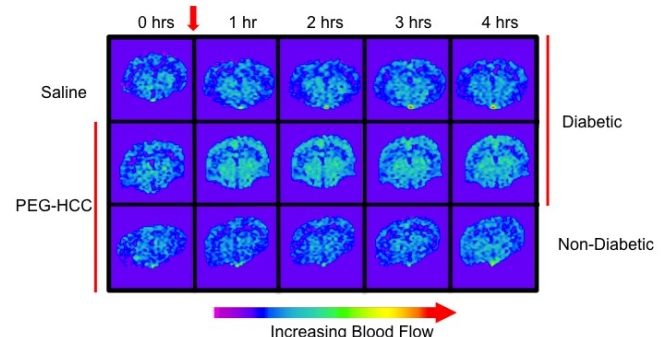


Figure 3: Blood flow maps demonstrate increase in rCBF over a 4 hour period of time in diabetic mice treated with PEG-HCC