EFFECT OF VITAMIN E ON THE PLATELET XANTHINE OXIDASE AND LIPID PEROXIDATION IN THE PATIENTS OF MYOCARDIAL INFARCTION

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ABSTRACT

Platelets play important role in precipitating ischaemic myocardial syndromes in many ways. One of the consequences of ischaemic diseases is excessive generation of oxygen derived free radicals that have numerous pathophysiological consequences. Platelet pro-oxidant enzyme, xanthine oxidase is one of the sources of generation of free radicals. In the present paper, we report the effect of administration of vitamin E along with aspirin on the levels of platelet xanthine oxidase and extent of free radical mediated damage in the patients reperfused after myocardial infarction.

Our findings show that administration of 400 mg. vitamin E for six days along with 80 mg. aspirin has an excellent anti-oxidant effect as evidenced by reduced platelet xanthine oxidase activity and lowering of malondialdehyde (MDA) levels which is an index of the extent of free radical mediated damage.

KEYWORDS

Vitamin E, Platelets, Myocardial infarction, Xanthine oxidase, Lipid peroxidation, Aspirin.

INTRODUCTION

Free radicals are thought to be the most important cause of the post reperfusion injury subsequent to ischaemia. Their role in ischaemia-reperfusion models have been comprehensively investigated and it has become clear that they are to be blamed for the bulk of post ischaemic injury (1,2). Oxygen derived free radicals trigger significant collagen damage and left ventricular dysfunction. During reperfusion, these changes extend beyond the ischaemic damage (3). The increased oxidative stress may cause endothelial dysfunction which may further aggravate the hypertensive process and may also accelerate atherogenesis (4).

Platelets are implicated, in many ways, in the pathophysiology of ischaemic myocardial disorders. Platelets, besides other important factors, have been demonstrated to possess their own free radical scavenging (eg. superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase) and free radical generating (xanthine oxidase) enzyme systems (5).

One of the major free radical generating systems, besides leakage of electrons from electron transport

chain, is xanthine oxidase. Under normal conditions, this enzyme exists as a dehydrogenase, converting hypoxanthine and xanthine into uric acid at the expense of NADP. Under ischaemic conditions, xanthine dehydrogenase undergoes selective proteolysis and is converted into xanthine oxidase. Latter also brings about conversion of hypoxanthine and xanthine into uric acid but using molecular oxygen with the resultant generation of oxy free radicals.

Ischaemia-reperfusion conditions are known to be associated with platelet aggregation and activation and consequently enhanced free radical generation. The free radicals generated should be effectively scavenged off by the free radical scavenging enzymes of the platelets. We have earlier shown that under myocardial ischaemia-reperfusion conditions, the activities of these enzymes are significantly lowered (5). Free radicals generated under these circumstances are not effectively scavenged off and may be leached out (6) and thus contribute to myocardial damage. This decreased efficacy of free radical scavenging system may be improved by external administration of non-enzymatic anti-oxidants in such cases. To test this surmise, the present investigation has been undertaken using one of the most effective non-enzymatic anti-oxidant, vitamin E.

MATERIAL & METHODS

All the biochemicals were procured from sigma chemical company, U.S.A. Other chemicals were of

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analytical grade of Qualigens or equivalent. Informed consent was obtained from each patient and the study was cleared by departmental ethical committee.

The patient group comprised of patients reperfused after myocardial infarction. A detailed clinical examination of all the patients was done to detect coexisting disorders. Cases with cerebrovascular accidents, diabetes mellitus and renal failure were excluded from the study. All the cases were subjected to routine investigations eg. hematocrit, urine analysis and blood chemistry (urea, creatinine, lipids, sugar, Na⁺ and K⁺) and electrocardiogram. All the patients were receiving β -blockers as routine post ischaemic therapy. The samples were collected within three hours of reperfusion. An age and sex matched group of healthy persons served as control. Both healthy and patient groups comprised of males, non smokers and within the age group of 50-75 years. The patients were distributed in three sub groups. Group 1 received β -blockers alone, Group 2 received 80 mg. aspirin along with β -blockers and Group 3 received 80 mg. aspirin and 400 mg. vitamin E along with β -blockers.

Preparation of platelets

9 ml. venous blood was drawn using poly propylene syringe and transferred in plastic tubes containing 1 ml. of 3.8% (w/v) citrate. The tubes were gently rotated to mix the contents. Platelets were prepared by the method described by Muenzer *et al.* (7). Blood samples were centrifuged at 800xg for 20 minutes at 8°C. The resulting supernatant was then spun at 4,500xg for 50 minutes. The platelet pellet, thus obtained, was taken up in 2 ml. chilled water and shaken vigorously. The suspension was left for two hours in ice before analysis.

Assay of xanthine oxidase activity

Assay of xanthine oxidase was carried out essentially according to the method described by Roussos (8). The assay mixture, in final volume of 3.0 ml, consisted of 0.30 ml. Tris-HCl buffer, 50 mM, pH 7.4; 0.30 ml. CuSO₄, 10mM; 0.05 ml xanthine, 2.58 m M per ml. in 0.05 M glycine buffer, pH 7.4; 0.1 ml platelet suspension and water to make up the volume. The change in absorbance was recorded at 290 nm at 5 seconds interval for 1 minute. Suitable controls were run simultaneously.

One unit of activity has been defined as change in absorbance at 290 nm in 1 minute by 1 ml. platelet suspension.

Protein estimation

Protein was estimated by the method of Lowry *et al.*, using Folin phenol reagent (9). Bovine serum albumin was used as standard.

Specific activity of the enzyme has been expressed as unit of activity/mg. protein.

Measurement of lipid per oxidation

Lipid peroxidation in the platelet preparations was assayed by the method of Ohkawa *et al.* (10) by measuring the levels of MDA (11-13). To 0.20 ml. of platelet suspension was added 0.8 ml. sodium dodecyl sulphate, 8.1%; 0.50 ml. glacial acetic acid and thiobarbituric acid, 0.8% to make up the volume to 3.0 ml. Contents of the tubes were mixed and heated over water bath maintained at 90°C. for 1 hour and immediately cooled thereafter under running water. To each tube 1.0 ml. water and 5.0 ml. solution of n-butanol and pyridine (15:1, v/v) was added and vortexed thoroughly and centrifuged at 800xg for 10 minutes. The upper layer was aspirated out and colour intensity

Table 1. Levels of xanthine oxidase and malondialdehyde (MDA) in human platelets of reperfused patients of myocardial infarction and healthy persons.

Cases	Specific activity of Xanthine oxidase	MDA (mMole/ml platelet suspension)
Healthy (n=15)	0.01±0.001	0.08±0.003
Patients Group 1 (n=10)	0.04±0.001	0.21±0.007
Patients Group 2 (n=8)	0.03± 0.001	0.15± 0.004
Patients Group 3 (n=7)	0.02± 0.0003	0.11± 0.003
n= the number of cases studied; Values reported are mean ± SE; Group 1: Untreated patients; Group 2: Patients given aspirin alone; Group 3: Patients given aspirin and vitamin E.		
p values: xanthine oxidase: healthy vs. group 1, p<0.0005; group 1 vs. group 2, p<0.005; group 1 vs. group 3, p<0.0005. MDA levels: healthy vs. group 1, p<0.0005; group 1 vs. group 2, p<0.05; group 1 vs. group 3, p<0.01. p> 0.05= insignificant p<0.05= significant.		

measured at 532 nm. The reference used was 1.1.3.3 tetra ethoxy propane.

Statistical analyses were carried out using Student's T test.

RESULTS

As evidenced from table 1, the specific activity of platelet xanthine oxidase is increased by about 300% in the patients (Group 1) as compared to healthy persons ($p < 0.0005$). MDA level was also increased by about 175% in the patients as compared to control ($p < 0.0005$).

Administration of aspirin alone to the patients (Group2) resulted in 25% decrease in the activity of xanthine oxidase ($p < 0.005$) and 28% decrease in MDA level ($p < 0.05$) compared to Group 1 patients.

Administration of combination of vitamin E along with aspirin to the patients (Group3) resulted in further decrease in the activity of the enzyme and the levels of MDA each by about 50% , p values < 0.0005 and < 0.01 respectively.

DISCUSSION

Platelets play major role in myocardial ischaemic syndromes (14,15). Platelets have their own pro-oxidant and anti-oxidant systems. Xanthine oxidase is one of the major sources of free radical generation.. During ischaemia adenosine pool is degraded to hypoxanthine and xanthine and simultaneously xanthine dehydrogenase is converted to xanthine oxidase. Ischaemic conditions, therefore, set a stage for copious production of free radicals upon reperfusion due to burst of oxygen consumption. The increased generation of free radicals due to enhanced activity of xanthine oxidase and decreased activities of free radical scavenging enzymes of platelets (5), results in an imbalance between rate of free radical generation and their scavenging.

Aspirin acts as an anti platelet agent (16) but its role as an anti-oxidant is not known. Vitamin E is the most reactive anti-oxidant (17,18). The most important function of vitamin E in the body is to protect polyunsaturated fatty acid from oxidation (19). There are a few reports that advocate administration of combination of low doses of aspirin and vitamin E as a preventive therapy in patients with risk factors for cardiovascular diseases (20,21).

To restore the oxidant-anti-oxidant balance we administered non-enzymatic anti-oxidant vitamin E from out side and observed its effect on one of the platelet pro-oxidant system and free radical mediated damage. Our results point out the definite beneficial role of administration of combination of vitamin E and aspirin in the post reperfusion patients of myocardial

infarction as this combination not only lowers the platelet xanthine oxidase activity but also controls free radical mediated damage.

The present findings point to significance of administration of vitamin E in the better management of post reperfusion cardiac conditions.

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