MR Contrast from Ascorbic Acid (Vitamin C) in Phantoms and In Vivo

C. D. Lascola[1], T. Venkatraman[1], B. Engstrom[1], and H. Wang[1]
[1]Department of Radiology and Brain Imaging and Analysis Center, Duke University Medical Center, Durham, NC, United States

Introduction: L-ascorbic acid (vitamin C) is the most abundant intracellular antioxidant and an essential co-factor for the biosynthesis of many important biochemicals. Intracellular levels of ascorbic acid (AA) are remarkably high, especially in tissues such as brain and tumor cells, where concentrations may reach 10-30 mM. In this study, we show that native AA in solution is capable of producing marked changes in T2 and T2* relaxivity at physiologically relevant concentrations. Susceptiblity effects are seen in both anaerobically and aerobically prepared solutions at acidic and neutral pH, but disappear after oxidation to dehydroascorbate. In animal experiments, a single bolus of dehydroascorbate (the form of Vitamin C transported across the blood-retina and blood-brain barriers before conversion to ascorbic acid) produces a significant change in T2 mapping in the mouse retina, where intracellular concentrations are known to rise significantly. These results raise two important possibilities: first, that endogenous AA may be an important contributor to native T2 and T2* contrast in CNS and other tissues; and second, that both oxidized and reduced forms of ascorbic acid may have utility as novel MR contrast probes.

Methods: AA phantoms were prepared under nitrogen (O_2-free) and O_2-enriched conditions. Control phantoms were composed of equimolar concentrations of Na citrate or Na Phosphate, which demonstrated equivalent T2 and T2 * relaxivities. Phantom and *in vivo* experiments were performed at 7T on a Bruker 70/30 MR scanner. For phantoms, T2 maps were acquired using MSME (TE/TR = 10/2000 ms) with varying TE. In *in vivo* experiments, T2 maps were generated with varying TE using RARE, 4 averages, with TR = 10000 ms. Dehydroascorbate (Sigma) was administered i.v. at 200mg/kg in buffered saline at pH 5.5. All data were processed using Paravision 4.0.

Results: In 50 mM AA phantoms at pH 7.0, a 21% drop in T2 is observed in anaerobically prepared solutions vs. control (281 +/- 4 ms vs. 373 +/- 3 ms) (Fig. 1). In O_2-enriched solutions at neutral pH, T2 drops 25% (295 +/- 4 ms) after 1 hour with identical starting concentrations, and drops 31% as compared to control after 24 hours. T2* changes in the same phantoms are even more marked at 1 hour, with 45% shortening in O_2-free and 54% shortening in O_2-enriched solution. Interestingly, although T2* shortening is further increased at 24 hours in both solutions, the deoxygenated solution shows T2* reductions of 63% in O_2-free vs. 52% in O_2 solutions as compared to control. In 50 mM O_2-free solutions with varying pH, T2 shortening is greatest at neutral pH vs. control (389 +/- 10 vs. 452 +/-10 ms), less dramatic at acidic pH 3.0 (399 +/- 10, 12% vs. control), and absent at pH 12.0, after oxidation of the solution is accelerated as evidenced by color change (Fig. 2). *In vivo* in mouse retina, where AA is known to accumulate at very high concentrations, T2 maps demonstrate a 9-11 ms (+/-3 ms) drop after single administration of i.v. dehydroascorbic acid at 200 mg/kg (Fig. 3).

Discussion and conclusion: AA is capable of significantly altering T2 and T2* relaxation in solution at physiologically relevant concentrations. Loading intracellular stores of ascorbate *in vivo* via pharmacological administration produces detectable changes in T2 maps of mouse retina. Given the magnitude of observed T2 and T2* susceptibility changes, it is reasonable to hypothesize that endogenous Vitamin C is an important contributer to overall T2 contrast in native tissues. The basis of T2 and T2* changes is not yet understood. One possiblity is the presence of a monodehydroascorbic acid radical, which is known to exist at equilibrium with the parent form of vinylogous L-ascorbate, and is detected even in the absence of oxygen or other oxidants. Another possible contribution may come from magnetic anisotropy arising from the aromatic ring of ascorbate, which is lost in the oxidized state.

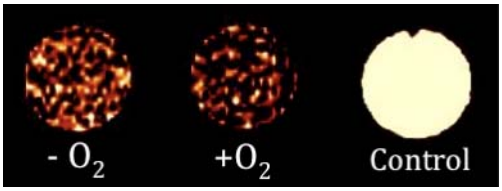

Fig. 1 T2 map of 50 mM ascorbic acid in anaerobic and aerobic solutions. solutions. At neutral pH (7.0) both O_2-free and O_2-enriched phantoms exhibit T2 shortening, although T2 and T2* effects are slightly increased in the presence of O_2.

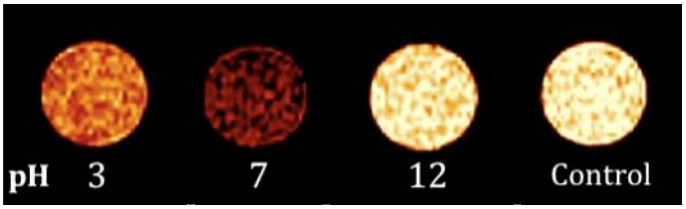

Fig. 2 T2 maps of ascorbic acid at different pH. T2 and T2* shortening are increased at neutral vs. acidic pH, but absent at alkaline pH, likely due accelerated oxidation of AA to its dehydroascorbate form.

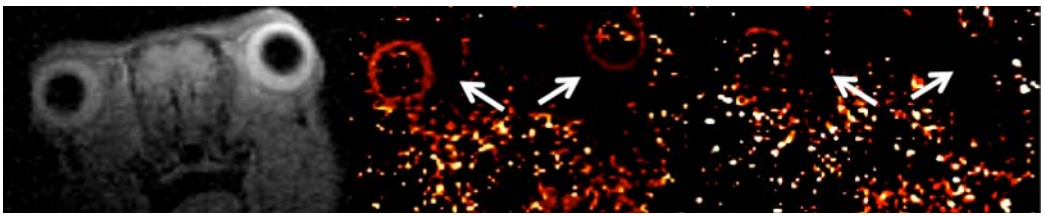

Fig. 3 *In vivo* imaging of mouse retina before and after administration of dehydroascorbic acid. T2 anatomic image (left) and corresponding T2 maps of eyes before and after (middle and right) administration of intravenous dehydroascorbic acid. Note the decreased conspicuity of retina following dehydroascorbic acid, consistent with T2 shortening.