Oxidative Stress in Patients with Chronic Heart Failure and Type 2 Diabetes Mellitus

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Parameters of oxidative stress were studied in patients with chronic heart failure and/or type 2 diabetes mellitus. Chronic heart failure was accompanied by severe oxidative stress, while in patients with type 2 diabetes mellitus the signs of oxidative stress were less pronounced. The intensity of free radical oxidation in patients with chronic heart failure and type 2 diabetes mellitus was not higher compared to patients with chronic heart failure.

Key Words: *chronic heart failure; diabetes mellitus; lipid hydroperoxides; malonic dialdehyde; antioxidant enzymes*

Oxidative stress (activation of free radical oxidation) is an important pathogenetic mechanism of cardiovascular pathology [1,5,6,10,13]. Activation of free radical processes is a general mechanism, which mediates the pathological effect of risk factors and proatherogenic disorders on endothelial function. These changes result in the development and progression of cardiovascular diseases, including chronic heart failure (CHF). However, the role of oxidative stress in the pathogenesis of CHF is poorly understood. Open, prospective, randomized clinical trial "Rational Effective Multicomponent Therapy for Heart Failure and Diabetes Mellitus" was performed at the Department of Myocardial Diseases and Heart Failure (A. L. Myasnikov Institute of Clinical Cardiology). The trial was aimed at evaluation of clinical characteristics of patients with CHF, type 2 diabetes mellitus (DM), and combined pathology and at dynamic evaluation of the intensity of oxidative stress in patients depending on the severity of these pathologies.

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MATERIALS AND METHODS

Group 1 included 82 patients with mild and moderate CHF of different etiology (functional classes II-III according to the New York Heart Association, NYHA) with left ventricular ejection fraction <45%, and type 2 DM. All patients received standard therapy for CHF (angiotensin-converting enzyme inhibitors, diuretics, β-adrenoblockers, and, if required, antiaggregants and digoxin) and DM (oral sugar-lowering drugs and insulin therapy). Group 2 included 25 patients with mild and moderate CHF of different etiology (functional classes II-III according to NYHA) with left ventricular ejection fraction <45%, and without changes in carbohydrate metabolism. All patients received standard therapy for CHF. Group 3 included 25 patients with type 2 DM without CHF symptoms and normal left ventricular ejection fraction. All patients received standard sugar-lowering drugs. Functional class of heart failure was estimated by clinical signs. All patients were subjected to transthoracic echocardiography with simultaneous measurement of the left ventricular ejection fraction (Simpson method). The state of carbohydrate metabolism was evaluated by the concentration of glycated hemoglobin (immu-

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noturbidimetry using Roche kits). The concentrations of cholesterol and triglycerides were measured enzymatically using Roche kits. Brain natriuretic peptide (BNP) was studied by enzyme immunoassay with BNP kits on an AxSYM System device (Abbott).

For isolation of low-density lipoproteins (LDL), the venous blood was taken from fasting patients and stabilized with 1 mg/ml EDTA as the anticoagulant. The plasma was centrifuged 2 times in a NaBr density gradient at 41,000 rpm for 2 h. Centrifugation was performed in a 50 Ti angle rotor at 4°C (Beckman L-8 refrigerator ultracentrifuge). Plasma samples were then dialyzed at 4°C for 16 h. Protein content in LDL was measured by the method Lowry. LDL were diluted with a solution containing 0.154 M NaCl and 50 mM phosphate buffered saline (pH 7.4) to a concentration of 50 μg protein/ml. LDL oxidation was induced by 30 μM $CuSO_4$ at 37°C. Lipid hydroperoxide accumulation was studied on a Hitachi 220 A spectrophotometer at 233 nm and fixed time intervals [3,4]. The kinetic curves of LDL oxidation were constructed from the results of the study. The lag phase was estimated (time for induction of oxidation proportional to antioxidant content in LDL) [3,4]. The content of lipid hydroperoxides in LDL was measured by a modified specific method with Fe^{2+}-xylene orange. The measurements were performed before and after reduction of organic hydroperoxides with triphenylphosphine [4,11]. The content of secondary products of free radical lipid oxidation (mainly malonic dialdehyde, MDA) was estimated in the reaction with thiobarbituric acid [7]. Erythrocyte Cu,Zn-superoxide dismutase (SOD) activity was determined by inhibition of nitroblue tetrazolium reduction with superoxide radical $O_2^{\bullet-}$ generated in the xanthine-xanthine oxidase system. The kinetics of formazan formation was recorded on a Hitachi-557 spectrophotometer at 560 nm [7,9]. Erythrocyte catalase activity was estimated from H_2O_2 utilization on a Hitachi 220A spectrophotometer [8]. Activity of Se-containing glutathione peroxidase in erythrocytes was measured in the glutathione reductase-coupled system. The rate of NADPH oxidation was estimated using tert-butyl hydroperoxide as the substrate at 340 nm. Kinetic studies were performed on a FP-901 Labsystems Oy chemical analyzer by the method described elsewhere [12] with our modifications [2].

The results were analyzed by means of Statistica 6.0 software. Independent variables were compared by nonparametric Mann—Whitney test. The differences between the samples were significant at $p<0.05$.

RESULTS

All groups of patients were age- and sex-matched. Groups 1 and 2 were comparable by the etiology and duration of CHF, functional class of the disease, clinical state score, left ventricular ejection fraction, and BNP content. Groups 1 and 3 were comparable by the duration and severity of CHF. Cholesterol concentration in group 2 patients was

TABLE 1. Characteristic of Patients by the Intensity of Oxidative Stress and Contents of Cholesterol and Glycated Hemoglobin

Parameter	Group			*p*		
	1 (CHF and DM), *n*=82	2 (CHF), *n*=25	3 (DM), *n*=25	between groups 1 and 2	between groups 3 and 1	between groups 2 and 3
Cholesterol, mmol/liter	5.1 (4.2; 5.8)	5.9 (4.2; 6.7)	5.0 (4.3; 5.6)	0.024	0.880	0.109
Glycated hemoglobin, %	7.1 (6.48; 8.2)	—	7.61 (6.94; 8.82)	—	0.122	—
Time of induction of Cu^{2+}-dependent LDL oxidation, min	10.29 (3.17; 29.49)	11.42 (3.06; 42.95)	42.90 (26.71; 71.76)	0.953	<0.001	0.002
LDL lipid hydroperoxides, μmol/mg protein	66 (10; 211)	32 (9; 110)	9 (7; 14)	0.249	<0.001	0.024
LDL MDA, nmol/mg protein	3.91 (1.31; 11.99)	2.03 (0.79; 5.69)	1.05 (0.63; 3.25)	0.059	<0.001	0.154
SOD, U/g hemoglobin	10 508 (6061; 13 599)	9237 (7578; 14 205)	15 467 (12 175; 17 504)	0.571	0.001	0.004
Glutathione peroxidase, U/g hemoglobin	2.38 (2.02; 2.78)	2.62 (2.13; 3.46)	3.55 (2.74; 4.35)	0.226	<0.001	0.010
Catalase, μmol/mg hemoglobin/min	710 (496; 1127)	647 (469; 870)	724 (409; 954)	0.313	0.510	0.712

Note. The data are presented as median values. The lower and upper quartiles are shown in brackets.

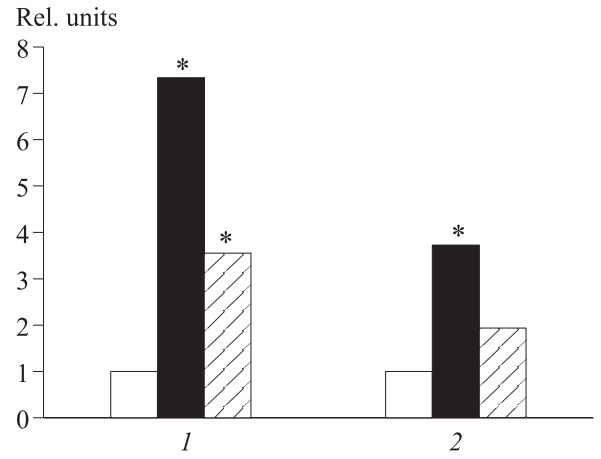

Fig. 1. Contents of lipid hydroperoxides (*1*) and MDA (*2*) in plasma LDL from examined patients. Here and in Fig. 2: light bars, group 3 (DM); dark bars, group 1 (CHF and DM); shaded bars, group 2 (CHF). Parameters in group 3 patients are taken as 1. *$p<0.05$ compared to group 3.

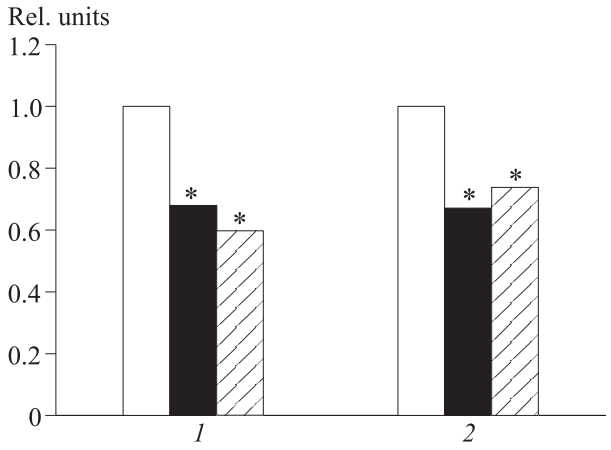

Fig. 2. Activities of SOD (*1*) and glutathione peroxidase (*2*) in erythrocytes from patients.

higher than in group 1 patients and was similar in groups 1 and 3 (Table 1).

The concentration of primary (lipid hydroperoxides) and secondary (MDA) free radical lipid oxidation products in LDL of group 1 patients considerably surpassed that in group 3 patients. Lipid hydroperoxide content in group 2 patients was higher than in group 3 patients. LDL oxidizability (lag phase in the kinetic curve) in group 1 and 2 patients was considerably higher than in group 3 patients. No intergroup differences were found in catalase activity. Activity of other antioxidant enzymes in group 3 patients was much higher than in patients of other two groups. LDL oxidizability, lipid hydroperoxide content, and antioxidant enzyme activity did not differ in patients of groups 1 and 2. However, MDA concentration in group 1 patients tended to increase compared to group 2 patients (p=0.059). Hence, the degree of oxidative stress in group 1 patients did not differ from that in group 2 patients, but surpassed that in group 3 patients.

Lipid hydroperoxide content in group 1 and 2 patients was higher than in group 3 patients (by 7.3 and 3.5 times, respectively). MDA concentration in group 1 patients was 3.7-fold higher than in group 3 patients (Fig. 1). Activities of SOD and catalase in the blood from group 1 and 2 patients were higher than in group 3 patients (Fig. 2).

Our findings suggest that CHF is accompanied by severe oxidative stress, manifested in a sharp increase in the content of lipid peroxidation products and decrease in the rate of enzymatic utilization of reactive oxygen species and lipoperoxides. The signs of oxidative stress were less pronounced in patients with type 2 DM. No sharp increase in the intensity of free radical oxidation was observed in patients with CHF and DM (group 1) compared to patients with CHF alone (group 3). These data suggest that the degree of oxidative stress is maximum during CHF and even type 2 DM (characterized, according to our previous data, by more pronounced intensification of free radical oxidation compared to severe chronic coronary heart disease [4]) does not aggravate this situation. The results of this study provide support for the hypothesis that free radical processes play an important role in the pathogenesis of endothelial dysfunction during CHF.

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