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Mini-Review

Defeating cancer with high concentrations of vitamin C: evidence from *in vitro* studies

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ABSTRACT

Vitamin C is an essential nutrient with several different functions in the human body. Humans and a few other animal species are incapable of producing it, and therefore depend on a balanced diet to ensure adequate plasma levels of this nutrient. The severe deficiency of vitamin C leads to scurvy, and a balanced diet alone can effectively prevent scurvy, but cannot guarantee a good health condition. This led to the conclusion that to be in good health man needs amounts of vitamin C in the order of grams (mega-doses) per day. Mega doses of vitamin C administered through intravenous injection can produce high plasma concentrations that *in vitro* show a powerful cytotoxic effect on a number of different human tumour cell lines. A number of different mechanisms have been advocated to explain the selective cytotoxicity of vitamin C to cancer cells, but whatever the mechanism may be, this observation has the potential for revolutionising the field of cancer chemotherapy.

KEYWORDS: vitamin C, ascorbic acid, sodium ascorbate, intravenous ascorbate, cancer, Warburg effect, GLUT, SVCT, human tumour cell lines

INTRODUCTION

Vitamin C (ascorbic acid or ascorbate) is an antioxidant and is a booster of the immune function. It is essential for the synthesis of collagen (one of the major components of connective tissue) and carnitine. Moreover, it shows antiviral and antibacterial effects *in vitro*, plays a role in microsomal hydroxylation reactions that catalyse cholesterol catabolism and detoxification of xenobiotic chemicals, and is involved in the metabolism of neurotransmitters [1-3].

Humans, as well as guinea pigs, some primates, a particular type of fruit-eating bat, the majority of fishes and birds [4], do not produce vitamin C, and therefore depend on diet for an adequate plasma concentration of this fundamental nutrient.

The severe deficiency of vitamin C leads to scurvy. Symptoms of scurvy include bleeding abnormalities (petechiae, perifollicular and sub periosteal haemorrhage, ecchymoses, purpura, bleeding gums, and hemarthrosis), bone pain, osteoporosis, arthralgias, myalgias, oedema, ascites, cardiomegaly, and electrocardiographic abnormalities suggestive of cardiac disease [5-7]. Fatigue, lassitude, and emotional changes (including depression and hypochondriasis) may precede the development of frank scurvy [8].

Thirty-six years ago, an important review by Pauling, Cameron, and Leibovitz presented the scientific basis to support the use of vitamin C as a therapeutic agent in the treatment of cancer [9].

Historical background

Frederik Klenner, in 1949, proposed treating cancer with vitamin C. He had also reported the successful treatment of bulbar poliomyelitis and a number of other viral diseases, with high doses of vitamin C,

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administered through mouth, intravenous and intramuscular injection [10].

In 1952, McCormick [11] proposed the use of vitamin C as a chemotherapeutic agent. Tamayo and Richardson [12] published a review on the use of vitamin C in cancer, but the first comprehensive review on this topic appeared in the scientific literature in 1979 [13]. However, Irwin Stone was the first to establish the rationale for the use of high doses of vitamin C in the treatment of cancer.

According to Irwin Stone, a biochemist who studied in depth the many functions and roles of ascorbate in man, a balanced diet alone, can effectively prevent scurvy, but cannot guarantee a good health condition. On this ground, he coined the term "hypoascorbemia" to define the inability of humans and a few other species to synthesize ascorbic acid because of the lack of the enzyme L-gulonolactone oxidase (GLO) [14] as a consequence of an "inborn error of carbohydrate metabolism" [15-17]. The lack of GLO in humans [18] led Stone [19-20] and other scientists, such as the two-time Nobel laureate Linus Pauling, to hypothesize that to be in good health man needs mega-doses of vitamin C (several grams a day), rather than doses in the order of milligrams, as stated by the Recommended Daily Allowances [21].

Stone also hypothesized that almost every known disease (including cancer, degenerative and chronic diseases, cardiovascular diseases, etc.) largely results from inadequate intake of vitamin C through food, and a consequent "hypoascorbemia", leading to the clinical condition he defined "Chronic Subclinical Scurvy" (CSS) [22, 23].

Based on this knowledge, in 1974, Cameron & Campbell published an article showing that, in "untreatable" cancer patients, vitamin C in high doses could bring about some significant improvement in morbidity and mortality [24]. Following this article, Cameron and Pauling showed that the survival of untreatable cancer patients increased by a factor of about 3 in the majority of cases, and about 20 in 10% of them, under treatment with 10 grams of vitamin C per day, starting with the intravenous route, followed by oral administration [25]. The same authors further confirmed these results in another article published by the same journal two years later [26]. Two later studies performed at Mayo Clinic [27, 28], failed in reproducing the results reported by Cameron & Pauling, but although severely flawed in the method, patients' selection, treatment procedure, and conclusions, they were accepted as the proof of the inefficacy of high doses of vitamin C in treating cancer [29].

Finally, in 2005, Chen and colleagues demonstrated that pharmacologic ascorbic acid concentrations selectively kill cancer cells by acting as a pro-drug to deliver hydrogen peroxide (H_2O_2) to cancerous tissues [30, 31], and since then, a number of other scientists throughout the world confirmed the anticancer properties of high (pharmacologic) concentrations of vitamin C at least *in vitro* and in animal studies [32].

Rationale

The rationale for the use of high doses of vitamin C in cancer, although largely investigated, is still matter of some controversy. Although widely considered an antioxidant [33], vitamin C in high concentrations can generate cytotoxic activity [34], and the majority of reports concur that the cytotoxic effect of vitamin C on cultured malignant cell lines is due to its pro-oxidant activity [30, 31]. In particular, at low ("physiologic") concentrations, vitamin C behaves as an antioxidant, while at high ("pharmacologic") concentrations, it behaves as a pro-oxidant [35]. Mixtures of ascorbic acid and copper or iron have been used for decades to induce oxidative modifications of lipids, proteins and DNA [36]. Ascorbic acid may contribute to oxidative damage formation by reducing ferric (Fe^{+++}) to ferrous (Fe^{++}) ions (and Cu^{++} to Cu^{+}), which in turn can reduce hydrogen peroxide (H_2O_2) to hydroxyl radicals. This has led to the idea that high (pharmacologic) doses of vitamin C act as a prodrug of hydrogen peroxide in biological systems [30, 31, 37-41]. However, some authors reject this view, as vitamin C-mediated Fenton reactions are controlled in the human body, due to the efficient sequestration of iron by iron-binding proteins (ferritin and transferrin), thus suggesting that the pro-oxidant effect of vitamin C may not be relevant *in vivo* [42, 43].

More recently, Yun and colleagues [44], by investigating the effects of high doses of vitamin C on KRAS and BRAF mutant colorectal cells, found that vitamin C in high concentration kills cultured human colorectal cancer (CRC) cells harbouring

KRAS or BRAF mutation and impairs tumour growth in Apc/KrasG12D mutant mice. From this experience, the authors conclude that "the oxidized form of vitamin C, DHA, is the pharmaceutically active agent, and that the selective toxicity of vitamin C to tumour cells stems from high glucose transporter (GLUT) expression combined with KRAS or BRAF oncogene-induced glycolytic addiction". In other words, DHA acts like a Trojan horse. Once inside, natural antioxidants within the cancer cell attempt to convert the DHA back to ascorbic acid; in the process, these antioxidants are depleted and the cell dies from oxidative stress [45].

These data warrant further investigations and clinical trials, but are not completely new. It is, in fact, a common notion that cancer cells with different mutations and histologies display increased glucose uptake and consumption due to the increased levels of GLUT receptors [46, 47]. More importantly, the fourteen members of the GLUT family identified so far exhibit different substrate specificities, and transport glucose as well as fructose, galactose, mannose, glucosamine, xylose, dehydroascorbic acid (DHA), urate and myoinositol with variable affinities and tissue expression, with DHA being transported by GLUT1, GLUT3, and GLUT4 [48].

Upregulation of GLUT expression, on the other hand, is the result of the so-called "Warburg Effect". Back in 1920, Otto Warburg and colleagues observed that tumours were taking up enormous amounts of glucose compared with what seemed to happen in the surrounding tissue. Moreover, glucose was fermented to produce lactate even in the presence of oxygen, hence the term "aerobic glycolysis" [49, 50]. Interestingly, the Warburg Effect is an early event in oncogenesis that is an immediate consequence of an initial oncogenic mutation, such as that of KRAS in pancreatic cancer or BRAF in melanoma; thus, it occurs not only before cell invasion, but also in benign and early-stage lesions [51, 52].

The *in vitro* cellular responses to high doses of ascorbate encompass:

- G1/S phase arrest [53, 54], and cell death by apoptosis [30, 55], autophagy [56, 57], and autoschizis [58].
- oxidative DNA breakage [59-61],
- lipid peroxidation of membranous organelles [62, 63],
- impaired mitochondrial function [64, 65],
- reduced levels of intracellular ATP [56], and Glutathione [66].

Vitamin C can also modulate the expression/ activation of a range of genes/proteins that regulate inflammation [67-71], angiogenesis [72-74], cell proliferation [75-77], and programmed cell death [78]. It may also inhibit the DNA methyltransferase (DNMT) activity in nuclear extracts of MeWo and BLM melanoma cells [79], thus indicating that under physiological pH conditions it influences the activity of the genome by regulating epigenetic processes [80]. Furthermore, vitamin C inhibits the Hypoxia Inducible Factor 1, which is important for the cell to adapt to environmental stress by upregulating glycolysis, angiogenesis, cell survival pathways, vascular control, erythropoiesis, and tissue remodelling [81]. Finally, vitamin C stimulates the immune system [82], and this is the rationale for its use in cancer treatment, according to Linus Pauling.

In vitro data

Since the first reports of its *in vitro* efficacy [30, 31, 55], it has become clear that vitamin C in high concentrations can kill a number of human tumour cells, both *in vitro* [30, 32, 55], and in tumour xenografts in rodents [83, 84].

In our experience, concentrations of vitamin C ranging from 3 to 7 mM, efficiently kill Y79 (retinoblastoma) [85], OCM1 and C918 (uveal melanoma) [86], and a number of myeloid leukaemia-derived cell lines, including HL60 [87], U937, NB4, NB4-R4 (retinoic acid [RA]-resistant), and NB4/AsR (ATO-resistant) human promyelocytic leukaemia [88].

Interestingly, in our human tumour culture experiments, we exposed human cell lines to vitamin C for no more than 1-2 hours, and then washed and cultured them for additional 18-24 hours before the evaluation of viability and apoptosis. As a result, while cell viability was only slightly decreased, at the end of the incubation period with vitamin C, both the percentage and total number of viable cells were substantially decreased, 18-24 hours after the complete removal of vitamin C from the culture medium. These results seem to indicate that vitamin C enters cancer cells almost immediately, when exposed, and exerts its cytotoxic effects from within the cells themselves rather than

from the outside (tissue culture media and/or extracellular fluids).

These observations seem to be in perfect agreement with the mechanistic explanation proposed by Chen and colleagues [31] according to which "pharmacologic" ascorbate (Vitamin C) is a prodrug of H_2O_2 in extracellular fluids (ECF), but not in blood. However, it does not explain how cell damage can be produced by H_2O_2, once ascorbate (Vitamin C) is removed by the culture medium, as we invariably found in our experiments on different human tumour cell lines.

Globally, our experiments demonstrate that:

- Vitamin C in high concentrations in the culture medium can efficiently kill human tumour cell lines derived from different types of cancer;
- The cytotoxic effects of high concentrations of vitamin C is progressive and can be observed even after the vitamin has been removed from the medium;
- No more than two hours exposure to high concentrations of vitamin C are needed to elicit a powerful cytotoxic effect;
- In the case of myeloid leukaemia, the normal myeloid precursors are not affected by the millimolar concentrations of vitamin C used in the experiments;
- 3 to 7 millimoles of vitamin C in the culture medium do not represent a very high concentration if we consider that, in the clinical setting the administration of 100 grams of vitamin C by intravenous infusion, three days a week for eight weeks, has led to plasma levels of more than 30 mM [89].

In vitro vs. *in vivo* data: filling the gap

Intravenous injection of high doses of vitamin C is a widely diffused, though not officially accepted practice, both in the United Stated and Europe. As shown in the previous section, high concentrations of vitamin C can both control and suppress cancer growth *in vitro* very efficiently by a number of different mechanisms, even if, as more recently noticed by Chen and colleagues [90], "the molecular mechanisms of its selective actions against cancer cells are not yet fully understood" and "…much work is still ahead".

However, the number and complexity of the potential mechanisms involved in the highly selective anticancer activity of vitamin C, which encompasses ROS-mediated cell damage, HIF inhibition, stimulation of the immune system, and epigenetic gene regulation, leave rooms for hope.

As reported by Chen and colleagues [90], "there are over 15 early phase clinical trials, with 8 of them using doses ≥50 g, and more observational studies and case reports with doses ranging from 1 g to 125 g per i.v. infusion" and "all showed good tolerability and profound safety. Indeed, when patients have normal glucose-6-phosphate dehydrogenase (G6PD) activity and adequate renal function, adverse events and toxicity are minimal".

All this should lead to the conclusion that vitamin C has the potential for revolutionizing the field of cancer chemotherapy, and although some scepticism remains [91], the evidence behind the efficacy of high doses of vitamin C in the treatment of cancer is overwhelming [90]. However, in order to translate efficiently the results of the *in vitro* studies to the clinics and fully exploit the complete anticancer potential of this molecule in clinical settings, much work is still ahead.

In a recent, excellent review on this topic, Gonzalez and colleagues [92], while reviewing the many beneficial effects of vitamin C in cancer treatment, suggests that three possible fields of intervention in order to improve the effectiveness of high doses of vitamin C *in vivo* are represented by:

The level of tissue oxygenation: it is widely known that tumour tissue oxygenation is very low, particularly when compared to the cell culture conditions used in the laboratory. Assuming that H_2O_2 formation is the main mechanism through which vitamin C destroys cancer cells, it is clear that low levels of oxygen, within the cancer tissue, may drastically decrease the efficacy of vitamin C. Improving cancer oxygenation might be realized through hyperbaric oxygen, ozone, sodium bicarbonate, and dichloroacetic acid;

The levels of blood glucose: excessive blood glucose may compete with vitamin C for the GLUT receptor sites, thus decreasing the amount of vitamin C, which can be effectively captured by the cancer cell. Reducing blood glucose levels with an appropriate diet, during vitamin C treatment, may provide a solution;

Physiological Red-Ox balance: the physiological Red-Ox state may also influence intravenous vitamin C effectiveness as an anticancer agent. An accurate evaluation of the Red-Ox state of each patients undergoing vitamin C treatment is recommended.

Practical guidelines concerning the intravenous infusion modalities and schedules, the type of vitamin to be used (ascorbic acid or sodium ascorbate), and the pharmacologic formulation, may also give a substantial contribution to the improvement of the clinical efficacy of vitamin C against cancer.

CONCLUSION

The evidence behind the anticancer effects of vitamin C in high concentration is no longer a matter of discussion among scientists. The focus of the debate, after the demonstration of the powerful anticancer effects of vitamin C *in vitro* in primary tumour cultures and animal tumour models, is mainly on the translation of the experimental data into a clear-cut clinical demonstration of efficacy. This can be achieved in a number of different ways, as mentioned in the previous sections.

One question remains open regarding the use of vitamin C in clinical cancer treatment: will there be anybody interested in investigating the clinical efficacy of a molecule which is natural, essential for many physiological functions, non-patentable, selectively active against cancer cells, completely harmless for normal cells, and with no side effects? This is, at present, the main question regarding the clinical use of vitamin C as an anticancer agent!

CONFLICT OF INTEREST STATEMENT

None to declare.

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