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FEATURES OF HIGHLY SPECIFIC FACTORS OF ENDOTHELIAL DYSFUNCTION AND INDICATORS OF THE HEMOSTATIC SYSTEM AND LIPID METABOLISM IN RATS UNDER VARIOUS EXPERIMENTAL CONDITIONS

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ОСОБЕННОСТИ ВЫСОКОСПЕЦИФИЧЕСКИХ ФАКТОРОВ ЭНДОТЕЛИАЛЬНОЙ ДИСФУНКЦИИ, ПОКАЗАТЕЛЕЙ СИСТЕМЫ ГЕМОСТАЗА И ЛИПИДНОГО ОБМЕНА У КРЫС В РАЗЛИЧНЫХ ЭКСПЕРИМЕНТАЛЬНЫХ УСЛОВИЯХ

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This study involved an investigation of lipidogram and coagulogram parameters with assessment of highly specific markers of endothelial dysfunction in 44 Wistar rats under various experimental conditions. Significant intersystem relationships were revealed between the content of circulating endothelial cells and the activated partial thromboplastin time, prothrombin time, platelet count, fractions of atherogenic lipoproteins, and atherogenic coefficient in animal models of hypercoagulation, dyslipidemia, and mild stress.

Keywords: endothelial dysfunction, lipid profile, coagulogram parameters, mild stress, experimental animals

Проведено исследование показателей липидограммы, коагулограммы с оценкой высокоспецифических маркеров эндотелиальной дисфункции у крыс линии Вистар, находящихся в различных экспериментальных состояниях. Выявлены значимые межсистемные взаимосвязи между содержанием циркулирующих эндотелиальных клеток с показателями АЧТВ, протромбинового времени, количеством тромбоцитов, фракциями атерогенных липопротеинов и коэффициентом атерогенности при моделировании у животных гиперкоагуляции, дислипидемии, а также состояния мягкого стресса.

Ключевые слова: эндотелиальная дисфункция, липидограмма, показатели коагулограммы, мягкий стресс, экспериментальные животные

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AC – Atherogenic coefficient
APTT – Activated partial thromboplastin time
CEC – Circulating endothelial cells
CMS – Chronic mild stress
CVD – Cardiovascular diseases
HDL-c – High-density lipoprotein cholesterol
INR – International normalized ratio
LDL-c – Low-density lipoprotein cholesterol
PLT – Platelet count
PT – Prothrombin time
TC – Total cholesterol
TGs – Triglycerides
VLDL-c – Very low-density lipoprotein cholesterol

Modern people experience daily effects that lead to stress on the body's adaptive systems. The neuroendocrine, cardiovascular, and circulatory systems provide the most important contributions to ensuring adequate responses to stress stimuli and normal functioning of the body's stress adaptation process [1]. These systems are the first to react to stressful effects, leading to qualitative and quantitative changes in the main reactivity parameters with the possibility of compensation and achieving homeostatic equilibrium [1, 2].

In recent years, multifaceted complex research efforts have been aimed at studying endothelial physiology and dysfunction with the goal of elucidating a universal mechanism through which the action of all risk factors for cardiovascular disease (CVD) is realized [3, 4]. Endothelial dysfunction develops when changes occur in the levels of lipoprotein fractions, C-reactive protein, hemostatic indices, and other biologically active substances [4, 5, 6]. Additionally, the study of the features of highly specific factors of endothelial dysfunction, which are a fairly significant component of preventive medicine, has been of great interest in recent years [7]. One of the most highly specific markers of endothelial dysfunction is the circulating endothelial cell (CEC) count. During the early manifestation of the pathological process, the number of CECs in the peripheral blood can become significantly higher than that in the state of physiological integrity of the endothelium [8, 9].

In view of the above, we considered it relevant to study the features of highly specific factors of endothelial dysfunction and indicators of the hemostatic system and lipid status in various experimental conditions as well as to search for multidimensional correlative intersystem relationships of the studied indicators.

Material and Methods. This comprehensive experimental study involved 44 mature male Wistar rats weighing 180 to 200 g. The animals were kept in a vivarium under natural light–dark conditions during the autumn period for 2 months.

In accordance with the goal of the experiment, the animals were divided into four groups: Group I (n=10) was the control group, Group II (n=12) comprised rats in a state of hypercoagulation achieved by daily administration of 0.6 mg of methionine for 45 days with limitation of water to 25 mL per day [10], Group III (n=10) comprised rats in a state of chronic mild stress achieved by periodic exposure to changing environmental factors («dirty cell,» tilting of cell by 30°, food deprivation, water deprivation, and «tight cell») for 45 days [11], and Group IV (n=12) comprised rats in a state of dyslipidemia achieved by administration of a diet with an increased total fat content (30 % of the weight of the usual vivarium diet) for 45 days.

At the beginning of the experiment, the animals were divided into cells according to the objectives of the study. Group II and IV rats were kept in single cells to facilitate control of the water regimen (establishment of hypercoagulation) and diet (establishment of dyslipidemia).

Biological materials (whole blood and plasma) were collected in the morning hours in accordance with ethical standards. The coagulogram was analyzed using a Coatron M1 coagulometer (TECO Medical Instruments, Lower Bavaria, Germany) with assessment of the activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR), and fibrinogen using dedicated kits (PZ Cormay S. A., Łomianki, Poland). The platelet (PLT) count was determined by the Fonio counting method. The lipid status was assessed using an Accent 200 biochemical analyzer (PZ Cormay S. A.) with determination of the total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglyceride, and very-low-density lipoprotein cholesterol (LDL-c) concentrations and calculation of the atherogenic coefficient (AC). Some of the most highly specific markers of endothelial dysfunction [12,13] were evaluated by flow cytometry (NovoCyte 3000 three-laser flow cytometer; Agilent, Santa Clara, CA, USA), by detecting clusters of CD45- and CD146+ differentiation using the same polyclonal fluorescein isothiocyanate- and phycoerythrin-labeled antibodies.

The study data were analyzed using statistical measures of variation. We used single-factor analysis of variance. Differences were considered statistically significant at $p<0.05$. Relationships were evaluated using Pearson correlation analysis. Mathematical processing was performed using SPSS Statistics Version 23.0 (IBM Corp., Armonk, NY, USA).

Results and Discussion. The body weight of the rats in Group IV increased by a mean of 43±12.7 g. The rats in Group III had wounds on their front paws by the end of the experiment and were prone to intragroup conflicts.

We analyzed the main parameters of the coagulogram in each experimental group of animals. Rats in Group II had significantly higher fibrinogen concentrations than those in Group I (4.48±0.7 vs. 3.5±0.38 g/L, respectively; $p<0.05$). Activation of the internal pathway of hemostasis as evidenced by the APTT was more pronounced in Group II (21.96±4.05 s) and Group IV (25.27±1.0 s) than in Group I ($p<0.05$ for both). The PT was significantly lower in Group II (9.78 s) than in Group I ($p=0.002$), but no significant differences were observed among the other groups. The INR was characterized by undetected variability, and the lowest values were found in Group II (Fig. 1). The PLT count did not exceed the reference

range except in Group II, consistent with the state of hypercoagulation (456.3±35.8×10^9/L).

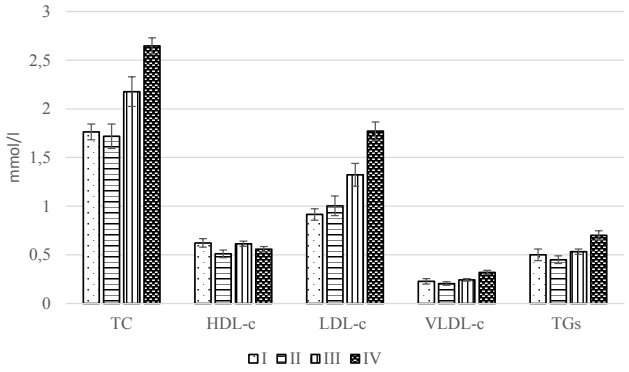

Fig. 1. Lipidogram parameters in experimental groups of animals. TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; VLDL, very-low-density lipoprotein cholesterol; TGs, triglycerides

In the analysis of the main indices of lipid metabolism, Group IV showed significantly higher concentrations of TC (2.65±0.23 mmol/L) and LDL-c (1.77±0.21 mmol/L) than Group I (1.76±0.15 and 0.91±0.12 mmol/L, respectively; p=0.001 for both). In Group III animals, the concentrations of TC (2.18±0.30 mmol/L) and LDL-c (1.32±0.23 mmol/L) were also significantly higher than those in Group I (p=0.001). Notably, the lowest concentration of anti-atherogenic HDL-c was observed in Group IV (0.55±0.06 mmol/L, p=0.06) (Fig. 2).

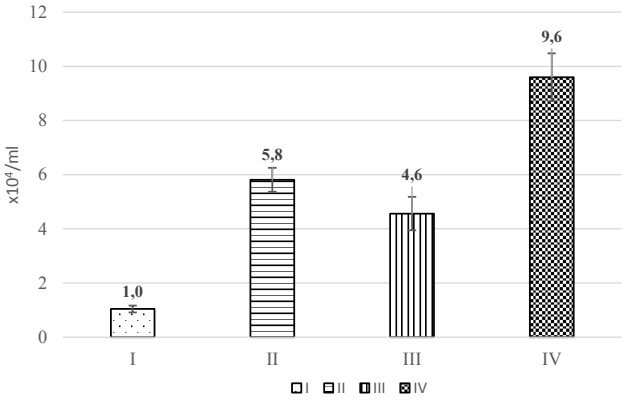

Fig. 2. Numbers of circulating endothelial cells in experimental groups of animals

The AC, which is the integral index of lipid metabolism, was significantly higher in Group III (2.54±0.24) and Group IV (3.79±0.56) than in Group I (1.85±0.18; p=0.001 for both) (Fig. 3).

The study of highly specific CECs in these experimental groups of animals allowed us to establish the reference range for the CEC count in the control group of animals (0.6–2.0×10^4/mL). A fairly wide range of the mean CEC count was noted among the experimental groups; however, regardless of the nature of the pathophysiological model used in this experiment, the CEC count was 4.5 to 9.5 times higher than that in the control group of rats (p=0.001).

The results of this experimental study provide a basis for conducting a correlation analysis to establish multidimensional intersystem relationships. We analyzed how the CEC count depends on parameters of the lipid spectrum and coagulogram in these four experimental groups of animals. The most statistically significant findings were the positive correlations of the TC concentration (r=+0.59, p=0.001), LDL-c concentration (r=+0.65, p=0.001), AC (r=+0.71, p=0.001), and PLT count (r=+0.47, p=0.001). We found negative correlations of the CEC count with the APTT (r=–0.36, p=0.016) and PT (r=–0.35, p=0.018).

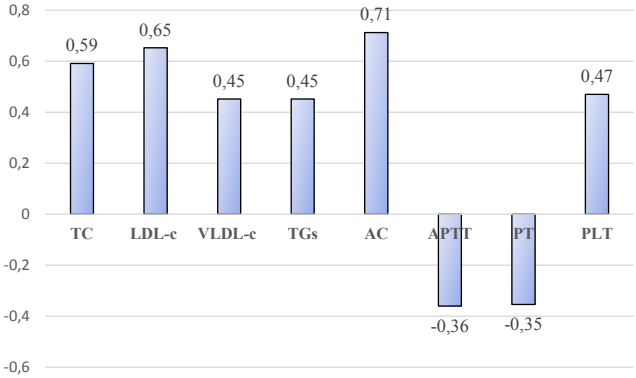

Fig. 3. Most significant correlative intersystem relationships of the circulating endothelial cell count with the studied parameters. TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; VLDL, very-low-density lipoprotein cholesterol; TGs, triglycerides; AC, atherogenic coefficient; APTT, activated partial thromboplastin time; PT, prothrombin time; PLT, platelet count

Conclusions. The results of this comprehensive experimental study reveal another new aspect of endothelial physiology and dysfunction in the search for early predictors of CVD. Undoubtedly, even small stressful events as well as the manifestation of lipid metabolism disorders and the state of hypercoagulation affect various physiological processes resulting in the formation of endothelial dysfunction, which is a leading factor in the development of CVD and age-related changes [14]. The present correlation analysis made it possible to establish significant intersystem relationships among highly specific markers of endothelial dysfunction. Activation of the blood clotting system, accompanied by a decrease in the APTT and PT and an increase in the PLT count, leads to an increase in CECs. Early metabolic changes in the lipid spectrum, characterized by an increase in the fractions of atherogenic lipoproteins and the AC, potentiate the formation of CECs. Multiparametric approaches can be used to evaluate the effectiveness of new pathogenetically and sanogenetically based methods for correcting endothelial dysfunction [15, 16].

Experimental animal procedures. the study was conducted in full compliance with the requirements of the Helsinki Declaration of the World Medical Association (1964), the «International Recommendations for Conducting Biomedical Research using Animals» (1985), the Rules of Laboratory Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 267 of 19.06.2003) and the positive conclusion of the local ethics committee.

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