SOME PATHOGENETIC ASPECTS OF ENDOCARDIAL ENDOTHELIUM DAMAGE OF RATS AS A RESULT OF STRESS EFFECT COMPlicated BY HYPERCHOLESTEROLEMIA

Ihor Luchko, Tetyana Huranych, Oksana Tuchak, Leonid Storozhuk
Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine

Abstract. The endothelial dysfunction is a predictor of occurrence for many diseases of cardiovascular system and a key link in their pathogenesis, formation and progression of clinical manifestations. Despite the large number of experimental and clinical researches, separate links of pathogenesis of heart endothelial cell damage under stress and in case of its combination with hypercholesterolemia require the further examination.

The aim of the work was to investigate the role of nitric oxide, prostaglandins and antioxidants in the pathogenesis of endocardial 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 and stress complicated by hypercholesterolemia.

An electro-impulse model was used to reproduce the stress. Alimentary hypercholesterolemia was modeled by keeping animals on an atherogenic diet for 2 months. To establish the role of individual pathogenetic factors in the mechanisms of endotheliocyte damage, animals were administered the following pharmacological drugs: L-arginine (a substrate for the synthesis of nitric oxide), a prostaglandin synthesis blocker indomethacin, and prostenon (a synthetic analog of prostaglandin E2, which has an antioxidant effect). The state of endocardial endothelium was examined by using the light microscopy, analyzing the smears-imprints from macropreparations of ventricles by counting the number of endothelial cells in them. The content of free fatty acids was determined by the radiochemical method.

It was shown that L-arginine significantly limits the damaging effect of the studied pathogenic factors on endocardial endothelium, reducing the number of exfoliated cells. The use of prostenon gives a slight, statistically unreliable positive effect. The use of indomethacin increases the damage of endothelial cells, which indicates the cytoprotective effect of prostaglandins under stress. All three studied preparations have no significant effect on the metabolism of free fatty acids both in case of “pure” emotional and pain stress and under stress complicated by hypercholesterolemia. The hypercholesterolemia of alimentary origin significantly limits the cytoprotective effect of L-arginine and prostenon on endocardial endothelium under stress action. In relation to indomethacin, in this situation, an increase of cell desquamation is observed, which indicates a decrease of prostaglandins protective effect.

Key words: endothelium, endocardium, stress, hypercholesterolemia.

Statement of the problem and analysis of the latest research

In accordance with the results of experimental and clinical studies, at the moment the endothelial dysfunction is considered an early predictor and a key etiopathogenetic link in the formation, progression, clinical manifestations of atherosclerosis and most diseases of cardiovascular system. Endothelial dysfunction is a violation of relationships between the factors that maintain homeostasis and regulate numerous functions of the endothelium [1, 9, 10, 16, 21]. It is important to note that endothelial cells are sensitive to the influence of various factors (both produced by cells of various body systems, and pharmacological agents introduced from the outside) [8, 9, 18].

From the list of main damaging factors that have a pronounced pathoendotheliotropic effect on the corresponding structures of the heart and blood vessels, one of the most important are stress factors of various genesis and an increase the level of free cholesterol (Ch) in the blood [1, 4, 12, 15]. The results of many studies related to the action of the mentioned factors show that a number of mechanisms are involved in the realization, in particular, the stress damage, the main of which are: the toxic effect of an increased catecholamines (CA) level in the blood, activation of lipid peroxidation (LP) processes, imbalance in the synthesis of prostaglandins (PG) [5, 6, 10, 13, 20, 26]. It was also established that the damaging effect of any stress factor on the heart is accompanied by the activation of lipolysis, an increase in the content of free fatty acids (FFA), which are characterized by the potentiation of the damaging effect of CA on the heart during stress reactions [3, 19, 25].

An important value in the regulation of vascular tone and the maintenance of aggregate state of blood has nitric oxide (NO), which is synthesized in the endothelium of vessels, including coronary, from the amino acid L-arginine [2, 27]. At the moment, there are data of experimental and clinical researches, which, on the one hand, indicate a violation of the function of vascular endothelium and NO synthesis by it under the influence of such factors as stress and hypercholesterolemia (HCE)
of animals was carried under chloroform anesthesia. Previously, we established the role of adrenoceptors and calcium ions in the mechanisms of endocardial endothelium damage of rats under the influence of stress on the background of HCE [23]. Taking into account the complexity of pathogenesis of the influence of mentioned factors on the structures of heart and vessels (in particular, the endothelial lining) and the insufficient research of all its aspects, we consider it appropriate the further study of this question.

The aim of the study. To explore the role of L-arginine, antioxidants and PG in the pathogenesis of endocardial endothelium damage (by the number of exfoliated cells and the content of FFA in it) as a result of the impact of emotional and pain stress (EPS) and under its action against the background of HCE.

Materials and methods

The research was carried on 54 outbred white male rats weighing 180-240 g, which were divided into three groups: 1st – control group, 2nd – animals that were exposed by EPS during three hours, 3rd – animals that were stressed against the background of HCE. In the 2nd and 3rd groups, the separate subgroups of animals were administered the drugs before the exposure of the stressor: L-arginine (100 mg/kg, “LAPHAL”, France, orally by a probe 1 hour before the exposure of stressor); indomethacin – a cyclooxygenase blocker (powder by 0.025 g, produced by “Zdorovya”, Ukraine) was administered by a probe into the stomach 90 minutes before the start of EPS action in the calculation of 10 mg/kg; prostenon – an antioxidant that is a pharmacological analogue of prostaglandin E2 (1 ml of 0.1 % solution in ampoules, produced by “Zdorovya”, Ukraine) was administered by the subcutaneous method 15 minutes before the stressor action.

The electro-impulse model of O. Desiderato [11] was used to reproduce EPS. Alimentary HCE in rats was reproduced according to the F.U. Peiler method [23]. The duration of the stress exposure was 3 hours, the animals were on an atherogenic diet for 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), Council Directive 86/609/EEC (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 endocardial endothelium state, the smears-imprints from macropreparations of the left ventricle of rats were prepared, applying them to the glass slides, which were stained by Romanovsky method. At the same time, in comparison with the cytoplasmin, the cell nuclei are clearly stained. The degree of endocardial endothelium damage was determined by the number of exfoliated cells per 1 mm² of the print area, which corresponds to 40 fields of view. The content of FFA was determined by the radiochemical method [23]. The obtained data were statistically processed using Microsoft Excel and Statistica 7.0 computer programs.

Research results and their discussion

The obtained research results show that after a three-hour stress reaction, the number of exfoliated endotheliocytes in the smear-imprints increases by 4.5 times compared to the control (p<0.001) (Table 1). In animals that were on an atherogenic diet, the pronounced damage of endocardial endotheliocytes under the action of EPS (increase by 6.5 times, p<0.001) was observed.

Table 1. The number of exfoliated endotheliocytes (cells/mm²) in smears-imprints from the endocardium of the left ventricle of rats after the use of L-arginine, indomethacin and prostenon under the conditions of EPS and EPS on the background of HCE action (M±m, n=6)

<table>
<thead>
<tr>
<th>Groups of animals and pharmacological drugs</th>
<th>Intact animals</th>
<th>EPS</th>
<th>EPS+HCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.67±1.69</td>
<td>107.50±4.92*</td>
<td>155.83±8.18*</td>
</tr>
<tr>
<td>L-arginine</td>
<td>–</td>
<td>84.33±4.79*</td>
<td>151.00±6.51*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>–</td>
<td>144.17±6.36*</td>
<td>234.67±6.51*</td>
</tr>
<tr>
<td>Prostenon</td>
<td>–</td>
<td>95.45±3.92*</td>
<td>137.25±5.81*</td>
</tr>
</tbody>
</table>

Note. * reliable compared to intact animals

Our data show that the use of exogenous L-arginine before the stress exposure in animals that were on a normal food diet, limits the endocardial endothelium damage by 22.2 % (p<0.001). Along with this, there is no statistically significant difference between the groups “EPS+HCE” and “EPS+HCE+L-arginine” according to this indicator.

The use of other pharmacological agents showed the following results. In case of indomethacin administration before the stressor action, the increase of desquamation of endothelial cells by 33.3 % (p<0.001) is observed. The use of prostenon does not give a significant statistically reliable cytoprotective effect, although the number of exfoliated cells decreases compared to the control. In case of stress complicated by HCE, the previous tendency is preserved in the action of the studied drugs, but their
pharmacological effect is less pronounced. Thus, under these conditions, a slight positive effect is observed after the use of prostenon (p<0.1), and the administration of indomethacin leads to an increase in the desquamation of endothelial cells by 50 % (p<0.001).

These results are consistent with the data of the number of experimental and clinical studies, which indicate that under the conditions of acute EPS there is a pronounced activation of sympatho-adrenal system and a toxic effect of an increased concentration of CA on various functional systems [5, 12, 14, 24]. In a previous study [23], we showed the role of adrenoceptors and Ca2+ ions in the implementation of such damage to endocardial endothelium.

The damage of endothelial lining of the cardiovascular system and, as a result, exfoliation of a significant number of altered and damaged cells has been established in a number of other researches of stress factors influence of various genesis [4, 13, 26].

It should be noted that the accumulation of Ch in the membranes of blood cells, aorta and myocardium is observed in animals that received an atherogenic diet [4, 7, 8]. There is also an increase of cholesterol-phospholipid index of the luminal part of the endotheliocytes plasmolemma of blood vessels and other cells [10, 21, 28]. The consequence of these changes is the development of endothelial dysfunction, one of the manifestations of which, in particular, is an imbalance between such endothelium-dependent regulators of vascular tone as endothelin-1 and NO [2, 9, 16, 17].

The analysis of the results obtained by us and their comparison with the data of other researchers gives a reason to say that the damage of endothelium in response to the action of one from atherogenesis factors – exogenous Ch has a systemic character. Structural and functional disorders of endotheliocytes under conditions of experimental HCE are also described in the microcirculatory bed of other organs: brain, liver, kidneys, lungs [5, 10, 16, 21, 28].

Table 2 represents the results of the FFA content determination in the endocardium under the action of mentioned pathogenic factors. The analysis of data shows that EPS and especially its combination with HCE leads to a significant increase of their content in the studied structure (by 39.9 % and 61.2 %, p<0.001 compared to the control). It is known that, during the stress reactions, FFA have the property to potentiate the damaging effect of CA on the heart and other internal organs due to: an increase in cellular oxygen requirement, a decrease the levels of adenosine triphosphate and creatine phosphate, promotion of the accumulation of platelets in the microcirculatory bed [3, 19, 22, 28]. In addition, their excessive accumulation has a direct toxic, detergent effect on various body cells [19, 25, 30], including endothelial cells [30].

### Table 2. The content of FFA (μg/g of dry tissue) in the endocardium of rats after the use of L-arginine, indomethacin and prostenon under the conditions of EPS and EPS on the background of HCE action (M±m, n=6)

<table>
<thead>
<tr>
<th>Groups of animals and pharmacological agents</th>
<th>Conditions of the experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact animals</td>
</tr>
<tr>
<td>Control</td>
<td>1.88±0.13</td>
</tr>
<tr>
<td>L-arginine</td>
<td>2.45±0.23</td>
</tr>
<tr>
<td></td>
<td>p1-2&lt;0.1</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2.73±0.17</td>
</tr>
<tr>
<td></td>
<td>p1-2&gt;0.05</td>
</tr>
<tr>
<td>Prostenon</td>
<td>2.35±0.16</td>
</tr>
<tr>
<td></td>
<td>p1-2&gt;0.1</td>
</tr>
</tbody>
</table>

This table shows that oral administration of L-arginine both in animals exposed by EPS and under the combined effect of EPS and HCE gives a slight, statistically unreliable decrease of FFA content. The same tendency is observed and after the administration of antioxidant prostenon before the stress reaction. The use of indomethacin as a blocker of cyclooxygenases is accompanied by an insignificant increase of FFA in the endocardium. Based on this, we can conclude that the pharmacological agents examined by us do not have a significant effect on the exchange of FFA in the endocardium under the action of stress and stress complicated by HCE.

To find out some pathogenetic links of the damaging effect of stress and stress complicated by HCE, we used three pharmacological agents: L-arginine, indomethacin and prostenon. What are the possible ways of influence of these drugs on the endothelium of endocardium in the mentioned conditions?

L-arginine belongs to the class of conditionally essential amino acids and is a cellular regulator of many vital body functions. In particular, due to its influence on the smooth muscle cells, it participates in the regulation of the tone of the walls of blood vessels, bronchi, and intestines. L-arginine is a substrate for nitric oxide synthase (NOS), which produces NO. NO, that is formed in the endothelium of vessels, is responsible for the relaxation of smooth muscles and is necessary for the decreasing of blood pressure. This amino acid is considered a priority in the production of NO, and therefore is important in the regulation of functions of cardiovascular and cerebrovascular systems. L-arginine slows down aging, inhibits platelet aggregation, regulates multiple metabolic pathways related to the metabolism of fatty acids, glucose, amino acids, and proteins [2, 27, 29]. The disrup-
tion of L-arginine – NO oxide metabolism is one of the most significant mechanisms of endothelial dysfunction. The changes of vascular endothelium function can be a consequence of both a decrease in the synthesis of NO by endothelial cells and the activation of its degradation processes under conditions of oxidative stress [27].

As we have established, oral use of L-arginine is manifested by a significant improvement of the structural and functional state of endocardial endotheliocytes under the action of EPS. The protective effect of L-arginine in relation to the endothelium is associated, obviously, with several mechanisms. First, it has the ability to activate cytoplasmic guanylate cyclase in endotheliocytes, which leads to an increase of cGMP concentration and, accordingly, to a decrease of intracellular Ca\(^{2+}\) concentration. Secondly, L-arginine suppresses the activation and adhesion of leukocytes and platelets to the surface of these cells, thereby prevent the damage of endothelial lining [9, 18]. The ability of this amino acid to reduce the negative impact of oxidative stress on the endothelium of coronary vessels has also been established [18]. In case of combined effect of emotional stress and alimentary HCE, the pathogenesis of endothelial damage includes, obviously, and another mechanisms and synthesis of NO from L-arginine is insufficient to limit the endothelial cell damage. In the clinical studies the effectiveness of L-arginine use as an additional therapy to the main treatment regimen for a number of cardiovascular and metabolic diseases (such as atherosclerosis, obesity, and diabetes) was proved [9]. Here it is shown that one of the most important pathophysiological connections between these conditions is the presence of NO. The development and progression of atherosclerosis is associated with dysfunction of endothelium and a decrease of the bioavailability of NO, due to which, obviously, the cytoprotective effect of L-arginine is neutralized under stress complicated by HCE.

An enhancement of the endothelial cells desquamation in case of cyclooxygenase blocker indomethacin administration before the start of stressor can serve as an indirect confirmation of the positive role of PG in the limitation of stress damage of cardiovascular system. In experimental and clinical studies, a significant increase in the levels of E\(_2\), F\(_{2\alpha}\), and I\(_2\) classes of PG under the influence of stressor factors of various genesis was established [6, 10, 26]. Increasing the activity of prostanoid-synthesizing systems has probably a protective nature and certain significance in limiting the damaging effect of CA on cardiovascular system in stressful situations. This protective role of these eicosanoids is determined by their three main properties: the ability to limit the activation of the adrenergic system, vasodilatory action and direct cytoprotective effect [6, 10, 31]. There is confirmation of the protective role of PG F\(_{2\alpha}\) in relation to the vascular endothelium during oxidative stress [31]. HCE limits the protective effect of PG in case of EPS. This is evidenced by the fact that when indomethacin is used in this case, the desquamation of cells is significantly increased (compared to animals that were exposed by EPS, but were on a normal diet).

Undoubtedly, the pathogenetic chain of endothe-liocyte damage includes LP processes, which is confirmed by us by the use of prostenon, a pharmacological analogue of prostaglandin E\(_2\), which has a pronounced antioxidant effect. However, it should be noted that, both under the conditions of EPS action and under the combined influence of both pathogenic factors, prostenon does not show a significant protective effect, although the number of exfoliated cells decreases. On the other hand, there are results of experimental and clinical studies that indicate the development of oxidative stress under the influence of various pathogenic factors, which contributes to endothelial damage [8, 13, 20]. This is accompanied by the intensification of free radical oxidation and the depletion of antioxidant system. An imbalance in the LP/antioxidant system leads to the damage of endothelial cells with the subsequent development of endothelial dysfunction, increase in the total number of circulating endothelial cells [13]. The criteria of the vascular endothelium damage are also: an increase the level of C-reactive protein, fibrinogen, Willebrand factor and a violation of the lipid spectrum of an atherogenic nature (an increase of low-density lipoproteins and total Ch along with a decrease of high-density lipoproteins) [7, 13]. It was also established that HCE increases the negative effect of oxidative stress on the vascular endothelium [8].

As we have established, all three studied pharmacological agents do not have a significant, statistically reliable effect on the exchange of FFA in the endocardium under the action of stress and stress complicated by HCE, although certain tendencies are observed. Thus, if L-arginine and prostenon in both experimental groups contribute to a decrease the content of FFA, then indomethacin, on the contrary, increases it.

A number of studies have shown a damaging effect of FFA on the endothelium under the influence of various pathogenic factors [22, 30]. It is established the fact of potentiation the FFA the damaging action of CA on the heart in stress reactions: an increase the myocardial oxygen demand, an increase the permeability of cell membranes, and a promotion of platelets accumulation in the microcirculatory bed of the myocardium [3, 19, 25]. In addition, and this is no less important, FFA stimulate the microsomal oxidation of adrenaline to adrenochromes, which lead to the activation of LP [3, 19].

**Conclusions**

1. The use of L-arginine and indomethacin proves that NO and PG are important in the pathogenesis of morphological and biochemical changes in endocar-dial endotheliocytes under the conditions of EPS action and under its influence against the background of HCE,
which limits the harmful effects of these pathogenic factors. The consequence of such changes is, obviously, the development of endothelial dysfunction and related disorders of various functional systems of the body. LP processes also have a cytotoxic effect under stress, because the use of prostenon (as an antioxidant) has a positive effect.

2. L-arginine, indomethacin, and prostenon do not have a significant effect on the exchange of FFA both in “pure” EPS and in case of stress complicated by HCE.

3. HCE of alimentary origin significantly limits the cytoprotective effect of L-arginine, antioxidants and PG on endocardial endothelium under the action of EPS.

Prospects for further research

Comparing the data obtained by us with the results of other experimental and clinical studies, it can be confirmed that the changes in the body that occur in response to the influence of stress factors and their effects against the background of HCE are systemic in nature and concern the endothelium of various areas of the vascular bed. In addition, the mechanisms of action of these pathogenic factors are quite complex and multifaceted. According to this, we consider that the further study of stress and HCE impact on the structural and functional state of the endothelium of vessels of various organs is advisable.

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.

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References


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