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Non-invasive Detection of M1 Activation in Macrophages using Hyperpolarized [1-^{13}C] Dehydroascorbic Acid

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Highlights (44 words)

The immune system plays an essential role in various diseases, and macrophage activation patterns can vary greatly - impacting intervention. We propose that hyperpolarized [1-^{13}C] Dehydroascorbic acid (DHA) can be used to probe M1 classically activated macrophages at a clinically-relevant field strength of 1.47T.

Abstract (452 words)

Introduction: Macrophage activation is a well-known response to injury, infection, and other pathologies. Activation is closely tied with inflammation, and has implications in a wide array of diseases such as Alzheimer's, multiple sclerosis, and traumatic brain injury. Not all macrophage activation is inflammatory however, and accurate determination of the type of response can lead to effective treatments and interventions. The two major activation patterns of macrophages are pro-inflammatory (M1) and anti-inflammatory (M2). Interestingly, M1-activated macrophages produces reactive oxygen species (ROS), increasing surrounding oxidative stress. Increased ROS production places pressure on the glutathione – glutathione disulfide (GSH and GSSG, respectively) buffer system, reducing GSH and consequently ascorbic acid formation. Here we hypothesize that hyperpolarized ^{13}C Magnetic Resonance Spectroscopy (MRS) of [1-^{13}C] Dehydroascorbic acid (DHA) can detect toxin-induced M1 activation of macrophages via detection of decreased ascorbic acid (AA) (**Figure 1A)** at the clinically-relevant field strength of 1.47 Tesla.

Methods: J774a.1 macrophages (ATCC) grown in complete Dulbecco's Modified Eagle's Medium (DMEM) were M1 activated using 100ng/mL lipopolysaccharide (LPS) for 24 hours. A spectrophotometric assay (Abcam) was performed to confirm increased ROS production in M1 activated (n=3) versus Control (n=3) cells. Data is normalized to control and reported as fold-change from control. 12.5uL [1-^{13}C] DHA (2.2M) was polarized for 1h on a Hypersense dDNP polarizer, then dissolved in 3.5mL buffer to yield a 7.86mM solution. Within 20s post-dissolution, approximately 400uL of [1-^{13}C] DHA was injected into a 5mm NMR tube containing a slurry of 20 million cells in DMEM (n=3 control, n=2 M1-activated). Hyperpolarized spectra were then acquired using the following parameters: Flip Angle = 5°, Repetition Time (TR) = 3s, for a total of 5 minutes on a 1.47T Oxford Pulsar NMR system. Analysis was performed with Mestrenova (Mestrelab) software, with the spectra summed and area under curve calculated. Data reported as mean $\pm$ standard deviation.

Results: As measured by spectrophotometric fluorescent assay, M1 activated macrophages exhibited a 2.5-fold increase in ROS compared to control (**Figure 1B**). On injection of HP [1-^{13}C] DHA to M1 activated J774a.1 macrophages at 1.47T, DHA is observed at 175ppm with a

noticeable buildup of AA at 179ppm (**Figure 1C**). Upon quantification, our preliminary results show that the AA/DHA AUC ratio from sum spectra was $1.21x10^{-2}$ in M1 activated cells compared to $1.46x10^{-2}$ control cells (**Figure 1D**). The decreasing trend observed is in accordance with literature descriptions of reduced AA under oxidative stress conditions.

Conclusions: We have shown the feasibility of detecting AA formation from HP [1-^{13}C] DHA in M1 activated macrophages at 1.47T. Future work includes further data acquisition of such AA formation, to complete an 80% power study (n=7 per group required). Parameters for data acquisition will also be optimized to maximize our ability to observe product formation.

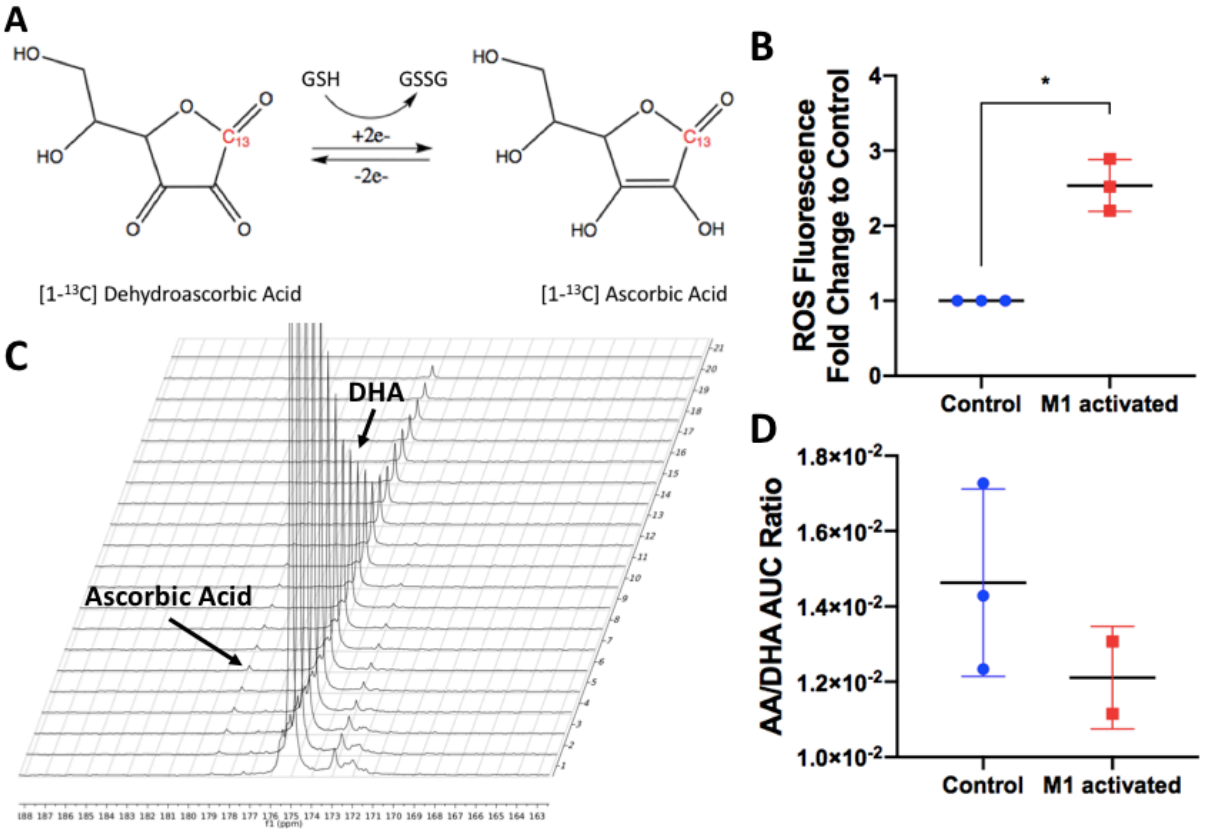

Figure 1. (**A**) [1-^{13}C] DHA reduction to [1-^{13}C] ascorbic acid by glutathione and ROS. (**B**) ROS detection by DCFDA Fluorescence assay with fold change to control (n=3,*$p < 0.05$, mean ± SD) (**C**) Dynamic acquisition of hyperpolarized [1-^{13}C] DHA (175ppm) injected into a 20million cell slurry at 1.47T with subsequent ascorbic acid (179ppm) formation, and (**D**) AA/DHA ratio of AUC from sum spectra of control (n=3) and LPS activated J774a.1 cells (n=2, p=0.24, mean ± SD).