EFFECT OF VITAMIN E ON PLATELET ENZYMATIC ANTI-OXIDANTS IN THE PATIENTS OF MYOCARDIAL INFARCTION

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ABSTRACT

Effect of administration of 600 mg. vitamin E each day, for six days, was observed on activity of some of the anti-oxidant enzymes and levels of malondialdehyde (as an index of free radical mediated damage) in the platelets of patients reperfused after myocardial infarction. It has been found that vitamin E administration significantly lowers the level of malondialdehyde in the patients. Vitamin E administration increases the activities of anti oxidant enzymes (viz. superoxide dismutase, glutathione reductase and catalase) tested both in the patients and healthy controls. Vitamin E administration causes general stimulation of anti-oxidant enzyme activities both in healthy persons and the patients, however, lowering of lipid per-oxidation upon administration of vitamin E is specific for patients. These findings exhibit beneficial role of vitamin E administration in the management of the patients reperfused after myocardial infarction.

KEYWORDS

Vitamin E, platelets, anti-oxidant enzymes, myocardial infarction, lipid per oxidation.

INTRODUCTION

Vitamin E is a group of about 8 naturally occurring tocopherols (1). This vitamin is essential in many species for normal reproduction, development of muscles, resistance of RBC's to hemolysis and a number of other physiological and biochemical functions (2). Vitamin E acts as a potent anti-oxidant, as it is capable of donating electron to free radicals and thereby quenching it. It is, therefore, capable of breaking free radical chain reaction by scavenging free radicals and thus protecting unsaturated fatty acids, especially of cell membranes, from oxidative damage.

Platelets are directly or indirectly involved in a variety of myocardial disorders such as angina pectoris, myocardial infarction and post reperfusion complications in the patients of myocardial infarction (3,4). Vitamin E is known to play significant role in the inhibition of platelet aggregation, adhesion, thrombosis and prevention of per-oxidative damage of low density lipoproteins (5). The vitamin has also been shown to reduce ischemia-reperfusion induced tissue damage

in many organs (6). However, there are also contradictory reports on these capabilities of vitamin E.

Reperfusion of the tissue, subsequent to ischemia, results in burst of oxygen consumption with consequent generation of oxygen derived free radicals; the oxidant-anti oxidant status of the tissue is thrown out of balance and multi dimensional free radical mediated damage ensues. Since vitamin E is a potent natural anti-oxidant and its administration is expected to restore the imbalance, therefore, the present study was designed to observe the effect of administration of vitamin E on the levels of some representative anti-oxidant enzymes and the free radical mediated damage in the platelets of patients reperfused after myocardial infarction.

MATERIALS AND METHODS

All the biochemicals used were procured from M/s Sigma Chemical Co., USA, other chemicals, purchased locally, were of analytical grade of Qualigens or equivalent.

The study was cleared by the departmental ethical committee and informed consent was taken from all the subjects employed in the study. The patient group comprised of 12 patients reperfused after myocardial infarction. Blood was collected within three hours of reperfusion. Both, the patient and the healthy groups were given 80 mg. aspirin at bedtime. The patients

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also received β -blockers as a routine post reperfusion therapy. The study excluded the patients suffering from any organic disease or the disease known to alter platelet function except aspirin, which was given to both healthy persons and the patients. Thirteen age and sex matched healthy persons served as the control. The effect of vitamin E was tested by oral administration of 600 mg. a tocopherol for six days to the same patients and healthy persons.

Preparation of platelets

Nine ml. venous blood was drawn using disposable sterile plastic syringe and transferred to polypropylene tubes containing 1 ml. 3.8% (w/v) sodium citrate and gently mixed by rotating the tubes. The platelets were prepared according to the method described by Muenzer *et al.* (7). Blood-citrate sample was centrifuged at 800xg for 25 minutes at 8°C. The supernatant was then centrifuged at 4,600xg for 45 minutes. The platelet pellet, thus obtained, was suspended in 2.0 ml. chilled water and left in cold for 2 hours before analysis. Protein was estimated by the method of Lowry *et al.* (8) using Folin phenol reagent. Bovine serum albumin was used as standard.

Enzyme assays

(1) Super oxide dismutase (SOD): Super oxide dismutase activity was assayed by the method of Misra and Fridovich (9). The reaction mixture consisted of 1.0 ml. carbonate buffer (0.2 M, pH 10.2), 0.8 ml. KCl (0.015 M), 0.1 ml. platelet suspension and water to make the final volume to 3.0 ml. The reaction was started by adding 0.2 ml. epinephrine (0.025 M). The change in absorbance was recorded at 480 nm at 15 second intervals for 1 minute at 25°C. Suitable control lacking enzyme preparation was run simultaneously. One unit of enzyme activity has been defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine.

(2) Catalase : Catalase activity was estimated by the method of Luck (10). The assay system consisted of 0.3 ml. phosphate buffer (0.2 M, pH 6.8), 0.1 ml. H_2O_2 (1 M) and water to make the final volume to 3.0 ml. The reaction was started by adding the suitable aliquot of enzyme preparation. The change in absorbance was measured at 15 seconds interval for 1 minute at 240 nm at 25°C. Suitable control was run simultaneously. One unit of catalase activity is defined as the amount of enzyme that liberates half of the peroxide oxygen from H_2O_2 in 100 seconds at 25°C.

(3) Glutathione reductase (GR): Glutathione reductase was measured by the method of Pinto and Bartley (11). In a final volume of 3.0 ml., 0.50 ml. phosphate buffer (0.25 M, pH 7.4); 0.1 ml EDTA (0.025 M, pH 7.4); 0.1 ml. NADPH (7.5 mM) and suitable aliquot of enzyme in the linearity range were added. Water was added to make

up the volume. Reaction was started by adding 0.25 ml. glutathione (25 mM). Change in absorbance was recorded at 340 nm at 25°C for 1 minute. Suitable control, lacking substrate, was run simultaneously.

One unit of enzyme activity has been defined as the formation of NADP in one minute by one ml. of enzyme preparation using extinction coefficient of 6.22.

Specific activities of the enzymes have been defined as enzyme activity per mg. protein.

Measurement of lipid per-oxidation: Free radical mediated damage was assessed by measurement of the extent of lipid per-oxidation in terms of malondialdehyde (MDA) formed, essentially according to Ohkawa *et al.* (13). To 0.25 ml. platelet suspension, added 0.20 ml. 8.1% sodium dodecyl sulphate; 1.5 ml of 20% acetic acid; 1.5 ml. of 0.8% thiobarbituric acid and water to make up the volume to 4.0 ml. The tubes were heated in water bath maintained at 90°C for 1 hour. The tubes were then cooled immediately under running tap water. To each tube was then added 1.0 ml. water and 5.0 ml. mixture of n-butanol and pyridine (15:1 v/v) and vortexed. The tubes were then centrifuged at 1,500xg for 30 minutes. The upper layer was aspirated out and absorbance measured at 532 nm. The reference used was 1,1,3,3- tetra ethoxy propane.

Student's T test was employed for statistical analyses.

RESULTS

Anti-oxidant parameters, under investigation, in the platelets of post reperfusion myocardial infarction patients and healthy persons have been compared and the results obtained are reported in Table 1. Upon administration of vitamin E to healthy persons, there is statistically significant increase in the activities of all the anti-oxidant enzymes under investigation (SOD 138% $p < 0.0001$; catalase 59% $p < 0.0001$; GR 85% $p < 0.0001$) with significant decrease in the levels of MDA 61% ($p < 0.0001$) Table 2. Compared to healthy persons in the patients (both without vitamin E treatment), there has been significant decrease in the activity of SOD (46%, $p < 0.05$) while the activities of other anti-oxidant enzymes varied statistically insignificantly. However, the levels of MDA in the patient group increased significantly (114%, $p < 0.0001$). Upon administration of vitamin E to the patients a significant increase in the activities of all the anti-oxidant enzymes was observed (SOD 182%, $p < 0.05$; catalase 82%, $p < 0.05$; GR 114%, $p < 0.005$) with marked decrease in the levels of MDA (48%; $p < 0.005$) Table 3.

To test whether vitamin E administration has the effect on the activities of the platelet enzymes and degree of lipid per-oxidation specifically on the patients or it is the generalized effect; the data of controls and patients, both given vitamin E, have been compared (Table 4).

The data show that the activities of anti-oxidant enzymes in both the cases change statistically insignificantly. Lipid per-oxidation remains increased, but as compared to the patients without vitamin E treatment, the values are significantly lowered (185%. $p<0.0005$).

DISCUSSION

During oxidative stress there is excessive generation of free radicals (13). The free radicals and their derivatives are capable of causing extensive damage at cellular and tissue levels resulting in a variety of untoward pathophysiological conditions (14). There are numerous reports to show preventive, protective and curative role of natural anti-oxidants, especially vitamin E, in a variety of oxidative stress mediated disorders including myocardial disorders (15,16). At

the same time, there are reports to show that vitamin E has no role in the prevention or cure of stress mediated disorders including myocardial disorders (17,18). Since platelets are involved, in more than one way, in precipitating ischemic and post reperfusion injury, it would be interesting to observe the effect of vitamin E administration on the pathophysiology of platelets. There are scanty and scattered reports on this aspect. It was shown in pigs that poly unsaturated fatty acid (PUFA) rich diet resulted in platelet dysfunction as assessed by platelet factor 3 activity (19) resulting ultimately to vasculitis and thrombosis (20). These findings have also been observed in humans (21). Later studies by Nordoy and Storm (22) have shown that a high intake of vitamin E for prolonged period of time does not interfere with platelet lipid metabolism or platelet function so long as there is no vitamin E deficiency. A more recent report by Meydani (2) indicates

Table 1. Antioxidant parameters in the platelets of healthy persons and the patients reperfused after myocardial infarction before and after vitamin E administration.

	Specific activity of superoxide dismutase	Specific activity of catalase	Specific activity of glutathione reductase	MDA (mM/ml platelet suspension)
Healthy persons without vitamin E administration (n=13)	0.63± 0.07	10.70± 0.83	5.14± 0.60	0.14± 0.012
Patients without Vitamin E administration (n=12)	0.34±0.06	11.21± 1.00	4.07± 0.53	0.30± 0.020
Healthy persons with vitamin E administration (n=13)	1.50± 0.20	17.00± 1.50	9.50± 0.92	0.05± 0.008
Patients with Vitamin E administration (n=12)	0.96± 0.27	20.40± 3.83	8.70± 1.24	0.15± 0.016
Values are mean ± SE; n= the number of cases.				

Table 2. Effect of vitamin E administration on the antioxidant parameters of platelets of healthy persons

	Specific activity of superoxide dismutase	Specific activity of catalase	Specific activity of glutathione reductase	MDA (nM/ml platelet suspension)
Healthy persons without vitamin E administration (n=13)	0.6298± 0.2525	10.7041±2.9952	5.1392± 2.0337	3.328x10 ⁶ ±1.067
Healthy persons with Vitamin E administration (n=13)	1.4894± 0.5868	16.9831± 5.2899	9.4723± 3.2982	2.077x10 ⁶ ± 0.572
P value	< 0.001	<0.001	< 0.001	>0.05
Values are mean ± SD; n= the number of cases; p< 0.01 highly significant, p>0.01 insignificant.				

that vitamin E may modulate platelet adherence and aggregation and thus prevent progression of fatty streak and cell proliferation to advanced lesions. Increased platelet aggregation contributes to development of atherosclerosis and increases the risk of myocardial infarction and stroke. High doses of vitamin E could reduce platelet hyperactivity and aggregation.

We have earlier reported the changes in the status of platelet enzymatic and non-enzymatic anti-oxidant systems and extent of lipid per-oxidation in ischemic myocardial syndromes (14, 23, 24). We could not find reports on the effect of vitamin E administration on the platelet anti-oxidant system hence undertaken this program where in some of the representative platelet anti-oxidant enzymes and the extent of lipid per-oxidation in the post ischemic patients of myocardial infarction and also the effect of administration of vitamin E on these parameters has been studied. Our findings show a significant decrease in the activity of superoxide dismutase, along with highly significant increase in the free radical mediated damage in the patients as compared to healthy persons. These findings suggest a definite post reperfusion injury in the platelets.

Vitamin E administration to the patients significantly improves the levels of platelet anti-oxidant enzymes

and lipid per-oxidation. These effects of vitamin E are, however, not specific to the patients at anti-oxidant enzyme level as evidenced by comparing the effect of vitamin E administration to healthy persons and the patients. Vitamin E administration significantly improves free radical mediated damage in the patients. Vitamin E thus appears to act as a preventive anti-oxidant as discussed by Halliwell and Gutteridge (25). Our findings suggest that vitamin E administration has similar effect on the activities of platelet anti-oxidant enzymes in healthy persons and the patients reperused after myocardial infarction. However, it is interesting to note that in the patients, there is enhanced lipid per-oxidation and free radical mediated damage and that vitamin E administration specifically and effectively reduces the free radical mediated damage of essential lipids responsible for the maintenance of cellular integrity in the patients.

These findings suggest the beneficial effect of vitamin E administration in the post reperused patients of myocardial infarction.

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Table 3. Antioxidant parameters in the platelets of patients before and after vitamin E administration.

	Specific activity of superoxide dismutase	Specific activity of catalase	Specific activity of glutathione reductase	MDA (nM/ml platelet suspension)
Patients before vitamin E administration (n=12)	0.3416±0.2187	11.2091±3.4702	4.0683±1.8372	7.774x10 ⁶ ±2.470
Patients after Vitamin E administration (n=12)	0.9609±0.9477	20.3700±13.2500	8.6525±4.3044	4.615x10 ⁶ ±0.484
P value	<0.01	<0.01	<0.0005	<0.0005
Values are mean ± SD; n= the number of cases; p<0.01 significant, p>0.01 insignificant.				

Table 4. Comparison of the effect of vitamin E administration on anti-oxidant parameters of the platelets in healthy persons and the patients

	Specific activity of superoxide dismutase	Specific activity of catalase	Specific activity of glutathione reductase	MDA (nM/ml platelet suspension)
Healthy Persons (n= 13)	1.4894±0.5868	16.9831±5.2899	9.4723±3.2982	2.077x10 ⁶ ±0.572
Patients (n=12)	0.9609±0.9477	20.3700±13.2500	8.6525±4.3044	4.615x10 ⁶ ±0.484
P value	>0.01	>0.01	>0.01	<0.01
Values are mean ± SD; n= the number of cases; p<0.01 significant, p>0.01 insignificant.				

REFERENCES

1. Marcus R, Coulstom AM. Fat soluble vitamin A, K, and E In : Hardman G , Limbird LE eds: therapeutic 9th ed. Elmsford, Newyork: perganon press, inc; 1996 . P. 1573-1590.
2. Mohsun Meydani (1995). Vitamin E. Lancet ; 345: 170-5
3. Bressler NM, Brockman MJ, Marcus AJ. (1979) Concurrent studies of oxygen consumption and aggregation in stimulated human platelets. Blood;53;167-78.
4. Fitzgerald DJ, Roy L, Catella F, Fitzgerald GA. (1986) Platelet activation in unstable coronary disease. New Eng. J. Med. : 315; 983-90.
5. Steiner M, Mower R. (1982) Mechanism of action of vitamin E on platelet function. Annal. New York Acad. Sci. : 393; 289-99.
6. Lehr HA, Vajkoczy P, Menger MD. (1998) Vitamin E: Focus on microcirculation. Microcirculation. : 5; 117-28.
7. Muenzer J, Wienbach EC, Wolfe SM. (1975) Oxygen consumption of human blood platelet I. Effect of thrombin. Biochim. Biophys. Acta. : 376; 237-42.
8. Lowry OH, Roseborough MJ, Farr AL, Randall AG. (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. : 193; 265-75.
9. Misra HP, Fridovich I. (1972) The role of superoxide anion in the auto-oxidation of epinephrine and a sample assay for superoxide dismutase. J. Biol. Chem. : 247; 3170-75.
10. Luck H. Catalase. In: Bergmeyer HU, editor. (1965) Methods in Enzymatic Analysis. Academic Press, NY. pp 885-94.
11. Pinto RE, Bartley W. (1969) The effect of age and sex on glutathione reductase and glutathione peroxidase activities and on aerobic glutathione oxidation in rat liver homogenate. Biochem. J. : 112; 109-15.
12. Ohkawa H, Ohishi N, Yagi K. (1979) Assay of lipid per oxidation in animal tissues by thiobarbituric acid reaction. Annal. Biochem. : 95; 351-58.
13. Toshikazu Yoshikawa, Yuji Naito. (2002) What is oxidative stress. J. Japan. Medi. Assoc. : 45; 271-76.
14. Pandey NR, Kaur G, Chandra M, Sanwal GG, Misra MK. (2000) Enzymatic oxidant anti oxidants of human blood platelets in unstable angina and myocardial infarction. Int. J. Cardiol. : 76; 33-8.
15. Vivekananthan DP, Penn Marc S, Shapp SK, Hsu A, Topol EJ. (2003) Use of anti oxidant vitamin E for the prevention of cardiac disease: Meta analysis of randomized trials. Lancet. : 361; 2017-23.
16. Routine vitamin supplementation to prevent cancer and cardiovascular disease: Recommendations and rationale. US preventive service task force. Annals. Int. Med. 2003; 139; 51-5.
17. Josefson D. (2003) Vitamin supplements do not reduce incidence of cancer or heart disease. Brit. Med. J. : 327; 70-8.
18. Halliwell B. (2000) The anti oxidant paradox. Lancet. : 355; 1179-80.
19. Nordoy A, Rodseth JM. (1971) The influence of dietary fats on platelets in man. Acta. Med. Scand.: 190; 27-34.
20. Nafstad I, Tollersrud S. (1970) The vitamin E deficiency syndrome in pigs I. Pathological changes. Acta. Vit. Scand. : 11; 450-80.
21. Nafstad I. (1974) Endothelial changes and platelet thrombosis associated with PUFA-rich, vitamin E deficient diet fed to pig. Thrombosis Research. : 5; 251-58.
22. Nordoy A, Strom E. (1975) Tocopherol in human platelets. J. Lipid. Res. : 16; 386-91.
23. Kaur G, Misra MK, Sanwal GG, Shanker K, Chandra M. (1999) Levels of glutathione reductase and glutathione peroxidase of human platelets in unstable angina and myocardial infarction. Boll. Chim. Farm. : 138; 437-39.
24. Kaur G, Pandey NR, Chandra M, Sanwal GG, Misra MK. (2000) Lipid peroxidation in human blood platelets during unstable angina and myocardial infarction. Boll. Chim. Farm. : 139; 217-19.
25. Halliwell B, Gutteridge JMC. (1990) The anti oxidants of human extra cellular fluids. Arch. Biochem. Biophys. : 280: 1-8.