Effect of vitamin E administration on blood pressure following reperfusion of patients with myocardial infarction

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BACKGROUND: Reperfusion of infarcted myocardium by thrombolysis is the major technique used to restore blood supply. Reperfusion, however, is associated with a burst of oxygen consumption, with the resultant excessive generation of free radicals causing reperfusion injury.

OBJECTIVE: In the present study, the effect of vitamin E administration on the status of free radical-mediated injury and blood pressure in post reperfusion hypertensive patients with myocardial infarction is assessed.

METHOD: Post reperfusion hypertensive patients were divided into three groups: those not receiving a beta-blocker (group I), those receiving acetylsalicylic acid (ASA) plus a beta-blocker (group II) and those receiving ASA, a beta-blocker and 400 mg vitamin E (group III). Groups II and III were comprised of patients from group I. Levels of malondialdehyde (MDA) and xanthine oxidase (XO) activity in blood were used as an index of oxidative stress. These parameters, along with blood pressure, were monitored in these groups.

RESULTS: Patients not receiving a beta-blocker had elevated levels of MDA and XO activity when compared with healthy persons ($P<0.0005$ both for MDA and XO). Compared with group I, patients receiving a beta-blocker plus ASA (group II) had a significant decrease in the activity of XO ($P<0.005$) and the levels of MDA ($P<0.005$). When vitamin E was incorporated in the treatment, there was highly significant decrease in these oxidative stress parameters compared with groups I and II ($P<0.0005$ for XO and MDA). Use of vitamin E as an adjuvant in hypertensive therapy (group III) resulted in better management of blood pressure (systolic $P<0.005$, diastolic $P>0.05$) when compared with group II.

CONCLUSION: Inclusion of vitamin E in antihypertensive therapy in post reperfusion hypertensive patients results in better management of blood pressure.

Key Words: *Acetylsalicylic acid; Blood pressure; Lipid peroxidation; Myocardial infarction; Reperfusion; Vitamin E; Xanthine oxidase*

Reactive oxygen species (ROS) and their derivatives are involved in a variety of human diseases (1,2). The oxygen free radicals are known to play an important role in the genesis of various cardiovascular disorders. Ischemia and reperfusion of ischemic tissues both lead to the generation of free radicals (3,4). ROS are produced continually in most tissues and are part of normal cellular function. Their generation may increase in various pathophysiological conditions including vascular diseases, in which enhanced formation of ROS may be pathogenic (5).

Various cellular antioxidant systems exist to defend against oxidant stress and maintain the redox balance of the cell. Excessive production of ROS, exceeding the rate of endogenous antioxidant defense mechanisms, is referred to as oxidative stress. The major damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins and nucleic acids (6). Oxidative stress has been identified throughout the process of atherogenesis, beginning at the early stage when endothelial dysfunction is barely apparent (7). As the process of atherogenesis proceeds, inflammatory cells as well as other constituents of the atherosclerotic plaque release large amounts of ROS, which further facilitate atherogenesis. In general, increased production of ROS may affect oxidation of low-density lipoproteins and increase endothelial cell dysfunction, which contribute to atherogenesis (8).

Control of blood pressure in patients reperfused after myocardial infarction by itself leads to attenuation of oxidative stress (9). Vitamin E functions as a powerful antioxidant by donating electrons to neutralize ROS and thereby preventing the oxidative damage that is thought to be responsible for atherosclerosis. In an attempt to reduce oxidative stress and control blood pressure in post reperfusion hypertensive patients, we administered the nonenzymatic antioxidant vitamin E along with acetylsalicylic acid (ASA) and a beta-blocker. Xanthine oxidase (XO) activity and lipid peroxidation were used as the markers of oxidative stress. XO is one of the major free radical-metabolizing enzymes that are elevated upon reperfusion of ischemic tissues (10).

PATIENTS AND METHODS

The biochemicals used in the present study were procured from Sigma Chemical Co, USA. Other chemicals were of analytical grade procured from Qualigens Fine Chemicals, India, or an equivalent company.

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TABLE 1
Systolic blood pressure (SBP) and diastolic blood pressure (DBP) of healthy subjects and patients

Groups	SBP, mmHg	DBP, mmHg
Healthy subjects, n=45 (mean ± SD)	118±7	78±7
Group I, n=35 (mean ± SD)	170±8	103±5
Group II, n=30 (mean ± SD)	144±8	85±6
Group III, n=30 (mean ± SD)	131±9	84±5
Group comparisons	**P (SBP)**	**P (DBP)**
Group I versus group II	<0.05	<0.05
Group I versus group III	<0.005	<0.05
Group II versus group III	<0.005	>0.05
Healthy subjects versus group I	<0.0005	<0.005
Healthy subjects versus group II	<0.0005	<0.1
Healthy subjects versus group III	<0.025	<0.1

Group I Post reperfusion myocardial infarction patients; Group II Post reperfusion myocardial patients receiving acetylsalicylic acid along with a beta-blocker; Group III Post reperfusion myocardial patients receiving vitamin E, acetylsalicylic acid and a beta-blocker. P>0.05 not significant

The patients included in the study were all Indian men between 45 and 65 years of age. Venous blood of 35 postinfarction patients with no history of antihypertensive therapy was aseptically collected in citrated vials immediately after reperfusion, and blood pressure was measured using a sphygmomanometer. This group served as the base line (group I). Thirty of these patients (five opted out of the study) were given 80 mg ASA along with 100 mg/day metaprolol for the five days immediately after thrombolysis (group II). On the morning of the sixth day, 12 h after fasting, venous blood was collected and blood pressure was measured before 400 mg vitamin E was included in the therapy for five days (group III). On the morning of sixth day of this therapy, another 12 h fasting blood sample was collected and blood pressure was measured again. All the medications were given through oral administration.

Forty-five age- and sex-matched healthy persons were also included in the study. Their sample collection and blood pressure measurement were done along with the patients in a similar manner.

Persons with cerebrovascular accidents, diabetes mellitus and renal failure, as well as smokers and alcoholics, were not included in the present study. Informed consent was obtained from each individual and the study was cleared by the Departmental Ethical Committee. All the biochemical parameters were studied in the blood and wherever needed, the blood was suitably diluted with water.

Statistical analysis

Statistical analyses were carried out using the Student's *t* test.

Assay of XO

XO activity was assayed by the Roussos method (11). The assay system, in final volume of 3.0 mL, consisted of 0.3 mL Tris-HCl, 50 mM, pH 7.4; 0.3 mL $CuSO_4$, 10 mM; and 0.05 mL xanthine, 2.58 µm/mL in 0.05 M glycine buffer, pH 7.4; in a suitable aliquot of the blood and water to make up the volume. Change in absorbance was recorded at 290 nm at 1 min intervals for 3 min. A control sample lacking enzyme was run simultaneously. Extinction coefficient of 10.03/cm at 290 nm was used to calculate conversion of xanthine to uric acid.

A unit of enzyme activity has been defined as the amount of the enzyme required to convert 1 µmol of substrate to product in 1 min at 25°C. Specific activity is the activity of enzyme/mg protein.

Protein estimation

Protein was estimated by using Folin phenol reagent (12). Bovine serum albumin was used as reference.

Measurement of lipid peroxidation

Lipid peroxidation was measured by monitoring the levels of malondialdehyde (MDA) (13). To 0.20 mL of blood, 0.8 mL sodium dodecyl sulphate, 8.1% (weight/volume); 0.50 mL glacial acetic acid; and thiobarbituric acid, 0.8% (weight/volume) were added, to make up a volume of 3 mL. The contents of the tubes were mixed and heated for 1 h over a water bath maintained at 90°C and then immediately cooled under running water. To each tube, 1 mL water and 5 mL solution of n-butanol and pyridine (15:1, by volume) were added, and vortexed and centrifuged at 800 × g for 10 min. The upper layer was aspirated out and colour intensity was measured at 532 nm. The reference used was 1,1,3,3-tetraethoxypropane.

RESULTS

Blood pressure

The blood pressure of patients under investigation is reported in Table 1. The average blood pressure of post reperfused myocardial infarction patients taken at base line (group I) was 170/103 mmHg. Upon administration of ASA and beta-blocker, the blood pressure was reduced (144/85 mmHg; P<0.05 for systolic and diastolic). After the addition of 400 mg vitamin E to the above medications, better control of blood pressure was observed (131/84 mmHg; group II versus group III, P<0.005 for systolic and P>0.05 for diastolic). The systolic blood pressure was significantly further lowered, whereas the diastolic blood pressure, which was already at normal value, did not further change significantly.

Lipid peroxidation

The MDA levels both in the blood of healthy persons and patients were measured and are reported in Table 2. In myocardial infarction patients (groups I, II and III), there was a statistically significant increase in the levels of MDA. In the present study, after treatment with ASA plus beta-blocker, the extent of lipid peroxidation was reduced significantly compared with group I (P<0.005). Supplementation of vitamin E decreased MDA levels significantly when compared with group I (P<0.0005). Upon comparing the MDA levels between vitamin E-treated and -untreated patients (group III versus group II), a highly significant decrease was observed (P<0.0005). Although treatment with a beta-blocker and ASA, and a beta-blocker, ASA and vitamin E both lowered MDA levels, they remained significantly higher than the levels seen in healthy persons.

XO

Compared with healthy persons, XO activity was significantly increased in the patients (P<0.0005). When a beta-blocker and ASA were administered, the enzyme activity in the blood of the patients was reduced (P<0.005), but a highly significant decrease was observable upon addition of vitamin E to the treatment regimen compared with group I (P<0.0005). In

addition, there was a significant decrease in the activity of the enzyme in the patients of group III compared with those in group II (P<0.0005). The enzyme activity in healthy persons, however, remained significantly lower than in any of the patient groups.

DISCUSSION

Our findings show a highly significant increase in the levels of MDA and the pro-oxidant enzyme XO in the blood of the post reperfusion patients. Increased levels of MDA and XO activity confirm free radical-mediated damage. The observations, in this respect, are similar to those of Bhakuni et al (14), who demonstrated that reperfusion of ischemic myocardium restores oxygen supply, but a sudden massive increase of oxygen supply results in a burst of oxygen consumption with consequent excessive generation of free radicals. Deleterious effects of reperfusion are manifold. There is excessive generation of free radicals, which are not effectively scavenged due to a concomitant decrease in the levels of antioxidant enzymes. Increased levels of free radicals may result in various types of damage including platelet activation and aggregation to block vessels and further aggravate the situation.

The findings reported show that in the patients, inclusion of vitamin E in the drug regimen, comprised of ASA and a beta-blocker, effectively reduces the MDA levels and XO activity. This observation strongly favours inclusion of vitamin E as an adjuvant in routine antihypertensive therapy in post reperfusion patients to effectively prevent excessive free radical generation and consequent damage as a result of myocardial reperfusion.

Our observations show that inclusion of vitamin E to the drug regimen in the management of blood pressure of patients is very effective. Beta-blockers are used to control elevated blood pressure. Low doses of ASA are also known to regulate blood pressure (15). How inclusion of vitamin E in this combination helps in better management of blood pressure is not completely understood. There appears to be some correlation between oxidative stress and blood pressure. Vaish et al (16) have shown that in uncontrolled hypertension, there is a significant increase in oxidative stress that decreases after reduction of blood pressure. Our findings are in support of an earlier report of Galley et al (17), who found similar results and are further confirmed by the work of Chen et al (18). Chen et al found that serum levels of vitamin E were positively and significantly associated with systolic and diastolic blood pressure and, and that adequate levels prevent hypertension. Recently, Vasdev et al (19) have also found that dietary supplementation of vitamin E significantly attenuated the increase in systolic blood pressure and associated biochemical and histopathological changes. It is known that oxidative stress and hypertension damage endothelial lining, thereby decreasing the synthesis of nitric oxide (NO), a potent vasodilator, causing a rise in blood pressure (20). Vitamin E is a powerful antioxidant and, thus, its administration reduces oxidative stress as shown by the data presented here. Reduction in oxidative stress causes restoration of endothelial lining and enhanced generation of NO, with consequent vasodilation and fall in blood pressure. This view is further supported by our earlier observation that in hypertension, NO levels are decreased and upon administration of vitamin E the levels are restored to almost normal values (21).

TABLE 2
Effect of the administration of vitamin E on the activity of xanthine oxidase and malondialdehyde (MDA) levels in blood

Cases	Specific activity of xanthine oxidase	MDA levels (nmol/mL deproteinized blood)
Healthy subjects, n=45 (mean ± SD)	0.0096±0.0008	29.79x10^6±1.437
Group I, n=35 (mean ± SD)	0.0479±0.0024	55.87x10^6±1.523
Group II, n=30 (mean ± SD)	0.0332±0.0013	48.65x10^6±2.108
Group III, n=30 (mean ± SD)	0.0216±0.0021	39.32x10^6±1.475
Group comparisons	**P**	**P**
Group I versus group II	<0.005	<0.005
Group I versus group III	<0.0005	<0.0005
Group II versus group III	<0.0005	<0.0005
Healthy subjects versus group I	<0.0005	<0.0005
Healthy subjects versus group II	<0.0005	<0.0005
Healthy subjects versus group III	<0.0005	<0.0005

Group I Post reperfusion myocardial infarction patients; Group II Post reperfusion myocardial patients receiving acetylsalicylic acid along with a beta-blocker; Group III Post reperfusion myocardial patients receiving vitamin E, acetylsalicylic acid and a beta-blocker. P>0.05 not significant

CONCLUSIONS

Our findings show that the administration of vitamin E along with ASA and a beta-blocker is most effective in the management of blood pressure and free radical-mediated damage in post reperfusion myocardial infarction patients, when compared with the administration of beta-blockers and ASA alone.

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