Hyperpolarized [1-^{13}C]dehydroascorbic acid

[1-^{13}C]DHA

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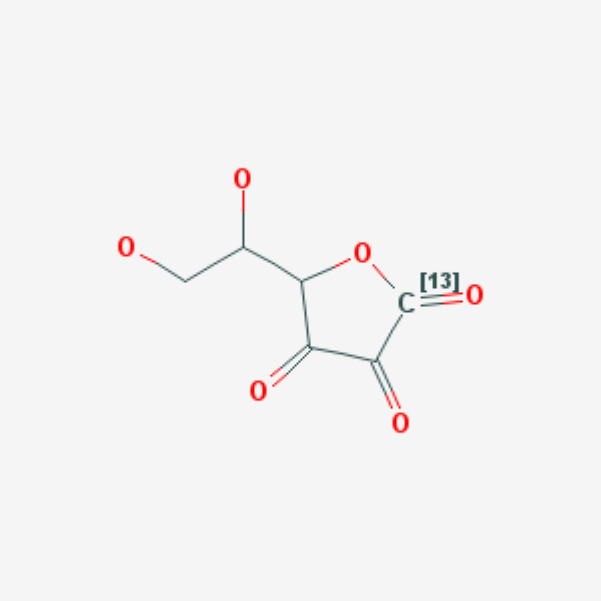
Chemical name:	Hyperpolarized [1-^{13}C]dehydroascorbic acid
Abbreviated name:	[1-^{13}C]DHA
Synonym:	
Agent Category:	Compound
Target:	Thoredoxin reductases
Target Category:	Enzyme
Method of detection:	Magnetic resonance imaging (MRI)/magnetic resonance spectroscopy (MRS)
Source of signal / contrast:	Hyperpolarized ^{13}C
Activation:	No
Studies:	• *In vitro* • Rodents



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Background

[PubMed]

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Magnetic resonance spectroscopy (MRS) has been noninvasively used to detect small molecules in tissues *in vivo* (1-4). Proton (^{1}H) and carbon-13 (^{13}C) are the most frequently investigated MRS applications (5-7). One technique has been often used to enhance ^{13}C nuclear spins with dynamic nuclear polarization (DNP), which dramatically increases sensitivities in MRS spectra of ^{13}C-labeled substrates in solutions and tissues (2, 3, 8). DNP transfers high electron-spin polarization to nuclear spins *via* microwave irradiation. The ^{13}C MRS signals from tissues are near background levels, whereas regions with hyperpolarized ^{13}C-labeled substrates provide strong signals. Thus, hyperpolarized ^{13}C-labeled substrates can provide >10,000-fold enhancement of the ^{13}C MRS signals from the substrate and its subsequent metabolites. ^{13}C-Labeled substrates must exhibit long spin-lattice relaxation time (T_1) values, be rapidly metabolized, and produce an observable change in the chemical shift (δ) value between the substrate and its metabolite(s). ^{13}C-Labeled substrates, such as [1-^{13}C]pyruvate, [2-1^{3}C]fructose, [^{13}C]bicarbonate, α-keto[1-^{13}C]isocaproate, and [1,4-$^{13}C_2$]fumarate have been studied using the DNP technique in tumors in preclinical studies to measure changes in metabolism *in vivo* through glycolysis, citric acid cycle, amino acid metabolism and fatty acid synthesis (1-3).

Vitamin C (ascorbic acid, AA) is reversibly oxidized to DHA, which is a substrate of glucose transporters GLUT1 and GLUT3 (9). Epithelial cells of the intestine, liver, and kidney transport AA directly through sodium-dependent transporters, whereas most normal tissues acquire AA through transport of DHA (10). Intracellular DHA is then converted to AA through reaction with glutathione (GSH) or GSH-dependent enzymes and through NADPH-dependent thioredoxin reductases (11). On the other hand, tumor cells have been found to lack the capacity to transport AA and to exhibit upregulation of the GSH and thioredoxin antioxidant systems, reflecting high oxidative stress in tumor cells (10). High oxidative stress has also been implicated in neurodegenerative and cardiovascular conditions (12). Bohndiek et al. (13) generated a hyperpolarized small molecule, [1-^{13}C]dehydroascorbic acid ([1-^{13}C]DHA), as an imaging agent of ^{13}C MRS for evaluation of intracellular reduction/oxidation (redox) status in tumors and other pathological conditions. [1-^{13}C]DHA has been evaluated as an intracellular redox probe using tumor cells *in vitro* and *in vivo*. Keshari et al. (14) have performed MRS imaging of redox status in normal organs and prostate tumor in mice using hyperpolarized [1-^{13}C]DHA.

Related Resource Links:

- Chapters in MICAD (Hyperpolarized ^{13}C)
- Gene information in NCBI (Thioredoxin reductase 1, thioredoxin reductase 2, GLUT1, GLUT3).
- Articles in Online Mendelian Inheritance in Man (OMIM) (Thioredoxin reductase 1, thioredoxin reductase 2, GLUT1, GLUT3)

Synthesis

[PubMed]

[1-^{13}C]DHA was prepared by charcoal oxidation of commercially available [1-^{13}C]AA. [1-^{13}C]AA 1 (2.9 mmol) was dissolved in 15 mL methanol, and then highly purified activated charcoal Norit (0.75 g) was added as a catalyst (13). The mixture was stirred vigorously for 90 minutes at room temperature, with oxygen bubbled through at 0.2 L/minute. Charcoal was removed from the product with filtration. The final product, [1-^{13}C]DHA, was then lyophilized to give a yield of 70% with >99% chemical purity. [1-^{13}C]DHA (8.2 ± 1.1%, n = 8) exhibited a significantly higher degree of polarization (P < 0.05) than [1-^{13}C]AA (5.1 ± 0.6%, n = 6) at pH 7 at 8 s after dissolution in phosphate-buffered saline (PBS) measured at 9.4 T. The δ values for [1-^{13}C]AA and [1-^{13}C]DHA were 179 ppm and 175 ppm, respectively. The T_1 values for [1-^{13}C]AA and [1-^{13}C]DHA were 15.9 ± 0.7 s and 20.5 ± 0.9 s, respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Bohndiek et al. (13) analyzed the conversion of [1-^{13}C]DHA to [1-^{13}C]AA in PBS containing 1, 5, 10, 25, and 50 mM GSH. ^{13}C MRS spectra were acquired at 9.4 T. The [1-^{13}C]AA signal (179 ppm) reached maximal intensity within 7–16 s. Reduction of [1-^{13}C]DHA to [1-^{13}C]AA was observed in RPMI medium, which contains 3.3 nM GSH and other factors. The rate of conversion observed in RPMI medium and EL4 mouse lymphoma cell suspension (1×10^8 cells in 2 mL) in RPMI medium were 99 ± 6 nmol/s (n = 2) and 223 ± 18 nmol/s (n = 3), respectively. On the other hand, no oxidation of [1-^{13}C]AA to [1-^{13}C]DHA was observed in either RPMI medium alone or in EL4 cell suspension in RPMI medium within 60 s.

Animal Studies

Rodents

[PubMed]

Bohndiek et al. (13) performed *in vivo* MRS studies in female C57BL/6 mice (number of mice not reported) bearing subcutaneous EL4 tumors. Hyperpolarized [1-^{13}C]AA or [1-^{13}C]DHA (6 mmol/mouse) was injected *via* tail vein catheter over a period of ~6 s. ^{13}C MRS spectra were acquired at 9.4 T. After injection of [1-^{13}C]DHA, the signals from [1-^{13}C]AA and [1-^{13}C]DHA were seen at 179 ppm and 175 ppm in the tumors, respectively. The apparent rate constant for the conversion of [1-^{13}C]DHA to [1-^{13}C]AA was 0.020 ± 0.004 s^{-1}. The ratio of the peak integrals of [1-^{13}C]AA to [1-^{13}C]DHA was 0.35 ± 0.08 with an average signal/noise ratio of 67 ± 20. The EL4 tumor concentration of GSH was determined to be 1,180 nmol/g. The calculated spontaneous reaction rate constant of [1-^{13}C]DHA to [1-^{13}C]AA with GSH was 0.0004 s^{-1} and would produce a

maximum relative signal of only 0.013 for [1-^{13}C]AA in the tumors. These data suggest that an enzymatic reaction is required to account for the high rate and level of [1-^{13}C]AA produced from [1-^{13}C]DHA observed in the tumors. On the other hand, no observable [1-^{13}C]DHA signal was detected in the tumors after injection of [1-^{13}C]AA.

Keshari et al. (14) performed *in vivo* MRS imaging (3 T) in normal mice (n = 5) and in transgenic mice (n = 4) with adenocarcinoma of the mouse prostate (TRAMP) model after injection of 17.5 mmol of hyperpolarized [1-^{13}C]DHA. *T2*-weighted images were obtained for 25 s at the start of [1-^{13}C]DHA injection. The conversion of [1-^{13}C]DHA to [1-^{13}C]AA was measured as a ratio of [AA]/[AA+DHA]. In normal mice, the conversions were rapid in the liver (0.41 ± 0.03) and kidney (0.25 ± 0.03), and were significantly different (P < 0.01). These ratios were similar in TRAMP mice. In tumors of TRAMP mice, the ratio was 0.23 ± 0.03 and significantly higher than that in the normal prostate (0.06 ± 0.03, P < 0.02). Hyperpolarized ^{13}C MRS imaging data acquired from rat brains (n = 3) demonstrated an even faster conversion (0.51 ± 0.10) with [1-^{13}C]AA localized to the brain and [1-^{13}C]DHA localized to the surrounding muscle tissue. In summary, the conversions of [1-^{13}C]DHA to [1-^{13}C]AA were rapid in the brain, liver, kidney, and TRAMP tumor. The conversions were slow in the normal prostate and muscle.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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