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Research Article

Some Structural and Chemical Changes in Endocardial Endothelium of Rats in Emotional and Pain Stress Complicated by Hypercholesterolemia

I.M. Luchko*, T.V. Guranych, N.M. Voronych-Semchenko, O.O. Shlyakhovenko, L.S. Storozhuk

Abstract

The objective of the research was to study the content of some neutral lipids of endocardial endothelium in rats in relation to structural changes occurring in it, in the co-existence of emotional and pain stress, as well as alimentary hypercholesterolemia.

Materials and methods. The electric-impulse model was used for stress modeling. Alimentary hypercholesterolemia was modeled feeding animals an atherogenic diet. The concentration of triacylglycerols, free and esterified cholesterol were examined using the method of thin-layer chromatography performed on silica gel. The concentration of free fatty acids was determined using the radiochemical method. The state of endocardial endothelium was studied with the help of light microscopy; the impression smears obtained from macro preparations of ventricle were analyzed.

Results. In co-existence of stress and hypercholesterolemia, significant increase in free cholesterol as well as free fatty acid concentration was noticed. This essentially exceeded the analogical indices under the action of stress only. Structural changes in the endocardium followed by desquamation of separate endotheliocytes were the result of stress reaction. In the action of both pathogenic factors, this process was intensified; layer-by-layer exfoliation of endotheliocytes was observed.

Conclusions. In acute emotional and pain stress, changes in lipid spectrum of membrane structures of endocardial endotheliocytes the main manifestation of which is the accumulation of free cholesterol in cells and increase in the levels of free fatty acids take place. The increase in the number of desquamated endothelial cells is the result of stress action as well. Alimentary hypercholesterolemia significantly increases such pathological changes.

Keywords
endothelium; endocardium; stress; hypercholesterolemia; lipids

Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine
*Corresponding author: fisiology@ifnmu.edu.ua

Problem statement and analysis of the recent research

Among various etiological factors possessing a significant damaged influence on the endothelium of the heart and vessels, stress situations of different genesis as well as increased free cholesterol levels are considered as one of the most important [2, 6, 7, 11]. These factors cause the development of endothelial dysfunction, which is the key link in the pathogenesis and clinical manifestations of most cardiovascular diseases [4, 5, 12]. Despite numerous experimental and clinical studies, some questions of the pathogenic effect of stress, and hypercholesterolemia (HCE) on morphology and function of cardiac endothelial lining, endothelial cell of the endocardium in particular, are not sufficiently studied. Damaging action of any stress factor on the heart is proven to be followed by activation of lipolysis, elevated levels of free fatty acids (FFA), degradation of membrane phospholipids with further accumulation of their isoforms, as well as intracellular accumulation of double-stranded fatty acids and acetyl coenzyme A [1, 6]. Since lipids are the basis of all biological membranes, the aforementioned disorders can obviously lead to significant morphological changes in different structures of cardiac endotheliocytes and vessels of mammals. In HCE, the excessive accumulation of cholesterol (C) in membranes of these cells is possible; as a result, the relationship between different classes of lipids is disturbed [2, 3]. It can significantly change the reaction of endotheliocytes to the action of neurohumoral factors during stress. At the same time, the literature data concerning the action of stress factors on lipid spectrum and morphological reconstruction of cardiac endothelial lining in HCE are quite limited.

The objective of the research was to study the content of some neutral lipids of endocardial endothelium in rats in relation to structural changes occurring in it, in the co-existence of emotional and pain stress (EPS), as well as alimentary hypercholesterolemia.

1. Materials and methods

The research was carried out on 22 nonlinearwhitemale rats weighting 180-240 g, that were divided into three groups: Group I – the control group, Group II– rats, that were under

the action of EPS for three hours, Group III – animals that were under the action of EPS on the background of HCE. The electric-impulse model of Desiderato O. was used for modelling EPS [8]. Alimentary HCE was modeled by daily administration of 250 mg of cholesterol in 1 ml of lard with obligatory adding of bile acids in the oral cavity using a probe. Rats were fed atherogenic diet for 60 days.

Animals were kept and manipulated in accordance with "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasburg, 1986), and "General Ethical Principles of Experiments on Animals", approved by the First National Congress of Bioethics (Kyiv, 2001). Euthanasia was carried out under nembutal anesthesia at a dose of 40 mg/kg of animal's weight. Lipids were extracted from the endocardial and myocardial tissues using a mixture of chloroform and methanol in the ratio of 2:1. The eluates were evaporated in an atmosphere of nitrogen and the method of thin-layer chromatography performed on silica gel (Woelm) was used. Free fatty acids, triacylglycerols (TAG) (Serva, Germany), cholesterol (Reanal, Hungary), cholesterol oleate (Amersham, England) were used as standards. The quantitative determination of fractions was made with the help of test-kits "Hemapol" (Czech Republic). The concentration of free fatty acids was determined applying the radiochemical method. For determination of cholesterol in blood plasma, there was used the qualitative reaction of Lieberman-Burchard which consists in adding a few drops of acetic anhydride and concentrated sulphuric acid to the chloroform solution of the sterol- the formation of a green colour after a few minutes is positive.

To study the state of endocardial endothelium, the impression smears obtained from macro preparations of the left and right ventricles were prepared. Ventricular walls of the endocardium were previously air-dried. Micro preparations were stained using a Romanovsky method. The degree of damage to endocardial endothelium was determined with the help of light microscope (E x 630), according to the number of desquamated cells per 1 mm2 of smear square, that equals to 40 fields of view. The obtained data were statistically processed with the help of mathematical programs Microsoft Excel, and Statistica 7.0.

2. Results and Discussion

The results of the research and their analysis showed, that acute three-hour EPS was followed by significant changes in the concentration of different classes of neutral lipids in endocardial endothelium (Table 1) being one of the signs of sympathoadrenal system activation, as well as the elevation of catecholamine levels in the blood [1].

Catecholamines are known to play a key role, since they have a strong lipomobilizing effect which is enhanced by glucocorticoids, adrenocorticotropic hormone and glucagon [1, 2, 10]. In addition, catecholamines increase the intensity of processes disintegrating membranes and promote lipid peroxidation. In stress, in rat endocardium, the level of non-etherified C and FFA increased by 13.9% ($p<0.01$) and 36.8%, respectively ($p<0.001$), while the level of etherified C and TAG decreased by 17.7% ($p<0.001$) and 11.2% ($p<0.05$). Such changes are obviously associated with the intensification of C and TAG ester hydrolysis under the influence of cholesterolases and triacylglycerollipases, the activity of which increases in stress reaction [1].

The results of the determination of the indices under the action of stress in animals being previously fed an atherogenic diet for 2 months were slightly different (Table 1). It is worth mentioning that in rats being fed an atherogenic diet, the level of total C in blood plasma increased by 1.8 time ($p<0.001$) as compared to the control group and the level of high-density lipoprotein C decreased by 27.4% ($p<0.001$), being a sign of elevation of plasma atherogenicity.

In the co-existence of stress and HCE, in the endocardium, there was observed a significant increase in the level of free C (by 32.4%, $p<0.001$) exceeding the analogical index during the action of EPS only by 13.9% ($p<0.001$) Thus, stress on the background of atherogenic diet considerably increases the accumulation of free C in membrane structures of endotheliocytes of rats. The increase in the levels of esterified C by 32.3% ($p<0.001$) was observed as well. Moreover, in this group of animals, a significant accumulation of FFA in the examined structure took place. In the endocardium, their concentration increased by 57.9% ($p<0.001$) while under the action of EPS only, their level increased by 36.8% ($p<0.001$). The endogenous source of FFA in this case, according to some authors, can be phospholipids, TAG and esters of C [10].

Summarizing the obtained data, we can conclude that stress on the background of alimentary HCE is very dangerous, since it is accompanied by the accumulation of free C and FFA in plasma membranes of endocardial endothelial cells which is one of the molecular mechanisms of their damage and can lead to formation of endothelial bodies (cytoplasts) and desquamation. Some other mechanisms including calcium, electrolyte-osmotic and acidic ones being activated by neurohumoral component of stress are involved in endotheliocyte damage [1, 4]. Stressful situations result in the occurrence of endogenous HCE, which together with exogenous HCE deepens the damaging effect [2, 9].

The analysis of the impression smears obtained from ventricular endocardium confirmed the increase in endotheliocyte damage through endothelial desquamation under the action of EPS on the background of HCE (Table 2).

After acute three-hour stress, the number of desquamated endotheliocytes in the impression smears obtained from the endocardium of the right ventricle and the left ventricle increased by 4.3 times ($p<0.001$), while on the background of HCE, such factor led to the increase of this index by 6.4-6.5 times ($p<0.001$) as compared to the control group of animals. Clusters of 20 and more desquamated endotheliocytes could be seen in the microscopes field of view (Fig. 1) indicating their possible layer-by-layer exfoliation.

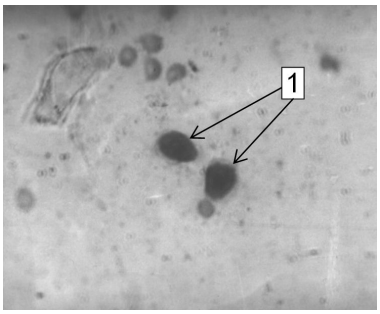
Thus, alimentary HCE considerably potentiates the dam-

Table 1. Lipid concentration in endocardial endotheliocytes of rats in emotional and pain stress and emotional and pain stress in case of hypercholesterolemia (M±m)

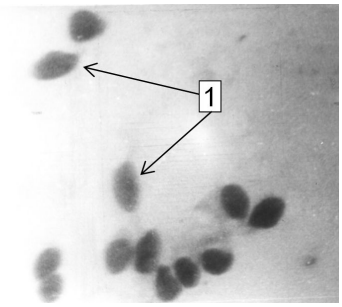
Research object	#	Experimental conditions	Indices			
			Free C, mkg/g of dry tissue	Esterified C, mkg/g of dry tissue	FFA, mkg/g of dry tissue	TAG, mkg/g of dry tissue
Endocardium	I	Control group, n=6	37.4±0.78	25.4±0.57	1.9±0.13	17.0±0.41
	II	EPS, n=8	42.6±0.89 $p_{1-2}<0.01$	20.9±0.73 $p_{1-2}<0.001$	2.6±0.08 $p_{1-2}<0.001$	15.1±0.62 $p_{1-2}<0.05$
	III	EPS on the background of HCE, n=8	49.5±1.16 $p_{1-3}<0.001$ $p_{2-3}<0.001$	33.6±0.82 $p_{1-3}<0.001$ $p_{2-3}<0.001$	3.0±0.14 $p_{1-3}<0.001$ $p_{2-3}<0.05$	16.4±0.62 $p_{1-3}>0.05$ $p_{2-3}<0.2$

Table 2. Number of endotheliocytes in impression smears (cells/mm^2) obtained from ventricular endocardium in emotional and pain stress and emotional and pain stress in case of hypercholesterolemia (M±m)

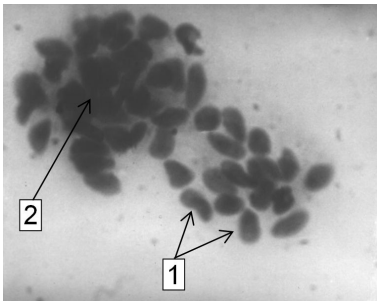
#	Groups of animals and experimental conditions	Research object	
		Endocardium of the right ventricle	Endocardium of the left ventricle
I	Control group, n=6	22±1.0	24±1.7
II	EPS, n=8	94±3.5 $p_{1-2}<0.001$	102±5.6 $p_{1-2}<0.001$
III	EPS on the background of HCE, n=8	140±5.8 $p_{1-3}<0.001$ $p_{2-3}<0.05$	156±8.2 $p_{1-3}<0.001$ $p_{2-3}<0.001$



(A) The control group;



(B) After three-hour stress;



(C) After the action of three-hour EPS on the background of HCE Mg: 630.

Figure 1. Endotheliocytes in impression smears obtained from the endocardium of the left ventricle of rats
Notes. 1 – separate endotheliocytes, 2 – layer of endotheliocytes.

aging effect of stressor on the endothelial layer of ventricular endocardium which once more confirms the danger of such factor combination.

3. Conclusions

- In acute emotional and pain stress, changes in lipid spectrum of membrane structures of endocardial endotheliocytes the main manifestation of which is the accumulation of free C in cells and increase in the levels of FFA take place.
- Along with changes in lipid composition, the effect of EPS, and, especially its combination with HCE, is accompanied by structural reconstruction of endothelial cells, the extreme manifestation of which is desquamation of the latter.

4. Prospects for further research

The obtained results, as well as the data of another experimental studied, allow us to talk about the systemic character of damage to cardiac endothelium and vessels of different caliber [3, 7, 9] in response to the action of one of the atherogenic factors – exogenous cholesterol. Therefore, in our opinion, future study of the effects of stressor and hypercholesterolemia on the structure and function of vascular endothelium of various organs will promote the discovery of new links in the pathogenesis of endothelial dysfunction in case of different diseases.

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