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STUDY OF ENDOTHELIAL DYSFUNCTION AND ASYMMETRIC DIMETHYLARGININE LEVELS

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Abstract

The aim of the study was to analyze changes in the level of endothelin-1 and asymmetric dimethylarginine in the development of endothelial dysfunction in experimental diabetic retinopathy and various methods of its correction.

The study was performed on white Wistar rats weighing 180-200 g. According to the tasks of the animal were divided into 7 groups:

As a result of our study proved a violation of the structural and functional state of the endothelium in experimental diabetic retinopathy, as evidenced by elevated levels of ADMA

and endothelin-1 in 2nd group (p <0.001), most pronounced in the 3rd stage. It was confirmed that the correction of the studied complication of diabetes mellitus only with a hypoglycemic drug, even with long-term administration, does not correct the development of endothelial dysfunction (p <0.001).

It was found that the addition of aflibercept and a solution of L-arginine in the correction to hypoglycemic drugs significantly (p <0.001) improves the condition of the endothelium, but does not solve the problem completely. It is observed that the correction of the simulated pathological condition by reducing hyperglycemia, administration of aflibercept and bromfenac (group № 5) has a positive effect on the normalization of endothelial function markers (p <0.001), but the effect is less pronounced than in the following groups. It was found that in rats in which diabetic retinopathy was simulated with subsequent correction of hyperglycemia, administration of aflibercept, L-carnitine and bromfenac (group № 6), the reduction of pathologically elevated levels of markers of endothelial dysfunction is more pronounced compared to the 3rd group, which indicates the feasibility of this method of correction. It was found that the most effective method of correction was in the 7th group of the experiment in which hyperglycemia was corrected, aflibercept, L-arginine and citicoline were obtained to normalize the levels of endothelial dysfunction markers - endothelin 1 and asymmetric dimethylarginine.

Key words: experimental diabetic retinopathy; endothelial dysfunction; endothelin-1; Asymmetric dimethylarginine; correction; metformin; aflibercept; L-arginine; citicoline; L-carnitine; bromfenac.

Introduction

Diabetes mellitus (DM) remains a threatening problem in the 21st century, and despite the availability of a wide range of modern treatments for type 1 and type 2 diabetes is steadily progressing [1]. Among patients with type 2 diabetes at the time of diagnosis, about 50% of patients already have signs of vascular pathology [2]. Due to the high risk of vascular complications, type 2 diabetes is classified by the American Heart Association as Cardiovascular Disease (CVD) [3]. In various diseases in which the cardiovascular system is damaged, the main pathogenetic links are vascular lesions - micro and macroangiopathy, which is mainly due to vascular endothelial disorders [4]. Macro- and microvascular complications, which significantly complicate the prognosis of such patients, due to the development and progression of endothelial dysfunction [5]. The vast majority of antidiabetic drugs do not have endothelioprotective effect. nitrogen [5]. Diabetes mellitus is characterized

by impaired production of nitric oxide. Decreased levels of free nitric oxide lead to insufficient capillary dilatation, vasospasm and the predominance of the effect of vasoconstrictor factors [6, 7]. The main factor that determines the biological effect of NO is its local concentration. Nitric oxide, which is produced in small / physiological quantities, provides regulation of important body functions [8]. At high concentrations of NO due to long-term excess production begin to dominate its indirect effects, which cause cytotoxic effects [8].

The purpose of the study: analysis of changes in the level of endothelin-1 and asymmetric dimethylarginine in the development of endothelial dysfunction in experimental diabetic retinopathy and various methods of its correction.

Materials and methods. The study was performed on white Wistar rats weighing 180-200 g. According to the tasks, the animals were divided into 7 groups:

Group 1 - 60 intact animals;

Group 2 - 60 animals in which diabetic retinopathy was simulated without further correction.

Group 3 - 60 animals in which diabetic retinopathy was simulated with subsequent correction of hyperglycemia.

Group 4 - 60 animals, which simulated diabetic retinopathy with subsequent correction of hyperglycemia, administration of aflibercept and L-arginine solution.

Group 5 - 60 animals in which diabetic retinopathy was simulated with subsequent correction of hyperglycemia, administration of aflibercept and bromfenac.

Group 6 - 60 animals in which diabetic retinopathy was simulated with subsequent correction of hyperglycemia, administration of aflibercept, L-carnitine and bromfenac.

Group 7 - 60 animals, which simulated diabetic retinopathy with subsequent correction of hyperglycemia, administration of aflibercept, a solution of L-arginine and citicoline.

Type 2 diabetes mellitus and diabetic retinopathy were modeled by intraperitoneal administration of streptozotocin (Sigma, USA) dissolved in 0.1 M citrate buffer with a pH of 4.5 [9]. The dose of streptozotocin 55 mg / kg body weight was divided into two injections. The introduction of streptozotocin was preceded by a high-fat diet for 28 days.

Doses of drugs:

Hypoglycemic drug - metformin (Merck Sante, manufactured in France) - at a dose of 300 mg / kg body weight in drinking form [10] in 0.9% sodium chloride solution through a syringe with an intragastric tube daily.

Administration of L-arginine solution, which is a donor of NO, (SIMESTA, made in China, USP32 quality standard) was carried out by intragastric administration of L-arginine solution in 0.9% sodium chloride solution at a dose of 500 mg / kg [11] through a syringe with intragastric tube. The volume of the solution depended on the weight of the animal and did not exceed 1 ml. The drug was administered once a day before morning feeding, daily for 10 days [12].

Aflibercept (anti-VEGF therapy) was administered in the form of subconjunctival injections at a dose of 0.08 ml (25 mg / ml) [12].

Bromfenac - instillation of 0.09% solution of eye drops once a day.

L-carnitine (Sigma, USA) was administered in the form of an aqueous solution through a syringe with an intragastric tube at a dose of 25 mg / 100 g of animal weight [13, 14].

Citicoline - 81.8 mg / kg (0.33 ml / kg) was administered intramuscularly once a day.

Withdrawal of animals from the experiment was carried out in three stages:

1st stage of the study - the 30th day after the start of modeling diabetes mellitus;

2nd stage of the study - the 60th day after the start of modeling diabetes;

Stage 3 of the study - the 180th day after the start of modeling diabetes.

Animals were removed from the experiment by decapitation under light ether anesthesia in accordance with the "Rules for performing work using experimental animals", approved by the Order of the Ministry of Health of Ukraine № 249 from 01.03.2012 and the Law of Ukraine № 3447-IV "On protection of animals from cruel treatment" (as amended from 15.12.2009 and from 16.10.2012).

Blood was taken from the retroorbital venous plexus, which lies in orbit behind the eyeball. The puncture was performed in a circular motion with a glass pipette with an extended capillary, the tip of which is ground at an angle of 45°. A conjunctival sac was punctured in the medial corner of the eye between the eyeball and the orbit. After puncture, the pipette was inserted to a depth of 2-4 mm behind the eyeball. Control of entry into the venous plexus - filling the pipette capillary with blood (Dyakonov A.V., Khrikina I.S., Hegai A.A., etc., 2013).

Determination of endothelin-1 and asymmetric dimethylarginine content was performed by enzyme-linked immunosorbent assay in blood serum.

Statistical processing of the obtained results

To detect changes in the studied parameters (activity of endothelial and inducible NO synthases) between different groups and at different stages, we used parametric statistical

methods, which are based on the operation with the parameters of statistical distribution (mean and variance).

The methods used are designed for normally distributed data, so we performed a check of all data for normality using the criterion of asymmetry and excess E.I. Pustylnyk. According to this criterion, the distribution does not differ from normal if the calculated empirical values of asymmetry and excess do not exceed critical, ie $A_{emp} < A_{cr}$, $E_{emp} < E_{cr}$, where A_{emp} and E_{emp} are calculated values of asymmetry and excess, and

$$A_{cr} = 3 \cdot \sqrt{\frac{6 \cdot (n-1)}{(n+1) \cdot (n+3)}}, \quad E_{cr} = 5 \cdot \sqrt{\frac{24 \cdot n \cdot (n-2) \cdot (n-3)}{(n+2)^2 \cdot (n+3) \cdot (n+5)}},$$

respectively, their critical values [15].

All the data we consider were normally distributed, so you can compare the average values of the samples in pairs. Note that in subsequent comparisons, we perform comparisons in independent samples. These will be comparisons between different groups of animals or comparisons between the same group of animals (but since there is no correspondence between animals in the samples, they will also be independent).

Before comparing the averages of the two samples, it should be ascertained whether the variances are homogeneous. For this purpose it is necessary to carry out check for homosketicity (homogeneity of dispersions).

Statistical hypotheses will be as follows:

H_0: the variance in group 1 does not differ from the variance in group 2.

H_1: the variance in group 1 is greater than the variance in group 2. The

hypotheses in the criterion are directed, so the criterion is one-sided. Hypothesis H_0 is rejected when $F_{emp} > F_{cr}$. This is evidenced by the p -value - the probability of error to reject the null hypothesis when it is correct. In various experiments, take $H_0$when p -value (set significance level), and reject $H_0$when p -value $<$. In all subsequent calculations, we chose a standard level of significance $= 0.05$.

The comparison of the averages is performed using t Student's-test. When comparing the average directional hypotheses will be as follows:

H_0: the average of group 1 does not differ from the average of group 2.

H_1: the average of group 1 is greater than the average of group 2.

To decide the absolute value of the calculated $t\ is$ compared with one-sided critical. If $|t_{emp}| < t_{cr}$, the null hypothesis can not be rejected. Here it is similarly possible to draw a conclusion and on $p -$ value.

All tests will be performed in the statistical package PASW Statistics 18. We will use the t-test procedure for independent samples, which immediately compares variances and means.

In subsequent tests, we will note whether the average values differ. If they are different, you need to specify this difference. The results of the t-test give an answer about the equality or difference of the mean values, but they do not allow to accurately measure the difference between the mean values. Note that this difference is quite conditional. We will calculate this difference as a percentage, ie the percentage difference between the average values of the 1st and 2nd group will be equal to $\left(\frac{-20.60_4}{104.7_9}\right) \cdot 100\% = 19.66_{\%}$.

Thus, we demonstrated a comparison of the mean values between different groups of animals.

The results of the study and their discussion:

The results of the study of endothelin-1 in the blood of experimental animals, which simulated non-proliferative diabetic retinopathy and its correction (Table 1).

Table 1. - The level of endothelin-1 in the blood of experimental animals with simulated diabetic retinopathy and with different methods of its correction on the 30th, 60th and 180th day (M ± m), (pkg / l)

Stages of Group	I Stage	Stage II	III Stage
Group 1	3.03 ± 0.18	3.03 ± 0.16	3.03 ± 0.18
Group 2	7.02 ± 0.22	8.05 ± 0.2	8.34 ± 0.2
Group 3	5.31 ± 0.21	5.9 ± 0.2	6.42 ± 0.18
Group 4	5.18 ± 0.15	5 ± 0.19	4.93 ± 0.18
Group 5	5.04 ± 0.18	5.16 ± 0.2	5.21 ± 0.2
Group 6	4 ± 0.21	3.84 ± 0.21	3.64 ± 0.2
Group 7	4.12 ± 0.16	3.58 ± 0.16	3.11 ± 0.13

Under pathological conditions there is a significant increase in endothelin-1, which in interaction with B-receptors vasospastic effect [16]. Endothelin-1 is also stimulated by hypercholesterolemia, low-density lipoprotein, hypoxia, hyperglycemia, angiotensin-2, cortisol, and thrombin [17]. Endothelin-1 stimulating receptors on smooth muscle cells leads

to persistent vasoconstriction and proliferation of the middle membranes of small vessels. It should be noted that endothelin-1 is 100 times more powerful than angiotensin-2 and during its introduction there is a decrease in coronary blood flow by 90% [16]. Due to the activation of cytokines, endothelin-1 enhances the inflammatory process, as well as the synthesis and secretion of factors such as fibroblast growth factor, which through the formation of extracellular matrix causes the development of vascular pathologies [16, 18]. During the development of endothelial dysfunction, endothelin01 not only takes an active part in this process, but can also lead to insulin resistance due to an increase in reactive oxygen species, primarily superoxidanion. It should be noted that closely related to active forms of oxygen E-1 indirectly initiates cardiovascular dysfunction and diabetic complications [19].

This marker is considered a classic vasoconstrictor, which during experiments also exhibits mitogenic functions. There is a close correlation between the rate of endothelial dysfunction and the concentration of endothelin-1 in blood plasma. A number of researchers have also noted high levels of endothelin in experimental animals during simulated hypercholesterolemia. The same trend was observed in patients with hypercholesterolemia. In cardiovascular pathology, in particular unstable angina or acute myocardial infarction, patients also had an increased concentration of this indicator in blood plasma [20].

Already at the first stage a significant increase in the studied indicator in the group with simulated pathology in comparison with the data of intact animals was established. The level of the marker of endothelial dysfunction is increased at this stage by 56.8% ($p < 0,001$). In the second stage, the level of E1 in group № 2 increased by 62.37%, compared with group № 1 ($p < 0.001$). It was also found that in the second group on the 60th day the value of this indicator increased by 12.88% compared with the results of the same group in the previous stage ($p < 0.001$), which indicates the further development of endothelial dysfunction in DR, and confirms the aggravating effect vasoconstriction in the pathogenesis of this complication of diabetes. In the third stage, the following results were obtained: between the group in which diabetic retinopathy was modeled without further correction and intact animals on the 180^{th} day, differences in the level of significance $p < 0.001$ (endothelin 1 level in the blood of experimental animals was higher by 63.66%). Analyzing the dynamics of E1 during all stages of the experiment, we found that compared with the 30^{th} day, the value of this indicator increased by 15.88% ($p < 0.001$), when comparing the data of the 2^{nd} and 3^{rd} stages obtained data indicating insignificant, but there is an increase in endothelial dysfunction, the progression of structural and functional disorders of the endothelium and an increase in pathological vasoconstriction on the background of the development of experimental diabetic

retinopathy. Analyzing the data of the marker of vasoconstriction (endothelin-1 level) and vasodilation (content of S-nitrosothiols) in the 2nd group, we can say about the pathological shift of vasoconstrictor-vasodilation potential, which was more pronounced at each subsequent stage of the experiment.

In the third group at the first stage the level of the studied marker is 42.92% ($p < 0.001$) higher than the value of intact animals. Compared with group № 2, its level is lower by 32.14% ($p < 0.001$). In the second stage, the content of E1 is 9.88% ($p < 0.05$) higher compared to the previous stage. Relative to the group of intact animals, it is higher by 48.56% ($p < 0.001$), and compared with the group without correction is lower by 36.69% ($p < 0.001$). In the third stage, the level of this marker pathologically increased by 17.27% ($p < 0.001$) compared with the 1st stage and by 8.2% ($p < 0.05$) compared with the 2nd. This suggests that the correction of hyperglycemia alone is not enough to normalize vascular tone in experimental diabetic retinopathy and vasoconstriction progresses steadily. Relative to the first group, the value of E1 is higher by 52.74% ($p < 0.001$), and relative to the second - lower by 29.97% ($p < 0.001$).

In group № 4 the level of endothelin-1 at the first stage is higher by 41.48% ($p < 0.001$) compared with the intact group, relative to the 2nd group it is less pronounced by 35.47% ($p < 0.001$). No statistically significant differences were found compared with group № 3. In the second stage, the content of E1 prevails by 39.37% ($p < 0.001$) compared with the intact group, compared with the group № 2 it is lower by 61.10% ($p < 0.001$), and compared with the 3rd lower by 17, 86% ($p < 0.001$). At the third stage, the level of the studied marker is 38.48% ($p < 0.001$) higher relative to the group № 1. Compared with the group № 2, it is lower by 69.19% ($p < 0.001$), and in comparison with the 3rd - by 30.18% ($p < 0.001$).

In the blood of animals of the fifth group in the first stage, the level of E1 is increased by 39.87% ($p < 0.001$) relative to these intact rats, compared with group № 2 it is lower by 39.20% ($p < 0.001$). There were no statistically significant differences in comparison with groups № 3 and № 4. In the second stage the contents of endothelin-1 to 41.22% ($p < 0.001$) higher compared to the 1st group, relatively lower in group 2 rats at 56.18% ($p < 0.001$) and 14.26% ($p < 0.001$) relative to the 3rd group. No statistically significant differences were found compared with group № 4. In the third stage, the values of the studied marker are higher by 41.75% ($p < 0.001$) relative to the intact group. Compared with group № 2, the level is lower by 60.20% ($p < 0.001$), compared with group № 3 - by 23.26% ($p < 0.001$). Differences in comparison with group № 4 are not established.

In group № 6 at the first stage the level of vasoconstriction is higher by 24.29% ($p < 0.001$) compared to group 1. Relative to all the following groups, it is lower: compared to the 2nd - by 75.26% ($p < 0.001$), compared with the 3rd by 32.63% ($p < 0.001$), relative to the 4th group of animals - by 29, 37% ($p < 0.001$) and relative to the 5th by 25.91% ($p < 0.001$). At stage № 2 (60th day of the experiment) the level of E1 is higher by 21.24% ($p < 0.01$) compared with the first group. In comparison a group № 2 is lower at 109.26% ($p<0.001$), compared with the third - by 53.09% ($p < 0.001$) compared to the 4th lower by 29.9% ($p < 0.001$) and relative to the 5th lower by 33.99% ($p < 0.001$). At the third stage, the content of the indicator is 16.66% ($p < 0.05$) higher than the norm, 129.22% ($p < 0.001$) lower compared to the data of group № 2. The increase is much less pronounced relative to all groups with correction: relative to the 3rd by 76.36% ($p < 0.001$), relative to the 4th by 35.48% ($p < 0.001$), and relative to the 5th by 43.08 % ($p < 0.001$).

In the seventh group, the level of E1 is 26.49% ($p < 0.001$) higher than the intact group in the 1st stage. Compared with the 2nd group, it is lower by 70.18% ($p < 0.001$), compared with the 3rd - by 28.78% ($p < 0.001$), relative to the 4th - by 25.62% ($p < 0.001$)), and relative to the 5th by 22.26% ($p < 0.001$). No statistically significant differences in comparison with group №6 were detected at this stage. In the second stage, the level of the marker is 15.22% ($p < 0.05$) lower compared to the previous one. Compared to the first group, it is higher by 15.3% ($p < 0,01$). Compared with the 2nd - lower by 125.06% ($p < 0.001$), compared with the 3rd - by 64.65% ($p < 0.001$), relative to the 4th and 5th groups lower by 39.7% ($p < 0.001$) and 44.1% ($p < 0.001$), respectively. Compared with the sixth group, the differences are not statistically established. At the third stage, the level of the indicator is lower by 32.62% ($p < 0.001$) compared to the 1st stage and by 15.1% ($p < 0.05$) compared to the 2nd. It is noteworthy that there are no differences between the data of intact animals and the 7th group, which indicates the normalization of endothelin-1 levels in the blood of rats. Compared to the 2nd group, the result is better by 168.3% ($p < 0.001$), relative to the 3rd - by 106.43% ($p < 0.001$), compared with the 4th - by 58.58% ($p < 0.001$)), compared with the 5th by 67.48% ($p < 0.001$), relative to the 6th group showed an improvement of 17.05% ($p < 0.05$). The obtained data indicate the normalization of vascular tone in rats of the 6th and 7th groups, a more pronounced improvement is observed in the seventh group.

Study of the dynamics of asymmetric dimethylarginine in the blood of experimental animals, which simulated diabetic retinopathy and its correction

A key role in the regulation of endothelial function is played by nitric oxide, for the synthesis of which the amino acid L-arginine is required. An important inhibitor of NO synthase is, as noted, asymmetric dimethylarginine, which blocks the binding of L-arginine to the enzyme. The concentration of this inhibitor in the blood varies and depends on many reactions, both at the cellular and tissue levels [21]. Arginine residues of proteins, which under the action of S-adenosyl-methionine dependent methyltransferases are subject to posttranslational methylation, play a key role in the metabolism of these enzymes. Since methylation is an irreversible process, it can be influenced only by proteolysis, so methylated proteins are characterized by active metabolism [21]. Free ADMA, which is released during proteolysis by intracellular dimethylarginine dimethylaminohydrolase, which is influenced by several factors, but primarily glycosylated proteins [22].

Inhibition of dimethylarginine dimethylaminohydrolase is accompanied by inhibition of NO synthesis and increase in ADMA content [21, 23]. Approximately 10% of the total amount of ADMA entering the bloodstream is partially hydrolyzed intracellularly and partially excreted by the kidneys. ADMA has a negative effect on cells, contributing to oxidative stress, shortening telomeres, inhibiting the release of NO, increasing the secretion of Interleukin-8 and monocyte chemotaxis factor 1 [24]. Its effect extends to the body as a whole: an increase in blood pressure, increased pulmonary and general peripheral vascular resistance, decreased cardiac output [21]. Atherogenesis processes such as expression of proinflammatory and chemotactic cytokines, monocyte adhesion [25] and accumulation of oxidized low-density lipoproteins activate ADMA in macrophages [26]. Patients with risk factors for atherosclerosis, such as diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia, and obesity, have elevated plasma ADMA levels [27, 28]. This correlates with indicators of initial atherosclerosis, such as the thickness of the intima-media carotid arteries [21]. The results of the study of this marker are presented in Table 2.

In the analysis of the level of ADMA at the first stage of our study found a significant (65.57%) increase in the group in which the simulated diabetic retinopathy ($p < 0.001$). At the second stage of the experiment, the following data were obtained: there is an increase in the level of the studied marker of endothelial dysfunction: an increase of 71.98% compared with intact animals ($p < 0.001$) and 18.6% compared with the experimental group without correction ($p < 0.001$). On the 180^{th} day, an even more pronounced increase in ADMA was detected in rats with simulated diabetic retinopathy: compared with the data of group № 1, its

level was pathologically increased by 75.85% (p <0.001). Compared with the data of the first stage, the level of ADMA is higher by 29.85% (p <0.001), compared with the results of stage № 2 - an increase in the concentration of endothelial NO synthase inhibitor by 13.82% (p <0.001).

Table 2. - The level of asymmetric dimethylarginine in the blood of experimental animals with simulated diabetic retinopathy and with different methods of its correction on the 30th, 60th and 180th day (M ± m), (μM / l)

Stages of Group	I stage	II stage	III stage
1 group	20.8 ± 1.28	20.8 ± 0.86	20.8 ± 1.25
2 group	60.4 ± 1.16	74.2 ± 1.35	86.1 ± 1.07
3 group	40.3 ± 1.2	49.1 ± 1.21	51.6 ± 1.09
4 group	38.2 ± 1.34	34.1 ± 1.47	30.9 ± 1.43
5 group	46.1 ± 1.47	49.3 ± 1.22	46.9 ± 1.43
6 group	36.8 ± 1.43	31.2 ± 1.2	28.7 ± 1.02
7 group	32.2 ± 1.26	26.8 ± 1.21	21.9 ± 1.11

In the 3rd group, in which only the correction of hyperglycemia was performed, at the first stage the study marker was increased by 48.40% (p <0.001) compared with the values of intact animals, and relative to the group without correction the level rise is less pronounced by 49, 88% (p <0.001). In the second stage, compared to the intact group, the level of the indicator is higher by 57.65% (p <0.001), and compared to the group № 2 - lower by 51.14% (p <0.001). Compared with the previous stage, there is a pathological increase in ADMA by 17.92% (p <0.001). In the third stage, the progression of the increase in the content of asymmetric dimethylarginine was established - its level is 59.69% (p <0.001) higher than the value of the intact group, and 21.90% (p <0.001) higher compared with the results of the 1st stage of its group. Compared with the data of the group without correction, the increase is less pronounced by 66.88% (p <0.001). The results of this group confirm that for normalization of the functional state of the endothelium and physiological activity of eNOS correction only by hypoglycemic agents is insufficient.

In the 4th group, in which aflibercept and nitric oxide donor were involved in the correction, at the first stage an increase of 45.56% detected (p <0.001) relative to the values of intact animals was. Compared with the 2nd group, the level of the marker is lower by 58.13% (p <0.001), no differences compared with the 3rd group were detected. In the second stage, there is a less pronounced rise in the level of asymmetric dimethylarginine - 12.02%

(p <0.05) lower compared to the first stage. 1st group at stage № 2, its content is higher by 39.03% (p <0.001). Compared to the 2nd group - lower by 117.61% (p <0.001), and compared to the 3rd group. At this stage, the differences are already present - the level of ADMA is lower by 43.98% (p <0.001). In the third stage, the content of the indicator is 32.69% (p <0.001) higher compared to the results of the intact group. Regarding the group № 2, the level is lower by 178.70% (p <0.001), and relative to the 3rd - by 67.01% (p <0.001). Compared with the first stage, the level of this marker is less elevated by 23.64% (p <0.001). The above indicates the feasibility of using L-arginine to normalize the level of ADMA and balance the functional state of the endothelium.

In group № 5, in which the simulated pathological condition was corrected with metformin, administration of aflibercept and bromfenac, in the first stage revealed an increase in ADMA levels by 54.89% (p <0.001) relative to the intact group. Compared with the 2nd group, the marker content is lower by 31.04% (p <0.001). But relative to the two previous groups, which corrected the experimental pathology, the level of inhibitor of endothelial nitric oxide synthase is increased - relative to the 3rd group by 12.57% (p <0.01), and relative to the 4th - by 17.13% (p <0.001). In the second stage, the difference compared to the intact group is slightly larger - by 57.82% (p <0.001), which indicates the absence of a positive effect of correction. Compared with the 2nd group, the level of the indicator is lower by 50.52% (p <0.001). Compared with group № 3, no statistically significant differences were found, and compared with group № 4 in the 5th efficiency is lower by 30.83% (p <0.001). In the third stage, the content of ADMA is 55.65% (p <0.001) higher than the value of intact animals (slightly less than on the 60th day, although the step-by-step analysis of the dynamics of statistically significant differences were not detected). Compared with the 2nd group, the level is lower by 83.61% (p <0.001), compared with the 3rd - by 10.03% (p <0.01), and compared with group № 4 the marker content is higher at 34.12% (p <0.001). The above indicates that this method of correction is not effective for normalizing the level of the studied inhibitor of endothelial nitric oxide synthase and a marker of endothelial dysfunction.

In the 6th group, in which the experimental DR was corrected by normalization of hyperglycemia, administration of aflibercept, L-carnitine and bromfenac, in the first stage the level of ADMA is higher by 43.49% (p <0.001) relative to group № 1; lower by 64.14% (p <0.001) compared with group № 2, lower by 9.51% (p <0.05) compared with the 3rd group. When compared with the data of the 4th group, no statistically significant differences were found. Compared with the 5th group, the marker level is better (less pronounced) by

25.26% (p <0.001). In the second stage, the pathological increase in the level of ADMA is less pronounced by 17.95% (p <0.01) compared to the first. Regarding the group № 1, the content of the indicator is higher by 33.36% (p <0.001), compared to the 2nd group it is lower by 57.36% (p <0.001), compared to the 5th - lower by 58.01 % (p <0.001). Compared with the 4th group, no statistically significant differences were found. In the third stage, compared with the first, the level of ADMA is more normalized by 28.25% (p <0.001). Relatively intact group content marker is higher at 27.52% (p<0.001) compared with the group № 2 is lower at 200.09% (p<0.001) relative to the 3rd group at 79.82% lower (p <0.001), venous 5th by 63.44% (p <0.001), compared with the 4th stat. significance was not detected. Characterizing the data of the 6th group as a whole we can say about the presence of pronounced positive dynamics, but complete normalization of the marker level is not established. The absence of differences between groups 4 and 6 throughout the experiment suggests the equivalence of efficacy from the introduction of L-arginine (group 4) and L-carnitine and bromfenac (group 6) to normalize the level of asymmetric dimethylarginine (metformin and aflibecept was administered in both groups in the same doses).

In the seventh group, in which the simulated non-proliferative therapy was corrected with metformin, the introduction of aflibercept, a solution of L-arginine and citicoline obtained the following results: relative to group 1, the level of ADMA is higher by 35.42% (p <0.001); compared to the 2nd it is lower by 87.60% (p <0.001), compared to the 3rd lower by 25.16% (p <0.001), relative to the 4th lower by 18.63% (p <0, 01), relative to the 5th by 43.16% (p <0.001), and compared to the 6th group is also less pronounced - by 14.29% (p <0.05). In the second stage, the level of the studied marker is 20.14% (p <0.01) lower than in the previous stage. Compared with group № 1, the marker content is higher by 22.41% (p <0.001), and relative to all subsequent groups of the experiment it is lower: by 176.90% (p <0.001) compared with the 2nd, by 83.20% (p <0.001) compared with the 3rd, by 27.24% (p <0.001) compared with the 4th, by 83.96% (p <0.001) compared with the 5th, and by 16.42% (p <0,01) relative to the 6th group. In the third stage, the level of ADMA is lower by 47.06% (p <0.001) compared to the 1st stage and 22.40% (p <0.01) compared to the 2nd. No differences compared with the data of intact animals were found, which indicates the normalization of the studied indicator under the influence of the proposed method of correction. With respect to group 2 is the best result in 293, 27% (p<0.001) relative 3rd - by 135.67% (p<0.001) compared to 4th - by 41.11% (p <0.001), in comparison with the 5th group - by 114.19% (p <0.001) and in comparison with the sixth group - by 31.05% (p <0.001). That is, we can say that the introduction of a donor of nitric oxide in combination with

citicoline gives the most pronounced of the studied methods of corrective effect, normalizing the level of asymmetric dimethylarginine.

ADMA, which is a structural analogue of L-arginine, inhibits the activity of all isoforms of NO synthases, thereby inhibiting the formation of nitric oxide in tissues and blood plasma. ADMA has been shown to significantly inhibit NO synthesis [29]. There is a clear correlation between the level of nitric oxide in plasma in physiological conditions, at the shift of which the development of vascular pathologies is observed [30]. Studies have shown that L-arginine activates vasomotor responses in vivo [31]. This is despite the fact that the content of endogenous L-arginine is 30 times higher in physiological concentrations in the plasma of the Michaelis-Menten constant for L-arginine in the reaction catalyzed by NO synthase [31-33]. Initially, the effect of L-arginine on vascular tone was considered somewhat paradoxical, as NO synthase was completely saturated with the substrate and the additional effect of exogenous arginine could not affect the intensity of nitric oxide synthesis [34]. Somewhat later, endogenous L-arginine analogues such as N-monomethyl-L-arginine (NMMA), asymmetric NN-dimethyl-L-arginine (ADMA) and symmetric NN-dimethyl-L-arginine (SDMA) were discovered [29, 34]. Two of them, ADMA and NMMA, are able to inhibit NO-synthase activity [35]. This allows us to explain the "arginine paradox", because a higher concentration of substrate is required for the activation of NO synthase in the presence of its endogenous inhibitors. Under physiological conditions, ADMA is a stronger inhibitor than NMMA, because its concentration in blood plasma is 5 times higher [29].

Given the above, further analysis of markers of physiological nitric oxide synthesis and the use of L-arginine to correct pathological conditions caused by sharply elevated levels of ADMA in the blood is informative.

Conclusions:

1. As a result of our study proved a violation of the structural and functional state of the endothelium in experimental diabetic retinopathy, as evidenced by an increase in ADMA and endothelin-1 in group 2 ($p < 0,001$), most pronounced in stage 3.

2. It is confirmed that the correction of the studied complication of diabetes mellitus only by hypoglycemic drug, even with long-term administration does not correct the development of endothelial dysfunction ($p < 0,001$).

3. It was found that the addition of aflibercept and a solution of L-arginine in the correction to hypoglycemic drugs significantly ($p < 0.001$) improves the condition of the endothelium, but does not solve the problem completely.

4. It is followed that the correction of the simulated pathological condition by reducing hyperglycemia, administration of aflibercept and bromfenac (group № 5) has a positive effect on the normalization of markers of endothelial function ($p < 0.001$), but the effect is less pronounced than in the following groups.

5. It was found that in rats in which diabetic retinopathy was simulated with subsequent correction of hyperglycemia, administration of aflibercept, L-carnitine and bromfenac (group № 6), the reduction of pathologically elevated levels of markers of endothelial dysfunction is more pronounced in comparison with data 3 groups, which indicates the feasibility of this method of correction.

6. It was found that the most effective method of correction was in the 7^{th} group of the experiment in which hyperglycemia was corrected, aflibercept, L-arginine and citicoline solution were obtained to normalize the levels of endothelial dysfunction markers - endothelin 1 and asymmetric dimethylarginine.

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