BioLogical NEWS

A Biological Therapies Newsletter.

Iron and Vitamin C

Introduction

There persists in medical literature the idea that vitamin C, acting as a reducing agent, causes reactions with iron (and other metals) that generate damaging free radicals. These free radicals then go on to produce oxidative damage to cells and molecules, the products of which can be measured in blood tests. The idea goes that vitamin C is a powerful reducing agent which reduces Fe (III) to Fe (II), the reduced iron then participates in the Fenton reaction which produces hydroxyl radicals. A considerable amount of literature can be found that links these ideas together, and the obvious conclusion reached by most of these researchers is a warning not to take vitamin C with metals and not to take vitamin C in iron overloaded conditions (e.g. haemochromatosis).

While these arguments on the surface are alarming and appear convincing, *they must of course stack up against clinical observations to be valid*. The fact is they don't.

The fact is that literally millions of people have taken or been administered very high doses of vitamin C, even considerable numbers of patients with iron overload disorders, and none of these people has ever "rusted". None of these patients have ever demonstrated damage to DNA or any other cells or tissues (except cancer cells) by any valid test. If you have any doubt about this, try and find cases in the medical literature.

What emerges from this is that the conclusions drawn by various researchers into the vitamin C/metal connection are largely based on flawed evidence. If a conclusion does not tie in with clinical observations, then there is something wrong. Without fail, the methods underpinning the "vitamin C/Fenton" paranoia are in vitro methods, i.e. isolated tissue cultures and blood samples exposed to the air. These methods inevitably produce results that are not necessarily related, if related at all, to in vivo conditions.

Correspondence to: Dr. Ian Dettman. PO Box 702, Braeside VIC 3195 Australia. +61 3 9587 3948
biol@biol.com.au

There is a considerable amount of in vivo research into this vitamin C/Fenton question. Without fail, in vivo research has NEVER been able to demonstrate that vitamin C is dangerous because of Fenton like reactions. Without fail, in vivo research ALWAYS demonstrates a positive effect of vitamin C on oxidative markers and damage in tissues.

Without fail, 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 – these are IN VITRO ARTEFACTS.

The real nail in the coffin for the Fenton idea is that almost every living higher organism on earth, plant or animal, makes its own vitamin C. Some animals and plants make enormous amounts of vitamin C (compared to what a human living in Western culture gets daily). These organisms have been exposed to metals right throughout evolution and they have not disappeared due to oxidative damage wrought by vitamin C.

There are enough clinical runs on the board for vitamin C to be able to make prescribing decisions based on prior clinical use. Positive clinical outcomes are just that – they cannot be "undone" by unfavourable press releases.

1. Linus Pauling Institute (and others)

The whole Vitamin C/Fenton in vitro/in vivo controversy has been studied in depth for many years now by several researchers, including those associated with the Linus Pauling Institute (LPI) in Oregon, headed by Professor Balz Frei. The issue of vitamin C acting as a damaging

agent in vivo due to Fenton effects has come up time and time again and repeatedly been debunked, yet it still persists. Biological Therapies published a newsletter on this issue in 2001[1], in relation to a paper finding that NAC and vitamin C caused muscle damage. This discussion contains correspondence between Dr Ian Dettman and Prof. Frei; Prof. Frei's comments are summarized here:

"One has to be extremely careful in the interpretation of these kinds of studies and critically evaluate experimental procedures, methodology, and ex vivo oxidation artefacts before jumping to conclusions (as so many authors do in this field). For example, once a plasma sample is extracted with acid or an organic solvent, iron may be released from its natural binding proteins (transferrin, ferritin, etc.), and together with exposure of the sample to ambient oxygen (20%), one can create all kinds of ex vivo artefacts. In addition, many authors use non-specific markers of lipid peroxidation, in particular TBARS, which can be derived from a variety of reactions, not just lipid peroxidation, and are easily formed ex vivo.

Literally millions of people have taken or been administered very high doses of vitamin C, even considerable numbers of patients with iron overload disorders, and none of these people has ever "rusted".

As you indicated, we have done quite a few studies on the possible interactions between vitamin C and iron under physiological conditions and in vivo, using specific and sensitive assays to measure lipid peroxidation. We always found anti-, rather than pro-oxidant effects of vitamin C under these conditions. I am currently working on another manuscript where we "pushed" the oxidation conditions even further, i.e., in addition to iron also added hydrogen peroxide to human plasma (the so-called complete Udenfriend system), and again found no pro-oxidant effects of ascorbate towards lipids as well as proteins under these conditions."

There are several well designed studies looking at the relationship between vitamin C, iron and in vivo oxidation. These studies have sought, as much as is possible, to control for ex vivo artefacts. A paper published in FASEB journal in 1999 by Carr and Frei[2] is an excellent review of the research into this question comparing in vitro, animal and human studies. "Vitamin C readily scavenges reactive oxygen and nitrogen species and may thereby prevent oxidative damage to important biological macromolecules such as DNA, lipids, and proteins. Vitamin C also reduces redox active transition metal ions in the active sites of specific biosynthetic enzymes. The interaction of vitamin C with 'free', catalytically active metal ions could contribute to oxidative damage through the production of hydroxyl and alkoxyl radicals; whether these mechanisms occur in vivo, however, is uncertain. To examine this issue, we reviewed studies that investigated the role of vitamin C, both in the presence and absence of metal ions, in oxidative DNA, lipid, and protein damage. *We found compelling evidence for antioxidant protection of lipids by vitamin C in biological fluids, animals, and humans, both with and without iron co supplementation.* Although the data on protein oxidation in humans are sparse and inconclusive, the available data in animals consistently show an antioxidant role of vitamin C. The data on vitamin C and DNA oxidation in vivo are inconsistent and conflicting, but some of the discrepancies can be explained by flaws in experimental design and methodology."

An experiment published in 1997 by Berger et al[3] looked at the antioxidant activity of vitamin C in iron overloaded plasma. "Vitamin C (ascorbic acid, AA) can act as an antioxidant or a pro-oxidant in vitro, depending on the absence or the presence, respectively, of redox-active metal ions. Some adults with iron-overload and some premature infants have potentially redox-active, bleomycin-detectable iron (BDI) in their plasma. *Thus, it has been hypothesized that the combination of AA and BDI causes oxidative damage in vivo.* We found that plasma of preterm infants contains high levels of AA and F2-isoprostanes, stable lipid peroxidation end products. However, F2-isoprostane levels were not different between those infants with BDI (138 +/- 51 pg/ml, n = 19) and those without (126 +/- 41 pg/ml, n = 10), and the same was true for protein carbonyls, a marker of protein oxidation (0.77 +/- 0.31 and 0.68 +/- 0.13 nmol/mg protein, respectively). Incubation of BDI-containing plasma from preterm infants did not result in detectable lipid hydroperoxide formation (</=10 nM cholesteryl ester hydroperoxides) *as long as AA concentrations remained high*. Furthermore, when excess iron was added to adult plasma, BDI became detectable, and endogenous AA was rapidly oxidized. *Despite this apparent interaction between excess iron and endogenous AA, there was no detectable lipid peroxidation as long as AA was present at >10% of its initial concentration. Finally, when iron was added to plasma devoid of AA, lipid hydroperoxides were formed immediately, whereas endogenous and exogenous AA delayed the onset of iron-induced lipid peroxidation in a dose-dependent manner. These findings demonstrate that in iron-overloaded plasma, AA acts an antioxidant toward lipids. Furthermore, our data do not support the hypothesis that the combination of high plasma concentrations of AA and BDI, or BDI alone, causes oxidative damage to lipids and proteins in vivo.*"

Another paper By Chen et al[4] looked again at this question in guinea pigs, and found that iron caused problems when there is a LOW ascorbate status. "Ascorbate is a strong antioxidant; however, it can also act as a prooxidant in vitro by reducing transition metals. To investigate the in vivo relevance of this prooxidant activity, we performed a study using guinea pigs fed high or low ascorbate doses with or without prior loading with iron dextran. Iron-loaded animals gained less weight and exhibited increased plasma beta-N-acetyl-D-glucosaminidase activity, a marker of tissue lysosomal membrane damage, compared with control animals. The iron-loaded animals fed the low ascorbate dose had decreased plasma alpha-tocopherol levels and increased plasma levels of triglycerides and F(2)-isoprostanes, specific and sensitive markers of in vivo lipid peroxidation. In contrast, the two groups of animals fed the high ascorbate dose had significantly lower hepatic F(2)-isoprostane levels than the groups fed the low ascorbate dose, irrespective of iron load. *These data indicate that 1) ascorbate acts as an antioxidant toward lipids in vivo, even in the presence of iron overload; 2) iron loading per se does not cause oxidative lipid damage but is associated with growth retardation and tissue damage, both of which are not affected by vitamin C; and 3) the combination of iron loading with a low ascorbate status causes additional pathophysiological changes, in particular, increased plasma triglycerides*."

Another paper (in vitro) published in 2003 by Suh et al[5] went even further and loaded tissues with hydrogen peroxide to increase the oxidative environment. This is the work referred to earlier in the letter from Prof. Frei. "The combination of ascorbate, transition metal ions, and hydrogen peroxide (H_2O_2) is an efficient hydroxyl radical generating system called "the Udenfriend system." Although the pro-oxidant role of ascorbate in this system has been well characterized in vitro, it is uncertain whether ascorbate also acts as a pro-oxidant under physiological conditions. To address this question, human plasma, used as a representative biological fluid, was either depleted of endogenous ascorbate with ascorbate oxidase, left untreated, or supplemented with 25 microM-1 mM ascorbate. Subsequently, the plasma samples were incubated at 37 degrees C with 50 microM-1 mM iron (from ferrous ammonium sulfate), 60 or 100 microM copper (from cupric sulfate), and/or 200 microM or 1 mM H_2O_2. Although endogenous and added ascorbate was depleted rapidly in the presence of transition metal ions and H_2O_2, no cholesterol ester hydroperoxides or malondialdehyde were formed, i.e., ascorbate protected against, rather than promoted, lipid peroxidation. Conversely, depletion of endogenous ascorbate was sufficient to cause lipid peroxidation, the rate and extent of which were enhanced by the addition of metal ions but not H_2O_2. Ascorbate also did not enhance protein oxidation in plasma exposed to metal ions and H_2O_2, as assessed by protein carbonyl formation and depletion of reduced thiols. Interestingly, neither the rate nor the extent of endogenous alpha-tocopherol oxidation in plasma was affected by any of the treatments. Our data show that even in the presence of redox-active iron or copper and H_2O_2, ascorbate acts as an antioxidant that prevents lipid peroxidation and does not promote protein oxidation in human plasma in vitro."

Another excellent review has been published by Fraga and Oteiza[6] in 2002, which looks at the relationship between iron toxicity and antioxidants. "Iron is an essential nutrient for the growth, development, and long-term survival of most organisms. High tissue iron concentrations have been associated with the development and progression of several pathological conditions, including certain cancers, liver and heart disease, diabetes, hormonal abnormalities, and immune system dysfunctions. In this review we discuss the relevance of iron toxicity on free radical-mediated tissue damage, and how iron interactions with nutrient antioxidants and other metals can affect the extent of oxidative damage to different biomolecules. *It can be concluded that the ingestion of antioxidant rich foods may prevent or delay primary and secondary effects associated with iron overload-related diseases*."

All of these papers show quite clearly that trouble brews when ascorbate is DEPLETED, and that its effect (at least on oxidation) is PROTECTIVE in the presence of metal ions, EVEN IN THE PRESENCE OF IRON OVERLOAD.

2. There are clinical trials of ascorbate in iron overload (in haemodialysis patients)

One problem that occurs commonly in haemodialysis treatment in iron overload disorders is a functional iron deficiency. This is due to erythropoietin resistance. A clinical trial by Gastadello et al[7] (and many others published since) has looked at this. "Haemodialysis patients with iron overload sometimes develop resistance to erythropoietin therapy due to 'functional iron deficiency'. It is known that this resistance may be overcome by iron supplementation; however, the latter could worsen haemosiderosis. Therefore, we treated four iron-overloaded haemodialysis patients who had developed relative resistance to erythropoietin (among whom three had features of 'functional iron deficiency') with ascorbic acid (500 mg intravenously after haemodialysis, 1-3 times a week). The erythropoietin doses were voluntarily kept unchanged during the study.

After a latency of 2-4 weeks, haematocrit and haemoglobin had increased respectively from 26.5 +/- 0.7 to 32.7 +/- 0.4 vol% and from 8.8 +/- 0.3 to 10.8 +/- 0.2 g/dl (means +/- SEM, P < 0.001). While serum ferritin remained unchanged, transferrin saturation increased from 27 +/- 7 to 54 +/- 12% (P < 0.05), suggesting that ascorbic acid supplementation had allowed mobilization of iron from tissue burdens. In one patient, haematocrit declined after withdrawal of vitamin C and increased again after rechallenge. Also, ascorbate supplementation was continued after the study in two patients and allowed the erythropoietin doses to be decreased, 8 and 11 weeks, respectively, after the start of the trial. When a control group of seven patients with normal iron status and without resistance to erythropoietin were challenged in the same manner with ascorbate, no elevation of haematocrit or transferrin saturation was noted. We conclude that ascorbate supplementation may circumvent resistance to erythropoietin that sometimes occurs in iron-overloaded patients, in particular, in the setting of 'functional iron deficiency"

The combination of iron loading with a low ascorbate status causes additional pathophysiological changes, in particular, increased plasma triglycerides.

3. Ascorbate and other antioxidants are depleted in "iron overload" disorders

A review by Chan et al[8] looks at the effects of various genetic iron overload disorders on antioxidants. "Sickle cell anemia, thalassemia, and glucose-6-phosphate-dehydrogenase deficiency are all hereditary disorders with higher potential for oxidative damage due to chronic redox imbalance in red cells that often results in clinical manifestation of mild to serve hemolysis in patients with these disorders. The release of hemoglobin during hemolysis and the subsequent therapeutic transfusion in some cases lead to systemic iron overloading that further potentiates the generation of ROS……The totality of the evidence suggests a notion of interdependence among antioxidants and that depletion of one will likely lead to a reduction of others. The electrons that fuel these recycling reactions are ultimately derived from the oxidation of foods and as such, the repairing pathways are closely linked to the overall energy status of an individual. It is equally clear that patients with these genetic defects suffer from chronic oxidative stress and have an altered redox state characterized by a gross depletion of antioxidant nutrients. Most of the clinical events in patients with these disorders were precipitated directly by severe antioxidant depletion resulting in inadequate protection."

An earlier paper by Chapman et al[9] describes ascorbate depletion in β-thalassaemia major and iron overload. "The incidence of ascorbic acid (AA) deficiency and its effect on serum ferritin concentration relative to body iron stores was studied in 61 unchelated patients with beta-thalassaemia major. Thirty-nine (64%) of patients had subnormal leukocyte ascorbate concentrations without clinical evidence of scurvy. The lowest leukocyte ascorbate concentrations tended to occur in the most transfused patients. No correlation was found between the units transfused and serum ferritin concentration in the AA-deficient patients but a close correlation (r = +0.82; p less than 0.005) existed for the AA-replete group. Similarly a close correlation (r = +0.77; p less than 0.005) was obtained between liver iron concentration and serum ferritin in AA-replete patients but only a weak correlation (r = +0.385; p less than 0.025) existed for the AA-deficient group. When AA-deficient patients were treated with ascorbic acid, serum iron and percentage saturation of iron binding capacity rose significantly; serum ferritin rose in 13 of 21 patients despite the simultaneous commencement of desferrioxamine therapy. In contrast all three measurements tended to fall in AA-replete patients with ascorbic acid and desferrioxamine therapy. Thus, AA deficiency is commonly present in beta-thalassaemia patients with iron overload and may give rise to inappropriate serum ferritin concentrations in relation to body iron stores."

4. Blood artefacts and the bleomycin backflip

It has been recognised recently that the standard bleomycin assay for detecting free iron is unreliable. New methods have been proposed, and a good review of this material has been published by Burkitt et al[10]. "In the presence of ferrous ions (Fe(2+)), the anti-tumour agent bleomycin will induce DNA degradation. Degradation of DNA into substances detectable by the thiobarbituric acid test has been used previously for the detection of iron in a form that is capable of catalysing the formation of the potentially harmful hydroxyl free radical…. Agreement between the two versions of the assay in the identification of plasma samples containing bleomycin-detectable iron was good, but agreement on the actual concentrations of such iron in the positive samples was poor. This discrepancy is believed to be due to interference with the thiobarbituric acid assay by plasma. *Consequently, it was not possible to obtain reliable estimates of free iron concentrations in plasma*

when using the conventional version of the bleomycin assay."

The credibility of results from studies using the bleomycin assay pre 2000 is highly questionable and as such these results can't be used as reasonable arguments about the effects of vitamin C on free iron.

As Prof. Frei has stated and as Dr. Dettman has repeatedly warned, EXTREME care must be taken when taking, storing and analyzing blood. The formation of ex vivo oxidation products and artefacts must be rigorously controlled and conclusions drawn from these types of studies must be considered in light of in vivo and clinical evidence.

It is blatantly obvious that in vitro tests (including blood tests) done on isolated or homogenised cells or DNA have little to do with in vivo situations. Almost ANYTHING will damage DNA and cell membranes in a test tube or on a slide, including vitamin C and exposure to air, rupture of cells and release of contents (including iron) and reagents used in tests.

5. What about all the other endogenous reducing agents?

The Fenton type literature seems to be, for some reason, preoccupied with vitamin C. There are many other extremely important antioxidants and redox pairs in biochemistry, all of which in vivo serve as reducing agents at some stage, such as GSH/GSSG, ALA/DHLA, NADP/NADPH, uric acid, cholesterol, vitamin E etc, some of which have stronger reducing potentials than vitamin C (i.e. stronger than the ascorbate/ascorbate radical pair). Many of the above agents, whether they are "stronger" than vitamin C or not also notably produce "undesirable results" in test tube studies. Reducing couples and systems are highly compartmentalised in biological systems, i.e. some are cytosolic, some are membrane bound etc. and in general there is not a great mish mash of chemical species banging into each other as occurs in isolated systems in typical in vitro situations. Biochemistry is *highly* compartmentalised and the concentration of substrates is tightly controlled by enzymes and transporters in living systems.

Vitamin C, if it is reducing a metal ion (e.g. Fe (III) to Fe (II)) is acting as a reducing agent. End of story. That metal ion is now an oxidising agent *by definition*. If Fenton type reactions do occur in cells then the hydroxyl radicals produced are immediately quenched by other vitamin C (ascorbate or ascorbate free radical) molecules, assuming the vitamin C molecules are there. This is the normal state of affairs in most cells. There is a reason why vitamin C is actively transported into cells against a concentration gradient – *it is largely to confer antioxidant protection to cells*. Vitamin C has a very high concentration in the brain (which uses 20% + of the body's energy) and the nucleus of cells (which of course contains DNA). This is no accident. Vitamin C is transported rapidly into cells as ascorbate (using SVCT transporters) or as the doubly oxidised dehydroascorbate (using GLUT glucose transporters). *Once inside cells, vitamin C is actively defended by enzymatic systems designed to reduce it and return it to its ascorbate form.* Reduced vitamin C is in MUCH higher concentration than other reducing agents and as such is typically immediately available to quench hydroxyl radicals (and other radicals). Giving vitamin C as a supplement or therapy supports these tissue concentrations and systems and is essentially similar to taking the large amounts of vitamin C found in foods in the wild (pre-agriculture and especially pre-processing).

The is fact that, almost without exception, most higher animals and plants manufacture reducing agents in *large* amounts, especially vitamin C, and that *depletion* of these substances has again and again been associated with clinical manifestations of oxidative damage and disease.

References

[1] Biological News, Vitamin C does NOT cause muscle damage. Dec 05, 2001. www.biologicaltherapies.com

[2] Carr A, Frei B. Does vitamin C act as a pro-oxidant under physiological conditions? FASEB J. 1999 Jun;13(9):1007-24. Review.

[3] Berger TM, Polidori MC, Dabbagh A, Evans PJ, Halliwell B, Morrow JD, Roberts LJ 2nd, Frei B. Antioxidant activity of vitamin C in iron-overloaded human plasma. J Biol Chem. 1997 Jun 20;272(25):15656-60.

[4] Chen K, Suh J, Carr AC, Morrow JD, Zeind J, Frei B. Vitamin C suppresses oxidative lipid damage in vivo, even in the presence of iron overload. Am J Physiol Endocrinol Metab. 2000 Dec;279(6):E1406-12.

[5] Suh J, Zhu BZ, Frei B. Ascorbate does not act as a pro-oxidant towards lipids and proteins in human plasma exposed to redox-active transition metal ions and hydrogen peroxide. Free Radic Biol Med. 2003 May 15;34(10):1306-14.

[6] Fraga CG, Oteiza PI. Iron toxicity and antioxidant nutrients. Toxicology. 2002 Oct 30;180(1):23-32. Review.

[7] Gastaldello K, Vereerstraeten A, Nzame-Nze T, Vanherweghem JL, Tielemans C. Resistance to erythropoietin in iron-overloaded haemodialysis patients can be overcome by ascorbic acid administration. Nephrol Dial Transplant. 1995;10 Suppl 6:44-7.
[8] Chan AC, Chow CK, Chiu D. Interaction of antioxidants and their implication in genetic anemia. Proc Soc Exp Biol Med. 1999 Dec;222(3):274-82. Review.
[9] Chapman RW, Hussain MA, Gorman A, Laulicht M, Politis D, Flynn DM, Sherlock S, Hoffbrand AV. Effect of ascorbic acid deficiency on serum ferritin concentration in patients with beta-thalassaemia major and iron overload. J Clin Pathol. 1982 May;35(5):487-91.
[10] Burkitt MJ, Milne L, Raafat A. A simple, highly sensitive and improved method for the measurement of bleomycin-detectable iron: the 'catalytic iron index' and its value in the assessment of iron status in haemochromatosis. Clin Sci (Lond). 2001 Mar;100(3):239-47.

Notes

5, 20-30 Malcolm Rd. Braeside, VIC, 3195. Australia www.biologicaltherapies.com biol@biol.com.au 61 3 9587 3948

Notes

Biological Therapies
A division of Orthomolecular Medisearch Laboratories Pty Ltd. ACN 006 897 856
Manufacturers and distributors of oral and parenteral nutritional supplements. Suite 5, 20-30 Malcolm Rd. Braeside, VIC, 3195. Australia. Phone Int: 61 3 9587 3948
Fax 61 3 9587 1720 biol@biol.com.au