Vitamin C and cancer revisited

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In this issue of PNAS, Chen et al. (1) show that i.p. injection of "pharmacologic doses" of vitamin C decreases the growth and weight of human, rat, and murine tumor xenografts in athymic, nude mice. This work follows a number of articles by the same group, led by Mark Levine at the National Institute of Diabetes and Digestive and Kidney Diseases, showing that millimolar concentrations of extracellular vitamin C kill cancer cells but not normal cells in a hydrogen peroxide (H2O2)-dependent manner (1–3). Such millimolar concentrations of vitamin C can be achieved in humans by i.v. infusion but not by diet or supplements (4). Hence, vitamin C is postulated to exert local pro-oxidant effects in the interstitial fluid surrounding tumor cells, killing them or inhibiting their growth, while leaving normal cells intact (1–3).

It is well known that vitamin C, or ascorbic acid, is an effective biologic antioxidant and does not act as a pro-oxidant under normal conditions (5) because it does not readily autoxidize, i.e., react with oxygen (O2) to produce reactive oxygen species, such as superoxide radicals (O2−) or H2O2. However, ascorbate readily donates an electron to redox-active transition metal ions, such as cupric (Cu2+) or ferric (Fe3+) ions, reducing them to cuprous (Cu+) and ferrous (Fe2+) ions, respectively (Reaction 1). In fact, reduction of copper or iron in the catalytic site of certain enzymes underlies ascorbate’s well known biologic function as a co-substrate in procollagen, carnitine, and catecholamine biosynthesis (6). Reduced transition metal ions, in contrast to ascorbic acid, readily react with O2, reducing it to superoxide radicals (Reaction 2), which in turn dismutate to form H2O2 and O2 (Reaction 3):

\[
\text{AscH}^- + \text{Me}^{(n+1)+} \rightarrow \text{Asc}^- + \text{Me}^{n+} + \text{H}^+ \quad \text{[Reaction 1]}
\]

\[
\text{Me}^{n+} + \text{O}_2 \rightarrow \text{Me}^{(n+1)+} + \text{O}_2 \quad \text{[Reaction 2]}
\]

\[
\text{HO}_2^- + \text{O}_2^- + \text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \quad \text{[Reaction 3]}
\]

[Sum of Reactions 1–3]

The H2O2 produced this way (Reactions 1–3) seems to be key to ascorbate’s antitumor effect because H2O2 causes cancer cells to undergo apoptosis, pyknosis, and necrosis (2). In contrast, normal cells are considerably less vulnerable to H2O2. The reason for the increased sensitivity of tumor cells to H2O2 is not clear but may be due to lower antioxidant defenses (7). In fact, a lower capacity to destroy H2O2—e.g., by catalase, peroxiredoxins, and GSH peroxidases—may cause tumor cells to grow and proliferate more rapidly than normal cells in response to low concentrations of H2O2. It is well known that H2O2 exerts dose-dependent effects on cell function, from growth stimulation at very low concentrations to growth arrest, apoptosis, and eventually necrosis as H2O2 concentrations increase (8). This dose-dependency may be shifted to the left in tumor cells, making them more sensitive to both the growth stimulatory and cytotoxic effects of H2O2. Whatever the exact mechanism, the increased sensitivity of tumor cells to killing by H2O2 may provide the specificity and “therapeutic window” for the antitumor effect of extracellular ascorbate (1, 2).

The chemical reactions linking ascorbate to H2O2, as explained above (Reactions 1–3), require a redox-active transition metal—without it, ascorbate cannot exert pro-oxidant effects. Chen et al. (2) speculate that there is an extracellular “metalloprotein catalyst” of between 10 and 30 kDa in size that interacts with ascorbate. Identification of this metal-containing protein will be critical because it seems to be the cause for millimolar concentrations of ascorbate to act as a pro-oxidant in interstitial fluid. In contrast, the protein must be absent or inactive in blood, otherwise ascorbate would become oxidized to the ascorblyl radical or be unstable, which is not observed (1). If this putative metalloprotein can be identified and characterized, it may serve as an additional target for anticancer therapy. For example, other naturally occurring reducing agents, such as certain flavonoids or thiol compounds, may be particularly effective in reducing the protein’s metal center, or drugs may be developed specifically targeting this center.

Although Chen et al. (1) provide no direct evidence for the existence of the metalloprotein or the formation of reduced transition metal ions by extracellular ascorbate, they measure the other reaction product formed between ascorbate and the putative metal center, i.e., the ascorbyl radical (Reaction 1). They show formation of this radical in a time-dependent and ascorbate-dose-dependent manner in interstitial fluid of tissues, including tumor xenografts, but not in blood (1, 3). They also show that the concentration of the ascorbyl radical correlates with the concentration of H2O2 in interstitial fluid, whereas no H2O2 can be detected in blood or plasma (3, 9). These observations, combined with the inhibitory effect on xenograft growth, provide the proof of concept that millimolar concentrations of extracellular ascorbate, achievable by i.p. injection or i.v. infusion in experimental animals and humans, respectively, exert pro-oxidant, antitumor effects in vivo.

Perspective

Why is it important to understand how vitamin C can produce H2O2 and kill cancer cells but not normal cells? Because without this detailed knowledge, we do not have a scientific rationale to revisit the question of whether i.v. infusion of vitamin C may have value in treating cancer patients. The potential cancer-therapeutic activity of vitamin C has a long and controversial history. In 1973, Linus Pauling and Ewan Cameron (10) postulated that vitamin C inhibits tumor growth by enhancing immune response and stabilizing glycosaminoglycans of the extracellular matrix by inhibiting hyaluronidase. Cameron and

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Clinic (14, 15). In both trials, patients controlled, double-blind trials of vitamin C-spon\nd two randomized, placebo-\nfed more than 1 year. Overall, 22% of vitamin C-treated pa\ntients but only 0.4% of controls survived \nto Ehrlich tumor cells and \nected i.p. with the copper-containing \ntype copper:glycylglycylhistidine (Cu:GGH) and vitamin C, 40% survived \n days, whereas no controls survived for longer than 30 days. The \nination of Cu:GGH and vitamin C was also \ntoxic to Ehrlich tumor cells in \n vitro, but the cytotoxicity was abrogated by cata\n lase, suggesting that H2O2 was the cyto\ntoxic species. The work of Chen et al. (1–3) also strongly suggests that H2O2 is \nponsible for the anticancer activity of vitamin C.

Survival rates were essentially the same for all groups. Plasma concentrations of \nvitamin C were not measured in either \n study, and vitamin C was given only \norally. In retrospect, the Mayo Clinic \ntrials may have failed to properly evalu\nate the clinical efficacy of vitamin C in \ncancer because of insufficient plasma \nconcentrations of vitamin C attained \nwith oral supplementation (4). Pauling and colleagues (16) empha\nsized host resistance to cancer but rec\nognized the anticancer role of redox chemistry, especially reactive oxygen \npecies formed from the reaction of vi\ntamin C with copper. When mice were \nioculated with Ehrlich tumor cells and \nnected i.p. with the copper-containing \ntype copper:glycylglycylhistidine (Cu:GGH) and vitamin C, 40% survived \n days, whereas no controls survived for longer than 30 days. The \nination of Cu:GGH and vitamin C was also \ntoxic to Ehrlich tumor cells in \n vitro, but the cytotoxicity was abrogated by cata\n lase, suggesting that H2O2 was the cyto\ntoxic species. The work of Chen et al. (1–3) also strongly suggests that H2O2 is \nponsible for the anticancer activity of vitamin C.

Interestingly, Chen et al. (1) noted \nthat metastases were present in ~30% of athymic mice grafted with glioblas\ntoma tumors, whereas no metastases \nwere detected in similar mice injected \ni.p. with ascorbate. This observation \narrants further investigation because \nmetastases account for a substantial \npercentage of cancer mortality.

Recent Clinical Studies
Two Phase 1 clinical trials of cancer and \nvitamin C have recently been published \nthat demonstrated remarkable tolerance and safety for high-dose (up to 1.5 g/kg) \ni.v. vitamin C in patients screened to \neliminate hyperoxaluria, glucose-6- \nphosphate dehydrogenase deficiency, \nand other medical conditions (17, 18). Additionally, a series of case reports \nicated high-dose i.v. vitamin C was \nrelated with long-term tumor regression in three patients with ad\nanced renal cell carcinoma, bladder \ncarcinoma, or B-cell lymphoma (19). \nClinical plausibility has been repeatedly \nsuggested, and Chen et al. (1–3) now \nve convincingly demonstrated bio-
logic plausibility and are poised to \ne the potential value of “pharmaco\nlogic ascorbate in cancer treatment” in humans.


