A rational approach for improving the ascorbate antineoplastic activity

AUTHOR QUERY SHEET

Author(s): V. Bocci, I. Zanardi, and V. Travagli

Article title: A Rational Approach for Improving the Ascorbate Antineoplastic Activity

Article no: 877477

Enclosures: 1) Query sheet
2) Article proofs

Dear Author,

Please check these proofs carefully. It is the responsibility of the corresponding author to check against the original manuscript and approve or amend these proofs. A second proof is not normally provided. Informa Healthcare cannot be held responsible for uncorrected errors, even if introduced during the composition process. The journal reserves the right to charge for excessive author alterations, or for changes requested after the proofing stage has concluded.

The following queries have arisen during the editing of your manuscript and are marked in the margins of the proofs. Unless advised otherwise, submit all corrections using the CATS online correction form. Once you have added all your corrections, please ensure you press the "Submit All Corrections" button.

AQ1 Please review the table of contributors below and confirm that last names are structured correctly and that the authors are listed in the correct order of contributions.

Contrib. No.	Given name(s)	Surname	Suffix
1	V.	Bocci	
2	I.	Zanardi	
3	V.	Travagli	

AQ2. Au: A declaration of interest statement reporting no conflict of interest has been inserted. Please confirm whether the statement is correct.

AQ3. Au: Please check and provide complete list of authors as per journal style in the reference list.

AQ4. Au: Please provide volume number and page range in references [2, 21].

AQ5. Au: Please provide end page number in references [8, 19, 28, 38, 39].

Cancer Investigation, 00:1–3, 2014
ISSN: 0735-7907 print / 1532-4192 online

DOI: 10.3109/07357907.2013.877477

informa
healthcare

COMMENTARY

A Rational Approach for Improving the Ascorbate Antineoplastic Activity

V. Bocci,[1] I. Zanardi,[2] andV. Travagli[2]

[1]*Dipartimento di Fisiologia,* [2]*Dipartimento di Biotecnologie, Chimica e Farmacia – Università degli Studi di Siena, Siena, Italy*

INTRODUCTION

There is no doubt today that pharmacological concentrations of ascorbate can be reached in the interstitial fluids after intravenous administration of ascorbate (50–110 g/m^2 per patient), while oral route fails to do so (1, 2). Consequently, 10–30 mM for 5–6 hr of ascorbate in interstitial fluids may generate cytotoxic levels of H_2O_2 in cancer-sensitive cells. However, even the combination of H_2O_2 with either chemotherapy (3) or oncothermia (4) remains unable to kill all cancer cells. There are several explanations for this failure and one is that the H_2O_2 concentration is too unstable. In order to make H_2O_2 more effective, the peritoneal route of high dose of ascorbic acid is proposed. Daily administration for at least 3 months may improve the outcome.

Gram doses of vitamin C administered orally had been proposed as a potential cancer therapy by Cameron and Pauling since 1976 (5). However, successive clinical trials performed in cancer patients treated with 10 g of once-a-day oral dosage of vitamin C showed no benefit (6). It is understandable why oral dosages of vitamin C do not yield high blood levels owing to a modest bioavailability and renal excretion so that the concentration of H_2O_2 at the levels of neoplastic cells are ineffective (7, 8).

Chen et al. deserved the merit to have clarified this problem: they have shown that, while the maximum tolerated oral dose of even 3 g every 4 hr yields a plasma vitamin C concentration of 220 μmol/L, a 50–100 g dose administered intravenously yields a minimal plasma level of 13,400 μmol/L (and up to 30 mmol/L), that is 61–150-fold higher (9–12). They also proposed that after intravenous administration of vitamin C, this compound, after transfer into the extravascular tissues, generates H_2O_2 and ascorbyl radicals (13). Consequently, the H_2O_2 levels becomes high enough to oxidize and possibly kill some neoplastic cells if these are not protected by catalase or by GSH peroxidase. Verrax and Calderon (14) have amply confirmed that oral and parenteral administration of ascorbate are not comparable, supporting Levine's *et al.* studies.

It is well established that H_2O_2 can exert a cytotoxic effect on either normal or particular neoplastic cells (15–17). In physiological conditions, normal cells produce most of the H_2O_2 into the mitochondria and this oxidant acts as an important signaling molecule that is quickly reduced to H_2O by antioxidants. In the case of neoplastic cells, H_2O_2 production is quite variable and some type of neoplasms can be killed by H_2O_2 produced outside the cell. Such a condition appears to happen when ascorbate, in the presence of a so far unknown protein fraction, probably within the alpha 2 globulins (ceruloplasmin or others globulins binding copper or iron) releases H_2O_2 and ascorbyl radicals in sufficient amount able to induce an irreversible oxidation of neoplastic cells (17–19). The H_2O_2 concentration has a paramount relevance and if it is below 10 μM it is usually not noxious, whereas concentrations from 40 μM to 1 mM cause growth arrest, apoptosis, and/or necrosis (20). H_2O_2 readily diffuse across aquaporin channels and, when inside the cell, it is rapidly degraded by scavenging enzymes such as catalase, GSH-peroxidase, or by peroxiredoxins. In normal cells, 80–90% of H_2O_2 is rapidly reduced, but what happens in the great variety of neoplastic cells remains uncertain. It is possible that some neoplastic cells, being unable to reduce intracellular H_2O_2, are killed and it seems that this is the case when extracellular H_2O_2 reaches a high concentration depending upon the ascorbate level in the neoplastic capillary environment. Recently, as a concomitant mechanism, it has been observed that ascorbate itself promotes lymphocyte development via an epigenetic arrangement giving an insight into the part of ascorbate-mediated enhancement of immune function (21). It appears obvious that at least some types of cancer must be sensitive to H_2O_2, but some species produce themselves H_2O_2 and have an antioxidant defence.

Such a composite situation emphasizes the importance to deliver not only a large dose of ascorbate (80–100 g) but above all to maintain a constant level of H_2O_2 in the neoplastic environment. It has been already proved that ascorbate acts as a prodrug and cannot generate H_2O_2 in whole blood (9). In any case, formation of H_2O_2 in plasma is promptly reduced. What is important therefore is the concentration of H_2O_2 and ascorbyl radical as inducer of peroxidation in the extracellular environment.

Owing to the so-far observed killing effect on some neoplastic cells, the crucial question is whether the intravenous

Correspondence to: Velio Bocci, MD, Emeritus Professor of Physiology, Università degli Studi di Siena, Viale Aldo Moro, 2, 53100 Siena, Italy, email: velio.bocci@unisi.it; or Valter Travagli, PharmD, Chief of the Post-graduate School of Hospital Pharmacy, Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Viale Aldo Moro, 2, 53100 Siena, Italy, email: valter.travagli@unisi.it

infusion of ascorbate is the best method to achieve an extracellular concentration of H_2O_2 as high or higher than 20 mM. The elimination of ascorbic acid is variable and dose-dependent because of its nonlinear pharmacokinetics. The half-life is obviously influenced by the rapid ascorbate transfer from blood to extravascular fluids, by renal filtration, several organs uptake and some degradation. In such a case the level of H_2O_2 in extracellular fluids raises quickly but remains sustained for a rather short time. The half-life of ascorbate in plasma of participants in a trial was approximately between 60 and 80 min (7), while in the culture medium it was of about 3 hr (22). Owing to the normal schedule of ascorbate administration every other day, a relevant problem is that a significant concentration of H_2O_2 among neoplastic cells last a too shorter time, while to be really effective it should remain at high levels for longer periods. An approximate calculation of the area under curve shows that high concentrations of H_2O_2 are brief, possibly unable to both kill all the neoplastic cells and prevent the insurgency of a resistance mechanism.

Is there a better administration route?

Between 1985 and 1992, evaluating the pharmacokinetic and pharmacodynamic of interferons, it was realized that the most proficient route of administration of these compounds was their administration via lymphatics (23–25). At that time, the subcutaneous route was selected because, by injecting interferons mixed with 60 mg human albumin in a 3 mL volume, it was possible to markedly enhance the absorption via cutaneous lymphatics. This so-called intralymphatic administration was found to decrease and delay the renal elimination of interferons, to enhance their biological activity and reduce general side effects. However, this route is not feasible in the case of ascorbate.

Due to the massive ascorbate dosages, a potential alternative to the intravenous administration is the use of the intraperitoneal route. In fact, the peritoneal membrane is a dynamic dialyzer, and the peritoneal cavity for at least five decades has been widely used in patients with a partial renal dysfunction (in such a case the patient becomes able to replace the peritoneal fluid a few times a day).

For the delivery of ascorbate solution, only a light and small intraperitoneal catheter appears necessary and the patient himself may learn how to replace the liquid under antiseptic technique for preventing peritoneal infection. The ascorbate solution must be prepared by the hospital pharmacists, according to validated standard operative procedures. Summarily, the scheduled amount of pure ascorbic acid should be solubilized in the adequate volume of water for injections, and the resulting hypertonic solution must be neutralized and sterilized to be used at once. Physiologically, the peritoneal cavity is almost virtual but it has a surface of not less than 1700 cm^2, hence 1 L of ascorbate, if homogeneously distributed, yields an almost imperceptible liquid layer. The liquid is absorbed mostly by great capacity lymphatics, which preferentially drain into the left thoracic duct ending into the angle of the junction of the left subclavian vein with the left internal jugular vein. Ascorbate will then mix with the blood pool and it will be redistributed into all organs. Renal loss of ascorbate will diminish and its half-life will be prolonged and the H_2O_2 levels in neoplastic organs, although at a slightly lower concentration then after IV infusion, will be sustained for longer time. In order to maintain a fairly constant and effective H_2O_2 levels in the neoplastic environment, the patient ought to perform one infusion daily. In order to ascertain the efficacy of this route, the peritoneal infusion should be continued for at least three months. This is possible as pharmacological levels of ascorbate are well-tolerated with the exception of renal impairment or glucose-6-phosphate dehydrogenase deficiency (26–28). Moreover, the H_2O_2 may prove to be far more effective in the case of peritoneal carcinomatosis as it occurs for ovarian cancer. This may avoid the need of extensive and traumatic peritonectomy and the use of toxic chemotherapy (29). Other abdominal tumors such as pancreatic cancer, liver, gastric, colon, and prostatic cancer may be usefully treated provided that the interstitial concentration of H_2O_2 is maintained for long periods at an effective concentration. By considering the pioneering and intensive efforts by Levine's group in showing the ascorbate efficacy in some neoplasms, it may be worthwhile to evaluate the intraperitoneal approach.

In conclusion, beside Levine and collaborators, several other groups have advocated the use of intravenous high dosages of ascorbate in neoplastic patients on the basis of the killing activity of H_2O_2 on tumoral cells (16, 30–33). Recently, the combination of ascorbate and chemotherapy has been evaluated (2, 34), but so far clinical results have been meagre. This may depend upon either the various sensitivity to H_2O_2 and malignancy of neoplastic cells or on their ability to become resistant during the discontinuous presence of H_2O_2 in the neoplastic environment (35). If this hypothesis is correct, the daily administration of ascorbate via intraperitoneal route for a long period may change the outlook that at the moment remains uncertain. However, whenever possible, it would be proficient to first evaluate the sensitivity of different neoplasms to H_2O_2 as well as the most effective combination with chemotherapeutic drugs (36, 37). So far, intravenous ascorbic acid appears able to reduce fatigue (38) and to prevent cancer-associated sepsis (39).

It is hoped that the oncologists may be interested in evaluating the intraperitoneal route and in any case we would be glad to help anyone else.

DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper. AQ2

The writing of this paper was not supported by any fund.

REFERENCES

1. 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 Chemother Pharmacol 2013;72:139–146. AQ3
2. Parrow NL, Leshin JA, Levine M. Parenteral ascorbate as a cancer therapeutic: A reassessment based on pharmacokinetics. Antioxid Redox Signal (in press). doi:10.1089/ars.2013.5372.

3. Welsh JL, Wagner BA, van't Erve TJ, et al. Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): Results from a phase I clinical trial. Cancer Chemother Pharmacol 2013;71:765–775.
4. Kovago C, Meggyeshazi N, Andocs G, Szasz A. Report of the pilot study done for the proposed investigation on the possible synergic effect between high-dose ascorbic acid application and oncothermia treatment. Conf Papers Med, vol. 2013, Article ID 386913, 2 pp. doi:10.1155/2013/386913
5. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. Proc Natl Acad Sci USA 1976;73:3685–3689.
6. Creagan ET, Moertel CG, O'Fallon JR et al. Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial. N Engl J Med 1979;301:687–690.
7. Levine M, Padayatty SJ, Espey MG. Vitamin C: A concentration-function approach yields pharmacology and therapeutic discoveries. Adv Nutr 2011;2:78–88.
8. Chen Q. Vitamin C in cancer treatment: Where pharmacokinetics speaks. J Drug Metab Toxicol 2012;3:e107.
9. Chen Q, Espey MG, Krishna MC et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues. Proc Natl Acad Sci USA 2005;102:13604–13609.
10. Chen Q, Espey MG, Sun AY et al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. Proc Natl Acad Sci USA 2007;104:8749–8754.
11. Chen Q, Espey MG, Sun AY et al. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. Proc Natl Acad Sci USA 2008;105: 11105–11109.
12. Hoffer LJ, Levine M, Assouline S et al. Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. Ann Oncol 2008; 19:1969–1974.
13. Levine M, Espey MG, Chen Q. Losing and finding a way at C: New promise for pharmacologic ascorbate in cancer treatment. Free Radic Biol Med 2009;47:27–29.
14. 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.
15. Davies KJ. The broad spectrum of responses to oxidants in proliferating cells: A new paradigm for oxidative stress. IUBMB Life 1999;48:41–47.
16. Symons MC, Rusakiewicz S, Rees RC et al. Hydrogen peroxide: A potent cytotoxic agent effective in causing cellular damage and used in the possible treatment for certain tumours. Med Hypotheses 2001;57:56–58.
17. Finkel T. Signal transduction by reactive oxygen species. J Cell Biol 2011;194:7–15.
18. Du J, Cullen JJ, Buettner GR. Ascorbic acid: Chemistry, biology and the treatment of cancer. Biochim Biophys Acta 2012;1826:443–457.
19. Carosio R, Zuccari G, Orienti I, et al. Sodium ascorbate induces apoptosis in neuroblastoma cell lines by interfering with iron uptake. Mol Cancer 2007;6:55. doi:10.1186/1476-4598-6-55.
20. Antunes F, Cadenas E. Cellular titration of apoptosis with steady state concentrations of H_2O_2: Submicromolar levels of H_2O_2 induce apoptosis through Fenton chemistry independent of the cellular thiol state. Free Radic Biol Med 2001;30:1008–1018.
21. Manning J, Mitchell B, Appadurai DA et al. Vitamin C promotes maturation of T-cells. Antioxid Redox Signal, in press (doi:10.1089/ars.2012.4988.)
22. Duarte TL, Almeida GM, Jones GD. Investigation of the role of extracellular H_2O_2 and transition metal ions in the genotoxic action of ascorbic acid in cell culture models. Toxicol Lett 2007;170:57–65.
23. Bocci V In Interferon, In vivo and clinical studies, vol. 4, NB Finter, R Oldham (eds.). (Amsterdam: Elsevier), 1985, 47–72.
24. Bocci V. Immunomodulators as local hormones: New insights regarding their clinical utilization. J Biol Response Mod 1985;4:340–352.
25. Bocci V. In Interferon. Principles and medical application, S Baron et al. (ed.). Galveston: UTMB Press, 417–425.
26. Pru C, Eaton J, Kjellstrand C. Vitamin C intoxication and hyperoxalemia in chronic hemodialysis patients. Nephron 1985;39:112–116.
27. Alkhunaizi AM, Chan L. Secondary oxalosis: A cause of delayed recovery of renal function in the setting of acute renal failure. J Am Soc Nephrol 1996;7:2320–2326.
28. Padayatty SJ, Sun AY, Chen Q et al. Vitamin C: Intravenous use by complementary and alternative medicine practitioners and adverse effects. PLoS One 2010;5:e11414.
29. Bocci V, Zanardi I, Pérez Olmedo JC et al. A technically feasible treatment for peritoneal carcinomatosis. Int J Ozone Ther 2012;11:85–89.
30. González MJ, Miranda-Massari JR, Mora EM et al. Orthomolecular oncology review: Ascorbic acid and cancer 25 years later. Integr Cancer Ther 2005;4:32–44.
31. Duconge J, Miranda-Massari JR, Gonzalez MJ et al. Pharmacokinetics of vitamin C: Insights into the oral and intravenous administration of ascorbate. P R Health Sci J 2008;27:7–19.
32. Ohno S, Ohno Y, Suzuki N et al. High-dose vitamin C (ascorbic acid) therapy in the treatment of patients with advanced cancer. Anticancer Res 2009;29:809–815.
33. Du J, Martin SM, Levine M et al. Mechanisms of ascorbate-induced cytotoxicity in pancreatic cancer. Clin Cancer Res 2010;16:509–520.
34. Monti DA, Mitchell E, Bazzan AJ et al. Phase I evaluation of intravenous ascorbic acid in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. PLoS One 2012;7:e29794.
35. Shatzer AN, Espey MG, Chavez M et al. Ascorbic acid kills Epstein-Barr virus positive Burkitt lymphoma cells and Epstein-Barr virus transformed B-cells in vitro, but not in vivo. Leuk Lymphoma 2013;54:1069–1078.
36. Pathi SS, Lei P, Sreevalsan S et al. Pharmacologic doses of ascorbic acid repress specificity protein (Sp) transcription factors and Sp-regulated genes in colon cancer cells. Nutr Cancer 2011;63:1133–1142.
37. Olney KE, Du J, van 't Erve TJ et al. Inhibitors of hydroperoxide metabolism enhance ascorbate-induced cytotoxicity. Free Radic Res 2013;47:154–163.
38. Suh SY, Bae WK, Ahn HY et al. Intravenous vitamin C administration reduces fatigue in office workers: A double-blind randomized controlled trial. Nutr J 2012;11:7. doi: 10.1186/1475-2891-11-7.
39. Ichim TE, Minev B, Braciak T et al. Intravenous ascorbic acid to prevent and treat cancer-associated sepsis? J Transl Med 2011;9:25. doi: 10.1186/1479-5876-9-25.