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Adenosine deaminase in ischemia reperfusion injury in patients with myocardial infarction

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Abstract
A comparative study on the levels of erythrocyte adenosine deaminase and lipid peroxidation has been undertaken in patients with myocardial infarction before and after thrombolysis along with matched healthy individuals. Our findings show that adenosine deaminase activity is highly elevated in post-reperfused patients when compared to pre- thrombolysed and healthy persons. Malondialdehyde(MDA) levels are also significantly increased in post-thrombolysed patients. The study reveals an important role of adenosine deaminase in reperfusion injury in patients with myocardial infarction.

Keywords: *Ischemia-reperfusion injury, adenosine deaminase, myocardial infarction, adenosine, MDA*

Introduction

Biological and mechanical stressors such as ischemia, hypoxia, cellular ATP depletion, Ca^{2+} overload, free radicals, pressure and volume overload may independently cause reversible and/or irreversible cardiac dysfunction [1]. Reperfusion of coronary blood flow to the infarcted myocardium is necessary to resuscitate the ischemic or hypoxic myocardium. Timely reperfusion facilitates cardiomyocyte salvage and decreases cardiac morbidity or mortality. Reperfusion of an ischemic area may result, however, in paradoxical cardiomyocyte dysfunction, a phenomenon termed as reperfusion injury. Modalities for reperfusion include thrombolysis as well as percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG) and cardiac transplantation. Several mechanisms and mediators of reperfusion injury have been described, but the most frequently cited include oxygen free radicals, intracellular calcium overload, endothelial and microvascular dysfunction and altered myocardial metabolism [2].

The production of excessive amounts of reactive oxygen species is an important mechanism of reperfusion injury. Molecular oxygen when reintroduced into a previously ischemic myocardium as after thrombolysis, undergoes sequential reduction leading to the formation of oxygen free radicals [2]. These oxy free radicals limit the benefits of reperfusion by thrombolysis [3].

Adenosine is a nucleoside that is present in different body tissues. It is produced by various cell types and behaves in general, as a regulatory substance with many organ-specific functions, that includes an important role in local metabolic regulation of coronary circulation [4]. Adenosine, a degradation product of ATP, acts as an endogenous cardioprotective substance [5,6,7]. In heart, adenosine not only plays a role in regulating growth and differentiation, angiogenesis, coronary blood flow, cardiac conduction and heart rate but may play a role as an endogenous determinant of ischemic tolerance. Elevation in adenosine formation with reduced oxygen delivery is consistent with the idea that myocardial hypoxia is a prerequisite for stimulated adenosine formation [8]. However, it has been shown that elevation in oxygen consumption under normoxic conditions can

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markedly enhance adenosine formation also. This leads to the concept that formation of adenosine is triggered by an imbalance between myocardial energy supply and demand reflected by the so called O_2 supply to demand ratio [9,10].

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) catalyzes the irreversible hydrolytic deamination of adenosine to inosine and ammonia [11]. The short half-life of adenosine is a consequence of its rapid catabolism into pharmacologically inactive inosine and hypoxanthine in endothelial cells and erythrocytes, which possess high activities of adenosine deaminase [12].

This study has been aimed to understand the relationship that exists between adenosine deaminase activity and its effect on myocardial ischemic patients. In the present study we have investigated erythrocytic adenosine deaminase activities in patients with myocardial infarction, before and after thrombolysis and compared them with healthy persons serving as control. Also, we have measured lipid peroxidation levels (as an index of free radical mediated damage) in their erythrocytes.

Materials and methods

All the chemicals employed, were of analytical grade of Qualigens or equivalent. All the biochemicals used were procured from Sigma Chemical Co., USA.

The study was approved by the Departmental Ethical Committee. Informed consent was taken from healthy persons as well as patients employed in the study. The patient group comprised of 17 male patients, between the ages of 30–70 years. Patients with known organic diseases were excluded from the study. Blood samples of these patients were collected before reperfusion and within 6 h after reperfusion. Twenty age and sex matched healthy persons served as control. The patients were thrombolysed with streptokinase as per the set protocol of the Department of Medicine, K.G.'s Medical University, Lucknow.

Preparation of Hemolysate

Venous blood (3.5 mL) was withdrawn before thrombolysis and within 6 h after reperfusion from the patients and transferred into polypropylene tubes containing 0.5 mL 3.8% sodium citrate, pH 7.2. The tubes were gently rotated to mix the contents and centrifuged at 2000 × g for 20 min at 4°C. The supernatant was discarded. The pellet containing RBC's was washed thrice with ice cold 0.85% NaCl on a centrifuge at 200 × g for 10 min at 4°C. The final pellet was taken up in 4.0 mL chilled water, left in the cold for 1 h and then the suspension centrifuged at 2000 × g for 20 min. The supernatant thus obtained, was used for analysis after suitable dilution.

Adenosine deaminase assay

The enzyme assay was carried out as described by Mustafa et al. [13]. The assay system consisted of 1.0 mL of phosphate buffer 50 mM, pH 7.4 and 0.1 mL of enzyme preparation, in the linearity range, and the volume adjusted to 1.9 mL with water. The reaction was started by addition of 0.05 μmole of adenosine in 0.1 mL and carried out at 30°C for 30 min. The reaction was then stopped by addition of 1.0 mL of 10% (v/v) perchloric acid and the solution was centrifuged at room temperature at 2000 × g for 20 min. The absorbance of the supernatant was read at 265 nm. A control was simultaneously run in which the substrate was added after stopping the reaction. For calculation of the activity, the molar extinction coefficient of adenosine was taken to be 8100.

A unit of enzyme activity was defined as the conversion of 1 nmole of adenosine to inosine per minute per mL enzyme under the assay conditions.

Protein estimation

Protein estimation was done by the method of Lowry et al., using Folin phenol reagent [14] with bovine serum albumin used as standard.

The specific activity of the enzyme was defined as enzyme activity per mg protein.

Measurement of lipid peroxidation

Lipid peroxidation measurement was done in terms of malondialdehyde (MDA) formed, according to the method of Ohkawa et al. [15]. To 0.05 mL of the hemolysate preparation was added 0.2 mL sodium dodecyl sulphate, 8.1% (w/v), 1.5 mL glacial acetic acid and thiobarbituric acid, 0.8% (w/v) to make up the volume to 3.0 ml. The contents of the tubes were mixed vigorously, heated over a water bath at 90°C for 1 h and then immediately cooled under running tap water. To each tube 1.0 mL of distilled water and 5.0 mL of a mixture of n-butanol and pyridine (15:1, v/v) was added and the tube vortexed thoroughly and centrifuged at 800 × g for 20 min. The upper layer was aspirated out and the color intensity measured at 532 nm. The reference used was 1,1,3,3 tetraethoxypropane (TEP).

Statistics

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

Results

As shown in Table I, the specific activity of adenosine deaminase in post-reperfused patients is increased by 125% when compared to healthy persons

Table I. Levels of adenosine deaminase and malondialdehyde (MDA) in erythrocytes of healthy persons, pre-reperfused and post-reperfused patients with myocardial infarction.

Cases	Specific activity of adenosine deaminase (unit/mg protein)	MDA (nmole × 10^4/mL RBC lysate)
Healthy (n = 20)	0.1382 ± 0.0475	6.639 ± 1.23
Pre-reperfused (n = 17)	0.1258 ± 0.0589	8.153 ± 2.809
Post-reperfused (n = 17)	0.3115 ± 0.0892	9.699 ± 3.822
Statistical analysis of the data	Specific activity of adenosine deaminase	MDA
Healthy vs Pre-reperfused patients	$p < 0.4$	$p < 0.1$
Healthy vs Post-reperfused patients	$p < 0.0005$	$p < 0.01$
Pre-reperfused patients vs Post-reperfused patients	$p < 0.0005$	$p < 0.2$

Values are mean ± S.D; n = no. of cases. $p > 0.01$ not significant, $p < 0.01$ significant, $p < 0.0005$ highly significant.

($p < 0.0005$) and MDA levels were also found to be significantly increased in post-reperfused patients by 46% when compared to healthy controls ($p < 0.01$).

The specific activity of adenosine deaminase in post-thrombolysed patients is very significantly increased (146%) when compared to pre-thrombolysed patients ($p < 0.0005$), but the MDA levels do not show any significant increase between the two patient groups.

The specific activity of adenosine deaminase in healthy persons is more or less unchanged when compared to pre-thrombolysed patients($p < 0.4$) The MDA levels in pre- thrombolysed is 22% higher than healthy controls ($p < 0.1$).

Discussion

Reactive oxygen species (ROS) are produced in living organisms from partial reduction of molecular oxygen under various physiological and pathological conditions [16]. These highly detrimental species are effectively scavenged off or neutralized by free radical scavenging enzymes and antioxidants. An imbalance between ROS production and antioxidant cell defenses has been reported to occur in several pathophysiological conditions as in ischemia and reperfusion [17]. Myocardial reperfusion injury is also attributed to the excessive generation of ROS during reperfusion of ischemic myocardium [2].

The integrity and several parameters of red blood cell functions are negatively affected by increased oxidative stress. In fact, changes of erythrocyte membrane ionic permeability [18], increase of lipid peroxidation [19], oxidation of protein sulphydryl groups[20] and activation of proteolysis [21] have all been described following the challenge of red blood cells with different oxygen radical generating systems.

It has been clearly indicated that energy metabolism and lipid peroxidation of human erythrocytes are both affected by oxidative stress, which is also responsible for hemolysis, but only if lipid peroxidation is higher than the threshold tolerance compatible with cell integrity.

The brevity of the cardiovascular effects of adenosine has been ascribed to its rapid deamination [22]. The disappearance rate of adenosine in whole blood depends on the amount of adenosine deaminase present in the red cells and on the permeability of the erythrocyte membrane to adenosine and it has been demonstrated that human red blood cell membrane is highly permeable to adenosine. The disappearance of adenosine from blood is principally due to its degradation into inosine and hypoxanthine by the enzyme present in the erythrocytes [23]. It is, therefore, postulated that the concentration of adenosine that reaches the effective site, the coronary arterioles, is reduced by virtue of continuing deamination and utilization by the red cells as the blood containing adenosine courses through the cannula and coronary arteries[24].

In myocardial ischemia-reperfusion, xanthine oxidase has been shown to be a major source of oxygen derived free radical generation [25].The substrate for this enzyme is provided by adenosine deaminase which deaminates adenosine to inosine, ultimately leading to the formation of hypoxanthine and xanthine. Zweier et al. have found that inhibition of adenosine deaminase decreased the formation of oxygen free radicals which in turn would prevent myocardial reperfusion injury [25].

We have observed significantly enhanced activity of adenosine deaminase in erythrocytes of post reperfused patients, which leads to the depletion of adenosine. Adenosine, as is reported, is an endogenous activator of cellular antioxidants [26]. The depletion of adenosine may lead to enhanced production of free radicals which may be the major contributor to reperfusion injury.

Oxygen-derived free radical generation in myocardial reperfusion causes cellular damage by peroxidation of polyunsaturated membrane phospholipids [27] and erythrocytes are very susceptible to free radical-induced lipid peroxidation which happen to be the basic element involved in ischemia reperfusion injury.

High MDA levels are also found in post thrombolysed patients when compared to healthy persons.

It has been observed that there is a rise in lipid peroxidation levels after successful thrombolysis and the free radical mediated damage at the time of reperfusion leads to the occurrence of reperfusion injury [28]. A statistically significant increase in MDA levels was not observed in pre thrombolysed patients when compared to healthy persons which may indicate that there is lower ROS generation prior to reperfusion which leads to lower lipid peroxidation.

Our study has tried to bring into focus the implication of adenosine deaminase and its contribution to reperfusion injury. The activity of adenosine deaminase lowers the cardioprotective adenosine levels and may lead to the generation of substrates for the pro-oxidant enzyme which could result in enhanced generation of superoxide free radicals and their more toxic derivatives.

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