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The Activity of Antioxidative Enzymes of Neutrophils in Patients with Unfavorable Outcome of Acute Coronary Syndrome

Andrey Kratnov, Elena Timganova

Department of Therapy of Yaroslavl State Medical University, Yaroslavl, Russia
Email: kratnov@mail.ru

Abstract

Background: There is a necessity for an establishment of specific markers of oxidative stress for screening in populations of high risk and an estimation of efficiency antioxidative therapies. **Objective:** Studying the activity of antioxidative enzymes at neutrophils in patients with acute coronary syndrome depending on outcome during the year. **Methods:** Intracellular metabolism of neutrophils was studied in 108 patients, of whom, in 58 persons has been diagnosed acute coronary syndrome, and 50 individuals were without coronary heart disease. **Results:** In patients with acute coronary syndrome, in comparison with patients without coronary heart disease, growth of production of superoxide anion on background reduction of glutathione reductase activity in neutrophils was revealed. The greatest reduction in parameters of glutathione reductase and catalase at simultaneous growth of activity of NAD(P)H oxidase and myeloperoxidase at neutrophils was observed in patients with fatal outcome during the year. **Conclusion:** The decrease of efficiency antioxidative protection of neutrophils associates with risk of fatal outcome in patients with acute coronary syndrome.

Keywords

Antioxidance, Reactive Forms of Oxygen, Neutrophils, Acute Coronary Syndrome

1. Introduction

Despite of presence convincing proofs that free radicals play an important role in development of atherosclerotic defeat, till now does not exist standard marker of oxidative stress and estimation of efficiency of antioxidant defence at cardiovascular diseases [1]. There are proofs, that the increase in peripheral blood of neutrophils which play an important role in reperfusion damage of myocardium is prognostic risk marker of cardiac death [2] [3].

In the recent years was revealed association the changes of intracellular metabolism of neutrophils with development of endothelial dysfunction which also is risk marker of fatal outcome at coronary heart disease [4] [5].

The objective was to study the activity of antioxidative enzymes at neutrophils in patients with acute coronary syndrome depending on outcome during the year.

2. Participants and Methods

2.1. Participants

The study included 58 patients with acute coronary syndrome aged 34 to 85 years (mean ± SD age: 62.5 ± 10 years), of them, 31 (53.4%) patients had unstable angina and 27 (46.6%) had acute myocardial infarction. 50 patients without coronary heart disease aged 18 to 69 years (mean ± SD age: 42.2 ± 13.4 years) which were hospitalized in hospital for professional survey have been examined. In order to exclude coronary heart disease in all patients, the electrocardiography was performed, along with bicycle ergometry, Holter electrocardiography monitoring, and echocardiography.

The patients with acute coronary syndrome were followed for 12 months from the date of entry into the study. Cases of repeated anginal attacks at rest (unstable angina), repeated or new nonfatal myocardial infarction, and cardiac death were recorder.

2.2. Methods

The material for this study was performed at admission of patients to the hospital with the consent of the Ethical Committee. Analysis of peripheral blood was performed at admission to the cardiology intensive care unit. Neutrophils were isolated from heparinised blood in Ficoll-Verographin double density gradient 1.077 and 1.092 g/cm^3. The second interface cells contained 95% of neutrophils. The number of neutrophils in the cell suspension was counted in a cell with Goryaev vivo staining with methylene blue in 3% acetic acid (Turk dye) to determine viable cells. Cell viability, estimated by trypan blue, was more than 90%. The cell suspension was diluted with medium 199, adjusting the concentration of 5×10^6 neutrophils per 1 ml. The final result was calculated per 1×10^6 neutrophils, proceeding from the amount of 5×10^6 cells in the studied suspension.

The test of spontaneous and stimulated nitroblue tetrasolium reduction (**NBT-test**) was performed by means of quantitative spectrophotometric method of Gentle T. A. & Thompson R. A. [6] using 0.2% nitro blue tetrasolium in phosphate buffer pH 7.2, fixing neutrophils with methanol and solubilising reduced diformasan in 2 M potassium hydroxide and dimethylsulfoxide 3:5 volume mixture. The calibration was performed by direct solubilisation of nitroblue tetrasolium in potassium hydroxide and dimethylsulfoxide at same ratio. The photometry was performed at 495 nm wavelength using spectrophotometer. For evaluation of neutrophils C3-receptor activity, suspension of phytohemagglutinin (*Phaseolus vulgaris*, "PanEco", USA) was used. The suspension was added to the cuvettes with neutrophils in amount of 0.2 ml for activated NBT-test.

Myeloperoxidase activity in neutrophils was studied using a 0.04% solution ortofenilendiamin in phosphate buffer pH 5.0, with the addition of 0.33% hydrogen peroxide solution at a ratio of 20:1 by volume. The reaction was stopped after 10 minutes of 10% sulphuric acid solution. The photometry was performed at 492 nm [7].

The determination of glutathione reductase activity in neutrophils was evaluated by method on degree of oxidation of NADH. In the control and skilled tests containing 0.5 ml of suspension neutrophils 1.5 ml of distilled water, 0.1 ml NADH and 0.3 ml buffer pH 8 was added. The reaction started by addition 0.2 ml of 0.033 mM solution oxidized glutathione. The tests were left at temperature 37°C for 30 minutes. To stop the reaction the test was placed in refrigerator for 10 minutes at temperature 4°C. The photometry was performed at 340 nm [8].

The determination of catalase activity in neutrophils was based on the ability of hydrogen peroxide to form salts with molybdenum stable colored complex. The reaction was run by adding 0.03% hydrogen peroxide solution and was stopped after 10 minutes, adding a 4% solution of ammonium molybdate. In control test instead of neutrophils distilled water was brought. The color intensity was measured at $\lambda = 410$ nm against a control sample of distilled water [9].

2.3. Statistical Analyses

Statistical data processing was performed using the package Statistica 8.0 (StatSoft, Inc., USA). The data for categorical variables are expressed as absolute values and percents, and the data for continuous variables are ex-

pressed as the median with the 25% - 75% inter quartile range. Differences between groups were considered statistically significant at $P < 0.05$.

3. Results

3.1. Characteristic of the Patients with Acute Coronary Syndrome

Repeated coronary events in patients with acute coronary syndrome during the year are presented in **Table 1**. Unfavorable outcome occurred in 34 (58.7%) patients. Of them repeated anginal attacks at rest were recorder in 20 (58.8%) patients, repeated or new myocardial infarction in 8 (23.6%) patients, and coronary death in 6 (17.6%).

3.2. Intracellular Metabolism of Neutrophils of the Study Participants

In patients with acute coronary syndrome in comparison with people without coronary heart disease authentically higher the parameters of the spontaneous NBT-test (103.6 > 92.8 mM NBT reduced; $P = 0.02$) and lower activity of glutathione reductase (42.7 < 67.7 nM·l^{-1}·sec^{-1}; $P = 0.003$) at neutrophils were observed (**Table 2**). Also in patients with acute coronary syndrome the parameters of the stimulated NBT-test were higher (116.3 > 109.2 mM NBT reduced), that testifies not only activation of NAD(P)H oxidase and production of superoxide anion, but the changes of structure glycoprotein receptors on surface of plasmatic membrane of neutrophils.

3.3. Intracellular Metabolism of Neutrophils in Dependence from Repeated Coronary Events in Patients with Acute Coronary Syndrome

At studying intracellular metabolism of neutrophils in process of weighting unfavorable outcome not only activation of production of superoxide anion radicals and more the prompt reply to stimulus of neutrophils, but also decrease in activity of antioxidative enzymes in cell were observed. The greatest changes intracellular metabolism of neutrophils in patients with fatal outcome during the year was revealed. In the given patients the decrease of glutathione reductase (13.3 < 57.1 nM·l^{-1}·sec^{-1}) and catalase activity (424.4 < 522.2 mCat/l) was accompanied by the greatest growth of NAD(P)H oxidase activity according to the parameters of the spontaneous (114.7 > 100.4 mM NBT reduced) and stimulated NBT-test (138.8 > 106.1 mM NBT reduced), and myeloperoxidase activity (19.4 > 15.5 SED) at neutrophils (**Table 3**).

Table 1. Repeated coronary events in patients with acute coronary syndrome.

Parameter	Acute coronary syndrome (n = 58)
Repeated coronary events	34 (58.7)
Repeated anginal attacks	20 (58.8)
Myocardial infarction	8 (23.6)
Cardiac death	6 (17.6)

Table 2. Intracellular metabolism of neutrophils of the study participants.

Parameter	No coronary heart disease (n = 50)	Acute coronary syndrome (n = 58)	P
Spontaneous NBT-test, mM	92.8 (75; 111.3)	103.6 (90; 118.5)	0.02
Stimulated NBT-test, mM	109.2 (105.4; 117.3)	116.3 (101.7; 133.1)	> 0.05
Myeloperoxidase, SED	15.3 (11.5; 17.9)	14.5 (7.5; 22)	> 0.05
Glutathione reductase, nM·l^{-1}·sec^{-1}	67.7 (26.8; 80.4)	42.7 (12.6; 59.8)	0.003
Catalase, mCat/l	494.1 (359.6; 532.8)	484.7 (313; 607.3)	> 0.05

Table 3. Intracellular metabolism of neutrophils in patients with acute coronary syndrome.

Parameter	Favorable outcome (n = 24)	Repeated anginal attacks (n = 20)	Myocardial infarction (n = 8)	Coronary death (n = 6)
Spontaneous NBT-test, mM	100.4 (87.4; 119.9)	103.1 (89.7; 113.8)	106.3 (99; 116)	114.7 (109.9; 118.5)
Stimulated NBT-test, mM	106.1 (93.8; 118.5)	121.2 (104.5; 135.1)	123.4 (114.6; 133.1)	138.8 (124.9; 152.7)
Myeloperoxidase, SED	15.5 (8.5; 22.5)	12.9 (7.1; 18.3)	11.6 (5.7; 14.1)	19.4 (12; 25.1)
Glutathione reductase, $nM \cdot l^{-1} \cdot sec^{-1}$	57.1 (15.9; 77.1)	35.1 (3.9; 43.8)	48.7 (33.2; 66.5)	13.3 (7.9; 18.6)
Catalase, mCat/l	522.2 (350.9; 696.5)	492.3 (303; 686.6)	398.2 (243.7; 491.4)	424.4 (313; 414.2)

4. Discussion

For today the doctrine about phagocytosis is a set of representations about the free and fixed cells of marrow which possessing powerful cytotoxic potential, exclusive reactance and high mobilization readiness are the first line of mechanisms immunological homeostasis. Among phagocytes the most mobile cells are neutrophils which before all are involved in any inflammatory process. However phagocytes in struggle for constancy of the internal environment of organism are capable not only to destroy microbes, but also to damage own tissue under certain conditions [5].

Connection of neutrophils to damage of myocardium begins with the first minutes after occlusion of coronary artery [10]. At reperfusion of the myocardium the adhesion of neutrophils to endothelium of coronary arteries leads to the development of the "no-reflow phenomenon" capillaries including collaterals. Production of destructions factors in neutrophils quickly accrues at their migration from vessels in myocardium that results to its damage [11]. At autopsy of patients with acute myocardial infarction was revealed that neutrophil infiltration of coronary arteries is actively associated with acute coronary events [12]. Believe that neutrophils exert an aggravating effect in arrhythmogenesis during ischemia/reperfusion, and inhibition of neutrophils accumulation can at least partially reduce arrhythmogenesis [13].

The brightest display of reactance neutrophils is the production of reactive forms of oxygen by means of which cells carry out the functions of effector. Reactive oxygen species initiate the process non fermentative peroxide oxidation. Uncontrollable formation of products peroxide oxidation in neutrophils can promote destruction of cellular membranes of phagocytes and damage of muscular tissue. The process of reactive oxygen species production in neutrophils is supervised by the ramified system antioxidative protection which functional full value substantially determines current of disease and probability of recovery [14]. The meta-analysis of numerous researches testifies the reduction of antioxidative protection in patients with coronary heart disease and its risk factors [15].

In the given research the development of fatal outcome in patients with acute coronary syndrome associated with the decrease of activity of glutathione reductase and catalase in neutrophils. The revealed reduction antioxidative protection of neutrophils in patients with coronary death was observed on background of increase of activity NAD(P)H oxidase and myeloperoxidase at cells that could promote destabilization of cells and development of endothelial dysfunction. It is possible, that in comparison with other patients with acute coronary syndrome the people with fatal outcome had additional factors of activation of oxygen-dependent metabolism of neutrophils which promoting the greater exhaustion antioxidative protection.

5. Conclusion

Thus, the decrease of activity of antioxidative enzymes (glutathione reductase and catalase) at neutrophils in patients with acute coronary syndrome associates with risk of fatal outcome in during the year.

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