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Vitamin C Against Cancer

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Abstract

The selective anticancer properties of vitamin C are known since at least four decades. However, only recently *in vitro* studies have shown that vitamin C, in high enough concentrations, can efficiently and selectively kill a number of different human tumor cell lines, and these data have been confirmed in experimental animal tumor models. The first human clinical trials revealed that high doses of vitamin C administered by intravenous injection are not only very well tolerated but also substantially improve the quality of life of patients with clinically advanced cancer. However, the clinical evidence of the effectiveness of vitamin C in fighting off cancer is still controversial. The present chapter outlines the importance of vitamin C for a number of physiological functions, within the human body, and shows that there is a solid rationale for its use in the routine treatment of cancer, either alone or in combination with conventional treatment.

Keywords: vitamin C, sodium ascorbate, cancer, oxidative stress, free radicals, high dose intravenous ascorbate

1. Historical background

The anticancer effects of vitamin C (ascorbic acid) are known since 1969, when Benade et al. published a paper showing that the sodium salt of this nutrient (sodium ascorbate) is highly toxic or lethal to Ehrlich ascites carcinoma cells *in vitro* [1].

A few years before this discovery, the American biochemist, Irwin Stone, had already published some interesting reports on the genetic origin of scurvy, the lethal disease produced by severe deficiency of vitamin C. He had also coined the term "*hypoascorbemia*," to define the

inability of humans and a few other species to synthesize vitamin C because of the lack of the enzyme L-gulonolactone oxidase (GLO), due to an "*inborn error of carbohydrate metabolism.*"

After decades of research in this field, Stone became convinced that given the lack of GLO, and the low amount of vitamin C introduced with food, man easily undergoes a condition of "*chronic subclinical scurvy* (CSS)," and CSS is our most widespread disease. The long-term biochemical outcome of CSS, according to the scientist, sets the stage for the development of the serious medical problems of later life, including, among others, cardiovascular diseases (CVDs), collagen diseases, and cancer. Clinical tests reveal that mega levels of vitamin C are useful in the prevention and treatment of cancer and other diseases. Moreover, to correct CSS at least 10 g of vitamin C per day depending upon the incident stresses is required. Under stress, the daily requirement of vitamin C may be up to 200–300 g/day.

Stone noted that in the past decades, "micro" daily levels of vitamin C had wiped out acute frank scurvy, but did not prevent the epidemic incidence of CSS, the more insidious and more dangerous, relatively asymptomatic form of scurvy. The full correction of CSS is, therefore, the first step in any preventive medicine procedure. Even though the recommended dietary allowances (RDA) [2] prescribe daily amounts of vitamin C in the order of a few milligrams, these, according to Stone, will only prevent the appearance of the terminal symptoms of scurvy, but will not do much else.

On this ground, Stone concluded that "vitamin C" is not a real "vitamin," and proposed the use of the term "ascorbate," to better define this missing human liver metabolite [3, 4].

Therefore, according to the scientist, cancer, as well as almost any other known human diseases, depends on both the inability of humans to synthesize vitamin C and the insufficient amount of the nutrient normally assumed with food, leading to a deficiency ("*hypoascorbemia*"), which, in the long term, transforms into CSS, thereby predisposing to all kind of diseases.

The evidence that among the mammals producing their own vitamin C, an unstressed 70 kg goat is capable of producing 13 g of this liver metabolite, [5] and much more under stress had convinced Stone that vitamin C RDAs were largely underestimated. Therefore, he proposed the use of mega doses or doses ranging from 300 to several thousand times, the amount suggested by the RDAs of the nutrient to treat and prevent different diseases including cancer [6].

However, although formally proposed by Stone, the therapeutic use of mega doses of vitamin C was not really a novelty.

In 1949, Frederick Klenner had reported the successful treatment of 60 cases of bulbar poliomyelitis, with high doses of vitamin C, administered by mouth and, simultaneously, by intramuscular and intravenous injection, continuously, for 72–96 hours, until the complete remission of the symptoms [7]. Klenner proposed his anti-Polio, vitamin C–based treatment after reading a series of studies published by Jungeblut, between 1935 and 1937 [8], but he also treated with success, a number of other viral diseases, by using the same high dose vitamin C protocol.

The plea for a substantial revision of the dosage of vitamin C used in clinics had already come from the Nobel Prize Albert Szent-Györgyi, the discoverer of vitamin C, who, in the

introduction to the Stone's book, "The healing factor," wrote, *The medical profession itself took a very narrow and wrong view. Lack of ascorbic acid caused scurvy, so if there was no scurvy there was no lack of ascorbic acid. Nothing could be clearer than this. The only trouble was that scurvy is not a first symptom of lack but a final collapse, a premortal syndrome, and there is a very wide gap between scurvy and full health … But nobody knows what full health is! … Full health, in my opinion, is the condition in which we feel best and show the greatest resistance to disease. This leads us into statistics, which demand organization. However, there is another, more individual difficulty. If you do not have sufficient vitamins and get a cold, and as a sequence pneumonia, your diagnosis will not be "lack of ascorbic acid" but "pneumonia." So you are waylaid immediately* [3].

Therefore, Szent-Györgyi had already warned the medical establishment about the need to radically review vitamin C RDAs (in the order of milligrams) that he considered sufficient to prevent scurvy, but largely insufficient to grant a condition of "full health."

The twofold Nobel Laureate, Linus Pauling later formalized this concept, in an article published in 1974 [9]. In this article, Pauling, after illustrating in detail the arguments in favor of the use of mega doses of vitamin C to treat a number of different diseases, suggests that the RDA of 45 mg/day, be renamed minimum dietary allowance, and the recommended dietary intake be introduced, ranging from 250 to 4000 mg/day for adults.

In the same year, Cameron and Campbell published an article concerning the treatment of 50 patients with advanced cancer, with 10 g of vitamin C administered by vein for the first few days, and then by mouth, for the rest of their lives. The results of this study indicated that high doses of vitamin C are useful as a routine supportive measure to reinforce standard treatment of earlier and more favorable cases [10].

Two years later, Cameron and Campbell demonstrated that the use of the protocol proposed by Cameron and Pauling significantly prolonged the survival and improved the quality of life of terminal cancer patients [11], but their work raised a number of criticisms, especially focused on the randomization procedure. To respond to the critics, the authors decided to undertake a second investigation, but this new study further confirmed that patients on mega doses of vitamin C lived, on average, 251 days longer than the untreated controls [12]. The same authors [13] and a group of Japanese clinicians [14] later confirmed the results formerly obtained by Cameron and Pauling.

In an attempt to either duplicate or refute the results reported by Cameron and Pauling, the Mayo Clinic initiated another investigation, which seemed to disprove the efficacy of the mega doses of vitamin C against cancer [15]. However, according to Pauling, the inclusion criteria used by the Mayo Clinic scientists were not conformed to the ones he had used. In fact, the Mayo Clinic study included patients previously treated with chemotherapy that compromises the immune response, while a functioning immune system is, according to Pauling, a fundamental prerequisite for an effective anticancer action of mega doses of vitamin C.

Therefore, based on this and other criticisms, the Mayo Clinic group undertook a second clinical investigation that substantially confirmed the results of the first one [16], and this study represented, for the scientific community, the definitive evidence of the inefficacy of mega doses of vitamin C against cancer.

As an undoubted evidence of the biases affecting this study, it will be worth mentioning that the second clinical trial performed at Mayo included only patients affected by colorectal cancer (CRC), that are clearly not representative of the entire complex and variegated range of cancer types affecting humans. However, to remain confined to just CRC as a paradigm of cancer, it will be worth mentioning the recent reports showing that vitamin C in high doses kills BRAF and KRAS mutants of CRC, which are resistant to the standard chemotherapeutic regimens, thus, substantially disproving the results of the second Mayo Clinic investigation [17–19].

2. Mechanistic explanation of the anticancer properties of vitamin C

Vitamin C is an essential nutrient with a number of beneficial functions, for the organism, since it

- helps the metabolism of tyrosine, folic acid, and tryptophan;

- increases the elimination of cholesterol;

- contributes to the synthesis of catecholamines;

- helps the body to absorb and breakdown histamine;

- enhances the absorption of nonheme iron;

- promotes the synthesis of collagen (its most widely known physiological function);

- neutralizes free radicals (it is a reducing agent, "scavenger" of free radicals, and a founder among the natural antioxidants);

- protects the DNA from damage due to free radicals and mutagens;

- reduces the risk of premature death;

- fights off widespread environmental pollutants;

- prevents the development of nitrosamines, and much more.

Vitamin C is ubiquitous, but humans, guinea pigs, some primates, a particular type of fruit-eating bat, the majority of fishes and birds do not produce it, and therefore they depend on diet for the assumption and use of this fundamental nutrient [20].

Regarding the anticancer properties of vitamin C, different authors have proposed various mechanistic explanations; among others:

2.1. The prooxidant pathway

As mentioned in the previous section, the hypothesis that vitamin C in high concentrations, administered by intravenous infusion, acts as a prooxidant, rather than antioxidant, leading to the formation of H_2O_2, with consequent oxidative damage to cancer cells, was formerly

proposed in 1969 [1]. According to this hypothesis, vitamin C kills cancer cells through the intracellular generation of toxic hydrogen peroxide (H_2O_2) produced upon its oxidation by the cells themselves.

Although relevant for clinical cancer treatment, this discovery remained "hidden" for more than four decades, and the theory of the prooxidant activity of vitamin C in high concentration was proposed again 36 years later, though with no mention of the original work done in 1969 [21, 22].

The theory was further investigated, very recently, by Yun et al. [17–19] who defined the chain of events leading vitamin C to behave as a prodrug of H_2O_2 thereby killing selectively cancer cells, both *in vitro* and *in vivo*. Briefly, vitamin C in high doses administered by intravenous injection exerts its selective cytotoxic effect on cancer cells, because, after parenteral administration, it behaves as a peroxide delivery system for the generation of sustainable ascorbate radical and H_2O_2 in the extracellular space, with consequent oxidative damage to cancer cells (**Figure 1**). The selectivity of the cytotoxic effect of parenteral vitamin C depends on the fact that cancer cells, compared to their normal counterpart, show a reduced level of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. The reduced level of antioxidant enzymes leads to cellular damage by accumulation of H_2O_2, with consequent intracellular redox imbalance and oxidative damage to different cellular structures.
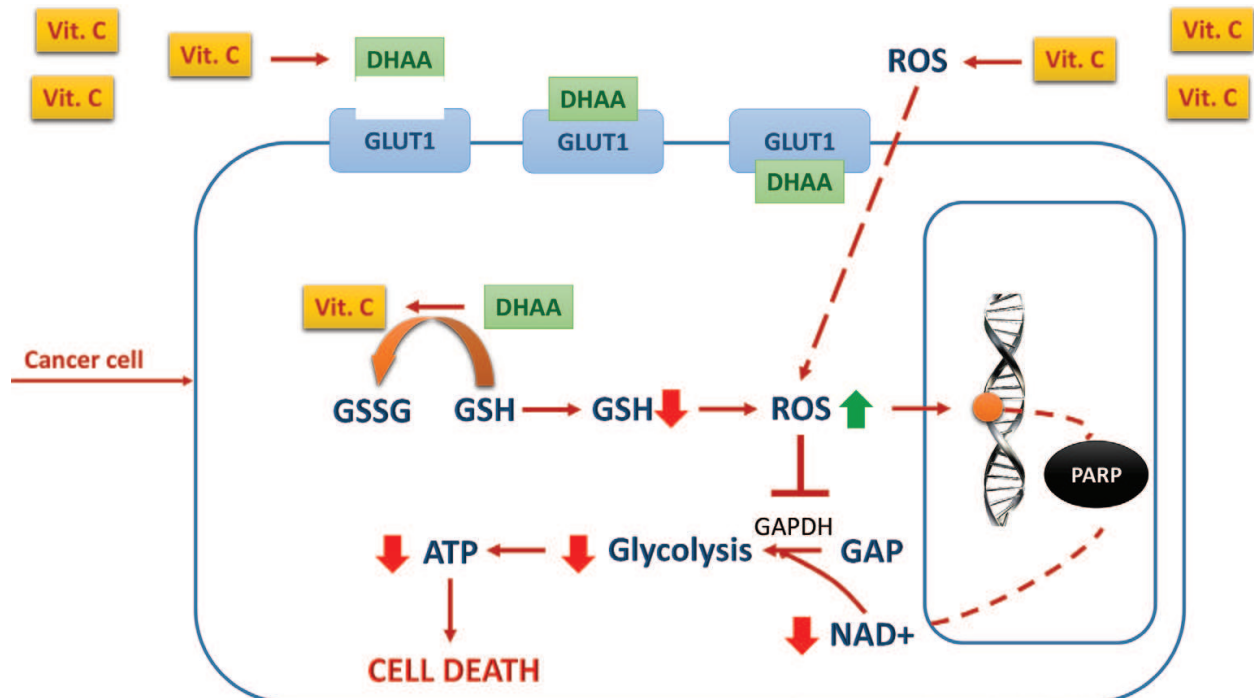
Figure 1. Prooxidant effect of vitamin C (see text). Legend: Vit. C = vitamin C, ATP = adenosin triphosphate, DHAA = dehydroascorbic acid, GSH = glutathione, GSSG = glutathione disulfide, GAP = glyceraldehyde 3-phosphate, GAPDH = glyceraldehyde 3-phosphate dehydrogenase, GLUT = glucose transporter, NAD = nicotinamide adenine dinucleotide, ROS = reactive oxygen species, PARP = poly ADP-ribose polymerase.

Yun et al. [17–19] have recently showed that the death of KRAS and BRAF cell mutants of CRC is imputable to the oxidized form of vitamin C: dehydroascorbic acid (DHAA). DHAA competes with glucose, for intracellular uptake by glucose transporters (GLUTs), mainly one and four subtype receptors. Interestingly, both KRAS and BRAF activating mutations are responsible of the upregulation of GLUT1 expression in different types of cancer, including CRC, although the upregulation of GLUT1 expression is not always associated with increased sensitivity of tumor cell lines to the cytotoxic effects of DHAA.

Investigation into the metabolic makeup of KRAS and BRAF CRC-derived cell lines shows that there is an accumulation of glycolytic intermediates upstream glyceraldehyde-3-phos-phate-dehydrogenase (GAPDH) and a contemporary depletion of the metabolites down-stream GAPDH, indicating an inhibition or severe reduction of its enzymatic activity, which appears to be the key of the cytotoxic effect of DHAA.

In summary, the data reported by Yun et al. on the effect of DHAA on CRC cell lines indicate that in glycolysis-addicted KRAS and BRAF mutated cell lines, high amounts of DHAA enter the cancer cells, thanks to the overexpressed GLUT-1 receptors. DHAA is then reduced again to vitamin C inside the cells. The reduction of DHAA to vitamin C scavenges glutathione (GSH), thus inducing redox imbalance and oxidative stress. Oxidative stress, in turn, leads to inactivation of GAPDH, inhibition of glycolysis, and energetic crisis, which leads to cancer cell death.

According to this mechanistic explanation, vitamin C, functioning as a prooxidant, would induce an increase in the intracellular reactive oxygen species (ROS), which leads to increased DNA damage, with consequent activation of poly ADP-ribose polymerase (PARP), an enzyme necessary to repair damaged DNA. PARP activation would in turn consume NAD+, with NAD+ depletion and consequent ADP depletion, leading to energetic crisis and death of cancer cells [23].

The theory according to which vitamin C in high doses would act as a prodrug of H_2O_2, beyond being criticized by different authors, seems somewhat controversial and overlooks a few important aspects as follows:

- Although reported in 2005 by Chen et al. [21, 22], it is not new, since, as we have seen, it had been already proposed by Benade et al. in 1969 [1]. Interestingly, while according to Benade, H_2O_2 forms inside the cell, starting from vitamin C, Chen et al. conclude that H_2O_2 is formed outside the cell, staring from DHAA. Our experience with high concentrations of vitamin C to treat, *in vitro*, retinoblastoma (Y79) (**Figures 2** and **3**) [24], uveal melanoma (C918, OCM1) [25], human promyelocytic leukemia (HL60) [26], and different human my-eloid leukemia (HL60, U937, K562, NB4, NB4-R1, and NB4/As) cell lines [27] indicates that H_2O_2 forms inside the cells, rather than outside. In fact, in our experiments, the cytotoxic effect of vitamin C on cancer cells in culture persists for hours/days after the removal of vitamin C from the culture medium.

- H_2O_2 is a metabolite normally produced by the cells of the body and usually overproduced by cancer cells. Therefore, H_2O_2 itself could be an optimal substitute for vitamin C, as an an-ticancer compound. In this regard, it could be useful remark that Reginald Holman, in 1957, published a paper in "Nature" showing that rat implanted with Walker 256 adenocarcinoma
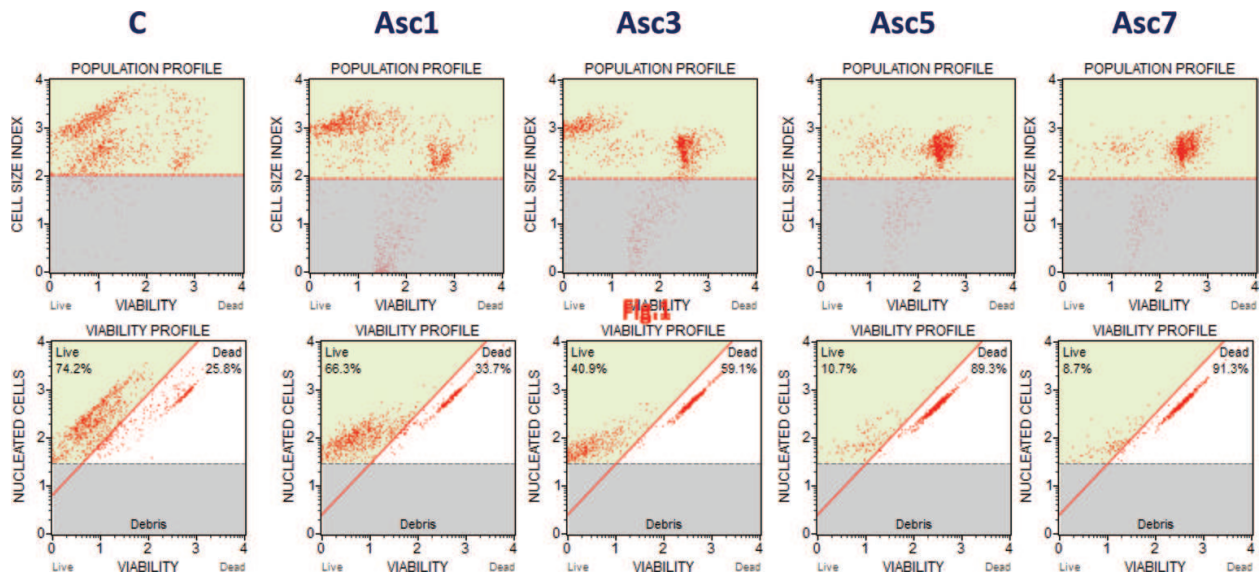
Figure 2. Flow cytometric analysis of Y79 human retinoblastoma cell line viability, after treatment with increasing concentrations of vitamin C (Asc) *in vitro*. C = control sample, Asc1 = vitamin C 1 mM, Asc3 = vitamin C 3 mM, Asc5 = vitamin C 5 mM, Asc7 = vitamin C 7 mM. Starting from a viability of about 74% (control sample), the percentage of viable cells after 1 hour of treatment with vitamin C and 18–24 hours of incubation are about 66% at 1 mM, 41% at 3 mM, 11% at 5 mM, and 9% at 7 mM of vitamin C.
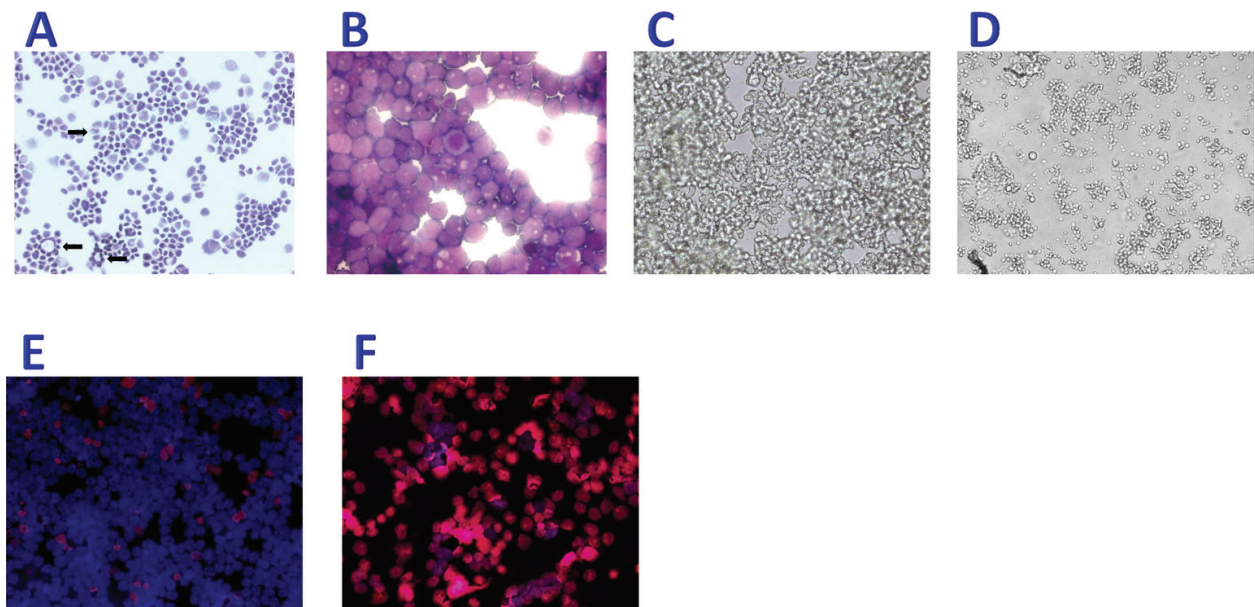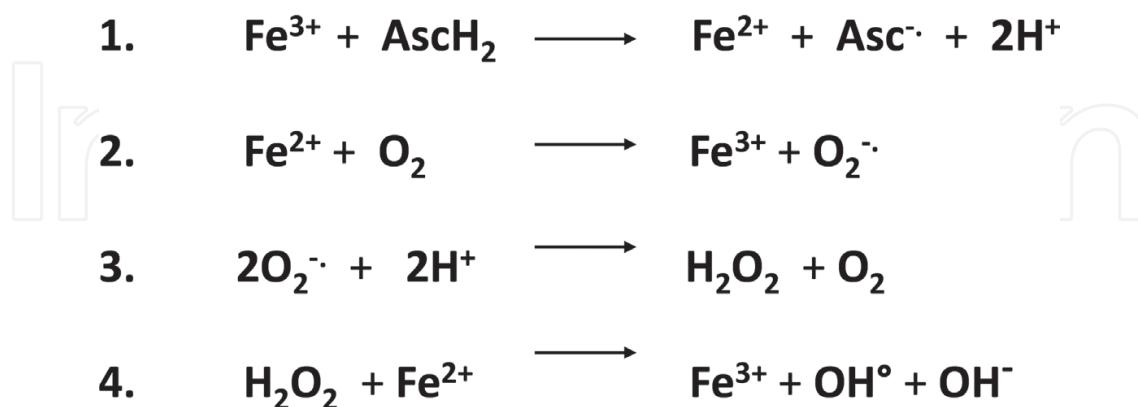

Figure 3. Microphotographs of Y79 human retinoblastoma cell lines: A: hematoxylin/eosin staining of cultured Y79. Interestingly, cells in culture tend to form the typical Flexner-Wintersteiner «rosettes» (black arrows) commonly seen in pathology specimends (original magnification: 200×). B: May-Grunwald Giemsa staining showing morphological details: large cells with loose chromatin and highly basophilic cytoplasm (original magnification: 400×). C: Contrast phase microphotographs of Y79 human retinoblastoma cell lines in culture (control sample): cells tend to form clusters in culture (original magnification: 200×). D: Contrast phase microphotograph of Y79 human retinoblastoma cell lines after treatment with vitamin C 7 mM. At a concentration of 7 mM, vitamin C destroys the vast majority of cells in culture. A few residual cells appear swollen and necrotic (original magnification: 200×). E and F: Hoechst/PI staining of Y79 human retinoblastoma cell line in the culture. With Hoechst/PI, live cells not distinguishable in the black/withe caption. In the control sample (E), there is a clear prevalence of cells stained by the Hoechst dye (alive) while in the sample treated with 7 mM of vitamin C (F), the prevalent color is given by PI (dead cells)(original magnification: 200×).

and treated by simply replacing their drinking water with 0.45% hydrogen peroxide showed a rate of cure of 50–60% [28]. The time for complete disappearance of the tumor varied from 15 to 50 days depending on the tumor size at the beginning of treatment. Holman's work was based on the assumption (later confirmed by studies on vitamin C) that malignant cells are deficient in catalase, and as such unable to detoxify high fluxes of H_2O_2. As a consequence, an *in vivo* measurement of catalase activity in tumors would represent a useful diagnostic tool to predict which cancers will respond to pharmacological doses of vitamin C therapy (or H_2O_2) [29].

- According to the prooxidant theory, vitamin C in high concentrations induces the production of H_2O_2 through a Fenton-like reaction (**Figure 4**). This reaction is the oxidation of organic substrates by iron and hydrogen peroxide. Trivalent iron (Fe^{3+}) is fundamental for the reaction, but, since Fenton-like reactions are usually controlled, *in vivo*, because of iron sequestration by metal-binding proteins, the prooxidant effect of vitamin C, *in vivo*, is considered scarcely significant by different authors [30, 31], and other mechanisms should be hypothesized.

- Cancer cells produce high amounts of H_2O_2, and high levels of this metabolite have been associated with key features in cancer, such as DNA alterations, cell proliferation, apoptosis resistance, metastatic spread, angiogenesis, and hypoxia-inducible factor 1 (HIF-1) activation. On the contrary, decreasing the cellular levels of H_2O_2 may reverse the malignant phenotype. Therefore, H_2O_2 can be either proapoptotic or antiapoptotic, either carcinogenic or anticarcinogenic, depending on its concentration and localization within the cell [32, 33];

VITAMIN C AND FENTON REACTION

1. $\quad Fe^{3+} + AscH_2 \longrightarrow Fe^{2+} + Asc^{-\cdot} + 2H^+$

2. $\quad Fe^{2+} + O_2 \longrightarrow Fe^{3+} + O_2^{-\cdot}$

3. $\quad 2O_2^{-\cdot} + 2H^+ \longrightarrow H_2O_2 + O_2$

4. $\quad H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH° + OH^-$

Figure 4. Fenton reaction mediated by vitamin C. (1) Vitamin C (ascorbic acid, AscH2) reduces ferric ions (Fe3+) to ferrous ions (Fe2+). (2) Ferrous ion reacts with oxygen to produce superoxide. (3) Dismutation of superoxide leads to hydrogen peroxide. (4) Hydrogen peroxide reacts with ferrous ions to form hydroxyl radicals. Legend: Asc = ascorbic acid, vitamin C, Fe = iron, O = oxygen, H_2O_2 = hydrogen peroxide, OH = hydroxyl radical.

- According to the "Fenton chemistry," invoked to explain the selective cytotoxic effect of vitamin C against cancer cells, trivalent iron (Fe^{3+}) is necessary for the formation of H_2O_2, starting from vitamin C. However, some literature data seem to demonstrate that the exact opposite is true. In particular, Mojic et al. using two prostate cancer cell lines (LNCaP and PC-3) showed that iron at physiological concentrations in the cell culture medium and human plasma abrogates the anticancer/cytotoxic effects of vitamin C. According to these authors, iron at physiological concentrations promotes both production and decomposition of H_2O_2, the latter being mediated by a Fenton reaction, which prevents the accumulation of H_2O_2, thus abolishing the cytotoxic effect of vitamin C. Therefore, as the authors conclude, the *in vitro* investigations on the anticancer properties of vitamin C may have been overestimated because all suffered the bias of a low amount of Fe^{3+} in the culture medium, if compared to normal plasma and body fluids. To repeat *in vivo* the results obtained *in vitro*, the authors suggest that the simultaneous administration of vitamin C and chelating agents remove iron [34].

- Vitamin C (ascorbate) readily undergoes pH-dependent autoxidation producing hydrogen peroxide, and catalytic metals accelerate the oxidation process. This means that catalytic iron is not strictly necessary for the production of H_2O_2, starting from vitamin C, and therefore, the Fenton reaction may not be essential for this purpose. The autoxidation, i.e., oxidation in the absence of catalytic metals, occurs via the ascorbate dianion (Asc^{2-}). In particular, that at pH 7.0, 99.9% of ascorbate (vitamin C) is in the form of monoanion ($AscH^-$). $Asc2^-$ increases by a factor 10, with one unit increase in the pH. Therefore, while the production of H_2O_2 may be scarcely relevant in the absence of catalytic iron (as in the "Fenton chemistry"), it may become considerable when the concentration of ascorbate is in the order of the millimoles, as in the case of the use of vitamin C as an anticancer compound. To give an example, an aqueous solution containing 20 mM of vitamin C in the form of sodium ascorbate (the sodium salt of the ascorbic acid) will contain 1 μM of Asc^{2-} which, in turn, will result in a flux of H_2O_2 on the order of 10 nM/s in a typical cell culture experiment [35].

- In most laboratory settings, autoxidation of vitamin C is due to adventitious catalytic metals, as part of the buffers used or contaminating of lab equipment. It is not by chance that the methods underpinning the "Fenton Paranoia" are *in vitro* methods using either isolated tissue cultures or d samples exposed to the air. Indeed, as reported by some authors: "… *unless extreme care is taken, every time vitamin C is added to blood samples (and urine samples) outside the body there are a whole host of oxidative products and markers produced …*" [36].

- Both the antioxidant and prooxidant activities of vitamin C in high doses may not necessarily be mutually exclusive. Studies on chelation therapy have shown that 5 g of the sodium salt of vitamin C added to the ethylenediaminetetraacetic acid (EDTA) chelation cocktail results in acute oxidative stress, but this effect is transitory, and after multiple sessions of EDTA-based chelation treatment, a prolonged, protective, antioxidant effect of the treatment becomes evident [37]. These data confirm the evidence reported by Mojic et al.[34] regarding the inhibitory effect of iron on the prooxidant activity of vitamin C, and also the idea, formerly illustrated by Klenner, according to which vitamin C in high concentration may act as a "flash oxidizer" [38].

In summary, increasing the concentration of vitamin C with mega doses of the nutrient injected in vein may lead to a substantial increase in the spontaneous generation of H_2O_2, with toxic consequences to cancer cells, according to the chain of biochemical reactions more recently described by Yun et al., and briefly summarized herein. This does not mean that the administration of vitamin C in high doses by intravenous injection abrogates its antioxidant properties, but most probably that both prooxidant and antioxidant effects coexist. It rather implies that both antioxidant and prooxidant properties are simultaneously present in the molecule, the latter resulting more pronounced when the concentration of the nutrient reaches values in the order of millimoles (20 mM).

2.2. The antioxidant pathway

"Collagen" is a collective name to designate group of fibrous proteins that occur in vertebrates as the chief constituent of connective tissue fibrils and in bones. Many of the symptoms of scurvy (the syndrome of acute deficiency of vitamin C) depend on the defective production of collagen. Since the beginning of the history of this nutrient, scientists know that vitamin C is essential for the synthesis of collagen. Indeed, vitamin C is a cofactor of collagen prolyl-4-hydroxylase (C-P4H), the enzyme responsible for the formation of hydroxyproline, the essential component of collagen. Under conditions of vitamin C deficiency, C-P4H loses its activity, and the organism does not form collagen properly, with consequent connective tissue deterioration, as it happens in scurvy (**Figure 4**).

Prolyl-hydroxylases are an entire family of enzymes, also known as 2-oxoglutarate-dependent dioxygenases (2-OGDDs) with a wide range of biological functions [39], and members of this family include HIF-hydroxylases [40]. These vitamin C–dependent enzymes are of extreme importance in tumor biology since hypoxia and induction of HIFs are a hallmark of many tumors [41].

HIF is a heterodimeric transcription factor discovered in 1991 [42]. In normal oxygen pressure conditions ("normoxia"), the HIF-1α unit is downregulated by vitamin C–dependent hydroxylases, while in hypoxic conditions, such as those so frequently found in solid tumors, there is a repression of the hydroxylation of HIF-1α, with consequent increase of HIF-dependent gene transcription, neoangiogenesis, and tumor growth and progression. The important consequence of the central role of vitamin C in the synthesis of HIF-1α hydroxylases is that low levels of vitamin C promote tumor growth and progression (as already hypothesized by Stone, Szent-Györgyi, and Pauling), by reducing HIF-1α hydroxylation, thereby stabilizing HIF-1α [43], and high levels of HIF render cancer cells more sensitive to vitamin C–induced toxicity [44].

Recently, Kuiper et al. have confirmed the above data by finding an inverse relationship between intratumor levels of vitamin C and HIF activity in both endometrial cancer and CRC [45–47].

For a better understanding of the centrality of the relationship between hypoxia and HIF in tumor biology, we must consider that cancer hypoxia (a very common feature in cancer) is associated with HIF activity that mediates angiogenesis, epithelial-mesenchymal transition

(EMT), stem cell maintenance, invasion, metastasis, and resistance to radiation therapy and chemotherapy [48]. Therefore, attempts to downregulate HIF synthesis and activity may represent a step forward in the search of an effective and selective anticancer drug [49, 50].

Is vitamin C such a molecule?

The current evidence shows that vitamin C has a close relationship with the function of HIF, and therefore, being a natural compound, it is the best-suited, natural molecule for the purposes of inhibiting cancer growth through HIF-mediated mechanisms.

Tian et al. note that the overexpression of HIF greatly enhances vitamin C–induced toxicity on cancer cells, since HIF increases the intracellular uptake of oxidized vitamin C through its transcriptional target GLUT1, synergizing with the uptake of its reduced form through sodium-dependent vitamin C transporters (SVCTs) [44].

Other authors, working with human leukemia cell lines, showed that vitamin C inhibits the growth of human leukemic cells not only through the generation of H_2O_2 but also and more importantly through the downregulation of HIF-1α transcription [51].

Further important insights into the role of HIF have come from studies of three tumorigenic models *in vivo*, showing that the antitumorigenic effects of antioxidants such as N-acetylcysteine (NAC) and vitamin C are not due to their ability to squelch DNA damage and genomic instability mediated by ROS but due to their capacity to downregulate HIF levels [52].

These results are of extreme interest for the following reasons:

- Whether we consider its prooxidant activity, leading to cancer cell damage through the generation of H_2O_2 or its antioxidant (more typical) activity, leading to the enzymatic breakdown, and nonenzymatic downregulation of the HIF, vitamin C in high doses always shows the characteristics of a simple, natural, and effective anticancer molecule;

- Antioxidants (all of them!) have anticancer effects;

- Vitamin C and other antioxidants may have a role as adjuvant therapy to prevent progression or recurrence of HIF-dependent tumors;

- Vitamin C may show anticancer properties even when administered by mouth, with caution about the dose, which must be sufficiently high.

Recent investigations have shown that ascorbate therapy has a significant effect on the expression of several genes relevant to the development or inhibition of cancer. In particular, the reduced expression of tumor-promoting genes, such as HIF, and the increased expression of tumor-suppressor genes, such as p53, support the notion that vitamin C can act as a potential agent for the suppression of tumor development [53].

2.3. The epigenetic pathway

As we have seen, vitamin C has a number of beneficial biological functions, many of which are related to its action as an electron donor for adjusting the redox state of iron-containing

enzymes. Recent studies have implicated Fe^{2+}-dependent oxidative modification activities in normal tissue homeostasis and experimentally induced reprogramming. The loss of these activities is associated with epigenetic defects and compromised cell differentiation or developmental potential (**Figure 5**).

One of the most important properties of Fe^{2+}-2OG-dependent dioxygenases is their susceptibility to environmental factors (as we have seen in the case of HIF-hydroxylases). Vitamin C is necessary to maintain the function of these enzymes, and different oncometabolites can inhibit their activity.

Recent studies show that vitamin C may enhance reprogramming of pluripotent stem cells, and the available data suggest a strong link between vitamin C, dioxygenase function, and stem cell differentiation, that is of great relevance for human disease [54]. In fact, vitamin C (in the form of sodium ascorbate), as a member of Fe^{2+}- and 2OG-dependent dioxygenases, plays a critical role in the demethylation of DNA and histone, as a cofactor for a group of enzymes termed methylcytosine dioxygenase ten-eleven translocation (TET, including TET1, TET2, and TET3) and some Jumonji-C (JmjC) domain-containing histone demethylases [55, 56].

(**Note 1**: TET1 catalyzes the conversion of the modified DNA base 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC). TET1 produces 5-hmC by oxidation of 5-mC in an iron and alpha-ketoglutarate-dependent manner [8]. The conversion of 5-mC to 5-hmC has been proposed as the initial step of active DNA demethylation in mammals.)

(**Note 2**: TETs belong to the Fe^{2+}- and 2OG-dependent dioxygenase superfamily.)
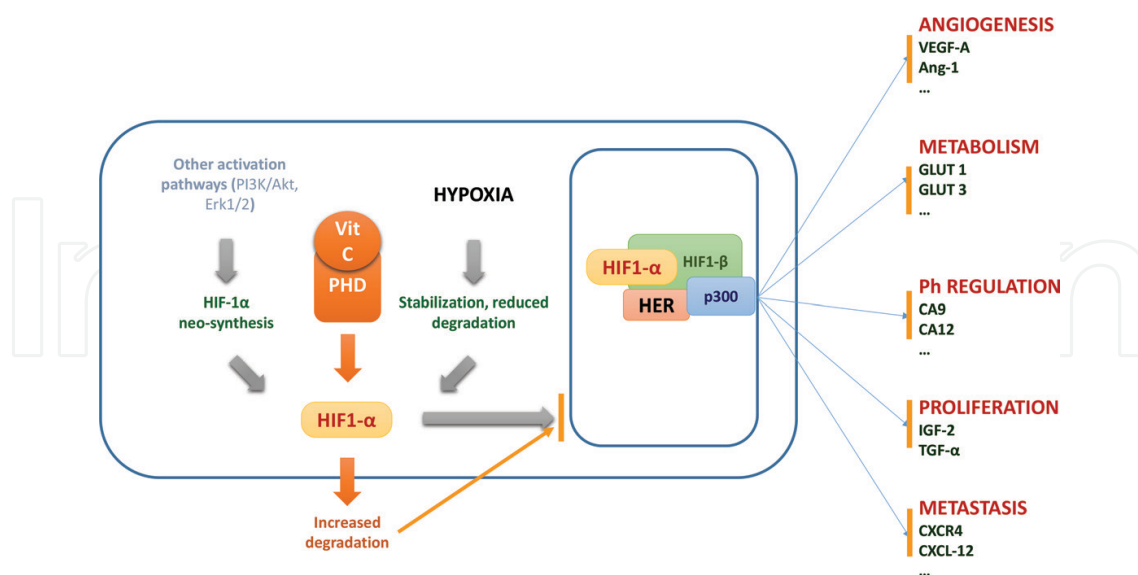
Figure 5. Hypoxia stabilizes HIF-1α as the rate of prolyl-hydroxylases (PHD)-mediated hydroxylation of a subunit is limited. HIF-1α accumulates in the cytoplasm and passes into the nucleus, where it binds to HIF1-β. Cofactor p300 allows the binding to hypoxia response elements (HREs) of target genes involved in angiogenesis, metabolism, pH regulation, proliferation, and metastasis. Vitamin C, as a cofactor of PHD, determines increased degradation of HIF1-α and, as a consequence, inhibits the expression of genes activated by HIF1-α.

Given its role as an essential cofactor of enzymes involved in epigenetic gene regulation/expression, vitamin C can be involved in embryonic development, postnatal development, aging, cancer, and other diseases [57].

In summary, the epigenetic gene regulation functions of vitamin C encompass:

- The regulation of DNA demethylation as an essential cofactor for TET dioxygenases;

- The regulation of histone demethylation as an essential cofactor for JmjC domain-containing histone demethylases;

- The role of interface between the genome and environment;

- The critical role in maintaining the epigenome, especially at early embryonic stages;

- The contribution to different disease in the case of deficiency, which causes reduction of the catalytic activity of TET dioxygenases and JmjC domain-containing histone demethylases [58].

According to some authors, vitamin C causes widespread, consistent, and remarkably specific DNA demethylation of 1847 genes in human embryonic stem cells (hESCs), including important stem cell genes, with a clear bias toward demethylation at CpG island boundaries [59].

Other epigenetically relevant effects of vitamin C include:

- The development of dopamine neuron differentiation in fetal midbrain [60], the induction of pluripotent state in mouse embryonic stem cells [61, 62];

- The enhancement of the demethylating activity of 5-azacytidine, and induction of cytotoxicity [63, 64];

- The inhibition of the malignant phenotype on melanoma cells *in vitro*, by partially reestablishing the global content of 5-hydroxymethylcytosine (5-hmC) and the consequent alteration in the transcriptome [65];

- The upregulation of several microRNA (miRNA) involved in tumor suppression and drug resistance, the most prominent of which correlates with increased overall survival of breast cancer or nasopharyngeal carcinoma [66];

- The inhibition of the proliferation, migration, and epithelial-mesenchymal-transition (EMT) of lens epithelial cells by destabilizing HIF-1α [67].

2.4. The immunologic pathway

Vitamin C concentrations in the plasma and leukocytes rapidly decline during infections and stress, and vitamin C supplementation improves a number of different immunologic functions, including, among others: antimicrobial and natural killer (NK) activities, lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity. Furthermore, by maintaining the cellular redox integrity, vitamin C protects the cells of the immune system against ROS [68, 69]. Immunocompetent cells such as lymphocytes, neutrophils, and monocytes have vitamin C levels 10-–100-fold higher than the plasma, and accumulate it against a concentration gradient (**Figure 6**) [70].
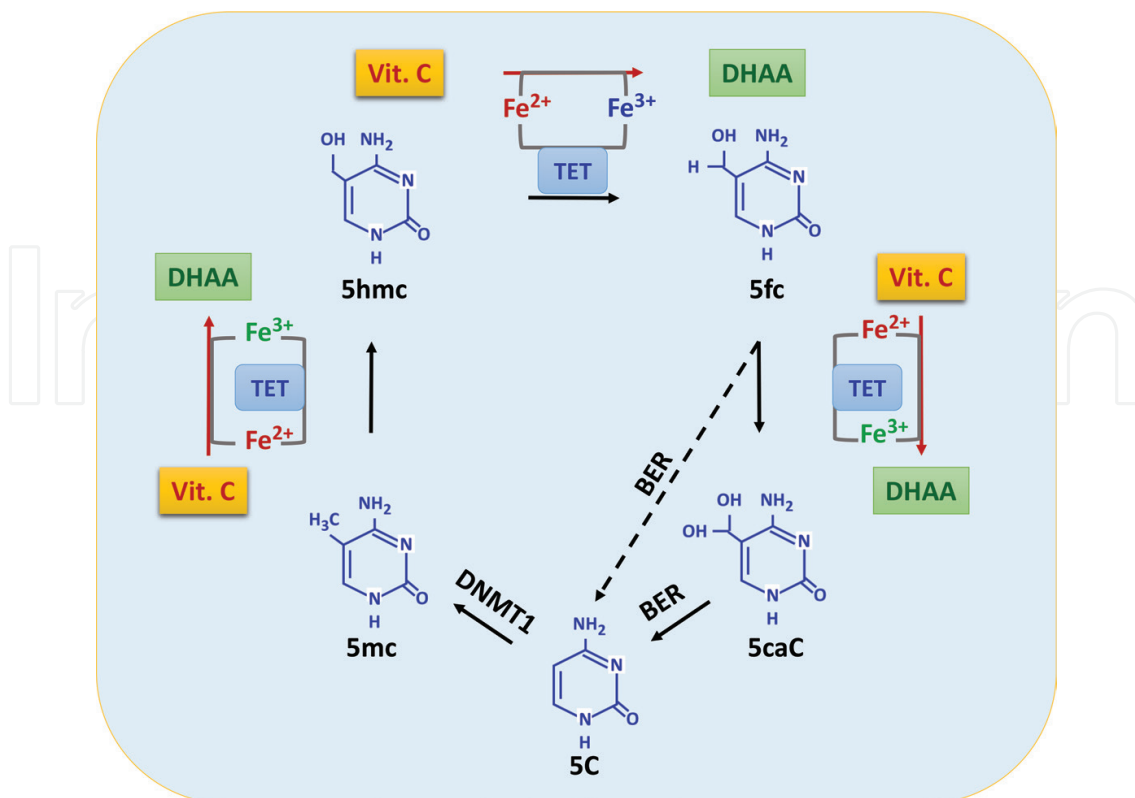
Figure 6. As a cofactor for TET dioxygenases, vitamin C participates in the conversion of 5-mC to 5-hmC, and further 5fC and 5caC, thus modulating DNA demethylation. Legend to figure: 5C = cytosine, 5-mC = 5-methylcytosine, 5-hmC = 5-hydroxymethylcytosine, 5fC = 5-formylcytosine, 5caC = 5-carboxylcytosine, BER = base excision repair, DNMT1 = DNA methyltransferase-1, DHAA = dehydroascorbic acid, Vit. C = vitamin C.

A decrease in the intracellular content of vitamin C may result in locally increased apoptosis of immune cells and immunosuppression [71].

Vitamin C is essential for immunoglobulin synthesis [72] and active phagocytosis [73] enhances the production of interferon [74] and suppresses the production of interleukin-18 (IL-18), a key regulator in malignant skin tumors, including melanomas, squamous cells carcinomas, and a number of other tumors [75].

The concept that the immune system can help fighting cancer has deep roots. In 1909, the German scientist Paul Ehrlich proposed that the incidence of cancer would be much higher, were it not for the action of our immune system in recognizing and eliminating tumor cells [76].

Half a century later, two scientists, Lewis Thomas and Frank Macfarlane Burnet, took Paul Ehrlich's original idea a step further and proposed the model of "immune surveillance," according to which the cells of the immune system actively patrol the body looking for cancerous cells and eliminate them as they arise. This idea became a grounding principle of the new field of cancer immunology that took shape beginning in the 1950s [77].

More recently, the relationship between cancer and the host immune system has become clear with the introduction of the concept of "immune editing," a three-phase process leading cancer cells to escape the control of the immune system, with consequent cancer progression [78].

Therefore, since no doubts exist, nowadays, about the role of the immune system in controlling cancer development, progression and spread, natural substances such as vitamin C, whose action spreads over a wide range of effectors of both the innate and adaptive immunity, represent a new promising therapeutic tool against cancer.

Much of the "booster" action of vitamin C on the immune system depends on its antioxidant nature. Indeed, vitamin C downregulates ROS-dependent expression of pro-inflammatory interleukin genes, via inhibition of transcription of NF-κB (nuclear factor kappa-light chain-enhancer of activated B cells), which, in turn, regulates the expression of pro-inflammatory cytokines, such as IL-1 and tumor necrosis factor-alpha (TNFα) [79]. Recent evidence shows that vitamin C enhances antioxidant defenses of T-cells [80] and increases T-cell responsiveness to antigens, thus suggesting that it has a definite role in regulating immune function [81].

Confirm of the role of vitamin C in regulating the immune system function come from studies showing that increased vitamin C concentrations inhibit antigen-induced, withdrawal-induced, steroid-induced, and spontaneous T-cell apoptosis [82], fas-induced apoptosis of monocytes [83], and increased cytotoxic activity of natural killer cells in humans [84]. All these data indicate that it can modulate the immune system by inhibiting T-cell apoptosis signaling pathways [85]. Similar results apply to monocyte-derived dendritic cells (DCs) [86].

As mentioned in the "historical background," more than four decades ago, Pauling attributed to vitamin C the role of a "booster" of the immune system. This was his main argument against the negative results reported by the Mayo Clinic scientists, since they should not have included, in their clinical trials, cancer patients previously treated with chemotherapy, because chemotherapy destroys immunocompetent cells and severely impairs the individual immune response to cancer.

The Mayo Clinic scientists apparently did not understand this argument and, in reply to the legendary scientist wrote "… *Any contention that previous chemotherapy prevented our patients from achieving the extraordinary survival increase claimed by Drs. Cameron and Pauling must be considered highly speculative at best. Our patients were entered into the study only when they were well past any acute immune-suppressive effects of previous therapy*" [87].

The evidence, today, indicates that a healthy immune system is necessary to control the malignant disease and that immune suppression associated with cancer and cancer chemotherapy contributes to its progression [88]. Therefore, in agreement with Pauling and the current evidence, it is likely to assume that since cancer chemotherapy produces long-lasting deleterious effects on the immune system [89, 90], patients previously treated with cytotoxic chemotherapy should not expect positive outcomes from the treatment with vitamin C as a "booster" of the immune system.

2.5. The collagen pathway

William McCormick first proposed, about 6 decades ago, the hypothesis that cancer could be due to a defect in the metabolism of collagen [91]. Given the fundamental role of vitamin C, in

the synthesis of collagen [92], the scientist concluded that a deficient intake of vitamin C (as in full blown or chronic subclinical scurvy) could determine cancer by disrupting the synthesis of collagen, thus creating a local permissive environment for cancer to grow and spread to other organs and tissues (**Figure 7**) [93].

As we have seen in the previous sections, vitamin C is required for collagen synthesis by acting as a cofactor for nonheme iron α-ketoglutarate-dependent dioxygenases such as prolyl-4-hydroxylase. It stimulates all types of collagen synthesis by donating electrons required for hydroxylation reactions of proline and lysine in procollagen, normally performed by specific hydroxylating enzymes.

In recent years, the role of basement membrane (BM) in the dynamic regulation of cell behavior and cell-signaling pathways has clearly emerged. The BM defines the tumor microenvironment and provides significant host-derived regulatory signals during progression of tumor growth and metastasis. The major component of basement membrane is type IV collagen, and recent studies indicate that in cancer progression there is a disruption of the normal assembly and organization of the basement membrane [94].

These studies support the notion that the loss of collagen type IV chains may provide a permissive microenvironment for cancer invasiveness, as hypothesized by McCormick, more than 5 decades ago [95].

Metastatic spread is a crucial event in the evolution of cancer, being responsible of the majority of cancer-related deaths. The steps leading to metastatic spread of cancer encompass the detachment of cancer cells from the primary tumor, the disruption of the BM, the invasion of the surrounding stroma, cancer cell entry into and transport through the vascular or lymphatic
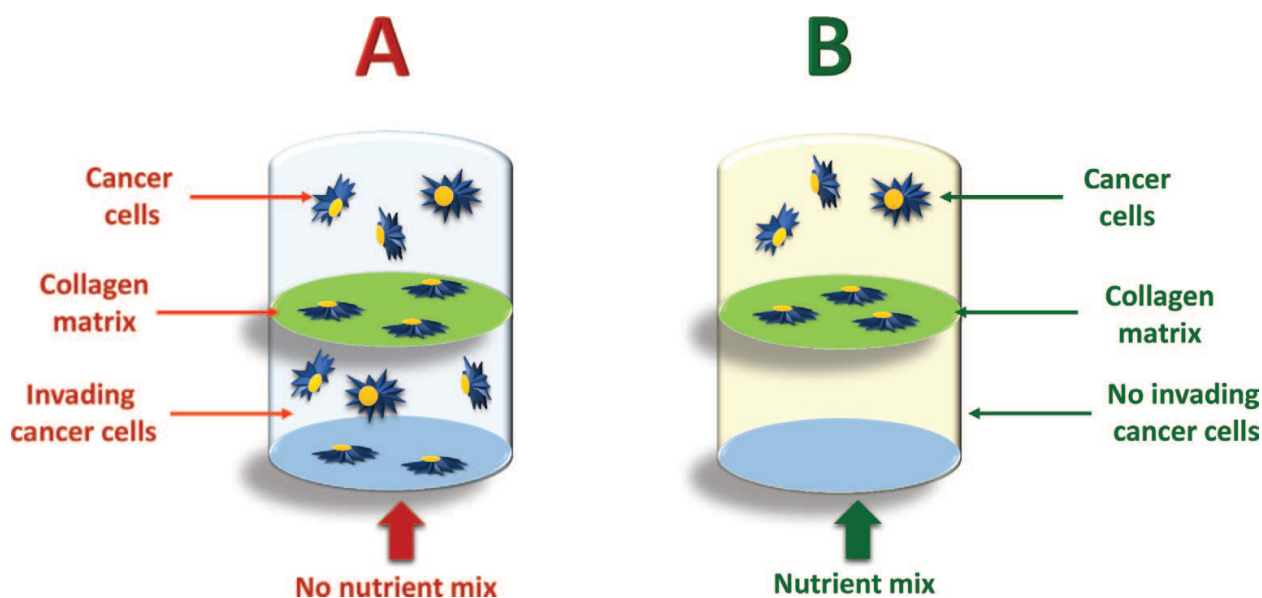
Figure 7. The "nutrient mix" used is a mixture of vitamin C, L-lysine, L-proline, and epigallocatechin gallate (EGCG). Cancer cells can destroy enzymatically the collagen matrix (A) between the two chambers of the vial, and spread over the second chamber. When the nutrient mix is added (B), cancer cells are no longer able to destroy the collagen matrix and therefore they remain confined to the superior chamber (see Refs. [99, 100]).

system to distal sites (liver, lungs, brain, etc.), and extravasation, tumor cell proliferation and angiogenesis at distal sites [96].

A critical event in tumor cell invasion is the degradation of the extracellular matrix (ECM), a complex network of extracellular macromolecules such as collagen, proteoglycans, fibronectin, laminin, and other glycoproteins that act as a barrier to the spread of cancer cells to distal sites by restricting tumor growth and invasion.

Matrix metalloproteinases (MMPs) are calcium-dependent endopeptidases, which require coordination of a zinc ion to mediate catalysis. As implied by their name, MMPs operate on a variety of substrates belonging to the ECM [97], and owing essentially to their vast diversity, MMPs participate in nearly every biological process, involving the remodeling of the ECM, from implantation of an embryo into the uterine wall to tissue necrosis.

A major structural protein for ECM and basement membrane is type IV collagen. Therefore, type IV collagenases MMP-2 (72-kd gelatinase A) and MMP-9 (92-kd gelatinase B), usually overexpressed in malignancy, are the focus of research in this field.

All types of cancer cells form tumors and spread in the body by degrading the ECM by means of various matrix MMPs. The activity of these enzymes correlates with the aggressiveness of tumor growth and invasiveness of the cancer.

In 1992, Pauling and Rath hypothesized that natural compounds such as lysine and vitamin C could inhibit ECM proteolysis and, as such, had the potential to modulate cancer growth and spread [98]. These nutrients would exert their anticancer activity by both inhibiting MMPs and strengthening the connective tissue surrounding the tumor.

Several lines of evidence support an indispensable role for vitamin C in maintaining good-quality collagen. Vitamin C assists the posttranslational modification of collagen by reducing iron in the participating enzymes, lysyl-hydroxylase, and prolyl-hydroxylase. Experimental *in vitro* data show incubation of cancer cells with a nutrient mixture containing vitamin C, L-lysine, L-proline, and epigallocatechin gallate (EGCG), they are no longer able to invade the collagen matrix [99, 100] and spread at distant sites (**Figure 7**).

Although the mechanisms through which the nutrient mixture used in these experiments inhibits MMPs deserves further investigation, it is quite clear that the role of vitamin C is to stabilize collagen, and contribute to tumor cell toxicity, through one or more of the mechanisms illustrated in the previous sections.

Experiments on Gulo-knockout (GULO-KO) mice challenged with murine B16FO cancer cells show that vitamin C–supplemented mice developed smaller tumors with more collagen encapsulation and fibrous capsule inter digitation. On the contrary, Gulo-KO mice deprived of ascorbate hosted large tumors with poorly defined borders showed more necrosis and mitosis, [101], thus reinforcing the notion that vitamin C plays a central role in the prevention and control of tumor growth, progression, and metastatic spread.

To further confirm their data, the author showed that vitamin C-supplemented GULO-KO mice injected with B16FO melanoma cells demonstrated a significant reduction (by 71%, $p = 0.005$) in tumor metastasis compared to GULO-KO mice on the control diet [102].

2.6. The antitoxic/chemopreventive pathway

"*Ascorbic acid is a potent detoxicant which counteracts and neutralizes the harmful effects of many poisons in the body. It will combat various inorganic poisons, such as mercury and arsenic, and it neutralizes the bad reactions of many organic poisons, drugs, and bacterial and animal toxins. Ascorbic acid detoxifies carbon monoxide, sulphur dioxide, and carcinogens, so it is the only immediate protection we have against the bad effects of air pollution and smoking*" [3].

This sentence defines yet another way through which vitamin C can prevent and fight off cancer, i.e., by neutralizing chemical carcinogens. Whether this effect depends on its antioxidant, anti-inflammatory effect, or yet other mechanisms, it is clear that vitamin C is an essential factor in cancer chemoprevention, and this cancer-preventive capacity is more likely associated with its protective effect against oxidative stress mediated by ROS [103].

Free radicals and other ROS are molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. Free radicals derive either from normal metabolic processes or from external sources, such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals.

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. Antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property.

Given this peculiarity, vitamin C, as noted by Stone, can combat the toxic effects of different organic and inorganic poisons, thus preventing and fighting cancer due to environmental pollutants.

Recently, the US Department of Agriculture and the National Cancer Institute recommend the consumption of a minimum of five servings of fruit and vegetables to prevent cancer, with vitamin C being able to reduce the risk of stomach, mouth, pharynx, esophagus, lung, pancreas, and cervical cancers [104]. Furthermore, both epidemiologic and observational studies based on food intake provide evidence for a strong, protective role of vitamin C against cancer [105, 106].

Different studies show that the vitamin C is capable of preventing nitrosation and interfere with experimentally induced carcinogenesis [107, 108].

Vitamin C administered together with diethylnitrosamine (NDEA) shows an inhibitory effect on the experimental esophageal carcinogenesis in Wistar rats [109]. It also protects against the toxic effects of a number of pesticides/insecticides, including malathion [110], imidacloprid [111], endosulfan [112], dimethoate [113], fenvalerate [114], and many others.

More importantly, vitamin C reduces the toxic effects of different anticancer agents, including cisplatin [115, 116], cyclophasphamide [117], selenium-cisplatin conjugates [118] radiation [119], arsenic [120], doxorubicin [121], alkylating agents [122], and many others.

2.7. The "adjuvant" anticancer effect

As shown in the previous section, vitamin C protects normal cells from the oxidative, genotoxic effects of chemotherapeutic agents, but this does not imply that it counteracts the cytotoxic effects of cancer chemotherapy and radiotherapy.

Regarding the clinical use of high doses of vitamin C in combination with standard anticancer chemotherapy, for a long time, and even today, detractors of vitamin C (that are still a multitude, within the scientific community!) warn against the risk that antioxidants (such as vitamin C) may enhance cancer cell proliferation.

The role of micronutrients with antioxidant properties (including vitamin C) as a useful adjunct to conventional chemotherapy and /or radiotherapy has been controversial, essentially because they could protect cancer cells from the deleterious effects of free radicals generated by the therapy, thereby preventing cancer cell death.

After an exhaustive review of the literature, encompassing more than 44 scientific articles on the effectiveness of vitamin C alone, or with other vitamins, with chemotherapy, the authors concluded that: "… *antioxidants [including vitamin C] do not protect cancer cells against free radical and growth-inhibitory effects of standard therapy. On the contrary, they enhance its growth-inhibitory effects on tumour cells, but protect normal cells against its adverse effects*" [123]. This literature review suggests that the use of vitamin C alone with chemotherapy results in increased survival, enhancement of chemotherapy, inhibition of tumor growth, decrease in the overall toxicity, modulation of genotoxicity linked to chemotherapy, distinct potentiating effect of chemotherapy, improved quality of life, and a whole series of other positive effects on the outcome of treated patients.

Recent evidence suggests that vitamin C can efficiently

- aid low-dose methotrexate (MTX) in inducing cell death in Hep3B cells [124],

- synergize arsenic trioxide in acute promyelocytic leukemia [26],

- improve chemosensitivity of ovarian cancer, reducing, at the same time, the toxicity of chemotherapy [125],

- sensitize tumor cells toward cytostatic drugs [126], and

- improve the quality of life of patients undergoing chemo/radiotherapy [127, 128].

In a position paper published in the *Journal of the American College of Nutrition* in 2001, the authors highlight that "… none of the published data on the effect of antioxidants in combination with radiation or chemotherapeutic agents on tumour cells supports this hypothesis" [129]. As the authors observe, normal and tumor cells differ in their responses to antioxidants; low-dose and high-dose antioxidants differ in their effect on tumor cells, some actions of antioxidants on tumor cells are unrelated to scavenging of free radicals, and antioxidants have profound effects on the regulation of gene expression in tumor cells.

3. What to do next

As we have seen, the expectations generated from *in vitro* and animal studies on vitamin C still wait for the confirmation of clinical studies. The discrepancy between *in vitro* and *in vivo* results is due to several factors presented in the next.

3.1. The confusion about the dose

Among the many mystifying (and sometimes pseudoscientific) data regarding the anticancer effects of vitamin C, the most blatant is surely the one concerning the dose used. While it is clear that therm "high" designates doses of vitamin C (generally administered by intravenous injection) leading to plasma concentrations in the order of millimoles (from one to several), current "institutional" clinical trials pass off as "high" doses of vitamin C of 1000 mg (1 g)! This is the case of a number of clinical trials, among which we could mention:

• A phase II trial of arsenic trioxide and ascorbic acid with temozolomide in patients with metastatic melanoma with or without central nervous system metastases [130],

• A clinical experience on the combination of arsenic trioxide and ascorbic acid in patients with refractory metastatic colorectal carcinoma [131],

• A phase I study on combination of decitabine, arsenic trioxide, and ascorbic acid for the treatment of myelodysplastic syndrome and acute myeloid leukemia [132], and many others. Clinical trials designed as the ones reported above are of no value in verifying the role of high doses of vitamin C in the treatment of cancer, since 1000 mg of the nutrient, even if administered in vein, is not a high ("pharmacologic") dose.

3.2. The level of tissue oxygenation

If we assume that one of the main mechanisms through which vitamin C in pharmacologic doses is toxic to cancer cells is the production of H_2O_2, then oxygen becomes a fundamental part of the cytotoxic activity of vitamin C against cancer. Solid tumors often contain areas subjected to acute or chronic hypoxia. Although severe or prolonged hypoxia is deleterious, adaptation to a hypoxic microenvironment, allows cancer cells to survive and proliferate in a hostile milieu. More importantly, since cell culture experiments are usually performed in an oxygen-rich environment, while solid tumors usually show a very low content of oxygen, this difference in oxygen content may explain the different outcome in vitamin C cancer cell killing *in vitro*, compared to what happens *in vivo* [133]. Overcoming cancer hypoxia may therefore represent one of the main ways to improve the anticancer activity of high doses of vitamin C in clinical settings, as commonly realized with either hyperbaric oxygen (HBO) or ozonated autohemotherapy.

3.3. The pharmaceutical preparation

Sodium ascorbate, rather than ascorbic acid, may be the preferred preparation for intravenous injection. Ascorbic acid produces a very acidic solution, when dissolved in water or saline solution, and, as such, unsuitable for intravenous injection. Therefore, in order to obtain a neutral solution, it is necessary to buffer it with either sodium bicarbonate or sodium hydroxide. However, adding a buffer may represent a major problem, in terms of stability of the solution, and therefore the sodium salt of vitamin C, which produces a pH of around 7.0, is clearly preferable.

3.4. The administration schedule

According to some author, a constant "flow" of vitamin C in the blood works as if the body would produce the nutrient on its own (the "dynamic flow" hypothesis) [134]. The slow, constant infusion of vitamin C is the best option to maintain a stable plasma level of the nutrient, by intravenous injection [135] even though this approach is not common in the treatment of cancer patients with intravenous high doses of the nutrient. In fact, the great majority of the cancer clinical trials performed so far with intravenous vitamin C use the infusion of vitamin C on alternate days, withdrawing the treatment during the weekend. With this treatment modality, systemic conditioning (the accelerated metabolism or disposal of ascorbic acid) may occur after prolonged supplementation of high doses of vitamin C. Thus, if vitamin C supplementation were to cease abruptly, the accelerated disposal of the nutrient may create a deficiency state ("rebound scurvy"), and this may represent a serious inconvenience, when treating cancer patients. This is why, since Klenner's experience with multiple administration routes, it may turn out to be useful to combine both intravenous and oral administration of large doses of vitamin C [7].

3.5. The glucose-ascorbate antagonism (GAA)

John Ely first proposed the glucose-ascorbate antagonism (GAA) theory in the 1970s [136]. According to this theory, the chemical structure of vitamin C and glucose is very similar and therefore they compete for the same transport system to enter the cells. As a consequence, both vitamin C and its oxidized form, DHAA, transported into different cell types (including adipocytes, erythrocytes, granulosa cells, neutrophils, osteoblasts, and smooth muscle cells), are inhibited by high blood glucose. Although *in vivo* studies are missing, investigations on diabetic patients have confirmed the theory. Therefore, given the inverse relationship between glucose and vitamin C blood levels, maintaining blood glucose levels within the normal range may greatly enhance the anticancer effect of vitamin C.

4. The efficacy of vitamin C in high doses against cancer: the facts

As a necessary premise to an evaluation of the anticancer properties of vitamin C, a realistic look at the state of the art on cancer chemotherapy can be helpful. The "war on cancer," officially declared by President Richard Nixon, with the National Cancer Act, in 1971, has been largely considered a failure, by the experts [137, 138], because of the following:

- The major improvements in survival rates mainly concern cancers of children and young adults, which account for 1.3% of all known cancers, and this has a little impact on the overall picture. Therefore, in most cases and for most forms of cancer, the war (the "war" metaphor) has been lost [139, 140].

- Targeted therapies (the "magic bullet" metaphor) are not curative because cancer usually adapts itself, becoming resistant to every new "weapon" used [141].

- Since the overwhelming majority of cancer is due to environmental, particularly lifestyle, factors, prevention, rather than cure, should be the foremost aim [142].

- The industry continues to be developed and the institutional organisms approve new cancer drugs, based on marginal improvements in survival at an unsustainably high financial cost [143].

- Furthermore, cancer chemotherapy has an inherent toxicity, which, in many instances, encompasses, among others, nausea, vomiting, mucositis, hair loss, bone marrow toxicity, cardiac, neurologic, and renal toxicity, and, in the long term, sterility and secondary malignancy.

- Finally, both early and recent reports demonstrate that cancer chemotherapy can be either ineffective/useless [144, 145] or definitely harmful [146, 147].

Compared to the current chemotherapeutic agents, vitamin C in high ("pharmacologic") concentration has the following advantages:

- It is a natural compound that is usually produced by the vast majority of plants and animals, but not (no longer!) by humans. As any other natural product, it is neither patentable nor commercially exploitable.

- Both tissues and plasma of cancer/leukemia patients show reduced levels of this nutrient and therefore the routine administration of adequate amounts of vitamin C to these patients is not only warranted, but highly desirable.

- High concentrations of vitamin C within tumor cells are associated with extended disease-free survival, while low concentrations are associated with aggressive tumor phenotype.

- It has no relevant side effects, with the exception of a slight diarrhea, a "guiding symptom" that indicates that the body "saturation" with the nutrient ("bowel tolerance"). To control the mild diarrhea that follows the body saturation threshold, it is sufficient to reduce or fractionate the doses, but in clinical practice, it is useful to maximize the effects of vitamin C, when assumed by mouth. Given the almost total absence of side effects, undue and sometimes laughable attempts to warn against its use have come, from time to time, from detractors of vitamin C, dealing with the possibility that several grams per day could lead to oxalate stone formation. In this regard, it will suffice to mention the European Food Safety Authority (EFSA) report n. EFSA-Q-2003-018, which, on this matter, clearly affirms: "*No significant relationships were found in an analysis of data from 5214 men and 5785 women between serum vitamin C concentrations and the prevalence of kidney stones*" [148]. Moreover, the hyperoxaluria associated with the use of high-dose vitamin C is primarily due to a laboratory artifact, resulting from the conversion of vitamin C to oxalate *ex vivo* (i.e., after it has left the body, while it is in the collection bottle) [149]. The only contraindication to the treatment with high doses of intravenous vitamin C is the deficiency of glucose-6-phosphate dehydrogenase (G6-P-D), a rare genetic disorder in which a number of different drugs are usually contraindicated.

- It is inexpensive.

Beyond all these advantages, vitamin C in high doses is clearly cytotoxic for a large number of human tumor cell lines. At plasma concentrations achieved by intravenous administration, vitamin C induces death in 75% of 48 cancer cell lines tested *in vitro* [150], but has no toxic effect on human peripheral white blood cells, fibroblasts, or epithelial cells. This represents the realization of the dream of the "magic bullet," even though the "scientific community" seems to continue to ignore it!

Regarding the anticancer efficacy of vitamin C *in vivo*, although the results of the first clinical trials have been rather disappointing, an unbiased analysis of the data currently available reveals an excellent safety profile, a clear-cut improvement of the quality of life [127], and a potentially important antitumor activity even though further, well-designed, controlled studies are strongly required.

5. Still looking for a "rationale": is too much of it good for nothing?

Almost 50 years after the discovery of the anticancer properties of vitamin C, scientists are still looking for a rationale for the use of this nutrient in the treatment of cancer. However, as we have seen, this rationale not only exists, but it is also evidence-based, well-founded, complex, and variegated, given the many extraordinary benefits of vitamin C for human health. Therefore, faced with such an overwhelming evidence in favor of the efficacy of vitamin C against cancer, the question may become "why mega doses of vitamin C have not yet entered the routine clinical treatment of cancer?"

As sad as it may appear, the many advantages of vitamin C as an anticancer agent represent likewise limitations to its use in clinical practice. In fact, vitamin C is a natural compound, and this implies that no pharmaceutical company can effectively exploit it for commercial purposes. Drug companies must patent the molecular structure of the active ingredient of their products in order to make a profit. Natural substances, such as vitamin C, cannot undergo any patent submission procedure, because they exist in nature.

Another important issue may be the price: compared to the high costs of cancer drugs, some of which may reach the 30,000 USD for a single dose [151], vitamin C with its price ranging from 20 to 40 USD per kilogram (depending on the country) represents a real outsider within such an expensive market. No pharmaceutical company would ever invest in the clinical development of such an inexpensive product!

Going deeper into this apparent "lack of interest" for vitamin C as an anticancer compound, we can find the outstanding issue of its selectivity of action (the "magic bullet" principle); an aspect that still fascinates the clinical oncologists. As we have seen, contrary to the chemotherapeutic agents, vitamin C kills cancer cells by exploiting a substantial metabolic difference between them and their normal counterpart; a property virtually unknown to the vast majority of the chemotherapeutic agents of common use in clinical practice!

The ethical implications of the above considerations are clear, and we will not discuss them herein. However, there are aspects, in this incomprehensible "indifference" to vitamin C as an anticancer molecule, which go far beyond plausibility and common sense.

One is surely the evidence that vitamin C deficiency is common in patients with advanced cancer, and, at the same time, patients with low plasma concentrations of vitamin C almost invariably show shorter survival, if compared to those with normal/higher concentrations [152]. Should not this evidence alone compel the clinicians to use vitamin C supplementation in cancer patients on a routine basis? A vitamin C deficiency, as we have seen in the first section of this chapter, is most probably in play in the genesis and development of cancer, and traces of this deficiency often remain, unless patients use supplements on their own, in blood and tissues of affected individuals. Is not this data alone sufficient to warrant the routine administration of vitamin C to cancer patients?

The other, not less relevant, aspect is the safety of vitamin C. The LD50 for a mouse (who normally produces its own vitamin C), is more than 3.3 g/kg of body weight, but most probably, even more than that, for mammals not producing their own vitamin, such as humans. This has clearly emerged from clinical studies on intravenous injection of mega doses of the nutrient, which have also showed a definite and unequivocal improvement in the quality of life of the treated patients.

The last aspect, regarding the importance of vitamin C in the treatment of cancer, is the demonstration of the capacity of this nutrient to reduce the side effects and improve the anticancer activity of conventional chemotherapeutic agents, when combined with them [125].

Do we really need more information to introduce high doses of vitamin C in the routine treatment of cancer?

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References

[1] Benade L, Howard T, Burk D. Synergistic killing of Ehrlich ascites carcinoma cells by ascorbate and 3-Amino-1, 2, 4-triazole. Oncology. 1969;**23**:33-43

[2] Panel on Dietary Antioxidants and Related Compounds; Subcommittee on Upper Reference Levels of Nutrients; Subcommittee on Interpretation and Uses of DRIs; Standing Committee on the Scientific Evaluation of Dietary Reference Intakes; Food and Nutrition Board; Institute of Medicine. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. 2000; 529 pages, ISBNs: Paperback: 978-0-309-06935-9; Hardcover: 978-0-309-06949-6. DOI:https://doi.org/10.17226/9810

[3] Stone I. The Healing Factor. "Vitamin C" Against Disease. New York: Grosset and Dunlap Inc.; 1972

[4] Stone I. Eight decades of scurvy. The case history of a misleading dietary hypothesis. Orthomolecular Psychiatry. 1979;**8**:58-62

[5] Chatterjee IB. Evolution and the biosynthesis of ascorbic acid. Science. 1971;**182**:1271-1272

[6] Stone I. The genetics of scurvy and the cancer problem. Journal of Orthomolecular Psychiatry. 1976;**5**:183-190

[7] Klenner F. The treatment of poliomyelitis and other virus diseases with vitamin C. Southern Medicine and Surgery. 1949;**111**:209-214

[8] Jungeblut CW. Further observations on vitamin C therapy in experimental poliomyelitis. Journal of Experimental Medicine. 1937;**66**:459-477

[9] Pauling L. Are recommended daily allowances for vitamin C adequate? Proceedings of the National Academy of Sciences of the United States. 1974;**71**:4442-4446

[10] Cameron E, Campbell A. The orthomolecular treatment of cancer II: Clinical trial of high dose ascorbic acid supplement in advanced human cancer. Chemico-Biological Interactions. 1974;**9**:285-315

[11] Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. Proceedings of the National Academy of Sciences of the United States. 1976;**73**:3685-3689

[12] Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: Reevaluation of prolongation of survival times in terminal human cancer. Proceedings of the National Academy of Sciences of the United States. 1978;**75**:4538-4542

[13] Cameron E, Pauling L. Cancer and Vitamin C. New York: W. W. Norton & Co.; 1979. [Updated and Expanded Cancer and Vitamin C, Cameron E, Pauling L, Camino Books, Inc., Phila., PA 19102; 1993]

[14] Murata A, Morishige F, Yamaguchi H. Prolongation of survival times of terminal cancer patients by administration of large doses of ascorbate. International Journal for Vitamin and Nutrition Research Supplement. 1982;**23**:103-113

[15] Creagan ET, Moertel CG, O'Fallon JR, Schutt AJ, O'Connell MJ, Rubin J, et al. Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. The New England Journal of Medicine. 1979;**301**:687-690

[16] Moertel CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ, Ames MM. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. The New England Journal of Medicine. 1985;**312**:137-141

[17] Yun J, Mullarky E, Lu C, Bosch KN, Kavalier A, Rivera K, et al. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. Science. 2015;**350**:1391-1396

[18] Van der Reest J, Gottlieb E. Anti-cancer effects of vitamin C revisited. Cell Research. 2016;**26**:269-270

[19] Reczek CR, Chandel NS. Revisiting vitamin C and cancer: A high dose of vitamin C kills certain colon cancer cells. Science. 2016:**350**:1317-1318

[20] Iqbal K, Khan A, Khattak MMAK. Biological significance of ascorbic acid (vitamin C) in human health. A review. Pakistan Journal of Nutrition. 2004;**3**:5-13

[21] Chen Q, Espey MG, Sun AY, Lee JH, Krishna MC, Shacter E, et al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid *in vivo*.Proceedings of the National Academy of Sciences of the United States. 2007;**104**:8749-8754

[22] Chen Q, Espey MG, Krishna MC, Mitchell JB, Corpe CP, Buettner G, et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues. Proceedings of the National Academy of Sciences of the United States. 2005;**102**:13604-13609

[23] Uetaki M, Tabata S, Nakasuka F, Soga T, Tomita M. Metabolomic alterations in human cells by vitamin C-induced oxidative stress. Scientific Reports. 2015;**5**:1-9

[24] Mastrangelo D, Massai L, Micheli L, Muscettola M, Cevenini G, Grasso G. High doses of ascorbate kill Y79 retinoblastoma cells in vitro. Journal of Clinical & Experimental Ophthalmology. 2013;**4**:268. DOI: 10.4172/2155-9570.1000268

[25] Mastrangelo D, Massai M, Valyi-Nagy K, Muscettola M, Aglianò M, Lodi L, et al. Megadoses of ascorbate as a new chemotherapeutic approach in uveal melanoma: A preliminary *in vitro* investigation. Journal of Clinical & Experimental Ophthalmology. 2013;**4**:6. DOI: org/10.4172/2155-9570.1000315

[26] Mastrangelo D, Massai L, Fioritoni G, Iacone A, Di Bartolomeo P, Accorsi P, et al. Megadoses of sodium ascorbate efficiently kill HL60 cells in vitro: Comparison with arsenic trioxide. Journal of Cancer Therapeutics and Research. 2013;**4**:1366-1372

[27] Mastrangelo D, Massai L, Lo Coco F, Noguera NI, Borgia L, Fioritoni G et al. Cytotoxic effects of high concentrations of sodium ascorbate on human myeloid cell lines. Annals of Hematology. 2015;**94**:1807-1816

[28] Holman RA. A method of destroying a malignant rat tumour *in vivo*. Nature. 1957;**179**:1033

[29] Doskey CM, Buranasudja V, Wagner BA, Wilkes JG, Duc J, Cullen JJ, et al. Tumor cells have decreased ability to metabolize H_2O_2: Implications for pharmacological ascorbate in cancer therapy. Redox Biology. 2016;**10**:274-284

[30] Hacisevki A. An overview of ascorbic acid biochemistry. Journal of the Faculty of Pharmacy of Ankara. 2009;**38**:233-255

[31] Carr AC, Frei B. Does vitamin C act as pro-oxidant under physiological conditions? FASEB Journal. 1999;**13**:1007-1024

[32] Lennicke C, Rahn J, Lichtenfels R, Wessjohann LA, Seliger B. Hydrogen peroxide-production, fate and role in redox signaling of tumor cells. Journal of Cell Communication and Signaling. 2015;**13**:39. DOI: 10.1186/s12964-015-0118-6

[33] López-Lázaro M. Dual role of hydrogen peroxide in cancer: Possible relevance to cancer chemoprevention and therapy. Cancer Letters. 2007;**252**:1-8

[34] Mojić M, Bogdanović Pristov J, Maksimović-Ivanić D, Jones DR, Stanić M, Mijatović S, et al. Extracellular iron diminishes anticancer effects of vitamin C: An *in vitro* study. Scientific Reports. 2014;**4**:5955. DOI: 10.1038/srep05955

[35] Du J, Cullen JJ, Buettner GR. Ascorbic acid: Chemistry, biology and the treatment of cancer. Biochimica et Biophysica Acta. 2012;**1826**:443-457

[36] Dettman I. Iron and Vitamin C. Biological Therapies Newsletter. 2003; 1-8

[37] Hininger I, Waters R, Osmana M, Garrel C, Fernholz K, Roussel AM, et al. Acute prooxidant effects of vitamin C in EDTA chelation therapy and long-term antioxidant benefits of therapy. Free Radical Biology & Medicine. 2005;**38**:1565-1570

[38] Klenner F. Observations on the dose and administration of ascorbic acid when employed beyond the range of a vitamin in human pathology. Journal of Applied Nutrition. 1971;**23**:3&4

[39] Loenarz C, Schofield CJ. Physiological and biochemical aspects of hydroxylations and demethylations catalyzed by human 2-oxoglutarate oxygenases. Trends in Biochemical Sciences. 2011;**36**:7-18. DOI: 10.1016/j.tibs.2010.07.002

[40] Ozer A, Bruick RK. Non-heme dioxygenases: Cellular sensors and regulators jelly rolled into one? Nature Chemical Biology. 2007;**3**:144-153. DOI: 10.1038/nchembio863

[41] Aprelikova O, Chandramouli GVR, Wood M, Vasselli JR, Riss J, Maranchie JK, et al. Regulation of HIF Prolyl hydroxylases by hypoxia-inducible factors. Journal of Cellular Biochemistry. 2004;**92**:491-501

[42] Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. Nature Reviews Molecular Cell Biology. 2004;**5**:343-354

[43] Knowles HJ, Raval RR, Harris AL, Ratcliffe PJ. Effect of ascorbate on the activity of hypoxia-inducible factor in cancer cells. Cancer Research. 2003;**63**:1764-1768

[44] Tian W, Wang Y, Xu Y, Guo X, Wang B, Sun L, et al. The hypoxia-inducible factor renders cancer cells more sensitive to vitamin C-induced toxicity. Journal of Biological Chemistry. 2014;**289**:3339-3351

[45] Kuiper C, Vissers MCM. Ascorbate as a co-factor for Fe- and 2-oxoglutarate dependent dioxygenases: Physiological activity in tumor growth and progression. Frontiers in Oncology. 2014;**4**:1-11

[46] Kuiper C, Molenaar IG, Dachs GU, Currie MJ, Sykes PH, Vissers MC. Low ascorbate levels are associated with increased hypoxia-inducible factor-1 activity and an aggressive tumor phenotype in endometrial cancer. Cancer Research. 2010;**70**:5749-5758

[47] Kuiper C, Dachs GU, Munn D, Currie MJ, Robinson BA, Pearson JF. Increased tumor ascorbate is associated with extended disease-free survival and decreased hypoxia-inducible factor-1 activation in human colorectal cancer. Frontiers in Oncology. 2014;**4**:1-10

[48] Semenza GL. Hypoxia-inducible factors: Mediators of cancer progression and targets for cancer therapy. Trends in Pharmacological Sciences. 2012;**33**:207-214

[49] Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia (Auckl). 2015;**11**:83-92

[50] Masoud GN, Li W. HIF-1α pathway: Role, regulation and intervention for cancer therapy. Acta Pharmaceutica Sinica B. 2015;**5**:378-389

[51] Kawada H, Kaneko M, Sawanobori M, Uno T, Matsuzawa H, Nakamura Y, et al. High concentrations of l-ascorbic acid specifically inhibit the growth of human leukemic cells via downregulation of HIF-1α transcription. PLoS One. 2013;**8**:e62717

[52] Gao P, Zhang H, Dinavahi R, Li F, Xiang Y, Raman V, et al. HIF-dependent antitumorigenic effect of antioxidants *in vivo*. Cancer Cell. 2007;**12**:230-238

[53] Mikirova N, Scimeca RC. Gene expression response to ascorbic acid in mice implanted with sarcoma S180 cells. Journal of Translational Science. 2016;**2**:145-153

[54] Montfort A, Wuts A. Breathing-in epigenetic change with vitamin C. EMBO Reports. 2013;**14**:337-346

[55] Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;**324**:930-935. DOI: 10.1126/science.1170116. PMC 2715015Freely accessible. PMID 19372391

[56] Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science. 2011;**333**:1300-1303. DOI: 10.1126/science.1210597. PMC 3495246Freely accessible. PMID 21778364

[57] Camarena V, Wang G. The epigenetic role of vitamin C in health and disease. Cellular and Molecular Life Sciences. 2016;**73**:1645-1658. DOI: 10.1007/s00018-016-2145-x

[58] Young JI, Züchner S, Wang G. Regulation of the epigenome by vitamin C. Annual Review of Nutrition. 2015;**35**:545-564. DOI: 10.1146/annurev-nutr-071714-034228

[59] Chung TL, Brena RM, Kolle G, Grimmond SM, Berman BP, Laird PW et al. Vitamin C promotes widespread yet specific DNA demethylation of the epigenome in human embryonic stem cells. Stem Cells. 2010;**28**:1848-1855

[60] He XB, Kim M, Kim SY, Yi SH, Rhee YH, Kim T, et al. Vitamin C facilitates dopamine neuron differentiation in fetal midbrain through TET1 and JMJD3-dependent epigenetic control manner. Stem Cells. 2015;**33**:1320-1332

[61] Gao Y, Han Z, Li Q, Wu Y, Shi X, Ai Z, et al. Vitamin C induces a pluripotent state in mouse embryonic stem cells by modulating microRNA expression. FEBS Journal. 2015;**282**:685-699

[62] Blaschke K, Ebata KT, Karimi MM, Zepeda-Martınez JA, Goyal P, Mahapatra S, et al. Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. Nature. 2013;**500**:222-228

[63] Sajadian SO, Tripura C, Samani FS, Ruoss M, Dooley S, Baharvand H, et al. Vitamin C enhances epigenetic modifications induced by 5-azacytidine and cell cycle arrest in the hepatocellular carcinoma cell lines HLE and Huh7. Clinical Epigenetics. 2016;**8**:46

[64] Liu M, Ohtani H, Zhou W, Due Ørskov A, Charlet J, Zhang YW, et al. Vitamin C increases viral mimicry induced by 5-aza-2'-deoxycytidine. Proceedings of the National Academy of Sciences of the United States. 2016;**113**:10238-10244

[65] Gustafson CB, Yang C, Dickson KM, Shao H, Booven D, Harbour JW, et al. Epigenetic reprogramming of melanoma cells by vitamin C treatment. Clinical Epigenetics. 2015;**7**:51

[66] Venturelli S, Sinnberg TW, Berger A, Noor S, Levesque MP, Böcker A, et al. Epigenetic impacts of ascorbate on human metastatic melanoma cells. Frontiers in Oncology. 2014;**4**:227. Available from: https://doi.org/10.3389/fonc.2014.00227

[67] Zhao L, Quan Y, Wang J, Wang F, Zheng Y, Zhou A. Vitamin C inhibit the proliferation, migration and epithelial-mesenchymal-transition of lens epithelial cells by destabilizing HIF-1α. International Journal of Clinical and Experimental Medicine. 2015;**8**:15155-15163

[68] Wintergerst ES, Maggini S, Hornig DH. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. Annals of Nutrition and Metabolism. 2006;**50**:85-94

[69] Sorice A, Guerriero E, Capone F, Colonna G, Castello G, Costantini S. Ascorbic acid: Its role in immune system and chronic inflammation diseases. Mini-Reviews in Medicinal Chemistry. 2014;**14**:444-452

[70] Combs Jr GF. The Vitamins. 4th ed. Academic Press; 2012. p. 598. Paperback ISBN: 9780128102442, eBook ISBN:9780123819819,Hardcover ISBN: 9780123819802

[71] Pavlovic V, Sarac M. A short overview of vitamin C and selected cells of the immune system. Central European Journal of Medicinal Chemistry. 2010;**6**:1-10

[72] Lewin S. Vitamin C. Its Molecular Biology and Medical Potential. New York, NY: Academic Press; 1976. p. 244

[73] Goetzl EJ, Wasserman SI, Gigli I, Austen KF. Enhancement of random migration and chemotactic response of human leukocytes by ascorbic acid. Journal of Clinical Investigation. 1974;**53**:813-818

[74] Dahl H, Degre M. The effect of ascorbic acid on production of human interferon and the antiviral activity *in vitro*. Acta Agriculturae Scandinavica, Section B. 1976;**84**:280-284

[75] Cho D, Hahm E, Kang JS, Kim YI, Yang YH, Park JH, et al. Vitamin C downregulates interleukin-18 production by increasing reactive oxygen intermediate and mitogen-activated protein kinase signaling in B16F10 murine melanoma cells. Melanoma Research. 2003;**13**:549-554

[76] Ehrlich P. Ueber den jetzigen Stand der Karzinomforschung. Ned Tijdschr Geneeskd. 1909;**5**:273-290

[77] Burnet FM. Immunological surveillance in neoplasia. Transplantation Reviews. 1971;**7**:3-25

[78] Dunn GP, Old LJ, Schreiber D. The immunobiology review of cancer immunosurveillance and immunoediting. Immunity. 2004;**21**:137-148

[79] Schwager J, Schulze J. Modulation of interleukin production by ascorbic acid. Veterinary Immunology and Immunopathology. 1998;**64**:45-57

[80] Pavlovic V, Pavlovic D, Kocic G, Sokolovic D, Sarac M, Jovic Z. Ascorbic acid modulates monosodium glutamate induced cytotoxicity in rat thymus. Bratislavske Lekarske Listy. 2009;**110**:205-209

[81] Wu CC, Doriarajan T, Lin TL. Effect of ascorbic acid supplementation on the immune response of chickens vaccinated and challenged with infectious bursal disease virus. Veterinary Immunology and Immunopathology. 2000;**74**:145-152

[82] Pavlovic V, Cekic S, Bojanic V, Stojiljkovic N, Rankovic G. Ascorbic acid modulates spontaneous thymocyte apoptosis. Acta Medica International. 2005;**44**:21-23

[83] Perez-Cruz I, Carcamo JM, Golde DW. Vitamin C inhibits FAS-induced apoptosis in monocytes and U937 cells. Blood. 2003;**102**:336-343

[84] Vojdani A, Bazargan M, Vojdani E, Wright J. New evidence for antioxidant properties of Vitamin C. Cancer Detection and Prevention. 2000;**24**:508-523

[85] Campbell JD, Cole M, Bunditrutavorn B, Vella AT. Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis. Cellular Immunology. 1999;**194**:1-5

[86] Tan PH, Sagoo P, Chan C, Yates JB, Campbell J, Beutelspacher SC, et al. Inhibition of NF-kappa B and oxidative pathways in human dendritic cells by antioxidative vitamins generates regulatory T cells. The Journal of Immunology. 2005;**174**:7633-7644

[87] Richards E. Vitamin C and Cancer: Medicine or Politics? McMillan Professional & Academic LTD; 1991. p. 269. ISBN-13:978-0312052423, ISBN-10:0312052421

[88] Whiteside TL. Immune suppression in cancer: Effects on immune cells, mechanisms and future therapeutic intervention. Seminars in Cancer Biology. 2006;**16**:3-15

[89] Verma R, Foster RE, Horgan K, Mounsey K, Nixon H, Smalle N, et al. Lymphocyte depletion and repopulation after chemotherapy for primary breast cancer. Breast Cancer Research. 2016;**18**:10. DOI: 10.1186/s13058-015-0669-x

[90] Grossman SA, Ellsworth S, Campian J, Wild AT, Herman JM, Laheru D, et al. Survival in patients with severe lymphopenia following treatment with radiation and chemotherapy for newly diagnosed solid tumors. Journal of the National Comprehensive Cancer Network. 2015;**13**:1225-1231

[91] McCormick WJ. Cancer: A collagen disease, secondary to a nutritional deficiency. Archives of Pediatric. 1959;**76**:166-171

[92] Grosso G, Bei R, Mistretta A, Marventano S, Calabrese G, Masuelli L, et al. Effects of vitamin C on health: A review of evidence. Frontiers in Bioscience Landmark Edition. 2013;**18**:1017-1029

[93] McCormick WJ. Is cancer a collagen disease attributable to vitamin C deficiency? Union Medicale Du Canada. 1959;**88**:700-704

[94] Ikeda K, Iyama K, Ishikawa N, Egami H, Nakao M, Sado Y, et al. Loss of expression of type IV collagen 5 and 6 chains in colorectal cancer associated with the hypermethylation of their promoter region. The American Journal of Pathology. 2006;**168**:856-865

[95] Tanjore H, Kalluri R. The role of type IV collagen and basement membranes in cancer progression and metastasis. The American Journal of Pathology. 2006;**168**:715-717

[96] Roomi MW, Monterrey JC, Kalinovsky T, Rath M, Niedzwiecki A. Inhibition of invasion and MMPs by a nutrient mixture in human cancer cell lines: A correlation study. Experimental Oncology. 2010;**32**:243-248

[97] Cathcart J, Pulkoski-Gross A, Cao J. Targeting matrix metalloproteinases in cancer: Bringing new life to old ideas. Genes and Diseases. 2015;**2**:26-34

[98] Rath M, Pauling L. Plasmin-induced proteolysis and the role of apoprotein(a), lysine and synthetic analogs. Orthomolecular Medicine. 1992;**7**:17-23

[99] Roomi MW, Monterrey JC, Kalinovsky T, Rath M, Niedzwiecki A. Comparative effects of EGCG, green tea and a nutrient mixture on the patterns of MMP-2 and MMP-9 expression in cancer cell lines. Oncology Reports. 2010;**24**:747-757

[100] Niedzwiecki A, Roomi MW, Kalinovsky T, Rath M. Anticancer efficacy of polyphenols and their combinations. Nutrients. 2016;**8**(552):1-17. DOI:10.3390/nu8090552

[101] Cha J, Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Ascorbate depletion increases growth and metastasis of melanoma cells in vitamin C deficient mice. Experimental Oncology. 2011;**33**:226-230

[102] Cha J, Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Ascorbate supplementation inhibits growth and metastasis of B16FO melanoma and 4T1 breast cancer cells in vitamin C-deficient mice. International Journal of Oncology. 2013;**42**:55-64. DOI: 10.3892/ijo.2012.1712

[103] Lee KW, Lee HJ, Surh YJ, Lee CY. Vitamin C and cancer chemoprevention: Reappraisal. American Journal of Clinical Nutrition. 2003;**78**:1074-1078

[104] World Cancer Resarch Fund/American Institute for Cancer Research. Food Research and the Prevention of Cancer: A Global Perspective. Washington, DC: American Institute for Cancer Research; 1997. p. 517

[105] Vecchia CL, Altieri A, Tavani A. Vegetables, fruit, antioxidants and cancer: A review of Italian studies. European Journal of Nutrition. 2001;**40**:261-267

[106] Henson DE, Block G, Levine M. Ascorbic acid: Biologic functions and relation to cancer. Journal of the National Cancer Institute. 1991;**83**:547-552

[107] Bollschweiler E, Wolfgarten E, Nowroth T, Rosendahl U, Monig SP, Holscher AH. Vitamin intake and risk of subtypes of esophageal cancer in Germany. Journal of Cancer Research and Clinical Oncology. 2002;**128**:575-580

[108] Mirvish SS, Wallcave L, Eagen M, Shubik P. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. Science. 1972;**177**:65-68

[109] Ramos AC, Araujo MR, Lopes LR, Andreollo NA. Role of the vitamin C in diethyl-nitrosamine-induced esophageal cancer in Wistar rats. Acta Cirurgica Brasileira. 2009;**24**:183-188

[110] Ismail SM. Protective effects of vitamin C against biochemical toxicity induced by malathion pesticides in male albino rat. Journal of Evolutionary Biology. 2013;**5**:1-5

[111] Eissa OS. Protective effect of vitamin C and glutathione against the histopathological changes induced by imidacloprid in the liver and testis of Japanese Quail. Egyptian Journal of Hospital Medicine. 2004;**16**:39-54

[112] Takhshid MA, Tavasuli AR, Heidary Y, Keshavarz M, Kargar H. Protective effect of vitamins E and C on endosulfan-induced reproductive toxicity in male rats. Iranian Journal of Medical Sciences. 2012;**37**:173-180

[113] Dubey S, Shrivastava R, Binjhade A, Chouhan R, Shrivastava VK. Antagonistic role of vitamin-C against dimethoate toxicity on kidney in air breathing fish Clarias batrachus (Linn.). International Journal of Current Research. 2015;**3**:25-34

[114] Hussein HK. Elnaggar MH, Al-Dailamy JM. Protective role of vitamin C against hepatorenal toxicity of fenvalerate in male rats. Garjest. 2012;**1**:060-065

[115] Appenroth D, Fröb S, Kersten L, Splinter FK, Winnefeld K. Protective effects of vitamin E and C on cisplatin nephrotoxicity in developing rats. Archives of Toxicology. 1997;**71**:677-683

[116] Antunes LM, Araujo MCP, Darin JDAC, Bianchi MLP. Effects of the antioxidants curcumin and vitamin C on cisplatin-induced clastogenesis in Wistar rat bone marrow cells. Mutation Research. 2000;**465**:131-137

[117] Ghosh S, Ghosh D, Chattopadhyay S, Debnath J. Effect of ascorbic acid supplementation on liver and kidney toxicity in cyclophosphamide-treated female albino rats. The Journal of Toxicological Sciences. 1999;**24**:141-144

[118] Blasiak J, Kowalik J. Protective action of vitamin C against DNA damage induced by selenium-cisplatin conjugate. Acta Biochimica Polonica. 2001;**48**:233-240

[119] Sato T, Kinoshita M, Yamamoto T, Ito M, Nishida T, Takeuchi M, et al. Treatment of irradiated mice with High-Dose ascorbic acid reduced lethality. PloS One. 2015;10:e0117020. DOI. org/10.1371/journal.pone.0117020. PubMed PMID: 25651298. PubMed PMCID: 4317183

[120] Mondal R, Biswas S, Chatterjee A, Mishra R, Mukhopadhyay A, Bhadra RK, et al. Protection against arsenic-induced hematological and hepatic anomalies by supplementation of vitamin C and vitamin E in adult male rats. Journal of Basic and Clinical Physiology and Pharmacology. 2016;**27**:643-652. DOI: 10.1515/jbcpp-2016-0020

[121] Ludke A, Sharma AK, Bagchi AK, Singal PK. Subcellular basis of vitamin C protection against doxorubicin-induced changes in rat cardiomyocytes. Molecular and Cellular Biochemistry. 2012;**360**:215-224

[122] Aidoo A, Lyn-Cook LE, Lensing S, Wamer W. Ascorbic acid (vitamin C) modulates the mutagenic effects produced by an alkylating agent in vivo. Environmental and Molecular Mutagenesis. 1994;**24**:220-228

[123] Stoute JO. The use of vitamin C with chemotherapy in cancer treatment: An annotated bibliography. Journal of Orthomolecular Medicine. 2004;**19**:198-245

[124] Yiang GT, Chou PL, Hung YT, Chen JN, Chang WJ, Yu YL, et al. Vitamin C enhances anticancer activity in methotrexate-treated Hep3B hepatocellular carcinoma cells. Oncology Reports. 2014;**32**:1057-1063. DOI: 10.3892/or.2014.3289. Epub Jun 25, 2014

[125] Ma Y, Chapman J, Levine M, Polireddy K, Drisko J, Chen Q. High-Dose parenteral ascorbate enhanced chemosensitivity of ovarian cancer and reduced toxicity of chemotherapy. Science Translational Medicine. 2014;**6**:222ra18

[126] Frömberg A, Gutsch D, Schulze D, Vollbracht C, Weiss G, Czubayko F, et al. Ascorbate exerts anti-proliferative effects through cell cycle inhibition and sensitizes tumor cells towards cytostatic drugs. Cancer Chemotherapy and Pharmacology. 2011;**67**:1157-1166

[127] Carr AC, Vissers MCM, Cook JS. The effect of intravenous vitamin C on cancer- and chemotherapy-related fatigue and quality of life. Frontiers in Oncology. 2014;**4**:283

[128] Stephenson CM, Levin RD, Spector T, Lis CG. Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. Cancer Chemotherapy and Pharmacology. 2013;**72**:139-146

[129] Prasad KN, Cole WC, Kumar B, Prasad KC. Scientific rationale for using High-Dose multiple micronutrients as an adjunct to standard and experimental cancer therapies. Journal of the American College of Nutrition. 2001;**20**:450S-463S

[130] Bael TE, Peterson BL, Gollob JA. Phase II trial of arsenic trioxide and ascorbic acid with temozolomide in patients with metastatic melanoma with or without central nervous system metastases. Melanoma Research. 2008;**18**:147-151. DOI: 10.1097/CMR.0b013e3282f2a7ae

[131] Subbarayan PR, Lima M, Ardalan B. Arsenic trioxide/ascorbic acid therapy in patients with refractory metastatic colorectal carcinoma: A clinical experience. Acta Oncologica. 2007;**46**:557-561

[132] Welch JS, Klco JM, Gao F, Procknow E, Uy GL, Stockerl-Goldstein KE, et al. Combination decitabine, arsenic trioxide, and ascorbic acid for the treatment of myelodysplastic syndrome and acute myeloid leukemia: A phase I study. American Journal of Hematology. 2011;**86**:796-800. DOI: 10.1002/ajh.22092. Epub Aug 3 2011

[133] Carreau A, El Hafny-Rahbi B, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. Journal of Cellular and Molecular Medicine. 2011;**15**:1239-1253

[134] Hickey DS, Roberts HJ, Cathcart RF. Dynamic flow: A new model for ascorbate. Journal of the Minerals. 2005;**20**:237-244

[135] Duconge J, Miranda-Massari JR, Gonzalez MJ, Jackson JA, Warnock W, Riordan NH. Pharmacokinetics of vitamin C: Insights into the oral and intravenous administration of ascorbate. Puerto Rico Health Sciences. 2008;**27**:7-19

[136] Hamel EE, Santisteban GA, Ely JT, Read DH. Hyperglycemia and reproductive defects in non-diabetic gravidas: A mouse model test of a new theory. Life Sciences. 1986;**39**:1425-1428

[137] Haines I. The war on cancer: Time for a new terminology. Lancet. 2014;**383**:1883

[138] Hanin L. Why victory in the war on cancer remains elusive: Biomedical hypotheses and mathematical models. Cancers. 2011;**3**:340-367

[139] Sporn BM. The war on cancer. Lancet. 1996;**347**:1377-1381

[140] Temple NJ, Burkitt DP. The war on cancer-failure of therapy and research: Discussion paper. Journal of the Royal Society of Medicine. 1991;**84**:95-98

[141] Huang S. The war on cancer: Lessons from the war on terror. Frontiers in Oncology. 2014;**4**:1-4

[142] Hanahan D. Rethinking the war on cancer. Lancet. 2014;**383**:558-563

[143] Mailankody S, Prasad V. Comparative effectiveness questions in oncology. The New England Journal of Medicine. 2014;**370**:1478-1481. DOI: 10.1056/NEJMp1400104

[144] Abel U. Chemotherapy of advanced epithelial cancer-a critical review. Biomedicine & Pharmacotherapy. 1992;**46**:439-452

[145] Morgan G, Ward R, Barton M. The contribution of cytotoxic chemotherapy to 5-year survival in adult malignancies. Clinical Oncology Journal (The Royal College of Radiologists). 2004;**16**:549-560

[146] Wise PH. Cancer drugs, survival, and ethics. British Medical Journal. 2016;**355**:i5792

[147] Godlee F. Too much chemotherapy. British Medical Journal. 2016;**355**:i6027

[148] Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Vitamin C (L-Ascorbic acid, its calcium, potassium and sodium salts and L-ascorbyl-6-palmitate). The EFSA Journal. 2004;**59**:1-21

[149] Gaby AR. Nutritional Medicine. Fritz Perlberg Publishing; 2011. p. 1358. ISBN-13:978-0982885000, ISBN-10:0982885008

[150] Chen Q, Espey MG, Sun AY, Pooput C, Kirk KL, Krishna MC, et al. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xeno-grafts in mice. Proceedings of the National Academy of Sciences of the United States. 2008;**105**:11105-11109

[151] Siddiqui M, Rajkumar SV. The high cost of cancer drugs and what we can do about it. Mayo Clinic Proceedings. 2012;**87**(10):935-943

[152] Mayland CR, Bennett MA, Allan K. Vitamin C deficiency in cancer patients. Palliative Medicine. 2005;**19**:17-20