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**Hypothesis**

# A new method for the activation of the cellular antioxidant system

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**Abstract**

The result that pharmacological ascorbic acid concentration in blood releases hydrogen peroxide and ascorbyl radicals when ascorbic acid is transferred into interstitial fluids is reviewed. The hydrogen peroxide concentration in some interstitial fluids (liver, gut, pancreas, kidneys) may be responsible for some killing activity of neoplastic cells deprived of antioxidant defences. However a real anti-tumour effect limited to some neoplasms remains an open problem not explored here. On the other hand reactive oxygen species (ROS) may induce peroxidation of lipids and, in this case, peroxidation compounds and alkenals returning into the general circulation may represent a moderate oxidative stress able to activate and free nuclear related factor 2 (Nrf2), which, after binding to antioxidant response elements (ARE), switch on the upregulation of the innate antioxidant system. This situation can be achieved by repeated intravenous infusion of ascorbic acid at the dose of 0.36-0.64 g/kg in patients with chronic cardiovascular diseases, macular degeneration (dry form), type 2 diabetes and chronic infectious diseases where, in spite of effective orthodox drugs, the diseases are complicated by a chronic oxidative stress. Consequently a prolonged orthodox therapy integrated by repeated ascorbic acid infusions may lead to a real improvement due to the combined approach proposed in this paper.



## INTRODUCTION

Cardiovascular diseases (chronic heart failure, peripheral obstructive arterial diseases, stroke) type 2 diabetes, age related macular degeneration (dry form), chronic viral hepatitis and human immunodeficiency virus (HIV) infections, chronic obstructive pulmonary diseases (COPD), sepsis and cancer are affections widely diffused in the population. Their progression is accompanied by an enhancement of metabolic processes such as the activation of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidases and xantine oxidase leading to an excessive release of reactive oxygen and nitrogen species (ROS and RNS), nitrogen oxidative species, superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), hydroxyl radical (•OH), nitric oxide (NO), peroxynitrite ($ONOO^-$). These oxidants leads to an indiscriminate lipid peroxidation with the final formation of α and ß-unsaturated aldehydes 4-hydroxynonenal (4-HNE), 4-oxononenal, 2-hexenal, acrolein and malonyldialdeyde (MDA), which are the subject of Michael addition reaction with the side chain of lysine, histidine and cysteine residues, hence to protein carbonylation [1]. Moreover oxidation of carbohydrates induces the formation of advanced glycation end products (AGEs), some of which are reactive towards hemoglobin. All of these toxic products aggravates the basic pathology and leads to irreversible damages such as insulin resistance associated with type 2 diabetes, macular degeneration with death of photoreceptors, immune dysfunction, impaired cell respiration associated with metabolic dysregulation and to a further decline of antioxidant defences [2-5]. During the previous decades it was thought that the oxidative stress could be corrected by the administration of several antioxidants but results have been disappointing because the antioxidants in most cases result unable to quench the variety and the damaging effects of ROS [6-8]. It is a passive therapy and either a scarce antioxidants bioavailability or their inability to reach the target or to actively stimulate the innate antioxidant response explains the failure.

In many of the above-mentioned pathologies, orthodox medicine has provided efficacious drugs such as statins, antihypertensive and anti-aggregant drugs, or antidiabetics or chemotherapeutics but they are usually unable to properly quench the chronic oxidative stress. Moreover either caloric restriction, or a daily exercise stress or the oral administration of selected chemical compounds such as curcumin, resveratrol and sulphoraphane have been proposed because they seem able to display some activity when administered in healthy individuals [9]. However during the last two decades the application of ozonetherapy has clarified that the use of a small dose of ozone added to human blood *ex vivo* can be effective [10-12]. Ozone acts in a hormetic fashion and as a mild stressor: it elicits the calculated formation of two messengers: $H_2O_2$ and lipoperoxide compounds such as 4-HNE. This aldehyde forms an adduct with either glutathione (GSH) or the unpaired thiol residue (Cys34) present in human albumin: after the infusion of ozonated blood in the donor patients, 4-HNE is able to activate a master molecular mechanism based on the release of the inactive nuclear related factor 2 (Nrf2) from the cytoplasm. Its translocation into the cell nucleus followed by its interaction with the antioxidant response element (ARE) represents the paradigmatic step leading to the activation of the synthesis of a great variety of antioxidant enzymes able to reactivate the redox system. Today the ozonated autohemotherapy is used in many countries but only by private physicians. Unfortunately it is frequently used in an empirical form without a precise knowledge of the biochemical steps and the molecular reactions which express the well tolerated oxidative stress able to induce the antioxidant response able to correct the chronic oxidative stress. It is very unfortunate that orthodox medicine, not only has not accepted this complementary approach but continues to refuse its application as an integrative medication. There is a diffuse scepticism and disregard towards the value of ozonetherapy due to the old concept that ozone is always toxic, a dogma that now is untenable [13]. Moreover, this is in open contrast with the fact that other potentially toxic gases, such as NO, carbon monoxide (CO), hydrogen sulphide ($H_2S$) and hydrogen ($H_2$), used in therapeutic dosages are now finding relevant medical applications [14]. The problem is further complicated by other practical reasons because valid ozone generators are expensive and often underdeveloped countries, where ozonetherapy could be used, lack electricity and medical oxygen. Moreover, the application of cheap procedures like the use of ozonated saline has further compromised the classical procedure.

**Proposal of a new method**

It appears necessary to describe an alternative, safe and effective procedure which has the following advantages and can be accepted by orthodox medicine:

1) there is no need of withdrawing blood from the patient that, although being a real auto-transfusion, is objected by transfusion specialists;
2) the aqueous solution to be infused is composed of pure ascorbic acid (vitamin C; 0.36-0.64 g/kg), dissolved in pure, sterile water buffered at pH 7. The solution is ultrafiltered and is within the biochemical concept of orthodox medicine because ascorbic acid at far higher concentrations is already used in oncology as a pro-antitumor agent;
3) another advantage is that the bulk purchase of ascorbic acid is very cheap and therefore this approach becomes amenable to be used in underdeveloped countries.

For several decades the daily use of oral ascorbic acid. (60-75 mg) has insured the recommended daily allowance (RDA). The average concentration in human plasma ranges between 0.5 and 1.5 mg/dl, equivalent to about 50 μM, which is about 8-16 times lower than that of uric acid, the other relevant reducing agent. Although Cameron and Pauling [15] suggested that the oral administration of 10 g of once a day of vitamin C may be useful in cancer, this postulation was shown wrong by two successive clinical trials performed with doubling the oral dose of ascorbic acid. [16, 17]. It goes to the merit of Levine's team [18-22] to have clarified this issue: the maximum tolerated oral dose of even 3 g every four hours yields a plasma vitamin C concentration of about 200 μmol/l, while a 100 g dose administered by intravenous infusion yields a plasma level as high as 30 mmol/l or about 150 fold higher. This high plasma level has a pharmacological relevance and may be able to kill sensitive neoplastic cells. This new aspect has been demonstrated also by other authors [23-29], who have evaluated the pharmacokinetics of mega doses of ascorbate. After a rapid mixing within the blood pool, ascorbic acid is partly filtrated by the kidneys with tubular uptake but variable amounts pass into the extravascular fluid, which is volumetrically more than twice the blood volume. Ascorbic acid diluted in the interstitial fluids, in contact with either traces of $Fe^{3+}$ or $Cu^{2+}$ probably transported by some unknown globulins or by albumin, becomes a pro-oxidant as shown in the next section. This approach, in a preliminary form, was discussed earlier by the author [30].

**Biochemistry of ascorbic acid.**

Ascorbic acid is one of the most potent reducing agents in biological systems. It has a major role in the synthesis of collagen because without it, collagen is insufficiently hydroxylated. Ascorbic acid is an essential vitamin because guinea pigs and primates have lost the ability to synthesize it and they must acquire it form fresh fruits in spite of the fact that a

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good deal of dehydroascorbic acid (DHA) is rapidly recycled to ascorbic acid [31, 32]. The lack of ascorbic acid in the diet for months causes scurvy, a deadly disease described by the French explorer Jacques Cartier (1491-1557) in 1536. James Lind (1716-1794), a Scottish physician, advised that lemons should be included in the diet of sailors in 1753. Albert Szent-Gyorgyi (1893-1986), a Hungarian-born biochemist, identified ascorbic acid in 1933.

Ascorbic acid has many virtues [33-35]:

1) it scavenges the superoxide anion, hydroxyl radicals, thiyl (RS•) and sulphenyl (RSO•) radicals, NO, singlet oxygen ($^1O_2$), $ONOO^-$, and $O_3$;
2) it slows down or blocks the formation of hypochlorous acid (HOCl);
3) it inhibits lipid peroxidation and prevents heme breakdown, hence the release of $Fe^{2+}$ that may favor •OH formation by the Fenton reaction;
4) it regenerates alpha-tocopherol by reducing alpha-tocopheryl radicals in lipoproteins and membranes;
5) it can reduce nitrosamines to inactive compounds thus inhibiting carcinogenesis in the stomach;
6) it protects uric acid from hydroxyl radical attack;
7) it seems to protect against ROS present in cigarette smoke in the lungs [36];
8) it allows the hydroxylation of proline to hydroxyproline by maintaining prolyl and lysil hydroxylases in an active form (with $Fe^{2+}$), a step indispensable for the synthesis of collagen.

These activities of ascorbic acid ($AscH_2$) are due to the transfer of electrons in one or two steps, with the formation of either semidehydroascorbate radical anion ($Asc•^-$), a poorly-reactive radical, or dehydroascorbic acid (DHA): $AscH_2 < > Asc•^- < > DHA$

The ascorbyl radical can be reconverted to ascorbic acid by NADH-semidehydroascorbate reductase, while dehydroascorbate either decomposes irreversibly to 2,3-diketogulonic acid or is recycled to ascorbic acid by GSH-dependent enzymes. While ascorbic acid is accumulated in cells by $Na^+$-dependent vitamin C transporters (SVCT1 and 2), DHA is absorbed by human erythrocytes via $Na^+$-independent facilitative glucose transporters (GLUTs) followed by intracellular reduction to ascorbate via a GSH-dependent reductase that oxidizes GSH to GSSG (oxidized glutathione). The development of this reaction allows to restore ascorbic acid several times.

Regarding the proposed method, it is important to note that when ascorbic acid is transferred into interstitial fluids, critical chemical reactions occur because ascorbic acid becomes a pro-oxidant. These reactions are variably taking place in the extracellular fluids of all organs and are somewhat dependent upon the structural features of different kind of blood capillaries. There are three types of endothelium: the first type has no fenestrations and capillaries are protected by a complete and continuous basement membrane. This occurs in muscle, central nervous system (CNS), lungs and dermis. The second type endothelium has intracellular fenestrations present in the kidneys, intestinal villi and some endocrine organs. The third type has discontinuous endothelium equal to intercellular gaps and is present in liver, spleen and bone marrow. Consequently, while the protein content of plasma has a concentration of about 70-78 mg/ml, it may range from as little as 20 mg/dl in the cerebrospinal fluid (CSF) (first type) to as much as 5 g/dl *i.e.* 60-70% of the plasma protein concentration in hepatic and intestinal lymph (third type) [37]. Obviously leaky capillary walls allow the passage of a far higher concentration of plasma proteins. Moreover there are other subtle differences: the CSF contains an albumin fraction which has an electrophoretic mobility slightly higher than plasmatic albumin, most probably due to fatty acids [38]. Mouithys-Mickalad *et al* [39] have reported that human interstitial fluids contain some ceruloplasmin with bound $Cu^{2+}$ and albumin, possibly binding $Cu^{2+}$ or/and $Fe^{3+}$ able to enhance the oxidation of ascorbic acid with an increased formation of $H_2O_2$ and ascorbyl free radical. A first conclusion is that organs of the second and third type capillaries will allow a transfer of more ascorbic acid than organs with tight closed endothelium. In these fluids ascorbic acid becomes a pro-oxidant and catalyzes the following reactions:

1) $AscH_2 + Fe^{3+} \rightarrow Asc•^- + Fe^{2+}$
2) $AscH_2 + O_2 \rightarrow Asc•^- + O_2•^-$
3) $O_2•^- + 2H^+ \rightarrow H_2O_2$
4) $O_2•^- + Fe^{2+} + 2H^+ \rightarrow H_2O_2 + Fe^{3+}$

The Fenton reaction takes place with the formation of •OH:

5) $H_2O_2 + Fe^{2+} \rightarrow •OH + OH^- + Fe^{3+}$

The Haber-Weiss reaction may also occur:

6) $H_2O_2 + {}^{\cdot}O_2•^- \rightarrow {}^{\cdot}•OH + OH^- + O_2$

It must be noted that a load of ascorbic acid administered to iron-overloaded patients (hemochromatosis, ß-thalassemia) not treated with iron chelators can be toxic. Moreover lack of glucose-6-phosphate dehydrogenase, renal insufficiency and a high oxalate concentration proscribe the use of ascorbic acid in about 5% of patients and therefore a great number can be infused with ascorbic acid either for a neoplastic problem or because they had a chronic diseases or a viral disease. A pharmacological dose of 25 up to 45 g ascorbic acid administered intravenously will yield a plasma concentration ranging from about 5 to 13 mmol/l which is about 100 fold higher than the one measured after oral administration of 10 g. While

hydrogen peroxide formation in blood is minimal and transitory, it will be significant in at least some of the interstitial fluids and sufficient for achieving the desired biochemical stimulation.

Mega dosages of ascorbic acid must be avoided because the oxidative stress induced by $H_2O_2$ must act in a hormetic fashion. There will not be a toxic effect of hydrogen peroxide on normal parenchymal cells because even a concentration of 40-50 μM will be rapidly reduced by the normal cell antioxidant system represented by catalase, GSH peroxidise (GPx) and peroxyredoxins. On the other hand, peroxidation of the either free or albumin-bound lipids will release lipoperoxides and eventually alkenals, particularly 4-HNE. Another possible aspect is the effect of hydrogen peroxide on either hepatic cells chronically infected with B and C hepatitis viruses or lymphoid cells with HIV. If infected cells become defective in antioxidants, they undergo apoptosis and therefore a simultaneous treatment with ascorbic acid and either interferon alpha, or other chemotherapeutics or with the highly active anti-retroviral therapy (HAART) may display a synergistic therapeutic effect. Historically, it is worth remember that Klenner [40] with apparent great success, used consistently the administration of 6-22 g oral vitamin C therapy every day in polio, pneumonia and hepatitis patients in 1948. Obviously it remains unknown if the therapeutic effect was due to ascorbic acid or to hydrogen peroxide released in interstitial fluids or to a placebo effect.

**The relevance of transitory and calculated peroxidative bouts in stimulating the antioxidant defence**

Peroxidation generates a variety of compounds in the interstitial fluids and these will rapidly return into the blood pool via the two lymphatic ducts or venous capillaries. Some will be metabolized by several enzymes [41-43], some will be excreted via the bile and kidneys [44], and some, such as 4-HNE and other minor alkenals, at submicromolar levels, will form adducts with GSH or with the Cys34 of albumin. The relevant feature is that both albumin and GSH adducts, not only interact with the endothelium thus activating NO synthase, but they will be transported into many organs from liver to the hypothalamus and the endocrine system. In this way 4-HNE becomes the hormetic ascorbic acid messenger as it will be released at many sites and informs a variety of cells of a transitory and acceptable oxidative stress. Once internalized in the cell cytosol, by reacting with sulfhydryl (SH) groups of Kelch-like ECH-associated protein-1 (Keap1), it allows the release of Nrf2. ROS and other electrophiles, by binding to two critical SH groups of Keap1 (Cys272 and Cys288), modify this protein and prevent the proteasomal breakdown of the Nrf2-Keap1 complex [45-48]. Once translocated into the nucleus, Nrf2 heterodimerizes with small Maf protein and binds to the ARE on DNA. This event induces the transcription of antioxidant proteins, such as superoxide dismutase (SOD), catalase, GSH reductase, GPx, GSH transferase, UDP-glucuronosyl transferase (UGT), NADPH quinone-oxidoreductase 1 (Nqo1), heat shock protein 70, heme-oxygenase-1 [49] and phase II enzymes [50, 51]. It also stimulates GSH synthesis thus enhancing the cell protective level [51]. The consequence of this process is that the use of either the best cardiovascular drugs or antidiabetics or antivirals associated with the intravascular infusion of ascorbic acid can be able to restore the normal redox potential thus blocking the progression of several diseases. The increased ascorbic acid levels and possibly the release of adrenocorticotropic hormone (ACTH)-cortisol will improve the patient's mood [12].

**A possible study design for evaluating the therapeutic effect following the intravenous infusion of ascorbic acid with chronic diseases**

Sterile solution of pharmaceutical grade ascorbic acid from 25 up to 45 g per each infusion of 150-200 ml of solution, at pH 7, twice weekly in patients with chronic diseases (vascular, degenerative, type 2 diabetes, COPD), for five months (about 45 times). The dose of 25 g is initially selected for achieving a mild, tolerable stress. However the ascorbic acid dose will be progressively increased in steps of 5 g (hence 25, 30, 35, 40 and 45 g) during the successive months.

Patients with infectious diseases will be also treated with progressively increasing dosages of ascorbic acid (25 up to 45 g for each infusion) three times weekly (*e.g.* Monday, Wednesday and Friday) for five months (about 67 times) by using the same step by step increase every month.

For analysis, the first blood sample has to be collected before starting the infusions as the control sample. Then other samples will be collected at the end of each month for evaluating levels of peroxidation markers, levels of antioxidants in plasma and blood cells. Levels of C-reactive protein (CRP) and levels of typical markers of the disease and viral load will also be determined.

## DISCUSSION AND CONCLUSION

After initial difficulties, the possibility that ascorbic acid may become an antineoplastic therapy has been almost achieved by its intravenous infusion of 80-100 g [18-22]. Only this method yields pharmacological concentrations (20-30 mmol/l of ascorbic acid in the plasma. However, in the interstitial fluids ascorbic acid behaves as a pro-oxidant and allows the generation of $H_2O_2$ and an ascorbyl radical. If tumor cells have a weak antioxidant defence, they may be killed by $H_2O_2$.

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The problem is complex because not only the neoplasm must be sensitive to $H_2O_2$ but also the location of the neoplasm is important because different organs have different $H_2O_2$ concentrations. It would be necessary to establish the effective dose and the correct timing because the present schedule of two infusions weekly may not be ideal as neoplastic cells may recover and become resistant. So far clinical results have been encouraging but they have to improve once the association with the right chemotherapeutic drug has been determined. This problem may also be improved by using another administration route, which is a problem evaluated in another paper [52].

The present suggested study has been concerned with a simpler problem, which is the use of ascorbic acid as a pro-oxidant able to cause a moderate oxidative stress when $H_2O_2$, ascorbyl radical and possibly oxydril radicals are formed in the interstitial fluids and start a limited peroxidation of polyunsaturated fatty acids (PUFA) present in the fluid or albumin-bound. It is now well clarified that 4-HNE or other electrophiles, transported as adducts, reach a multitude of cells and induce the activation of the innate antioxidant system. This molecular mechanism of action was also activated by the reactions elicited by ozone added to human blood *ex vivo* and was quite effective if only ozonetherapy had been accepted as a valuable integration of the orthodox medications. The activity of ascorbic acid closely resembles the mechanism of action of ozone because the active messengers are identical. It seems now possible to use the intravenous infusion of ascorbic acid in patients with chronic diseases complicated by a chronic oxidative stress. Mikirova *et al* [53] have somewhat preceded the present proposal and have already used ascorbic acid in cancer patients, not for the therapy of cancer, but for reducing inflammation. They found that both inflammation as well as CRP is markedly abated by the treatment as theoretically envisaged. Plasma C-reactive protein has also been found to be significantly reduced in cardiovascular patients treated with ascorbic acid [54]. These results encourage to evaluate the proposed scheme in patients with chronic diseases complicated by a chronic oxidative stress.

## ABBREVIATIONS

4-HNE: 4-hydroxy-2,3-nonenal; ARE: antioxidant response element; CNS: central nervous system; COPD: chronic obstructive pulmonary disease; GSH: glutathione (reduced form); GPx: glutathione peroxiase; GSSG: oxidized glutathione; GLUTs: $Na^+$-independent facilitative glucose transporters; HIV: human immunodeficiency virus; MDA: malonyldialdehyde; NADPH: nicotinamide adenine dinucleotide phosphate; NqO1: NADPH quinone-oxidoreductase 1; Nrf2: nuclear related factor 2; ROS: reactive oxygen species; PUFA: polyunsaturated fatty acids; SVCT1 and 2: $Na^+$-dependent vitamin C transporters

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The author declares to have no competing interests. The paper was written by the author without any funds. After retiring at 74, he is now 84. Without neither sponsors, nor funds he cannot perform a clinical trial but he hopes that other scientists will be interested in performing a study for verifying and perfecting the schedule and treatments dosages of ascorbic acid in patients with chronic diseases. While he is ready to help anyone, he will be very grateful for clarifying the issue eventually improving the treatment for the sake of patients.

## REFERENCES

1. Dalle-Donne I, Aldini G, Carini M, Colombo R, Rossi R, Milzani A. Protein carbonylation, cellular dysfunction, and disease progression. J Cell Mol Med 2006; 10:389-406.
2. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39:44-84.
3. Finkel T. Signal transduction by reactive oxygen species. J Cell Biol 2011; 194:7-15.
4. Graves DB. The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. J Phys D Appl Phys 2012; 45:263001.
5. Chen F, Haigh S, Barman S, Fulton DJ. From form to function: the role of Nox4 in the cardiovascular system. Front Physiol 2012; 3:412.
6. Halliwell B. The antioxidant paradox. Lancet 2000; 355:1179-80.
7. Firuzi O, Miri R, Tavakkoli M, Saso L. Antioxidant therapy: current status and future prospects. Curr Med Chem 2011; 18:3871-88.
8. Moyer MW. The myth of antioxidants. Sci Am 2013; 308:56-61.
9. Bocci V, Valacchi G. Free radicals and antioxidants. How to re-establish redox homeostasis in chronic disease? Curr Med Chem 2013; in press.
10. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: Ozone is a strong oxidant as wee as a medical drug. Medicinal Research Review 2009; 29 (4): 646-682..
11. Bocci VA, Zanardi I, Travagli V. Ozone acting on human blood yields a hormetic dose-response relationship. J Translat Med 2011; 9:66-77.
12. Bocci V. How a calculated oxidative stress can yield multiple therapeutic effects. Free Radic Res 2012; 46:1068-75.
13. Bocci V. Is it true that ozone is always toxic? The end of the dogma. Toxicol Appl Pharmacol 2006; 216:493-504.
14. Nakao A, Sugimoto R, Billiar TR, McCurry KR. Therapeutic antioxidant medical gas. J Clin Biochem Nutr 2009; 44:1-13.
15. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer. Proc Natl Acad Sci USA 1978; 75:4538-42.
16. Creagan ET, Moertel C. Vitamin C therapy of advanced cancer. N Engl J Med 1979; 301:1399-407.
17. 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. A randomized double-blind comparison. N Engl.J Med 1985; 312:137-41.

**18.** Chen Q, Espey MG, Krishna MC, Mitchell JB, Corpe CP, Buettner GR, Shacter E, Levine M. Pharmacologic ascorbic acid concentrations kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tisuues. Proc Natl Acad Sci USA 2005; 102:13604-9.

**19.** Chen Q, Espey MG, Sun A, Lee JH, Krishna MC, Shacter E, Choyke PL, Pooput C, Kirk KL, Buettner GR, Levine M. Ascorbate in pharmacological concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. Proc Natl Acad Sci USA 2007; 104:8749-54.

**20.** Chen Q, Espey MG, Sun AY, Pooput C, Kirk KL, Krishna MC, Khosh DB, Drisko J, Levine M. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. Proc Natl Acad Sci USA 2008; 105:11105-9.

**21.** Padayatty SJ, Levine M. Reevaluation of ascorbate in cancer treatment: emerging evidence, open minds and serendipity. J Am Coll Nutr 2000; 19:423-5.

**22.** Levine M, Padayatty SJ, Espey MG. Vitamin C: a concentration-function approach yields pharmacology and therapeutic discoveries. Adv Nutr 2011; 2:78-88.

**23.** Gonzalez MJ, Miranda-Massari JR, Mora EM, Jimenez IZ, Matos MI, Riordan HD, Casciari JJ, Riordan NH, Rodriguez M, Guzman A. Orthomolecular oncology: a mechanistic view of intravenous ascorbate's chemotherapeutic activity. P R Health Sci J 2002; 21:39-46.

**24.** 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. P R Health Sci J 2008; 27:7-15.

**25.** Hoffer LJ, Levine M, Assouline S, Melnychuck D, Padayatty SJ, Rosadiuk K, Rousseau C, Robitaille L. Miller WH Jr. Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. Ann Oncol 2008; 19:1969-74.

**26.** Ohno S, Ohno Y, Suzuki N, Soma G, Inoue M. High-dose vitamin C (ascorbic acid) therapy in the treatment of patients with advanced cancer. Anticancer Res 2009; 29:809-15.

**27.** Verrax J, Calderon PB. Pharmacologic concentrations of ascorbate are achieved by parenteral administration and exhibit antitumoral effects. Free Radic Biol Med 2009; 47:32-40.

**28.** Du J, Martin SM, Levine M, Wagner BA, Buettner GR, Wang SH, Taghiyev AF, Du C, Knudson CM, Cullen JJ. Mechanisms of ascorbate-induced cytotoxicity in pancreatic cancer. Clin Cancer Res 2010; 16:509-20

**29.** Du J, Cullen JJ, Buettner GR. Ascorbic acid chemistry, biology and the treatment of cancer. Biochim Biophys Acta 2012; 1826:443-57.

**30.** Bocci V. Is the ascorbate saline solution a possible substitute of ozonetherapy? In: Ozone: A New Medical Drug, 2nd edition, Springer, Dordrecht, Netherlands, pp 49-51, 2001.

**31.** Mendiratta S, Qu ZC, May JM. Erythrocyte ascorbate recycling: antioxidant effects in blood. Free Radic Biol Med 2008; 24:789-97.

**32.** Mendiratta S, Qu ZC, May JM. Enzyme-dependent ascorbate recycling in human erythrocytes: role of thioredoxin reductase. Free Radic Biol Med 1998; 25:221-8.

**33.** Frei B. On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction. Proc Soc Exp Biol Med 1999; 222:196-204.

**34.** Halliwell B. Vitamin C: poison, prophylactic or panacea? Trends Biochem Sci 1999; 24:255-9.

**35.** McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Radic Biol Med 1999; 26:1034-53.

**36.** Polidori MC, Mecocci P, Levine M, Frei B. Short-term and long-term vitamin C supplementation in humans dose-dependently increases the resistance of plasma to ex vivo lipid peroxidation. Arch Biochem Biophys 2004; 423:109-15.

**37.** Schultze HE, Heremans JF. Molecular Biology of Human Proteins. Elsevier, Amsterdam, Netherlands, Vol 1 pp 589-669, 1966.

**38.** Bocci V, Gammack DB. Some physicochemical properties of albumins isolated from human serum and cerebrospinal fluid. J Neurochem 1966; 13:875-8.

**39.** Mouithys-Mickalad A, Deby C, Deby-Dupont G, Lamy M. An electron spin resonance (ESR) study on the mechanism of ascorbyl radical production by metal-bindin proteins. Biometals 1998; 11:81-8.

**40.** Klenner FR. Virus pneumonia and its treatment with vitamin C. South Med Surg 1948; 110:36-8.

**41.** Siems W, Grune T. Intracellular metabolism of 4-hydroxynonenal. Mol Aspects Med 2003; 24:167-75.

**42.** Petersen DR, Doorn JA. Reactions of 4-hydroxynonenal with protein and cellular targets. Free Radic Biol Med 2004; 37:937-45.

**43.** Awasthi YC, Ansari GA, Awasthi S. Regulation of 4-hydroxynonenal mediated signalling by glutathione S-transferase. Methods Enzymol 2005; 401:379-407.

**44.** Laurent A, Alary J, Debrauwer L, Cavedi JP. Analysis in the rat of 4-hydroxynonenal metabolites excreted in bile: evidence of enterohepatic circulation of these byproducts of lipid peroxidation. Chem Res Toxicol 1999; 12:887-94.

**45.** Motohashi H, Yamamoto M. Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med 2004; 10:549-57.

**46.** Zhang DD. Mechanistic studies of the Nrf2-Keap1 signaling pathway. Drug Metab Rev 2006; 38:769-89.

**47.** Li W, Khor TO, Xu C, Shen G, Jeong WS, Yu S. Activation of Nrf2 antioxidant signalling attenuates NF kappa B-inflammatory response and elicits apaptosis. Biochem Pharmacol 2008; 76:1485-9.

**48.** Kwak MK, Kensler TW. Targeting Nrf2 signaling for cancer chemoprevention.Toxicol Appl Pharmacol 2010; 244:66-76.

**49.** Abraham NG, Kappas A. Pharmacological and clinical apects of heme oxygenase. Pharmacol Rev 2008; 60:79-127.

**50.** Itoh K, Chiba T, Takahashi S, Ishi T, Igarashi K, KatohY, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophysical Res Comm 1997; 236:313-22.

**51.** Sagai M, Bocci V. Mechanisms of action involved in ozone therapy: is healing induced via a mild oxidative stress? Med Gas Res 2011; 1:29.

**52.** Bocci V, Zanardi I, Perez Olmedo JC, Travagli V. A technically feasible treatment for peritoneal carcinomatosis. Int J Ozone Ther 2012; 11:85-9.

**53.** Mikirova N, Casciari J, Rogers A, Taylor P. Effects of high-dose intravenous vitamin C on inflammation in cancer patients. J Translat Med 2012; 10:189-99.

**54.** Block G, Jensen C.D, Dalvi T.B, Norkus E.P, Hudes M, Crawford PB, Holland N, Fung EB, Schumacher L, Harmatz P. Vitamin C treatment reduces elevated C-reactive protein. Free Radic Biol Med 2009; 46:70-7.




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