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The Effects of Vitamin E on Catalase Activies in Various Rat Tissues

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Abstract: Vitamin E and other antioxidants prevent or minimize oxidative damage in biological systems. The level of antioxidant defense sufficient to protect the organism from high free radical concentrations is one of the new horizons for Vitamin E research. The role of Vitamin E as a protective agent aganist oxidative stress was evaluated by measuring the antioxidant enzyme catalase (CAT) activities in different tissues of Swiss male albino rats aged two months.

In this study, rats were divided to three groups; control (n=10), E_1 (n=5) which received 25 mg/kg. per day of Vitamin E, IM. for 10 days and E_2 (n=7) which received 50 mg/kg. per day of Vitamin E, I.M. for 25 days. Catalase activities of cortex cerebrum,cerebellum, liver, kidney, heart and lung tissues were determined by a modified method of Luck and Aebi.

Comparing the Vitamin E supplemented group (E1 +E2) with the controls, no significant difference could be detected in tissue CAT activities. However, in group E2 both renal and cardiac CAT activities were found to be higher when compared to the controls ($p<0.01$) as well as to the E1 group ($p<0.01$).

Since beneficial effects of Vitamin E has been observed on human health, this issue is very important for basic science and for the rapeutic approaches as well.

Key Words: catalase, Vitamin E, kidney, heart, rats

Introduction

Oxidative stress in biological systems originates as the result of an imbalance between the generation of oxidizing species and cellular antioxidant defenses (1-3). Numerous enzymatic and nonenzymatic mechanisms take place to protect the cell against oxidative damage (3). The radical chain reaction of lipid peroxidation appears to be a continuous physiological process. This process, if out of control, can alter essential cell functions and lead to cell death (4-6).

A major contributor to non-enzymatic protection against lipid peroxidation is Vitamin E (Vit E), a known free radical scavenger (3,7). Vit E as a lipid soluble, chain-breaking antioxidant (7-10) plays a major protective role against oxidative stress (4) and prevents the production of lipid peroxides by scavenging free radicals in biological membranes (11). Since the discovery of Vit E in 1922 by H.M. Evans, when it was first described as an anti-sterility agent, many scientists and physicians have sought to elucidate its biochemistry, health benefits and clinical applications (8).

Recently, Vit E is being widely investigated due to its action against oxidative stress (12-18), its protective role on biological membranes (19) and also related to its effect on delaying the symptoms of aging (20). The in vivo function of Vit E as an antioxidant has not yet been fully elucidated (12). Recent studies have revealed that Vit E possesses an antioxidative activity in protecting cells from damage by highly reactive superoxide free radicals (21). In the tissues of Vit E deficient animals, it is reported that lipid peroxidation is enhanced suggesting that Vit E plays a role as a physiological antioxidant based on its chemical properties (11).

Since it is known that Vit E prevents or minimizes oxidative damage in biological systems, at what level the antioxidant defense should be to protect the body from the high free radical concentrations is one of the many new horizons for Vit E research (8).

This study is planned to evaluate the role of Vit E as a protective agent against oxidative stress by measuring the antioxidant enzyme catalase (EC 1.11.1.6)

(CAT) activity in different tissues of Swiss male albino rats.

Materials and Method

Reagents and solutions: All reagents were analytical grade and purchased from Sigma Chem Co (St. Louis) and Merck Darmstadt (Germany).

Animals: In this study Swiss male albino rats (n=22) aged two months were divided to three groups. The E1 group (n=5) received 25 mg/kg Vit E I.M. per day for 10 days, while E2 group (n=7) received 50 mg/kg Vit E I.M. per day for 25 days. Food and water were permitted to all animals ad libitum. Two animals, one from the controls and the other from the Vit E groups (E1 or E2) were guillotined on the same day. Their brains and organs were removed immediately and chilled in ice cold 0.9% w/v NaCl.

Assays: Cortex cerebri and cerebellum were dissected from the surrounding tissue on a chilled dissection board and rinsed in ice cold 0.9% w/v NaCl to remove blood. Tissue samples being blotted were weighed and afterwards placed into a phosphate buffer solution (pH=7.0) in a ratio of 1/10 (w/v) and homogenised at 0°C using a Braun homogenisator. After centrifugation at 600 g for 10 minutes, the supernatant was removed and measurement of catalase activity was made duplicate with a LKB spectrophotometer.

For the estimation of CAT, the enzymatic decomposition of H_2O_2 was followed directly at 240 nm with a modified method of Aebi (22) and Luck (23). Tissue protein concentrations were measured according to Lowry's method (24).

Statistical analysis: The results reported represent the mean ± SEM. Differences between the groups were evaluated using Student's t test. A p value <0.05 was considered to be statistically significant.

Results

In Table 1, the CAT activities (mean±SEM) of the control, Vit E1 and Vit E2 groups are presented. In comparison to the controls no significant difference could be detected in cortex cerebri, cerebellum, liver and lung catalase activities in both Vit E1 and Vit E2 groups (Table 1). As to renal and cardiac tissues, although CAT activities of both tissues manifest no significant differences in Vit E1 group, in Vit E2 group CAT activities of both tissues show a prominent increase with respect to the controls ($p<0.01$) as well as to E1 group (<0.01). The increase in renal tissue CAT activity is demonstrated in Figure 1, while the increase in cardiac tissue CAT activity is demonstrated in Figure 2.

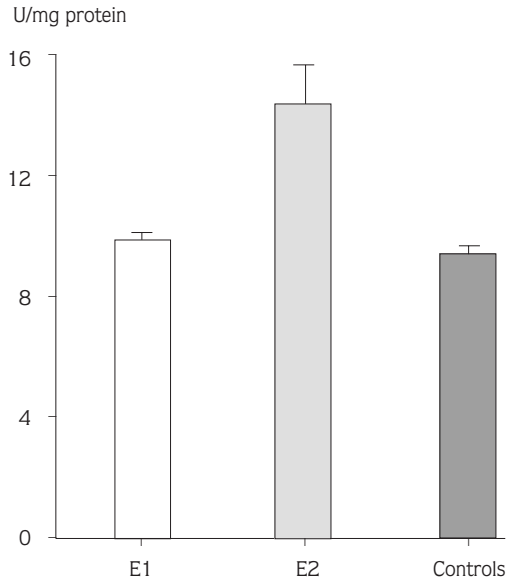

Figure 1. Renal tissue catalase activities (mean±SEM values) in controls and Vit E groups.

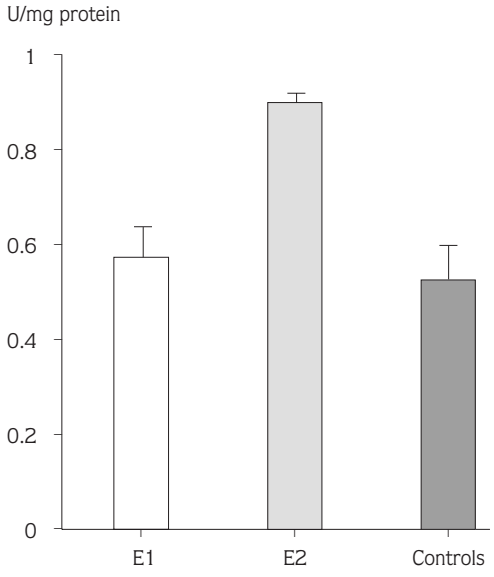

Figure 2. Cardiac tissue catalase activities (mean±SEM values) in controls and Vit E groups.

Table 1. The mean ± SEM values of the CAT activities in the control, Vit E1 and Vit E2 groups.

	Control (n=10)	Vit E1 (n=5)	Vit E2 (n=7)
Cortex cerebri	0.26±0.01	0.29±0.04	0.27±0.03
Cerebellum	0.44±0.03	0.37±0.06	0.39±0.05
Liver	20.82±3.71	22.5±1.52	19.97±2.39
Lung	2.48±0.19	3.30±0.65	2.87±0.11
Kidney	9.51±0.25	9.88±0.19	14.4±1.30
Heart	0.52±0.08	0.57±0.07	0.90±0.02

Discussion

Epidemiological studies provide increasing evidence related to the importance of the human antioxidant defense system in assessing the risk of chronic and degenerative diseases. In recent years, several such investigations have provided strong circumstantial evidence for the beneficial effects of Vit E and have shown a highly significant correlation between lower risk to ischemic heart disease mortality and higher plasma Vit E levels. Beneficial effects of Vit E supplementation on human health are also noted in various chronic diseases and some acute clinical conditions (8).

Recently, it has become clear that, Vit E is also necessary for the maintenance of normal neurological structure and function. In vivo, Vit E is the only-well-recognized, lipid soluble, chain-breaking antioxidant and may therefore be expected to play an important role in protecting lipid rich structures such as the brain from free radical damage (25). It has been reported that enrichment of brain membranes with Vit E by dietary supplementation provides a higher protection of brain membranes against free radical oxidation (10).

Chaudiere J et al. (26) reported that despite the fact that Vit E concentration was 12 times lower in the brain of Vit E deficient rats, no significant change in CAT activity in cerebral tissue was found between the controls and the Vit E deficient groups. These results suggest that the central nervous system (CNS) is still substantially protected when its Vit E content has been decreased to 3 µg/g fresh weight (26).

In a recent study Matsuo M et al. (27) reported that, since the Vit E concentration in brain was found fairly stable, there might be a mechanism by which brain antioxidant capacity is maintained and optimized despite the possible infucence of oxidative stress. In concordance with the results mentioned above, in our study no significant difference could be detected in cortex cerebri and cerebellum CAT activities in the Vit E groups when compared to the controls (Table 1).

As to the liver, Dabholkar et al. (28) have reported hepatomegaly and marked increases in the activities of peroxisomal enzymes such as CAT in Vit E deficient rats. Recently Matsuo M et al. (27) have also observed increased CAT activities especially in the liver of old rats.

In contrast with the studies above, Suga et al. (11) reported that among the peroxisomal enzymes of the liver, the activity of CAT decreased in Vit E deficient rats and restored to the control levels with Vit E supplementation. The decrease in CAT activity in Vit E deficient rats may be due to the suppression of heme biosynthesis (11). Hauswirth JM et al. (29) also suggested that Vit E might have a role in the biosynthesis of heme due to the fact that Vit E deficient animals show a decrease in the activity of hepatic heme proteins, CAT and microsomal cytochromes p-450 and b5. However Fraga CG et al. (4) could not detect a change in liver CAT activities of rats fed with different Vit E deficient diets. Similarly, our results also show no significant change in liver CAT activities in both of the Vit E supplemented groups (Table 1).

Discussing our results with respect to lung tissue CAT activity, no significant variation could be noted in the Vit E supplemented rats compared to the controls. Contrary to our Vit E supplemented rats, Matsuo M et al. (27) also could not detect any changes in Vit E deficient rats.

In our study among all of the tissues investigated, one of the most prominent changes in CAT activity were noted in renal tissue.

Ramsammy LS et al. (30) applying 600 mg/kg Vit E per day for 6 days to protect against gentamycine nephrotoxicity in the rat noted a failure of inhibition of lipid peroxidation by Vit E supplementation. They postulated that Vit E did not prevent or even reduce the severity of gentamycine-induced proximal tubular cell lesions and necrosis.

Parallel to Ramsammy LS et al (30) our result with respect to kidney CAT activities of the E1 group

were similar. However, deviating from the above investigators (30), a striking increase in kidney CAT activity was detected in the E2 group compared to the E1 group and the controls. It must be stressed that the duration of application of Vit E is limited to 6 days in Ramsammy's study (30) and to 10 days in our E1 group whereas in our E2 group this interval lasts for 25 days.

Based on the fact that it usually takes several days or weeks to substantially increase the Vit E content of membranes (8) and also taking into consideration the marked influences of the health status, life style and environment on the requirements of the organism for Vit E (8), therefore Vit E supplementation sufficient to protect the organism from toxic agents and free radical damage is a time consuming process. It is concluded that Vit E is an essential component of the kidney for the protection of this tissue against peroxidative damage. Since Vit E is the least toxic among fat-soluble vitamins and no toxicity has been observed at high doses (31), our preliminary results indicate that long term and high dose Vit E supplementation may be beneficial in the prevention of nephrotoxicity.

Besides renal tissue, another tissue manifesting a prominent variation in antioxidant enzyme activity after the supplementation of high doses of Vit E was cardiac tissue. L. Packer (8) indicates that, there is a highly significant correlation between ischemic heart disease, angina pectoris and low plasma Vit E levels. He stresses that Vit E is inversely related to the risk of angina, independent of the other antioxidants. These observations which are in concordance with our results postulate the fact that log term and high dosage Vit E administration might be beneficial in patients carrying the risk of coronary heart disease. Fur-

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