Oxidative Resistance of the Plasma and Electrophysiological Remodeling of the Myocardium in Coronary Patients

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The diagnostic and prognostic potentialities of the parameters of free-radical processes and high resolution ECG in coronary disease were evaluated. A relationship between oxidative resistance of the plasma (MDA concentration after 24-h copper-induced oxidation) and clinical characteristics of coronary disease (functional class of angina and high-resolution ECG parameters) was detected. Changes in ECG parameters directly correlated with MDA levels, the frequency of their registration reflects the severity of coronary disease. The absolute values of ECG, MDA, and their dynamics correlated with the severity of coronary disease and outcome of acute coronary syndrome, the prognosis was unfavorable for patients with MDA level>100 nmol/ml. The level of MDA increased by days 5-7 of observation in all patients with acute coronary syndrome, indicating exhaustion of the plasma antioxidant system during exacerbation of the coronary syndrome. Hence, evaluation of the plasma oxidative resistance and high resolution ECG can serve as a new diagnostic complex approach for detecting coronary patients with an unfavorable prognosis.

Key Words: *oxidative resistance of plasma; myocardial ischemia; electrophysiological remodeling; high-resolution electrocardiography; coronary heart disease*

Disorders in lipid metabolism and ischemic injury to cardiomyocytes with myocardial dysfunction resulting from the endothelial involvement in vessels of different diameters serve as the pathogenetic basis for the progress of coronary disease. Myocardial ischemia is paralleled by activation of free-radical processes, which leads to dysfunction of ionic channels, changes in the transmembrane potential, and excitability of the conduction system cells and cardiomyocytes, formation of zones with disturbed electrophysiological properties in ischemic myocardium [11].

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The role of activation of free-radical processes in the pathogenesis of complications of coronary disease became the object of special studies, in which it was evaluated mainly in patients with reperfusion arrhythmias by measuring generation of active oxygen forms and stationary concentration of LPO products [7]. At present, evaluation of oxidative resistance of isolated lipoproteins and plasma is considered as a more adequate approach. Introduction of evaluation of oxidative resistance is explained by the fact that stationary LPO products have a short life span in the blood flow, because lipoproteins are absorbed by the reticuloendothelial system cells. Using the index of plasma oxidative resistance (oxidizabilitty) it is possible to differentiate coronary patients by the severity of coronary dysfunction of the myocardium [5].

The appearance of various forms of transient myocardial ischemia is one of the main manifestations of chronic coronary disease. Clinical functional methods for its diagnosis and evaluation of disorders in cardiomyocyte membranes, used in everyday clinical practice, are as a rule based on clinical signs and *ST* segment shift on ECG at rest or after functional tests.

On the other hand, well-grounded data suggest the use of individual ECG parameters (high resolution EG, HR ECG), their alteration and dispersion, spectral components, and other parameters obtained on the basis of analysis of averaged ECG signal for the diagnosis of ischemia and disorders in electrophysiological characteristics of the myocardium in coronary disease [1,3,13].

These approaches are based on the assumption that electrophysiological alteration of cells and their membranes is associated with remodeling after ischemic episode or myocardial infarction, contributes to arrhythmogenesis and development of "electromechanical disagreement" in zones of myocardial dysfunction. Electrical remodeling in these cases anticipates structural geometrical changes in the myocardium and is a more sensitive marker of the pathological processes.

Study of the relationship between biochemical tests, reflecting activation of free-radical processes, endothelial dysfunction of vessels and cell membranes in various forms of coronary disease and variants of ischemia ("hybernated" and "stunned" myocardium), on the one hand, and changes in the electrophysiological characteristics of the myocardium, on the other, seems to be an important aspects in the search for new diagnostic tests for evaluation of the severity of myocardial ischemia and course of coronary disease.

We studied the potentialities of a new biochemical test evaluating the oxidative resistance of the plasma, intended for the use as an indicator of the severity of ischemic changes in the myocardium and evaluated its relationship with HR ECG values, reflecting electrophysiological remodeling of the myocardium in coronary patients with various forms of the disease.

MATERIALS AND METHODS

The study was carried out in 155 patients with various forms of coronary disease. Groups 1-3 (n=99) consisted of men and women aged 43-72 years (mean age 59±6 years) with verified (positive exercise treadmill test or coronarography data) coronary disease, with and without history of myocardial infarction (MI) and arterial hypertension, receiving standard antianginal and hypotensive therapy. Group 1 consisted of patients without effort angina (n=32), group 2 included patients with stable effort angina, functional class (FC) II (n=37), and group 3 consisted of 30 patients with FC III angina. A total of 56% patients had a history of arterial hypertension or MI (no earlier than 6 months before the study). When selected into the study groups, the patients underwent standard examinations, including echocardiography, daily Holter ECG monitoring, and treadmill test. Group 4 consisted of patients aged 44-69 years (63±3 years; n=56) with acute coronary syndrome (ACS). The dynamics of *ST* segment and *T* wave depression and of enzyme values indicated the absence of MI (or confirmed the non-*Q*-type MI). Acute MI was diagnosed on the basis of a typical anginal attack longer than 20 min, which could not be arrested with nitroglycerin; *ST* segment depression in point *J* in 2 concordant precardial leads by 2 mm and more and/or in 2 concordant frontal leads by 1 mm and more with subsequent typical MI dynamics without formation of the *Q* wave; laboratory data (2-fold increase of plasma concentration of creatine phosphokinase and its myocardial fraction 2-fold *vs.* the upper threshold normal value). Ventricular extrasystoles of high grade after Lown—Wolf were referred to potentially hazardous arrhythmias: solitary, if their number was more than 10 per hour, polytopic, 2 and more successive extrasystoles, *R* to *T* extrasystoles, and unstable (<30 sec) and stable (>30 sec) ventricular tachycardia. The frequencies of ciliary arrhythmia paroxysms and frequent supraventricular extrasystoles were evaluated. The patients received standard therapy including nitrates, β-adrenoblockers, angiotensin converting enzyme inhibitors, and aspirin. Patients with ACS were examined according to the following protocol: the first 6-12 h of disease (stage 1); the end of the first 24 h (stage 2); days 5-7 of disease (stage 3). The final terms of studies for ACS group were repeated MI, hospitalization for an exacerbation of coronary disease, documented potentially hazardous arrhythmias, and lethal outcome during 1 year of observation. The groups were similar by the mean age and sex of patients. Echocardiogram parameters also virtually did not differ, left-ventricular ejection fraction being at least 50%.

Control group (n=32) consisted of healthy men and women aged 42-67 years (mean age 55.5±6.9 years). Their examinations included standard ECG at rest, treadmill test, 24-h Holter ECG monitoring, 24-h monitoring of arterial pressure, common and biochemical blood analysis, and analysis of blood thyroid hormones. Control group consisted of sub-

jects without pathological changes in ECG at rest, negative results of the treadmill test, arrhythmias during Holter monitoring, and with no more than 10 and 15 supraventricular and ventricular extrasystoles, respectively, and without arterial hypertension (24-h monitoring of arterial pressure was carried out in doubtful cases).

Subjects with gross disorders in blood parameters, thyroid abnormalities, and suffering from acute and chronic diseases, with severe arrhythmias and congestive cardiac insufficiency were not included in the study groups.

The presence of late atrial and ventricular potentials (LAP and LVP, respectively), individual HR ECG parameters were analyzed. Time analysis included evaluation of the duration of unfiltered *QRS* complex, filtered *QRS* complex, low-amplitude (<40 μV) signals at the end of filtered *QRS* complex, total spectral *QRS* density, and the mean quadratic amplitude of the latest 40 msec. Late ventricular potentials were diagnosed from the presence of at least 2 of 3 pathological values of HR ECG time analysis: longer than 120 msec filtered *QRS* complex, longer than 38 msec low-amplitude signals, and less than 20 μV root mean square voltage of the terminal 40 msec. The duration of unfiltered and filtered *P* wave, duration of signals below 5 μV, root mean square voltage of the entire *P* wave, and root mean square voltage of the terminal 20 msec were determined. More than 125 msec duration of filtered *P* wave was considered as the quantitative criterion of LAP.

The intensity of free-radical processes was evaluated by the level of active oxygen forms generation by peripheral blood leukocytes (by the chemiluminescent method) and oxidative resistance (oxidizability) of the plasma. Chemiluminescence (CL) kinetics was registered by the luminol-dependent method with barium sulfate [2]. Oxidative resistance of the plasma was determined by accumulation of LPO secondary product (MDA) after 24-h incubation with copper ions. Coronary patients were differentiated by the severity of myocardial ischemia by means of this method [4]. Serum levels of total cholesterol (CH), triglycerides, and HDL CH were measured on a Centrifichem-400 automated analyzer with enzyme kits (Boehringer Mannheim GmbH). HDL CH was measured after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid and $MgCl_2$ [8]. The content of LDL CH was calculated by the formula presented previously [9].

The significance of differences between the data in various groups of patients was evaluated using two-way Student's *t* test, nonparametric Mann—Whitney *U* test, and Wilcoxon's test. The relationship between the parameters was studied in the analysis of regression after Pierson or Spearman. The differences were considered significant at $p<0.05$.

RESULTS

In groups 1-3 the intensity of CL gradually increased with increasing the severity of angina (Table 1). Leukocyte CL in group 1 was 84.4% ($p<0.05$) higher than in the control, in group 2 it was 3.2 times higher ($p<0.01$) in comparison with the control group and 73% ($p<0.05$) higher than in group 1. In FC III angina (group 3) the CL intensity was 4-fold higher ($p<0.01$) than in the control group and 23.6% ($p<0.05$) higher than in group 2 patients. Hence, changes in activity of free-radical processes and angina FC was noted, which confirms our previous data on the relationship between plasma oxidizability and severity of angina pectoris [4].

Plasma oxidizability in coronary patients is significantly higher (in other words, oxidation resistance of the plasma is lower) than in normal subjects, which is seen from analysis of plasma concentration of MDA, the degree of plasma oxidizability depending on the presence and severity of angina. Plasma MDA/ml value in patients without angina pectoris was comparable to that in the control group. Plasma MDA/ml level gradually increased as angina augmented: in group 2 patients it was 37.8% higher than in the controls ($p<0.05$) and significantly (28.4%; $p<0.05$) higher than in group 1; in group 3 plasma MDA/ml value was 74.2% ($p<0.001$) higher than in the controls and 26.3% ($p<0.05$) higher than in group 2. A significant direct relationship between plasma concentration of MDA and angina FC was detected (Spearman rank correlation $r_S=0.63$; $p<0.0001$). No appreciable differences in the CH and LDL CH values in these groups were detected (Table 1).

Changes of the same direction were detected between angina FC and HR ECG parameters. The incidence of LAP and LVP detection was also associated with the severity of the coronary syndrome in coronary patients. The incidence of LAP in patients without angina pectoris was 1.4 times, of LVP 1.6 times higher than in the control group (Table 1). LAP was recorded 2.7 times and LVP 2.6 times more often in group 2 than in group 1. In group 3 the detection rates were even higher (Table 1). A relationship was found between angina FC and HR ECG parameters: the duration of filtered *P* wave ($r_S=0.28$; $p<0.01$), duration of filtered *QRS* complex ($r_S=0.28$; $p<0.01$), and duration of low-amplitude

TABLE 1. Activity of Free-Radical Processes, Blood Lipid Level, and Incidence of LAP and LVP in Coronary Patients and Healthy Individuals (*M*±*m*)

Group	CL, arb. units	MDA, nmol/ml plasma	CH, mg/dl	LDL CH, mg/dl	LAP, %	LVP, %
Control	13.5±1.8	72.3±2.8	186.0±6.4	120.0±5.4	5	3
1	24.9±3.0*	77.6±5.1	230.0±12.5	160.0±10.7	12	14
2	43.1±3.2**+	99.7±3.7*+	229.0±4.8	158.0±4.5*	33	37
3	53.3±6.7**	126.0±6.7***o	243.0±3.9	167.0±5.5*	49	41

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the control; +p<0.05 compared to group 1; op<0.05 compared to group 2.

signals at the end of filtered *QRS* complex (r_S=-0.51; p<0.0001). The HR ECG parameters correlated with plasma oxidizability and generation of active oxygen forms, CL index with the duration of filtered *P* wave (R=0.23; p<0.05), and plasma MDA/ml value with the duration of low-amplitude signals at the end of filtered *QRS* complex (R=-0.25; p<0.01).

A similar trend was observed in analysis of groups of coronary patients with a history of MI and patients with a history of MI and with potentially hazardous arrhythmias (Fig. 1).

Plasma concentration of MDA in patients with a history of MI and FC II (n=18) and FC III (n=13) was higher (p<0.01) than in patients without angina pectoris (n=32). Prolongation of filtered *QRS* complex and *P* wave correlated with a history of MI and with the severity of angina. Several relationships were detected in patients with a history of MI: between the incidence of LAP, LVP, and severity of angina, between angina FC and plasma oxidizability (r_S=0.73; p<0.0001); between angina FC and HR ECG parameters (duration of filtered *P* wave (r_S=0.36; p<0.01), duration of filtered *QRS* complex (r_S=0.35; p<0.05), and total spectral density of *QRS* (r_S=0.27; p<0.05)). The parameters of free-radical processes correlated with HR ECG parameters: CL index correlated with the duration of filtered *QRS* complex (R=0.27; p<0.05), while MDA level correlated with the duration of low-amplitude signals at the end of *QRS* (R=-0.37; p<0.01).

The difference in LAP and LVP detection rate was significant in the subgroups of patients with a history of MI and with potentially hazardous arrhythmias (Fig. 1). In these subgroups LAP was detected 2.9 times and LVP 1.4 times more often in patients with FC II (n=6) than in patients without angina pectoris with a history of MI and potentially hazardous arrhythmias (n=17). The incidence of LAP in FC II and III angina (n=8) was comparable. The incidence of LVP registration in patients with FC III was 1.6 times higher than in FC II in these subgroups. Plasma concentration of MDA in these patients with FC III was 50% higher (p<0.05) than in those with FC II and 115% higher (p<0.01) than in patients without angina pectoris (43% difference; p<0.05).

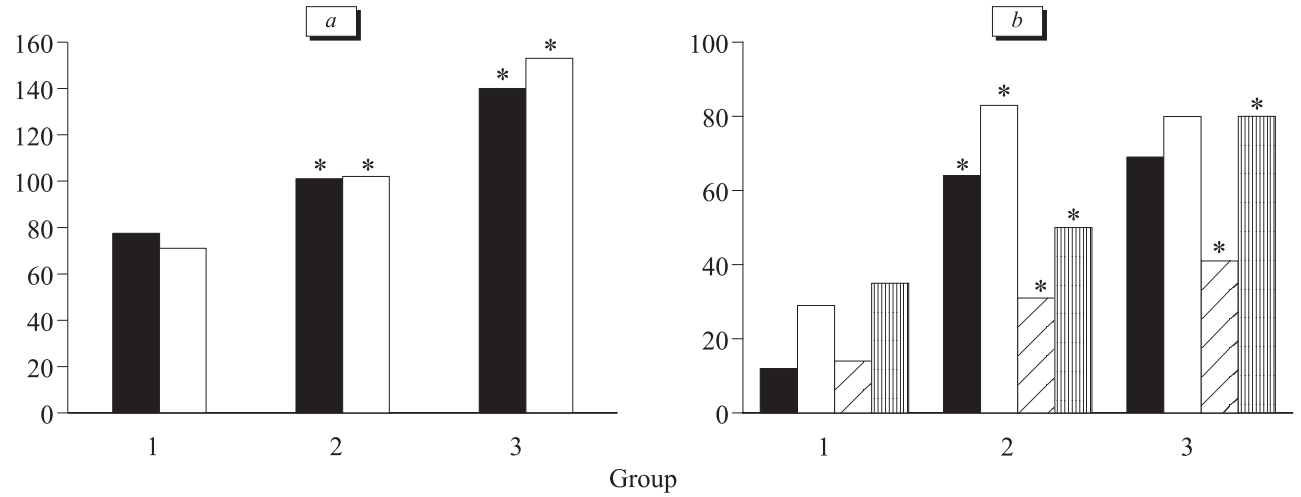

Fig. 1. Plasma oxidizability (*a*) and incidence of LAP and LVP in coronary patients (*b*). *a*) ordinate: nmol MDA/ml plasma after 24-h incubation with 20 μM copper sulfate. Dark bars: history of MI; light bars: history of MI and arrhythmias. *b*) dark bars: history of MI (LAP); light bars: history of MI and arrhythmias (LAP); cross-hatched bars: history of MI (LVP); vertically-hatched bars: history of MI and arrhythmias (LVP). *p<0.05 compared to group 1.

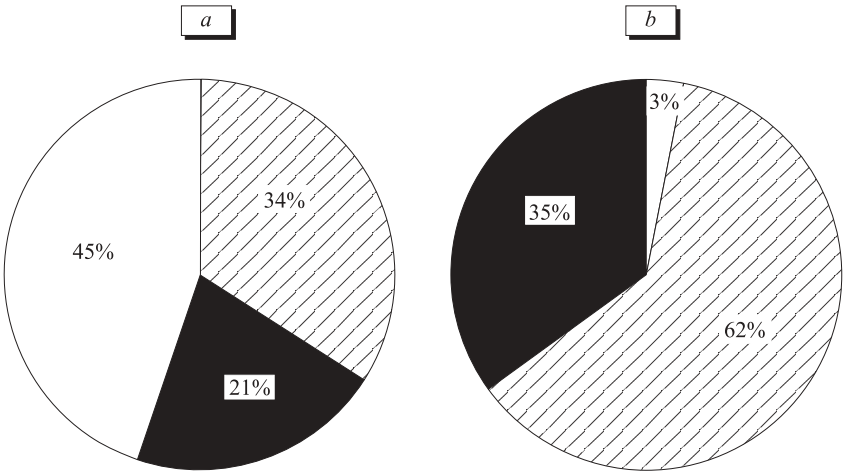

Fig. 2. Incidence of LAP and LVP in coronary patients with plasma MDA concentrations above (*a*) and below 100 nmol/ml (*b*) after 24-h incubation with 20 μM copper sulfate. Light sectors: no potentials; dark sectors: LVP; cross-hatched sectors: LAP.

All coronary patients (n=99) included in the study were divided into 2 large subgroups with MDA levels below (n=43) and above 100 nmol/ml plasma (n=56). Comparative analysis of LAP and LVP incidence in these patients confirmed the relationship between oxidative resistance of the plasma and electric instability of the myocardium (Fig. 2). In patients with MDA>100 nmol/ml LAP was registered 2-fold more often and LVP 1.7 times more often than in the group with MDA<100 nmol/ml. The results refer the patients with high oxidizability (in other words, with low oxidation resistance) of the plasma (MDA>100 nmol/ml) to a group at a high risk of potentially hazardous arrhythmias.

The results obtained in patients with ACS (group 4) were subdivided depending on MDA level: <100 nmol/ml (n=20) or >100 nmol/ml (n=36). The distinguished subgroups of ACS patients were comparable by age, sex, duration of coronary disease, number of patients with concomitant arterial hypertension, and history of MI.

The incidence of favorable outcomes in subgroup of ACS patients (without MI) with MDA<100 nmol/ml was 79%, while in the subgroup with high MDA level 44%. The incidence of MI development and *ST* depression during the first 24 h were higher in the patients with MDA>100 nmol/ml. A shorter period of prehospital deterioration of clinical status in the group with high MDA level is worthy of note. Patients with MDA<100 nmol/ml after 24 h and 5-7 days developed a trend to a reduction of the length of filtered *QRS*, total spectral density, and a significant reduction of the mean quadratic amplitude after 5-7 days in comparison with the values after 24 h (Table 3). The incidence of LVP decreased from 25 to 15% by days 5-7 of the disease.

In group 4 with MDA>100 nmol/ml the mean MDA values were higher than in group 1 at all stages of the study. A significant prolongation of filtered *QRS* complex and reduction of its spectral density were observed after 24 h. After 5-7 days *QRS* spectral density reduced significantly and the mean quadratic amplitude increased. The incidence of LVP was higher in patients with MDA>100 nmol/ml at all stages, mainly at the expense of reduced amplitude of *QRS* spectral density.

The incidence of repeated hospitalizations with exacerbation of coronary disease, repeated MI, and arrhythmic complications was 2-fold higher during the first 6 months of observation in the group of patients with MDA>100 nmol/ml than in the group with MDA<100 nmol/ml, and during the period between months 6-12 the incidence of repeated hospitalizations was significantly higher.

A high prognostic significance of the MDA> 100 nmol/ml value during 1 year of observation was revealed in patients with ACS. High sensitivity

TABLE 2. HR ECG Parameters of *QRS* Complex and *P* Wave in Patients with Angina Pectoris ($M\pm m$)

Group	Duration of filtered complex, msec	Total spectral density, μV	Filtered *P* wave, msec	Root mean square voltage of the entire *P* wave, μV
1	103.0±3.3	89.6±3.0	120.0±3.6	3.8±0.6
2	104.1±2.0	75.3±4.7*	129.0±3.0*	4.6±0.3*
3	112.1±2.4*	80.9±6.8**	132.3±3.7**	4.8±0.4**

Note. p<0.05 compared to *group 1, **group 2.

TABLE 3. Parameters of HR ECG *QRS* Complex and MDA Concentrations in Patients with ACS with Different MDA Levels (*M*±*m*)

Parameter	Period of study		
	6 h	24 h	5-7 days
MDA<100 nmol/ml (*n*=20)			
Duration of filtered complex, msec	99.5±2.2	95.3±2.5	96.4±2.1
Duration of low-amplitude signals at the end of filtered *QRS* complex, msec	40.7±2.4	42.1±2.8	42.2±2.6
Total spectral density, μV	77.0±3.4	72.2±3.2	74.2±3.1
Root mean square voltage of the terminal 40 msec, μV	19.5±1.7	16.1±1.8	14.7±1.8*
LVP, %	5 (25%)	4 (20%)	3 (15%)
LAP, %	3 (15%)	3 (15%)	3 (15%)
MDA, nmol/ml	76.0±4.5	69.2±4.0	86.2±4.6**
MDA>100 nmol/ml (*n*=36)			
Duration of filtered complex, msec	87.0±2.3	93.7±2.3*	94.0±2.5
Duration of low-amplitude signals at the end of filtered *QRS* complex, msec	42.9±2.1	41.8±1.9	39.2±2.3*
Total spectral density, μV	118.0±3.0	104.0±2.1*	98.0±2.7*,**
Root mean square voltage of the terminal 40 msec, μV	20.4±2.0	21.7±1.9	19.2±2.4*
LVP, %	7 (19%)	6 (17%)	10 (28%)
LAP, %	10 (27%)	7 (19%)	8 (22%)
MDA, nmol/ml	125.6±3.3	137.9±3.0	148.5±3.5*,**

Note. The percentage of patients is shown in parentheses. $p<0.05$ compared to the data for *6 h, **24 h.

of values was determined for 3 selected checkpoints: repeated hospitalization (79%), MI (83%), and potentially hazardous arrhythmias (89%).

The diagnosis of the type and severity of ischemic changes in the myocardium by simple and noninvasive methods of examination remains an important problem of cardiology. Many reports discuss this problem. Reduction of coronary blood supply to the myocardium induces a series of interwoven events: disorders in perfusion, metabolism, systolic and diastolic functions (regional and global), clinical and ECG signs of ischemia ("ischemic cascade"). The sequence of functional and morphological disorders varies and depends on the type of disorders in myocardial perfusion.

The relationship between changes in HR ECG and plasma oxidizability with the progress of coronary disease, detected in our study, reflects the progress of ischemic dysfunction and the severity of the "ischemic" substrate with disorders of electrophysiological characteristics in examined patients. Two variants of changes in the absolute amplitudes and time parameters of *P* wave and *QRS* complex are worthy of note. One of the causes of this phenomenon is presumably the difference in changes of the electrophysiological characteristics of "hybernated" myocardium in chronic ischemia and "stunned" myocardium after transitory ischemia. From the biochemical viewpoint, the detected ischemic dysfunction of the myocardium is a result of pronounced changes in capillary endothelium and activation of xanthine oxidase mechanism of superoxide anion radical generation [6].

Both variants of ischemic dysfunction are observed in stable angina. Activation of free-radical processes is paralleled by initiation of LPO, which leads to lipoprotein oxidation. Oxidized lipoproteins, specifically LDL, are assumed to play the key role in impairment of endothelium-dependent relaxation and changed endothelial permeability [6]. Three causes are responsible for this: disordered interactions between agonists and the receptor, mediated by G proteins, reduced activity of NO synthase, and decreased content of NO at the expense of its interaction with superoxide radical. Oxidized LDL stimulate the production of vasoconstrictors by the endothelium [6], including prostaglandin $F_{2\alpha}$, and reduction of synthesis of vasoprotective prostaglandin F_{12} [10], which leads to endothelial dysfunction.

Lipid peroxides toxic for cells are formed in the endothelium: MDA, unsaturated fatty acid peroxides, free radicals (superoxide anion radical, peroxinitrite). These substances modify functional characteristics of the endothelium: the cells loose the glycocalyx, the endothelium swells, its permeability for LDL and monocytes increases [10]. A long course of coronary disease is characterized by the development of microangiopathy with massive formation of subendocardial and intramural cicatrices. Microcirculatory disorders resultant from the above processes augment heterogeneity of electrophysiological characteristics of tissues under conditions of acute or chronic myocardial ischemia and create the substrate for the development of complications of coronary disease and emergence of potentially hazardous arrhythmias. This is reflected by high correlation between plasma oxidizability and HR ECG values (amplitude and time) in patients with high MDA values.

The detected relationship between plasma oxidizability, on the one hand, and severity of clinical forms of coronary disease and HR ECG values, on the other, suggests regarding plasma oxidizability as a new additional diagnostic sign reflecting the severity of ischemic endothelial dysfunction of the myocardium and disorders in its electrophysiological characteristics. Our results prompt regarding coronary patients with high oxidizability (in other words, with low oxidative resistance) of the plasma (MDA>100 nmol/ml) as a group with more severe course of the disease and high risk of complications,

Comparative analysis of the incidence of LAP and LVP and individual parameters of *P* wave and *QRS* complex lengths and amplitude in coronary patients with plasma MDA values below and above 100 nmol/ml indicated more pronounced disorders in electrophysiological properties of the myocardium in high MDA values. Our data are in line with published data [12] indicating a direct relationship between the duration of filtered *QRS* complex and index of disordered local contractility, detected by analysis of correlations. Multifactorial analysis showed that the presence of "hybernated" and "stunned" myocardium in coronary patients after MI more reliably predicts future complications than the number of atherosclerotically modified coronary arteries [12].

The pattern of changes in HR ECG parameters and plasma oxidizability indicated that the negative changes in their absolute values were associated with a high risk of complications, such as nonfatal MI, repeated hospitalizations, and arrhythmias. The severity of disorders in free-radical processes depended on the severity of coronary disease, while the detected correlation between HR ECG values and plasma oxidizability reflected the presence of ischemic dysfunction of the myocardium and severity of myocardial electrophysiological disorders during the progress of ischemia and coronary disease.

Evaluation of the dynamics of absolute HR ECG values and free-radical processes can be used for monitoring ischemic injuries and electrophysiological remodeling of the myocardium. On the other hand, further studies are needed for more precise evaluation and further investigation of the detected regularities.

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