REDUCTION OF [1-13C]-DEHYDROASCORBIC ACID TO [1-13C]-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 [1-13C]-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 status1,2. What limits the rate of reduction of hyperpolarized [1-13C]-DHA to [1-13C]-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 [1-13C]-DHA1,3; 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 [1-13C]-DHA to [1-13C]-AA in etoposide-treated tumors is highly variable and shows no correlation with the levels of glutathione.

Methods
Hyperpolarization and dissolution of [1-13C]DHA: [1-13C]-DHA was polarized on a prototype 3.35 T polarizer magnet (Oxford instruments), as described previously1,4. 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 13C (100 MHz) was placed over the tumor. The mouse was transferred into a quadrature ¹H 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 [1-13C]-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-MS5.

Results
The rate of reduction of hyperpolarized [1-13C]-DHA to [1-13C]-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 [1-13C]-DHA (Figure 1D). There was no correlation between [1-13C]-DHA reduction rate and either total glutathione content in the tumors or the ratio of GSSG/GSH (Figure 2).

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

References

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