In vivo reduction of Hyperpolarized [1-^{13}C]-Dehydroascorbic Acid is affected by glucose transporter expression

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Background and Motivation

The ability of cancer cells to maintain a highly reduced intracellular environment is strongly correlated with aggressiveness of the disease and drug resistance (1,2). Non-invasive imaging of tumor redox status may therefore indicate prognosis and give an early readout of treatment response. We demonstrated recently that the rate at which the oxidized form of vitamin C, [1-^{13}C]-Dehydroascorbic Acid (DHA), is reduced to [1-^{13}C]-Ascorbic Acid (AA) may serve as a hyperpolarized imaging biomarker of tumor redox status (3). DHA is taken up by the glucose transporters (4), in particular GLUTs 1 and 3 (5,6), therefore we hypothesized that the rate of reduction of DHA *in vivo* would be influenced by changes in the expression of the glucose transporters, in addition to sensing alterations in the intracellular redox state. To verify this hypothesis, we treated mice bearing EL4 lymphoma tumors with diethyl maleate, which complexes glutathione leading to depletion of the antioxidant but has also been shown to inhibit DHA membrane transport in cell culture (7). We imaged both the rate of DHA reduction with hyperpolarized ^{13}C MRSI and uptake of the glucose analog, ^{18}F-fluoro-2-deoxyglucose (FDG), with PET.

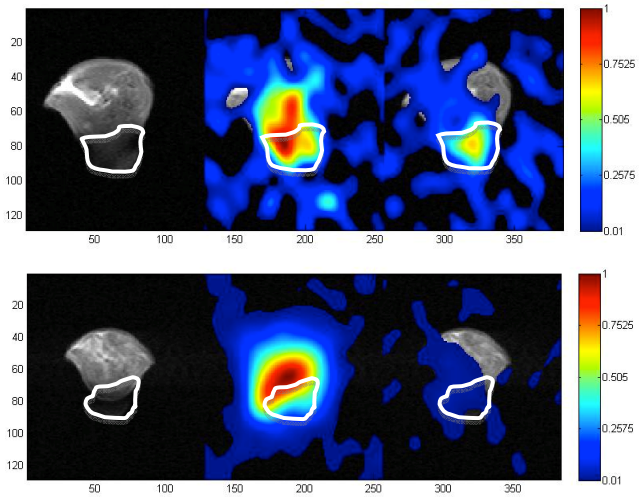

Figure 1: Tumor reduction of hyperpolarized [1-^{13}C]-Dehydroascorbic acid before (upper panel) and after (lower panel) treatment with diethyl maleate. Left: ^{1}H reference image with tumor delineated in white. Center: CSI of [1-^{13}C]-Dehydroascorbic acid at 175ppm Right: CSI of the resulting [1-^{13}C]-Ascorbic acid at 179ppm. Both images are displayed with a low threshold value to allow visualization of [1-^{13}C]-AA post DEM on the same scale

Methods

Mice bearing subcutaneous EL4 lymphoma tumors were examined at baseline (day 0) and then at 2 h post treatment with 1.6g/kg diethyl maleate (DEM), administered i.p. at 24 h after the first imaging study (day 1). Biochemical assays of glutathione content and redox enzymes were performed in one cohort (n=4), where tumors were freeze clamped. [1-^{13}C]-Dehydroascorbic Acid (DHA) was hyperpolarized as described previously [1] and chemical shift images were acquired using a 7T horizontal bore magnet (Varian; Palo Alto, CA) in a second cohort (n=3). Finally, PET studies were performed (n=2) following administration of 3 MBq FDG at baseline and again after diethyl maleate treatment. PET data were acquired between 60 and 90 minutes after tracer injection using a NanoPET/CT scanner (Bioscan-Mediso; Washington, DC). A volume of interest was drawn around each tumor and standardized uptake values (SUV75) were calculated in the most FDG-avid portion of the tumor (75% of the maximum intensity voxel). For all imaging studies, mice were anaesthetized using isoflurane and euthanized at study completion, when tumors were excised for histopathological analysis.

Results and Discussion

Biochemical assays revealed that DEM treatment at this dose resulted in significant depletion of tumor glutathione levels, by nearly 40% ($p<0.01$) relative to control levels. Upregulation of glutaredoxin and glucose-6-phosphate dehydrogenase activities were also observed, although this was not statistically significant ($p<0.1$). Injection of hyperpolarized [1-^{13}C]-DHA resulted in readily detectable signals from both the injected substrate and the reduced form of vitamin C, [1-^{13}C]-AA. Prior to treatment with DEM, both forms of the vitamin were seen predominantly within the tumor volume, indicating that [1-^{13}C]-DHA was avidly transported and reduced in EL4 tumors relative to normal tissue (Figure 1, upper). At 2 h after administration of DEM, however, little reduction of [1-^{13}C]-DHA to [1-^{13}C]-AA was observed (Figure 1, lower). The level of [1-^{13}C]-AA, normalized to the [1-^{13}C]-DHA peak, fell from 0.37±0.16 to 0.039±0.018 after treatment. Given the data from biochemical assays, this decrease could not be explained by down-regulation of intracellular antioxidant systems, which with the lower glutathione content tended to be upregulated. Furthermore, DEM treatment did not induce tumor shrinkage, or tumor cell necrosis according to histopathological analysis using H&E staining so these factors also could not have influenced DHA reduction. However, FDG PET studies (Figure 2) revealed a 56% decrease in the SUV mean calculated over 75 minutes, suggesting that DEM treatment caused a decline in glucose transporter expression and as hypothesized, this affected DHA reduction. These results suggest that changes in glucose transporter expression may influence hyperpolarized [1-^{13}C]-DHA measurements and that care should be taken to assess the effects of DHA transport in an imaging study of tumor redox status.

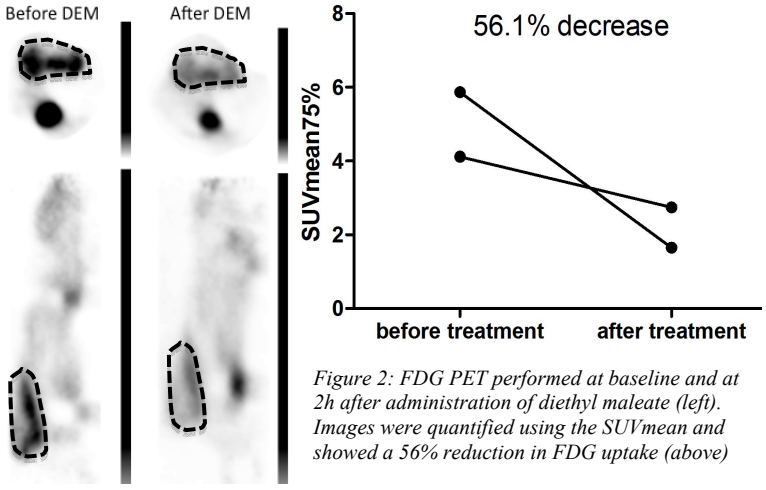

Figure 2: FDG PET performed at baseline and at 2h after administration of diethyl maleate (left). Images were quantified using the SUVmean and showed a 56% reduction in FDG uptake (above)

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References (1) Balendiran *et al* (2004) *Cell Biochem Funct* **22** 343-52 (2) Ballatori *et al* (2009) *Biol Chem* **390** 191-214 (3) Bohndiek *et al* (2011) *JACS* **133** 11795-11801 (4) Corti *et al* (2010) *Arch Biochem Biophys* **500** 107-115 (5) Vera *et al* (1993) *Nature* **364** 79-82 (6) Rumsey *et al* (1997) *J Biol Chem* **272** 18982-9 (7) Savini *et al* (2000) Biochem J **345** 665-672