REDUCTION OF (1-^{13}C)-DEHYDROASCORBIC ACID TO (1-^{13}C)-ASCORBIC ACID IS NOT CORRELATED TO GLUTATHIONE IN A TREATMENT RESPONSE MODEL OF MURINE LYMPHOMA *IN VIVO*

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Target audience

The data will concern researchers in the field of dissolution DNP and those interested in tissue redox state and cancer biology.

Purpose

Hyperpolarized [1-^{13}C]-dehydroascorbic acid (DHA), the oxidized form of vitamin C, can be used both *in vitro* and *in vivo* as a magnetic resonance (MR) marker of redox status[1,2]. What limits the rate of reduction of hyperpolarized [1-^{13}C]-DHA to [1-^{13}C]-AA *in vivo* and hence which metabolic process it directly reports on is still poorly understood. Glutathione is thought to play a key role in the reduction of [1-^{13}C]-DHA[1,3], however, the extent to which other reducing equivalents such as NADPH contribute has not been reported to date. We show here that the reduction of hyperpolarized [1-^{13}C]-DHA to [1-^{13}C]-AA in etoposide-treated tumors is highly variable and shows no correlation with the levels of glutathione.

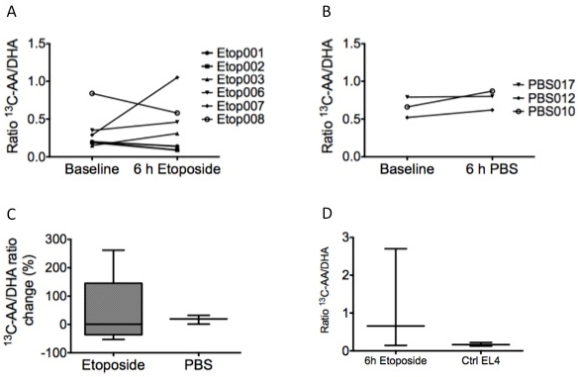

Figure 1 [1-^{13}C]-AA/DHA ratio in EL4 tumours 6 h post etoposide treatment. Hyperpolarised [1-^{13}C]-AA/DHA ratio before and 6 h post etoposide (A) or PBS (B) treatment. Average ratio change post treatment (C). Thermal carbon-13 spectra of untreated and etoposide treated EL4 tumours 150 s post injection of [1-^{13}C]-DHA (D). Ctrl = control.

Methods

Hyperpolarization and dissolution of [1-^{13}C]-DHA: [1-^{13}C]-DHA was polarized on a prototype 3.35 T polarizer magnet (Oxford instruments), as described previously[1,4]. ***In vivo MRS*:** EL4 tumor-bearing mice treated for 6 h with either PBS or etoposide (67 mg kg^{-1}) were anaesthetized and a 24-mm diameter home-built surface coil tuned to ^{13}C (100 MHz) was placed over the tumor. The mouse was transferred into a quadrature ^{1}H volume coil in a 9.4 T magnet (Varian). Two hundred µL of dissolution fluid (28 mM DHA) was injected into the tail vein and 200 tumor slice-selective spectra were acquired with a flip angle of 10° and a TR of 1 s. ***Thermal MRS:*** EL4 tumor-bearing mice, either untreated or treated for 6 h with etoposide (67 mg kg^{-1}), were anaesthetized and injected with a 28 mM solution of [1-^{13}C]-DHA. Mice were sacrificed after 150 s and the tumors were excised and rapidly frozen with liquid nitrogen cooled tongs. Metabolites were extracted with ice-cold perchloric acid (7% v/v) and the samples analyzed using a 500 MHz spectrometer (Bruker) ***Glutathione measurements:*** The reduced (GSH) and oxidized forms (GSSG) in tumor tissue were quantified by LC/MS-MS[5].

Results

The rate of reduction of hyperpolarized [1-^{13}C]-DHA to [1-^{13}C]-AA was highly variable in etoposide-treated EL4 tumors (**Figure 1A-C**). The same result was obtained in thermal NMR measurements on tumors injected with [1-^{13}C]-DHA (**Figure 1D**). There was no correlation between [1-^{13}C]-DHA reduction rate and either total glutathione content in the tumors or the ratio of GSSG/GSH (**Figure 2**).

Discussion

Etoposide, a topoisomerase II inhibitor, has been shown previously to rapidly increase the amounts of reactive oxygen species (ROS) in EL4 murine lymphoma cells[6]. This may explain the highly variable rates of reduction of hyperpolarized [1-^{13}C]-DHA to [1-^{13}C]-AA in EL4 tumors *in vivo*, when the levels of cell death this early following treatment are not discernibly increased[7]. However, these rates of reduction were not correlated with glutathione levels, suggesting a contribution of other factors to the reduction of hyperpolarized [1-^{13}C]-DHA, such as the rate of the pentose phosphate pathway (PPP), which produces NADPH.

Conclusions

The reduction of hyperpolarized [1-^{13}C]-DHA *in vivo* was not dependent on glutathione levels suggesting a contribution from other factors, namely the rate of NADPH production by the PPP.

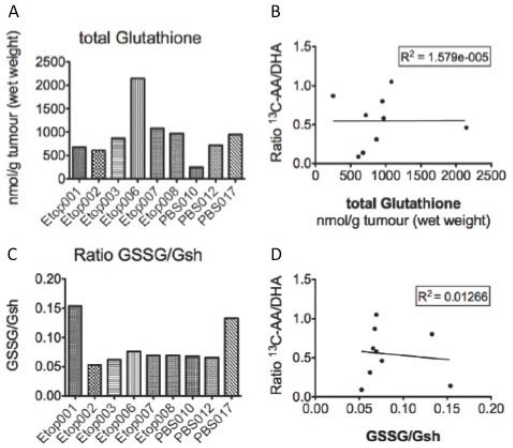

Figure 2 Glutathione levels in etoposide treated EL4 tumors. (A) Total glutathione levels or (C) GSSG/GSH ratio in etoposide or PBS-treated tumors and correlation to [1-^{13}C]-AA/DHA ratio in DNP MRS experiments (B, D). Etop = etoposide.

References

1. Bohndiek S. E., Kettunen I. M., Hu D. E. *et al.* Hyperpolarized [1-^{13}C]-Ascorbic and Dehydroascorbic Acid: Vitamin C as a probe for imaging redox status in vivo. *J Am Chem Soc* 2011;133:11795-801
2. Keshari K. R., Kurhanewicz J., Bok R. *et al*. Hyperpolarized ^{13}C dehydroascorbate as an endogenous redox sensor for in vivo metabolic imaging. *Proc Natl Acad Sci U S A* 2011;108(46):18606-11
3. Keshari K. R., Sai V., Wang Z. J. *et al.* Hyperpolarized [1-^{13}C]Dehydroascorbate MR Spectroscopy in a Murine Model of Prostate Cancer: Comparison with 18F-FDG PET. *J Nucl Med* 2013;54(6):922-8
4. Ardenkjaer-Larsen J. H., Golman K., Gram A. *et al.* Increase of signal-to-noise of more than 10,000 times in liquid state NMR. *Discov Med* 2003;3(19):37-39
5. Zhu P., Oe T. and Blair I. A. Determination of cellular redox status by stable isotope dilution liquid chromatography/mass spectrometry analysis of glutathione and glutathione disulfide. *Rapid Commun Mass Spectrom* 2008;22(4):432-40
6. Boren J. and Brindle K. M. Apoptosis-induced mitochondrial dysfunction causes cytoplasmic lipid droplet formation. *Cell Death Differ* 2012;19:1561-70
7. Hu D. E. and Brindle K. M. Immune cell-induced synthesis of NO and reactive oxygen species in lymphoma cells causes their death by apoptosis. *FEBS* 2005;579:2833–2841

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