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PECULIARITIES OF DISORDERS OF NITROGEN OXIDE SYSTEM IN THE BLOOD AT ADRENALIN-INDUCED MYOCARDIAL INJURY IN CONDITIONS OF IMMOBILIZATION STRESS AND THEIR CORRECTION BY L-ARGININE

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The aim: *of the study was to elucidate the changes to nitric oxide activity in the blood during adrenaline-induced myocardial injury under immobilization stress and to establish the corrective effect of L-arginine.*
Methods: *determination of free arginine was conducted by the method of Aleinikova T.L., total nitric oxide products in the blood by the method of Schmidt H.H., the total activity of nitric oxide synthase by the method of Sumbaiev V.V. Immobilization stress was reproduced by the method of Horizontov P.D. Adrenaline-induced myocardial injury was reproduced by the method of Markova O.O. L-arginine was injected based on scientific data by Kiryanova N.A.*
Results. *Studies have shown that on days 1 and 3 with adrenaline-induced myocardial injury under immobilization stress there was an increase in nitric oxide products in the blood, respectively, according to control. The use of L-arginine on the 5th day, led to a decrease in levels of NO products in the blood by less than, lower against the group of animals with MI and IS, to treatment.*
Conclusions. *Thus, biochemical studies of NO system in the dynamics of IS and MI showed an increase in food content and total synthase activity of NO on the background of reduced levels of L-arginine, which were detected at all stages of the study and especially expressed on the 1st day before treatment. The use of the drug L-arginine, made it possible to identify its corrective effect on impaired metabolic processes in MI and IS*
Keywords: *Free arginine, nitric oxide system indicators, adrenaline-induced myocardial injury, immobilization stress*

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1. Introduction

Arginine metabolism is depend of the activity of nitric oxide syntetase and arginase enzymes. Nitric oxide syntetase oxidases arginine to citrulline and NO, and arginase hydrolyzes arginine into ornithine and urea [1]. In recent decades, comorbid pathology is observed quite often in the clinic of internal diseases. For the most part, concomitant pathology complicates the course of the underlying disease, worsening the survival prognosis of patients. The stress system is a regulatory system, which is represented by three constituents: nervous, endocrine and immune, the action of which is aimed at maintaining homeostasis [2]. The mechanisms of stress in the organs of cardiovascular system are associated with increased production of stress hormones by the adrenal glands, mainly catecholamines, which by affecting α-adrenoceptors lead to vasoconstriction, impaired blood supply to tissues and induce the development of nitrosoxidative processes [3]. Catecholamines released in response to activation of the sympatho-adrenal system, together with the autonomic nervous system, have a regulatory effect on the cardiovascular and immune systems, lungs, liver and skeletal muscles [4, 5].

Under physiological conditions in blood vessel endotheliocytes, NO is synthesized in low concentrations by constitutive NOS, then diffuses into vascular smooth muscle, where it reacts with soluble guanylate cyclase heme (sGC) resulting in the formation of a secondary messenger – cyclic guanosine monophosphate (sGMP) [6]. The latter acts through protein kinase G and leads to relaxation of smooth muscle cells and vasodilation and increased blood flow to organs [7, 8]. Nitrogen oxide (NO) is an important intracellular and intercellular signaling molecule involved in the regulation of various physiological and pathophysiological processes in the cardiovascular, nervous, immune and digestive systems [9]. L-arginine is a chemical developing block called “an amino acid”. It is obtained from the diet and is necessary for the body to make proteins. L-arginine is found in red meat, poultry, fish, and dairy products. It could also be made in a laboratory and used as medicine. L-arginine is converted in the body into a chemical called nitric oxide. Nitric oxide causes blood vessels to open wider for improved blood flow. L-arginine also stimulates the release of growth hormone, insulin, and other substances in the body [10].

These days, the issue of the peculiarities of NO system in the dynamics of adrenaline-induced myocardial injury (MI) and immobilization stress (IS) before and after the use of L-arginine remains unstudied.

The aim of our study was to elucidate the changes of NO system in the dynamics during adrenaline-induced myocardial injury under immobilization stress and to establish the corrective effect of L-arginine.

2. Planning (methodology) of research

After analyzing the literary sources [11, 12], it was decided to use the L-arginine drug as an angioprotector with endothelium stabilizing, vasodilating, antiischemic and antioxidant properties, as a drug that prevents oxidative stress and vascular endothelial damage, improves rheological properties of blood.

Two models were chosen for the experiment: the immobilization stress (IS) model and the model of adrenaline myocardial infarction (MI). These models are scientifically justified [13, 14].

The obtained results of experimental studies of the L-arginine drug on rats will help to apply the results in clinical practice.

Stages:

1. Literary sources analysis;
2. Defining the object of the study;
3. Experimental IS and MI models on rats and study of the drug effect on the reduction of comorbid pathology in rats;
4. Biochemical studies of the NO system parameters in the dynamics of comorbid pathology development (IS and MI);
5. Obtained data analysis.

3. Materials and methods of research

The research was conducted based on Danylo Halytsky Lviv National Medical University, during 2019–2020.

The medicine Tivomax-Darnitsa, produced by PRJSC PHARMACEUTICAL company DARNITSA, Ukraine, was used for research purposes in the solution form with arginine hydrochloride 42 mg/ml.

The experiments were performed on white male Wistar rats weighing 180–200 g, which were divided into 3 groups of 10 animals each (one control group and two experimental ones). Rats of the control group were injected with saline at a dose of 1 mg per 1 kg of body weight intraperitoneally. Animals of the first experimental group were subjected to prolonged immobilization stress (3h) and injected intraperitoneally with 0.18 % adrenaline solution 1 mg per 1 kg of body weight, and then were removed from the experiment on the 1st, 3rd and 5th day of the experiment. The second experimental group of animals underwent MI under IS on the 5th day after treatment with L-arginine, which was injected at a dose of 150 mg/kg intraperitoneally daily from the 1st to the 5th day of the experiment.

The research was conducted based on Danylo Halytsky Lviv National Medical University Laboratory of Industrial Toxicology Certificate No. RL 086/17 from 26.06.2017 on the conformity of the measurement control system in accordance with DSTU ISO 10012:2005 Photoelectrocolorimeter KFK-3, No. 9004484. Calibration certificate No. 753/E from 15.11.2019, Spectrophotometer СФ-46, No. 901672. Calibration certificate No. UA/37/191114/001803 from 12.11.2019, pH-metr pH-150MI, No. 0137, ECK-10606, No. 00093 Calibration certificate No. 708/E from 12.11.2019 for the this article.

All experimental animals were kept in standard conditions at the vivarium of Danylo Halytsky Lviv National Medical University. The research was conducted in compliance with scientific and practical recommendations for keeping and working with laboratory animals and the provisions of the European Convention on the protection of vertebrate animals used for experimental and scientific purposes. Study was approved by the Commission on Bioethics of Danylo Halytsky Lviv National Medical University (Protocol No 2 from 17.02.2020) for this article. Animals were decapitated under anesthesia using diethyl ether and removed from the experiment on the 1st, 3rd and 5th day.

L-arginine was injected based on scientific data by Kiryanova N. A. [11]. Immobilization stress (IS) was reproduced by the method of Horizontov P. D., Belousova O. I., Fetodov M. I. (1983) [13]. Adrenaline-induced myocardial injury (MI) was reproduced by the method of Markova O. O. (1998) [14]. Determination of free arginine was conducted by the method of Aleinikova T. L., Rubtsova H. V., Pavlova N. A. [15]. Determination of total nitric oxide products (nitrite and nitrate ions) in the blood by the method of Schmidt H. H [16]. Determination of the total activity of nitric oxide synthase by the method of Sumbaiev V. V., Yasinskaya V. V. [17]. Processing of digital data was performed by the method of variation statistics using Student's criterion [18].

4. Results

Biochemical studies have shown that on days 1 and 3 with adrenaline-induced myocardial injury under immobilization stress there was an increase in NO system in the blood, respectively, by 251.9±3.9 % ($p<0.05$) and 110.3±3.7 % ($p<0.05$) according to control. Subsequently, on the 5th day of immobilization stress and adrenaline-induced myocardial injury, a slight increase in NO system in the blood was found above the control by 44.9±1.6 % ($p<0.05$), which indicated cell damage and compensatory output (Fig. 1).

The conducted biochemical study of the total activity of nitric oxide synthases showed an increase in their activity in the blood by 1, 3 and 5 days, respectively, by 204.3±4.2 % ($p<0.05$) and 160.9±3.6 %, ($p<0.05$), and 139.1±3.2 %, ($p<0.05$) (Fig. 2), under the combined effects of adrenaline-induced myocardial injury under immobilization stress during the specified period of the experiment, against control. Obtained results indicated damage to endothelial cells and their release into the blood.

The level of free arginine in the blood with adrenaline-induced myocardial injury under conditions of immobilization stress on the 1st, 3rd and 5th days of the experiment decreased by 80.7±1.6 %, ($p<0.05$), 66.1±1.4 %, ($p<0.05$) and 67.9±1.4 %, ($p<0.05$) before treatment with L-arginine compared with control (Fig. 3).

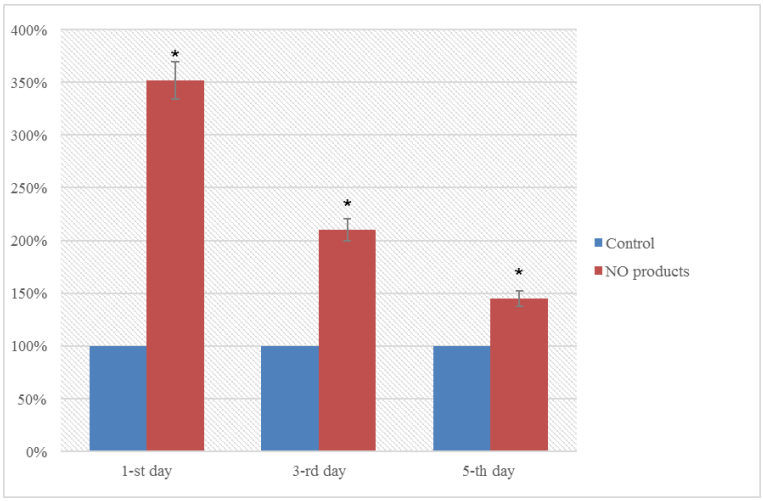

Fig. 1. NO products in the blood of animals with adrenaline-induced myocardial injury under immobilization stress (in % of control): * – p<0.05 compared to the indicator in the control group

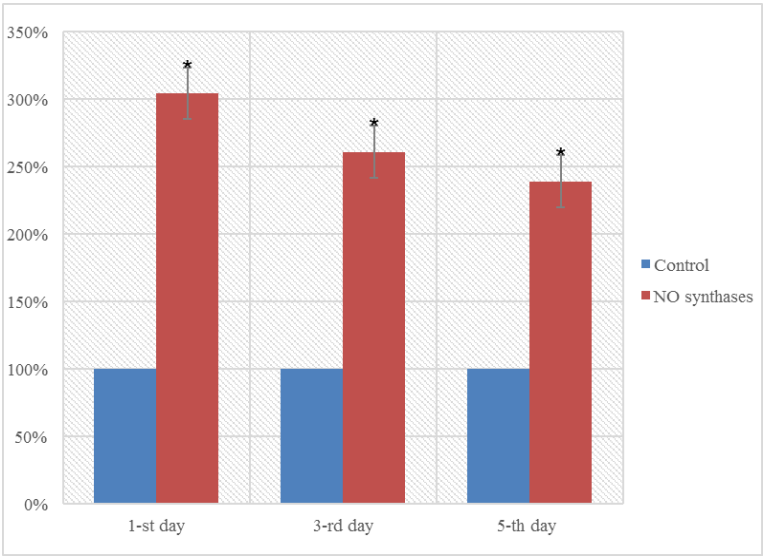

Fig. 2. NO synthases systems in the blood in adrenaline-induced myocardial injury under immobilization stress (in % of control): * – p<0.05 compared to the indicator in the control group

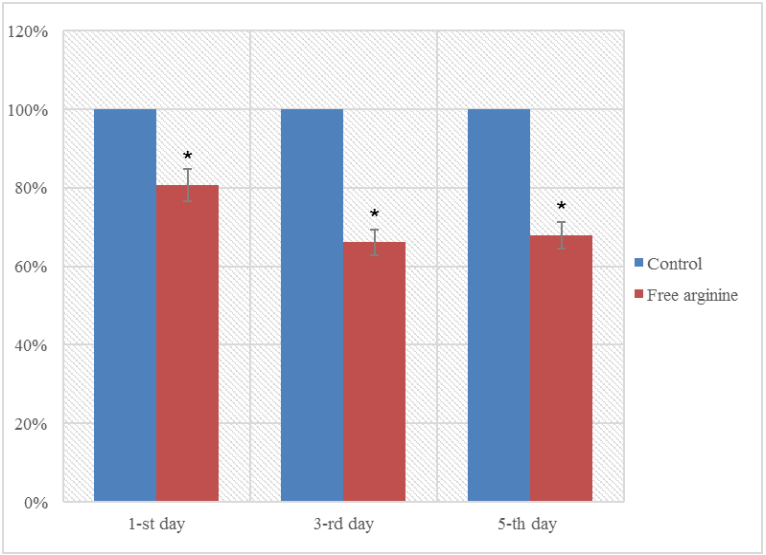

Fig. 3. The level of free arginine in the blood in adrenaline-induced myocardial injury under immobilization stress (in % of control): * – p<0.05 compared to the indicator in the control group

The use of L-arginine on the 5th day, led to a decrease in levels of NO products in the blood by less than 25.8±1.1 % ($p<0.05$), lower against the group of animals with MI and IS, to treatment, indicating on the stabilization of cell membranes under the influence of this drug. Comparing the analysis of the results of animal studies before and after treatment for adrenaline-induced myocardial injury under immobilization stress with L-arginine on day 5 of the experiment, we obtained a decrease in NO synthases in the blood by 49.1±2.6 %, ($p<0,05$) (Fig. 4). This allows us to conclude that this amino acid as a hepatoprotector and antioxidant has a positive effect on these indicators. The results of our study confirm similar studies that were conducted in Lviv (Havrona O.P.). [12], who found an increase in nitric oxide metabolites in the tissues of the skin, kidneys and blood in humans and rats and changes in the functioning of the L-arginine / NO system in the serum of rats in other pathologies.

After application of the drug L-arginine on the 5th day, the level of free arginine increased by 100.0±2.4 % ($p<0.05$), compared with its content before treatment, under the conditions of IS and MI (Fig. 4). Thus, in comorbid pathology, the level of free arginine in the blood drops sharply, indicating damage to the vascular endothelium, and with the introduction of exogenous L-arginine, the concentration of endogenous free arginine is adjusted above physiological levels to confirm improved vascular endothelial function and antioxidant activity.

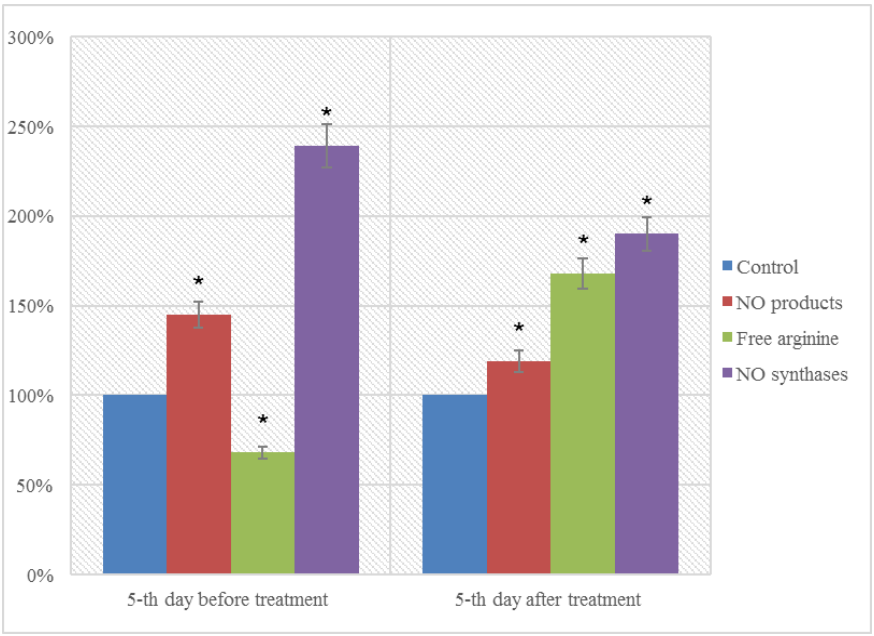

Fig. 4. The effect of the drug L-arginine on the activity of the NO system in the blood in adrenaline-induced myocardial injury under immobilization stress (in % comparison for 5 days before and after treatment): * – $p<0.05$ compared to the indicator in the control group

5. Discussion

Thus, biochemical studies of NO system in the dynamics of IS and MI showed an increase in food content and total synthase activity of NO on the background of reduced levels of L-arginine, which were detected at all stages of the study and especially expressed on the 1st day before treatment. The use of the drug L-arginine, made it possible to identify its corrective effect on impaired metabolic processes in MI and IS.

Abroad, similar studies were conducted jointly by U. Forstermann (Mainz, Germany) and W.C. Sessa (New Haven, USA), who studied the regulatory functions of NO synthase and proved that unchanged NOS contributes to the pathophysiology of inflammatory diseases and septic shock. Endothelial NOS (eNOS, NOS III) is mainly expressed in endothelial cells. It supports vasodilation, controls blood pressure and many other vasoprotective and antiatherosclerotic effects. Many cardiovascular risk factors lead to oxidative stress, dissociation from eNOS, and endothelial dysfunction in the vascular system [19]. Elsewhere in the world, Indian scientists S. Habib and A. Ali studied the biochemistry of NO in 2011, pointing out that the main reactions of NO can be divided as the direct effect of the radical where it itself plays a role in either damage or protection of the cellular environment, and in an indirect effect in which NO by-products formed by the convergence of two independent pathways of radical formation play a role in biological reactions, which mainly include oxidative and nitrosative stress [20].

Study limitations. Our studies are limited to morphological studies in the blood tissue.

Prospects for further research. Further study of the effect of stable metabolites NO, total activity NOS and level of L-arginine in the blood and myocardial tissue in adrenaline-induced myocardial injury under immobilization stress before and after use of the drug L-arginine is needed.

6. Conclusions

Studies indicate changes in metabolic processes in the blood, damage to the plasma membrane and vascular endothelial function in combined pathology of the MI under conditions of immobilization stress, manifested by

increased NO system and NO synthase system in the blood. Data on reduced levels of free arginine could also be used in coronary heart disease, which results in acute coronary syndrome. Research work and in the educational process, and the use of the drug L-arginine led to the correction of NO products and free arginine, which indicates a positive corrective effect of the drug on these indicators and the prospects for its further study in the experiment and in the clinic to improve identified disorders of nitric oxide systems for adrenaline-induced myocardial injury under stress and the development of guidelines cardiovascular disease.

Conflict of interests

The authors declare that they have no conflicts of interest.

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