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REVIEW/RESEARCH ARTICLE/SHORT COMMUNICATION

Involvement of endothelial NO synthase expression regulators in the mechanisms of endothelial dysfunction development against the background of cobalt chloride and L-NAME exposure in experiment

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Abstract

Literature representations about the toxicity of heavy metals, one representative of which is cobalt, are not exhaustive. Insufficient attention is paid to the violation of nitroxide-producing endothelial function as a risk factor for vascular complications. The aim of the study was to investigate the characteristics of the effect of cobalt chloride and its complex with L-NAME on indicators of LPO system - AOS, NO-producing function of the endothelium, including the expression of endothelial NO synthase. To realize the goal, experimental studies were conducted on Wistar rats with exogenous load of cobalt chloride and its combination with endothelial NO synthase inhibitor - L-NAME. The following parameters were determined in the experiment: malondialdehyde concentration, catalase activity and ceruloplasmin concentration, nitric oxide content, and the level of endothelial NO synthase expression. The data showed a significant increase in oxidants in the body and their ability to inhibit the production of nitric oxide (NO), the main vasodilator. Against the background of combined administration of cobalt chloride and L-NAME revealed even greater activation of free-radical reactions, more pronounced inhibition of nitric oxide formation. When administering cobalt and modified L-arginine, there is a violation of the internal molecular structure of endothelial NO synthase (eNOS) and the ability of the enzyme to produce active oxygen metabolites (AMCs). In contrast, L-arginine stimulates the expression level of eNOS, the formation of nitric oxide and inhibits lipoperoxidation. This type of research is highly relevant because only knowledge of the mechanisms of endothelial dysfunction will allow a predictive approach to preventive measures.

Keywords: cobalt chloride, lipid peroxidation, endothelial dysfunction, nitric oxide, L-NAME, L-arginine.

Introduction

Study of mechanisms of negative influence of environmental factors, in particular, heavy metals, does not cease to attract attention of scientists, both in our region and in Russia as a whole. This problem, dealing with the study of the mechanisms of toxicity of ecopathogenic factors, mainly coming from

industrial facilities into the ecosystem, is very relevant and significant for both fundamental and clinical medicine. In this regard, we consider a widely used in the national economy metal - cobalt, which has a direct effect on the body under production conditions [1,2]. Cobalt ions, affecting hemoglobin iron, contribute to disruption of oxygen-transport function of erythrocytes, which results in AMC formation and activation of lipoperoxidation process [3,4]. AMK also disrupt the activity of antioxidant system enzymes (AOS), which also contributes to the development of oxidative stress. Disturbance of redox reactions is accompanied by inhibition of NO-forming function of endothelium. The most important cause of this disorder is a change in the level of expression and activity of endothelial NO synthase (NOS-3), which generates physiological levels of nitric oxide (NO) [5]. Under toxic conditions the impairment of nitroxide-producing endothelial function is promoted by the increased content of eNOS expression inhibitor asymmetrical dimethylarginine (ADMA) in blood and deficiency of L-arginine substrate, which is an inducer of NOS-3 expression [5,6,7]. Deficiency of L-arginine and NO, the main vasodilatory factor, is accompanied by endothelial dysfunction under conditions of the model induced by cobalt intoxication and L-NAME (L-nitro-arginine-methil-ester-NG arginine methyl ester). At the same time, it should be noted that impaired cholesterol metabolism and preterogenic changes in the endothelium participate in limiting the bioavailability of L-arginine for eNOS.

The foregoing implies the necessity to study the effect of ecopathogenic factors on the functional state of the endothelium under the conditions of NOS-3 inhibitor use. The studies devoted to the study of the mechanisms of endothelium NO-producing function impairment under the influence of ecopathogenic factors and regulators of endothelial NO synthase expression are rather insufficient in the literature.

Therefore, the aim of the study was to investigate the mechanisms of involvement of NO synthase expression regulators in the toxic effects caused by exposure to cobalt chloride in the experiment.

Materials and Methods

Endothelial dysfunction was modeled on linear Wistar rats, control n=15 and intoxicated + L-NAME (25mg/kg) n=60. Cobalt chloride 2 mg/kg was administered parenterally for 30 days. Depending on the experimental conditions, experimental animals were divided into the following groups: Group 1 - intact, i.e. control animals injected with physiological solution in an equivalent volume with other substrates; Group 2 - intact rats with administration of eNOS inhibitor - L-NAME; Group 3 - with an experimental model of cobalt angiopathies; Group 4 - with eNOS inhibitor L-NAME injected against the background of cobalt chloride intoxication; Group 5 - rats with cobalt chloride intoxication and L-arginine parenterally injected at the dose of 10 mg/kg for 30 days. Compliance with international requirements of work with experimental animals, including humane treatment with them, is confirmed by the decision of the Ethical Committee of the IBMI All-Russian Research Center of RAS (protocol № 6 from 26.12.2018).

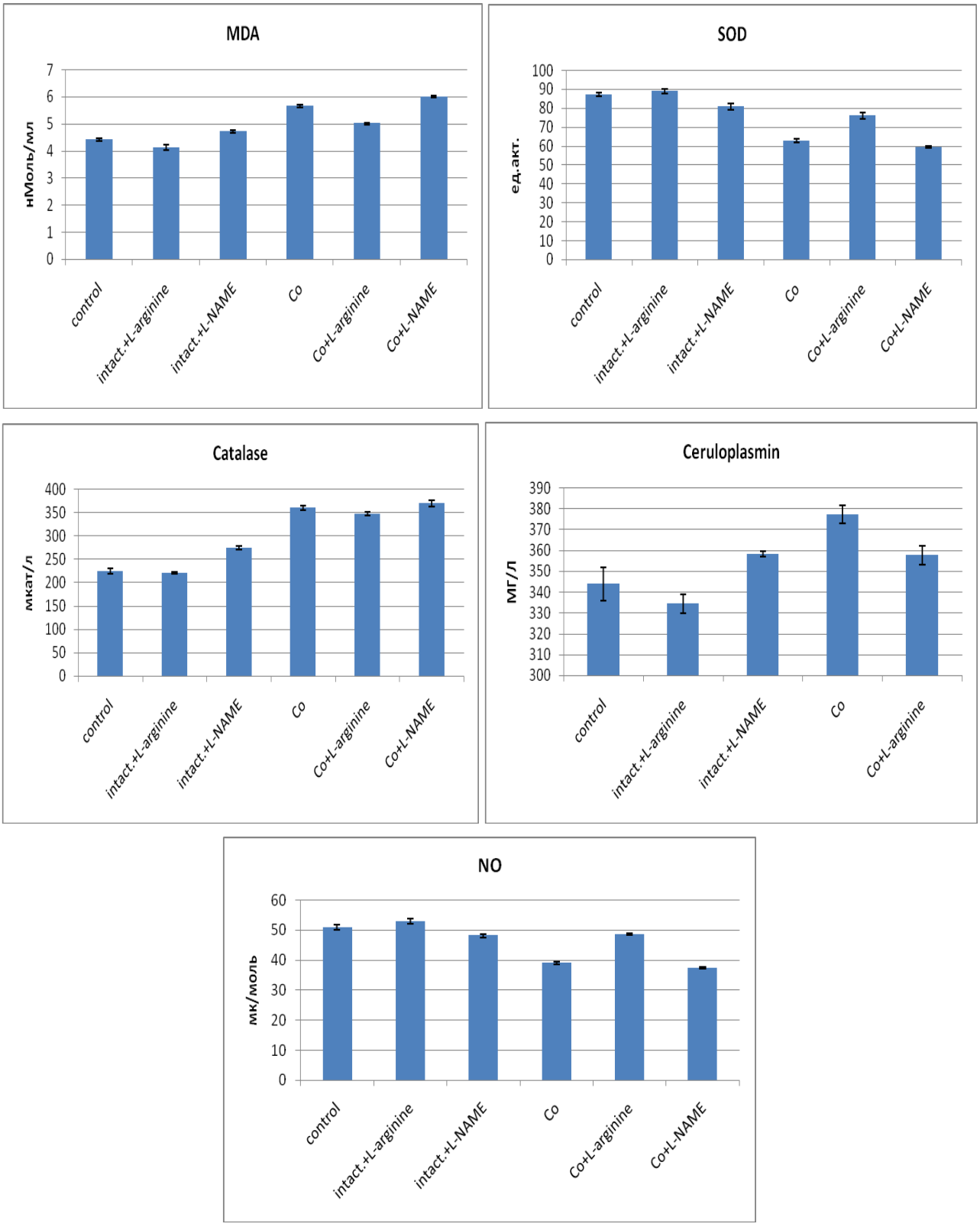
At the end of the experiment, the intensity of lipid peroxidation in erythrocytes, kidney and myocardial tissues was studied by malonic acid aldehyde (MDA) [8]. The activity of cellular antioxidant protection (AOP) was judged by the activity of catalase [9], superoxide dismutase (SOD) [10], ceruloplasmin (CP) concentration [11]. Cholesterol (CH) metabolism was assessed by total cholesterol concentration, its content in low-density lipoproteins (LDL) and high-density lipoproteins (HDL). The concentration of total nitric oxide (NOx) metabolites was determined [12]. Membrane mechanisms of organ cell damage were judged by the activity of sodium- and potassium-activated ATPase in renal and myocardial cells. The effect of L-arginine and L-NAME on biochemical parameters, including the expression level of eNOS, was used in a separate variant. The eNOS expression level was studied at the National Medical Research Center for Preventive Medicine of the Ministry of Health of Russia. The results were processed by correlation analysis using Statistika and Microsoft Office Excel programs.

Results and Discussion

Endothelial dysfunction was simulated by a combination of cobalt chloride and L-NAME. The studies were performed under intoxication conditions with parenteral administration of cobalt chloride. The duration of the experiment was 30 days. The results showed impairment of redox reactions according

to increased concentration of MDA in erythrocyte hemolysate, renal and myocardial tissues, as well as impaired activity of AOS enzymes. Statistically significant decrease of SOD functional activity in erythrocytes, increase of catalase activity and CP concentration in blood serum were shown (Fig. 1).

Figure 1: Dynamics of changes in parameters of LPO - AOS and the content of nitric oxide in intoxication syndrome.



The multidirectional nature of changes in adaptive enzymes is most likely due to their different molecular structure. Catalase, which has 4 heme groups in its molecule, appears to be more stable than SOD. Disturbances in redox reactions were accompanied by a decrease in the concentration of NOx, the main vasodilating factor. Since the causes of insufficient formation of nitric oxide may be a deficiency in the substrate L-arginine and violation of its bioavailability for eNOS, in a separate version of the experiments we administered L-arginine during intoxication syndrome in experimental rats against the background of exposure to cobalt chloride for 30 days. The data showed the ability of the NO synthase inducer L-arginine to increase the nitric oxide concentration against the background of a decrease in the intensity of lipid peroxidation (LPO). The adaptive system under these conditions showed an increase in the activity of SOD, as well as positive dynamics with regard to catalase and CP (Fig.1).

In intoxication syndrome, L-arginine application promotes competitive inhibition of ADMA action on NO synthase and the resulting nitric oxide has an antioxidant effect. In the model experiment against the background of cobalt chloride and modified L-arginine, on the contrary, intensification of free-radical oxidation (FRO) and increase of MDA level was observed. Disruption of redox reactions is accompanied by an even more pronounced decrease in NOx (Fig. 1).

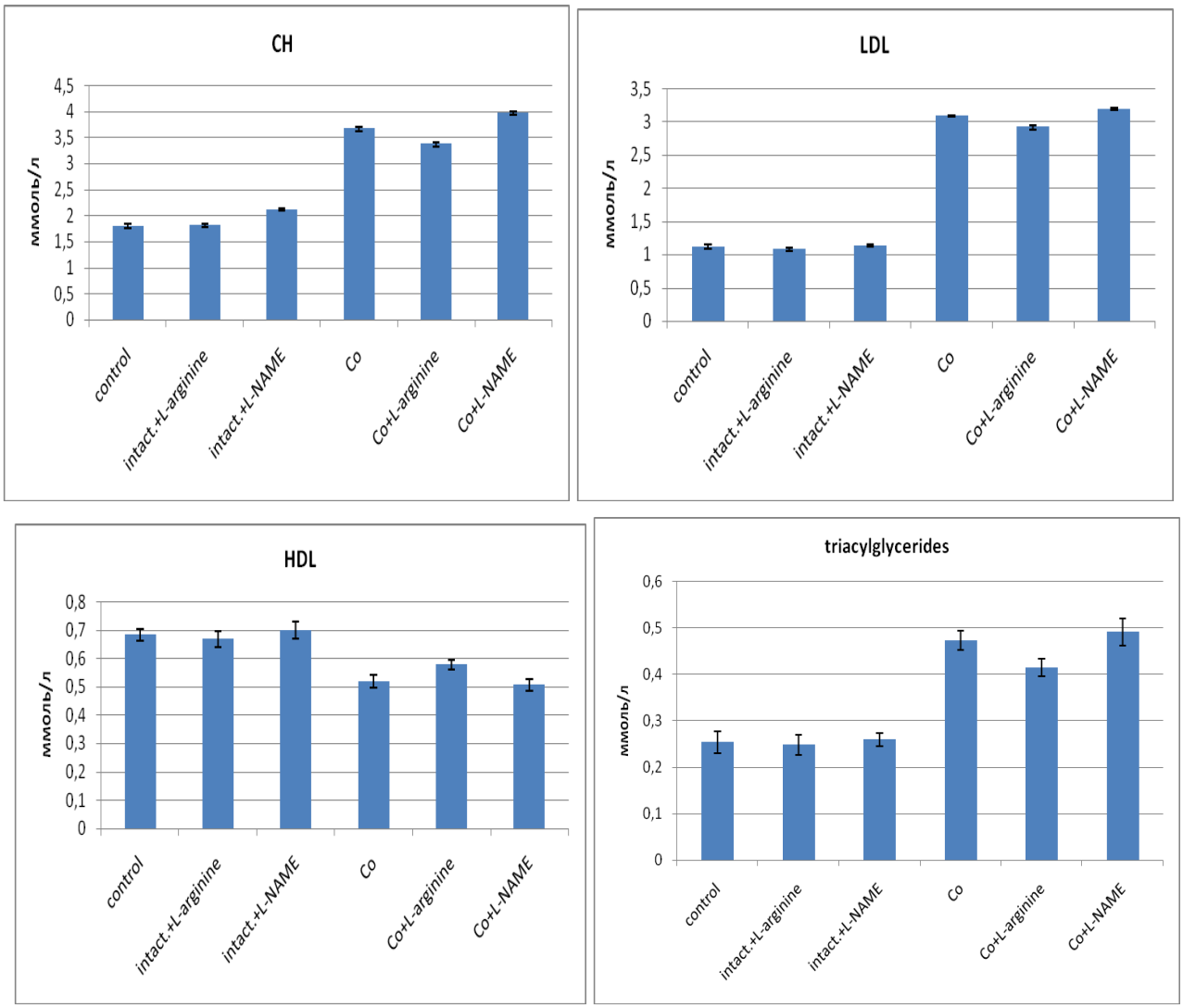
Under the conditions of the experiment induced by the eNOS inhibitor L-NAME, there was a disruption of the enzyme molecule structure, manifested by a 23.9% inhibition of eNOS expression level, whereas L-arginine increased the enzyme expression level by 29.5%. Hypercholesterolemia and hyper-β-lipoproteinemia contribute to the impaired availability of the L-arginine substrate to eNOS. Moreover, oxidative modification of LDL and apo-B100 leads to a decrease in the affinity of LDL particles for their receptors. There occurs interaction of lipoproteins with receptors "scavenger" phagocytes, they enrich with lipids and form "frothy" cells - the beginning of atherogenesis. It should be assumed that under these conditions there is a disruption of the transport mechanism for L-arginine to NO synthase, i.e., its bioavailability. Moreover, the functional ability of eNOS is impaired by modified L-arginine, which is an inhibitor of eNOS expression.

The occurring activation of lipoperoxidation caused qualitative changes in cell membrane phospholipids, which caused a decrease in the functional activity of Na,K-ATPase in the cortical and medullary interstitium of renal tissue and myocardium by 43.7% and 36.9%, 65.04%, respectively. It should be noted that L-arginine, exhibiting antioxidant properties, contributes to the normalization of the lipid matrix of the cytoplasmic membrane and increases the activity of ATPase.

Thus, exposure to cobalt chloride and L-NAME impairs the redox potential in cells and reduces the content of total nitric oxide metabolites, a major vasodilatory factor. In contrast, L-arginine inhibits SRO and increases the adaptive mechanism under the influence of AMC. Moreover, L-arginine induces eNOS expression, whereas L-NAME, on the contrary, inhibits it. The risk factors of atherogenesis - hypercholesterolemia, hyper-β-lipoproteinemia - contribute to the formation of endothelial dysfunction, limiting the availability of L-arginine to eNOS.

Against the background of L -arginine in experimental rats, the data showed a statistically significant decrease in the concentration of total cholesterol, an increase in HDL cholesterol and a decrease in LDL cholesterol (Fig. 2).

Figure 2. Data on changes in cholesterol metabolism in intoxication syndrome.



Thus, L-arginine, by inhibiting lipoperoxidation, causes positive dynamics in cholesterol metabolism, which contributes to a better bioavailability of the arginine substrate and, accordingly, an increase in NOx concentration. Consequently, the restoration of the L-arginine-NO synthase-NO signaling pathway plays an important role in mediating the vasodilatory action of NO. Biochemical markers of endothelial and visceral dysfunction are increased content of LPO products in erythrocytes and internal organ homogenates, decreased NOx concentration and sodium and potassium activated ATPase activity. L-arginine had a stimulating effect on the production of total NO metabolites, and their content significantly increased (Fig. 1).

Consequently, the studied drug L-arginine had a positive effect on the metabolic indicators capable of changing the content of nitric oxide, the main vasodilatory metabolite, and, accordingly, on the tone of the vascular wall.

Conclusion

The data obtained in cobalt chloride and L-NAME intoxication indicate the ability of oxidative stress to inhibit nitroxide-producing endothelial function, due to decreased expression levels of endothelial NOS (NOS-3), deficiency of the enzyme inducer L-arginine and impaired bioavailability for the enzyme. Oxidatively modified LDL, which has become unrecognizable by its LDL receptors, has contributed to this effect. The aggregate of these biochemical changes can be characterized as a general pathological process called endothelial dysfunction, which can have a negative impact on the function of internal organs. The use of L-arginine for the correction of disorders has demonstrated very proven results in restoring endothelial function, as well as positive dynamics on the part of internal organs: kidneys and myocardium

according to the inhibition of LPO in them and increasing the activity of sodium pump enzyme - Na,K-ATPase.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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