TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2006; 36 (3): 141-146
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr

The Effect of Pre-Injury Supplementation with Selenium or Vitamin E on Lipid Peroxidation and Antioxidant Enzymes in Burn Injury

Ramazan MEMİŞOĞULLARI[1]
Kenan GÜMÜŞTEKİN[2]
Şenol DANE[2]
Fatih AKÇAY[1]

[1] Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum - TURKEY

[2] Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum - TURKEY

Aim: The purpose of this experimental study was to investigate the effect of pre-injury supplementation with vitamin E or selenium on antioxidant enzyme and malondialdehyde levels, as markers of lipid peroxidation, after thermal injury to rats.

Materials and Methods: The animals were divided into 3 groups: Group 1 (n = 15): controls, no supplementation; Group 2 (n = 14): received vitamin E (100 mg/kg per day) for 10 days pre-burn; Group 3 (n = 14): received selenium (4 ppm in drinking water) for 10 days pre-burn. All animals were given second-degree burns. Serum and liver tissues of the rats were sampled 3 d after being burned. Serum and liver tissue superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione S-transferase (GST) activity and malondialdehyde (MDA) levels were measured.

Results: Group 2 had significantly higher serum and liver tissue SOD, GSH-Px, and liver tissue CAT activity when compared to Group 1. In contrast, serum and tissue MDA levels and tissue GST activity were significantly lower in Group 1. Group 3 had greater serum and liver tissue SOD and GSH-Px activity, and lower MDA levels compared to Group 1.

Conclusions: These data revealed that supplementation with vitamin E or selenium during the pre-injury period decreased lipid peroxidation and increased liver tissue SOD, GSH-Px, CAT, serum SOD, and GSH-Px activity following thermal injury to rats. Thus, supplementation with vitamin E or selenium, at appropriate doses, for patients at high risk of oxidative damage, such as surgical patients, may have beneficial effects.

Key Words: Selenium, vitamin E, antioxidant enzyme, malondialdehyde, burn injury

Yanık Hasarında Hasar Öncesi Selenyum veya E Vitamini Desteğinin Lipid Peroksidasyonu ve Antioksidan Enzimler Üzerine Etkisi

Amaç: Bu deneysel çalışmanın amacı hasardan önce E vitamini veya selenyum verilmesinin termal incinme sonrası lipid peroksidasyonunun bir göstergesi olarak malondialdehid düzeyleri ve antioksidan enzimler üzerine etkisini araştırmaktı.

Yöntem ve Gereç: Grup 1 (n=15) yanıklı kontrol sıçanlar, grup 2 (n=14) yanık öncesi 10 gün 100 mg/kg/gün E vitamini alan sıçanlar ve grup 3 (n=14) yanık öncesi 10 gün, içinde 4 ppm selenyum bulunan içme suyunu kullanan sıçanlar olmak üzere hayvanlar 3 gruba ayrıldı. Serum ve karaciğer dokusu süperoksid dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GSH-Px) ve glutatyon s-transferaz (GST) aktiviteleri ve malondialdehid düzeyleri (MDA) ölçüldü.

Bulgular: E vitamini grubu kontrol grubu ile karşılaştırıldığında anlamlı şekilde artmış serum ve karaciğer SOD, GSH-Px ve karaciğer katalaz aktivitelerine sahipti. Serum ve karaciğer dokusu MDA düzeyleri ve karaciğer GST aktivitesi ise aksine E vitamini grubunda anlamlı şekilde azalmıştı. Selenyum grubu, kontrol grubuyla karşılaştırıldığında artmış serum ve karaciğer dokusu SOD ve GSH-Px aktiviteleri ve azalmış MDA düzeylerine sahipti.

Sonuç: Bu veriler, yanmış sıçanlarda hasardan önceki periyotta E vitamini veya selenyum verilmesinin, termal incinme sonrası karaciğer dokusu SOD, GSH-Px, CAT, serum SOD ve GSH-Px aktivitelerini artırdığı ve lipid peroksidasyonunu azalttığını göstermektedir. Böylece cerrahi gibi oksidatif hasar için yüksek riske sahip bireylere uygun dozlarda E vitamini veya selenyum verilmesi yararlı etkiler sağlayabilir.

Anahtar Sözcükler: Selenyum, vitamin E, an tioksidan enzimler, malondialdehit, yanık

Received: January 03, 2005
Accepted: March 24, 2006

Correspondence
Ramazan MEMİŞOĞULLARI
Department of Biochemistry,
Abant İzzet Baysal University,
Düzce Medical School,
Düzce - TURKEY

rmemisogullari@hotmail.com

* This study was presented as a poster at 'Klinik Biyokimya Uzmanları Derneği 3. Ulusal Biyokimya Kongresi, 21-25 September 2005, Antalya'.

Introduction

In the literature, data from experimental and clinical studies support the role of reactive oxygen species (ROS) in systemic inflammatory response syndrome and post-burn organ dysfunction (1,2). ROS include superoxide radical (O_2), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2). ROS, particularly OH, interact with lipids, proteins, and nucleic acids, resulting in loss of membrane integrity, structural or functional changes in proteins, and genetic mutations, respectively. To overcome the toxic effect of ROS, the body utilizes several antioxidant defense systems, including both enzymatic [such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione S-transferase (GST)] and nonenzymatic (such as glutathione, vitamin A and vitamin E) components (3,4). $O_2^{\cdot}$ is generated in mitochondria and is converted into H_2O_2 by SOD. H_2O_2, in the presence of O_2 and iron, forms an $^{\cdot}OH$, a more reactive form, which is then converted into lipid peroxide. CAT is present in peroxisomes in eukaryotic cells and it can transform H_2O_2 into H_2O and O_2. GSH-Px is a selenoprotein, which reduces lipidic and non-lipidic hydroperoxides, as well as H_2O_2, while oxidizing glutathione (2,3). GST catalyzes the conjugation of glutathione with toxic metabolites, which subsequently results in detoxification of toxic metabolites (5).

The process of lipid peroxidation involves oxidative conversion of polyunsaturated fatty acids into malondialdehyde (MDA), which is a marker of lipid peroxidation and is usually measured as thiobarbituric acid reactive substances (TBARS), or lipid peroxides (3).

Selenium (Se) is an essential micronutrient, and small amounts of it are needed for tissue oxygenation and for protection against lipid peroxidation. It is involved in one of the main antioxidant defense systems of the body with GSH-Px, which is the key plasma selenoprotein consisting of 4 identical subunits, each containing one covalently bound Se atom (6).

Vitamin E is a well-known antioxidant agent that protects cell membranes, especially fatty acids, against oxidative damage caused by various pollutants, peroxides, and free radicals formed during metabolic processes. It is known to decrease oxygen radical release from neutrophils (2).

During a literature review, we found that although some papers about the effect of antioxidant supplementation after injury on blood and tissue antioxidant enzymes or lipid peroxidation levels exist, we did not find any studies dealing with antioxidant supplementation before injury occurs. Therefore, we planned this experimental study to investigate the effect of supplementation with vitamin E or Se, before burn injury, on SOD, CAT, GSH-Px, and GST activity, and MDA levels post thermal injury in rats, since it is interesting to investigate the influence of pre-injury, supra-normal antioxidant supplementation on the evolution of antioxidant parameters. Supplementation with vitamin E or Se before burn injury may not occur in reality, but our aim was to use burn injury as a standard model of oxidative stress. Our purpose was to evaluate pre-injury supplementation with antioxidants on planned oxidative stress (like surgery).

Materials and Methods

Animal experiments

Male Sprague Dawley rats, weighing 230-260 g, were used for the experiment. All animals received humane care in compliance with the guidelines of the criteria of The Atatürk University Research Council. The Atatürk University Medical Faculty Ethics Committee approved the study protocol. The rats were fed standard laboratory chow and water, and were housed in a temperature-controlled environment (22 ± 2 °C); 4-5 rats were housed in each cage.

The animals were divided into 3 groups. There were 15 rats in Group 1, 14 in Group 2, and 14 in Group 3.

Group 1 (n = 15): controls, received only intragastric 0.9% NaCl solution for 10 days pre-burn,

Group 2 (n = 14): received intragastric vitamin E (100 mg/kg per day) (7) for 10 days pre-burn,

Group 3 (n = 14): received selenium (4 ppm in drinking water) for 10 days pre-burn.

Vitamin E (Ephynal, 300 mg capsule, Roche, France) was dissolved in corn oil (30 mg/ml) and administered orally through a stomach tube. Se (sodium selenite anhydrous, 44%-46% Se, Acros Organics, Belgium) was added to drinking water at a concentration of 4 ppm.

Full skin thickness burns were produced with the method of Arons et al.'s burn model (8). Under light ether anesthesia, rats were injected intraperitoneally with

ketamine-HCl (Ketalar, 20 mg/kg). After carefully shaving the surface of the gluteal region, each rat was exposed for 20 s to a metal apparatus 1 cm in diameter heated to 90 °C. Thus, a second-degree burn approximately 1 cm^2 was formed on the surface of the gluteal region. The animals in all 3 groups were sacrificed 3 days later and intracardiac blood and liver tissues were sampled.

Biochemical analyses

Reduced glutathione (GSH), NADPH, glutathione disulfide reductase, H_2O_2, xanthine, xanthine oxidase, CuCl2, nitroblue tetrazolium, trichloroacetic acid, thiobarbituric acid, and 1-chloro-2,4-dinitrobenzene were obtained from Sigma (St. Louis, MO, USA). All other chemicals and reagents used were of the highest quality available.

Blood samples were collected in vacutainers without additive, allowed to clot for 30 min at room temperature, and were then centrifuged at 3000 x g for 5 min to get serum. The serum was divided into aliquots. Serum aliquots were stored for 3 months at -80 °C until biochemically analyzed.

Tissues were homogenized in a homogenizer (Omni TH International, USA). After centrifugation at 10,000 x g for about 60 min, the upper clear layer was taken. The homogenate was divided into aliquots. These aliquots of homogenate were stored for 3 months at -80 °C until biochemically analyzed. Each tissue and serum sample had 5 sections analyzed (each parameter was measured in one sitting). Protein was identified using the Bradford method (9).

MDA in serum and liver tissue was measured by the thiobarbituric acid method (10). Aliquots of 0.2 ml of supernatant fraction/serum were mixed thoroughly with 0.8 ml of phosphate-buffered saline (pH 7.4) and 0.025 ml of butylated hydroxytoluene solution. After adding 0.5 ml of 30% trichloroacetic acid, the samples were placed on ice for 2 h and then centrifuged at 2000 x g at 25 °C for 15 min. One milliliter of supernatant was mixed with 0.075 ml of 0.1 mol/l ethylenediamine tetra acetic acid and 0.25 ml 1% thiobarbituric acid in 0.05 N sodium hydroxide and placed on boiling water for 15 min; when cooled to room temperature, absorbance at 532 nm was determined. TBARS were expressed as MDA, using a molar extinction coefficient for MDA of 1.56 x 105 cm^{-1} M^{-1}. Serum and tissue MDA levels were expressed as nmol/ml and nmol/mg protein, respectively.

SOD activity was calculated according to Sun and co-workers (11). Serum and tissue SOD activity were expressed as U/ml and U/mg protein, respectively.

GSH-Px activity was measured according to the Paglia and Valentina method (12). Serum and tissue GSH-Px activity are expressed as U/ml and U/mg protein, respectively.

CAT activity was measured in supernatant at 20 ºC according to the method of Aebi (13). Using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}, the rate of the first 30 s was used to calculate the unit of activity as U/mg protein.

The activity of GST was determined with the method of Habig et al. (14) by following the increase in absorbance at 310 nm using 1-chloro-2, 4-dinitrobenzene as substrate.

All biochemical measurements were performed with a spectrophotometer (CECIL CE 3041, Cambridge, UK).

Statistics

SPSS for Windows (version 10.0, Chicago, IL, USA) was used for statistical analyses. The results were expressed as means ± SD. Results were analyzed with Mann-Whitney U test and Pearson's rank correlation test. The accepted level of significance was $P < 0.05$.

Results

All parameters are shown in the Table.

When Group 2 was compared to Group 1: serum and liver tissue SOD ($P < 0.001$, for both) and GSH-Px ($P < 0.005$, for both) activity were significantly greater in Group 2, and liver tissue CAT activity ($P < 0.05$) was higher in Group 2; conversely, serum and tissue MDA levels ($P < 0.001$, $P < 0.005$, respectively), and tissue GST activity ($P < 0.05$) were significantly lower in Group 2.

Group 3 had greater serum and liver tissue SOD ($P < 0.005$, for both) and GSH-Px ($P < 0.001$, for both) activity, and lower MDA ($P < 0.005$, for both) levels than Group 1.

On the other hand, liver tissue SOD activity ($P < 0.05$) was lower and GSH-Px activity ($P < 0.05$) was higher in Group 3 than in Group 2.

Table. Liver tissue and serum antioxidant enzymes activity, and malondialdehyde levels in the study groups.

	GROUP 1 (Control, n = 15)	GROUP 2 (Vitamin E group, n = 14)	GROUP 3 (Selenium group, n = 14)
Liver Tissue			
SOD (U/mg prot)	1.54 ± 0.25	2.51 ± 0.76 [a]	1.95 ± 0.41 [b, Φ]
GSH-Px (U/mg prot)	37.53 ± 7.46	45.85 ± 8.29 [b]	47.57 ± 7.13 [a]
CAT (U/mg prot)	145.13 ± 38.85	163.07 ± 22.76 [d]	159.57 ± 30.95
GST (U/mg prot)	0.51 ± 0.08	0.40 ± 0.10 [c]	0.47 ± 0.11
MDA (nmol/mg prot)	7.09 ± 1.26	5.11 ± 1.62 [b]	5.29 ± 1.40 [b]
Serum			
SOD (U/ml)	0.48 ± 0.15	0.68 ± 0.10 [a]	0.66 ± 0.10 [b]
GSH-Px (U/ml)	22.69 ± 9.8	31.36 ± 10.3 [b]	45.07 ± 18.5 [a, Φ]
MDA (nmol/ml)	23.23 ± 5.06	16.66 ± 3.39 [a]	17.05 ± 4.44 b

a: $P < 0.001$; b: $P < 0.005$; c: $P < 0.01$; d: $P < 0.05$ when compared to the control group.

[Φ]: $P < 0.05$ when compared to Group 2

In Group 1, negative correlations were noted between liver tissue SOD and MDA ($r = -0.55$, $P < 0.05$), liver tissue CAT and MDA ($r = -0.55$, $P < 0.05$), liver tissue GSH-Px and MDA ($r = -0.58$, $P < 0.01$), serum GSH-Px and MDA ($r = -0.67$, $P < 0.01$), and serum SOD and MDA ($r = -0.69$, $P < 0.05$). Additionally, a positive correlation was found between liver tissue GST and MDA ($r = 0.52$, $P < 0.05$).

In Group 2, a negative correlation was present between liver tissue SOD and MDA ($r = -0.69$, $P < 0.01$), liver tissue CAT and MDA ($r = -0.62$, $P < 0.05$), and liver tissue GSH-Px and MDA ($r = -0.59$, $P < 0.05$).

Furthermore, in Group 3, significant negative correlations were observed between liver tissue SOD and MDA ($r = -0.65$, $P < 0.05$), liver tissue CAT and MDA ($r = -0.70$, $P < 0.005$), liver tissue GSH-Px and MDA ($r = -0.71$, $P < 0.005$), serum GSH-Px and MDA ($r = -0.66$, $P < 0.01$), and serum SOD and MDA ($r = -0.64$, $P < 0.05$).

Discussion

Under normal conditions, there exists a balance between free radicals and the natural scavengers of the body; but, during trauma, the balance is lost and reactive oxygen metabolites predominate (15). Increased generation of ROS in extracellular spaces is seen during inflammation, when relatively low concentrations of SOD and CAT increase the susceptibility of extracellular components to oxygen radical injury, which may stimulate chemotaxis for other inflammatory cells. The generation of free radicals in the absence of scavenging defenses might be the major cause of increased lipid peroxidation in injured rats (16). Various studies have shown that there is a close relationship between the intensity of lipid peroxidation and secondary complications following burns. Therefore, modulation of lipid peroxidation has become an important issue, and both endogenous and exogenous antioxidants have been used to decrease this phenomenon (17-20). Previous studies claimed that antioxidant vitamin supplements significantly increased plasma antioxidant levels (20-22). It has been reported that MDA production significantly increases in burned rats (19,23), and supplementation with vitamin E resulted in an increase in SOD (23,24) and CAT (25) activity, and a decrease in lipid peroxide (MDA) levels (23,24); moreover, an inverse correlation between vitamin E and lipid peroxides has been observed (24). Our study confirmed these papers' results. Contrary to the above papers though, we administered vitamin E prior to injury. Nevertheless, in another study, it is reported that vitamin E supplementation had no effect on erythrocyte CAT, SOD, and GSH-Px activity, either in male smokers or nonsmokers (26). It has been speculated that the protective effect of post-burn vitamin E supplementation to burned rats showed that it acted as an efficient free radical scavenger and protected neutrophil function by

elevating SOD levels and lowering MDA levels. The data of the present study are in agreement with the literature cited above.

Vitamin E is found throughout the body, in both cell and sub-cellular membranes, and is the major scavenger of oxygen radicals in membranes. Levels of vitamin E in plasma and tissues have been found to be reduced following tissue injury and immediately after burn injury. However, supplementation of vitamin E, through its antioxidant actions have been shown not only to reduce the generation of ROS and lipid peroxides after tissue injury, but also to prevent the accumulation of neutrophils within burned tissue (20). An increase in GSH-Px activity caused by vitamin E supplementation in baby hamster kidney fibroblast cell lines has also been reported (6). The present study confirmed this effect of pre-burn supplementation with vitamin E.

In skin ulcers or severely inflamed and erosive lesions due to burn wounds, lipid peroxides are markedly increased, and, during this stage, topical application of SOD extracted from a bovine source was dramatically effective in treating skin lesions (1).

Vitamin E terminates free radical reactions by donating its reactive hydrogen atom at the carbon-6 position to the oxygen radical to form a tocopheroxy radical. This radical is thought to be either recycled to a-tocopherol, by interacting with soluble antioxidants, or irreversibly oxidized into a-tocopherylquinone. Thus, a-tocopherol appears to be a crucial cellular defense agent against loss of normal membrane integrity due to oxidative attack (2,20,27,28). The fact that GST activity was inhibited in the presence of a-tocopherylquinone, as it is by other non-substrate ligands for GST, confirms that GST and a-tocopherylquinone interact directly. a-tocopherylquinone binds to GST and may be transported to the site of metabolism or excreted in bile as other non-substrate ligands for GST (27,28). This may explain the reduced GST activity in liver tissue of burned rats in Group 2 of the present study.

Berger et al. (29) examined Se losses and balances in 10 patients with burns, and observed lower Se and plasma GSH-Px concentrations that were indicative of a deficiency state, which could not be explained by the measured losses. Sanstrom et al. (30) reported that Se supplementation does not affect CAT activity in mammalian cells. However, there are studies reporting decreased CAT activity and increased GSH-Px activity in Se supplemented L1210 cells cultured in fetal bovine serum (31,32).

Our results indicated that supplementation with vitamin E or Se during the pre-injury period prevented lipid peroxidation and increased liver tissue SOD, GSH-Px, CAT, serum SOD, and GSH-Px activity in injured rats. Hence, supplementation with vitamin E or Se, at appropriate doses in patients at high risk for oxidative damage due to surgery, angiography, endoscopy, biopsy, cauterization, and radiotherapy may have beneficial effects.

References

1. Cetinkale O, Senel O, Bulan R. The effect of antioxidant therapy on cell-mediated immunity following burn injury in an animal model. Burns 1999; 25: 113-118.
2. Latha B, Babu M. The involvement of free radicals in burn injury: a review. Burns 2001; 27: 309-317.
3. Memisogullari R, Taysi S, Bakan E et al. Antioxidant status in type II diabetes mellitus. Cell Biochem Func 2003; 21: 291-296.
4. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001; 54: 176.
5. Sheweita SA, Abd El-Gabar M, Bastawy M. Carbon tetrachloride-induced changes in the activity of phase II drug-metabolizing enzyme in the liver of male rats: role of antioxidants. Toxicology 2001; 165: 217.
6. Gul M, Temocin S, Hanninen O. Selenium supplementation sensitizes renca cells to tert-butylhydroperoxide induced loss of viability. Indian J Exp Biol 2000; 38: 1020-1025.
7. Helen A, Krishnakumar K, Vijayammal PL et al. Antioxidant effect of onion oil (Allium cepa. Linn) on the damages induced by nicotine in rats as compared to alpha-tocopherol. Toxicol Lett 2000; 116: 61.
8. Arons MS. Burn wound infection--a review. Conn Med 1965; 29: 718-722.
9. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248.
10. Jain SK, McVie R, Duett J et al. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 1989; 38: 1539-1543.
11. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497-500.

12. Paglia DE, Valentina WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-169.

13. Aebi H. Catalase. In: Methods of Enzymatic Analysis. Ed. HU Bergmeyer, Verlag Chemie, Weinheim, 1974; Chap 2, pp 673-678.

14. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-7139.

15. Elsayed NM. Antioxidant mobilization in response to oxidative stress: a dynamic environmental-nutritional interaction. Nutrition 2001; 17: 828-834.

16. Latha B, Ramakrishnan M, Jayaraman V et al. The efficacy of trypsin: chymotrypsin preparation in the reduction of oxidative damage during burn injury. Burns 1998; 24: 532-538.

17. Demling RH, Lalonde C. Systemic lipid peroxidation and inflammation induced by thermal injury persists into the post-resuscitation period. J Trauma 1990; 30: 69-74.

18. Daryani R, LaLonde C, Zhu D et al. Effect of endotoxin and a burn injury on lung and liver lipid peroxidation and catalase activity. J Trauma 1990; 30: 1330-1334.

19. Berger MM, Cavadini C, Chiolero R et al. Copper, selenium, and zinc status and balances after major trauma. J Trauma 1996; 40: 103-109.

20. Chao JC, Huang CH, Wu SJ et al. Effects of beta-carotene, vitamin C and E on antioxidant status in hyperlipidemic smokers. J Nutr Biochem 2002; 13: 427-434.

21. Steinberg FM, Chait A. Antioxidant vitamin supplementation and lipid peroxidation in smokers. Am J Clin Nutr 1998; 68: 319-327.

22. Jialal I, Grundy SM. Effect of combined supplementation with alpha-tocopherol, ascorbate, and beta carotene on low-density lipoprotein oxidation. Circulation 1993; 88: 2780-2786.

23. Bekyarova G, Yankova T, Kozarev I et al. Reduced erythrocyte deformability related to activated lipid peroxidation during the early postburn period. Burns 1996; 22: 291.

24. Zhang MJ, Wang QF, Gao LX et al. Comparative observation of the changes in serum lipid peroxides influenced by the supplementation of vitamin E in burn patients and healthy controls. Burns 1992; 18: 19.

25. Brown KM, Morrice PC, Arthur JR et al. Effects of vitamin E supplementation on erythrocyte antioxidant defence mechanisms of smoking and non-smoking men. Clin Sci (Lond) 1996; 91: 107-111.

26. Duthic GG, Arthur JR, James WPT et al. Antioxidant status of smokers and nonsmokers: effects of vitamin E supplementation. Ann New York Acad Sci 1989; 570: 435-438.

27. Van Haaften RI, Evelo CT, Penders J et al. Inhibition of human glutathione S-transferase P1-1 by tocopherols and alpha-tocopherol derivatives. Biochim Biophys Acta 2001; 1548: 23-28.

28. Arita M, Sato Y, Arai H et al. Binding of alpha-tocopherylquinone, an oxidized form of alpha-tocopherol, to glutathione-S-transferase in the liver cytosol. FEBS Lett 1998; 436: 424-426.

29. Berger MM, Cavadini C, Bart A. Selenium losses in 10 burned patients. Clin Nutr 1992; 11: 75.

30. Sandstrom BE, Marklund SL. Effects of variation in glutathione peroxidase activity on DNA damage and cell survival in human cells exposed to hydrogen peroxide and t-butyl hydroperoxide. Biochem J 1990; 271: 17-23.

31. Lin F, Thomas JP, Girotti AW. Hyperexpression of catalase in selenium-deprived murine L1210 cells. Arch Biochem Biophys 1993; 305: 176-185.

32. Geiger PG, Thomas JP, Girotti AW. Lethal damage to murine L1210 cells by exogenous lipid hydroperoxides: protective role of glutathione-dependent selenoperoxidases. Arch Biochem Biophys 1991; 288: 671-680.