Time- and dose-dependent differential regulation of copper-zinc superoxide dismutase and manganese superoxide dismutase enzymatic activity and mRNA level by vitamin E in rat blood cells

Maliheh Hajiani[1], Farideh Razi[2], Aboualfazl Golestani[1], Mehdi Frouzandeh[3], Ali Akbar Owji[4], Shahnaz Khaghani[1], Naghmeh Ghannadian[5], Ahmad Shariftabrizi[1], Parvin Pasalar[1,6]

[1]Department of Biochemistry, Tehran University of Medical Sciences, Tehran, Iran, [2]Endocrinology and Metabolism Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran, [3]Department of Biotechnology, Tabiat Moddares University, Tehran, Iran, [4]Department of Biochemistry, Shiraz University of Medical Sciences, Shiraz, Iran, [5]Students' Scientific Research Center (SSRC), Tehran University of Medical Sciences, Tehran, Iran, [6]Center for Research on Occupational Diseases (GRAD), Tehran, Iran

Background: Vitamin E is the most important lipid-soluble antioxidant. Recently, it has been proposed as a gene regulator, and its gene modulation effects have been observed at different levels of gene expression and cell signaling. This study was performed to investigate the effects of vitamin E on the activity and expression of the most important endogenous antioxidant enzyme, superoxide dismutase (SOD), in rat plasma.
Methods: Twenty-eight male Sprauge–Dawley rats were divided into four groups: control group and three dosing groups. The control group received the vehicle (liquid paraffin), and the dosing groups received twice-weekly intraperitoneal injections of 10, 30, and 100 mg/kg of vitamin E ((±)-α-Tocopherol) for 6 weeks. Quantitative real-time reverse transcription-polymerase chain reaction and enzyme assays were used to assess the levels of Cu/Zn-SOD and Mn-SOD mRNA and enzyme activity levels in blood cells at 0, 2, 4, and 6 weeks following vitamin E administration. Catalase enzyme activity and total antioxidant capacity were also assessed in plasma at the same time intervals.
Results: Mn-SOD activity was significantly increased in the 100 and 30 mg/kg dosing groups after 4 and 6 weeks, with corresponding significant increase in their mRNA levels. Cu/Zn-SOD activity was not significantly changed in response to vitamin E administration at any time points, whereas Cu/Zn-SOD mRNA levels were significantly increased after longer time points with high doses (30 and 100 mg/kg) of vitamin E. Catalase enzyme activity was transiently but significantly increased after 4 weeks of vitamin E treatment in 30 and 100 mg/kg dosing groups. Total antioxidant status was significantly increased after 4 and 6 weeks in the 100 mg/kg dosing group.
Conclusion: Only the chronic administration of higher doses of alpha-tocopherol is associated with the increased activity and expression of Mn-SOD in rats. Cu/Zn-SOD activity and expression does not dramatically change in response to vitamin E.

Keywords: Vitamin E, Superoxide dismutase, Real-time PCR, Oxidative stress, Antioxidant

Introduction

An estimated 1–3% of oxygen molecules consumed in the mitochondria are converted into one or more types of reactive oxygen species (ROS).[1] In low/moderate concentrations, ROS are involved in normal physiological processes[2] and influence diverse cellular functions by altering the activity of different serine/threonine and tyrosine kinases, mitogen-activated protein kinases, and various protein tyrosine and serine/threonine phosphatases. ROS also affect the activity of myriad transcription factors, including AP-1, NF-kappa B, HIF-1, fork head transcription factors, and p53.[3] When ROS are produced in excessive amounts, they cause oxidative stress. Oxidative stress is implicated in the etiology and progression of

Correspondence to: Parvin Pasalar, Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran 14176-13151, Iran. Email: Pasalar@tums.ac.ir

DOI 10.1179/1351000212Y.0000000008

over a hundred pathological conditions. Organisms have developed a series of defense mechanisms to overcome free radical-induced oxidative stress. These involve preventive mechanisms, repair mechanisms, physical defenses, and antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Non-enzymatic antioxidants include ascorbic acid, tocopherol, carotenoids, flavonoids, and other antioxidants. Both the activities and the intracellular levels of these antioxidants should be in balance for the organism to function normally.[2] Antioxidant enzymes act cooperatively and synergistically to scavenge ROS, as none of them can single handedly clear all forms of ROS.[4]

SODs are the first and most important line of antioxidant enzyme defense systems against ROS, particularly superoxide anion radicals.[5] SODs function by a so-called 'ping-pong' mechanism as it involves the sequential reduction and oxidation of the metal center, with the concomitant oxidation and reduction of superoxide radicals.[6] These reactions typically require no external source of redox equivalents and are self-contained components of the antioxidant machinery. SODs are thus able to function in a variety of intracellular and extracellular environments.[7] In addition, if the actions of SOD and hydrogen peroxide-consuming enzymes are not in concert, an increased production of hydrogen peroxide is expected from SOD activity, which then might facilitate the production of hydroxyl radicals and their consequential damage.[8] Three distinct isoforms of SOD have been identified in mammals. Two isoforms of SOD have Cu and Zn in their catalytic center: intracellular cytoplasmic Cu/Zn-SOD (SOD1) and extracellular EC-SOD (SOD3). SOD2 (Mn-SOD) has manganese in its catalytic center and is localized in the mitochondria of aerobic cells. Many *in vitro* and *in vivo* studies have established Mn-SOD as the only SOD vital for life in an oxygen-rich environment. Increases in the resistance to oxygen toxicity are due to the oxygen-stimulated expression of Mn-SOD and over-expression of Cu/Zn-SOD does not rescue Mn-SOD$^{-/-}$ mice from early death caused by a lack of Mn-SOD.[3,9]

Among the non-enzymatic anti-oxidants, vitamin E protects the organism against the attack of ROS by acting as a lipid-based free radical chain-breaking molecule. Apart from free its radical scavenging activity, vitamin E engages in non-antioxidant actions by regulating gene expression and cell signaling.[10] Vitamin E regulates the transcription of several genes, including the genes involved in the uptake and degradation of tocopherols, lipid uptake, and atherosclerosis, as well as the expression of extracellular proteins, inflammation, cell adhesion, and platelet aggregation. It also regulates the genes involved in cell signaling and cell cycle regulation, which might be mediated through the inhibition of protein kinase C and phosphatidylinositol 3-kinase.[11–13]

In recent years, much attention has been directed toward the oxidant-antioxidant balance and how it might affect different pathological states. These studies are largely based on the assumption that taking antioxidants or augmenting the antioxidant defense system can prevent several diseases.[14,15] Thus, supplementation of antioxidants and free radical scavengers via diet or pharmacological delivery has been examined in numerous trials, with variable success.[16] However, a substantive compendium of negative effects of antioxidant use, especially concerning dietary supplementation with higher doses of vitamins C and E, has developed.[1]

We have previously shown[17,18] that vitamin E can exert both *in vivo* pro-oxidant and antioxidant effects in rats, depending on the dose and duration of administration. In this study, we followed a similar study design to investigate which isoform of SOD is responsible for the enhancement of antioxidant defense by chronic vitamin E administration in rats.

Materials and methods

Animals and experimental protocols

Adult male Sprauge–Dawley rats ($n = 28$) weighing 220–250 g were randomly assigned to four groups of seven rats each: one control and three dosing groups. They were held two per cage in a room that was controlled for temperature (22 ± 2°C), humidity, and light (12 hours light: dark cycle). All the experiments reported here comply with the university regulations on the care and handling of experimental animals. Vitamin E ((±)-α-Tocopherol, Sigma, Cat. No. T3251, St Louis, MO, USA) was dissolved in liquid paraffin. The control group received only the vehicle, and the three dosing groups received twice weekly intraperitoneal injections (50 µl) of 10, 30, and 100 mg/kg body weight vitamin E for 6 weeks. Blood samples were taken from the tails of the rats before the administration of vitamin E (week 0) and at the end of 2, 4, and 6 weeks; these samples were collected in polyethylene tubes containing 0.5-mM ethylenediaminetetraacetic acid. Plasma was separated by centrifugation, and the pellets were washed three times with cold isotonic saline and stored at −20°C until analysis.

Total RNA extraction

Total RNA was isolated from 50 µl of fresh blood cells, using Trizole reagent (Roche, Cat. No. 1166715, Mannheim, Baden-Württemberg, Germany) according to the manufacturer's protocol. RNA was resuspended in 50 µl of RNase-free (diethyl pyrocarbonate, DEPC) water. The integrity of the total

RNA was checked on agarose gels and its quantity and purity was assessed spectrophotometrically. RNA samples were stored at −80°C until analysis.

Primers design

Intron-spanning primer pairs were designed to minimize genomic DNA contamination and generate an amplicon between 150 and 350 bp. Primers were used as follows: beta-actin forward and reverse primers were AACTCCCATTCCTCCACTT and GAGGGCCTCTCTCTTGCTCT, respectively; Cu/Zn-SOD forward and reverse primers were GCGGTGAACCAGTTGTGGTG and AGCCACA TTGCCCAGGTCTC, respectively; and Mn-SOD forward and reverse primers were AGCTGCACCA CAGCAGCAC and TCAACAAGCCACCCGAAA CA, respectively.

Real-time quantitative polymerase chain reaction

Real-time quantitative polymerase chain reaction (PCR) was performed with the Rotor-Gene 3000 real-time PCR detection system (Bio-Rad, Australia). According to the manufacturer's instructions, a master-mix of the following reaction mixture was prepared: 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), 12.5 µl 2X Quantitect SYBR Green RT-PCR Master Mix (Qiagen, CA, USA), and 0.25 µl Quantitect RT mix (Qiagen). 500 ng RNA was added to each reaction tube. The final volume was brought to 25 µl by the addition of DEPC water. The following real-time reverse transcription-PCR protocol was used: reverse transcription (50°C for 30 minutes) and the PCR initial activation step (95°C for 15 minutes), followed by 40 cycles of 15 seconds of denaturation at 94°C, 30 seconds of annealing at 63°C, and 30 seconds for extension at 72°C. Amplification specificity was verified by performing a melting curve analysis of the PCR products for 3 minutes at 90°C and then lowering the temperature to 55°C in 0.2°C increments at 1 second per increment. Optical data were collected throughout the duration of the temperature drop, with a dramatic increase in fluorescence seen when the strands re-annealed. The relative expression of the real-time reverse transcription-PCR products was determined using the ΔΔCt method. This method calculates relative expression using the following equation: fold induction $=2^{-[\Delta\Delta C_t]}$, where Ct = the threshold cycle, i.e. the cycle number at which the sample's relative fluorescence rises above the background fluorescence, and ΔΔCt = [Ct gene of interest (unknown sample) − Ct housekeeping gene (unknown sample)]/[Ct gene of interest (calibrator sample) − Ct housekeeping gene (calibrator sample)]. One of the control samples was chosen as the calibrator sample and used in each PCR.

SOD activity assay

SOD activity was determined using a Ransod commercial assay kit (Randox, Antrim, UK). This method employs xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-introphenol)-5-phenyl tetrazolium chloride to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. For the assay of Mn-SOD activity, potassium cyanide (final concentration of 3 mM) was added to the incubation mixture in order to inhibit Cu/Zn-SOD activity. The activity of Cu/Zn-SOD was derived by the subtraction of Mn-SOD from the total SOD activity.[12] The units of SOD activity were derived from a standard curve generated using purified SOD (Ransod) from bovine erythrocytes. The results were expressed as SOD units per gram hemoglobin. Hemoglobin concentration was determined by the cyanomethemoglobin method.[13]

CAT activity assay

CAT activity was measured as described by Klotz *et al.*[19] The decrease in optical absorption observed at 240 nm was measured at time 0 and 30 seconds following addition of hydrogen peroxide to erythrocyte hemolysates. A higher level of CAT activity resulted in a lower level of observed absorption.

Total antioxidant assay

Plasma antioxidant capacity was evaluated using a total antioxidant assay kit (Randox, UK). This assay uses antioxidants as reducing agents in a redox-linked colorimetric method. In this assay, ABTS (2,2′-azino–di-[3-ethylbenz thiazonoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and hydrogen peroxide to produce the cation radical ABTS*. This cation has a relatively stable blue-green color that is measured at 600 nm. The antioxidant is then added to the tube which causes the suppression of this color to a degree proportional to its concentration.

Statistical analysis

All data are presented as the mean ± SD for each group. We estimated the differences among the different treatments by *t*-test. Differences were considered statistically significant at $P < 0.05$.

Results

Effects of vitamin E on the activity of different SOD isoforms

The Mn-SOD activity of the rats receiving 10 mg/kg of vitamin E did not show any significant difference with respect to the corresponding control group after 2 and 4 weeks of treatment. The Mn-SOD activity was significantly increased after 6 weeks of treatment as compared with that of the control group (513 ± 30

versus 469 ± 31 U/gHb; $P < 0.03$). There was also a significant increase in the Mn-SOD activity from weeks 4 to 6 of treatment in this group (480 ± 17 versus 513 ± 30 U/gHb; $P < 0.03$). The Mn-SOD activity of rats in the 30-mg/kg dosing group was significantly increased compared with that of the control group after 4 weeks (450 ± 49 versus 580 ± 90 U/gHb; $P < 0.01$) and 6 weeks (469 ± 31 versus 953 ± 39 U/gHb; $P < 0.001$) of vitamin E treatment. A significant increase in the Mn-SOD activity from weeks 4 to 6 of treatment was observed in this group (580 ± 90 versus 953 ± 39 U/gHb; $P < 0.0001$). The same pattern was observed in the Mn-SOD activity of the rats receiving 100 mg/kg of vitamin E. The difference was statistically significant after 4 weeks (450 ± 49 U/gHb versus 620 ± 50 U/gHb; $P < 0.001$) and 6 weeks (469 ± 31 U/gHb versus 1050 ± 18 U/gHb; $P < 0.0001$) compared with the respective values in the control group. Similarly, a significant increase in the Mn-SOD activity from weeks 4 to 6 of treatment was observed in this group (620 ± 50 U/gHb versus 1050 ± 18 U/gHb; $P < 0.0001$) (Fig. 1).

In contrast, the activity of the other isoform, Cu/Zn-SOD, did not follow the same pattern. Values of Cu/Zn-SOD activity generally ranged between 1000 and 1300 U/gHb, and there was no significant change at any time point in any vitamin E treatment schedule compared with the control or the preceding time point (Fig. 2).

Effects of vitamin E on the mRNA level of different SOD isoforms

Mn-SOD mRNA did not show any significant change during the 6-week experimental course in the 10-mg/kg dosing group. In the 30-mg/kg dosing group,

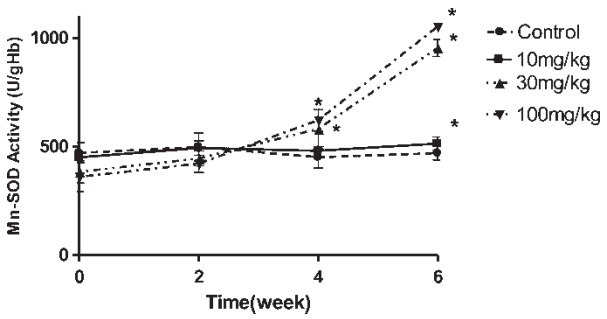

Figure 1 Dose–response curve of Mn-SOD activity. Mn-SOD activity (U/gHb) was significantly increased by vitamin E treatment in the 100-mg/kg dosing group after 4 weeks ($P < 0.001$) and 6 weeks ($P < 0.0001$). In the 30-mg/kg dosing group, vitamin E exerted a weaker, yet eventually enhancing effect on Mn-SOD activity which was statistically significant after 4 weeks ($P < 0.01$) and 6 weeks ($P < 0.001$). The lowest dose (10 mg/kg) had a slightly enhancing effect on Mn-SOD activity only after 6 weeks of treatment ($P < 0.03$). Error bars indicate the standard deviation. *Statistically significant differences compared with control group at the same time point.

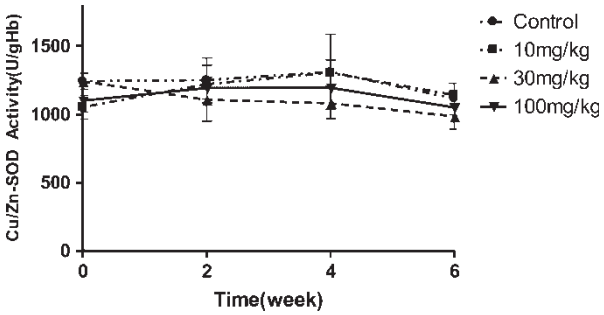

Figure 2 Dose–response curve of Cu/Zn-SOD activity. Cu/Zn-SOD (U/gHb) activity was not significantly changed in the 10-, 30-, or 100-mg/kg dosing groups in response to vitamin E administration. Error bars indicate the standard deviation.

Mn-SOD mRNA was significantly increased after 4 (1.88 ± 0.11 versus 0.95 ± 0.11; $P < 0.0001$) and 6 weeks of treatment (1.65 ± 0.18 versus 1.23 ± 0.21; $P < 0.001$). In the 100-mg/kg dosing group, the changes were more robust compared with those of the 30-mg/kg group, and the mRNA level was significantly increased after 4 weeks (2.11 ± 0.14 versus 0.95 ± 0.11; $P < 0.0001$) and 6 weeks (2.23 ± 0.12 versus 1.23 ± 0.21; $P < 0.0001$) of vitamin E injection (Fig. 3).

Vitamin E did not have any effect on the Cu/Zn-SOD mRNA in the 10-mg/kg dosing group at any time points. Vitamin E did cause a significant increase in Cu/Zn-SOD mRNA after 6 weeks in the 30-mg/kg dosing group (1.79 ± 0.13 versus 1.40 ± 0.06; $P < 0.001$). Vitamin E induced a significant increase (1.76 ± 0.24 versus 1.15 ± 0.10; $P < 0.001$) in Cu/Zn-SOD mRNA levels after 4 weeks in the 100-mg/kg vitamin E treatment group (Fig. 4).

Effects of vitamin E on CAT enzyme activity

The CAT activity in rats receiving 10 mg/kg of vitamin E did not show any significant difference

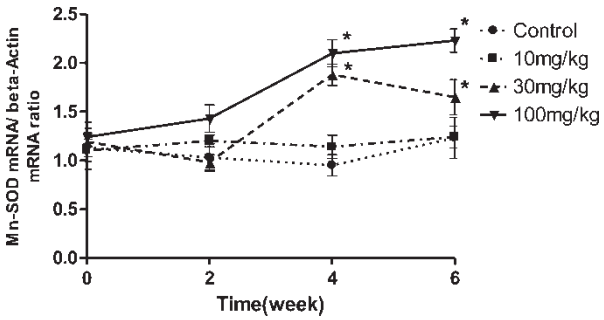

Figure 3 Effect of different doses of vitamin E on Mn-SOD mRNA compared with the correspondent housekeeping gene in rat blood cells in the control and three dosing groups. Mn-SOD mRNA was significantly increased after 4 weeks ($P < 0.0001$) and 6 weeks ($P < 0.001$) in the 30-mg/kg dosing group. Vitamin E had a similar effect in the 100-mg/kg group with statistically significant increase after 4 weeks ($P < 0.0001$) and 6 weeks ($P < 0.0001$). Error bars indicate the standard deviation. *Statistically significant differences compared with control group at the same time point.

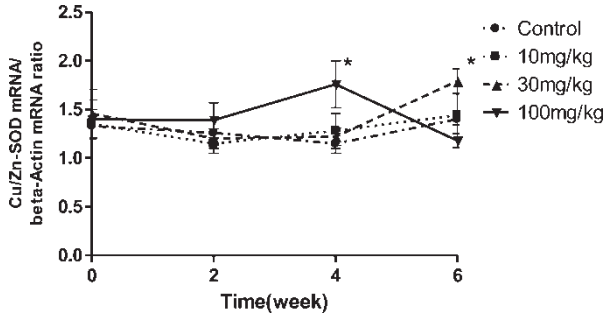

Figure 4 Effect of different doses of vitamin E on Cu/Zn-SOD mRNA compared with the correspondent housekeeping gene in rat blood cells in the control and three dosing groups. Cu/Zn-SOD mRNA was weakly but significantly increased after 6 weeks of treatment in the 30-mg/kg dosing group ($P < 0.001$) and after 4 weeks in the 100-mg/kg dosing group ($P < 0.001$). Error bars indicate the standard deviation. *Statistically significant differences compared with control group at the same time point.

compared with the corresponding control group after 2, 4, and 6 weeks of treatment. The CAT activity in rats in the 30-mg/kg dosing group was significantly increased compared with the control group after 4 weeks (43.2 ± 9.2 U/gHb versus 15 ± 4.9 U/gHb; $P < 0.0001$) of vitamin E treatment. A significant increase in the CAT activity from weeks 2 to 4 of treatment was observed in this group (43.2 ± 9.2 U/gHb versus 20.14 ± 5.2 U/gHb; $P < 0.0001$). Rats receiving 100 mg/kg of vitamin E showed the same pattern of changes in the CAT activity. The difference was statistically significant after 4 weeks (37.5 ± 11.3 U/gHb versus 15 ± 4.9 U/gHb; $P < 0.001$) compared with the control group (Fig. 5).

Effects of vitamin E on total antioxidant defense

Plasma ABTS reactivity, representing the total plasma antioxidant defense, did not change

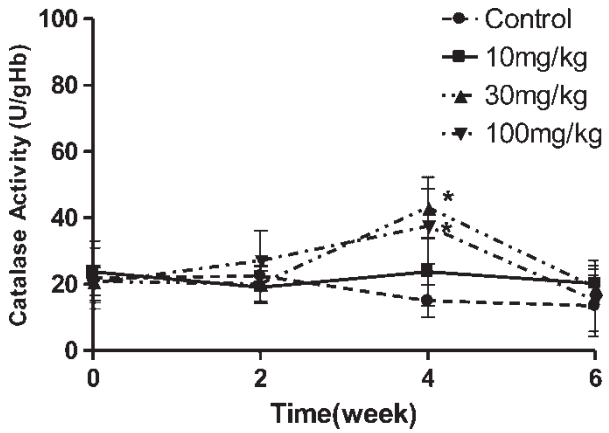

Figure 5 Dose–response curve of CAT activity. CAT activity (U/gHb) was significantly and transiently increased by vitamin E treatment in the 30-mg/kg ($P < 0.0001$) and 100-mg/kg ($P < 0.001$) dosing groups after 4 weeks of treatment. The lowest dose of vitamin E (10 mg/kg) did not have any effect on CAT activity. Error bars indicate the standard deviation. *Statistically significant differences compared with control group at the same time point.

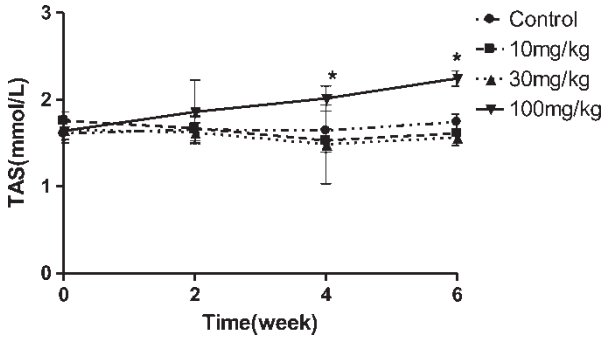

Figure 6 Total plasma antioxidant status (TAS) showed a continuous increase from 2 weeks until the end of the study in the 100-mg/kg dosing group, which was statistically significant after 4 weeks ($P < 0.0001$) and 6 weeks ($P < 0.0001$) of treatment. Total antioxidant defense was unchanged in the groups receiving 10 and 30 mg/kg of vitamin E. Error bars indicate the standard deviation. *Statistically significant differences compared with control group at the same time point.

significantly during the 6-week study period in the control, 10-, and 30-mg/kg dosing groups. However, total antioxidant defense showed a continuous increase from week 2 until the end of the study in the 100-mg/kg dosing group, which was statistically significant after 4 weeks (2.01 ± 0.14 versus 1.64 ± 0.04; $P < 0.0001$) and 6 weeks (2.24 ± 0.08 versus 1.74 ± 0.09; $P < 0.0001$) compared with the control group (Fig. 6).

Discussion

It was shown in this study that vitamin E enhances Mn-SOD expression in a time- and dose-dependent manner. Vitamin E can up-regulate Mn-SOD mRNA via a direct increase in the transcriptional activity of the SOD gene, as it is a known modulator of several of the SOD transcription factors such as AP-1 and NF-kappa B,[20,21] located in the promoter region of the Mn-SOD.[22,23] Vitamin E can also up-regulate SOD mRNA by inhibiting protein kinase C (PKC)-alpha which is shown to be involved in the regulation of Mn-SOD levels in some models,[24–27] and vitamin E is a known inhibitor of PKC.[12] In addition, through changing the intracellular redox state, vitamin E can indirectly change the activity of several transcription factors involved in the transcriptional regulation of Mn-SOD, such as NF-kappa B.[19,28,29] Furthermore, as ROS are potent inhibitors of SOD, vitamin E can up-regulate SOD activity through its superoxide scavenging activity.[30–32]

Differential regulation of Mn-SOD and Cu/Zn-SOD by vitamin E has been previously shown in a few biological systems other than the blood.[33–36] It has been shown previously that transgenic mice expressing Mn-SOD in pulmonary tissues are more resistant to oxygen toxicity,[37] and transgenic mice expressing Mn-SOD in the heart are protected

against adriamycin-induced cardiac toxicity[38] or myocardial ischemia-reperfusion injury.[39] On another note, over-expression of the Cu/Zn-SOD has been implicated in the pathogenesis of the rapid aging featured in the brains of patients with Down's syndrome.[40] Over-expression of Cu/Zn-SOD gene in mice is also associated with the increased generation of ROS and oxidative damage.[41] On the basis of the above-mentioned literature, our results imply that the effects of vitamin E on enhancing Mn-SOD activity and the lack of any effect on Cu/Zn-SOD activity are both beneficial for the antioxidant-induced defensive mechanisms of the system.

Induction of antioxidant enzymes in mammalian cells are generally accompanied by an increased tolerance to the oxidative stress.[42–44] In our study, CAT activity had a transient increase after 4 weeks of treatment, which was predictable as CAT and GPx both act to scavenge SOD products (H_2O_2). In the absence of adequate amounts of CAT, hydrogen peroxide might be expected to undergo conversion to highly toxic hydroxyl radicals by way of the Fentontype reaction.[45] Subsequently, when the H_2O_2 product of SOD was probably more scavenged by other enzyme systems, such as GPx, CAT activity was eventually decreased. This effect can still be in line with potentiating the anti-oxidant capacity, considering that relative level of SOD activity compared with CAT activity is a key factor for efficient SOD activity, and that the combined activity of CAT and SOD could potentially lead to either positive or negative effects on the anti-oxidant defense potential.[46]

We have previously shown in a comparable study design that a dose of 100 mg/kg of vitamin E administered bi-weekly results in a maximum plasma level of 35 μM in rats and doses above this level will have pro-oxidant activity.[17,18] The upper limit of total oral consumption of vitamin E by humans is considered to be 1000 mg (1400 IU) per day which can increase the plasma level of vitamin E up to 85 μM.[18] In this study, doses below 100 mg/kg did not significantly increase antioxidant defense. We also did not see any pro-oxidant effect in the 100-mg/kg dosing group. Thus, it is tempting to conclude that this dosage (100 mg/kg twice weekly) might be considered in the design of clinical trials as an optimum dosage of vitamin E. Nevertheless, the relationship between plasma antioxidant status and other target tissues should not be overestimated. For example, after an initial increase in the tissue concentration of vitamin E, it reaches a saturated plateau in the central nervous system and brain tissues.[47] Therefore, it might still be questionable whether the doses proven to be effective in enhancing the antioxidant status in blood, will have the same effect in tissues with a pathologic state such as tumors or vascular cells.

Considering that SODs function as master keys controlling cellular ROS levels, SODs and SOD mimetics may have potential uses as therapeutic agents in oxidative stress-related diseases.[4] For instance, SOD can be used in treating mustard gas burns.[48] Furthermore, the positive effects of SOD on the recovery of human hematopoietic stem cells have been successfully shown.[49] In addition, up-regulation of SOD expression inhibits breast cancer growth.[50] Gene therapy approaches have been undertaken with some success in order to increase the level of SOD before radiotherapy.[51] On the basis of our results, vitamin E administration in an optimum dosage is a safe method to enhance the expression of Mn-SOD, and thus can be considered as a therapeutic strategy in the pathological conditions in which over-expression of SOD is desirable.

Acknowledgement

This study was financially supported by Grant Number 123-456-781 from Tehran University of Medical Sciences.

References

1 Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. J Nutr Biochem 2007;18(9):567–79.
2 Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39(1):44–84.
3 Holley AK, Dhar SK, St Clair DK. Manganese superoxide dismutase vs. p53: regulation of mitochondrial ROS. Mitochondrion 2010;10(6):649–61.
4 Yan F, Mu Y, Yan G, Liu J, Shen J, Luo G. Antioxidant enzyme mimics with synergism. Mini Rev Med Chem 2010;10(4):342–56.
5 Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med 2002;33(3):337–49.
6 Abreu IA, Cabelli DE. Superoxide dismutases-a review of the metal-associated mechanistic variations. Biochim Biophys Acta 2010;1804(2):263–74.
7 Vertuani S, Angusti A, Manfredini S. The antioxidants and pro-antioxidants network: an overview. Curr Pharm Des 2004; 10(14):1677–94.
8 Johnson F, Giulivi C. Superoxide dismutases and their impact upon human health. Mol Aspects Med 2005;26(4–5):340–52.
9 Holley AK, Dhar SK, Xu Y, St Clair DK. Manganese superoxide dismutase: beyond life and death. Amino Acids 2012;42(1): 139–58.
10 Azzi A, Gysin R, Kempa P, Ricciarelli R, Villacorta L, Visarius T, *et al.* Regulation of gene and protein expression by Vit E. Free Radic Res 2002;36:30–6
11 Azzi A, Breyer I, Feher M, Pastori M, Ricciarelli R, Spycher S, *et al.* Specific cellular responses to alpha-tocopherol. J Nutr 2000;130(7):1649–52.
12 Azzi A, Ricciarelli R, Zingg JM. Non-antioxidant molecular functions of alpha-tocopherol (Vit E). FEBS Lett 2002; 519(1–3):8–10.
13 Azzi A, Gysin R, Kempná P, Munteanu A, Villacorta L, Visarius T, *et al.* Regulation of gene expression by alpha-tocopherol. Biol Chem 2004;385(7):585–91.
14 Niki E. Vitamin C as an antioxidants. Simopoulus AP. Vitamins, minerals, and functional consequences of maternal malnutrition. World Res Nutr Diet 1991;64:4–30.
15 Cases N, Sureda A, Mastre I, Tauler P, Cordova A, Roche E, *et al.* Response of antioxidant defences to oxidative stress induced by prolonged exercise: antioxidant enzyme gene expression in lymphocyte. Eur J App Physiol 2006;98(3):263–9.

16 Gutteridge JM, Halliwell B. Antioxidants: molecules, medicines, and myths. Biochem Biophys Res Commun 2010;393(4):561–4.
17 Golestani A, Rastegar R, Shariftabrizi A, Khaghani S, Payabvash SM, Salmasi AH, *et al.* Paradoxical dose- and time-dependent regulation of superoxide dismutase and antioxidant capacity by Vit E in rat. Clin Chim Acta 2006;365(1–2):153–9.
18 Hajiani M, Golestani A, Shariftabrizi A, Rastegar R, Payabvash S, Salmasi AH, *et al.* Dose-dependent modulation of systemic lipid peroxidation and activity of anti-oxidant enzymes by Vit E in the rat. Redox Rep 2008;13(2):60–6.
19 Klotz MG, Klassen GR, Loewn PC. Phylogenetic relationships among prokaryotic and eukaryotic catalases. Mol Biol Evol 1997;14:951–8.
20 Rimbach G, Minihane AM, Majewicz J, Fischer A, Pallauf J, Virgli F, *et al.* Regulation of cell signalling by Vit E. Proc Nutr Soc 2002;61(4):415–25.
21 Stäuble B, Boscoboinik D, Tasinato A, Azzi A. Modulation of activator protein-1 (AP-1) transcription factor and protein kinase C by hydrogen peroxide and D-alpha-tocopherol in vascular smooth muscle cells. Eur J Biochem 1994;226(2):393–402.
22 Fernández V, Tapia G, Varela P, Castillo I, Mora C, Moya F, *et al.* Redox up-regulated expression of rat liver manganese superoxide dismutase and Bcl-2 by thyroid hormone is associated with inhibitor of kappaB-alpha phosphorylation and nuclear factor-kappaB activation. J Endocrinol 2005;186(3): 539–47.
23 Miao L, St Clair DK. Regulation of superoxide dismutase genes: implications in disease. Free Radic Biol Med 2009; 47(4):344–56.
24 Das KC, Guo XL, White CW. Protein kinase Cdelta-dependent induction of manganese superoxide dismutase gene expression by microtubule-active anticancer drugs. J Biol Chem 1998; 273(51):34639–45.
25 Niwa Y, Ozaki Y, Kanoh T, Akamatsu H, Kurisaka M. Role of cytokines, tyrosine kinase, and protein kinase C on production of superoxide and induction of scavenging enzymes in human leukocytes. Clin Immunol Immunopathol 1996;79(3):303–13.
26 Suzuki K, Tatsumi H, Satoh S, Senda T, Nakata T, Fujii J, *et al.* Manganese-superoxide dismutase in endothelial cells: localization and mechanism of induction. Am J Physiol 1993; 265(4 Pt 2):H1173–8.
27 Liu M, Wallmon A, Olsson-Mortlock C, Wallin R, Saldeen T. Mixed tocopherols inhibit platelet aggregation in humans: potential mechanisms. Am J Clin Nutr 2003;77(3):700–6.
28 Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 2007; 292(1):R18–36.
29 Ji LL, Gomez-Cabrera MC, Vina J. Role of nuclear factor kappaB and mitogen-activated protein kinase signaling in exercise-induced antioxidant enzyme adaptation. Appl Physiol Nutr Metab 2007;32(5):930–5.
30 Bray RC, Cockle SA, Fielden EM, Roberts PB, Rotilio G, Calabrese L. Reduction and inactivation of superoxide dismutase by hydrogen peroxide. Biochem J 1974;139:43–8.
31 Meier B, Sehn AP, Michel C, Saran M. Reactions of hydrogen peroxide with superoxide dismutase from Propionibacterium shermanii – an enzyme which is equally active with iron or manganese – are independent of the prosthetic metal. Arch Biochem Biophys 1994;313:296–303.
32 Fujii J, Taniguchi N. Down regulation of superoxide dismutases and glutathione peroxidase by reactive oxygen and nitrogen species. Free Radic Res 1999;31:301–8.
33 Koch O, Farré S, De Leo ME, Palozza P, Palazzotti B, Borrelo S, *et al.* Regulation of manganese superoxide dismutase (MnSOD) in chronic experimental alcoholism: effects of Vit E-supplemented and -deficient diets. Alcohol 2000;35(2):159–63.
34 Sulekha S, Madhavi J, Venkateshwari A, Yasmeen S, Pratibha N. Superoxide dismutase phenotypes in duodenal ulcers: A genetic marker? Indian J Hum Genet 2006;12(3):125–8.
35 Frank S, Zacharowski K, Wary GM, Thiemermann C, Pfeilschifter J. Identification of copper/zinc superoxide dismutase as a novel nitric oxide regulated gene in rat glomerular mesengial cells and kidneys of endotoxemic rats. FASEB J 1999;13:869–82.
36 Sugino N, Telleria CM, Gibori G. Differential regulation of copper zinc superoxide dismutase and manganese superoxide dismutase in rat corpus luteum: induction of manganese superoxide dismutse messenger ribonucleic acid by inflammatory cytokines. Biol Reprod 1998;59:208–15.
37 Wispé JR, Warner BB, Clark JC, Dey CR, Neuman J, Glasser SW, *et al.* Human Mn-superoxide dismutase in pulmonary epithelial cells of transgenic mice confers protection from oxygen injury. J Biol Chem 1992;267(33):23937–41.
38 Yen HC, Oberley TD, Vichitbandha S, Ho YS, St Clair DK. The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice. J Clin Invest 1996;98(5):1253–60.
39 Chen Z, Siu B, Ho YS, Vincent R, Chua CC, Hamdy RC, *et al.* Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. J Mol Cell Cardiol 1998;30(11):2281–9.
40 Ceballos-Picot I, Nicole A, Briand P, Grimber G, Delacourte A, Defossez A, *et al.* Neuronal-specific expression of human copper-zinc superoxide dismutase gene in transgenic mice: animal model of gene dosage effects in Down's syndrome. Brain Res 1991;552(2):198–214.
41 Ditelberg JS, Sheldon RA, Epstein CJ, Ferriero DM. Brain injury after perinatal hypoxia-ischemia is exacerbated in copper/zinc superoxide dismutase transgenic mice. Pediatr Res 1996;39(2):204–8.
42 Chow CK. Biochemical responses in lungs of ozone-tolerant rats. Nature 1976;260(5553):721–2.
43 Clerch LB, Massaro D. Tolerance of rats to hyperoxia. Lung antioxidant enzyme gene expression. J Clin Invest 1993;91(2): 499–508.
44 Rahman I, Clerch LB, Massaro D. Rat lung antioxidant enzyme induction by ozone. Am J Physiol 1991;260(6 Pt 1):L412–8.
45 Mao GD, Thomas PD, Lopaschuk GD, Poznansky MJ. Superoxide dismutase (SOD)-catalase conjugates. Role of hydrogen peroxide and the Fenton reaction in SOD toxicity. J Biol Chem 1993;268(1):416–20.
46 Michiels C, Raes M, Toussaint O, Remacle J. Importance of Seglutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic Biol Med 1994;17: 235–48.
47 Martin A, Janigian D, Shukitt-Hale B, Prior RL, Joseph JA. Effect of Vit E intake on levels of vitamins E and C in the central nervous system and peripheral tissues: implications for health recommendations. Brain Res 1999;845(1):50–9.
48 Eldad A, Ben Meir P, Breiterman S, Chaouat M, Shafran A, Ben-Bassat H. Superoxide dismutase (SOD) for mustard gas burns. Burns 1998;24:114–9.
49 Ma EP, Liu XZ, Liu MD, Han Y, Lui X, Wu ZZ. The effect of superoxide dismutase on the recovery of human bone marrow hemopoietic stem cells stored at 4°C. Cryobiology 1998;37: 372–5.
50 Weydert CJ, Waugh TA, Ritchie JM, Iyer KS, Smith JL, Li L, *et al.* Overexpression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. Free Radic Biol Med 2006;41(2):226–37.
51 Greenberger JS, Epperly MW. Antioxidant gene therapeutic approaches to normal tissue radioprotection and tumor radiosensitization. In Vivo 2007;21(2):141–6.