Pharmacology, Kinetics, Dosing and Safety of Intravenous Ascorbic Acid

Prepared as Data for Safe use of Ascorbic Acid in Human Research

As performed at Anderson Medical Specialty Associates in support of the Bastyr University Integrative Oncology Outcomes Study.

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As an adjunct to our previously published guidelines as well as our ongoing use of intravenous ascorbic acid in patients with cancer I have prepared this document with appropriate references to answer common questions which are both mechanistic and practical. The purpose of this document is to organize the available peer reviewed data regarding the use, monitoring and biochemistry of intravenously administered ascorbic acid as employed in a pro-oxidative manner. All of this with the intention being to synthesize often disparate and misunderstood data as this therapy is one of many integrative oncology therapies employed in our general clinic and with study participants.

What potential mechanisms would the administration of intravenous ASC employ to create pro-oxidant effects?

"Strikingly, ascorbate may also lead to pro-oxidant effects, especially through the reduction of transition metal ions such as iron and copper… These reactions between ascorbate and transition metals are thought to be responsible for the pro-oxidant and cytotoxic properties of ascorbate observed in vitro … Ascorbate is also known to induce the release of iron bound to ferritin or haemosiderin, which could take part in the lipid peroxidation process driven by the Fenton reaction … Transition metals are not the only compounds that react with ascorbate. Indeed, quinoid compounds can be reduced by ascorbate … leading to the generation of a semiquinone radical that is readily reoxidized by molecular oxygen…" [2]

The cell paradox: Does the ASC need to enter the tumor cell?

"Extracellular but not intracellular ascorbate mediated cell death, which occurred by apoptosis and pyknosis/necrosis. Cell death was independent of metal chelators and absolutely dependent on H(2)O(2) formation. Cell death from H(2)O(2) added to cells was identical to that found when H(2)O(2) was generated by ascorbate treatment… Taken together, these data indicate that ascorbate at concentrations achieved only by i.v. administration may be a pro-drug for formation of H(2)O(2), and that blood can be a delivery system of the pro-drug to tissues. These findings give plausibility to i.v. ascorbic acid in cancer treatment, and have unexpected implications for treatment of infections where H(2)O(2) may be beneficial." [6]

If ASC is a prodrug for H2O2 production and pro-oxidant surge where does this take place?

Hoffer et.al. reported in 2008 "… doses up to 1.5 g/kg have been injected i.v. to cancer patients [4] with minimal adverse effects. This protocol achieved plasma ascorbic acid concentrations>10 mM for more than 4 h, which is largely sufficient to induce cancer cell death in vitro." [2] "The data show that

pharmacologic ascorbate is a prodrug for preferential steady-state formation of Asc• and H2O2 in the extracellular space but not blood." [3] So the H2O2 surge created by ASC is primarily limited to the extracellular space which gives the H2O2 access to the cells.

What potential mechanisms can the H2O2 generated by the ASC (as well as the metabolism of the ASC itself) have in the damage and death of a cancer cell?

Pharmacologic ascorbic acid concentrations produce extracellular H2O2, which can diffuse into cells, deplete ATP in sensitive cells, and thereby cause cell death. ATP may be depleted by three mechanisms. (i) DNA damage induced by H2O2 activates PARP. Activated PARP catabolizes NAD+, thereby depleting substrate for NADH formation and consequent ATP synthesis. (ii) H2O2 is catabolized by concurrent oxidation of GSH to GSSG. To reduce GSSG back to GSH, GSH reductase utilizes NADPH, which is provided by the pentose shunt from glucose. Glucose used to reduce NADP+ to NADPH cannot be used for glycolysis or NADH production so that ATP generation is decreased. (iii) H2O2 may directly damage mitochondria, especially ATP synthase, so that ATP production falls. Some cancer cells rely primarily on glycolysis rather than on oxidative phosphorylation respiration for ATP production (the Warburg effect). Compared with oxidative phosphorylation, ATP generation by glycolysis is inefficient. In glycolysis-dependent cancer cells, decreased glycolysis may lower intracellular ATP. Cancer cells that are glycolysis-dependent may be particularly sensitive to pharmacologic ascorbic acid concentrations, compared with cells that use oxidative phosphorylation. [3]

As a note of caution watch the form of ASC being measured when reading the available literature, as the amount of plasma or ECF ASC required to create oxidation is different from the level of ascorbate radical generated:

With an infusion of 0.5 g/kg plasma ascorbate concentrations reached 8 mM with ascorbate radical concentrations reaching 250 nM (with the threshold for H2O2 production being 100 nM). "After i.v. injection, ascorbate (ASC) baseline concentrations of 50–100 micro M in blood and extracellular fluid increased to peaks of >8 mM… With i.v. administration of ascorbate, ascorbate radical (Asc•_) concentrations were as much as 12-fold greater in extracellular fluid compared to blood and approached 250 nM. Asc•_ concentrations of _100 nM in extracellular fluid were the threshold concentration for detectable production of H2O2." [3]

What is the safety profile in humans of intravenously administered ASC at levels which achieve these concentrations?

Hoffer et.al. reported in 2008 "In the first phase I trial published this year, doses up to 1.5 g/kg have been injected i.v. to cancer patients with minimal adverse effects." [4] In 2010 Padayatty et.al. reviewed over 50,000 administrations of intravenous ASC showing an excellent safety record with the only high grade adverse events being completely preventable by proper screening. [9] The results of our own human trials (some included in the NIH funded research into integrative oncology outcomes) initially showed an excellent safety profile in our 2010 report [7] as well as in our update in 2014 [8]. In our

experience with patients ranging from One year of age to 93 years of age this excellent safety profile with no high grade adverse events has been maintained [7,8].

Have any improvements in the protocol been made through this research to make the ASC more stable and as biochemically neutral as possible while it is infused into the patient?

Yes. In the earlier days of our human research we completed an interventional trial using the original oncology patients receiving intravenous ASC to answer the question "is it possible to alter the ASC IV formula so that the oxidant capacity is preserved and patient blood chemistry (specifically electrolytes) is as unaffected as possible?" After this trial completed we had over 400 data points including pre and post IV electrolyte levels. Our changes in the base IV ASC formula proved to be as neutral to electrolyte shifts in the patient before and after the IV as possible, while maintaining a stable solution that did not degrade the oxidant potential of the ASC. [10] These formulas were presented at a scientific meeting in 2012. [7]

What practically speaking is required as to dose of the infusion to reach at least 10 mM as described above[2] for over 4 hours? (In other words 10 mM (10,000 microM)is equal to what in SI laboratory measurement?):

Ascorbic acid (ASC) conversions from SI to Traditional (lab) units: [1]

ASC converted from mg/dl to micromolar (microM) = mg/dl X 56.78

ASC converted from microM to mg/dl = microM X 0.0176

[Calculation note: The above gives micromolar (mciroM) concentrations from mg/dL. After finding micromoles convert to millimolar (mM – the units most used in the ASC studies) by multiplying microM X 0.001.]

Therefore doses to yield the following ranges in plasma should be sufficient to meet the above criteria for H2O2 production and other actions of ASC:

ASC Plasma concentrations of 300 to 400 mg/dl = ASC [17.04 - 22.7 mMol/L] exceed the 10 mM oxidative threshold originally discussed [2] and should therefore create a stead state of H2O2 production in the extracellular space for an extended period of time.

And finally: Can the assessment of plasma ASC be done without sending the plasma sample to a lab?

Yes. In a study group comparing oncology patients, diabetic non-diabetic patients (all receiving intravenous ASC) plasma levels were compared to the glucometer assessment (below) which validated this simple assessment. [5] Using a glucometer the simple reading calculation of:

[Glucometer reading post IV] – [Baseline glucometer reading] = <u>Post IV ASC estimation</u>

References:

[1] http://www.ctdslab.co.uk/conversion.html

[2] Verrax J, Calderon PB. The controversial place of vitamin C in cancer treatment. Biochemical Pharmacology 76 (2008) 1644–1652

[3] Chen, et.al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo .www.pnas.org doi:10.1073:pnas.0702854104 PNAS, May 22, 2007. vol. 104. no. 21; 8749–8754

[4] Hoffer L, LevineM, Assouline S, Melnychuk D, Paddayatty S, Rosadiuk K, et al. Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. Ann Oncol 2008.

[5] Ma Y, Sullivan G, Schrick E, et.al. A Convenient Method for Measuring Blood Ascorbate Concentrations in Patients Receiving High-Dose Intravenous Ascorbate. Journal of the American College of Nutrition. Volume 32, Issue 3, pages 187-193. 2013 DOI:10.1080/07315724.2013.791167

[6] Chen Q, 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 U S A. 2005 Sep 20;102(38):13604-9. Epub 2005 Sep 12.

[7] Anderson P. “Intravenous Vitamin C in Naturopathic Oncology.” Scientific Presentation. Oncology Association of Naturopathic Physicians. Scottsdale, Arizona. 2012.

[8] Standish L, Cochran B, Anderson P, et.al. Can Integrative Oncology Extend Life in Advanced Disease? 10th International Conference of the Society for Integrative Oncology (SIO): Abstract 79. Presented October 21, 2013. (As reported in Medscape October 2014)

[9] Padayatty SJ, Sun AY, and Chen Q, et al. (2010) Vitamin C: Intravenous Use by Complementary and Alternative Medicine Practitioners and Adverse Effects. PLoS ONE 5(7): e11414:1-8. PMID: 20628650.

[10] Anderson P., Naydis E., Standish L. (2011, November). High Dose IV Ascorbic Acid Therapy: the Bastyr Experience. Poster session presented at the Society for Integrative Oncology, Cleveland, OH.

Appendix:

Unit Conversion

0.001 mM = 1 microM = 1000 nM