Hyperpolarized ^{13}C Ascorbates in the Anesthetized Rat Brain

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INTRODUCTION: Reduction and oxidation (redox) chemistry is involved in both normal and abnormal brain function, in processes as diverse as circadian rhythms and neurotransmission. Intracellular redox is maintained by coupled reactions involving NADPH, glutathione (GSH), and vitamin C (VitC), as well as their corresponding oxidized counterparts. The reducing agents GSH and VitC are maintained at high concentrations in the brain, and have a critical role in dealing with reactive oxygen species (ROS) seen as culprits in aging, neurodegenerative disease, and ischemic injury. We have developed [1-^{13}C] dehydroascorbate [DHA], the oxidized form of VitC, as an endogenous redox sensor for *in vivo* imaging using hyperpolarized (HP) ^{13}C spectroscopy [1]. In contrast to VitC, DHA readily crosses the blood-brain-barrier (BBB) and may play a key role in maintaining cerebral ascorbate levels (**Figure 1**). The goal of this study was to compare HP [1-^{13}C] DHA and HP [1-^{13}C] VitC in the normal brain.

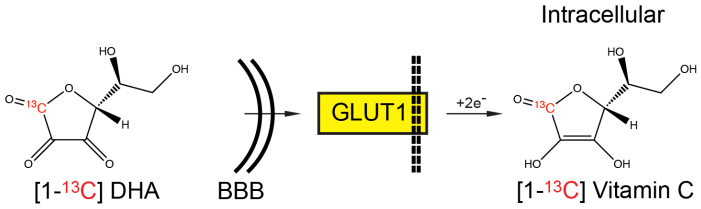

Figure 1. ***In vivo*** **reduction of [1-^{13}C] DHA following transport across the blood-brain-barrier, transport via GLUT family transporters, and intracellular reduction (thought to be GSH-mediated)**

METHODS: ***Synthesis of [1-^{13}C] DHA:*** [1-^{13}C] DHA (Isotec, Miamisburg, OH) was synthesized using a published method [2]. ***Hyperpolarization and dissolution of [1-^{13}C] DHA and [1-^{13}C] Vitamin C:*** A 2.2M solution of [1-^{13}C] DHA in dimethyacetamide (DMA) containing 15mM OX063 trityl radical (Oxford Instruments) was hyperpolarized on a HyperSense DNP instrument (Oxford Instruments) as previously described [3]. Similarly, a 2.2M solution of [1-^{13}C] Vitamin C (Omicron, South Bend, IN) was prepared as a sodium salt in NaOH/ water/ dimethyl sulfoxide (DMSO) containing 15mM OX063. This compound was polarized by an identical method and dissolved in 100 mM phosphate buffer, pH 7.0. ***3T Studies:*** *In vivo* 2D ^{13}C MRSI studies were performed using a 3T MRI scanner (GE Healthcare, Waukesha, WI) equipped with the MNS (multinuclear spectroscopy) hardware package as previously published [4] with 5mm x 5mm in-plane resolution (slab thickness 20mm). 2 mL of 15 mM HP [1-^{13}C] DHA solution were injected similar to previously described methods for [1-^{13}C] pyruvate. Similar experiments were performed using HP [1-^{13}C] Vitamin C at 50 mM. In all cases the imaging sequence was initiated 10 s following completion of a 15 s injection (representing a total delay of 25 s). ***Data Processing and Analysis:*** MRSI data was processed using custom software written in IDL 8 (ITT Visual Information Solutions, CO, USA) and Matlab 2009b (MathWorks, MA, USA). DHA and Vitamin C resonances were integrated and peak heights were used to calculate relevant ratios. Average metabolite ratios (VitC/ [VitC + DHA]) were calculated for voxels corresponding to brain and surrounding tissues.

RESULTS: The transport of DHA *in vivo* occurs by a mechanism analogous to that of glucose, by facilitated diffusion using GLUT1, GLUT3, and GLUT4. Since glucose is known to compete with the uptake of DHA, *in vivo* experiments were performed on rats fasted overnight. Animals were also pre-treated with a DHA dose identical to that administered during the imaging experiment, 1-hour prior, which has been shown to reduce the physiologic effects of DHA administration [5]. Data obtained are summarized in **Figure 2**. For HP [1-^{13}C] DHA studies (**Figure 2a**), voxels corresponding to brain were compared with those within in the surrounding soft tissues. For brain voxels (n=16) the average VitC/ [VitC + DHA] was 0.51 ± 0.10 and average VitC/ DHA was 0.51 ± 0.10 (**Figure 2c**). Remarkably, no VitC resonances were observed in voxels outside the brain. In contrast, following injection of HP [1-^{13}C] VitC, no observable oxidation to HP [1-^{13}C] DHA (or other metabolite) was observed. In these studies, the magnitude of the [1-^{13}C] VitC resonance was higher in surrounding tissues than in the brain (**Figure 2b**).

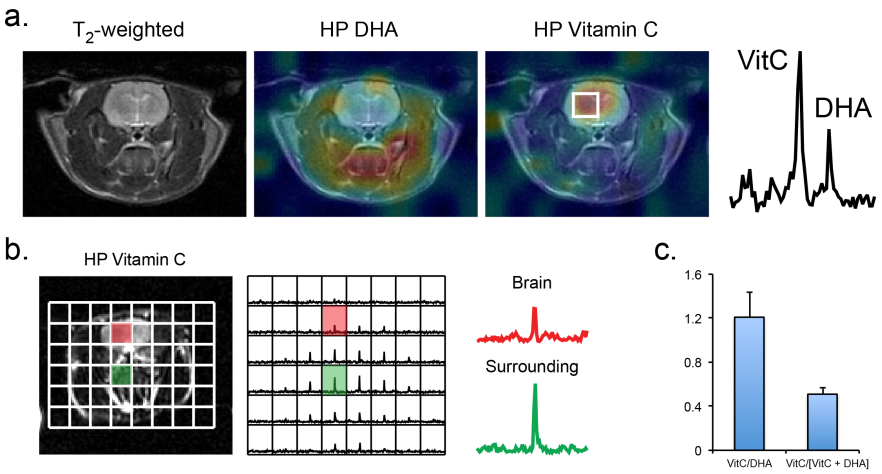

Figure 2. ***In vivo*** **2D ^{13}C MRSI studies in the normal rat brain. (a) HP [1-^{13}C] DHA study demonstrating reduction to [1-^{13}C] VitC within brain. No observable VitC signal was observed in voxels corresponding to surrounding tissue. (b) HP [1-^{13}C] VitC study demonstrating no oxidation to [1-^{13}C] DHA. The HP [1-^{13}C] VitC signal is diminished in brain voxels, consistent with limited blood-brain-barrier penetration. (c) Average metabolite ratios calculated for brain voxels (n=16) following injection of HP DHA**

DISCUSSION: The steady-state concentration of Vitamin C in the brain is remarkably high, estimated to be 10 mM in neurons, while glia harbor high concentrations of GSH [6]. This high Vitamin C level is generally attributed to the high rate of oxidative metabolism in neurons, making the brain particularly vulnerable to ROS and ischemia/reperfusion injury. Total brain Vitamin C levels are under strong homeostatic regulation, with extracellular Vitamin C concentrations mediated in part by heteroexchange with glutamate. High levels of brain Vitamin C are neuroprotective, and may be enhanced by administration of DHA, which readily crosses the blood-brain barrier. On injection of HP [1-^{13}C] DHA into a normal rat, significant reduction to [1-^{13}C] Vitamin C was observed within the brain, with no background conversion observed in surrounding tissues. Given the limited spatial resolution of this study, we were not able to determine whether this reduction took place primarily in gray matter (dominated by neurons) or white matter (predominantly glia). Rapid reduction of HP [1-^{13}C] DHA within the brain suggests a role for this probe in functional imaging (GLUT transporter density), determining local glutamate concentrations, as well as predicting vulnerability to ROS.

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