Free radicals in myocardial injury: experimental and clinical studies

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Abstract

The exposure of cardiac cells to OFR generated artificially, showed a marked decrease (p < 0.01) in cellular utilization of glucose alongwith a significant decrease in calcium uptake (p < 0.05). We have also provided evidence for a direct relationship of neutrophil OFR production with the extent of myocardial ischemia in patients of myocardial infarction. Our data provides evidence for implication of OFR in myocardial injury and the pivotal role played by modulators like calcium, ECGF and prostaglandins in potentiating damage to the myocardium. (Moll Cell Biochem 111: 71–76, 1992)

Key words: neutrophil, free oxygen radicals, calcium, endothelial cell growth factors, prostaglandins, indomethacin

Introduction

In recent years oxygen free radicals have been implicated in cell damage in diverse organs such as brain, kidney, liver, gut, myocardium and blood vessels [1-3]. Out of these, the major thrust has been in the area of vascular and myocardial injuries [4]. The initial injury is because of the ischemic changes [5-7]. But later on the aggravation of injury is through inflammatory cells [7-9]. There have been ample evidences in literature both in clinical and experimental settings that the inflammatory cells play a key role in myocardial and vascular damage [8-11]. The depletion of the neutrophils or use of anti-inflammatory agents have also been shown to decrease the myocardial injury [10-12]. However, pathophysiology of atherosclerosis and myocardial infarction is multifactorial. There may be many modulators which affect the end result of myocardial injury, one of the key modulators is calcium which apart from modulating myocyte functions, is also necessary for neutrophils to function [13, 14]. Another important substance is Prostaglandins which takes an additional part in many important metabolic pathways of inflammatory cells such as Protein Kinase C mediated pathway [15]. During the atherosclerotic process the endothelial cells generate several key products [16], which also may have important interactions in this phenomenon. Two of them are prostacyclin which has an ameliorating effect on TxB2 liberated by platelets and ECGF which may have a chemotactic effect on inflammatory cells. Implication of some of these key modulators in the process of atherosclerosis and myocardial injury is the theme of the study.
Materials and methods

Subjects

Patients of Myocardial Injury admitted to the wards or attending the clinic services of PGIMER, Chandigarh, were taken for the study. The patients were subgrouped as follows;

Group-I Acute myocardial infarction – 18 patients, Controls twelve normal healthy adults matched for age and sex.

The diagnosis of acute myocardial infarction was based on (1) clinical setting of typical chest pain and associated features (2) serial ECG showing the presence of abnormal waves with ST segment elevation and T wave inversion. (3) Elevation of CKMB isoenzyme.

Exclusion Criteria

i) Patients/Controls who had any other inflammatory conditions at or two weeks preceding their inclusion in the study.

ii) Patients/Controls receiving steroidal or non steroidal anti inflammatory agents at the time of the study.

iii) Patients/Controls taking calcium channel blockers or beta blockers during the preceding 2 weeks.

Methods

Serial venous blood samples were drawn from each subject. Fist sample was at presentation varying from 6-24 hours of the onset of ischemia and second sample at 72 hours later.

CK-MB:

This was assayed by using standard Boehringer Mannheim Kits. cat. No. 126322.

Isolation of neutrophils and monocytes

Heparinized venous blood was subjected to dextran sedimentation. Leukocyte rich plasma obtained was processed by density gradient centrifugation on a ficollhypaque column as described by Boyum [17]. Viability was assessed by trypan blue exclusion.

Chemiluminescence

The neutrophils were suspended in MEM without indicator (pH 7.4) and taken in polyethylene luminometric cuvettes and incubated for 15 min at 37°C in CO₂ incubator. 20μl of luminol from a stock solution of (5 mg/ml of 0.1 N NaOH) was added and further stimulated by 20μl latex as required by the method of Cheung et al. [18]. The chemiluminescence (CL) values were recorded in counts per minute.

Animal studies

Male albino mice weighing [30–35 gm) were used for the study.

Myocyte isolation

In brief hearts were removed from mice. Myocytes were obtained by perfusing the hearts with minimal essential medium supplemented with 0.2% hyaluronidase and 0.1% collagenase type II following by mincing of the hearts and disintegration by gentle strokes with loose tolerance glass tissue homogenizer. Cells were purified in MEM containing 1% Bovine Serum Albumin, (essentially fatty acid free) [19]. Viability was determined by light microscopy using both Trypan blue exclusion and elongated, clear striated, non-granular appearance as the criteria for normal morphology.

These isolated myocytes were then subjected to FOR, generated artifically by using a enzyme substrate system involving 0.2 mM xanthine and 27 mU xanthine oxidase by the method of McDonough et al. 1987.

Glucose oxidation

This was studied by the method of Babior and Cohen [21].

Calcium uptake

Cardiac membrane vesicles (CMV) were prepared as described by Reeves and Sutko [22]. 40μg of CMV was taken and preloaded with 160 mM NaCl and Sodium dependent calcium transport determined in presence of superoxide generating system.

Effect of indomethacin

Rhesus monkeys weighing 4–5 kg were taken for the study. Groups I and III were maintained on stock pellet diet only while groups II and IV were put on atherogenic diet providing 1.8 mg cholesterol/kilo calorie of the diet per animal per day. After six months monkeys from Gp. III and IV were given indomethacin at the dosage of 2.5 mg orally every alternate day. The duration of