

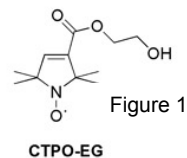
Nitroxide Compound CPTO-EG as an MRI contrast Agent

A. Nencka¹, X. Liu¹, J. Jeseoph¹, S-J. Li¹, and B. Kalyanaraman¹

¹Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, United States

Introduction: The conventional T1 contrast agent, gadolinium-DTPA complex, does not cross the blood brain barrier (BBB) in the absence of injury or tumor. Nitroxides possess a single unpaired electron but generally do not cross the BBB. The compound we developed can cross the BBB and provide MRI contrast in brain imaging by shortening the longitudinal relaxation time (T1) of water protons (1). Present study reports the progress of the development of a contrast agent, 2,2,5,5-tetramethylpyrroline 1-oxide 3-ethyleneglycolcarboxylate (CTPO-EG) (figure 1), which can cross the BBB and has significantly greater relaxivity.

Methods: Four mice were processed individually and were subsequently averaged for analysis. Data were acquired on a Bruker 30 cm bore 9.4T animal imager, in accordance with institutional procedures. Scan parameters for the spoiled gradient recalled echo pulse sequence included: 2.4 cm field of view, 96 × 96 acquisition matrix, 1 mm slice thickness, 3.9 ms echo time, 104 ms repetition time, 10 s effective repetition time, 25 kHz receiver bandwidth, 40° flip angle, 7 sequential slices, and 120 repetitions. A dosage of 300 mg/kg CTPO-EG was administered to the mice three minutes into the scan. Processing was performed by: reconstructing to reduce motion and flow artifacts, registering each data set to the first repetition, generating a mask of voxels within the brain, and smoothing the data with a 2 mm FWHM Gaussian filter. Group analysis was performed by registering each processed run to one run, averaging the processed data sets, and fitting a gamma-variate nonlinear model to the average data set. The contrast agent relaxivity was found through a phantom study to be 3.59 s⁻¹/[mg/ml]. Contrast agent concentration was calculated to be [agent]=(R₁-R₁₀)/relaxivity, where R₁ is 1/T₁ of the enhanced tissue at a time point and R₁₀ is 1/T₁ for the unenhanced tissue.



Results: Global enhancement of nearly 8% was observed in the brain after the agent injection, as seen in Figure 2. Figure 3 illustrates the signal enhancement over time at two minute intervals, starting one minute after injection. Figures 4 and 5 illustrate the observed T₁ time series in the thalamus and cortex. Figures 6 and 7 include the average calculated agent concentration time series in the cortex and thalamus. It is apparent in these figures that the CTPO-EG contrast agent accumulated more strongly in the thalamus than the cortex.

Discussion: We have demonstrated that 1) The contrast agent nitroxide CPTO-EG is water soluble and can pass the BBB of the mice brain; 2) The agent is a T1 relaxer with highly detectable sensitivity; 3) The nitroxide agent is very stable in the brain and was not reduced too quickly. There is a need to develop a contrast agent to measure the redox status because increased reactive oxygen species and decreased antioxidant defense may contribute to numerous brain disorders such as stroke, amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. Development of a blood-brain barrier permeable nitroxide contrast agent is a promising strategy to monitor brain disorders caused by oxidative stress.

Reference:

1. Fuminori Hyodo et al., *Journal of Cerebral Blood Flow & Metabolism* (2008) 28, 1165–1174.
2. V. Calabrese et al. *Nature Review Neuroscience* 8, 766, 2007.

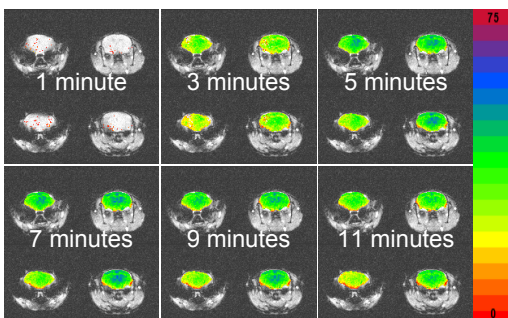
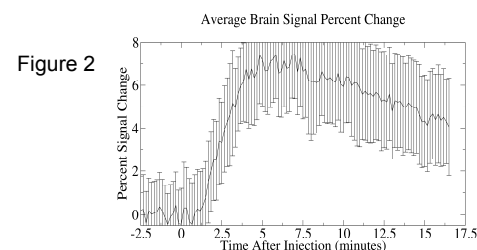


Figure 3

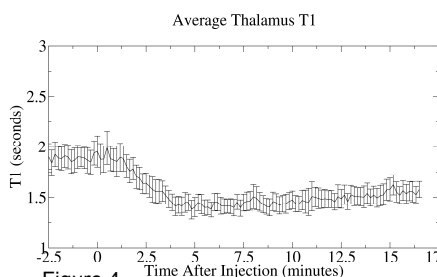


Figure 4

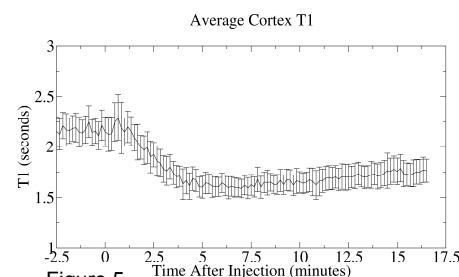


Figure 5

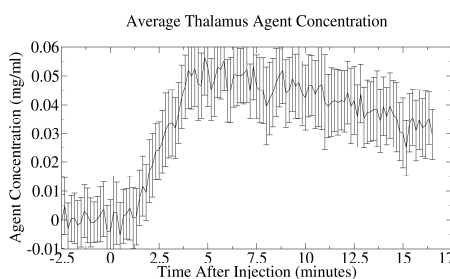


Figure 6

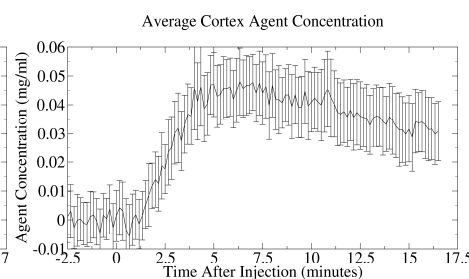


Figure 7