The Trials and Tribulations of Vitamin C

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Introduction

Ascorbic acid is a small water-soluble molecule that functions as a vitamin in a few species, including guinea pigs, humans, and other primates. Although citrus fruits were recognized as a dietary imperative to prevent scurvy during sea voyages of the 18th century, the anti-scorbutic factor, vitamin C, was not isolated until 1928, when Albert Szent-Györgyi extracted vitamin C from animal adrenal glands. The chemical structure of vitamin C, which has the formula $C_6H_8O_6$, was determined by Haworth and Hirst in 1933.[1] Vitamin C functions as an electron donor for a number of enzymes, including those involved in the synthesis of collagen, carnitine, hormones, and neurotransmitters. Vitamin C also enhances the detoxification of xenobiotics and carcinogens by cytochrome P450 enzymes, may be required for immunocompetence, and is involved in the conversion of cholesterol to bile acids. Vitamin C is the most effective aqueous antioxidant in plasma,[2] scavenging reactive oxygen species and reactive nitrogen species.[3,4]

The Media and the Safety of Vitamin C

In the last few years, a number of reports have appeared in the scientific literature that raised concerns about the safety of vitamin C. Despite the many published safety reviews that have found few, if any, problems associated with the intake of supplemental vitamin C.[5-8] the media have unduly emphasized this spate of negative reports without examining them in the context of the accumulated scientific literature. The public has been left with uncertainty about the safety of the routine use of large doses of vitamin C for prophylactic or therapeutic purposes.

Recently, the news media mischaracterized the information on vitamin C in the report by the British Expert Group on Vitamins and Minerals (EVM) on the safe upper levels for vitamins and minerals.[9] For instance, on May 7, 2003, the BBC stated that "people who take large doses of certain vitamins and minerals risk permanently damaging their health, a government watchdog has warned." The article continued, "Experts from the UK's Food Standards Agency say high levels of minerals like beta-carotene [sic] and zinc over a long period may have irreversible harmful effects...In addition, they reiterated warnings that high doses of vitamin C, calcium and iron can harm health but said long-term damage can be avoided if people stop taking them." The following day, the BBC reported that "The FSA is also warning that too much vitamin C, calcium and iron in supplement form may be damaging." An examination of the EVM report reveals that no such strong warnings on vitamin C were issued. The EVM report stated that "The available data suggest that vitamin C is not associated with significant adverse effects and there are no obvious specific key toxic endpoints for vitamin C dose given orally to healthy subjects." The report continued, "There are insufficient data to set a Safe Upper Level for vitamin C. The vitamin may be of low toxicity, though adverse effects, in particular on the gastrointestinal system, may occur in subjects consuming quantities of vitamin C greater than 1000 mg/day... It should be noted that higher levels of vitamin C may be without adverse effects in many individuals." This viewpoint is concordant with that expressed by the U.S. Institute of Medicine in 2000, which could not identify any serious side effects of vitamin C and set the tolerable upper intake level (UL) at 2,000 mg/day based solely on the possible laxative effect of vitamin C at larger doses, a side effect easily rectified by

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decreasing the dose.[7] The EVM report concluded with the speculation that subjects with hemochromatosis, thalassemia, or disordered gastrointestinal function may be harmed by large doses of vitamin C.

Johnston evaluated biomarkers for setting a UL for vitamin C in 1999.[10] After carefully reviewing the potential adverse effects of large doses of vitamin C on systemic conditioning, kidney stone formation, pro-oxidant activity, iron overload, hemolysis in subjects with glucose-6-phosphate dehydrogenase deficiency, and destruction of vitamin B_{12}, Johnston concluded that "the available data indicate that oral intakes of very high amounts of vitamin C (2-4 g/day) are well tolerated biologically. Presently, strong scientific evidence to define and defend a UL for vitamin C is not available."

A recurrent concern about supplemental vitamin C is its putative role in kidney stone formation. This has been very effectively addressed by two large-scale prospective studies that investigated the association between vitamin C intake and kidney stone formation.[11,12] The first cohort of 45,251 men aged 40-75 years with no history of kidney stones was followed for 6 years. There was no increased risk for stone formation in men with the highest daily intake of vitamin C (≥1,500 mg) compared to those with the lowest daily intake (<250 mg). The second cohort of 85,557 women aged 34-59 years with no history of kidney stones was followed for 14 years. Again, there was no significant difference in stone formation between those women with the highest daily intake of vitamin C (≥1,500 mg) and those with the lowest daily intake (<250 mg).

The U. S. Preventive Services Task Force published its recommendations on the prevention of CVD and cancer with antioxidant vitamins in the *Annals of Internal Medicine* on July 1, 2003,[13,14] which generated more interest in the media. The Task Force reviewed relevant English language papers in the Cochrane Controlled Trials Registry and MEDLINE published between 1966 and 2001 and selected 38 articles for analysis that met certain criteria. This report reviewed the evidence for vitamin C and noted that no primary prevention trials for vitamin C supplementation alone on cancer or CVD have been published and that the observational studies have not revealed impressive and consistent protective effects for vitamin C. However, the Task Force acknowledged that "with the exception of vitamins for which there is compelling evidence of net harm (for example, ß-carotene supplementation in smokers), there is little reason to discourage people from taking vitamin supplements." While the safety of vitamin C was not a focus of the Task Force report, it should be noted that no toxicity associated with vitamin C intake was reported in the analyzed studies.

The Food and Nutrition Board of the United States Institute of Medicine issued its authoritative *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* in 2000.[7] Since this publication influences public health policy and food industry initiatives, it is a carefully and conservatively drawn study that comprehensively evaluated the major safety issues of these antioxidants. In establishing the tolerable upper intake level (UL) for vitamin C, the panel assessed putative adverse effects, including gastrointestinal problems, increased oxalate and uric acid excretion, excessive iron absorption, the destruction of vitamin B_{12}, systemic conditioning (rebound scurvy), prooxidant effects, and others. No adverse effects of large doses of vitamin C were reliably substantiated and, as previously mentioned, the UL was set at 2,000 mg/day based solely on the potential laxative effect of larger doses in some people. Of other published safety reviews, none has identified a significant problem associated with supplemental vitamin C. Therefore, consensus about the safety of vitamin C has remained unaltered for many years. Indeed, if supplemental vitamin C is harmful, clear indications would have emerged by now, since thousands of people have been consuming large doses for decades.

Infectious Diseases

A wealth of clinical evidence supporting the efficacy of vitamin C in treating disease, especially infectious diseases like polio and the common cold, emerged in the 1940s and 1950s before the advent of randomized, placebo-controlled, double-blind methodology, which has become the gold standard of clinical trials. Because this clinical literature consists mainly of anecdotal reports and non-randomized, non-controlled studies and is not retrievable by searching standard electronic databases, such as MEDLINE, many clinicians and scientists are unaware, or unconvinced, of the therapeutic value of large doses of vitamin C. Pauling[15, 16] Stone,[17] Werbach,[18] Hoffer,[19] Sheffrey,[20] and Levy[21] have provided access to this historical evidence and excellent bibliographic references in their respective books and papers. When Linus Pauling published *Vitamin C and the Common Cold* in 1970, a seminal book that introduced the concept of the optimal intake of vitamin C for better health to much of the public, only four clinical studies on the common cold that used one or more grams of supplemental vitamin C per day had been published.[15] Hemilä reviewed the 21 placebo-controlled clinical trials of vitamin C and the common cold that used at least one gram of vitamin C per day published between 1971 and 1994.[22] In these studies, vitamin C reduced the duration of the cold and ameliorated symptoms by an average of 23%, although there was no consistent effect on incidence. Mechanistically, it remains undetermined if the beneficial effects are mainly due to vitamin C's immunomodulatory function, antihistamine function, antiviral activity, or a combination of these. More recently, Hemila reviewed the published clinical studies on vitamin C and other infectious diseases, such as pneumonia, tuberculosis, bronchitis, hepatitis, rubella, and herpes.[23] Many of these studies reported benefits from supplemental vitamin C but suffered from methodological flaws, such as too few subjects to achieve statistical power or relatively low doses of vitamin C. Nevertheless, when combined with case reports, anecdotal reports, and the decades of clinical experience of physicians in this field, such as Klenner[24] and Cathcart,[25] these studies provide a basis for the plausibility of therapeutic efficacy. Additionally, an antiviral activity of high concentrations of vitamin C against HIV in vitro has been reported.[26] Using T-lymphocytes infected with HIV, Harakeh et al found a dose-dependent suppression of HIV replication at noncytotoxic vitamin C concentrations of 50-150 μg/ml, which are physiologically attainable by oral or intravenous administration.

Pro-oxidant Effects?

The first of the recent papers to raise alarms about vitamin C was published by Podmore et al in 1998, who claimed that modest supplementation with 500 mg/day of vitamin C exerted pro-oxidant effects and may cause genetic damage in humans.[27] Podmore's group used gas chromatography-mass spectrometry to measure 8-oxoadenine and 8-oxoguanine, markers of DNA damage, in lymphocytes from the blood of 30 healthy volunteers who were given a placebo for six weeks, 500 mg/day of vitamin C for the next six weeks, and neither the placebo nor vitamin C for the following seven weeks. Levels of 8-oxoadenine increased during vitamin C supplementation period, while levels of 8-oxoguanine, which is an order of magnitude more mutagenic than 8-oxoadenine, decreased. This paradox raised important questions about the methodology, i. e. the results may have been due merely to artifactual oxidation of the samples during processing and, therefore, not reflective of the effect of vitamin C on lymphocyte DNA. Indeed, others have reported that levels of 8-oxoguanine determined by more sensitive assays that eliminate ex vivo artifacts are actually about two orders of magnitude less than those reported by Podmore. The Eu-

ropean Standards Committee on Oxidative DNA Damage compared various analytical assays used to measure markers of oxidative DNA damage and concluded that there is very substantial quantitative variation in measurements between methods and laboratories.[28] Additionally, Levine demonstrated that the maximal level of vitamin C in human lymphocytes is about 3.5-4 mM, which can be achieved with a daily intake of as little as 100-400 mg of vitamin C.[29,30] Therefore, increasing the intake to 500 mg/day should have no discernable effect on the vitamin concentrations in lymphocytes above what can be achieved dietarily. One recent study demonstrated a decrease in micronuclei, markers of DNA damage, in human lympho-cytes from subjects supplemented with 1,000 mg/day of vitamin C for one week followed by co-supplementation with 1,000 mg/day of vitamin C and 335.5 mg/day of vitamin E for one week.[31] Another recent study found significant decreases in 8-oxoguanine and 5-OH Me uracil in white blood cells from subjects supplemented with 260 mg/day of vitamin C for six weeks, although there were no significant changes in other markers of oxidative DNA damage.[32]

A subsequent report on the pro-oxidant effects of vitamin C was published in *Science* in 2001.[33] In this in vitro study, vitamin C was found to react with lipid hydro- peroxides to form genotoxic compounds, including 4,5-epoxy-2(*E*)-decenal, a precursor of etheno-2'-deoxyadenosine. Although the concentration of vitamin C used in these in experiments was physiological (25 µM-2 mM), the concentration of lipid hydroperoxide was about four orders of magnitude greater than its physiological concentration in plasma. Vitamin C is the most powerful antioxidant in plasma[2] and prevents the formation of lipid hydroperoxides *in vivo* by direct and indirect mechanisms, such as recycling vitamin E. Furthermore, the reaction rate of glutathione peroxidase with lipid hydroperoxides is much faster than the reaction rate between vitamin C and lipid hydroperoxides, so the 2-hour incubation period reported in *Science* would provide ample time for the decomposition of lipid hydroperoxides by glutathione peroxidase in plasma.[34,35]

While the pro-oxidant function of vitamin C has been classically demonstrated in vitro whereby vitamin C reduces transition metal ions like Fe^{3+} and Cu^{2+}, which then, in the presence of hydrogen peroxide, generate the hydroxyl radical, the putative pro-oxidant activity of vitamin C *in vivo* has been controversial. Indeed, recent evidence suggests that vitamin C acts as a physiological antioxidant even in the presence of free metal ions. In an ex vivo experiment, Suh et al. showed that ascorbate inhibited lipid oxidation in human plasma treated with ferrous ammonium sulfate or cupric sulfate and H_20_2 and that ascorbate did not promote protein oxidation in plasma treated with metal ions, as determined by protein carbonyl formation.[36] In an in vivo study, Chen et al showed that supplemental vitamin C given to guinea pigs injected with iron to produce iron overload actually inhibited the formation of liver and plasma F_2-isoprostanes, sensitive markers of lipid oxidation.[37] Proteggente et al. showed that co-supplementation with 260 mg/day of vitamin C and 14 mg/day of iron for six weeks in 20 healthy subjects did not significantly affect most markers of DNA damage in white blood cells.[32] Given these results, the concerns about the possible pro-oxidant activity of vitamin C in vivo seem unwarranted.

Atherosclerosis

Another media attack on vitamin C occurred in 2000, when it was reported that Dwyer et al had demonstrated in a study of 573 subjects aged 40-60 years that the use of vitamin C supplements for at least one year was associated with an increased thickening of the carotid artery wall.[38] Dwyer's study was not published in a peer-reviewed journal, so its methods and data were not subject to critical examination by other experts. However,

there are several apparent limitations. First, vitamin C intake was estimated by recall and food frequency questionnaires, often used in epidemiological studies but notoriously imprecise. Second, the carotid artery wall measurements were made using high resolution B-mode ultrasound, which is a very sensitive but difficult technique. Perhaps the biggest problem, however, was the failure of the media to compare Dwyer's results with those of other studies that addressed the relationship between vitamin C and atherosclerosis. For example, a well-controlled, peer-reviewed study of 11,307 subjects, using methodology similar to that of the Dwyer study and published five years earlier, found that men and women aged 55-64 years in the highest quintile of daily vitamin C intake (upper cutpoints: 982 mg for men and 728 mg for women) had reduced carotid artery wall thickness compared to those in the lowest quintile of daily vitamin C intake (upper cutpoints: 56.4 mg for men and 64.2 mg for women).[39] More recently, a randomized trial in 40 cardiac-transplant patients found that one-year supplementation with 1,000 mg/day of vitamin C and 800 IU/day of vitamin E very significantly retarded the progression of arteriosclerosis, as measured by intimal index, compared to placebo-treated patients.[40] Additionally, a randomized, placebo-controlled six-year intervention trial with 440 hypercholesterolemic men and women aged 45-69 years found that supplementation with 272 IU/day of vitamin E and 500 mg/day of slow-release vitamin C significantly retarded atherosclerotic progression in men by 37% compared to the placebo group, as assessed by ultrasonographic measurement of carotid artery intima-media thickness.[41] There were no significant differences in adverse events between the placebo and supplemented groups. The initial three-year trial found a trend toward protection in men taking only vitamin C supplements, but it was not statistically significant.[42] In a secondary prevention trial, an antioxidant cocktail consisting of 800 IU of vitamin E, 1,000 mg of vitamin C, 25 mg of beta-carotene, and 100 μg of selenium given daily for 3 years to patients with confirmed coronary disease retarded proximal artery stenosis compared to placebo, but also blunted the effectiveness of a combination of simvistatin and niacin in decreasing clinical events.[43] The independent effect of vitamin C was not ascertained.

Dwyer's study is the only one among many epidemiological studies designed to assess the effect of vitamin C on cardiovascular disease to find a harmful effect. If Dwyer's results are valid, it follows that adhering to the ubiquitous recommendation to consume a diet with abundant fruit and vegetables, which may provide an intake of 500 mg/day of vitamin C, would promote atherosclerosis. A recently published epidemiological study, discussed below, confirms that a high intake of vitamin C from supplements significantly reduces the risk of CHD in women.[44]

Relatively few studies have investigated the association between vitamin C and C-reactive protein (CRP), a marker of inflammation that has emerged as a strong predictor of clinical events, e.g. myocardial infarction and stroke, in CVD patients. One study of 85 patients with peripheral arterial disease (PAD) found low serum vitamin C levels in PAD patients (27.8 μM/L) compared to 113 healthy subjects (51.7 μM/L).[45] Serum vitamin C levels in PAD patients were also inversely correlated with serum CRP levels (r=-0.742). However, an intervention study with healthy men in the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) trial found no effect on CRP levels of daily supplementation with 500 mg of vitamin C and 272 IU of vitamin E for 3 years.[46]

More Evidence

Epidemiological and biochemical studies have suggested that diets rich in vitamin C offer some protection against heart disease and cancer. A comprehensive review by Carr and Frei in 1999 examined the published evidence on the prophylactic

functions of vitamin C on lipid oxidation, oxidative DNA damage, cardiovascular disease, cancer, and cataract in humans, as well as the effect of supplemental vitamin C on endothelium-dependent vasodilation.[47] Of the twelve in vivo studies on lipid oxidation in which vitamin C was the only supplement given (60-6,000 mg/day), only one showed an increase in lipid oxidation as measured by thio-barbituric acid-reactive substrates (TBARS) in plasma. Six studies showed no change in TBARS, conjugated dienes (CD), or F_2-isoprostanes, and seven demonstrated decreased lipid oxidation as measured by TBARS, CD, and/or malonaldehyde.

Of seven in vivo studies on oxidative DNA damage assessed by determination of oxidatively modified DNA bases by HPLC with electrochemical detection, gas chromatography-mass spectrometry, or the comet assay, five showed a significant decrease in one or more markers of oxidative DNA damage in subjects supplemented with vitamin C (60-1,000 mg/day).

Of twelve prospective cohort studies on CVD risk in which the actual intake of vitamin C was determined, seven showed an inverse association between vitamin C intake and CVD risk. Vitamin C intake was not found in any study to be associated with an increased risk of CVD. In most studies that measured plasma concentrations of vitamin C, a reduced risk of CVD was associated with higher plasma concentrations. Recently, Osganian et al reported the results of a prospective study of the relationship between vitamin C intake and CHD in 85,118 female nurses followed for 16 years.[44] Confirming the earlier NHANES I study that reported decreases in CVD mortality of 42% and 25% in men and women, respectively, who consumed about 300 mg/day of vitamin C from diet and supplements,[48] Osganian et al. found that the adjusted risk for CHD was reduced by 28% in women who consumed vitamin C supplements, with the greatest risk reduction in those taking at least 400 mg/day of vitamin C. Somewhat surprisingly, there was no consistent difference in CHD risk related to the duration of supplementation, except for a period of less than two years, in which no risk reduction was observed.

Of the fourteen prospective cohort studies of vitamin C and cancer in which dietary intakes of vitamin C were stated, four reported significant risk reductions in subjects whose intake was ≥80 mg/day. In those studies in which the lowest quantile of vitamin C intake was greater than 86 mg/day, no significant risk reductions were observed. Carr and Frei speculated that this may be due to tissue saturation at an intake of 80-110 mg/day with no further benefit expected above these values, which result in plasma concentrations of about 50 μM/L or more.

Interestingly, higher intakes of vitamin C in two studies (>300 mg/day or >490 mg/day) were strongly associated with protection against cataract. In one study of 50,828 women, long-term supplementation (≥10 years) with vitamin C was also associated with a 45% reduced risk of cataracts, whereas shorter duration of vitamin C use was not. More recently, the ARED study of 4,757 subjects reported no difference in age-related cataract incidence or progression in those taking a daily antioxidant cocktail containing 500 mg of vitamin C, 400 IU of vitamin E, 15 mg of beta-carotene, and with or without 80 mg of zinc oxide and 2 mg of cupric oxide for an average of 6.3 years compared to the placebo group.[49] However another arm of the ARED study with 3,640 subjects found that the supplemented group had a significantly reduced risk for the development of advanced age-related macular degeneration.[50]

Finally, Carr and Frei reviewed the effect of vitamin C given orally (500 or 2,000 mg) or by intraarterial infusion (10-25 mg/min, 1,000 mg or 3,000 mg) on vasodilation. All twelve of the clinical studies reported significantly improved vasodilation following vitamin C administration, probably due

to the stabilization of tetrahydrobiopterin, which increases the synthesis of nitric oxide in endothelial cells. Improved vasodilation following vitamin C supplementation may also be useful in preventing some of the complications of diabetes. In a number of intervention studies, supplemental vitamin C in doses of 500 mg or 1,000 mg has also been found to significantly lower systolic and/or diastolic blood pressure in borderline hypertensive subjects,[51-54] although one study of patients with Type II diabetes did not find any effect of 1,500 mg/day of vitamin C on blood pressure or endothelial function.[55] One non-randomized depletion/repletion study of 68 healthy subjects, including 4 hypertensives, found that plasma vitamin C was inversely associated with diastolic blood pressure.[56] Subjects in this study were given a relatively small daily dose of vitamin C (117 mg) for one month.

Pauling reported three case studies in which the provision of 3-6 grams/day each of L-lysine and vitamin C rapidly ameliorated severe exercise-induced angina pectoris in patients with severe coronary artery disease.[57,58,59] While L-lysine may have exerted effects unrelated to vasodilation, such as binding to Lp(a) as hypothesized by Pauling, some of the observed benefit may have been due to the vasodilatory effects of vitamin C.

There has been speculation about the utility of antioxidant vitamins used adjunctively in surgery. In a randomized, prospective study of 595 subjects, Nathens et al recently reported that the risk of pulmonary morbidity, organ failure and length of ICU stay were substantially reduced in surgical trauma patients who received 1,000 IU of vitamin E and 1,000 mg of intravenous vitamin C per day for the duration of admission to the ICU or 28 days, compared to similar patients not receiving the supplements.[60]

Cancer

In 1999, a paper on the accumulation of vitamin C in cancer cells raised speculation about the possible harmful effects of supplemental vitamin C in cancer patients. Agus et al. reported that dehydroascorbic acid (DHA), possibly formed pericellularly by the oxidative burst of neighboring activated neutrophils,[61] is taken up by human xenograft tumor cells in athymic mice by the facilitative glucose transporters (GLUTs) and is then reduced to ascorbic acid, which is trapped intracellularly and accumulates.[62] The investigators speculated that the high intracellular vitamin C concentration might be exploited by cancer cells. The import of these observations is questionable, since DHA is virtually undetectable in the plasma of healthy humans and may be out-competed for cellular transport by glucose.[63] Other research has shown that vitamin C, but not DHA, enters cells through two sodium-dependent vitamin C transporters with different tissue distribution, SVCT1 (present in epithelial cells of the intestine, kidney, and liver), whose efficiency appears to be age-related[64] and SVCT2 (found in a wide range of tissues and organs, including the eye and brain).[65,66] In 1997, Agus et al. showed that DHA crosses the blood-brain barrier in rats and mice, a process mainly mediated by facilitative glucose transporters.[67] In these experiments, 14C-labelled DHA and ascorbic acid were injected into the tail vein of animals. DHA was found in the brain after a short period, and ascorbic acid appeared after 30 minutes. Again, the physiological relevance of these observations is questionable, since DHA is not normally detectable in plasma. The relative contribution of the different transport mechanisms for vitamin C in different cell types remains unresolved.

Many *in vitro* and in vivo studies have demonstrated an anticancer effect of vitamin C and its metabolites, suggesting that the cytotoxic effect of vitamin C predominates. Of course, vitamin C in vitro is readily oxidized by trace amounts of free transition metal ions in the culture me-

dium, so recognition of the importance of the use of metal chelators is paramount in interpreting cell culture experiments using vitamin C. Tsao et al. investigated the cytotoxic effects of ascorbate derivatives on mouse leukemia cells and found that the cytotoxic moiety resides in the enediol lactone ring of the various derivatives.[68] Similarly, Kimoto et al. found that vitamin C in combination with a copper tripeptide (glycylglycylhistidine) was cytotoxic to Ehrlich ascites tumor cells in mice.[69] In a short monograph, Morishige presented a case report of a patient with osteosarcoma successfully treated with ascorbate and the copper tripeptide, which was designed to mimic the copper transport site of albumin.[70] The anticancer mechanism was hypothesized to be the release of copper locally by the high peptide-cleaving activity of tumor cells, thus generating cytotoxic radicals in the presence of ascorbate. A clever model using xenografts of human tumor fragments implanted in the subrenal capsule in mice has been used to demonstrate the immune-independent inhibitory effect of vitamin C alone or combined with transition metal ions.[71,72] Vitamin C was provided in the drinking water with copper or iron in order to generate oxidation products of vitamin C, which had more pronounced inhibitory effects than vitamin C itself.

Prasad et al published two reviews that argued for the essentiality of multiple antioxidants, including large doses of vitamin C, in enhancing the cytotoxicity of chemotherapeutic drugs and radiation.[73,74] In their in vitro experiments, the addition of vitamin C to the culture media increased the cytotoxicity of 5-fluorouracil, bleomycin, and sodium butyrate, as well as x-irradiation, in murine neuroblastoma cells compared to these agents without vitamin C. Vitamin C itself at low (5 μg/ml) and high concentrations (200 μg/ml) had no inhibitory effect on cell growth, although an inhibitory effect was observed in human melanoma cells at a high concentration of vitamin C (100 μg/ml), but not at a low concentration (50 μg/ml). Whereas the addition of single antioxidants to the culture medium had variable effects on tumor cell growth, the most impressive results were produced by the combination of vitamins C and E, beta-carotene, and 13-cis retinoic acid. Of course, cell culture or animal experiments do not faithfully predict the outcome of the clinical application of vitamin C. However, abundant clinical evidence has been presented by Cameron and Pauling,[75] Hoffer and Pauling,[76] and Riordan[77] detailing the therapeutic response of cancer patients to vitamin C given intravenously and/or orally or as part of a micronutrient regimen. Additionally, one small non-randomized clinical trial in Finland found that the provision of a micronutrient regimen, including large doses of vitamin C, in combination with chemotherapy and radiation significantly prolonged survival in patients with small-cell lung cancer compared to similar patients treated only with chemotherapy and radiation.[78] The in vitro and in vivo data amassed to date do not seem to support the concern about the possible interference of vitamin C with standard therapy, as discussed above. However, definitive clinical data are lacking.

In a commentary published in 2000, Padayatty and Levine called for a re-evaluation of vitamin C and cancer, especially when the vitamin is given intravenously, which results in plasma concentrations about ten times higher than orally administered vitamin C and comparable to media concentrations of vitamin C that demonstrated anticancer activity *in vitro*.[79] While the failed Mayo Clinic trials of vitamin C and cancer[80,81] have been criticized for other serious methodological flaws, Padayatty and Levine speculated that the critical difference between the work of Cameron and Pauling and the Mayo Clinic studies purportedly designed to replicate Cameron's clinical work was the mode of administration, i.e. Cameron gave 10 grams/day or more of sodium ascorbate intravenously for about ten days followed by

oral administration continued indefinitely, whereas the Mayo Clinic gave only oral vitamin C for a limited period. Recently, Tamayo and Richardson have reiterated the need for careful evaluation of vitamin C and cancer by carefully designed clinical trials that enroll patients with advanced common types of cancer and uncommon cancers and administer vitamin C intravenously or by bolus.[82]

Prevention or Treatment

It is important to distinguish primary prevention studies from secondary prevention studies, which may be better termed "treatment" studies. The media do not often make this distinction when reporting clinical trials. Once a chronic disease has been established and reaches the point of symptomatic or clinical manifestation, it is useless to ask if the intervention with large doses of vitamin C will prevent disease. Such intervention will address only therapeutic response and the prevention of subsequent clinical events, and the result may be a consequence of the stage of the disease and the dose, duration, and mode of administration of vitamin C, among other factors. Indeed, if one wants to answer definitively the question, "Does vitamin C prevent heart disease?", and if one plausibly assumes that vitamin C may be especially important in inhibiting the early stages of lesion development, a placebo-controlled, double-blind, randomized clinical trial with thousands of pre-adolescents (when lesions first appear) supplemented daily with various doses of vitamin C or placebo for three or four decades until clinical manifestations become apparent would need to be conducted. It is doubtful that such a study would ever be done. Likewise, one could determine if vitamin C prevents cancer in humans by designing a similar study and enrolling subjects before any cells have been initiated. Of course, it's impossible to determine if one has any initiated cells and their precise fate. Several large, well-controlled animal studies have demonstrated that vitamin C offers substantial protection against the development of mammary cancer in mice[83] and skin cancer in mice,[84,85] although it's difficult to extrapolate to humans.

Pharmacokinetics

Very few careful pharmacokinetic studies on vitamin C in healthy humans have been published. Two German studies from 1970 helped to inform Linus Pauling's opinion on the optimal intake of vitamin C.[86,87] The first study of 75 healthy adults (25 women, 50 men) used oral doses of 1.5, 3, 6, and 12 grams of vitamin C and showed absorption of 49.5%, 36.2%, 25.6% and 16.1%, respectively. The mean excretion was 62% of the absorbed dose. The maximal plasma concentration of vitamin C at the largest dose was more than 114 μM. The companion study investigated the pharmacokinetics of intravenously administered vitamin C in 11 healthy adult men and 1 woman.

Levine et al. published two pharmacokinetic studies, one with 7 healthy young men and the other with 15 healthy young women.[29,30] The 1996 study with men demonstrated considerable interindividual variability. The vitamin C doses (30-2,500 mg/day) were administered sequentially over the study period; 6 men remained enrolled for the 1,000 mg/day dose and only 3 were available for determinations at the 2,500 mg/day dose. Nevertheless, Levine showed that lymphocytes became saturated with vitamin C at a dose of 100 mg/day, which resulted in intracellular concentrations of about 3.5 mM. Neutrophil saturation also occurred at the 100 mg/day dose, which resulted in intracellular concentrations of 1.3 mM. Plasma levels of vitamin C increased rapidly between the 30 mg/day and 200 mg/day doses. Plasma concentrations of vitamin C were about 66 μM (range: 57.1-75.1 μM) at the 200 mg/day dose, 70 μM (range: 60.0-80.4 μM) at the 400 mg/day dose, and 77 μM (range: 70.5-84.3 μM) at the 1,000 mg/day dose. There was a non-statistically

significant increase at the 2,500 mg/day dose, although one of the three men showed an increase from 75.9 μM at the 1,000 mg/day dose to 91.8 μM at the 2,500 mg/day dose. Levine's recent study with women showed lymphocyte saturation at a daily dose of 200-400 mg, somewhat higher than the dose that saturated lymphocytes in men. The curve for plateau plasma vitamin C concentration as a function of dose in women was quite similar to that observed in men. Interestingly, over three weeks were required at a specific daily dose of vitamin C to achieve steady state plasma concentrations in men and over five weeks in women. It is not apparent that the investigators in the earlier German studies recognized this phenomenon. While these pharmacokinetic data are illuminating, they are not necessarily predictive of the pharmacokinetics in older or ill people whose need for ascorbate may be greater. A recent meta-analysis of 36 studies on the relationship between vitamin C intake and plasma concentrations found that the elderly (aged 60-96 years) exhibit a significantly lower plasma concentration of vitamin C than do adults (aged 15-65 years) for a given daily intake.[88] For example, to achieve a vitamin C plasma concentration of 50 μM/L in 50% of adults, an estimated daily intake of 78 mg is needed, but the elderly need 150 mg. To achieve 50 μgM/L in 80% of adults requires an estimated daily intake of 173 mg, whearas the elderly need 478 mg.

Indeed, it is difficult to reconcile some studies reporting benefits of large doses of vitamin C with Levine's pharmacokinetic model. For example, Dawson et al. showed that supplementation with 1,000 mg/day of vitamin C, but not with 200 mg/day, dramatically reduced blood levels of lead in adult male smokers by 81% after one week.[89] An observational study by Cheng et al. found that older men whose intake of vitamin C was at least 339 mg/day had lower lead levels in their blood than men whose intake was less than 109 mg/day.[90] Johnston reported that an intake of 2,000 mg/day of vitamin C, resulting in a plasma concentration of 1.02 mg/100 ml, substantially reduced blood histamine levels from 72.3 ng/ml at baseline to 43.1 ng/ml, whereas a 500 mg/day dose of vitamin C, resulting in a plasma concentration of 0.95 mg/100 ml, had little effect on histamine levels.[91] This observation may help explain why large doses of vitamin C have a salubrious effect on common cold symptoms, whereas small doses have little discernable effect. These reports also highlight the importance of dose and duration in evaluating the preventive role of vitamin C and, additionally, the mode of administration in assessing therapeutic response.

Special Experimental Considerations

A number of interesting observations about vitamin C used in in vitro and in vivo models are important to consider when designing or evaluating experimental studies. Many cultured cells without vitamin C added to the medium are scorbutic and do not reflect physiological status. For example, human endothelial cells cultured without vitamin C added to the medium show abnormally high levels of oxidative stress that affect other parameters, such as eNOS activity.[92] Small amounts of supplemental vitamin C (0.076% by weight) administered to mice, a species able to synthesize vitamin C, depress levels of vitamin C in the liver, lung, muscle, spleen, and plasma compared to unsupplemented mice, demonstrating the so-called "mouse effect."[93] To raise the vitamin C level in these tissues (except plasma) in mice, the amount of vitamin C added to the diet must be greater than 0.5% by weight. Additionally, guinea pigs, an excellent animal model for studying vitamin C because of their inability to synthesize the vitamin, maintained on vitamin C deficient diets and housed in cages with wood chips can remain ascorbutic by consuming the chips, which

provide enough vitamin C to prevent scurvy.[94]

The Real Problem

The magnitude of the precise prophylactic effect of vitamin C against heart disease and cancer remains unresolved, and we have little dose response data on the effect of vitamin C on its manifold biochemical reactions. Studies using the guinea pig model have demonstrated remarkable protective effects of high-dose vitamin C against liver damage and mortality caused by aflatoxin B_1 exposure[95] and against neuro-lathyrism or death induced by exposure to ß-*N*-oxalylamino-*L*-alanine, a potent neurotoxin.[96] These results, combined with the aforementioned human studies on lead and vitamin C, show the potential for substantial improvements in public health in regions with high toxicant exposure.

We know that vitamin C has an important and underappreciated role in clinical practice and that it is a very safe substance. Studies will continue to report the gamut of beneficial, neutral, or potentially harmful effects of supplemental vitamin C, which must be reviewed carefully and critically. Many, perhaps most, of these studies will be imperfect, containing insignificant flaws or serious problems that render the results questionable. In particular, the news media must endeavor to display much more scientific competence, perspective and technical precision in their coverage of scientific and medical reports on vitamin C. Otherwise, the public may become confused and needlessly suffer. As Wakimoto and Block pointed out, about 50% of older Americans ingest less than the RDA for vitamin C (90 mg/day for men, 75 mg/day for women), and about 25% ingest less than 50% of the RDA for vitamin C.[97] Clearly, inadequate daily intake of vitamin C is among the most serious problems that need to be addressed.

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