

Ascorbic Acid in Cancer: A Renewed Hope?

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Abstract

Ascorbic acid (AA), long known to treat scurvy, has had debatable use as an anti-neoplastic drug in the past. However recent *in vitro* and *in vivo* studies have revealed previously unexplored mechanisms through which AA selectively damages cancer cells without causing damage to normal cells. In view of newly emerging evidence, many clinical trials have been designed to study these effects in patients with different types of cancers. Promising results from these initial trials are giving renewed hope to the use of AA as an adjuvant to the conventional chemotherapeutic drugs to treat cancer, to alleviate toxicity from the treatment and to reduce patient morbidity.

Keywords: Ascorbic acid; Cancer

Introduction

Vitamin C in physiological and deficiency states

We have come a long way in our knowledge about ascorbic acid (AA), commonly referred to as vitamin C, from the year 1747, when Scottish naval surgeon James Lind discovered that a nutrient in citrus foods prevents scurvy. AA is a water soluble ketolactone which can undergo intracellular oxidation to form ascorbate and dehydroascorbic acid (DHA). Humans lack the ability to synthesize AA as they lack the vital enzyme L-gulonolactone oxidase, which is the final step in the synthesis [1]. Both AA and DHA present in dietary form are absorbed in the entire length of the human intestine. The intracellular concentration of AA is mediated by Sodium-dependent Vitamin C Transporters (SVCT1 and SVCT2). SVCT1 is present largely in the epithelia lining the intestine and in the renal epithelia, where its role is absorption of dietary AA and reabsorption from the glomerular filtrate, respectively. In addition, SVCT2 is metabolically active in some specialized tissues like the placenta, having the role of facilitation of transport of AA to the fetus [2]. DHA on the other hand is absorbed by sodium independent Glucose Transporters (GLUT1, GLUT3 or GLUT4), and subsequently undergoes intracellular reduction [3,4]. AA is an important co-factor in multiple hydroxylation reactions including collagen formation, mitochondrial fatty acid transport, maintenance of homeostasis in intracellular organelles like mitochondria, and endoplasmic reticulum, and along with other anti-oxidants like glutathione, is an important scavenger of ROS [1,5]. Thus, dietary supplementation of AA is essential to maintain normal physiology. The Recommended Daily Allowance (RDA) for AA in adults is 90 mg/day for men and women [6,7]. A daily dietary consumption of 100 mg of AA was found to be sufficient to prevent scurvy for 1 month even if dietary AA was abruptly stopped [8].

As a modality of treatment of cancer, studies in the literature regarding the use of AA have been controversial. Recent advances in the understanding of the effect of AA in cancer biology have reignited an interest in its use in cancer therapy or in mitigating the morbidity associated with treatment for cancer. To understand the highlights of recent advances in this field, we can consider the typical sequence of events seen in most untreated cancers (Figure 1; black text) and briefly review the role of AA in each of these phases (Figure 1; blue text).

Ascorbic Acid can Prevent DNA Damage

One of the most common causes of DNA damage is due to the

formation of reactive oxygen species (ROS) and thymidine dimers. The inability of the cell to repair this damage and / or undergo apoptosis is an important first step in tumorigenesis. As an anti-oxidant, AA protects DNA and mitochondrial proteins of the normal cell from the damage caused by ROS [9]. This role of AA in the prevention of cell damage by the scavenging of ROS can be considered as the first line of defense against cancer (Figure 1, unshaded box; AA actions in blue text).

Ascorbic Acid can Alter Intracellular Pathways and Cause Cancer Cell Death

Division of cells that retain damaged DNA results in the development of clones having tumorigenic potential. Emerging evidence has shown the close interaction between AA and important intracellular pathways including those of apoptosis, autophagy, and immune-activation, which play a vital role in clearing these tumorigenic clones.

Treatment with AA may directly induce cell death of these tumorigenic clones by altering the key proteins involved in the apoptotic pathway. In human colon cancer cells (HCT-8), AA treatment at a concentration of 2 mM resulted in the induction of apoptosis, 12 hours after the treatment, by increasing calcium influx into the endoplasmic reticulum, and upregulation of the expression of the pro-apoptotic factor Bax [10]. Similarly, when treated with AA, modulation of p53, p21, Bcl-2 and Bax by AA in adult T-cell leukemia cell lines (HuT-102, C91-PL, CEM and Jurkat) resulted in the induction of cancer cell death by apoptosis in a dose dependent manner [11]. AA was also found to upregulate the expression of p53 to enhance cisplatin induced apoptosis in human colon cancer cells (HCT116) [12]. When AA is combined with a glycolysis inhibitor, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3-PO), a synergistic induction of apoptosis via a ROS-dependent pathway in non-small cell carcinoma cell lines (H1299, H661

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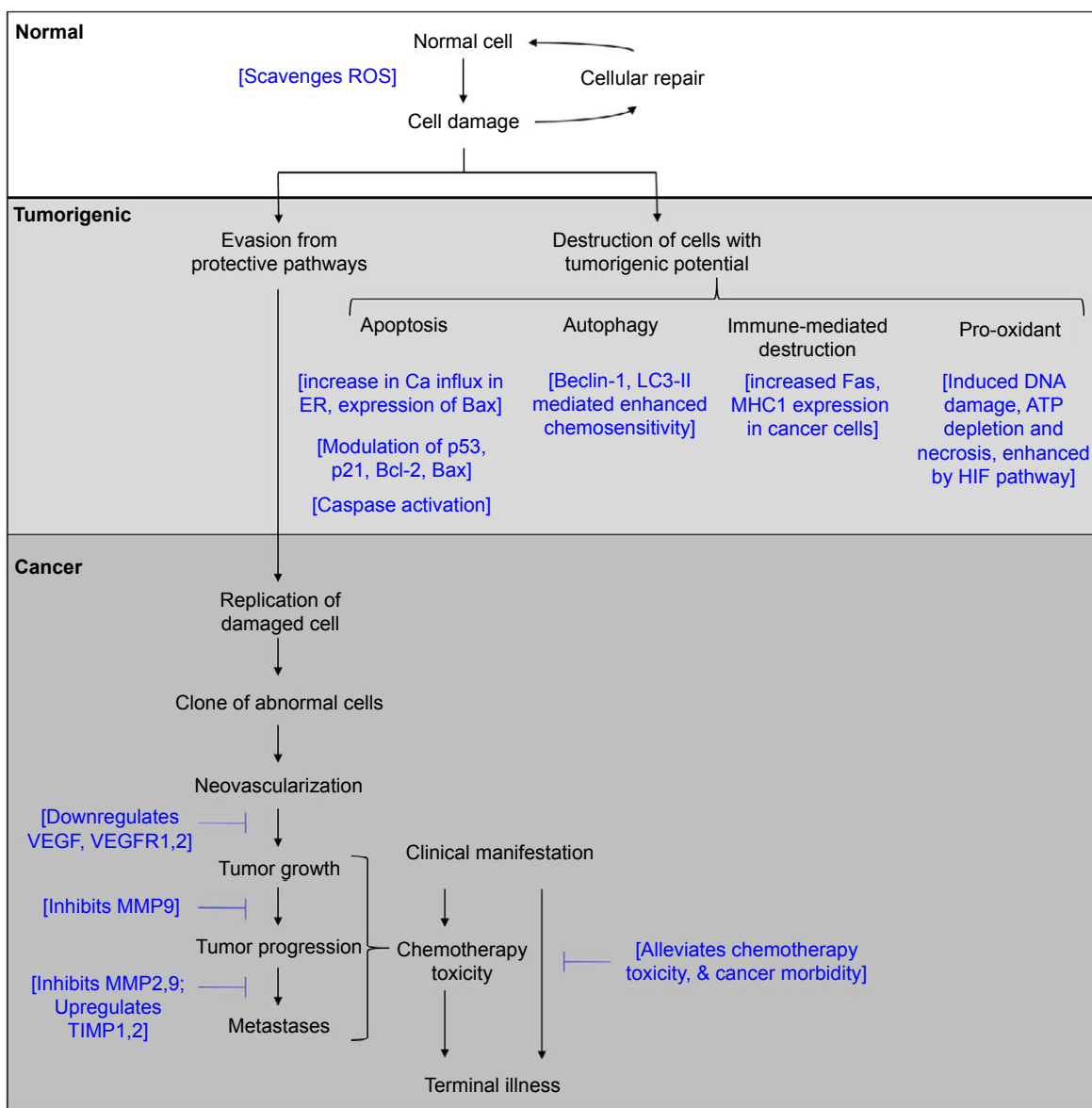


Figure 1: Role of Ascorbic Acid in cancer. Arrows illustrate the typical pathway (black text) seen in untreated cancers. Actions of normal cellular repair are shown in the clear box. Mechanisms associated with the initiation of cellular pathology that may result in a tumorigenic state are shown in the light grey box. Mechanisms of cancer cell biology and progression are highlighted in the dark grey box. Inhibition of or effects on a particular step by Ascorbic Acid is shown in bracketed blue text.

and A549) was observed [13]. In addition, AA was shown to potentiate low dose methotrexate in inducing cancer cell death by caspase 3 and caspase 9 activation in hepatocellular carcinoma cells (Hep3B) [14].

In human breast cancer cells (BT20 and MDA-MB231), treatment with AA affected the autophagic pathway by the induction of caspase-independent effectors including beclin-1 and LC3-II [15]. While AA was found to play an important role in the modulation of the proteins involved in essential intracellular pathways to eliminate the clones with tumorigenic potential, it also upregulated the expression of Fas and MHC class I, which in turn mark these abnormal clones as a target for host immune cells [16].

AA in the presence of catalytic metal ions can also act as a reducing agent by donating a single electron. This phenomenon is particularly important when it reduces the ferric ion to the ferrous form which

in turn reacts with the hydrogen peroxide molecule to produce the strongly oxidizing hydroxyl radical (Fenton reaction). By constantly renewing the ferrous form of iron, AA directly acts as a pro-oxidant [1,17-19]. In an elegantly conducted study, Tian et al showed that high levels of intracellular AA induced oxidative stress and DNA damage in a dose dependent manner in different cancer cell lines (VHL-defective RCC10, RCC4, RPTEC, Bel-7402, HeLa, HCT116, MDA-MB-435S, SK-OV-3, SW480, and U251), which in turn caused ATP depletion leading to necrosis. However, the normal cells were spared from this pro-oxidant effect (Warburg effect). This mechanism of selective destruction of cancer cells was further enhanced synergistically by the activation of hypoxia-inducible factor (HIF) [20]. Thus, directly altering intracellular pathways and marking the abnormal clones as targets for immune cells is the second line of defense conferred by AA (Figure 1, light grey box; AA actions in blue text).

Ascorbic Acid can Decrease Tumor Progression and Cancer Metastasis

As the clone of abnormal cells multiply, the cancer tissue requires additional blood supply for sustaining multiplication. Inhibition of neovascularization results in necrotic changes in the rapidly dividing cancer tissue. AA at pharmacologic doses (<5 mM) was found to restrict this critical step of neovascularization in colon cancer cells (RKO and SW480) by down-regulating the pivotal factor, vascular endothelial growth factor (VEGF), and VEGF receptors 1 and 2 [21]. As the cancer tissue grows, for it to invade neighboring tissues and metastasize, it is essential for the cancerous cells to develop the ability to breakdown extracellular matrix (ECM). Matrix metalloproteinases-2 and 9 (MMP-2 and 9) are proteases that play a key role in the breakdown of the ECM, which normally is an important step in wound healing, angiogenesis, and bone remodeling. Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a glycoprotein which naturally inhibits MMPs preventing excessive break down of the ECM. An intricate balance in the activity of MMPs and TIMP-1 maintains the integrity of the ECM under homeostasis [22]. However, many cancers are known to overexpress MMP-2 and 9, which results in excess digestion of the ECM, an important step involved in tumor progression and metastases. AA was found to directly inhibit the expression of MMP-2 and 9 and upregulate the expression of TIMP-1 and 2, which directly inhibited cancer growth and metastases in mouse models [23,24]. Inhibition of cancer neovascularization and maintaining tissue barrier integrity act as the third line of defense of AA against cancer (Figure 1, dark grey box; AA actions in blue text). Understanding the pharmacokinetics of AA and DHA is critical as DHA may not provide the same anti-cancer effects as AA [25,26].

Ascorbic Acid as a Palliative Therapy in Cancer

In addition to the effects of AA on cancer cell biology as seen by evidence from *in vitro* and *in vivo* studies, AA has also shown beneficial effects, alleviating the morbidity of cancer and its treatment in some clinical studies. Chemotherapeutic drugs given to treat cancers produce many debilitating side effects like pancytopenia, acute kidney injury, neuropathy, and cardiomyopathy. Cisplatin is an important anti-neoplastic drug used in the treatment of many solid organ cancers and the most important dose limiting toxicity that is seen in a significant number of patients is nephrotoxicity. It has been shown previously that AA, either when administered alone or when used in combination with cisplatin, alleviated this toxicity without altering the chemotherapeutic effects of cisplatin [27-33]. AA, used at a concentration ranging from 3 mM to 100 mM was also found to reduce doxorubicin-induced cardiomyopathy and paclitaxel-induced toxicity without altering efficacy of the anti-neoplastic effects of these drugs [34-36]. Reduced morbidity was shown in a study conducted in Korea, where terminal cancer patients who received high dose AA (10 g AA twice a day with a 3 day interval followed by an oral dose of 4 g daily for a week) had lesser symptoms of fatigue, nausea, vomiting, pain and appetite loss than those who did not [37]. However, further studies are required to understand the mechanisms involved in alleviating the toxic side effects of treatments in terminal cancer.

Clinical Trials Looking at Benefits of Ascorbic Acid Therapy in Cancer

Saturation of bioavailability is seen when AA is administered at a per oral dose of 400 mg daily, which corresponds to a plasma level of 60–100 μ M. This aspect could be one of the reasons for failure of previous clinical trials to show benefit in administering AA to cancer

patients [38,39]. However, intravenous (IV) administration of AA can bypass this saturation and can achieve plasma concentrations up to 50 mM, which have been well tolerated without side effects [36,40,41]. Recently concluded phase 1 clinical trials conducted on small groups of pancreatic cancer patients show that there is no toxicity observed in cancer patients treated with high dose IV AA (target concentration around 20 mM) [42,43]. Ma Y et al. have recently shown evidence that high dose IV AA given as an adjuvant therapy with carboplatin and paclitaxel, showed synergistic inhibition of ovarian cancer in a mouse model. An elegantly designed clinical trial in humans (NCT00228319) based on this mouse model by the same group did not show toxicity due to IV administration of high dose AA (target concentration of 20-23 mM) [36]. Many ongoing clinical trials are also looking at the safety of the use of high dose IV AA in cancer [NCT01833351, NCT01080352, NCT01754987 and NCT01555489]. As more evidence regarding safety of high dose IV AA is coming to light, new clinical trials are being conducted that explore the potential benefits of the use of AA in cancer when combined with standard chemotherapy [NCT01555489, NCT01550510, and NCT01364805].

Conclusion

Despite all the debates regarding the anti-cancer effects of AA, recent evidence suggests that AA has a role in checking cancer progression from the initial selection of a malignant clone to the metastases of cancer cells and alleviating chemotherapy toxicity and decreasing morbidity of terminal cancer [36,44]. A recently conducted study showed that AA can be incorporated into solid lipid nanoparticles, which can induce cancer cell death by targeted delivery [45]. Multiple studies discussed above have looked at AA concentrations ranging from 2 mM up to 100 mM, which is sufficient to cause cancer cell death *in vitro*. Improved bioavailability by IV AA administration and its corresponding safety profile to date, as well as the encouraging *in vitro* studies and the novel delivery by the use of nanotechnology all suggest further exploration of AA in cancer would be advantageous. Larger clinical trials are needed to confirm the safety of IV AA to maintain high plasma concentrations in different cancers, to establish the benefit of adding AA as adjuvant drug to different chemotherapy regimens for cancers, and to refine the efficacious dose.

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