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LEVELS OF MALONDIALDEHYDE AND MAGNESIUM AND THE ACTIVITY OF CATALASE AND GLUTATHIONE REDUCTASE AS MARKERS OF OXIDATIVE STRESS IN THE ERYTHROCYTES OF PATIENTS WITH ISCHEMIC HEART DISEASE

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СОДЕРЖАНИЕ МАГНИЯ И МАЛОНОВОГО ДИАЛЬДЕГИДА И АКТИВНОСТЬ КАТАЛАЗЫ И ГЛУТАТИОНРЕДУКТАЗЫ В ЭРИТРОЦИТАХ БОЛЬНЫХ ИШЕМИЧЕСКОЙ БОЛЕЗНЬЮ СЕРДЦА

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РЕЗЮМЕ

Показано, что в эритроцитах больных ишемической болезнью сердца возрастает содержание магния и малонового диальдегида. Вместе с этим, в эритроцитах повышается активность антиоксидантных ферментов: каталазы и глутатионредуктазы. Полученные данные свидетельствуют о возможности развития компенсаторных процессов, направленных на поддержание в эритроцитах больных прооксидантно-антиоксидантного равновесия.

ВМІСТ МАГНІЮ І МАЛОНОВОГО ДІАЛЬДЕГІДУ ТА АКТИВНІСТЬ КАТАЛАЗИ І ГЛУТАТИОНРЕДУКТАЗИ В ЕРИТРОЦИТАХ ХВОРИХ НА ІШЕМІЧНУ ХВОРОБУ СЕРЦЯ

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РЕЗЮМЕ

Показано, що в еритроцитах хворих на ішемічну хворобу серця зростає вміст магнію і малоного діальдегіду. Разом із цим, в еритроцитах підвищується активність антиоксидантних ферментів: каталази та глутатіонредуктази. Отримані дані свідчать про можливість розвитку компенсаторних процесів, спрямованих на підтримання в еритроцитах хворих прооксидантно-антиоксидантної рівноваги.

Keywords: erythrocytes, catalase, glutathione reductase, lipid peroxidation, oxidative stress, ischemic heart disease.

Oxidative stress is a term denoting imbalance between the production of oxidants and the related defence systems of an organism [9,13,15]. Oxidative stress appears to play an important role in the development of several diseases from their Ischemic Heart Disease (IHD). Oxygen free radicals, such as superoxide anion radical, singlet oxygen, hydroxyl radical, and perhydroxyl radical are together referred to as reactive oxygen species (ROS) and play an important role in the pathogenesis of several diseases [7,14].

It has been suggested that free radical damage compromises composition integrity of cell membranes, which decreases membrane fluidity [6]. Exposure to reactive oxygen species from a variety of sources has led organisms to develop a series of defence mechanism [17] that functions interactively and synergistically to neutralize free radicals. Thus, antioxidants are capable of stabilizing or deactivating, free radicals before they

attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being [9]. In previous studies was shown that, erythrocytes are involved in the pathological process of some diseases [2,3]. Therefore, the purpose of this work was to study some markers of the prooxidant-antioxidant system in erythrocytes of patients with IHD.

MATERIAL AND METHODS

The present study was performed on erythrocytes of seven healthy donors, and seven patients with IHD.

Blood samples were collected by venous puncture in heparinized tubes and the plasma was separated by centrifugation at 3000 g for 15 min.

Haemolysed erythrocytes obtained by the method of Drabkin [11]. Erythrocyte magnesium (Mg^{2+}) was determined spectrophotometrically using Xylidyl blue [8]. Lipid peroxidation was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in

hemolysate by the method described by Michishen et al [5]. After the reaction of MDA with thiobarbituric acid the reaction product was followed spectrophotometrically at 532 nm.

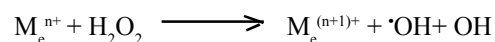
Catalase (EC.1.11.1.6) activity was measured spectrophotometrically according to Caraliok et al [4].

The glutathione reductase (EC. 1.6.4.2) activity was measured according to Agabeli [1]; using oxidized form of glutathione and NADPH. Glutathione reductase catalyzes the NADPH-dependent reduction of glutathione disulfide (GSSG) to glutathione (GSH).

RESULTS AND DISCUSSION

Magnesium levels were 120% higher in

erythrocytes of patients with IHD when compared to the controls ($P < 0.001$) (Table. 1). Magnesium is a bivalent metal which can play an important role in the formation of free radicals by participating in Fenton reaction:



ROS can attack polyunsaturated fatty acids and induce formation of lipid peroxidation products. MDA which is the final product of lipid peroxidation (LPO) was used as a marker to determine the oxidative stress.

MDA levels in the erythrocytes of patients with IHD were 20% higher by comparison with the control group ($P < 0.001$) (Table.1).

Table 1

Levels of magnesium and MDA in erythrocytes of patients with IHD

Group	Magnesium (mmol/l)	MDA (opt.den.)
Control	3.31 ± 0.24	0.127 ± 0.0005
IHD	7.27 ± 0.28*	0.154 ± 0.005*

Opt.den: optical density.

* Significant differences by comparison with the control ($P < 0.001$).

Increased MDA levels in coronary artery disease were demonstrated in several clinical studies. Pucheu et al. reported an increase in level of MDA in the serum of patients with acute myocardial infarction [16]. In the

present study MDA levels showed significant relationship with IHD. The antioxidant activity of catalase was 51% higher in the erythrocytes of patients with IHD by comparison with healthy group (Table 2).

Table 2

Activity of catalase and glutathione reductase in erythrocytes of patients with IHD

Group	Catalase (mmol/l.min)	Glutathione reductase (mmol/l.min)
Control	0.24 ± 0.04	0.105 ± 0.01
IHD	0.363 ± 0.03*	0.208 ± 0.01*

* Significant differences by comparison with the control ($P < 0.05$).

The glutathione reductase activity was 98% higher in the patients with IHD when compared to controls (Table 2). Catalase catalyses the decomposition of hydrogen peroxide to give water and molecular oxygen [10]. Glutathione reductase is the key enzyme of glutathione metabolism and is widespread in all tissues and blood cells.

This enzyme catalyses reduction of oxidized glutathione (GSSG) to glutathione (GSH) in the presence of NADPH and maintains a high intracellular GSH/GSSG ratio of about 500 in red blood cells [12]. In the present study the increase of the activity of catalase and glutathione reductase maybe as response on increasing concentrations of hydrogen peroxide.

The obtained results confirm that in patients with

IHD it happens a change in the prooxidant- antioxidant state of erythrocytes, in the erythrocytes increases the process of peroxidation. Therefore, the activity of antioxidant system increases, that may have compensatory effect.

CONCLUSION

1. In the erythrocytes of patients with Ischemic Heart Disease the concentration of Mg^{2+} was increased, that can lead to the formation of Reactive Oxygen Species.

2. In the erythrocytes of patients with Ischemic Heart Disease the reaction of lipid peroxidation were increased, as evidenced the increase of MDA levels.

3. Strengthening of peroxidation processes in erythrocytes of patients with IHD was accompanied with

the increase of the activity of antioxidant enzymes (catalase and glutathione reductase).

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