THE ROLE OF ADRENORECEPTORS AND CALCIUM IONS IN THE PATHOGENESIS OF ENDOCARDIUM ENDOTHELIAL DAMAGE OF RATS UNDER STRESS ACTION ON THE BACKGROUND OF HYPERCHOLESTEROLEMIA

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Abstract. Topicality. Endothelial dysfunction is a key link in the pathogenesis, formation and progression of clinical manifestations of a significant number of cardiovascular system diseases. Some pathogenetic links and structures associated with morphofunctional disorders of cardiac endothelial cells under the stress conditions and its combination with hypercholesterolemia need further examination.

The aim of the study was to investigate the role of adrenoreceptors and calcium ions (by using adrenoreceptors and Ca²⁺-channels blockers) in the pathogenesis of endocardium endothelium damage (by the content of free fatty acids in it and the number of exfoliated cells) as a result of emotional and pain stress action and its influence on the background of hypercholesterolemia.

Materials and methods of research. An electro-pulse model was used for stress reproduction. The alimentary hypercholesterolemia was simulated by keeping animals on an atherogenic diet during 2 months. To establish the role of the above-mentioned cellular structures in the mechanisms of endothelial cell damage, the following pharmacological drugs were administered to animals: β-adrenoblocker, α-adrenoblocker and Ca²⁺-channel blocker. The endocardium endothelium state was studied by using light microscopy, by analyzing the smear-imprints from macropropreparations of ventricles by counting the number of endothelial cells in them. The content of free fatty acids was determined by radiochemical method.

Research results. It has been shown that β-adrenoblocker and Ca²⁺-channel blocker significantly limit the damage of endocardium endothelium of left ventricle under the action of emotional and pain stress, and to a much lesser extent, the same effect has α-adrenoblocker. The protective effect of the mentioned medications under the action of stress factor against the background of alimentary hypercholesterolemia significantly decreases.

Conclusions. The data obtained, their analysis and comparison with the results of other experimental and clinical studies allow us to make certain conclusions about the pathogenesis of endothelial dysfunction under emotional stress, as well as under conditions of its combination with hypercholesterolemia. In particular, an important link in the development of structural and some biochemical changes of endocardial endothelial cells is the toxic effect of catecholamines, which affect the organism’s functional systems mainly by β-adrenoreceptors (involving Ca²⁺ ions), to a lesser extent – by α-adrenoreceptors.

Key words: endocardium, stress, hypercholesterolemia, adrenoreceptors, calcium.

Problem statement and analysis of recent research. Currently, in consequence of numerous experimental and clinical studies, it is proven that endothelial dysfunction is the key link in the pathogenesis, formation and progression of clinical manifestations of a significant number of cardiovascular system diseases [7, 8, 15, 18, 20]. The stressors of various genesis and disorders of lipid metabolism, in particular, an increasing of free cholesterole concentration in blood are the most important factors that have a significant pathoendotheliotrophic effect on the heart and blood vessels [4, 9, 10, 12, 14, 22, 25]. The results of many researches show that a number of mechanisms are involved in the implementation of stress injuries, the main from which are: toxic effect of elevated catecholamines (CA) blood level, activation of lipid peroxidation (LP), imbalance in prostaglandin synthesis [5, 7]. The cardiotoxic action of CA is associated with excessive stimulation of adrenoreceptors, which promotes the elevation of cyclic adenosine monophosphate (cAMP) intracellular concentrations and, apparently, leads to the activation of phosphoinositol metabolism. This effect on the cardiomyocytes membrane is mediated with the participation of two intracellular mediators: cAMP and Ca²⁺ ions [1, 16, 17, 24]. It was also found, that the damaging effect of any stress factor on the heart is accompanied by the activation of lipolysis, the increasing of free fatty acids (FFA) content, as well as intracellular accumulation of double-chain forms of fatty acids and acetyl-coenzyme A [14, 20, 21, 22, 25]. Inherent for FFA is potentiation of the damaging effects of CA on the heart during stress reactions: increasing the myocardial oxygen demand, decreasing adenosine triphosphate and creatine phosphate levels in the heart muscle, promoting platelet accumulation in the microcirculatory net of myocardium [23, 28]. However, it should be noted, that some pathogenetic links and structures, that are associated with morphofunctional disorders of cardiac endothelial cells under the stress conditions and its combination with hypercholesterolemia, need further examination.

The aim of the research. To determine the role of adrenoreceptors and calcium ions (by using of adrenoreceptors blockers and Ca²⁺-channels blockers) in the pathogenesis of endocardium endothelium damage...
(by the content of FFA in it and the number of exfoliated cells) under the action of emotional and pain stress (EPS) and its impact on the background of hypercholesterolemia (HCE).

**Materials and methods of research**

The studies were performed using 54 outbred white male rats, weighting 180-240 g, which were divided into three groups: 1st – control group, 2nd – animals that were exposed to three hours action of EPS, 3rd – animals that were exposed to stress action against the background of HCE. In the 2nd and 3rd groups, before the stress action certain subgroups of animals were administered some medications: phentolamine (α-adrenoblocker, powder of 0.025 g, manufactured by “Zdorovia”, Ukraine) and propranolol (β-adrenoblocker, 1 ml of 0.1 % solution in ampules, manufactured by “Zdorovia”, Ukraine) once intravenously at a dose of 0.5 mg/kg before the beginning of stress influence; finoptin – Ca²⁺-channels blocker (2 ml of 0.25 % solution in ampules, manufactured by „ORION”, USA) – at a dose of 2.5 mg/kg intramuscularly 30 minutes before the stress reaction.

The electro-pulse O. Desiderato model was used for EPS reproduction [13]. Alimentary HCE in rats was reproduced by F.U. Peiler method [26]. The duration of stress influence was 3 hours, the animals were kept on an atherogenic diet during 2 months. The keeping of animals and manipulations with them were carried out in compliance with the Council of “Europe Convention for the Protection of Vertebrate Animals Used in Experiments and Other Scientific Purposes” (1986) and “General Ethical Principles of Animals Experiments”, adopted by the First National Congress of Bioethics (Kyiv, 2001). Euthanasia of animals was carried under chloroform anesthesia.

For the examination of endocardium endothelium condition, the smear-imprints from macropreparations of left ventricle of rats were prepared, applying them to slides, which were stained by Romanovsky method. In this case, the cell nuclei are clearly stained in comparison with the cytoplasm. The damage degree of the endocardium endothelium was determined by the number of exfoliated cells per 1 mm² of the imprint area corresponding to 40 fields of view. The FFA content was determined by radiochemical method [26]. The data obtained were processed statistically by using computer programs Microsoft Excel and Statistica 7.0.

**Analysis and discussion of research results**

The analysis of our obtained experimental results shows that propranolol and finoptin have a significant cytoprotective effect in relation to the endocardium endothelium of the left ventricle of rats, reducing the desquamation of endothelial cells during emotional and pain stress at 63.9 % and 40.1 % (p<0.001), respectively (Table 1). The α-adrenoblocker phentolamine limits the endothelial damage to a much lesser extent (the number of exfoliated cells in micropreparations decreases at 16.7% (p<0.02).

These results have some correlations with the data of a number of experimental and clinical studies [2, 5], which indicate that there is a pronounced activation of the sympatho-adrenal system (SAS) under the conditions of acute EPS. This is accompanied by the toxic effect of CA high concentrations on various functional systems, including endothelial lining [2, 3, 21, 27].

The damage of the luminal part of endothelial cells plasmolemma, as well as the exfoliation of a significant number of altered and damaged cells have been found and in a number of other studies about the stressors action [4, 9].

It should be noted that in case of stress reaction complicated by HCE, the same trend in the action of drugs used is observed, but their pharmacological effect is less pronounced. Thus, under such conditions, the cytoprotective effect of propranolol and finoptin is weaker. Under their influence, the degree of the endocardium endothelium damage decreases (at 51.9 % and 26.9 %, p<0.001 respectively), but to a lesser extent than under the influence of isolated EPS. The administration of

**Table 1. The number of exfoliated endothelial cells (cells/mm²) in smears from the endocardium of left ventricle of rats after the using of finoptin, phentolamine and propranolol under the action of EPS and EPS on the background of HCE (M±m, n=6)**

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<th>№</th>
<th>Groups of animals and pharmacological agents</th>
<th>Experimental conditions</th>
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<td>1.</td>
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<td>3.</td>
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<td>4.</td>
<td>Propranolol</td>
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Note. * – probably compared to intact animals
Phentolamine reduces the desquamation of endothelial cells at 16.1 % (p<0.05).

It should be noted that in animals that were on an atherogenic diet accumulation of cholesterol (CS) in the membranes of blood cells, aorta and myocardium was found [20, 21, 22]. Also, at the same time the cholesterol-phospholipid index of the luminal part of endothelial cells plasmolemma of blood vessels and other cells increases [11, 22, 25, 28]. The consequence of these changes is the development of endothelial dysfunction, in particular, imbalance between the production of such substances and their action. The action of phentolamine (through β-adrenoreceptors and Ca2+-ion channels, respectively). In this situation, the effect of phentolamine is virtually unchanged.

Thus, it is obvious that HCE in case of EPS potentiates the damage of endocardium endothelium and significantly limits the protective effect of propranolol and finoptin (through β-adrenoreceptors and Ca2+-ion channels, respectively). In this situation, the effect of phentolamine is virtually unchanged.

Comparison of the presented above results with the data of many other authors gives grounds to talk about the systemic character of endothelial damage in response to one of the atherogenic factors – exogenous CS. Structural and functional disorders of endothelial cells in conditions of experimental HCE are described also in the microcirculatory net of other organs: brain, liver, kidneys, lungs [11, 21, 22, 28], as well as in the aorta and femoral artery [8, 9].

The second part of our research was connected with the determination of FFA content in the endocardium under the influence of the mentioned pathogenic factors. The analysis of data, which are represented in Table 2 shows that finoptin and propranolol have a pronounced effect on this index under stress conditions. The content of FFA, as products of lipid hydrolysis is reduced at 20.9% (p<0.01) and 22.8 % (p<0.01) respectively against the background of their action. The action of phentolamine is not accompanied by statistically significant restrictions of the elevation of these substances concentrations, but such trend is observed.

These results indicate the effect of CA on the lipid spectrum of cells under stress, which is apparently associated with the implementation of so-called lipid triad: activation of phospholipases and lipases, processes of LP and detergent action of lysophosphatides and excess of fatty acids. The lipomobilizing effect of CA is enhanced by glucocorticoids, adrenocorticotropic hormone and glucagon [2, 19, 21, 23].

As under the action of isolated stress, finoptin and propranolol are effective in conditions of EPS on the background of HCE. Under the influence of finoptin, the content of FFA in the endocardium decreases at 25.7% (p<0.05). Under such conditions propranolol inhibits their release at 21.5 % (p<0.02). The use of phentolamine leads to a slight decrease of the examined index.

Under the action of EPS, HCE potentiates the accumulation of FFA in the endocardium. The endogenous source of FFA in this case, according to the researches of some authors [19, 21, 23, 28], may be phospholipids, triacylglycerides and esters of CS. The elevation of FFA level in the cells is, probably, the result of increased hydrolysis of lipids due to increased activity of cholesterol esterases and triacylglycerol lipases.

Analyzing the above-mentioned, it can be concluded, that the endocardium endothelial cells damage under the action of EPS is realized primarily due to the effect of CA on α- and β-adrenoreceptors (mostly on β-adrenoreceptors) with the participation of Ca2+ ions, which is confirmed by other studies [1, 2, 3, 24, 27]. In addition, the ability of Ca2+ antagonists, α- and β-adrenoblockers to improve endothelial function has been established in a number of other researches [1, 2, 27].

**Conclusions**

1. The use of adrenoreceptors blockers and blockers of calcium channels proves that an important pathogenetic link of the morphological and biochemical changes of endocardial endothelial cells under the action of EPS and its influence on the background of HCE is the toxic effect of CA. It is realized mainly by β-adrenoreceptors, to a lesser extent by α-adrenoreceptors and with the participation of calcium ions. As a result of such changes, obviously, is the development of

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<td>Intact animals</td>
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<tr>
<td>1</td>
<td>Control</td>
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<tr>
<td>2</td>
<td>Finoptin</td>
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<tr>
<td>3</td>
<td>Phentolamine</td>
<td>-</td>
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<td>4</td>
<td>Propranolol</td>
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**Table 2. The content of FFA (μg/g of dry tissue) in the endocardium of rats when using finoptin, phentolamine and propranolol under the conditions of EPS and EPS on the background of HCE action (M±m, n=6)**
endothelial dysfunction and related to it disorders of various functional systems of the organism.

2. The increase of blood CS level intensifies the stress factor effect and significantly limits the cytoprotective effect of β-adrenoreceptor blockers and Ca²⁺-ion channels blockers on the endocardium endothelium, accompanied by an increase the number of exfoliated endothelial cells and FFA content in this structure.

**Prospects for further research.** Summarizing the results of our and other experimental and clinical studies, we can consider the fact, that changes in the organism, that occur in response to stressors influence and their effects on the background of hypercholesterolemia have systemic character. Therefore, it is advisable the further study of the mentioned pathogenic factors influence on the structural and functional state of the vascular endothelium of various organs.

**Ethics Policy.** All experiments were carried out according to the legislation of Ukraine (Law of Ukraine № 3447-IV “On protection of animals from cruel treatment”, 2006), the rules of European Convention for the protection of vertebrate animals used in experimental research and for other scientific purposes (Strasbourg, 1986) and approved by the Local Ethics Committee.

**Financial Disclosure.** The authors declare no financial support.

**Conflict of Interests.** The authors declare no conflict of interests.

**References**


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