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PATHOGENETIC FEATURES OF ENDOTHELIAL DYSFUNCTION IN EXPERIMENTAL TYPE 2 DIABETES MELLITUS

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Introduction. Diabetes mellitus (DM) is one of the most acute medical and social problems in modern society. The leading link in the pathogenesis of diabetes is a persistent increase in blood glucose and insulin resistance of target cells, which in turn leads to complications. Endothelial cells are the first to be affected by high blood glucose concentrations. Hyperglycaemia activates protein kinase C, leads to the accumulation of glycosylated proteins, an increase in the content of oxidised plasma lipoproteins, which causes disruption of vascular energy metabolism and endothelial cell damage

Materials and Methods. The experimental study was carried out on rats of both sexes of the Wistar line, weighing 180–200 g, aged 3 months. During the experiment, the animals were divided into two groups – 18 rats each: Group I – intact animals; Group II – animals with modelled diabetes mellitus.

Results. It has been established that diabetes mellitus increases vasoconstrictor mediators such as endothelin-1 against the background of a decrease in the activity of endothelial NO synthase, which indicates a violation of the physiological synthesis of nitric oxide, and accordingly leads to the development of endothelial dysfunction in the modelled pathological condition.

Conclusions. In type 2 diabetes mellitus, a whole cascade of pathological reactions in the vascular endothelium unfolds. As a result, persistent endothelial dysfunction develops, which leads to the fact that the vascular endothelium itself is involved in the pathogenesis of type 2 diabetes and causes a number of other complications

Keywords: endothelial dysfunction, diabetes mellitus, endothelin-1 nitric oxide, NO synthases.

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ПАТОГЕНЕТИЧНІ ОСОБЛИВОСТІ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ ПРИ ЕКСПЕРИМЕНТАЛЬОМУ ЦУКРОВОГО ДІАБЕТУ 2-ГО ТИПУ

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В експериментальному дослідженні на статевозрілих щурах із змодельованим цукровим діабетом 2-го типу було показано роль медіаторів пошкодження в розвитку ендотеліальної дисфункції. Встановлено, що при цукровому діабеті спостерігається підвищення вазоконстрикторних медіаторів, таких як ендотелін-1 на тлі зменшення активності ендотеліальної NO-синтази, що свідчить про порушення фізіологічного синтезу оксиду азоту, і відповідно призводить до розвитку дисфункції ендотелію на тлі змодельованого патологічного стану. Усі ці складові можуть призводити до прогресування мікроангіопатичних ускладнень.

Ключові слова: ендотеліальна дисфункція, цукровий діабет, оксид азоту ендотелін-1, NO-синтази.

Introduction. It is well known that diabetes mellitus (DM) is one of the most pressing medical and social challenges of modern society. The number of people with diabetes is continuously increasing due to the rapid pace of urbanization, the growing prevalence of obesity, and sedentary lifestyles [1, 3]. A key pathogenic mechanism of diabetes is the persistent elevation of blood glucose levels and target cell resistance to insulin, which in turn leads to complications such as retinopathy, neuropathy, nephropathy, and cardiovascular diseases [4, 5].

It has been demonstrated that endothelial dysfunction and impaired arterial elasticity are closely linked to the onset and progression of diabetic complications [6, 7]. Endothelial cells are the first to be affected by elevated blood glucose concentrations. Under hyperglycemic conditions, protein kinase C is activated, leading to the accumulation of glycosylated proteins and an increase in oxidized plasma lipoproteins. These processes disrupt vascular cell energy metabolism and cause endothelial damage [8]. Intracellular oxidative stress plays a key role in the pathogenesis of endothelial dysfunction by promoting the synthesis of vasoconstrictive, pro-inflammatory, and growth mediators, as well as coagulation factors, while simultaneously reducing nitric oxide production and fibrinolytic system components. Ultimately, these changes contribute to the development of vascular complications in diabetes, including both microangiopathies and macroangiopathies [9, 10].

The formation of diabetic microangiopathies and macroangiopathies is a major determinant of patient progno-

sis and life expectancy. It has been suggested that "diabetes begins as a metabolic disorder and ends as a vascular pathology" [11]. No other immune or metabolic disease causes such extensive damage to the entire vascular system [12]. Structural vascular changes occur, including thickening of the basement membrane, alterations in its selective permeability, and narrowing of the vascular lumen due to atherosclerotic changes in the vessel walls. These processes underlie the development of diabetic angiopathies and their associated pathologies [13].

Despite significant advancements in diabetes treatment, this pathology remains a focus of research worldwide due to the insufficient understanding of the mechanisms underlying its complications [14].

Objective. The aim of the present study is to investigate changes in markers of endothelial functional state in experimental diabetes mellitus.

Materials and Methods. The experimental study was conducted on Wistar rats of both sexes, weighing 180–200 g and aged 3 months, which were maintained on a standard vivarium diet.

During the experiment, the animals were divided into two groups of 18 rats each:

– Group I – intact animals;
– Group II – animals with induced diabetes mellitus.

To model type 2 diabetes mellitus (T2DM), the rats received a single intravenous injection of streptozotocin at a dose of 65 mg/kg, preceded by an intraperitoneal administration of nicotinamide at a dose of 230 mg/kg, 15 minutes prior. This model allowed for the development of moderate and stable hyperglycemia while preserving approximately 40% of pancreatic insulin reserves. The animals were euthanized on day 30 by decapitation under light ether anesthesia.

Animal housing, handling, and procedures were carried out in accordance with the "General Ethical Principles for Animal Experiments," adopted by the V National Congress on Bioethics (Kyiv, 2013). Additionally, the study adhered to the recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985) and the methodological guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine, "Preclinical Drug Research" (2001).

To assess endothelial functional status in blood plasma, the following parameters were measured: endothelin-1 concentration – determined using an enzyme-linked immunosorbent assay (ELISA) with "Biomedica gruppe" kits (Austria); von Willebrand factor activity – assessed by a photometric method using ristocetin cofactor time with Innovance VWF Ac reagents (Siemens Healthineers, Germany); endothelial and inducible nitric oxide synthase (eNOS and iNOS) levels – evaluated by a spectrophotometric method based on nitrite accumulation in a reaction mixture containing 50 mM potassium dihydrogen phosphate (pH 7), 1 mM magnesium chloride, 1 mM NADPH, and 2 mM calcium chloride, incubated for 15 minutes at 37°C.

Statistical analysis of the obtained data was performed using Statistica 8.0 software. The minimum level of statistical significance was set at $p < 0.05$.

Results and Discussion. It is well established that the concept of endothelial dysfunction as a disorder of NO-dependent regulation is based on the critical role of nitric oxide (NO) in modulating nearly all endothelial functions. The NO synthesis system is highly sensitive to various types of damage. Nitric oxide regulates the activity and sequential activation of all biologically active substances produced by endothelial cells. In addition to its vasodilatory effect, NO inhibits smooth muscle cell proliferation, prevents blood cell adhesion, and exhibits anti-aggregant properties [15, 16]. Given that the physiological levels of NO required for normal vascular function are maintained through the synthesis of NO from L-arginine by endothelial NO synthase (eNOS), it was reasonable to investigate changes in the activity of both endothelial and inducible NO synthase (iNOS) (Figure 1).

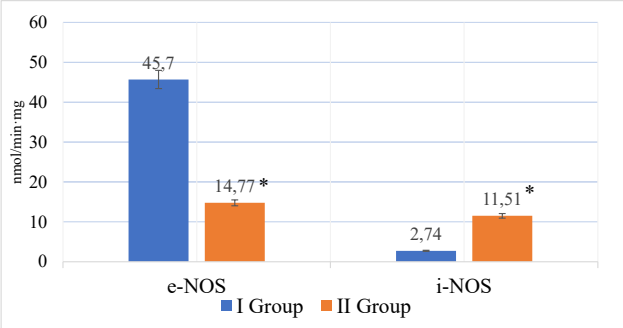

Fig. 1. NO synthase activity in experimental diabetes mellitus in rats

*Note: * – $p < 0.05$ compared to the intact animal group.*

During our study, a significant threefold decrease in eNOS levels was observed in rats with experimental diabetes mellitus compared to the intact animal group. Notably, a compensatory fourfold increase in iNOS content was detected in Group II animals compared to intact controls, rising from 2.74±0.09 to 11.51±0.67 nmol/min·mg, respectively.

The increase in iNOS levels in diabetes may indicate an enhanced NO synthesis, which, in turn, interacts with superoxide radicals, leading to endothelial cell dysfunction, inhibition of lymphocyte proliferation, and suppression of various enzymes [17]. These changes may also be influenced by biologically active substances such as interferon, interleukin-1, and other cytokines, further highlighting significant endothelial alterations in diabetes mellitus [18]. The aforementioned findings suggest an impairment of physiological nitric oxide synthesis, contributing to endothelial dysfunction in the context of an experimentally induced pathological state. These factors may ultimately lead to the progression of microangiopathic complications.

Our results confirm that disruptions in NO synthesis represent a critical pathogenetic link in diabetes development and serve as a key driver of endothelial dysfunction [15].

To gain deeper insights into endothelial functional status and its disturbances in diabetes, we selected endothelin-1 as a study target. Endothelin-1 is a classical representative of endothelins with strong vasoconstrictive properties [7, 19].

In laboratory rats with experimental type 2 diabetes mellitus, endothelin-1 levels were found to be elevated twofold (statistically significant differences at $p < 0.05$) (Figure 2).

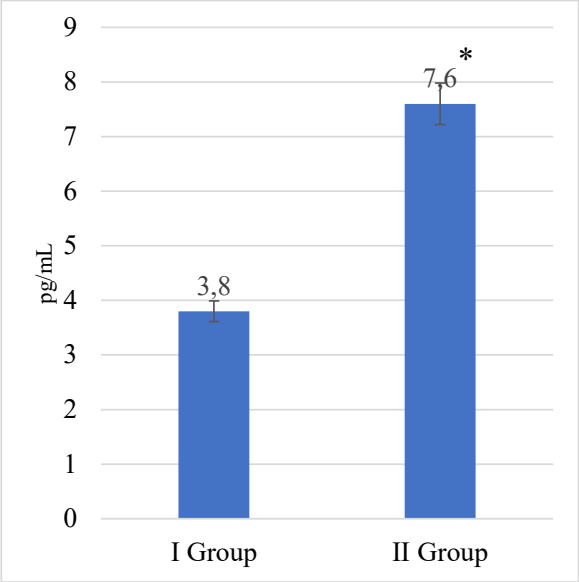

Fig. 2. Changes in endothelin-1 levels in rats with diabetes mellitus

*Note: * – p<0.05 compared to the intact animal group.*

Changes in endothelin-1 concentration during the progression of diabetes mellitus indicate an imbalance in endothelial factor secretion, leading to enhanced vasoconstriction. This may result in ischemia, further endothelial damage, and serve as a pathogenic mechanism in the development of endothelial dysfunction, contributing to both vascular impairment and insulin resistance [5, 20].

It is important to note that endothelin-1 plays a significant role not only in the pathogenesis of atherosclerosis, ischemic brain damage, and pulmonary hypertension but also in the development of diabetes mellitus and its complications.

An increase in plasma von Willebrand factor (vWF) levels was also observed in experimental animals, rising from 135.02±24.7% to 162.03±21.14% compared to intact animals. We hypothesize that the increased release of vWF may be due to continuous exposure to platelet agonists, which, in turn, leads to a depletion of granules or disruption of their release. Consequently, this enhances hemostatic system activity and intercellular interaction processes.

Our findings confirm literature data suggesting that endothelial dysfunction is a key component in the development of both micro- and macroangiopathies [10]. This is primarily associated with the sensitivity of endothelial cells to oxidative stress, hyperglycemia, and hyperlipidemia. These pathological factors contribute to endothelial cell damage, dysregulation of bioactive substance balance, and disturbances in intercellular interactions, vascular function, and hemostasis [14, 18].

Conclusions. Type 2 diabetes mellitus triggers a cascade of pathological reactions within the vascular endothelium, which is primarily affected by hyperglycemia, excessive exposure to hypertensive and inflammatory stimuli, thrombotic activators, increased leukocyte adhesion, and disruption of endothelial intercellular junctions. As a result, persistent endothelial dysfunction develops, leading to the direct involvement of the vascular endothelium in the pathogenesis of type 2 diabetes mellitus and the onset of various complications [15, 17].

Thus, endothelial dysfunction is an integral aspect of insulin resistance syndrome, contributing to increased vascular reactivity, the development of arterial hypertension, and the subsequent emergence of micro- and macrovascular complications [3].

Prospects for Further Research. Future research should focus on expanding our understanding of the pathogenesis of diabetic nephropathy and identifying the most diagnostically valuable biochemical markers of the pathological process. Based on the obtained results, further efforts will be directed toward developing novel pathogenetic treatment strategies aimed at halting disease progression and refining conservative treatment methods.

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