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Down-regulation of Superoxide Dismutase Gene Expression in Cultured Rat Aortic Smooth Muscle Cells (A7r5) after Long-term Incubation with Vitamin C

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Background : Oxygen free radicals have been linked to the process of cardiovascular disease and aging. Epidemiological studies supported the beneficial effect of supplementation of antioxidants such as vitamin C and vitamin E. Superoxide dismutase (SOD) is a endogenous enzyme system which can scavenge oxygen free radicals. This study investigated the effect of supplementation of ascorbic acid (vitamin C) on the changes of SOD.

Methods : Rat aortic smooth muscle cells (A7r5) were divided into 4 groups: a control group (without vitamin C) and treatment groups with vitamin C at 50 μM, 100 μM and 200 μM. After a short-term (2 days) or long-term (7 days) incubation, the enzyme activity and mRNA level of SOD were measured.

Results: After 7 days incubation, vitamin C resulted in a decrease in the activity and mRNA level of Mn-SOD (mitochondrial enzyme) in a concentration-dependent manner. However, vitamin C supplementation did not change the gene expression and activity of Cu,Zn-SOD (cytosolic enzyme) either after short-term or long-term incubation.

Conclusion: The results obtained suggest that 7 days incubation with relative high concentrations of vitamin C may down-regulate the gene expression and activity of antioxidant enzyme Mn-SOD in cultured smooth muscle cells.

Key Words: Oxygen free radicals; Superoxide dismutase; Vitamin C; Smooth muscle cells.

Introduction

The primary cause for most cardiovascular diseases is thought to be arteriosclerosis, a multifactoral disease. There is an increasing evidence that oxidative stress caused by reactive oxygen species, particularly oxidation of LDL, is a risk factor for coronary artery disease and plays a role in the pathogenic pathway.[1] These reactive oxygen species are responsible for the oxidative damage of biological target molecules of vascular endothelial cells and smooth muscle cells such as DNA, lipids, carbohydrates, or proteins; the reactive oxygen species include peroxyl radicals ($ROO^{\bullet}$), the nitric oxide radical ($NO^{\bullet}$), the superoxide anion radical ($O_2^{\bullet-}$), the hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$), and hydrogen peroxide (H_2O_2). LDL oxidation is due to a

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lipid peroxidation reaction initiated by free radicals. Separate investigations of the lipid and protein part of oxidized LDL (oxLDL) demonstrated that oxidative modifications contribute to the proatherogenic properties of oxLDL. To counteract the pro-oxidant load, a diversity of antioxidant defense systems are operative in biological systems, including enzymatic and non-enzymatic antioxidants.[2]

Superoxide dismutases (SODs) are key enzymes in the cellular defense against oxidative damage caused by free radicals.[3] Superoxide free radicals are a normal byproduct of metabolism, generated by oxidative phosphorylation and photosynthesis.[4] Oxidative damage caused by free radicals results in the breakdown of biological macro-molecules and thus contributes to adverse conditions such as aging, cancer, atherosclerosis and a variety of degenerative diseases.[5] SODs catalyze the breakdown of toxic superoxide radicals into hydrogen peroxide and molecular oxygen, thus providing protection against oxidative damage.[5]

Vitamin C (ascorbic acid) is one of the most powerful natural antioxidants.[6] Vitamin C is water-soluble and is found in high concentrations in many tissues; human plasma contains about 60 μmol ascorbate/l.[7,8] Upon reaction with reactive oxygen species it is oxidized in two one-electron steps to dehydroascorbate via the ascorbyl free radical. Dehydroascorbate is recycled back to ascorbate by the dehydroascorbate reductases.[8] As a scavenger of reactive oxygen species, ascorbate has been shown to be effective against superoxide radical anion, hydrogen peroxide, the hydroxyl radical, peroxynitrite, and singlet oxygen. Important sources of ascorbate in the diet are fruits, broccoli, cauliflower, Brussels sprouts, and cabbage; its content may exceed 100mg ascorbate/100g fresh weight. In recent years, interest in the possible role of antioxidant vitamins in the prevention of heart disease has awakened in the medical and research community. This study was undertaken to evaluate the effect of vitamin C supplementation on SOD activity and gene expression in rat aortic smooth muscle cells (A7r5).

Materials and Methods

Preparation of A7r5 Cells

Rat aortic smooth muscle cells (A7r5) were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Cells were washed in phosphate-buffered saline (PBS) containing 0.1% EDTA and 0.5% trypsin and centrifuged at 1000 rpm for 5 min. The supernatant was discarded and the cells were suspended in culture medium. Cell suspension (100 μL) was mixed thoroughly with 100 μmL PBS containing 0.04% trypan blue. The number of surviving cells was counted. Surviving cells were divided into 4 groups: a control group incubated with culture medium containing 0.5% ETOH (without vitamin C) and treatment groups (vitamin C) incubated with culture medium containing vitamin C at 50 μM, 100 μM, and 200 μM, respectively. The value of PH in solution was kept at 7.4 in each experiment group.

Assay of SOD Activity

The activity of SOD was measured using a commercial assay kit (Wako, Tokyo, Japan). Cells from dish were harvested and homogenized in 1 mL of 0.9% NaCl.[9] The crude homogenate was centrifuged at 10,000 × g for 1 h to separate into a supernate (cytosolic) and a pellet (particulate). For assay of Mn-SOD activity in the pellet, 1 mM potassium cyanide was added to the incubation mixture to inhibit Cu,Zn-SOD activity. The activity of Cu,Zn-SOD was obtained by subtraction of Mn-SOD from total SOD activity in the pellet. The units of SOD activity were derived from a standard curve constructed using purified SOD from bovine erythrocytes (S-2515; Sigma, USA). Results were expressed as unit/mg of protein determined.[10]

Northern Blotting Analysis

Cells for RNA isolation were kept frozen in liquid nitrogen immediately after removal and then stored at -80 °C. Total RNA was isolated as described previously.[11] RNA was then transferred to Hybond N

nylon membranes (Amersham, Manchester, UK) overnight in 2 volumes of saline-sodium citrate (SSC). The RNA was fixed on the membrane by UV transilluminator and stained with 0.05% methylene blue.[12] Filters were rapidly prehybridized at 65 °C in hybridization solution (Quikhyb®, Stratagene, CA, USA). The cDNA probes were also prepared. Plasmids containing cDNA of SOD were supplied by Dr. Y.S. Ho (Wayne State University,Detroit,MI, USA) and plasmids containing cDNA of catalase and glutathione peroxidase (GPX) were obtained from Dr. T.S. Chiou (Tzu-Chi Medical College, Hua-Lien, Taiwan). Transformation in Escherichia coli, plasmid preparation, and cDNA purification were performed according to standard methods.[13] Radioactive probes (P^{32}) were prepared using the multiprime DNA labeling system (Amersham, UK). The prepared cDNA inserts and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes were added directly into the prehybridization solution (Quikhyb®, Stratagene, CA, USA) at a radioactivity of 1×10^6 cpm/ml. Hybridization was performed at 68 °C for 70 min. After washing, the wet blot membranes were sealed in plastic foil and exposed to medium-sensitive medical x-ray film (Fuji, Tokyo, Japan) at -70 °C using intensifying screens.[14] Exposure times were 2-3 days for Northern blots. Hybridization intensity of autoradiographic signals was measured using two-dimensional densitometry. The obtained density (optical unit) was calculated versus the value of slot blot for GAPDH to make the internal control of quantification of mRNA.

Statistics

All values were presented as means ± standard error of mean (SEM) from each group. Statistical significance was evaluated using one-way ANOVA when multiple groups were compared; when only 2 groups were compared, Student's *t-test* was used. A value of $p < 0.05$ was considered to be significant.

Results

Effect of Vitamin C on Cell Number of Cultured A7r5 Cells

In the present study, the number of surviving cells was counted under a light microscope with a counter. The cultured A7r5 cells were divided into 4 groups: the control group incubated with culture medium containing 0.5% ETOH (vehicle) and treatment groups (vitamin C) incubated with culture medium containing vitamin C at 3 concentrations. The number of cells in the control after 7 days of culturing was $19.3 \pm 1.6 \times 10^6$ cells, which was not different from cell numbers cultured with 50 μM [$(18.9 \pm 1.5) \times 10^6$ cells], 100 μM [$(20.2 \pm 1.9) \times 10^6$ cells] and 200 μM of vitamin C [$19.4 \pm 1.8) \times 10^6$ cells].

Effect of Vitamin C on the Activity of Superoxide Dismutase (SOD) in Cultured A7r5 Cells

The activities of SOD showed divergent effect after incubation with different concentrations of vitamin C; the activities of Cu,Zn-SOD did not change after vitamin C incubation (Table 1), but the activities of Mn-SOD showed a tendency of a dose-dependent decrease when the dosage of vitamin C increased during the 2 day (short-term) incubation period. This phenomenon was even more remarkable at 7 day (long-term) incubation period (Table 1), where the Mn-SOD activity dropped from 30.5 IU/mg protein

Table 1. Change of SOD Activity (IU/mg protein) in A7r5 Cells after 2 Days' and 7 Days' of Incubation with Vitamin C

	2 days				7days			
Vitamin C	0 μM	50 μM	100 μM	200 μM	0 μM	50 μM	100 μM	200 μM
Cu,Zn-SOD	76.0±0.6	76.7±0.8	75.4±1.1	75.6±0.8	75.1±0.9	76.0±0.8	75.2±0.6	74.9±0.5
Mn-SOD	29.1±1.1	29.2±0.9	28.9±0.8	27.4±0.9	30.5±1.2	27.2±0.8*	24.6±0.4**	20.5±0.4**

Data are expressed as mean ± SEM (N=10). *P<0.05; **P<0.01; (compared with 0 μM vitamin C).

Table 2. Change of SOD mRNA (%) in A7r5 after 2 Days' and 7 Days' Incubation with Vitamin C

	2 days				7days			
Vitamin C	0 μM	50 μM	100 μM	200 μM	0 μM	50 μM	100 μM	200 μM
Cu,Zn-SOD	100	101.5±6.4	101.8±7.0	99.8±7.3	100	97.1±8.9	98.4±6.4	101±8.4
Mn-SOD	100	102.1±5.8	103.4±5.6	101.5±8.8	100	90.5±5.8*	81.2±5.2**	75.6±5.4***

Data are expressed as mean ± SEM (N=10). *p < 0.05; **p < 0.01; ***p< 0.001 (compared with 0 μM vitamin C).

to 20.5 IU/mg protein (p<0.01).

Effect of Vitamin C on the mRNA of SOD in Cultured A7r5 Cells

Similar results were also noticed in SOD mRNA after incubation of vitamin C with A7r5 cells (Fig. 1). The level of Cu,Zn-SOD remained unchanged, whereas Mn-SOD showed a dose-dependent decrease after incubation with vitamin C at different concentrations (Table 2). These data were compatible with the changes of SOD activities (Table 1).

Discussion

Coronary artery atherosclerosis is the single most important cause of morbidity and mortality in the Western world.[15] The initial stage of atherosclerotic lesion development is characterized by the local accumulation of oxidized low-density lipoprotein (oxLDL) and the recruitment of monocytes to the arterial wall.[16,17] Once on the arterial wall, the monocytes differentiate into resident macrophages and then gradually converted to lipid-laden foam cells. Foam cells are the hallmark of the early atherosclerotic lesion called the"fatty streak".

Thus, it appears that inhibition of LDL oxidation is either necessary or sufficient to inhibit atherosclerosis, and local effects of antioxidants on the vascular wall or non-antioxidant mechanisms may be equally important.[18] Animal studies with vitamin C have shown that vitamin C deficiency is associated with accelerated atherosclerosis in guinea pigs.[19] In addition, the majority of studies investigating vitamin C supplementation in cholesterol-fed rabbits have found significant inhibition of the development of atherosclerosis.[19]

Epidemiological and limited trial data suggest a protective role of dietary antioxidants against cardio-

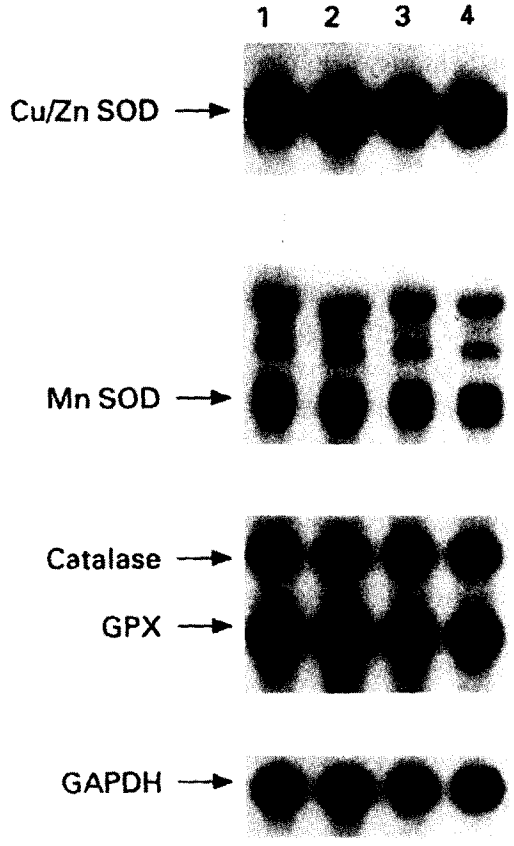

Fig. 1. Northern blot of total RNA (30 μg/lane) from control and vitamin C-incubated samples probed for Cu,Zn-SOD, Mn-SOD, catalase and glutathione peroxidase (GPX) to compare with the internal standard (GAPDH). Lane 1 shows the control and lanes 2-4 indicate the responses to a 2-day incubation with vitamin C at 50, 100 and 200 μM, respectively.

vascular disease (CAD) in humans, including vitamin E and C.[18, 20, 21] Currently, there is quite consistent support for a protective effect against CAD of vitamin E supplementation with more than 100 international units (IU) per day for at least 2 years.[20] In contrast, the evidence in support of beta-carotene acting as an anti-atherogenic agent has been weakened very substantially by recent trial data.[22,23] Vitamin C supplementation may be most important in reducing CAD risk among populations with near-deficient vitamin C intakes. For example, a recent study has found that individuals with plasma levels of vitamin C <11.4 μM have a 2.5-fold increased CAD risk (myocardial infarction) compared to individuals with higher vitamin C levels.[21] Furthermore, it has been reported that the degree of carotid artery atherosclerosis in humans is inversely correlated with vitamin C intake,[24] and several studies have found a significantly and substantially reduced risk of ischemic stroke with increased vitamin C consumption.[25] However, some reports found that opitimal dose of vitamin C supplementation is important, excess antioxidant may be harmful.[26,27] Although promising, currently available scientific evidence is insufficient to conclude that vitamin C can act as an antiatherogen or lower the risk of CAD in humans.

Superoxide dismutases (SODs) are key enzymes in the cellular defense against oxidative damage caused by free radicals.[2] In eukaryotes, copper, zinc superoxide dismutase (Cu,Zn-SOD) is a cytosolic, homodimeric metalloenzyme which degrades superoxide radicals at a rate limited by the diffusion of substrate into the enzyme active site.[2] The mitochondrial manganese superoxide dismutase (Mn-SOD) is a defensive antioxidant enzyme which protects cells against oxy-radical species generated during oxidative metabolism and exposure to several chemical (redox-cycling drugs), physical (radiation) and biological (cytokines) agents. Abnormalities of SOD level and gene expression are linked to some diseases which make cells to be susceptible to oxidative damage.[28,29]

In the present study, we found that long-term (7 days) incubation of ascorbic acid (vitamin C) with A7r5 cells resulted in decrease of both the activity and mRNA level of endogenous Mn-SOD within 7 days. The mitochondria is a major organelle where reactive oxygen species (ROS) are produced. So, Mn-SOD is more changeable than Cu,Zn-SOD during oxidative stress or after antioxidant supplementation.[30] The mechanism of down-regulation of Mn-SOD gene expression may be due to lower levels of oxygen free radicals which are effectively scavenged by long-term large doses of vitamin C. Recent evidence suggests reactive oxygen species such as superoxide anions and hydrogen peroxide, function as intracellular second messengers signaling transduction which could influence the transcripription of superoxide dismutase.[31] Vitamin C has been widely used in the world because of its possible antioxidant effects. Therefore, we believed that the examination of the effect of vitamin C on SOD in this study would be of considerable interest.

In conclusion, the present study shows that supplementation of vitamin C in cultured aortic smooth muscle cells at relatively long-term high concentrations may down-regulate the gene expression and activity of endogenous antioxidant enzyme Mn-SOD within 7 days. The observation is interesting, and whether it is deleterious in humans needs further investigation.

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長時間之維他命C投與會造成大白鼠主動脈平滑肌細胞中超氧化物轉化酵素的基因表現下降

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背景: 氧自由基的生成及對細胞的破壞是被認為與許多疾病的形成有關。體內的酵素系統，例如超氧化物轉化酵素(superoxide dismutase: SOD)可清除有害的氧自由基。流行病學的研究證實抗氧化劑如維他命 C，E 可減少氧自由基的傷害，但是也有文獻指出長時間大量的抗氧化劑投與未必有益。本研究即探討在正常生理狀況下，大量及長時間維他命 C 對於大白鼠平滑肌細胞的超氧化物轉化酵素活性及基因表現的影響。

方法: 培養中的大白鼠主動脈平滑肌細胞(A7r5)分為四組：一組為對照組不加入維他命 C；另外三組加入不同濃度的維他命 C (50 μM，100 μM，200 μM)。在投與第二天及第七天後測量兩種超氧化物轉化酵素：錳-超氧化物轉化酵素(Mn-SOD)銅，鋅-超氧化物轉化酵素(Cu,Zn-SOD)的活性及基因表現。

結果: 對照組及不同濃度實驗組的細胞數目並無差異，表示維他命 C 對於培養細胞的生長與死亡沒有特別影響。在投與 7 天的維他命 C 後，粒腺體中的錳-超氧化物轉化酵素的活性及基因表現皆呈現出有意義的濃度相關性下降；而細胞液中的銅，鋅-超氧化物轉化酵素活性及基因表現無論在第二天或第七天都無意義的變化。

結論: 本研究發現抗氧化劑維他命 C 在高劑量及長時間的投與後會下降調節(down-regulation)主動脈平滑肌細胞的內生性氧自由基清除物：錳-超氧化物轉化酵素基因表現及活性。這種作用的生理意義及對細胞的影響還須進一步的研究證明。

關鍵詞: 氧自由基；超氧化物轉化酵素；維他命C；平滑肌細胞。

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Comment

The Vitamin C Supplement Myth: What Role do They Play in Affecting the Free Radical Scavenging Enzyme System?

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Recent evidence suggests that oxidant stress plays a major role in several aspects of vascular biology. Oxygen free radicals are implicated as important factors in signaling mechanisms leading to vascular pathologies such as postischemic reperfusion injury and atherosclerosis.[15] Oxidative stress is increasingly recognized as a potentially important contributor to atherogenesis and restenosis after vascular intervention and injury. Indeed, studies in this area have renewed interest in the oxidative-modification hypothesis of atherogenesis. Superoxide is a "foundation" radical that may lead to the formation of the reactive oxygen species hydrogen peroxide,[30] hydroxyl radical,[2] and peroxynitrite.[3] Superoxide is produced intracellularly by electron leakage from mitochondria during oxidative phosphorylation and by activation of several cellular enzymes, including NADPH oxidases,[20] cyclooxygenase, nitric oxide synthase,[31] and xanthine oxidase. Antioxidant enzymes, including superoxide dismutase (SOD), catalase, and peroxidases protect cells by maintaining O^{2-} and H_2O_2 at low levels.

The superoxide dismutases (SODs) represent a major cellular defense against O_2^- and formation of peroxynitrite.[7] Three isozymes of SOD have been identified, including a cytosolic copper/zinc-containing form (Cu/ZnSOD), an extracellular isozyme (ECSOD), which is also a copper/zinc-containing enzyme, and a mitochondrial manganese form (MnSOD). In the vessel wall, one third to one half of the total vascular SOD is the ECSOD.[26] ECSOD has been reported to be a major antioxidant enzyme system of the arterial wall, located strategically between endothelium and vascular smooth muscle cells.[26] The ECSOD concentration within the arterial wall is high enough to suppress pathological effects of superoxide anions such as reaction with NO leading to formation of deleterious peroxynitrite.[26] Human arteries contain extraordinarily large amounts of ECSOD that are 100 times higher as compared with skeletal muscle or fat tissue,[18,26] pointing out the special function of this protein within the vessel wall. Inhibition of vascular SOD resulted in impairment of endothelium-dependent dilation in bovine coronary arteries in vitro, suggesting that SOD levels are critical for the ability of NO to modulate vascular tone.[22] This concept finds further support by the in vivo observation that SOD deficiency impaired endothelium-dependent dilation as the result of increased inactivation of NO.[17] ECSOD activity is decreased in atherosclerotic lesions of human aorta as compared with macroscopically normal segments of the same individual.[16] In contrast, ECSOD activity is increased in aortas of hyperlipidemic rabbits.[16] Human recombinant ECSOD, when administered exogenously, can reduce infarct size in pigs.[11] Likewise, chimeric enzymes in which heparin-binding domains have been added to Cu/ZnSOD and form mimics of ECSOD have been shown to reduce blood pressure in rats made hypertensive by angiotensin II.[8] These findings suggest that ECSOD likely plays a major role in preventing the pathophysiological effects of O_2^- in the vasculature. In addition, MnSOD[6] is located in the mitochondrial matrix, which is encoded by a nuclear

gene. Mitochondria are particularly prone to oxidative DNA damage because they metabolize over 95% of the oxygen,[10] but they lack histones and have a poor ability for DNA repair.[24] Thus, MnSOD is regarded as the primary defensive enzyme against oxidative stress within mitochondria. A number of studies demonstrated the induction of MnSOD in various cell lines and tissues following oxidative stress, such as treatments with tumor necrosis factor (TNF), interleukin-1, lipopolysaccharide, interferon, 12-O-tetradecanoylphorbol-13-acetate (TPA).[28,29] Most of these treatments could generate reactive oxygen species in cells that, in turn, could activate transcription factors, mainly nuclear factor-B (NF-B) and activator protein-1 (AP-1),[13] to allow their nuclear translocation and allow them to bind to genes involved in the stress responses. In accordance, the involvement of NF-B and AP-1 in activating MnSOD genes in response to TNF, TPA, H_2O_2, or thiol-reducing agents has been suggested in human and rat cells.[13] These studies correlated the conditions that changed the DNA binding activity of either NF-B or AP-1 with the induction of MnSOD, and thus concluded that the activation of the MnSOD gene is mediated by either of these factors.[13]

It is not surprising then that modifications of oxidation have been proposed for dealing with the atherosclerotic and mechanically manipulated vessel. Recently the wealth of epidemiological data linking dietary and supplementary intake of antioxidant vitamins with reduction in the clinical manifestation of atherosclerosis. At the same time, a range of studies have examined the impact of antioxidants on many of the isolated cellular effects thought to be associated with restenosis in cell culture and on intimal hyperplasia in animal models of disease. A considerable body of information exists regarding the role of vitamin C in mammalian physiology. Humans and other primates cannot synthesize vitamin C, whereas most mammals (e.g., rat and mouse) endogenously produce vitamin C in the liver.[5] Vitamin C is absorbed from the gastrointestinal tract in the form of ascorbic acid, while dehydroascorbic acid is reduced to ascorbic acid for gastrointestinal absorption.[25] Based on epidemiological data, the threshold levels for effective protection from cardiovascular disease by vitamin C has been estimated at 40-50 mmol.[9] Most ascorbic acid is excreted by the kidneys, but a limited amount is metabolized in the body. Ascorbic acid is oxidized to dehydroascorbic acid, which can undergo irreversible hydrolysis to 2,3-diketo-L-gulonic acid, with decarboxylation to CO_2 and components of the pentose phosphate cycle or oxalic acid plus threonic acid. Ascorbic acid is the main water-soluble antioxidant in human plasma, it also plays a central role in regulation of intracellular redox state.[19] Ascorbic acid is present in millimolar concentrations within the cell cytosol, and serve to scavenge free radical species and to detoxify reactive oxygen species that are produced by normal oxidative metabolism.[19] *In vitro* experiments have demonstrated that vitamin C has the capacity to scavenge superoxide anion.[21] The reaction rate constants are estimated to be 3×10^5 M/s between vitamin C and superoxide anion[27] and 2×10^9 M/s between SOD and superoxide anion.[27] Despite a 10^4 slower reaction rate observed with vitamin C, tissue vitamin C concentrations are 10^4 greater than that of SOD,[27] enabling vitamin C to competitively scavenge superoxide anion.

Although basic mechanisms support a role of vitamin C in cardiovascular disease, clinical data are less encouraging. Epidemiologic studies are inconsistent regarding vitamin C and coronary disease.[4] Large doses of vitamin C induce vasodilatation in the brachial and coronary arteries,[4] but whether this has any bearing on such actions at physiologic concentrations of vitamin C found in patients with coronary artery disease is not known. Inconsistencies in the literature may be due, in part, to the prooxidant properties of vitamin C[23]. Vitamin C is not exclusively an antioxidant and has been shown to be a prooxidant in some circumstances.[12] For example, supplementation with 500 mg vitamin C/d significantly increased DNA damage in volunteers.[23] A high dietary intake of vitamin C might reflect a healthy diet, or perhaps a healthy lifestyle. It is therefore uncertain whether the effects of vitamin C can be discerned clearly by epidemiologic studies alone.

In this issue of the Journal, Liu *et al.* report the results of down-regulation of MnSOD gene expres-

sion in rat aortic smooth muscle cells after long-term incubation with vitamin C. The ability of the antioxidant vitamin C to inhibit the MnSOD activity and gene expression deserves further attention. These effects were introduced by raising vitamin C concentration and treatment duration. Decreased MnSOD may reflect substrate inhibition, since superoxide anions are scavenged by vitamin C. On the other hand, insufficient MnSOD activity may contribute to increased lipid peroxidation with resultant mitochondrial damage. Thus, the chronic consumption of high concentration of vitamin C results in altered levels of SOD activities in vascular smooth muscle cells which in turn may contribute to vascular injury. On the basis of mechanistic, physiologic, and epidemiologic studies, the beneficial effects of vitamin C in coronary artery disease have not been resolved. Properly designed experimental studies can test this hypothesis. Before proceeding, we should be sure that the relation between SOD activity and high vitamin C concentrations is real. We must be very careful that artifacts are eliminated. Accurate measurement, then, is the heart of the matter. To resolve the vexatious issue of peripatetic heavenly bodies, Galileo put his faith in accurate measurements and said, "Measure what is measurable, and make measurable what is not so." We should remember Galileo's words but temper them with the analysts' reminder: "Vitamin C... is it there just like they say, or has it oxidized away..."

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