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Losing and finding a way at C: New promise for pharmacologic ascorbate in cancer treatment

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The wall of waves that nearly swamped vitamin C as an agent for cancer treatment began innocuously. Fifty-five years ago, Robert McCormick hypothesized that cancer metastases spread through weakened collagen and that metastases could be blocked by vitamin C, which made collagen stronger [1]. Nearly 2 decades later, the Scottish surgeon Ewan Cameron embellished the hypothesis by adding that ascorbate inhibited the enzyme hyaluronidase, which otherwise destroyed collagen so that cancers could metastasize [2]. Based on only minimal *in vitro* data, he and Allan Campbell began treating terminally ill cancer patients with high doses of vitamin C in the early 1970s, with at least one spectacular success [3]. Campbell and Cameron settled on a treatment dose of 10,000 mg daily. By comparison, at that time the maximum recommended dietary allowance (RDA) for vitamin C in many countries was 60 mg daily. Cameron contacted the two-time Nobel Laureate Linus Pauling, who had his own interests in high-dose vitamin C for treatment of colds and schizophrenia. Pauling joined and championed Cameron's efforts. Together, they published a retrospective case series in 1976 in the *Proceedings of the National Academy of Sciences* [4], a paper that created a typhoon in that journal, among cancer scientists and physicians and with the general public. A follow-up paper in the same journal intensified the controversy 2 years later, with additional cases [5]. In both papers, the authors concluded that patients who had ascorbate treatment benefited with enhanced quality and prolongation of life. Multiple scientific objections were raised: the lack of blinding inherent to a retrospective case-series design, the underlying susceptibility of the rural Scottish patient population to endogenous scurvy, the lack of independent pathologic confirmation of diagnosis, and the possibility of a placebo effect [6–8].

Charles Moertel and colleagues at the Mayo Clinic designed two prospective double-blind placebo-controlled treatment trials to attempt to restore scientific balance and civility. Neither, unfortunately, was restored. Enrolled patients in the first trial had prior chemotherapy [6] and in the second trial had none [7]. Both trials, using the same dose that Pauling and Cameron recommended, were published in the *New England Journal of Medicine* and showed no effect of ascorbate. Pauling had multiple objections, but they were drowned by passionate opinions, and the medical community concluded that vitamin C had no place in cancer treatment. Robert Wittes, in an editorial accompanying the second trial, wrote that ascorbate showed no utility in cancer treatment and should not be used. But he added a disclaimer: his conclusions could change if new evidence arose [9].

Indeed, new evidence has emerged, but from human physiology. In projects unrelated to cancer, NIH scientists were developing strategies to optimize nutrition as a new way to promote health

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and prevent disease [10,11]. They sought to define optimal nutrition and quantitative strategies that could be translated for use by the general public. Vitamin C was selected as a model nutrient. Superficially, it seemed that there already was in place an approach to ideal nutrition: the RDAs. But with more examination, it was apparent that the RDAs at the time were not suitable. Then, the bases of RDAs were prevention of deficiency with an added margin of safety, but neither had bearing on ideal intake. The strategy advanced by NIH scientists was characterization of vitamin (i.e., nutrient) function in relation to vitamin concentration. Maximum function without toxicity was a quantitative means to define optimal [10,11]. In essence, this was a classic biochemical kinetics approach, but with the key addition that kinetics had an in vivo component. This fresh approach, termed *in situ* kinetics, had its roots in the concept of defining conditions that allowed optimal bacterial growth [12]. Further, none other than Linus Pauling had proposed that enzymes with K_m's shifted rightward for nutrient substrates, based on enzyme mutations, could thereby have altered nutrient requirements for maximal function [13], a concept recently expanded upon in human disease [14]. The advance of the NIH group was to use kinetics principles, not for enzymes with mutations, but for normal enzymes that had requirements for vitamins as cofactors or cosubstrates. Graphically, the concept was that vitamin (nutrient) concentration was an *x*-axis display, and vitamin function in vivo was a *y*-axis display [10,11,15].

To test the hypothesis, there was a key prerequisite. What were the actual *x*-axis vitamin concentrations in humans that would impact vitamin function? For vitamin C as the model, it was essential to know what vitamin C concentrations were found *in vivo* as a function of dose. Thus, what was necessary was characterization in humans of vitamin C dose–concentration relationships. Surprisingly, the information available for all vitamins was either limited or nonexistent.

To proceed, the NIH investigators undertook detailed characterizations of vitamin C physiology and pharmacokinetics in healthy young adults [16,17]. They found that vitamin C concentrations in human plasma and cells were carefully, or tightly, controlled by multiple mechanisms acting together: bioavailability, or intestinal absorption; tissue accumulation; and renal reabsorption and excretion. Not studied, but also a probable contributor, was utilization rate as a function of homeostasis. Once oral intake of vitamin C exceeded 200 mg daily, it was difficult to increase plasma and tissue concentrations using higher oral doses. Characterizing bioavailability, which turned out to have key consequences, was accomplished using pharmacokinetics techniques [16,18]. After subjects were brought to steady state for each of seven doses, they received that dose first orally and then intravenously. As the dose increased, the percentage absorbed orally decreased. However, when ascorbate was administered intravenously, tight control was bypassed, until renal excretion restored equilibrium.

With unequivocal data showing that intravenous ascorbate transiently bypassed tight control of oral doses, the NIH investigators had a surprising realization [8]. Pharmacokinetics had been overlooked in the cancer studies. Although both the Mayo Clinic and the Cameron/Pauling studies used the same vitamin C dose, the route of administration was different. The Mayo Clinic patients received only oral vitamin C, but the Cameron patients received both oral and intravenous vitamin C. At the doses used, intravenous (iv) administration of ascorbate would produce concentrations that could never be achieved orally. Only iv ascorbate was a drug, producing concentrations that could be 70- to 100-fold higher than maximally tolerated oral doses [8,19].

If iv but not oral ascorbate was a drug, then the conclusion that ascorbate was not effective in cancer treatment was based on a false premise and deserved reexamination. The NIH investigators did so. They first characterized ascorbate pharmacokinetics with oral and intravenous administration in detail [19]. Building on the findings of other groups [20], the

cytotoxicity of ascorbate toward cancer cells was examined *in vitro*, identifying extracellular hydrogen peroxide formation as the effector species [21]. These data were extended *in vitro* to show that pharmacologic ascorbate concentrations produced both ascorbate radical and hydrogen peroxide in the extravascular space in both normal extracellular fluid [22,23] and tumor xenografts [23]. Importantly, these conditions resulted in decreased growth of aggressive tumor implants in mice [23]. These preclinical data are supported by studies showing that pharmacologic ascorbate concentrations could be administered safely to patients [24,25] and that ascorbate concentrations that were effective in slowing tumor growth could be achieved in humans [23,25].

In this issue of *Free Radical Biology & Medicine* Verrax and Calderon provide the first comprehensive confirmation of the pharmacologic ascorbate–cancer story [26]. The Calderon laboratory was well suited to extend these studies, because of its prior exploration of vitamin K3 plus ascorbate as cancer therapy [27,28]. It is especially noteworthy that Verrax and Calderon were able to corroborate a role for pharmacologic ascorbate using different sets of tumor models both *in vitro and in vivo*. Data from their xenograft models were particularly encouraging because the treatment dose used was fourfold lower than that used by the NIH investigators, yet still produced nearly a 50% reduction in tumor growth. As the authors noted, higher treatment doses were possible and could have reduced tumor growth further. Given the long-standing controversy enveloping ascorbate as a possible cancer treatment, the Verrax and Calderon findings are a welcome addition to the emerging pharmacologic ascorbate saga.

Verrax and Calderon extend this area of investigation in several ways. Control experiments using only oral ascorbate treatment were added and showed conclusively that only parenteral ascorbate was effective in decreasing tumor growth. Consistent with the contribution of transition metals in hydrogen peroxide cytotoxicity, Verrax and Calderon showed that cell death *in vitro* was diminished, although not fully prevented, by metal chelates. The possibility of adverse effects of pharmacologic ascorbate was investigated *in vitro*, by characterizing the effect of ascorbate with different classes of chemotherapeutic agents. Pharmacologic ascorbate concentrations did not inhibit chemotherapeutic agents, but rather enhanced cytotoxicity, a very promising finding.

In direct contrast to Verrax/Calderon, Heaney and coworkers just months ago reached exactly the opposite conclusion: that vitamin C antagonizes chemotherapeutic agents [29]. What explains this difference? Whereas Verrax and Calderon used ascorbate, Heaney et al. used dehydroascorbic acid (oxidized ascorbate), but called it vitamin C. This incorrect nomenclature leads down a slippery slope. All known actions of vitamin C are mediated by the reduced molecule ascorbate, not dehydroascorbic acid. If dehydroascorbic acid was equivalent to ascorbate, mice lacking the tissue ascorbate transporter slc23a2 should have normal tissue ascorbate concentrations. Instead, tissues are severely ascorbate deficient, and the mice die at birth [30]. Verrax and Calderon administered parenteral ascorbate to animals and measured pharmacologic ascorbate concentrations, findings that have clear translational application. By contrast, Heaney *et al.* administered dehydroascorbic acid at pharmacologic concentrations, which does not translate to people because dehydroascorbic acid is diabetogenic [31]. Verrax and Calderon also showed in their animal model that high doses of oral ascorbate did not increase tumor growth, a concern alluded to by Heaney *et al.* and now directly addressed.

Despite advances, there is much work ahead. The basis underlying either the sensitivity or the resistance of cancer cells to pharmacologic ascorbate and hydrogen peroxide is not well understood. Such understanding can guide decisions about which cancer patients might potentially benefit from pharmacologic ascorbate therapy. Hydrogen peroxide as a generator of reactive oxygen species (ROS) has promiscuous actions, making downstream molecular targets difficult to define. Such targets may differ widely depending on cell type. The effector

ROS themselves may vary, and may be very difficult to measure because of short half-lives. Using in vivo models, we need to explore whether there are synergistic or antagonistic interactions between ascorbate and chemotherapeutic agents [23,29].

These are difficult mechanistic problems, and some would argue that we must have full explanations before moving ahead clinically. We disagree. The uniqueness of pharmacologic ascorbate is its lack of known adverse side effects, with appropriate screening, compared to almost any other anti-cancer drug. As we are learning mechanisms, we can concurrently begin small targeted and rigorous clinical trials, adding ascorbate to conventional therapy for patients who have no alternatives. There is no substitute for testing pharmacologic ascorbate in the clinic: this is the same route by which many drugs became incorporated into oncology practice. We must be rigorous in both characterizing mechanisms and proceeding clinically. In cancer treatment, we still do not have a surfeit of effective therapies, cannot allow passion to blind us to promise, and should not again lose our way at C [8].

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