

Vitamin C and Oxidative DNA Damage Revisited

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Abstract

Reactive oxygen metabolites can promote carcinogenesis by damaging DNA. vitamin C is a redox chemical with antioxidant and pro-oxidant properties that may produce radical species depending on the presence of various conditions. Nevertheless, evidence presented herein discourages the consideration of any mutagenic or carcinogenic activity of vitamin C. In contrast, it substantiates a protective and anti carcinogenic effect of ascorbate in vivo.

Introduction

It is evident that reactive oxygen metabolites can promote carcinogenesis by damaging DNA,¹ especially when damage occurs to genes involved in cell cycle regulation.² Nevertheless, further damage to the cell (membranes, DNA or enzymatic machinery) may induce cell death. Ascorbic acid (vitamin C) is a redox chemical with demonstrated antioxidant and pro-oxidant properties.³ We will analyze if the pro-oxidant effect of vitamin C may produce DNA damage *in vivo*.

Discussion

Vitamin C has been accused of causing chromosomal aberrations that may produce increases in cell proliferation and mutations that may lead to cancer. However a number of reasons exist that contradict this concern:

1. Common logical sense and long evolutionary evidence. Logical sense and evolutionary evidence contradict that 200 mg of vitamin C will cause DNA damage and cancer. We know that most animals produce their own vitamin C endogenously.

The concentration of vitamin C made

by these animals is equal or greater than what would allegedly cause DNA damage and promote cancer.⁴ When vitamin C production of these animals is adjusted for body weight and in animals weighing 150 lb or more it averages 9,000 to 12,000 mg daily.

In 1998 Podmore⁵ reported that vitamin C could be an oxidant. This concern was substantiated by Blair⁶ in 2001, when his group reported that the daily equivalent of 200 mg of vitamin C could produce substantial amounts of DNA damage. It could be argued that the genotoxic effects of vitamin C *in vitro* are well-documented,^{1,6-8} It is reported to cause dose dependent increases in DNA damage⁷ and at high concentrations, to enhance the cytotoxicity of hydrogen peroxide.⁸ vitamin C's redox properties are concentration and environmentally dependent. It depends on quality and quantity of antioxidant molecules, quantity and free or bound status of divalent cations or metal ions, quantity and efficiency of antioxidant enzymes, membrane fatty acid composition, and on the presence of other redox agents. However, it is not known whether the concentrations attainable *in vivo* are sufficient or if the redox environment is suitable to facilitate or at least permit the expression of pro-oxidant properties under normal physiological conditions. Nevertheless, in a 1999 study by Crott and Fenech,¹ vitamin C supplementation (2 g) did not appear to cause DNA damage under normal physiological conditions. Moreover, it has been demonstrated that human serum (with a high content of antioxidant substances) inhibits the cytotoxic activity of vitamin C.⁹ This has been also observed by Riordan et al.¹⁰

Interestingly, the pro-oxidant effect of vitamin C is theorized to be its most important anti-cancer mechanism.¹¹

But it must be noted that malignancy is a pathological state in which normal physiological conditions are disturbed. This

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pathologic state of metabolic unbalance favors anaerobic respiration (fermentation) as the main energy fuel and uncontrolled cell proliferation as survival mechanism. Also quantitative metabolic differences have been detected in malignant tissue, such as the deficiency of the enzyme catalase,¹² which converts H_2O_2 to O and H_2O .

We need to remember that cells, in order to be able to divide, need to reduce cohesiveness and dismount part of their structure; in other words, dedifferentiate. This unstable state of cellular organization facilitates free radical damage in the malignant cell. With all this taken into account, vitamin C in high doses (intravenous), in the presence of divalent cations and oxygen can act as a chemotherapeutic pro-oxidant in malignant tissue (by generating hydrogen peroxide).

The precise role of vitamin C in relation to DNA mutations is controversial. Ascorbic acid has strong antioxidant properties, but it also has pro-oxidant effects in the presence of free transition metals. Vitamin C has the capacity to form radicals that interact with viral and bacterial DNA.^{3,13} This probably raised the suspicion about a possible mutagenic capacity in humans. However, antimutagenic effects of ascorbate have been reported. Norkus et al¹⁴ reported results of in-vivo studies indicating that vitamin C is not mutagenic or cytotoxic in guinea pigs even when fed at levels of 5,000 mg/kg body weight/day. Bruce et al¹⁵ observed that the levels of fecal mutagens are reduced by 60% in the feces of people consuming normal diets supplemented with 4 g of vitamin C per day. Similar results were reported by Dion et al¹⁶ using vitamin E in addition to vitamin C.

In another report¹⁷ daily doses of 1 gram vitamin C were given to a group of 35 coal tar workers occupationally exposed to polycyclic aromatic hydrocarbons and benzene during the processing of coal tar. Genetic analysis of peripheral blood lymphocytes revealed a significant drop in the frequency of aberrant cells. In addition vitamin C sup-

plementation has been reported to decrease gastric mucosal DNA damage in 28 out of 43 chronic gastritis patients.¹⁸ This study was corroborated by Drake et al.¹⁹

2. Epidemiological evidence of dietary vitamin C intake. A vast quantity of epidemiological evidence has shown (in over 100 studies) that vitamin C intake is inversely related to cancer, with protective effects shown for cancers of the pancreas, oral cavity, stomach, cervix, rectum, breast and lung.²⁰⁻²³

3. Inaccuracy and difficulty of DNA oxidation measurements. DNA damage is a very complicated process that is balanced out by efficient DNA repair mechanisms dependent on B vitamins. There are about 20 markers of DNA damage. Measurement of 8-oxoguanine and 8-oxoadenine are the most common methods for assessing DNA damage, but there is no consensus on what the true levels are in human DNA. They are difficult to measure because of the ease with which it is formed artifactually during isolation, hydrolysis and analysis. In the study by Podmore et al,⁵ 8-oxoguanine levels were decreased by vitamin C while the 8-oxoadenine increased. Of interest, 8-oxoguanine is at least ten times more mutagenic than 8-oxoadenine; therefore Podmore's study indicates that vitamin C supplementation is actually beneficial since the benefits of decreasing 8-oxoguanine levels outweigh the detrimental effects of increasing 8-oxoadenine levels. Nonetheless, the negative results of this study received considerable attention by the media, while in contrast a following report from this same group²⁴ suggesting a role for vitamin C in the regulation of DNA repair enzymes and thereby showing an antioxidant effect did not receive the same exposure. It should be pointed out that iron is imbedded in the center of the DNA double helix at certain loci where it seems to function as an oxygen sensor to activate DNA in response to oxidative stress. Because iron is a big molecule, it distorts the outer shape of the DNA helix. This distur-

tion depends on the oxidation state of iron. Under non-oxidized (reduced) conditions, the iron is present in the ferrous state and the DNA helix is fairly tight around the iron atom. But when the iron is oxidized to the ferric state, it opens up the DNA so that it is more easily expressed so transcription into RNA and then into proteins can be accomplished. Most likely the proteins expressed are enzymes like SOD, catalase, glutathione peroxidase, heat shock proteins, plus other proteins that help mobilize and regulate the antioxidant defense system. The ability of DNA to sense free radicals and oxidizing conditions in this way is an essential aspect of our capability to maintain homeostasis and adapt to stress. This temporary damage to DNA is necessary and a small price to pay in order to be able to have an enhanced capacity for adaptability and increased survival. It seems that in its activated state, the ferrous iron-DNA complex can react with vitamin C, dehydroascorbic acid, oxygen and/or hydrogen peroxide to produce reactive oxygen species (probably the hydroxyl radical) which can attack DNA at the iron binding site. This iron binding site is especially rich in A-T base pairs, explaining why more damage would occur to A and T residues than to the G and Cones.

4. Negative relevant human studies. It should be noted that most studies that show a vitamin C induced DNA damage or generation of genotoxins were done in vitro. In contrast, most human studies do not confirm that vitamin C causes any important DNA damage. There are at least six relevant human studies that disprove the notion that high dose vitamin C causes important DNA damage.²⁵⁻³⁰ Huang et al²⁵ found no evidence of a significant effect or interaction on oxidative DNA damage in non-smoking adults taking 500 mg/day vitamin C supplement. Schneider et al,²⁶ reported that taking 1,000 mg/day vitamin C (smokers and non-smokers) for 7 days did not produce DNA damage in blood

lymphocytes. Vojdani et al,²⁷ found that in 20 healthy volunteers that were divided into 4 groups and given either placebo or daily doses of 500, 1,000, or 5,000 mg, neither induce mutagenic lesions nor have negative effects on NK cell activity, apoptosis, or on the cell cycle. Proteggente et al²⁸ measured the effects of 260 mg/day vitamin C and vitamin C plus iron in humans and concluded that there was no compelling evidence for a prooxidant effect of vitamin C supplementation in the presence or absence of iron on DNA base damage. Proteggente reported no detrimental effects on oxidative damage to DNA, using healthy individuals with plasma ascorbate levels at the upper end supplemented with 12.5 mg iron for 6 weeks.²⁹ Brennan et al³⁰ gave 1,000 mg vitamin C to volunteers for 42 days and concluded that supplementation with vitamin C significantly decreased H₂O₂-induced DNA damage in peripheral blood lymphocytes.

Conclusion

Quality scientific research is characterized by findings which are clinically significant and/or advance our basic scientific knowledge. The articles discussed herein by Podmore et al⁵ and Lee et al⁶ do neither. In light of the evidence available and presented here, a conclusion that vitamin C is harmful is unwarranted since these studies are compromised by unproved assumptions, an unacceptable study design and inaccurate measurements. To date we do not even know the true significance or repercussions of nucleotide oxidation in the DNA nor the precise role of ascorbate in this process. Hundreds of studies strongly suggest a protective, risk reducing and even therapeutic role for ascorbate in cancer.

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