

Influence of Enos Expression Regulators on Changes in the Endothelium Function Under the Intoxication Syndrome

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ABSTRACT

The study was conducted on a model of endothelial dysfunction caused by intoxication with nickel chloride and an eNOS inhibitor – L-NAME. The results showed a significant increase in oxidants and their inhibitory effect on the development of nitric oxide (NO) – a biochemical marker of the endothelial dysfunction. When introducing nickel and modified L-arginine, the internal molecular structure of endothelial NO synthase (eNOS) and the ability of the enzyme to produce nitric oxide and active oxygen metabolites (AMA) is violated. L-arginine stimulates the expression of eNOS, the development of nitric oxide and inhibits lipoperoxidation. This experimental method is relevant, because only fundamental knowledge about the mechanisms of development of endothelial dysfunction will allow us to develop a predictive approach for the prevention of negative effects.

Keywords: nickel, lipid peroxidation, AOS, nitric oxide, endothelial dysfunction

1. INTRODUCTION

The study of toxic mechanisms of ecopathogenic factors, in particular heavy metals, is an urgent problem [1, 2]. In Russia, 2017 was declared the year of the struggle for environmental cleanliness.

Nickel can penetrate into the biological environment of the body [3, 4]. Its excess causes negative effects [5]. As a variable-valence ion, nickel promotes the development of reactive oxygen species that initiate lipid peroxidation (LPO). The toxicity of heavy metals is determined by the presence of an unpaired electron on the outer orbit of the atom, which allows it to react with oxygen and polyunsaturated fatty acids (PUFAs). The reaction products are a superoxidion radical, a hydroxyl radical, hydrogen peroxide, and singlet oxygen. Interaction with PUFA changes the structure of the cell membrane. Lipid peroxidation is a process that is characteristic of body cells [6, 7].

Damage to biomembranes caused by lipid peroxidation disrupts metabolic and functional processes. The depletion of regulatory mechanisms causes the endothelial dysfunction in which a number of biochemical systems are involved: lipid peroxidation, antioxidant system (AOS), NO-forming function, disturbance in the ornithine cycle, accompanied by hyperactivity of the arginase enzyme. The factors causing these negative influences are diverse and

toxic situations in the body. They can be caused by ecopathogenic factors, in particular heavy metals. Under the oxidative stress, endothelial dysfunction can be caused either by decreased production of nitric oxide (NO) by endothelial cells (EC), or by a violation of its bioavailability as an important vasodilating factor [8]. Nitric oxide acts as an intracellular messenger and is involved in the implementation of responses from cells. Nitric oxide is formed during the enzymatic oxidation of L-arginine by NO synthase (NOS); the source of the nitrogen atom is the nitrogen-containing group of the R chain. Reduced NO production may be associated with low expression of the enzyme endothelial NO synthase (eNOS), with its reduced activity or accelerated decomposition of NO, since the interaction of NO with the superoxidion radical (O₂⁻) occurs 3 times faster than the reaction O₂⁻ dismutations – superoxide dismutase (SOD). The resulting peroxynitrite is a damaging factor. Moreover, violations are systemic. Under toxic conditions, the NO-producing function of the endothelium is impaired [9].

This is facilitated by modified L-arginine – L-NAME (L-nitro-arginin-methyl-ester – NG arginine methyl ether), which is an inhibitor of endothelial NOS. There are three NOS isoforms, two of which are constant enzymes: neuronal (NOS I), endothelial (NOS III or eNOS) and inducible (iNOS or NOS II) NO synthases. Endothelial and neuronal NOS are constitutive and their trigger is Ca²⁺ + calmodulin. In contrast to nNOS and iNOS which are

localized in the cytoplasm, eNOS is associated with the structures of cell membranes and responds to signals from the internal environment. The nitric oxide produced by eNOS is a physiologically significant vasodilator, while a decrease in NO production and / or bioavailability synthesized in endothelial cells (EC) is one of the main causes of endothelial dysfunction. A key factor in the development of endothelial dysfunction is a L-arginine deficiency, which is used in the ornithine cycle. It is an inducer of NOS-3 expression. In contrast, L-NAME alters the molecular structure of eNOS so that the enzyme begins to produce AMA. A deficiency of L-arginine and NO, the main vasodilating factor, is accompanied by endothelial dysfunction in a model caused by nickel intoxication and L-NAME [10]. Changes in the cholesterol metabolism contribute to limiting the bioavailability of L-arginine for eNOS.

It is necessary to study metabolic changes that are crucial for the development of endothelial dysfunction caused by a combination of nickel intoxication + L-NAME.

The article aims to study the molecular mechanisms of violation of the NO-forming function of the endothelium in a model experiment with a combination of nickel chloride and L-NAME.

2. METHODS AND MATERIALS

Studies on male rats Wistar of one age group (10–14 months) weighing 220–250g were conducted. Depending on the experimental conditions, the experimental animals were divided into the following groups:

- 1) intact rats, i.e. control animals that were injected with saline in an equivalent volume with other substrates;
- 2) intact rats injected with an eNOS inhibitor – L-NAME (at a dose of 25 mg/kg of animal weight);
- 3) rats injected with nickel angiopathies (NiCl₂ x 6H₂O 0.5 mg/kg of animal weight for a month parenterally);
- 4) rats injected with an eNOS inhibitor – L-NAME against the background of intoxication with nickel chloride;
- 5) rats injected with nickel chloride and L-arginine at a dose of 10 mg/kg for 30 days.

The experiments were in compliance with international principles of humane treatment of animals (“Rules for the work using experimental animals”, Helsinki Declaration). At the end of the experiment, intensity of lipid peroxidation in erythrocytes, renal and myocardial tissues was studied using the secondary product, malonic acid aldehyde (MDA) [11]. The activity of antioxidant cell defense (AOD) was assessed by the activity of catalase [12], superoxide dismutase (SOD) [13], and the concentration of ceruloplasmin (CP) [14]. The concentration of total metabolites of nitric oxide (NOx) was determined [15]. The membrane mechanisms of organ cell damage were assessed by the activity of the LPO process. The activity of serum-specific enzymes was evaluated: transaminases (AlAT, AcAT), GGTP and

alkaline phosphatase with Vital kits. The effect of L-arginine and L-NAME on biochemical parameters, including the level of eNOS expression, was studied. Studies on the level of eNOS expression were carried at the National Medical Research Center for Preventive Medicine of the Ministry of Health of Russia. The results were processed in Statistika 6.

3. RESULTS

Simulation of the endothelial dysfunction was induced by a combination of nickel chloride and L-NAME. The studies were carried out under intoxication conditions with a parenteral administration of 0.5 mg/kg NiCl₂ x 6H₂O and 25 mg/kg L-NAME. The duration of the experiment was 30 days. The results showed a violation of redox processes. An increase in the concentration of MDA in the hemolysate of erythrocytes, in the renal and myocardial tissues was identified. An analysis of the activity of enzymes of the antioxidant system (AOS) showed a statistically significant decrease in the functional ability of SOD in red blood cells, an increase in catalase and the concentration of CP in serum. The multidirectional nature of the changes in adaptive enzymes is due to their different molecular structures. Thus, nickel intoxication with an inhibitor of eNOS expression under the impaired redox potential was accompanied by a decrease in the concentration of NOx, the main biochemical marker of endothelial dysfunction. The reasons for the insufficient formation of NO can be different: a deficiency of the L-arginine substrate and a violation of its availability for eNOS (Table 1).

The possibility of using L-arginine with the NO enzyme was determined in a separate variant in experimental rats on the background of exposure to nickel chloride for 30 days. We studied the state of the LPO – AOS system and the NO content. The results showed the ability of L-arginine to increase the concentration of NO in toxic conditions. In this case, a decrease in the LPO intensity according to the secondary product of malonic acid aldehyde was observed. Under the decrease in lipid peroxidation activity, the adaptive system showed an increase in SOD activity, as well as a positive trend for the catalase and CP (Table 1). These changes helped eliminate discrepancies in the LPO – AOS system. Against the background of modified L-arginine, on the contrary, intensity of free radical oxidation (SRO) and the content of MDA increased while inhibiting the activity of AOS. Violation of the redox potential of the cells was accompanied by a decrease in the content of NO (Table 1).

NO synthase is a complex molecule having reductase and oxygenase areas. The interaction between them is carried out by electrons. However, coenzymes play an important role, in which the restored state is disturbed under the oxidative stress. Modeling of the endothelial dysfunction disrupts the internal molecular structure of the enzyme and the level of eNOS expression. Our experimental data on the level of enzyme expression in the aortic endothelium showed the stimulating role of L-arginine and the inhibitory L-NAME.

Table 1 Data on oxidation and reduction and the content of nitrogen oxide

Indicators	Groups				
	Control	Intact + L-NAME	NiCl ₂ + L-NAME	Intact + L-arginine	NiCl ₂ + L-arginine
MDA nmol / ml	4.41±0.089	4.72±0.032 ¹¹¹¹⁾	6.25±0.04 ²²²²⁾	4.38±0.04	5.42±0.03 ³³³³⁾
SOD unit act.	88.8±1.77	86.8±1.16 ¹¹¹¹⁾	47.8±0.45 ²²²²⁾	88.8±1.43	58.78±0.32 ³³³³⁾
Catalase mkat / L	227.6±1.54	229.42±0.67 ¹¹¹¹⁾	352.8±2.59 ²²²²⁾	221.16±0.84	314.5±0.67 ³³³³⁾
CP mg / L	343.8±5.44	346.2±2.03 ¹¹¹¹⁾	451.54±1.19 ²²²²⁾	334.8±2.2	390.12±0.48 ³³³³⁾
NO µmol	51.54±0.48	50.4±0.46 ¹¹¹¹⁾	30.56±0.06 ²²²²⁾	51.46±0.31	40.136±0.01 ³³³³⁾

Note: ¹¹¹¹⁾ – p<0,001; ¹¹¹⁾ – p<0,01; ¹¹⁾ – p<0,02; ¹⁾ – p<0,05 – NiCl₂+ L-NAME relative to control, ²²²²⁾ – p<0,001; ²²²⁾ – p<0,01; ²²⁾ – p<0,02; ²⁾ – p<0,05 – NiCl₂+ L-NAME relative to intact + L-NAME, ³³³³⁾ – p<0,001; ³³³⁾ – p<0,01; ³³⁾ – p<0,02; ³⁾ – p<0,05 – NiCl₂+ L-arginine relative to NiCl₂+ L-NAME.

The activation of lipid peroxidation caused qualitative changes in phospholipids. Phospholipase A2 contributed to the conversion of phospholipids into lyso derivatives, which changed their molecular structure and permeability. The result of these effects was the development of endothelial dysfunction whose indicator is an increase in blood activity of organ-specific enzymes: AIAT, AsAT, GGTP and alkaline phosphatase (Fig. 1–4). An increase in transaminase activity indicates a damage to the cytoplasmic membranes of hepatocytes and cardiomyocytes. The combination of changes in the membrane enzyme GGTP and transaminases confirms the changes that occur in the cell membranes of internal organs during nickel intoxication and the combined effect of nickel chloride and L-NAME.

The introduction of an inducer of NO – L-arginine decreased the activity of organospecific enzymes: AIAT, AsAT, and GGTP. An increase in the content of nitric oxide has an antioxidant effect and helps restore the molecular structure of cell membranes and their hydrophobicity.

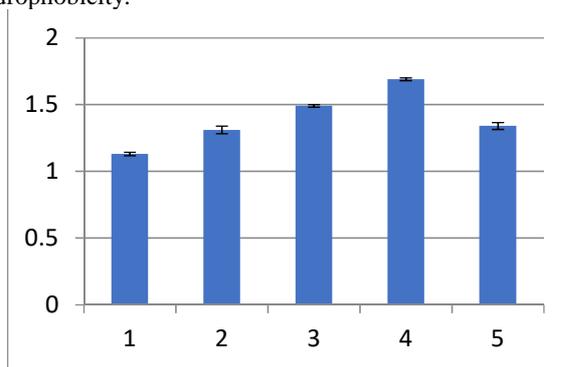


Figure 1 AIAT activity data under the intoxication syndrome (µmol / s * l). Note: 1 – control, 2 – L-NAME, 3 – NiCl₂, 4 – NiCl₂+L-NAME, 5 – NiCl₂+L-arginine.

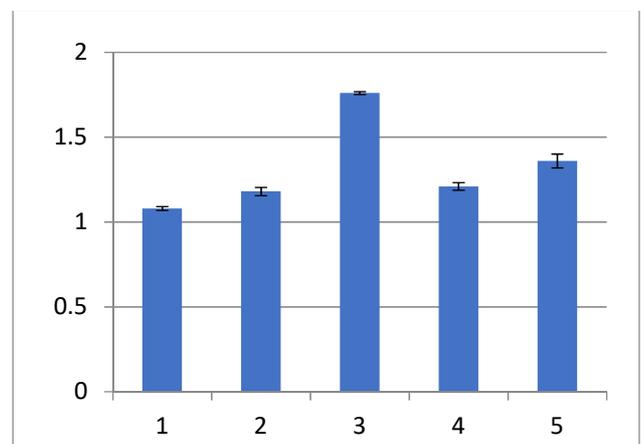


Figure 2 AsAT activity data under the intoxication syndrome (µmol / s * l). Note: 1 – control, 2 – L-NAME, 3 – NiCl₂, 4 – NiCl₂+L-NAME, 5 – NiCl₂+L-arginine.

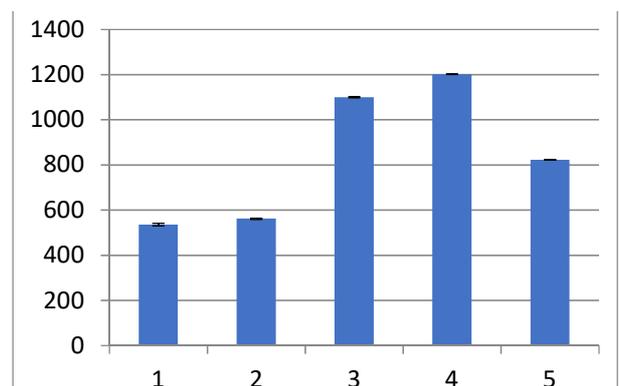


Figure 3 GGTP activity data under the intoxication syndrome (nmol / s * l). Note: 1 – control, 2 – L-NAME, 3 – NiCl₂, 4 – NiCl₂+L-NAME, 5 – NiCl₂+L-arginine.

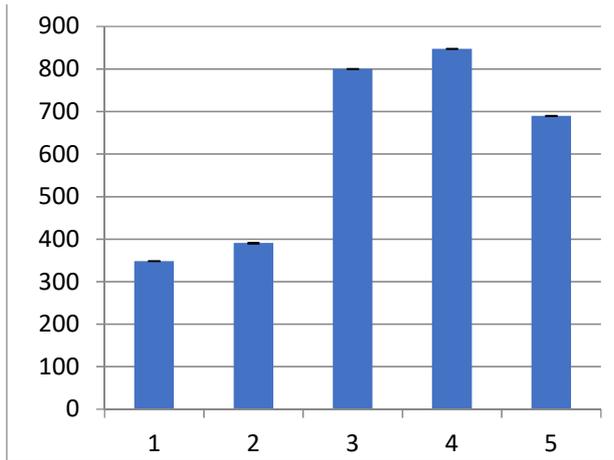


Figure 4 GGTP activity data under the intoxication syndrome in experiment (nmol / s * I). Note: 1 – control, 2 – L-NAME, 3 – NiCl₂, 4 – NiCl₂+L-NAME, 5 – NiCl₂+L- arginine

4. CONCLUSION

Thus, when exposed to nickel chloride and L-NAME, the redox potential is disrupted and the total metabolites of NO, the main vasodilating factor, are reduced. In contrast, L-arginine inhibits CPO and increases the adaptive mechanism of AMA. Moreover, L-arginine induces the expression of eNOS, while L-NAME inhibits. Atherogenesis risk factors, such as impaired transport of L-arginine substrate for endothelial NO synthase, limiting the availability of L-arginine to eNOS, contribute to the endothelial dysfunction. Biochemical markers of dysfunction of the endothelium and visceral systems are an increase in the content of lipid peroxidation products, a decrease in the content of total metabolites of nitric oxide, and an increase in the activity of organ-specific enzymes in blood serum.

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