CANCER

Ascorbate Combination Therapy: New Tool in the Anticancer Toolbox?

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The addition of high-dose ascorbate to existing anticancer treatment strategies can improve efficacy and decrease toxicity—but not in all patients or with all combination therapies (Ma *et al.*, this issue).

Over the past 50 years, biomedical research has contributed to greatly improved health outcomes in cardiovascular and infectious diseases, but the impact of research on overall cancer incidence and mortality has been less dramatic. Current anticancer therapeutic regimens often combine multiple tools from the oncologist's tool box—one or more chemotoxic drugs, ionizing radiation, and targeted therapies—depending on cancer type and stage. However, improved survival invariably comes with substantial toxicity. Therefore, new drug combinations are needed that deliver superior tumor control while minimizing toxicities. In this issue of *Science Translational Medicine*, Ma *et al.* investigate a new use for an old chemical—vitamin C. The authors show that vitamin C (ascorbate), as part of a combination therapy regimen, enhances chemosensitivity and reduces drug toxicity in human ovarian cancer, which often has a poor prognosis resulting from late diagnosis and inherent chemoresistance (*1*).

VITAMIN C CONTROVERSY

High-dose ascorbate has become increasingly popular as a supportive treatment for cancer patients in alternative- and integrated-medicine circles. However, its use is not currently supported by the medical profession after the failure of high-profile trials with oral ascorbate designed to validate earlier studies that used both oral and intravenous ascorbate (*1*). The controversy around ascorbate as an anticancer agent is based on a lack of understanding of the chemical's pharmacokinetics (absorption, transport, and excretion) and pharmacodynamics (mode of action). Ascorbate is generally considered as an antioxidant that protects cells against free radical damage. Here, we focus on the pro-oxidant role of ascorbate, which requires pharmacological (millimolar) rather than physiological (high micromolar) concentrations.

Pharmacological ascorbate therapy delivers high-dose ascorbate intravenously, in order to circumvent the tight physiological constraints surrounding ascorbate absorption from the gut and subsequent excretion; with oral delivery, ascorbate concentrations in serum do not exceed 100 to 200 micromolar (*1*). Most researchers in the field concur that the anticancer effect of pharmacological ascorbate is mediated by generating high concentrations of extracellular H_2O_2, which diffuses into cells and kills them via the generation of reactive oxygen species (ROS), such as hydroxyl free radicals (Fig. 1). Increased ROS concentrations create oxidative stress, overwhelming the cell's antioxidant defense system and resulting in DNA damage that cumulates in cell death. The role for H_2O_2 is supported by the fact that ascorbate does not cause oxidative stress, DNA damage, or cell death in culture when adminstered in the presence of excess catalase, which converts H_2O_2 to oxygen and water. In addition, high-dose ascorbate is well tolerated, with minimal toxicity in animals and people with normal renal function and glucose 6-phosphate dehydrogenase (G6PD) activity; G6PD maintains glutathione concentrations in cells, protecting them from oxidative damage.

As a single agent, high-dose ascorbate does not demonstrate anticancer activity in clinical trials. Although several studies in the last decade (*2*) have reported a cancer-specific toxicity of pharmacological ascorbate in laboratory studies and delayed tumor growth in rodent xenograft models, ascorbate therapy did not cure any cancers. However, ascorbate could be a useful addition to existing therapy as a combination agent because of its low toxicity profile. And indeed, the latest research efforts in this field now focus on combining high-dose ascorbate with other cytotoxic drugs and ionizing radiation. Figure 1 details the various mechanisms of action of these treatments.

Now, Ma *et al.* present an example of ascorbate combination therapy in ovarian cancer (*1*). The authors investigated the effects of high-dose ascorbate alone and in combination with the cytotoxic drugs carboplatin and paclitaxel in human ovarian cancer cell lines, an ovarian cancer mouse model, and a small phase I pilot study. The authors describe H_2O_2-mediated toxicity of ascorbate in seven human ovarian cancer cell lines in culture, with no effect in a noncancerous, immortalized human ovarian epithelial cell line. High-dose ascorbate caused both genotoxic (DNA damage) and metabolic (ATP depletion) stresses, similar to those produced by exposure of cancer cells to H_2O_2. The authors further showed a synergistic effect between high-dose ascorbate and the DNA-alkylating drug carboplatin with respect to DNA damage and cell survival in the ovarian cancer cell lines but not in the noncancerous ovarian epithelial cell line.

The authors validated their in vitro results using an ovarian cancer xenograft model in an athymic mouse. The animals received ascorbate (4 g/kg; injected intraperitoneally twice daily) in combination with carboplatin, and this cocktail reduced tumor growth more than either agent alone, cutting tumor weight by 94% compared with untreated animals. This effect was significantly better than the standard combination of carboplatin and paclitaxel, and the most potent antitumor effect was shown when all three drugs were combined. Ascorbate-treated tumors exhibited increased H2A histone family member X (H2AX) phosphorylation (a marker of double-stranded DNA breaks and ATP depletion, which led to adenosine monophosphate (AMP)–activated protein kinase (AMPK) activation and subsequent inhibition of mammalian target of rapamycin (mTOR), which regulates cancer cell proliferation and survival. Again, the response was strongest in tumors of mice that had been treated with all three drugs.

Last, the authors validated the lack of ascorbate toxicity in a phase I/IIa clinical trial in 25 evaluable patients with newly diagnosed stage III/IV ovarian cancer. Patients were randomized to either standard carboplatin plus paclitaxel ($n = 12$) or to car-

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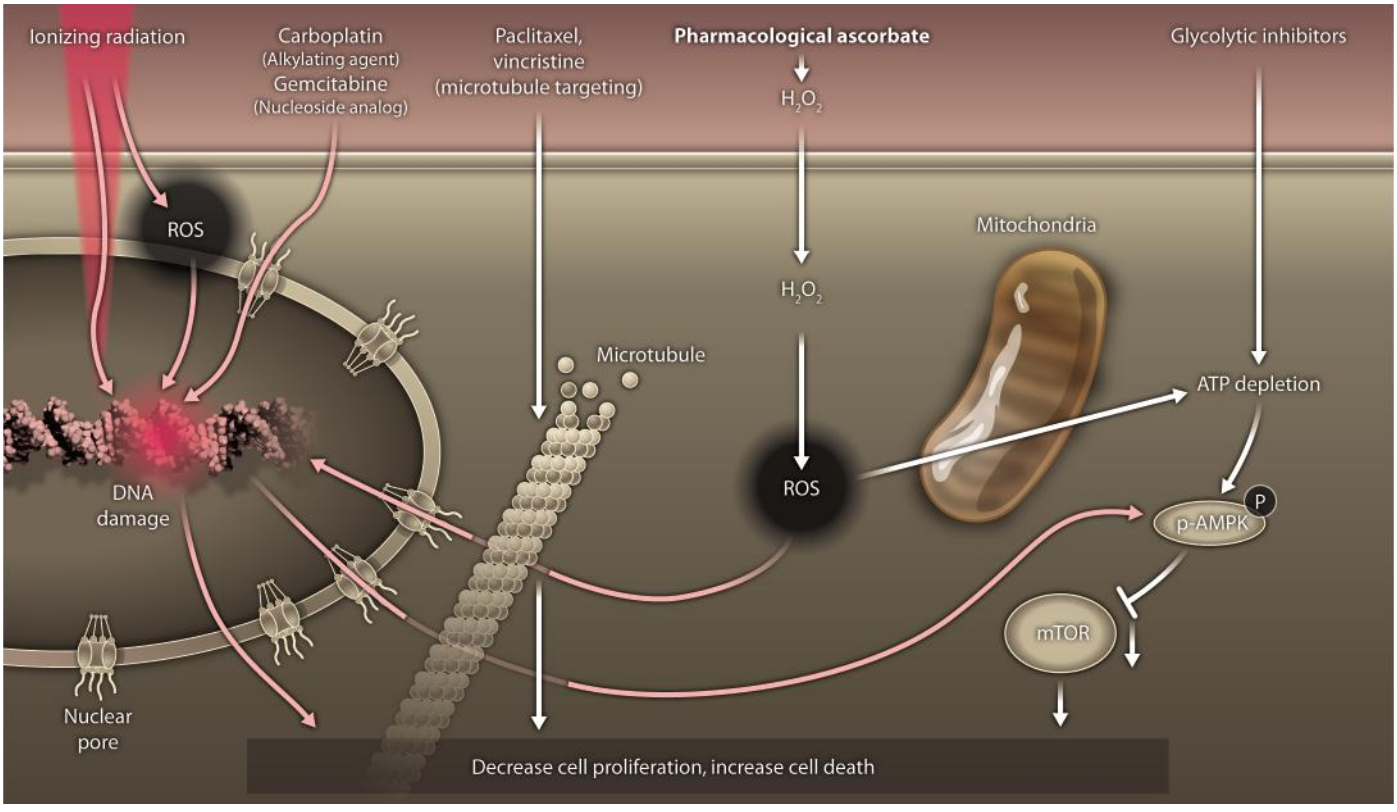

Fig. 1. Ascorbate synergy. Pharmacological ascorbate generates extracellular H_2O_2, which diffuses into the cells and produces double-stranded DNA breaks (DSBs), thus activating the DNA damage sensor protein ATM. It also depletes cellular ATP concentrations. Decreased ATP activates AMPK, which reduces mTOR activation, reducing the cell's survival and proliferative signals (*1*). The alkylating agent carboplatin contains reactive platinum complexes that bind to nucleophilic groups, such as GC-rich sites in DNA, producing cross-links in DNA and resulting in inhibition of cell proliferation and cell death. The mitotic inhibitors paclitaxel and vincristine bind to and stabilize tubulin in the nucleus, inhibit assembly of microtubules and thus spindle formation during mitosis, and halt the cell cycle in metaphase. The nucleoside analog gemcitabine is incorporated into DNA during replication and inhibits ribonuceotide reductase, depleting the base pool for DNA synthesis. Glycolytic inhibitors deplete ATP by preventing glycolysis. Ionizing radiation delivers high-energy radiation, which causes DNA damage by direct ionization and indirectly, and most frequently, by generation of ROS.

boplatin, paclitaxel, and ascorbate (n = 13). Standard chemotherapy was administered for the initial 6 months, and ascorbate treatment for 12 months, given in the therapeutic range of 75 or 100 g/infusion twice per week. Adding ascorbate to the standard treatment (carboplatin plus paclitaxel) decreased treatment-related toxicities. Ascorbate supplementation also appeared to improve overall survival and median time to disease progression; however, this increase was not statistically significant, as the trial was not powered to detect efficacy.

Other preclinical studies have evaluated the combination of high-dose ascorbate with cytotoxic treatments, including the nucleoside analog gemcitabine in pancreatic cancer (*3*), the mitotic inhibitor vincristine (*4*), glycolytic inhibitors for small-cell lung cancer (*5*), and ionizing radiation for glioblastoma multiforme (*6*). Two small phase I pilot studies have been conducted with pharmacological ascorbate and the nucleoside analog gemcitabine (*7*, *8*). One of these showed that administration of high-dose ascorbate concurrent with gemcitabine was well tolerated in 9 patients with stage IV (metastatic) pancreatic cancer. Fewer patients presented with grades 1 to 3 toxicities compared with gemcitabine treatment alone. Ascorbate was administered (50 to 125 g/infusion) twice a week for up to 6 months. The authors reported an increase in progression-free survival from 9 weeks to 26 weeks and an increase in overall survival from 6 months to 12 months (*8*). The second pilot study evaluated the effect of ascorbate addition (100 g/infusion three times a week for only 8 weeks) to a combination therapy of gemcitabine and erlotinib (an inhibitor of epidermal growth factor receptor tyrosine kinase) in 14 patients with metastatic pancreatic cancer. This study reported a decrease in tumor size in 8 of 9 patients, although patient survival was the same as that reported for patients treated with gemcitabine and erlotinib alone (*7*).

The synergy reported by Ma *et al.* for pharmacological ascorbate and carboplatin suggests that carboplatin concentrations may be decreased in combination with high-dose ascorbate, thus decreasing side effects without compromising tumor control. The three clinical studies described above all report either a decrease in toxicities when high-dose ascorbate was added to conventional treatment (*1*) or a lack of additional toxicities, an effect that could be attributed to ascorbate supplementation (*7*, *8*). Ma *et al.* and others have addressed the selective cytotoxic mechanism of ascorbate—ATP depletion and DNA damage—but not the mechanism behind the reduction in toxicity when combined with other cytotoxic agents. The apparent protective effect of high-dose ascorbate on normal tissue remains one

of the unanswered questions of ascorbate therapy but is likely to be unrelated to ascorbate's redox effects, as these cytotoxic drugs do not rely on ROS generation.

The same studies have also shown that adding pharmacological ascorbate to standard chemotherapy increases patient survival, even though this was not statistically significant in these small patient cohorts. This, together with results from the preclinical studies, particularly the synergy observed between carboplatin and pharmacological ascorbate (*1*), strongly suggests that the time has come to test ascorbate combination therapy in stage II/III clinical trials to evaluate its efficacy.

NOT FOR EVERYONE

Preclinical studies have shown a large variation in ascorbate sensitivity even within cancers of the same type. This variation may result from intrinsic differences between cancer cells or in organ-specific cancer microenvironments, both of which are currently poorly understood. Although it is widely accepted that pharmacological ascorbate kills cancer cells by generating extracellular H_2O_2, which causes oxidative stress, the downstream pathways altered by that oxidative stress may vary among cancer types, potentially explaining differential ascorbate sensitivity. If the initial damage is generated by excess ROS, then the cancer specificity of pharmacological ascorbate could be explained by the fact that cancer cells have a less robust antioxidant defense system (*9*). However, the antioxidant ability of cells depends on both intrinsic and extrinsic factors, which are likely to change over time. Cell-extrinsic differences in antioxidant capacity often are related to diet, lifestyle, disease progression, and disease treatment. The intrinsic antioxidant capacity of a cell depends on its genetic and epigenetic profiles, which determine the expression of cellular antioxidants and, particularly salient in this case, the sodium-dependent vitamin C transporters (SVCT1 and SVCT2) (*10*). A search of gene-expression databases reveals extensive variation in *SVCT* gene transcription in different cancers. Some cancers, such as colorectal cancer, generally lose *SVCT* expression compared to their normal-tissue counterparts, while other cancers, such as B cell leukemia, show increased *SVCT* expression relative to the normal tissue of origin (www.oncomine.org). The observation that *SVCT* expression can also differ widely among tumors of the same type further complicates the matter.

Cancers that do not express SVCT1 or SVCT2 are prime candidates for all forms of ascorbate combination therapy. However, cancers that overexpress these transporters could turn the ascorbate effect on its head by taking in ascorbate, rather than leaving it outside the cell to produce the prooxidant H_2O_2. In addition, the increased antioxidant capacity resulting from intracellular ascorbate would be counter-productive for any ROS-dependent therapy, such as ionizing radiation, potentially reversing the therapeutic effect. Therapies that cause DNA damage or cell death in a ROS-independent manner are less likely to be compromised in this manner. Once the mechanisms of ascorbate action have been fully elucidated, a rational approach can be taken to designing ascorbate combination therapy in the setting of highly variable ascorbate transporter expression.

As with all therapies, the efficacy of high-dose ascorbate will differ among patients. The key to successful anticancer therapy is selection of the best tool from the toolbox for the job at hand. Thus, it is critical to determine both the total antioxidant capacity of tumors and the likely fate of the pharmacological ascorbate in order to identify patients who are most likely to benefit from addition of ascorbate. Therefore, we strongly suggest that, at a minimum, patient tumors be tested for expression of ascorbate transporters before considering a patient for ascorbate combination therapy.

REFERENCES

1. Y. Ma, J. Chapman, M. Levine, K. Polireddy, J. Drisko, Q. Chen, High-dose parenteral ascorbate enhanced chemosensitivity of ovarian cancer and reduced toxicity of chemotherapy. *Sci. Transl. Med.* **6**, 222ra18 (2014).
2. N. L. Parrow, J. A. Leshin, M. Levine, Parenteral ascorbate as a cancer therapeutic: A reassessment based on pharmacokinetics. *Antioxid. Redox Signal.* **19**, 2141–2156 (2013).
3. M. G. Espey, P. Chen, B. Chalmers, J. Drisko, A. Y. Sun, M. Levine, Q. Chen, Pharmacologic ascorbate synergizes with gemcitabine in preclinical models of pancreatic cancer. *Free Radic. Biol. Med.* **50**, 1610–1619 (2011).
4. E. J. Song, V. C. Yang, C. D. Chiang, C. C. Chao, Potentiation of growth inhibition due to vincristine by ascorbic acid in a resistant human non-small cell lung cancer cell line. *Eur. J. Pharmacol.* **292**, 119–125 (1995).
5. S. B. Vuyyuri, J. Rinkinen, E. Worden, H. Shim, S. Lee, K. R. Davis, Ascorbic acid and a cytostatic inhibitor of glycolysis synergistically induce apoptosis in non-small cell lung cancer cells. *PLOS ONE* **8**, e67081 (2013).
6. P. M. Herst, K. W. Broadley, J. L. Harper, M. J. McConnell, Pharmacological concentrations of ascorbate radiosensitize glioblastoma multiforme primary cells by increasing oxidative DNA damage and inhibiting G2/M arrest. *Free Radic. Biol. Med.* **52**, 1486–1493 (2012).
7. D. A. Monti, E. Mitchell, A. J. Bazzan, S. Littman, G. Zabrecky, C. J. Yeo, M. V. Pillai, A. B. Newberg, S. Deshmukh, M. Levine, Phase I evaluation of intravenous ascorbic acid in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *PLOS ONE* **7**, e29794 (2012).
8. J. L. Welsh, B. A. Wagner, T. J. van't Erve, P. S. Zehr, D. J. Berg, T. R. Halfdanarson, N. S. Yee, K. L. Bodeker, J. Du, L. J. Roberts 2nd, J. Drisko, M. Levine, G. R. Buettner, J. J. Cullen, Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): Results from a phase I clinical trial. *Cancer Chemother. Pharmacol.* **71**, 765–775 (2013).
9. G. T. Wondrak, Redox-directed cancer therapeutics: Molecular mechanisms and opportunities. *Antioxid. Redox Signal.* **11**, 3013–3069 (2009).
10. A. J. Michels, T. M. Hagen, B. Frei, Human genetic variation influences vitamin C homeostasis by altering vitamin C transport and antioxidant enzyme function. *Annu. Rev. Nutr.* **33**, 45–70 (2013).

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