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 (H_2O_2)-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 (O_2) to produce reactive oxygen species, such as superoxide radicals ($O_2^{\bullet -}$) or H_2O_2. However, ascorbate readily donates an electron to redox-active transition metal ions, such as cupric (Cu^{2+}) or ferric (Fe^{3+}) ions, reducing them to cuprous (Cu^{+}) and ferrous (Fe^{2+}) 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 O_2, reducing it to superoxide radicals (Reaction **2**), which in turn dismutate to form H_2O_2 and O_2 (Reaction **3**):

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

$$Me^{n+} + O_2 \rightarrow Me^{(n+1)+} + O_2 \quad \textbf{[Reaction 2]}$$

$$HO_2^{\bullet} + O_2^{\bullet -} + H^+ \rightarrow H_2O_2 + O_2 \quad \textbf{[Reaction 3]}$$

$$2AscH^- + O_2 \xrightarrow{Me} 2Asc^{\bullet -} + H_2O_2 \quad \textbf{[Sum of Reactions 1–3]}$$

The H_2O_2 produced this way (Reactions **1–3**) seems to be key to ascorbate's antitumor effect because H_2O_2 causes cancer cells to undergo apoptosis, pyknosis, and necrosis (2). In contrast, normal cells are considerably less vulnerable to H_2O_2. The reason for the increased sensitivity of tumor cells to H_2O_2 is not clear but may be due to lower antioxidant defenses (7). In fact, a lower capacity to destroy H_2O_2—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 H_2O_2. It is well known that H_2O_2 exerts dose-dependent effects on cell function, from growth stimulation at very low concentrations to growth arrest, apoptosis, and eventually necrosis as H_2O_2 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 H_2O_2. Whatever the exact mechanism, the increased sensitivity of tumor cells to killing by H_2O_2 may provide the specificity and "therapeutic window" for the antitumor effect of extracellular ascorbate (1, 2).

Millimolar concentrations of extracellular vitamin C kill cancer cells but not normal cells.

The chemical reactions linking ascorbate to H_2O_2, 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 ascorbyl 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 H_2O_2 in interstitial fluid, whereas no H_2O_2 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 H_2O_2 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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See companion article on page 11105.

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Campbell (11) reported on the response of 50 consecutive patients with advanced cancer to continuous i.v. infusions (5–45 g/d) and/or oral doses (5–20 g/d) of vitamin C. No or minimal response was observed in 27 patients; 19 patients exhibited tumor retardation, cytostasis, or regression; and 4 patients experienced tumor hemorrhage and necrosis. The first clinical study by Cameron and Pauling (12) compared survival times between 100 patients with terminal cancer treated with i.v. and oral vitamin C, usually 10 g/d, and 1,000 comparable patients not given vitamin C. Patients treated with vitamin C survived approximately four times longer than controls, with a high degree of statistical significance ($P < 0.0001$). A follow-up study reported that patients given vitamin C had a mean survival time almost 1 year longer than matched controls (13). Overall, 22% of vitamin C-treated patients but only 0.4% of controls survived for more than 1 year.

The National Cancer Institute sponsored two randomized, placebo-controlled, double-blind trials of vitamin C and advanced cancer at the Mayo Clinic (14, 15). In both trials, patients were given 10 g/d vitamin C or placebo. Survival rates were essentially the same for all groups. Plasma concentrations of vitamin C were not measured in either study, and vitamin C was given only orally. In retrospect, the Mayo Clinic trials may have failed to properly evaluate the clinical efficacy of vitamin C in cancer because of insufficient plasma concentrations of vitamin C attained with oral supplementation (4).

Pauling and colleagues (16) emphasized host resistance to cancer but recognized the anticancer role of redox chemistry, especially reactive oxygen species formed from the reaction of vitamin C with copper. When mice were inoculated with Ehrlich tumor cells and injected i.p. with the copper-containing tripeptide copper:glycylglycylhistidine (Cu:GGH) and vitamin C, 40% survived 60 days, whereas no controls survived for longer than 30 days. The combination of Cu:GGH and vitamin C was also toxic to Ehrlich tumor cells *in vitro*, but the cytotoxicity was abrogated by catalase, suggesting that H_2O_2 was the cytotoxic species. The work of Chen *et al.* (1–3) also strongly suggests that H_2O_2 is responsible for the anticancer activity of vitamin C.

Interestingly, Chen *et al.* (1) noted that metastases were present in ≈30% of athymic mice grafted with glioblastoma tumors, whereas no metastases were detected in similar mice injected i.p. with ascorbate. This observation warrants further investigation because metastases account for a substantial percentage of cancer mortality.

Recent Clinical Studies

Two Phase 1 clinical trials of cancer and vitamin C have recently been published that demonstrated remarkable tolerance and safety for high-dose (up to 1.5 g/kg) i.v. vitamin C in patients screened to eliminate hyperoxaluria, glucose-6-phosphate dehydrogenase deficiency, and other medical conditions (17, 18). Additionally, a series of case reports indicated that high-dose i.v. vitamin C was associated with long-term tumor regression in three patients with advanced renal cell carcinoma, bladder carcinoma, or B-cell lymphoma (19). Clinical plausibility has been repeatedly suggested, and Chen *et al.* (1–3) now have convincingly demonstrated biologic plausibility and are poised to explore the potential value of "pharmacologic ascorbate in cancer treatment" in humans.

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