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Mechanisms of Endothelial Dysfunction in Pathologies of Various Genesis

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***Abstract* – Studies in the experiment and the clinic demonstrated the nature of changes in the system of POL – AOC, nitric oxide homeostasis and cholesterol as factors contributing to dysfunction of the vascular endothelium. In metabolic syndrome in pregnant women and in patients with myocardial ischemia, as well as in intoxication syndrome (cobalt chloride), the induction of the lipid peroxidation process according to malonic aldehyde and impaired AODS cells was detected. A deficit of nitric oxide (NO), a violation of its bioavailability and vasoconstriction develops. Consequently, lipid peroxidation-triggering factors: elevated blood glucose, impaired oxygen supply to the myocardium (ischemia), and exposure to cobalt chloride play an important role in disrupting the formation of nitric oxide and adaptive mechanisms from reactive oxygen species (ROS). Disruption of nitric oxide homeostasis (eNOS/NO) plays a decisive role in the genesis of vascular complications and can be considered a biochemical marker for the development and progression of various pathologies. Based on these data, it is possible to develop a methodology for correcting the pathogenetic orientation.**

Key words – endothelial dysfunction, pathologies, biomedicine, vascular endoteleum

I. Introduction

Modern biomedical science pays special attention to the study of the role of endothelial dysfunction in the pathogenesis of vascular complications in many pathologies: toxic effects, coronary artery disease, metabolic syndrome in pregnant women, etc. Summarizing the literature data, it should be noted that endotheliocytes under physiological conditions produce vasoactive substances: nitrogen oxide, angiotensin I and II, prostacyclin, endothelin and thromboxane [1, 2]; participate in hemocoagulation and activation of fibrinolysis; in reactions of innate and acquired immunity; perform an enzymatic function – the expression of angiotensin converting enzyme (ACE); regulate the proliferative processes of smooth

muscle cells (SMC) and its contractile function, provide the function of protection against vasoconstrictor influences, etc.

The depletion of these regulatory mechanisms leads to endothelial dysfunction, the development of which involves the POL system – AOS, NO-forming function and cholesterol metabolism. The reasons leading to these negative effects are diverse, and they include toxic situations in the body that can be caused by ecopathogenic factors heavy metals [3]. In addition to the intoxication syndrome, these biochemical systems are disturbed in case of socially significant pathologies: IHD and metabolic syndrome.

Atherosclerosis – hypercholesterolemia (GHS), leading to the development of endothelial dysfunction (DE), is a key factor initiating atherogenesis and contributing to its progression [4, 5]. Oxidized, modified LDL contribute to the disruption of NO synthesis and a decrease in the expression level of the NOS-3 gene and the enzyme endothelial NO synthase itself [6]. From the position of free radical theory, one can substantiate the main mechanisms of atherogenesis: the formation of foam cells and the development of aortic lipoidosis, the migration of smooth muscle cells from media to intima, the increase in their proliferation and the occurrence of complications of atherosclerosis with subsequent vascular thrombosis. Many different substances are involved in the course of free radical reactions, and an even greater number of components participate in the process of their regulation. Violation of any link in this system can lead to oxidative stress. The ratio of the main links of this system prooxidants and antioxidants, determines the development and progression of oxidative stress and, as a result, the development of free radical pathology [7].

II. Statement of the problem

Study of the involvement of lipid metabolism changes in the development of endothelial dysfunction in various angiopathies: against the background of heavy metal salts, coronary heart disease (CHD) and metabolic syndrome.

III. Materials and methods

Experimental studies were carried out on Wistar male rats, with chronic intoxication with cobalt chloride for 1 month. At the end of the experiment, samples were analyzed in rats. The intensity of lipid peroxidation was judged by the concentration of malonic acid dialdehyde (MDA) [8], as well as the activity of catalase enzymes [9], SOD [10], and the concentration of ceruloplasmin [11]. The concentration of the final metabolites of nitric oxide (total content of nitrates and nitrites (NOX)) was determined by the method of Metelskaya V.A. [12]. Studies were conducted on the background of pharmacological agents that affect the reproduction of eNOS – the substrate itself and the L-NAME inhibitor. Lipid metabolism was judged by the content of total cholesterol and its fractions.

In clinical studies, the state of lipid peroxidation, antioxidant protection was determined according to the functioning of SOD, catalase, and the concentration of ceruloplasmin. In patients with coronary artery disease and in pregnant women with metabolic syndrome, violations of biochemical parameters were determined, including changes in lipid and carbohydrate metabolism, as well as blood pressure values, as an indicator of endothelial dysfunction.

Statistical processing of the results was performed using Statistica 6.0. $p < 0.05$ was considered the level of statistical significance.

Clinical material was collected on patients with coronary artery disease and in pregnant women with metabolic syndrome. 75 patients were examined (45 men and 30 women aged 50.1 ± 10 years, whose cholesterol content in plasma was 6.4 mmol/l or more. The duration of the disease was 1–10 years. During an in-hospital examination, exertional angina All patients with coronary artery disease were observed with a frequency of attacks 4–8 times a week.

A survey of 24 pregnant women, 14 – with metabolic syndrome. Conditions for the inclusion of patients in the survey were: singleton pregnancy, age over 18 years, no indication of diabetes and severe somatic pathology. The control group consisted of 20 relatively healthy individuals, with normal blood levels of cholesterol (4.3 mmol/l), triglycerides – 1.23 mmol/l, LDL-C – 1.46 mmol/l, LDL-C – 2.68 mmol/l.

For typing of dyslipoproteinemia (DLP), A.N. Klimov and N.G. Nikulcheva (1984). The total cholesterol (cholesterol), triglycerides (TG), high-density lipoprotein cholesterol (cholesterol HDL) cholesterol were determined, the cholesterol atherogenic coefficient (cholesterol coefficient) according to A. Klimov (1984) was calculated using the Fredewald equation.

IV. Discussion of the results

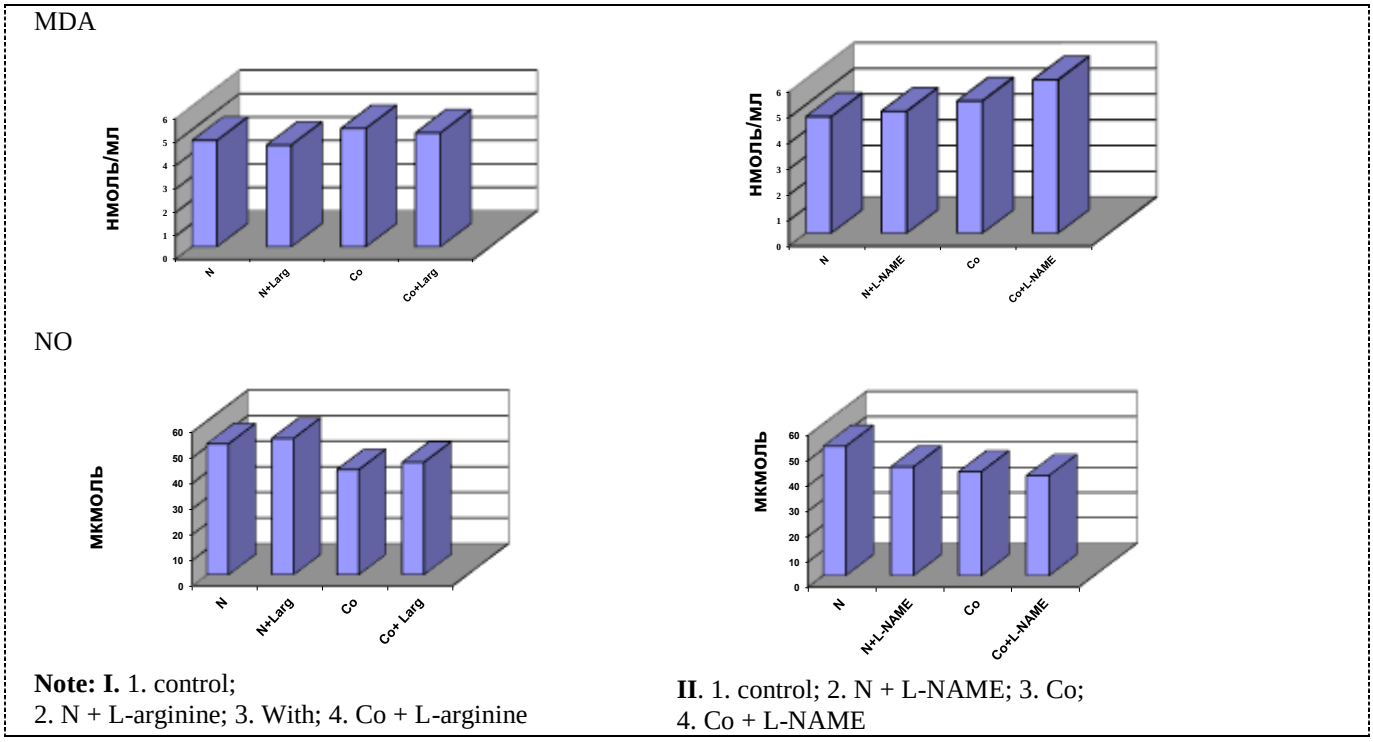
Experimental and clinical data showed that toxic angiopathies and vascular complications caused by heavy metal intoxication (cobalt chloride) are characterized by the formation of reactive oxygen species (ROS) and activation of free radical oxidation (FRO), as evidenced by a statistically significant increase in MDA concentration in hemolysate red blood cells. In patients with coronary artery disease and in pregnant women with metabolic syndrome, activation of free radical oxidation in erythrocytes was also detected. Analysis of AOS data showed that, in toxic pathologies in serum, SOD activity decreases, while catalase activity and ceruloplasmin concentration increase. Regarding clinical material, it should be noted a decrease in the activity of all AOS enzymes. In all pathologies under conditions of oxidative stress, the NO-producing function of the endothelium is disturbed, as evidenced by the concentration of NOx.

When intoxicated with cobalt chloride in rats against the background of developed oxidative stress, a decrease in the concentration of total NO metabolites was found by 19.7 % ($p < 0.001$) and an increase in the concentration of MDA – POL product by 10.9 % (figure 1).

The study of the relationship between these indicators revealed the presence of a strong inverse relationship ($r = -0.72$).

Administration of L-arginine to rats with cobalt intoxication showed an increase in NO with inhibition of lipid peroxidation. At the same time, there is an increase in the level of SOD functioning. Unlike L-arginine, the eNOS inhibitor L-NAME showed opposite results, i.e., the ability of the enzyme instead of NO to produce ROS.

In patients with coronary artery disease and in pregnant women with metabolic syndrome, a stable significant increase in plasma lipoperoxidation products was found according to MDA data. The concentration of MDA significantly increased, which amounted to 83.2% in relation to the data of the control group (table 1).



Note: I. 1. control;
2. N + L-arginine; 3. With; 4. Co + L-arginine

II. 1. control; 2. N + L-NAME; 3. Co;
4. Co + L-NAME

Fig. 1. The content of malonic aldehyde and NOx on the background of the inducer and inhibitor of endothelial NOS in a toxic situation

TABLE I. THE INDICATORS OF THE LIPID PEROXIDATION – ANTIOXIDANT SYSTEM IN PATIENTS WITH CORONARY ARTERY DISEASE II FC COMPARED WITH THE CONTROL GROUP.

lipid peroxidation rates	Control group n=20	Patients with coronary artery disease n=25
MDA, нмоль/мл	17.3±0.23	31.7±0.15
NO, мкМ	57.5±0.65	38.9±0.73
SOD, units Act	67.4±0.52	35.6±0.79
Catalase, mkat/l	228.8±2.33	160.4±1.53
CP mg/l	262.5±3.99	149.3±3.12

Analysis of the activity of anti-radical protection showed a significant decrease in the activity of the main enzymes – catalase on average by 70 % ($p < 0.001$) and SOD by 52.8 % ($p < 0.001$), CPU-56.8 % ($p < 0.001$).

Thus, the concentration of lipid peroxidation products and MDA, significantly increases in patients with angina II FC, due to the reduced activity of AOS enzymes.

So, hypoxia of cardiomyocytes leads to uncompensated amplification of lipid peroxidation processes in the myocardium, accompanied by a violation of the structure and function of myocardiocytes. Activation of lipid peroxidation is one of the causes of impaired coronary circulation and microcirculation, due to endothelial dysfunction, leading to vasospasm, enhanced platelet aggregation and thrombus formation.

Our research has established that oxidative stress leads to a violation of the NO-producing function of the endothelium and a decrease in the content of nitric oxide (NO). Nitric oxide is a messenger molecule that participates in many physiological and pathological processes and has both beneficial and destructive effects [13]. It is an endothelial relaxation factor, formed from L-arginine with the participation of the nitric oxide synthase enzyme (NO synthase). Several coenzymes are involved in this reaction, including nicotine (NADPH) + (NADP), flavin (FAD, FMN) nucleotides, tetrahydrobiopterin (BH4), Ca2 ions, calmodulin. NO synthases are divided into constantly functioning or constitutive: neuronal (NOS-1) and endothelial (NOS-3). For the expression of these two forms are necessary Sa^{2+}, calmodulin. Inducible NOS (NOS-1) is expressed and functions in response to the action of endotoxin and cytokines, bacterial lipopolysaccharides.

Against the background of an increase in the concentration of MDA in the blood of IHD patients, a significant decrease in the content of total NO metabolites from 57.5 μmol to 38.9 μmol, showed a decrease in activity of 67.6%. Nitric oxide deficiency was one of the causes contributing to spasm of coronary vessels and strokes.

It should be noted that in patients with CHD II FC, against the background of oxidative stress, endothelial dysfunction develops, which is promoted by POLO metabolites and a decrease in the content of NO – the main vasodilating factor. A manifestation of endothelial dysfunction is a violation of blood pressure – hypertension, a risk factor for myocardial ischemia. Another important factor in the development of endothelial dysfunction in cobalt intoxication and ischemic heart disease was metabolic cholesterol, hypercholesterolemia, as well as oxidative modification of atherogenic LDL during their formation or disruption in the bloodstream by lipid peroxidation products.

Therefore, under conditions of oxidative stress, apo-β 100 structure changes, as a result of which this LDL protein loses its affinity for receptors of LP particles. Modified LDL phagocytosis by macrophages in the subendothelial space and they are accumulated in areas of atherosclerotic vascular lesion [14].

Intensification of lipid peroxidation processes in the liver in atherosclerosis is accompanied by suppression of the enzymatic catabolism of cholesterol (cholesterol) in hepatocytes, which in turn helps to maintain elevated levels of cholesterol in blood plasma [7].

The dyslipoproteinemia (DLP) revealed by us was manifested by increased blood levels of total cholesterol, high levels of cholesterol-LDL and decreased cholesterol-free cholesterol. According to the characteristics of the lipid spectrum by the World Health Organization (1992), such DLP is classified as type II, subtype II-a.

Consequently, the accumulation of products of metabolic disorders of lipid peroxidation products, atherogenic lipoproteins and a decrease in the content of nitric oxide induces ischemia, and this pathological cascade can progress and become a risk factor for worsening IHD and the development of myocardial infarction. Adequate pathogenetic therapy is necessary to prevent this.

V. CONCLUSION

Thus, the pathogenetic link of endothelium dysfunction in chronic toxic angiopathies is the intensification of POL, caused by reactive oxygen species (ROS) and the inhibition of antioxidant protection of cells (AOD). Under conditions of oxidative stress, the expression of eNOS is disturbed, the production and bioavailability of nitric oxide – the main vasodilating factor, which is accompanied by impaired tissue metabolism. In patients with coronary artery disease and metabolic syndrome under conditions of oxidative stress, impaired nitric oxide production and cholesterol metabolism is accompanied by endothelial dysfunction, increased blood pressure during the day and hemodynamic changes in the myocardium. The change in the nitrooxide synthase system – NOX is an indicator of vascular disorders.

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