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ENDOTHELIAL DYSFUNCTION PATHOGENETIC SIGNIFICANCE AND ITS MARKERS PROGNOSTIC IMPORTANCE IN SPONTANEOUS ARTERIAL HYPERTENSION

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The purpose of the study was to investigate the diagnostic importance of main endothelial dysfunction biomarkers in experimental arterial hypertension with an inflammatory component in its pathogenesis. Blood levels of von Willebrand factor, plasminogen activator inhibitor 1, endothelin 1, and soluble vascular cell adhesion molecule 1 occurred to be significantly increased in rats with spontaneous arterial hypertension. The endothelial dysfunction markers selected for this study are the substances that evidently highlight its specific pathogenetic mechanisms and consequently the mechanisms of arterial hypertension development. The data obtained highlighted endothelial dysfunction complex mutually reinforcing pathogenetic mechanisms were detected, which showed its pathogenesis complexity and multi-component nature and reflected the diverse body systems pathological dysfunction systemic nature. The authors concluded that adequate and effective therapy for arterial hypertension should consider the identified pathogenetic mechanisms and be aimed at endothelial dysfunction development prevention and progression.

Key words: endothelial dysfunction, arterial hypertension, von Willebrand factor, plasminogen activator inhibitor-1, soluble vascular cell adhesion molecule-1, endothelin-1, pathogenesis.

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ПАТОГЕНЕТИЧНА ЗНАЧУЩІСТЬ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ ТА ПРОГНОСТИЧНА ВАЖЛИВІСТЬ ЇЇ МАРКЕРІВ ПРИ СПОНТАННІЙ АРТЕРІАЛЬНІЙ ГІПЕРТЕНЗІЇ

Метою дослідження було з'ясування діагностичного значення основних маркерів ендотеліальної дисфункції при експериментальній артеріальній гіпертензії із запальним компонентом у патогенезі. У щурів зі спонтанною артеріальною гіпертензією доведено суттєве зростання в крові концентрації фактору фон Віллебранда, інгібітору активатора плазміногену-1, ендотеліну-1 та розчинної молекули адгезії клітин судин-1. Вказані маркери ендотеліальної дисфункції є субстанціями, які висвічують певні її патогенетичні механізми, отже, і механізми розвитку артеріальної гіпертензії. Отримані дані розкривають складні взаємопідсилюючі патогенетичні механізми ендотеліальної дисфункції, що показує складність, багатокомпонентність патогенетичних механізмів та відображає системність патологічної дисфункції різних систем організму. Автори висловлюють про те, що адекватна та ефективна терапія артеріальної гіпертензії повинна враховувати виявлені патогенетичні механізми та бути спрямована на запобігання розвитку та прогресії ендотеліальної дисфункції.

Ключові слова: ендотеліальна дисфункція, артеріальна гіпертензія, фактор фон Віллебранда, інгібітор активатора плазміногену-1, розчинна молекула адгезії судинних клітин-1, ендотелін-1, патогенез.

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Experimental studies and clinical observations showed the crucial importance of the vascular wall endothelium in arterial pressure formation and maintenance in a closed vascular system [2, 10]. The relevance of this area of research is explained by the progressive increase in patients with primary and secondary hypertension [13, 14], which stimulated the focus on intensive research devoted to arterial hypertension early diagnosis, effective treatment, and decreased risk of complications.

The fundamental role of endothelial cells in regulating vascular tone in response to acetylcholine without the involvement of central regulatory mechanisms was discovered in the early 1980s [7]. Endothelial cells have since acquired the functions of a cardiovascular endocrine organ that is capable of providing interaction between blood and tissue in critical situations [11].

The endotheliocytes' primary function is to preserve and regulate blood flow to tissues to provide both oxygen and nutrients. Endothelial cells regulate vasodilation in physiological conditions. They also form a semipermeable barrier between blood and peripheral tissues, demonstrating thus their antithrombotic and anti-inflammatory properties, which allows to call vascular endothelium as the vascular health gatekeeper [2].

Endothelium was shown not to be a passive barrier between blood and tissues but is an active organ whose dysfunction is an essential component of numerous cardiovascular diseases pathogenesis, including

atherosclerosis, arterial hypertension, ischemic stroke, coronary heart disease, chronic heart failure, metabolic syndrome, etc. [11, 15].

Hypertension remains the key origin of cardiovascular complications leading to increased mortality. Their pathogenesis imperfections might probably explain the fact the cardiac-vascular diseases steadily increased mortality.

An important role of endothelial dysfunction (ED) in arterial hypertension development was shown [8]. Endothelial damage was registered in oxidative stress and inflammation conditions, transforming its function from vasoprotective to vasoconstrictor, prothrombotic and proapoptotic [9].

Renin-angiotensin-aldosterone system chronic hyperactivation is considered to be one of the leading factors in endothelial dysfunction manifestation [6, 8]. Endothelium's massive importance for cardiovascular disease development resulted from the very fact of angiotensin-converting enzyme main pool localization directly on the membrane of endothelial cells [6]. In the renin-angiotensin-aldosterone system, 90 % of activity is directed to organs and tissues, among which the vascular endothelium ranks first. Therefore, renin-angiotensin-aldosterone system hyperactivation is an indispensable attribute of endothelial dysfunction [8].

Important to understand that arterial hypertension development and subsequent life-threatening cardiovascular complications are extremely dangerous endothelial dysfunction consequences. We made efforts to perform experimental trials devoted to investigating the pathogenetic importance of inflammation in endothelial dysfunction. We consider it vitally important to elucidate the main markers of arterial hypertension in early diagnostics, considering the confirmed inflammatory element in this pathological pathogenesis.

The purpose of the study was to investigate the diagnostic importance of main endothelial dysfunction biomarkers in experimental arterial hypertension with an inflammatory component in its pathogenesis.

Materials and methods. Experimental trials were performed on 32 white Wistar line rats weighing 180–200 g of both sexes. The special line of animals with spontaneous arterial hypertension (SHR) was received from the vivarium of Kharkiv National Medical University. These rats are characterized by a persistent increase in blood pressure developed from the age of 5–6 months.

The animals were kept in standard vivarium conditions with a constant temperature (23–25 C) and sufficient natural lighting. They had free access to a standard diet and water. Experimental animals keeping, handling and manipulation was carried out in accordance with the “International Code of Medical Ethics” (Venice, 1983), the “General Ethical Principles of Animal Experiments” adopted by the “General Ethical Principles of Animal Experiments” adopted by the Fifth National Congress on Bioethics (Kyiv, 2013) and was guided by the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1986), Directive 2010/63/EU of the European Parliament and Council on protecting animals used for scientific purposes and guidelines of the of Ukraine “On protection of animals from cruel treatment” No. 440-IX of 14 January 2020.

Experimental animals were randomized as follows. Group 1 – intact rats with normal blood pressure (n=8); group 2 – rats with spontaneous arterial hypertension (SHR) which developed a persistent increase in blood pressure from the age of 5–6 months (n=24).

The ED presence in rats was checked according to the following indexes: von Willebrand factor (vWF) and plasminogen activator inhibitor 1 (PAI-1) content as hemostatic factors, soluble vascular cell adhesion molecule 1 (sVCAM-1) level as an endothelial inflammation marker and endothelin 1 concentration as an index of vasoconstriction.

The abovementioned compound concentration was determined in blood plasma received after the rat's euthanasia by sodium thiopental overdose (i.p., 50 mg/kg) three days after experimental trials started. The vWF blood level was determined using the “Willebrand-test” reagent kit (“Biomedica Group”, Austria) in citrated plasma. The method is based on its ability to exclude platelet agglutinin in the presence of the antibiotic rystomycin. The ability for such agglutination is preserved in platelets after their fixation with formaldehyde when the reaction to other aggregation inducers is completely lost.

The endothelin-1 concentration in blood was determined using the “Endothelin 1” (“Biomedica Group”, Austria) immunoenzyme kits aimed for endothelin-1 quantitative determination.

ELISA kits (“Biomedica Group”, Austria) were used to detect both PAI-1 and sVCAM-1 blood serum levels.

The data obtained were presented as mean (M) and the standard error of the mean (m) and were calculated statistically using the nonparametric Mann-Whitney U-test. The minimum statistical probability was determined at $p<0.05$.

Results of the study and their discussion. Spontaneous hypertension manifestation was characterized by a significant change in humoral substance content in rats' blood. These changes significantly differed from the course of this pathological condition with elevated blood pressure in normal rats.

The vWF concentration in rats with SHR was revealed to be 55 % higher pertaining to the same index in intact experimental animals ($p<0.05$; fig. 1 A).

The level of PAI-1 in SHR rats was equal to 17.9 pg/ml, which occurred to be in 1.7 times higher than the same index in control observations (10.4 pg/ml; $p<0.05$; fig. 1 B).

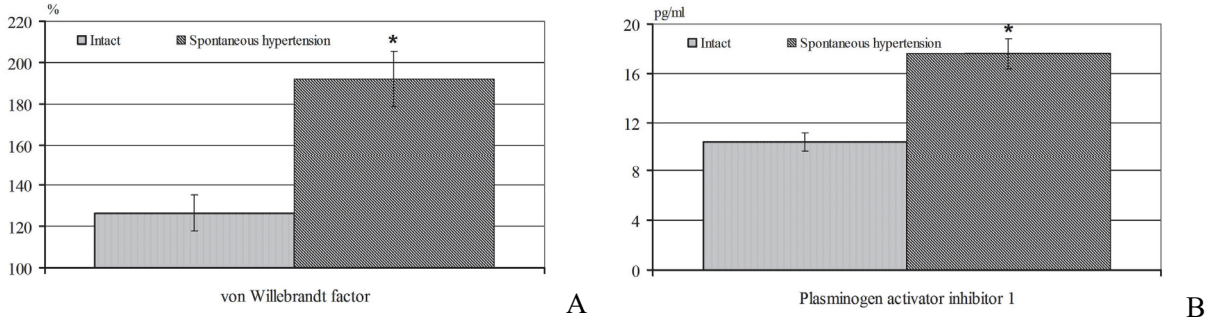

Fig 1. The level of plasminogen activator inhibitor 1 and von Willebrand factor in rats with spontaneous arterial hypertension. Note: * – $p<0.05$ – significant differences between the studied indexes compared to analogous control indices.

The data we received while investigating the concentrations of both endothelial inflammation (sVCAM-1) and vasoconstriction (endothelin-1) markers are shown in Fig. 2.

The level of endothelin-1 in SHR rats was equal to 12.4 pg/ml, which was 2.9 times higher pertaining to the same index in rats of the control group identical to 4.25 pg/ml ($p<0.01$; Fig. 2 A). The sVCAM Concentration in SHR rats was identical to 1215.6 ng/ml, which occurred to be 1.5 times greater compared with the analogous control index equal to 804.7 ng/ml ($p<0.05$; Fig. 2 B).

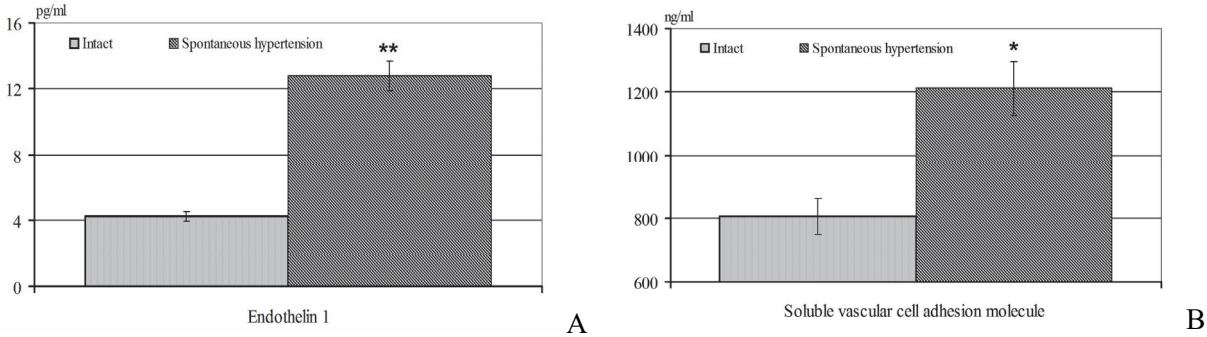

Fig. 2. The concentration of soluble vascular cell adhesion molecule and endothelin-1 in rats with spontaneous arterial hypertension. Note: * – $p<0.05$ and ** – $p<0.01$ – significant differences of the studied indexes compared to analogous control indices.

Thus, we will allow ourselves to interpret the data obtained in the following way. Firstly, we believe that arterial hypertension diagnostics or early diagnostics and any other pathological condition should have a pathophysiological background.

Secondly, we want to explain that the animals we used with spontaneous arterial hypertension are an adequate model of essential hypertension recognized as relevant for the experimental investigation of increased blood pressure features and methods of its correction.

In this aspect – the third block of data obtained discussion – we consider it important the data indicated the expressed changes in the content of the studied substance in the blood of rats with spontaneous arterial hypertension. A significant increase of vWF and PAI-1 levels and endothelin-1 and sVCAM-1 were detected.

Let us note that the ED markers selected for this study are the substances that evidently highlight its certain pathogenetic mechanisms and, consequently, the mechanisms of arterial hypertension development. Thus, significantly increasing blood plasma vWF and PAI-1 concentrations indicates blood viscosity enhancement in these pathological conditions. An endothelin-1 level increase shows the inflammatory process involvement, and sVCAM-1 hyperconcentration outlines vasoconstrictor effects in conditions ED. As a result, we reveal ED complex mutually reinforcing pathogenetic mechanisms which showed, from one side, its pathogenesis complexity and multicomponent nature, and, from the other side,

reflected the diverse body systems pathological dysfunction systemic nature in these pathological conditions.

These data are, in a certain way, related to the results of studies confirming endothelin-1 expression activation in systemic circulation vessels and the heart together with arterial pressure increase [11]. We consider the following sequence of pathological events in this case: arterial pressure increase → endothelial cells desquamation and/or damage → increased endothelin-1 expression which initially has a cushioning and adaptive importance according to the hemodynamic laws because the vascular tension decreases with its wall thickening [6]. However, as the damage increases, these changes can further stimulate endothelin-1 expression increase due to the following pathological effects – vasoconstriction → increased arterial pressure → vascular damage progression → progression of atherosclerosis.

In this aspect, blocking both the endothelin system and endothelin-1 binding receptors activities may provide a new therapeutic approach beyond blood pressure decrease in hypertension, thus preventing the progression of vascular damage and improving clinical prognosis. This was shown in experimental trials but remains to be proved in clinical conditions in patients with cardiovascular diseases [11].

The vWF plays an important role in blood coagulative and anticoagulative systems' dynamic activity regulation, and its hyper concentration in our experimental conditions indicates this functional system breakdown towards hypercoagulation. VWF expression is regulated at transcriptional and (post)translational levels, and its release into the bloodstream involves platelets after endothelial desquamation [5]. The vWF expression regulation is also determined by organisms' inflammatory and immune reaction severity, which has distinctive clinical manifestations in cardiovascular pathology [5, 11].

Direct dependence and frequent clinical registration of inflammation and clot formation combination are known. PAI-1 is an antifibrinolytic protein that regulates fibrin clot formation [4]. Although PAI-1 is associated with vascular risk factors, its function as a biomarker of adverse cardiovascular events prediction has yielded controversial results due to the heterogeneity of the patient populations studied and the methodologies used [4]. Our data suggest both vWF and PAI-1 pathogenetic importance in hypercoagulability development in SHR rats.

The sVCAM-1 role in ED pathogenesis in arterial hypertension is quite interesting. The decisive role of tumor necrosis factor-alpha was shown, which hyperactivation in the acute or chronic inflammatory process and conditions of immune dysfunction ensures sVCAM-1 release from leukocytes at the initial manifestations of endothelial cell destruction [1]. Vasoconstriction is one of its effects, and the increase in expression determines the clinical prognosis of arterial hypertension. Of interest are the clinical results which evidently showed sVCAM-1 level decrease in the blood of patients with arterial hypertension and chronic inflammatory diseases, using rheumatoid arthritis as an example, after treatment with tumor necrosis factor-alpha receptor blockers [1, 3]. Consequently, targeted anti-cytokine anti-inflammatory therapy helps to reduce the sVCAM-1 level in the blood of patients with arterial hypertension, thereby eliminating the ED concomitant alternative factor.

It should be noted in conclusion that the above-mentioned components of ED pathogenetic mechanisms in arterial hypertension indicate a severe systemic pathology and organ dysfunction in these pathological conditions, consisting at least in an inflammatory reaction presence, hypercoagulation development and imbalance in blood viscosity system regulation as well as the regulatory vasoconstrictor effects enhancement. It's logical to assume that even one of the above-listed alterative factors contributes to endothelial destruction, subsequently intensifying the clinical manifestation of arterial hypertension.

The clinical nature of arterial hypertension, endothelial functioning, and arterial stiffness, which are the ED-related clinical and laboratory characteristics [14, 15], are significantly improved with ED markers circulating in blood effective correction [12].

We are confident that arterial hypertension adequate and effective therapy should take into account the identified pathogenetic mechanisms and be aimed, among other things, at endothelial dysfunction development prevention and/or progression.

Conclusions

1. Blood levels of vWF, PAI-1, endothelin-1, and sVCAM occurred to be significantly increased in rats with spontaneous arterial hypertension.

2. The ED markers selected for this study are the substances that evidently highlight its certain pathogenetic mechanisms and, consequently, the mechanisms of arterial hypertension development. Blood

plasma vWF and PAI-1 concentrations significant increase indicate blood viscosity enhancement in these pathological conditions. An endothelin-1 level increase shows the inflammatory process involvement, and sVCAM-1 hyperconcentration outlines vasoconstrictor effects in conditions ED.

3. The ED complex mutually reinforcing pathogenetic mechanisms were detected which showed its pathogenesis complexity and multicomponent nature and reflected the diverse body systems pathological dysfunction systemic nature.

4. Even one of the detected alterative factors contributes to endothelial destruction, which subsequently intensifies the clinical manifestation of arterial hypertension.

5. Arterial hypertension adequate and effective therapy should consider the identified pathogenetic mechanisms and be aimed at endothelial dysfunction development prevention and/or progression.

Prospects for further research include investigation of possible markers of endothelial dysfunction use in this pathological condition early diagnosis in patients with arterial hypertension.

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