Medical Hypotheses

Intravenous Ascorbate as a Tumor Cytotoxic Chemotherapeutic Agent

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Abstract — Ascorbic acid and its salts (AA) are preferentially toxic to tumor cells in vitro and in vivo. Given in high enough doses to maintain plasma concentrations above levels that have been shown to be toxic to tumor cells in vitro, AA has the potential to selectively kill tumor cells in a manner similar to other tumor cytotoxic chemotherapeutic agents. Most studies of AA and cancer to date have not utilized high enough doses of AA to maintain tumor cytotoxic plasma concentrations of AA. Data are presented which demonstrate the ability to sustain plasma levels of AA in humans above levels which are toxic to tumor cells in vitro and suggests the feasibility of using AA as a cytotoxic chemotherapeutic agent.

Introduction

Cytotoxic drugs began to be considered consistently successful for therapy of some cancers around 1950 (1). A large jump in the cure rate for several cancer types – especially childhood, acute lymphoblastic leukemia, Hodgkin's disease, and testicular tumors – was seen between 1950 and 1990 (from 0% for all in 1950 to 75%, 80% and 90% respectively) (2). Other, relatively common, types of cancer (3), including head and neck, large bowel, stomach, pancreatic, liver, cervical, and melanoma, for the most part remain refractory to cytotoxic chemotherapy, with and without adjuvant chemotherapy, with no demonstrable prolongation of life (2).

Even though the term 'chemotherapy' generally includes hormonal and cytotoxic agents, this discussion is limited to cytotoxic agents. Whether they are alkylating agents, antimetabolites, or antibiotics, the rational for using chemotherapeutic agents in the treatment

of malignancy is to preferentially induce cytotoxicity of malignant cells. Because of the similarities between normal and malignant cells – both being born of the same host – a chemotherapeutic dose which is cytotoxic to cancer cells can also be toxic to normal cells. Oncologists must often push the limits of acceptable toxic side effects in order to effect a remission. Ideally, there should be a large gap between the lower dose required for efficacy and the higher dose of toxicity to the patient. Adverse effects of chemotherapy include hair loss, nausea and vomiting, cardiac toxicity, and secondary cancers (4). One of the most common toxic manifestations of many cytotoxic agents is bone marrow suppression (2) which can lead to immune suppression and hematopoietic dysfunctions. Because infectious complications are one of the major causes of death in cancer patients (5), more host-non-toxic compounds – particularly compounds without immune suppressive qualities – need to be investigated for their chemotherapeutic value.

There is a 10–100-fold greater content of catalase in normal cells than in tumor cells (6). This potentially creates a large gap between the toxic dose for normal cells and for tumor cells of agents which induce hydrogen peroxide generation. Ascorbic acid and its salts (AA) are preferentially toxic to tumor cells in vitro (6–13) and in vivo (14–19). This preferential cytotoxicity has been demonstrated to be related to intracellular hydrogen peroxide generation (6,8,14). AA thus belongs in a class of substances which, given at the correct dosage, can preferentially induce cytotoxicity of tumor cells with negligible toxic effects to the host.

This communication will demonstrate that plasma AA concentrations exceeding those required to kill 100% of tumor cells in vitro can be sustained in humans and that those levels can generally only be obtained by intravenous administration of AA. We propose that intravenous AA, administered in sufficient doses to achieve plasma concentrations that have demonstrable cytotoxic effects on tumor cells, be investigated as a chemotherapeutic agent on the basis of the above facts and the following positive qualities of AA:

1. Preferentially kills neoplastic cells.
2. Is virtually non-toxic at any dosage (20).
3. Does not suppress the immune system, unlike most chemotherapy agents.
4. Increases animal and human resistance to infectious agents by enhancing lymphocyte blastogenesis, enhancing cellular immunity, strengthening the extracellular matrix, and enhancing bactericidal activity of neutrophils and modulation of complement protein (21-31).
5. Strengthens the structural integrity of the extracellular matrix which is responsible for stromal resistance to malignant invasiveness (20).

Cellular in vitro studies

In 1969, researchers at the NCI reported AA was highly toxic to Ehrlich ascites cells in vitro. The goal of the study was to exploit the 10–100-fold lower catalase activity in tumor cells compared to normal cells. The proposed cytotoxic mechanism was generation of toxic hydrogen peroxide. The toxicity was greatly enhanced by concomitant administration of 3-amino-1,2,4-triazole (ATA), a catalase inhibitor. Catalase and glucose added to the culture medium and a low oxygen tension reduced the toxic effects of AA and ATA. The addition of vitamin K3 (menadione sodium bisulfite) to the medium overcame the protective effects of low oxygen tension and glucose (6). In

1977, Bram et al reported preferential AA toxicity for several malignant melanoma cell lines, including four human-derived lines. They found that catalytic concentrations of Cu^{2+} greatly increased the preferential toxicity for melanoma cells (7). Another French group also found that AA and Cu^{2+} were toxic to mouse melanoma cells in vitro. Noto et al reported that AA plus vitamin K3 had growth inhibiting action against three human tumor cell lines at non-toxic levels (8). Helgestad et al recently reported that new malignant T-cell line, isolated from a boy with malignant lymphoma, was very sensitive to AA in culture at concentrations achievable in human plasma (9). In 1980, Park reported that several leukemic cell cultures were sensitive to AA at concentrations attainable in vivo, while normal hemopoietic cells were not suppressed (10). Metabolites of AA have also shown antitumor activity in vitro (11,12).
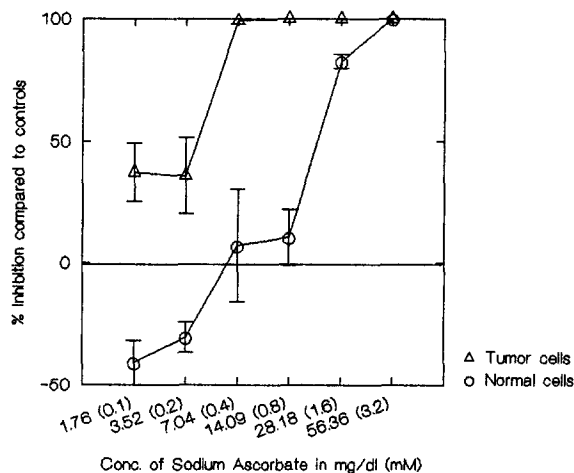

Fig. 1 Dose response (mean ±1 SD) of normal human colon fibroblasts (CCD-18 Co, ATCC, Rockville, MD) and newly established cell line from human colon carcinoma to sodium ascorbate. Results reflect total viable cells. n=8 for each concentration; cultured 4 days after supplementation of sodium ascorbate. Medium changed every 24 h. DMEM Hi-glucose culture medium (Irvine Sci.) w/10% heat inactivated fetal calf serum + antibiotics + Fungizone. 24-well (Nunc) tissue culture plates, 5% CO_2 humidified incubator at 37°C. 5000 cells/well seeded.

In support of these workers, we find that AA is toxic to several types of human tumor cells at concentrations which are non-toxic to normal cells. Figure 1 shows the AA percent dose-response of a newly established human colon tumor cell line and a normal human colon fibroblast cell line (ATCC CCD-18-Co). The AA begins to reduce cell proliferation in the tumor cell line at the lowest concentration, 1.76 mg/dl, and is completely cytotoxic to the cells at

7.04 mg/dl, while significant inhibition of the normal cells is demonstrated only at a dose of 28.18 mg/dl and 100% cell death is realized only at a dose of 56.36 mg/dl (8-fold higher dose than the tumor cells). In addition, the normal cells grew at an enhanced rate at the low dosages (1.76 and 3.52 mg/dl). Figure 2 also shows preferential toxicity of AA for tumor cells. >95% toxicity to human endometrial adenocarcinoma and pancreatic tumor cells (ATCC AN3-CA and MIA PaCa-2) occurred at 20 and 30 mg/dl, respectively. No toxicity or inhibition was demonstrated in the normal, human skin fibroblasts (ATCC CCD 25SK) even at the highest concentration of 50 mg/dl.
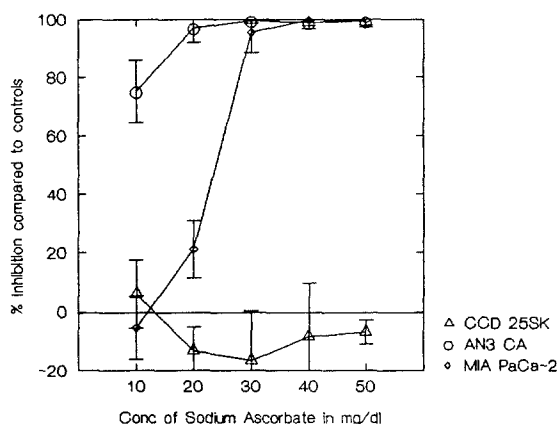

Fig. 2 Dose response (mean ±1 SD) of one normal human (CCD-25SK=skin fibroblast) and two human tumor (ATCC, Rockville, MD) (MIA PaCa 2=pancreatic carcinoma, AN3 CA=endometrial adenocarcinoma) cell lines to sodium ascorbate. Results reflect total viable cells. DMEM Hi-glucose culture medium (Irvine Sci.) w/10% heat-inactivated fetal calf serum + antibiotics + Fungizone. 5% CO_2 humidified incubator at 37^{0}C. For tumor cell lines, n=24 for each concentration and control; cultured 3 days after supplementation of AA. 96-well (Falcon) tissue culture plates, 3000 cells/well seeded. For normal cell line, 8 wells counted for each concentration and controls (4 wells pooled) from 24-well plates (Nunc). 10 000 cells/well seeded.

Animal studies

Subcutaneous injection of AA significantly potentiated the curative effects of chemotherapy on advanced Lewis lung carcinoma in mice (14). Orally administered AA inhibited DNA, RNA, and protein synthesis in epithelial neoplastic cells in mice and in rats (15), inhibited transplantable melanoma tumor development in mice (16), and enhanced carbidopa–levodopa methyl ester antitumor activity against pigmented B16 melanoma in mice (17). Administered to the drinking water of Swiss mice, AA (0.1%) inhib-

ited the growth of solid sarcoma 180 and increased the survival time in comparison to the controls (18). Tsao et al reported that AA in the drinking water of mice significantly inhibited the growth of human mammary tumor fragment xenografts implanted in immunocompetent mice. AA was also effective as a tumor inhibitor in this model when given in the diet along with cupric sulfate (19). As a dietary additive alone, AA was not effective. This finding supports the theory that the inhibitory action of AA is due to hydrogen peroxide (and hydroxyl radical) production because of Cu^{2+}'s known ability to catalyze the production of these substances in the presence of AA (32).

Human studies

Although the use of very high-dose intravenous AA for the treatment of cancer was proposed as early as 1971 (33), and Cameron published a protocol in this journal for the use of AA in the treatment for cancer (34) which included initial intravenous AA administration, to our knowledge there has been no large study of intravenous AA at levels high enough to maintain plasma levels above a level known to inhibit or kill tumor cells. Two of us (HDR and JAJ) reported apparent positive effects of intravenous AA on metastatic kidney adenocarcinoma (35). Cameron and Pauling have published extensive suggestive evidence for prolonged life in terminal cancer patients orally supplemented (with and without initial intravenous AA therapy) with 10 g/day of AA (36–44). Although both of these reports administered intravenous AA, plasma levels during infusion were not monitored, therefore it was not possible to determine if cytotoxic plasma levels of AA were achieved.

Morishige and Murata also reported evidence for increased survival and prolongation of life in terminal cancer patients with oral AA supplementation (45,46). In contrast, Creagan et al reported that oral AA had no effect on the survival of patients with advanced cancer and Moertal et al reported that oral AA did not increase the survival of another group of advanced cancer patients who had no prior chemotherapy (47,48). These negative results from controlled trials have been the subject of much debate centered mostly on protocol. These arguments will not be rehashed here because the long-term, oral dosage used in those experiments (10 g/day), while substantial and capable of producing immunostimulatory and extracellular matrix modulation effects, was not high enough to achieve plasma concentrations that are generally cytotoxic to tumor cells in culture.

An average of high and low normal plasma AA limits from five studies of adults yields a normal

range (95% range) of 0.39–1.13 mg/dl (49). The highest plasma level of AA that we have seen achievable in humans via oral supplementation is 4.5 mg/dl. The lowest cytotoxic level of AA in vitro for any of the cellular studies mentioned above was 0.88 mg/dl for a malignant lymphoma (9). This low cytotoxic level of AA is exceedingly rare. In our experience, 5–40 mg/dl of AA is required in vitro to kill 100% of tumor cells within 3 days. The 100% kill levels of 30 mg/dl for the endometrial carcinoma cells and 40 mg/dl for the pancreatic carcinoma cells in Figure 2 are typical.

For a chemotherapeutic agent to be effective, its plasma levels must reach the tumor cell toxicity range. The longer the plasma level can be kept in that range, the more effective the agent will be. For this reason, plasma pharmacokinetic studies are sometimes performed to assess the plasma level of chemotherapeutic agents over time.

Because AA is so readily cleared from the body, we decided to measure plasma levels of AA during extended intravenous infusions of AA in a few cancer patients. The AA determination method was that of Henry (50). A representative example is a pancreatic cancer patient, a male aged 69, weighing 70 kg. After initial screening for a toxic reaction to intravenous AA with small doses given intravenously during a 1 h infusion, he was given large doses of AA in 1000 cc Ringer's Lactate infused over an 8-h period.

1 h after beginning his first 8-h infusion of 115 g AA (Merit Pharmaceuticals, Los Angeles, CA), the plasma AA was 3.7 mg/dl and at 5 h was 19 mg/dl. During his fourth 8-h infusion, 8 days later, the 1 h plasma level was 158 mg/dl and 5 h was 185 mg/dl. Both values in the fourth 8-h infusion are well above the concentration required to kill 100% of human pancreatic tumor cells in our laboratory (Fig. 2).

Plasma levels during the first infusion were much lower than during the fourth infusion; this indicates an enormous capacity for destruction of ascorbate by this individual and highlights the need for measurements to ensure that adequate plasma levels of AA are achieved during therapy.

So far, plasma levels of over 100 mg/dl have been maintained in 3 patients for more than 5 h using continuous intravenous infusion. The patient cited above has, to date, received 39 of the 8-h infusions of AA, ranging in dose from 57.5 to 115 g, over a 13-week period. A recent CT scan revealed that there had been no progression of tumor growth during the treatment period.

Altogether, six patients have been infused intravenously with similar doses of AA over 8-h periods with no reported side-effects. In all cases, the patients had either been given no further therapeutic options by their oncologists, had refused further conventional treatment, or in one case, requested the use of AA in conjunction with standard chemotherapy. Intravenous AA administration in these cases was approved by our Institutional Review Board.

Discussion

Counter arguments

Many oncologists believe that the issue of AA and cancer is closed mainly due to the Mayo Clinic studies of Creagan and Moertel (47,48). Even those directly involved in cancer research with AA are skeptical of its therapeutic value due to the high levels required for cytotoxic effects – even if those effects are preferential. One unanswered question is whether plasma AA levels can be maintained above the levels that have a direct cytotoxic action on tumor cells in the human body. This has partially been answered by the example given above. Evidence that tumor cytotoxic effects have been achieved in cancer patients has been reported by Cameron and Pauling (20,37,51). In rare instances of patients with widely disseminated and rapidly proliferating tumors, intravenous AA administration (10–45 g/day) precipitated widespread tumor hemorrhage and necrosis, resulting in death. Although the outcomes were disastrous in these cases, they are similar to the description of tumor-necrosis-factor-induced hemorrhage and necrosis in mice (52) and seem to demonstrate the ability of AA to kill tumor cells in vivo.

Another unresolved question is to what extent the in vitro AA cytotoxicity may be extrapolated to in vivo conditions. In vitro cultures contain 'free' iron or copper ions. These ions, which are capable of catalyzing the oxidation of AA, could be responsible, at least in part, for the cytotoxicity of AA. The in vitro levels of these ions, particularly for copper, are probably unachievable in plasma. However, the work of Tsao suggests that some ionic copper is available in vivo (at least in mice) as a catalyst for ascorbate oxidation. Tsao reported that dietarily supplemented ionic copper potentiated the inhibitory effect of ascorbate on human mammary xenografts in mice (19). In the case of the cell culture data presented in Figure 1 and 2, it is unlikely that ionic copper was responsible for catalytic oxidation of ascorbate, as no copper was added to, or in the original formulation of, the medium. The above-mentioned necrosis and hemorrhage case reports, and the work of Tsao, suggest that the limitation of ionic catalysts is not always critically limiting, but this issue requires further clarification and study. It may be possible, for example, to find

ways to adjust in vivo conditions to ensure adequate vulnerability of tumor cells to high levels of AA.

We realize that extrapolation of in vitro data to in vivo therapeutic value is limited in multiple ways, one of which is the effect of serum concentration on toxic effects. Figure 3 demonstrates that 20% human serum added to the culture medium of human prostate tumor cells (ATCC PC-3) partially protects the cells from the inhibitory effects of AA. Begin reported a similar, dose dependent, protective effect of fetal calf serum on the toxicity of polyunsaturated fatty acids toward human breast cancer cells (53). This leaves us not knowing the exact toxic dose of AA for either normal or tumor cells. The combination of the data from Figure 2, in which toxic effects of AA on one normal cell line were observed at 58.36 mg/dl and the lack of side effects in patients maintaining >100 mg/dl plasma levels suggests that there is at least some negative shift of AA toxicity when moving from in vitro to in vivo. The precise preferential in vivo tumor toxic levels of AA have yet to be determined.
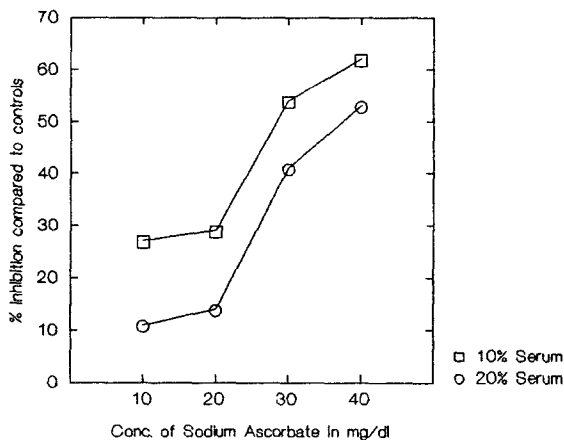

Fig. 3 Dose response (mean of pool of 8 samples) of human prostate tumor cell line (PC-3, ATCC, Rockville, MD) to sodium ascorbate. Results reflect total viable cells. 10 and 20% human serum from patient with prostate adenocarcinoma was added to DMEM Hi-glucose culture medium (Irvine Sci.) w/10% heat-inactivated fetal calf serum + antibiotics + Fungizone. 5% CO_2 humidified incubator at 37°C. Cultured for 7 days after supplementation of ascorbate. Seeded with 5000 cells/well in 24-well culture plates (Nunc).

One other factor which could influence how in vitro effects are extrapolated to in vivo effects is active transport of AA into certain tissues. Several tissues have been identified as containing greater than plasma concentrations of AA (wt of AA/unit of wet tissue), thus indicating an active transport of AA. In descend-

ing order, tissue levels of AA in humans rank as follows: adrenals, leukocytes, pituitary, brain, eye-lens, pancreas, kidney, liver, spleen, heart-muscle, and plasma (54). Plasma concentrations of AA required for toxicity of both normal and tumor cells in these tissues could potentially be lower.

Safety

Although it is very rare, tumor necrosis, hemorrhage, and subsequent death should be the highest priority concern for the safety of intravenous AA for cancer patients. While there is little data on the safety of the high doses of AA that we are proposing here other than the reports of Klenner, who reported no ill effects of dosages as high as 150 g intravenously over a 24-h period (33), many of the publications cited earlier describe the relative lack of toxicity of high dose oral AA, including Cathcart (55) who describes no ill effects with doses of up to 200 g/d in patients with various pathological conditions. According to Rivers, (56) high dose AA is generally safe with contraindications in the following circumstances: renal insufficiency, chronic hemodialysis patients, unusual forms of iron overload, and oxalate stone formers. Care should be taken to screen potential patients for the above-mentioned conditions prior to beginning any high-dose AA therapy. Screening for red cell glucose-6-phosphate dehydrogenase deficiency, which can give rise to hemolysis of red blood cells under oxidative stress (57), should also be performed. After screening, any cancer therapy should be started at a low dosage to ensure that tumor hemorrhage does not occur.

Cameron described a rebound effect that can occur in response to high circulating levels of AA. He proposed that abnormally low levels of AA can occur between intravenous infusions of AA and that his effect is caused by increased levels of hepatic enzymes responsible for degradation and metabolism of AA (34). We have not found that to be the case, at least when the patient is orally supplementing between infusions. All of our patients have supplemented between infusions, therefore we have no data that allows us to directly compare our results to Cameron's. Plasma levels of two patients who were receiving 15 and 22.5g infusions of AA 3 times weekly and orally supplementing to bowel tolerance with <10 g/d of AA were 2.4 and 2.8 mg/dl immediately prior to infusions. Another patient who was also supplementing with oral AA and receiving infusions 2 times weekly, recorded plasma AA levels of between 2.6 and 4.5 mg/dl on the 6 occasions prior to infusion. These examples indicate that a scorbutic rebound effect can be avoided with

oral supplementation. Because of the possibility of a rebound effect, measurement of plasma levels during the periods between infusions should be performed to ensure that no such effect takes place. Every effort should be made to monitor plasma AA levels when a patient discontinues intravenous AA therapy.

Conclusion

We believe that sufficient evidence exists to support the testing of intravenous AA for extended periods as a cytotoxic chemotherapy agent, in doses high enough to maintain plasma levels above those which have been found to be preferentially cytotoxic to tumor cells in vitro. AA is relatively non-toxic and is preferentially cytotoxic to tumor cells at levels attainable in human plasma, at least in vitro. Further studies on the in vivo effects and mechanism of AA's preferential cytotoxicity need to be performed.

Acknowledgements

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