ERYTHROCYTIC ADENOSINE DEAMINASE IN POST MYOCARDIAL INFARCTION ANGINA PATIENTS

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ABSTRACT

A comparative study on the levels of erythrocyte adenosine deaminase and lipid peroxidation has been undertaken in post myocardial infarction angina patients along with age and sex matched healthy individuals serving as control. Present findings show that levels of adenosine deaminase is highly elevated in post myocardial infarction angina patients compared to healthy persons. Malondialdehyde levels are also significantly increased in post myocardial infarction angina patients. The study shows that adenosine deaminase has an important implication in ischemic myocardial syndrome.

KEY WORDS

Adenosine deaminase, Post MI angina, Lipid peroxidation.

INTRODUCTION

Coronary artery disease is multifactorial in etiology and has spectrum of presentations ranging from stable angina, acute coronary syndrome to completely asymptomatic disease. Myocardial ischemia results from an imbalance between myocardial oxygen demand and supply. Adenosine, a degradation product of ATP, acts as an endogenous cardioprotective agent in conditions such as myocardial ischemia (1). Enhanced adenosine formation with reduced oxygen delivery is consistent with the idea that myocardial hypoxia is a prerequisite for stimulated adenosine formation. It leads to the concept that formation of adenosine is triggered by an imbalance between myocardial energy supply and demand, reflected by the O₂ supply to demand ratio (2). Adenosine acts as a coronary artery vasodilator to increase the blood supply to ischemic myocardium and is known to play a major role in the regulation of coronary blood flow in the ischemic heart (1).

Adenosine deaminase (adenosine aminohydrolase; EC 3.5.4.4) is one of the key enzymes of purine catabolic pathway and catalyses the hydrolytic deamination of adenosine to inosine and ammonia (3). The short half-life of adenosine is a consequence of its rapid catabolism into inosine and hypoxanthine in erythrocytes which possess high activities of adenosine deaminase.

In the present study we have measured the activity of adenosine deaminase and extent of lipid peroxidation in the erythrocytes of post MI angina patients and compared with that of healthy persons serving as control to understand a possible role of the enzyme in ischemic myocardial disorders.

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 each person included in the study. The patient group comprised of thirty one patients of the age group 45-65 yrs. Patients with any known organic disease were excluded from the study. Thirty five age and sex matched healthy persons served as control. All the cases were non-smokers, non-alcoholic and non-diabetic. The patients were receiving medications which included beta-blockers, statins, ACE inhibitors and aspirin.

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Preparation of Hemolysate: Venous blood (3.5ml) was collected and transferred into sterile polypropylene tubes containing 0.5 ml 3.8% (w/v) sodium citrate, pH 7.2. The tubes were gently rotated to mix the contents and centrifuged at 2000xg 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 200xg for 10 min at 4°C each. The final pellet was taken up in 4.0 ml chilled water, left in the cold for 1 hr for hemolysis and then centrifuged at 2000xg 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 (4). The assay system consisted of 1.0 ml of phosphate buffer 50 mM, pH 7.4; 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 mmole adenosine and the reaction carried out at 37°C for 30 min. The reaction was stopped by addition of 1.0 ml of 10% (v/v) perchloric acid and the mixture was centrifuged at room temperature at 2000xg for 20 min. The absorbance of the supernatant was read at 265 nm. A control lacking the substrate was run simultaneously. Millimolar extinction coefficient of adenosine at 265 nm was 8.1. A unit of enzyme activity has been defined as the conversion of 1 nmole of adenosine to inosine per minute under the assay conditions.

Protein estimation: Protein estimation was done by the method of Lowry et al. (5) using Folin phenol reagent. Bovine serum albumin was used as the standard. The specific activity of the enzyme has been defined as unit per mg protein.

Measurement of lipid per-oxidation: Lipid per-oxidation was estimated in terms of MDA formed, according to the method of Ohkawa, Ohishi and Yagi (6). To 0.05 ml of the hemolysate, 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 hr and then immediately cooled under running tap water. To each tube, 1.0 ml of water and 5.0 ml of a mixture of n-butanol and pyridine (15:1, v/v) was added and the tube vortexed and centrifuged at 800xg 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).

Estimation of CPK-MB and Troponin I levels: Three ml of blood was collected from all the subjects, under study, to estimate the serum CPK-MB and Troponin I levels. All the

estimations were carried out in an ISO-9001:2000 certified laboratory.

Statistical analyses were carried out by using Mann-Whitney test. A value of $p < 0.05$ has been considered statistically significant.

RESULTS

The personal profiles and clinical parameters of all the subjects under study are shown in Table 1. The specific activity of adenosine deaminase in post MI angina patients is very significantly increased when compared to healthy persons ($p < 0.0001$) and MDA levels are also very significantly increased when compared to healthy persons ($p < 0.0001$) as shown in Table 2.

Table 1: Personal profile and clinical details of healthy persons and post MI angina patients

	Healthy persons	Post MI angina
Age	54.0±7.07	59.2±5.03
No. of Cases	n = 35	n = 31
Sex Males	n = 29	n = 27
Females	n = 6	n = 4
Demographic data		
Urban background	n = 25	n = 20
Rural background	n = 10	n = 11
Details of myocardial ischemic disease	-	Myocardial infarction patients Males (n = 9) Failed reperfused patients Males (n = 18) Females (n = 4)
CPK-MB (ng/ml)	0.32±0.031	24.42±1.56
Troponin I (ng/ml)	0.12±0.028	6.86±0.44

Values are mean ± SD ; n = no. of samples; Lab reference range for serum CPK-MB : 0.100 – 4.9 ng/ml; Lab reference range for serum Troponin I : < 0.40 ng/ml

DISCUSSION

Myocardial ischemia is a potent stimulator of adenosine release from the heart. It has been reported that adenosine is released during and immediately after induced angina. Adenosine released from ischemic areas may diffuse to marginally ischemic zones where resultant increase in blood flow may help to protect the jeopardized cells (7).

The disappearance rate of adenosine in whole blood depends on the amount of adenosine deaminase present in the red cells and upon the permeability of erythrocyte membrane to

Table 2: Levels of adenosine deaminase and malondialdehyde (MDA) in the erythrocytes of healthy persons and post MI angina patients

Cases	Specific activity of adenosine deaminase (unit /mg protein)	MDA Levels (nmole/ml RBC lysate)
Healthy persons	0.1627±0.0625	77.57±16.83
Post MI angina patients	0.2985±0.0654	91.58±11.83
Healthy persons vs. post MI angina patients	p<0.0001	p<0.0001

Values are mean ± SD; $p > 0.05$ not significant, $p < 0.0001$ highly significant.

adenosine. It has been demonstrated that human red blood cell membrane is highly permeable to adenosine (8). The measurement of adenosine release is difficult owing to the low concentration of adenosine in blood and its relatively short half-life in erythrocytes due to adenosine deaminase. It is, therefore, postulated that the concentration of adenosine that reaches the effective site, the coronary arterioles, is reduced by continuing deamination and utilization by the red cells as the blood containing adenosine courses through the coronary arteries (9). In a recent study, dipyridamole, an inhibitor of both adenosine transport and adenosine deaminase, has been investigated as a possible candidate for the treatment of angina pectoris (10) which indicates that adenosine deaminase may have a crucial role in myocardial ischemic diseases. Adenosine, as is reported, is an endogenous activator of cellular antioxidants (11). The depletion of adenosine by adenosine deaminase may lead to enhanced production of free radicals which are implicated in the pathogenesis of myocardial ischemic injury. We have earlier shown the significance of adenosine deaminase in ischemic myocardial syndromes (12, 13). Oxygen derived free radical generation causes cellular damage by peroxidation of polyunsaturated membrane phospholipids and erythrocytes are very susceptible to free radical induced lipid peroxidation (14).

In the present study, we have found significantly enhanced level of adenosine deaminase activity in the erythrocytes of post MI angina patients which indicates that adenosine deaminase activity can be used in the advent of better therapeutic measures for the treatment of ischemic heart diseases. The study also implicates that adenosine deaminase activity may be useful as a supplemental index during angina to monitor the effects of regional ischemia on myocardial metabolism.

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