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Status of some free radical scavenging enzymes in the blood of myocardial infarction patients

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Abstract

Pro-oxidant and anti-oxidant systems and their levels have significant roles in occlusive vascular diseases. In the present communication, we have measured the levels of some representative anti-oxidant enzymes in the blood of the patients of myocardial infarction after reperfusion and compared them to age and sex matched healthy persons. Our findings show that the activities of anti-oxidant enzymes (viz. SOD, catalase and glutathione reductase) are significantly decreased whereas there is significant increase in the levels of malonaldialdehyde (a marker of free radical-mediated damage) in the patients. The findings point out that ischemic myocardial disorders are associated with excessive free radical generation and free radical-mediated damage of lipids.

Keywords: *Free radicals, anti-oxidant, blood, lipid per-oxidation, myocardial infarction*

Introduction

Paradoxical as it may seem, oxygen is both essential for life as well as, in some form, among the most toxic species. Oxygen in the environment is generally found either in its fully oxidized form (O_2) or in its reduced form (H_2O) [1]. A free radical is an ionic species that contains one or more unpaired electrons. Free radicals are continuously formed in the body, both by deliberate synthesis and by chemical side reactions [2–4]. The most active producers of reactive oxygen species are phagocytic cells. They respond to various foreign organisms with a metabolic burst in which oxygen is reduced to superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH°) [5]. These free radicals are involved in variety of disorders like cancer, liver diseases and various cardiovascular disorders [6,7]. The increased accumulation of free radicals cause toxic changes or even death of the cells [8]. Free radicals, particularly hydroxyl radical, react with lipids, nucleic acids and proteins resulting in the loss of membrane integrity, structural or functional changes in proteins and enzymes and genetic alterations respectively [9]. Free radicals seem to play a role in the formation of foam cells in atherosclerotic plaques [10]. Oxygen derived free radicals trigger collagen damage and left ventricular dysfunction. During reperfusion these changes extend beyond the ischemic damage [11]. The increased oxidative stress may cause endothelial dysfunction which may further aggravate the hypertensive processes and may also accelerate atherogenesis [12].

The aim of the present study has been to investigate the changes in the levels of some anti-oxidant enzymes in the blood after reperfusion of myocardial infarction patients.

Materials and methods

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

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The study was cleared by the Departmental Ethical Committee and informed consent was taken from each subject. A total of 62 subjects comprising of 37 patients and 25 age and sex matched healthy persons were included in the study. All the cases were males (age 45–70 years), nonsmoker, non-alcoholic, and non-diabetic. The patients were receiving normal post infarction medication including aspirin and routine antihypertensive therapy. Reperfusion was carried out by administration of streptokinase. The blood sample was collected within 3 h of reperfusion.

9 ml venous blood was drawn using a disposable plastic syringe and transferred to polypropylene tubes containing 1 ml of 3.8% (w/v) sodium citrate. Blood samples were diluted 15 times with chilled water, left for at least 1 h at 0–4°C and briefly centrifuged before analysis.

Enzyme assays

All enzyme assays were carried out at 25°C.

Superoxide dismutase (SOD)

SOD activity was assayed by the method of Misra and Fridovich [13]. The reaction mixture comprised of 1.0 ml sodium carbonate buffer (0.2 M, pH 10.2), 0.8 ml KCl (0.015 M), 0.1 ml diluted enzyme preparation in the linearity range and water to make the final volume to 3.0 ml. The reaction was started by adding 0.2 ml, (0.25M) of epinephrine. The change in absorbance was recorded at 480 nm at 15 s intervals for 1 min. A suitable control, lacking the enzyme preparation, was run simultaneously. One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto-oxidation of epinephrine.

Catalase

Activity of catalase was determined by the method of Lück [14]. The assay system consisted of 0.3 ml phosphate buffer (0.2 M, pH 6.8), 0.1 ml of (1 M) H_2O_2 and water to make up the volume to 3.0 ml. The reaction was started by addition of a suitable aliquot of enzyme. The change in absorbance was measured at 15 s intervals for 1 min at 240 nm. A suitable control, lacking the enzyme, was run simultaneously.

One unit of activity is defined as the amount of enzyme which liberates half the per-oxide oxygen from a H_2O_2 solution in 100 s. The unit of enzyme activity is, therefore, related to the first order half life.

Glutathione reductase

Glutathione reductase activity was measured by the method of Carlberg and Mannervik [15]. The reaction mixture consisted of 1.5 ml of potassium phosphate buffer (0.2 M, pH 7.0 containing 2 mM EDTA), 0.15 ml of 2 mM NADPH, 0.2 ml of 20 mM oxidized glutathione and added distilled water to make up the total volume to 3.0 ml. The reaction was started by adding a suitable aliquot of enzyme in the linearity range. The change in absorbance was recorded at 340 nm for 1 min at 15 s intervals. Control lacking enzyme was run simultaneously.

One unit of glutathione reductase activity is expressed as the amount of NADP formed in 1 min by one ml of enzyme preparation. Calculation of the enzyme activity has been done by using the extinction coefficient of NADPH as 6.22×10^3.

Protein measurement

Protein was measured by the method of Lowry et al. [16], using Folin phenol reagent. Bovine serum albumin was used as standard.

Specific activity of the enzymes has been defined as 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 according to the method of Ohkawa et al. [17]. To 0.2 ml of diluted blood was added 0.2 ml of 8.1% (w/v) sodium dodecyl sulfate, 1.5 ml of acetic acid (20% v/v, pH 3.5), 1.5 ml of thiobarbituric acid (0.8% w/v) and water to make up the volume to 4.0 ml. The tubes were boiled in a water bath at 95° C for 1 h, immediately cooled under running tap water and 5.0 ml mixture of n-butanol and pyridine (15:1 v/v) was added and vortexed. The tubes were then centrifuged at 3,500 rpm for 30 min. The upper layer was aspirated out and the color intensity measured at 532 nm. The reference used was 1,1, 3,3-tetraethoxy propane.

Statistical Analyses

Student's 'T' test was used for the analysis of data.

Results

The results obtained, are reported in Figures 1–4 which show statistically significant decrease in the activities of all the enzymes in the patients compared to controls. The decrease in catalase activity was 36% ($p < 0.0005$), glutathione reductase 36% ($p < 0.01$) and SOD 44% ($p < 0.05$). The levels of MDA showed an increase of 75% ($p < 0.005$) in the patients.

Discussion

Under normal conditions, there exists a fine balance between the rate of generation of free radicals and their detoxification/ elimination by the anti-oxidant system of the body.

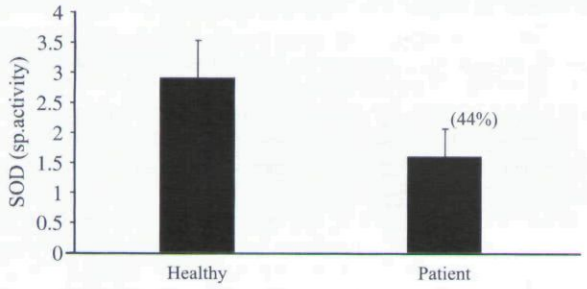

Figure 1. Levels of superoxide dismutase in healthy persons (n = 25) and the patients (n = 37). Values are expressed as mean ± SD. Value in parenthesis represents % change in patients compared to healthy persons.

Figure 3. Levels of glutathione reductase in healthy persons (n = 25) and the patients (n = 37). Values are expressed as mean ± SD. Value in parenthesis represents % change in patients compared to healthy.

Myocardial ischemia and subsequent reperfusion by thrombolysis or other means results in the burst of oxygen consumption with consequent enhanced generation of oxygen derived free radicals [18]. During ischemia, energy to the cells is supplied mainly by anaerobic mechanism; wherein generation of ATP is much less than needed. This results in breakdown of ATP ultimately into hypoxanthine and xanthine. Acidic conditions that prevail under anaerobic conditions cause selective proteolysis of xanthine dehydrogenase to produce xanthine oxidase [19]. Xanthine oxidase utilizes molecular oxygen as a substrate and produces urate and oxy-free radicals. Upon reperfusion, both xanthine oxidase and it's substrates hypoxanthine and xanthine are abundantly available. This situation is conducive for copious generation of oxy-free radicals and their derivatives. Moreover, reperfusion also initiates aerobic metabolism and suddenly mitochondrial electron transport chain becomes overloaded. This enhances the probability of leaching out of the electrons from the chain. The leached electrons from mitochondrial electron transport chain are another potent source of generation of free radicals [20].

Enhanced generation of free radicals during reperfusion of ischemic myocardium has been reported earlier also [21,22]. It has been shown that free radicals have a major role in the generation of atherosclerotic plaque [23] and mediating thrombotic episodes [24]. To adequately neutralize the free radicals, generated during reperfusion, the free radical scavenging systems should be active enough to prevent the damaging effects of these radicals. Of the free radical scavenging enzymes, SOD and catalase are very important because these two enzymes detoxify most toxic free radical derivatives (oxy-free radical, H_2O_2 and hydroperoxides). It has been shown that these two enzymes could prevent changes in myocardial functions and Ca^{+2} handling [25,26].

Findings reported in the present communication show a statistically significant decrease in the activities of free radical scavenging enzymes and a highly significant increase in the free radical-mediated damage (as evidenced by markedly enhanced levels of MDA) in the reperfused patients, compared to control. These observations point to the fact that there is enhanced generation of free radicals and consequent damage in the patients and the activities of the free radical scavenging enzymes are significantly decreased. It is not clear whether the decreased activities of these enzymes are the cause or the consequence of myocardial ischemia/reperfusion but the decreased activities of these enzymes result in inadequate scavenging of free radicals, thus levels of free radicals increase which can cause potential harm to the ischemic myocardium upon reperfusion. The decreased activities of these free radical

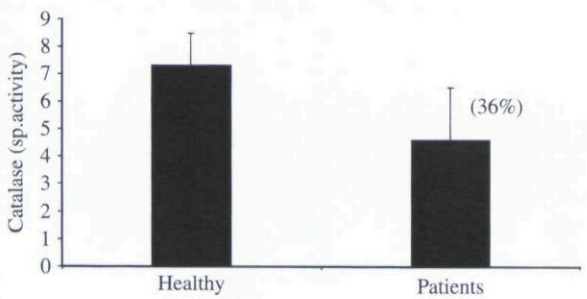

Figure 2. Levels of catalase in healthy persons (n = 25) and the patients (n = 37). Values are expressed as mean ± SD. Value in parenthesis represents % change in patients compared to healthy persons.

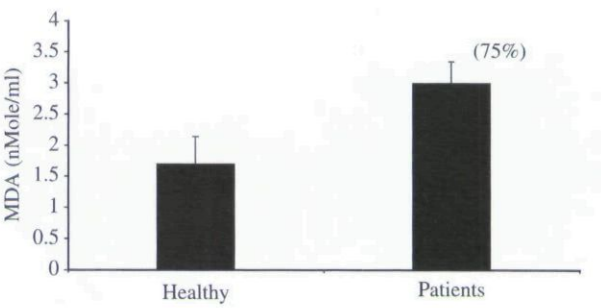

Figure 4. Levels of MDA in healthy (n = 25) and the patients (n = 37). Values are mean ± SD. Value in parenthesis represents % change in patients compared to healthy persons.

scavenging enzymes might be due either to (a) an effect on the alterations in the *de novo* synthesis of the enzymes, or (b) inhibition of the existing enzymes by the free radicals. Since the time taken for reperfusion is very short, alterations in the conformation of the enzymes undergoing biosynthesis to elicit reduced activity appears to be a remote possibility. Damage of the existing enzymes so as to exhibit reduced activities by the high levels of free radicals generated during reperfusion appears to be more probable. This view is further supported by the observation that administration of anti-oxidants such as vitamin E and C for a short time to the patients significantly restores the decreased levels of the enzymes back to near normal values and decreases the levels of MDA generation [27,28].

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