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STUDY OF VASODILATION PROCESSES

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Abstract

To date, the key role of endothelial dysfunction in the occurrence and progression of diabetes mellitus and diabetic retinopathy has been proven. The study was performed on white Wistar rats weighing 180-200 g. According to the tasks, the animals were divided into 7 groups. Our results indicate a violation of vasodilation on the 30th day of experimental diabetic retinopathy with subsequent progression of pathological changes on the 60th and 180th day of the study, as evidenced by a decrease in the content of S-nitrosothiols in group 2 ($p < 0,001$), most pronounced in the 3rd stage. When analyzing the data of group № 3, it was found that the correction of the pathological condition with the help of hypoglycemic agents

has some positive effect, but does not allow to significantly adjust the pathological development of reduced vasodilatory potential. The results of the 4th group indicate that the involvement of nitric oxide donor and aflibercept in the correction of diabetic retinopathy corrects the pathological changes and helps to restore the physiological pathway of nitric oxide synthesis and vascular tone, the maximum effect is observed on the 180th day of the experiment, but normative cannot be achieved. It is observed that the correction of the simulated pathological condition by reducing hyperglycemia, administration of aflibercept and bromfenac (group № 5) gives positive results in the first stage, but less pronounced than involvement in the complex correction of L-arginine solution in subsequent stages. It was found that rats in which diabetic retinopathy was simulated with subsequent correction of hyperglycemia, administration of aflibercept, L-carnitine and bromfenac (group № 6) have a pronounced tendency to normalize the studied marker of hypoxia in comparison with the previous methods in the second stage. nitrosothiols at the time of reaching the 3rd stage is reduced. The obtained data suggest that the method of correction chosen in group 7 (correction of hyperglycemia, aflibercept, L-arginine and citicoline solution) more pronouncedly normalizes the content of the vasodilation marker compared to other groups of our experiment, which is pronounced in long-term correction - on 180th day.

Key words: experimental diabetic retinopathy; endothelial dysfunction; vasodilation; S-nitrosothiols; correction; metformin; aflibercept; L-arginine; citicoline; L-carnitine; bromfenac.

Introduction

Diabetic retinopathy (DR) according to the WHO is the main cause of decreased vision and blindness in diabetes. This pathology is the main cause of visual impairment in the population of economically developed countries [1-4]. It should be noted that even with the compensation of carbohydrate metabolism, the development of DR continues, so hyperglycemia is not the only factor in the development of retinopathy in diabetes [5-9]. To date, the key role of endothelial dysfunction in the occurrence and progression of DR has been proven [10, 11]. The initial morphological signs of the studied pathological condition are endothelial cell proliferation, thinning of the basement membrane and loss of pericytes, which in turn leads to aneurysms and violation of the diameter of vascular capillaries and hemodynamics [4, 12, 13]. Endothelial cells are the first to "take the blow" of hyperglycemia, glucose toxicity and dyslipidemia and under its influence begin to synthesize atherogenic factors [10, 14]. There is an increase in the permeability of the vessel wall and violation of

their elasticity, which leads to hemorrhages and exudates. Transcapillary transport is disrupted, which in turn leads to retinal ischemia [14].

The aim of the study: analysis of changes in the content of S-nitrosothiols as a marker of vasodilation 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:

1st group - 60 intact animals;

Group 2 - 60 animals, which simulated diabetic retinopathy 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, which simulated diabetic retinopathy with subsequent correction of hyperglycemia, the introduction 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, the introduction 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 [15]. 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 [16] in 0.9% sodium chloride solution through a syringe with an intragastric tube daily.

Administration of a solution of L-arginine, which is a donor of NO, (SIMESTA, made in China, quality standard USP32) was carried out by intragastric administration of a solution of L-arginine in 0.9% sodium chloride solution at a dose of 500 mg / kg [17] 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 [17].

Aflibercept (anti-VEGF therapy) was administered in the form of subconjunctival injections at a dose of 0.08 ml (25 mg / ml) [18].

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 [19, 20].

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°. The 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 AV, Khrikina IS, Hegai AA, etc., 2013).

The content of S-nitrosothiols, which are known to be stable metabolites of NO, was determined by spectrofluorimetric method [21, 22]

Statistical processing of the results

To detect changes in the studied indicators between different groups and at different stages, we used parametric statistical methods, which are based on the operation of the parameters of statistical distribution (mean and variance).

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

A_{emp} and E_{emp} - calculated values of asymmetry and excess, and respectively, their critical values [25].

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

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 homoscedasticity (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 directed 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, the percentage difference between the average values of the 1st and 2nd groups will be equal to $\left(\frac{-20.604}{104.79}\right) \cdot 100\% = 19.66\%$.

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

Research results and their discussion:

Results of content research S-nitrosothiols in the experimental study are presented in Table 1.

Table 1 - The content of S-nitrosothiols 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), (μmol / l)

Stages of Group	I stage	II stage	III stage
1 group	0,38 ± 0,01	0,37 ± 0,01	0,38 ± 0,01
2 group	0,18 ± 0,01	0,16 ± 0,01	0,13 ± 0,01
3 group	0.24 ± 0.01	0.22 ± 0.01	0.18 ± 0.01
4 group	0.28 ± 0.01	0.3 ± 0.01	0.31 ± 0.01
5 group	0.31 ± 0.01	0.27 ± 0.02	0.24 ± 0.01
6 group	0.28 ± 0.01	0.33 ± 0.01	0.26 ± 0.01
7 group	0.31 ± 0.01	0.32 ± 0.02	0.36 ± 0.01

It is proved that S-nitrosothiols on a par with dinitrosotiol complexes of iron with glutathione or cysteine as thiol ligands are an endothelial relaxation factor [24]. Also, S-nitrosothiols perform the function of the main transport form of nitric oxide, which transfers it between cells. Subsequently, S-nitrosothiols in the zone with a high content of thiols and non-heme iron form dinitrosol complexes of iron, the catabolism of which releases nitric oxide. S-NO relaxes blood vessels and also act as stabilizers of nitric oxide and form a physiological depot NO, performing its transport function [25, 26].

One of the ways of nitric oxide formation is its release from S nitrosothiols. The latter are NO donors and, as noted, play a key role in the transport and secretion of this molecule in

the human body [27-29]. Catabolism of S-nitrolothiols is essential for understanding human metabolism. The key issue is to study the interregulation of nitric oxide with its derivatives or oxidation products, primarily nitrite ions and S-nitrosothiols to understand intracellular changes in the transition from physiological to pathological conditions in the cell. Nitric oxide, produced by endothelial cells, migrates to the intercellular space, where it is captured by erythrocytes and transported to muscle cells, causing them to vasodilate [30]. The general formula of S-nitrosothiols is RSNO, where R is a cysteinepeptide,

-containingRS is a piolatanion, and NO is a nitroso group [29]. Since the identification of S-nitrosothiols as key components of reactions that activate nitric oxide synthesis, interest in their metabolic reactions has increased significantly. Synthesized RSNOs play a key role in the study of NO-dependent signaling mechanisms and are used as nitric oxide donors. Studies focus primarily on SNO as an intermediate in the formation of nitric oxide and a marker of its synthesis [31]. S-nitrosothiols stabilize the level of nitric oxide on highly conserved cysteine in the beta chain of hemoglobin [32].

At the first stage, a marked decrease in the content of S-nitrosothiols in the blood of animals simulated diabetic retinopathy (the level of S-NO decreased in group 2 by 110.36% compared to with the data of the 1st group ($p < 0.001$) On the 60th day (II stage) revealed an even more pronounced decrease in vasodilation potential: the content of S-NO is lower by 139.87% compared with intact animals ($p < 0.001$). Comparing the data of the 2nd group of the 1st and 2nd stages revealed a weakening of vasodilation, but no statistically significant differences were found ovleno. In the third stage of the study, it was found that in the group in which diabetic retinopathy was simulated, the content of S-nitrosothiols decreased by 190.73% compared with intact animals ($p < 0.001$), which indicates an even more pronounced decrease in vasodilation and a significant violation of synthesis nitric oxide. Carrying out a stepwise analysis of S-nitrosothiols at each of the stages of the experiment in group № 2 revealed the following: differences between the data of the first and third stages were 37.84% ($p < 0.05$), statistical differences between the second and third stages are not detected.

In the third group in the first stage, the content of S-nitrosothiols is lower by 56.78% ($p < 0.001$) relative to the intact group, and higher by 25.47% ($p < 0.01$) compared with group № 2. In the second stage, it decreased by 69.93% ($p < 0.001$) relative to the 1st group and is 29.16% ($p < 0.001$) higher than in the group without correction. At stage № 3, the level of the marker is lower by 104.63% ($p < 0.001$) compared with the 1st group. Regarding the group № 2, it is higher by 29.43% ($p < 0.01$). Carrying out a step-by-step characterization of the proven

continuous decrease of vasodilation potential - by 30.52% ($p < 0.01$) compared with the 1st stage and by 19.62% ($p < 0.05$) compared with the 2nd.

In group № 4 the content of S-nitrosothiols in the first stage is 34.11% ($p < 0.001$) lower than in group 1. Compared with the group without correction, it is higher by 36.25% ($p < 0.001$) and compared with the group № 3 - by 14.46% ($p < 0.05$). In the second stage, the level of the marker is 24.33% ($p < 0.001$) lower relative to the intact group, compared with the 2nd group it is higher by 48.17% ($p < 0.001$), and relative to group № 3 better by 26.83% ($p < 0.001$). In the third stage, the content of the vasodilation marker is lower by 22.24% ($p < 0.001$) relative to the 1st group; compared with the 2nd group, its increase by 57.95% ($p < 0.001$), and compared with the 3rd - by 41.07% ($p < 0.001$). Compared to the first stage, the level of the indicator increased by 9.09% ($p < 0.05$).

In group № 5, the level of the vasodilation marker was 23.11% ($p < 0.001$) lower compared to the intact group. Compared to group № 2, the indicator is higher by 41.48% ($p < 0.001$), relative to the third group - by 21.48% ($p < 0.001$), compared with group № 4, no statistically significant differences were found. In the second stage, there is a decrease in the content of S-NO by 14.88% ($p < 0.05$) compared with the 30th day. The level of the marker is 40.49% ($p < 0.001$) lower relative to the intact group, compared with the 2nd and 3rd groups it is higher by 41.43% ($p < 0.001$) and 17.33% ($p < 0.05$) respectively. No significant differences were found with respect to the 4th group. In the third stage, the decrease in the content of S-nitrosothiols is even more pronounced - by 28.69% ($p < 0.001$) relative to the first stage. Compared with group № 1, the level was lower by 58.86% ($p < 0.001$). Compared with group № 2, the content of the vasodilation marker is higher by 45.36% ($p < 0.001$), with group № 3 - by 22.57% ($p < 0.01$). And for the group № 4 the level of the indicator is lower by 29.96% ($p < 0.001$).

In the study of the content of S-nitrosothiols in the blood of rats of the sixth group, it was found that the level is 32.22% ($p < 0.001$) lower than in intact animals, 37.15% ($p < 0.001$) higher compared to group № 2. Regarding the 3rd group, the level of the marker is also higher - by 15.67% ($p < 0.05$). And in comparison with the data of groups 4 and 5 no statistically significant differences were found. In the second stage, the content of S-NO is higher by 13.81% ($p < 0.05$) compared to the previous stage. Compared with group № 1, the marker content is lower by 13.20% ($p < 0.05$). Compared to the 2nd group, an increase of 52.81% ($p < 0.001$) was established, and relative to the 3rd - by 33.38% ($p < 0.001$). No statistically significant differences were found compared with group № 4. Compared with group № 5, the result is better by 19.42% ($p < 0.01$). In the third stage, the improvement of S-NO content is

slightly less pronounced - it is lower by 27.47% ($p < 0.001$) compared to the 60th day and 45.65% ($p < 0.001$) less compared to the intact group. For the group № 2 the level of the marker is higher by 49.90% ($p < 0.001$), and for the group № 3 - by 29.01% ($p < 0.001$). Compared with group 4 in the third stage, the correction used in group 6 is less effective by 19.15% ($p < 0,01$). Compared with group № 5 no significant differences were found.

In the seventh group in the first stage, the level of S-NO is lower by 22.71% ($p < 0.001$) relative to normal. Compared with the values of the 2nd group, the marker content is higher by 41.67% ($p < 0.001$). Regarding the group № 3, it is higher by 21.73% ($p < 0.001$). No differences were found compared with groups 4, 5 and 6. In the second stage, the content of S-NO by 15.66% ($p < 0.05$) is lower compared to the intact group. Relative to group № 2, the value of the marker is higher by 51.78% ($p < 0.001$), relative to the 3rd - by 31.94% ($p < 0.001$), and compared to the 5th - by 17.67% ($p < 0, 05$). Compared with the 4th and 6th groups, no statistically significant differences were found. In the third stage, there is a positive trend, which is manifested in an increase in the level of S-NO by 15.59% ($p < 0.01$) relative to the 1st and 11.03% ($p < 0.05$) relative to the 2nd stage. Regarding the intact group, no statistical differences were found, which indicates the normalization of the marker level in the group № 7. With respect to all these groups provlyayetsya rate increase, to 64.28% ($p < 0.001$) higher than in group 2, at 49.38% ($p < 0.001$) - than in the 3rd, at 15.03% ($p < 0.001$) compared with the 4th group, 34.62% ($p < 0.001$) compared with the 5th, and 28.69% ($p < 0.001$) relative to the group № 6. That is, we can say that the correction used in group 7 is the most effective in normalizing the pathologically reduced vasodilatory potential and is most pronounced in stage 3 of the experiment (Dynamics of the content of the studied marker is clearly illustrated in Fig. 1).

Conclusions:

1. Our results indicate a violation of vasodilation on the 30th day of experimental diabetic retinopathy with subsequent progression of pathological changes on the 60th and 180th day of the study, as evidenced by a decrease in the content of S-nitrosothiols in group 2 ($p < 0,001$), maximally expressed at the 3rd stage.

2. In the analysis of data of group № 3 it is established that correction of a pathological condition by means of hypoglycemic means has some positive influence, but does not allow to correct markedly pathological development of decrease in vasodilatory potential.

3. The results of the 4th group indicate that the involvement of the donor of nitric oxide and aflibercept in the correction of diabetic retinopathy corrects the pathological changes and helps to restore the physiological pathway of nitric oxide synthesis and vascular tone, the

maximum effect is observed on the 180th day of the experiment, but normative values cannot be reached.

4. It is observed that the correction of the simulated pathological condition by reducing hyperglycemia, administration of aflibercept and bromfenac (group № 5) gives positive results in the first stage, but less pronounced than involvement in the complex correction of L-arginine solution in subsequent stages.

5. It was found that rats in which diabetic retinopathy was simulated with subsequent correction of hyperglycemia, administration of aflibercept, L-carnitine and bromfenac (group №6) have a pronounced tendency to normalize the studied marker of hypoxia compared with previous methods, but in the second stage S-nitrosothiols at the time of reaching the 3rd stage is reduced.

6. The obtained data suggest that the method of correction chosen in group 7 (correction of hyperglycemia, administration of aflibercept, L-arginine and citicoline solution) more pronouncedly normalizes the content of the vasodilation marker compared to other groups of our experiment, which is pronounced in long-term correction - on the 180th day.

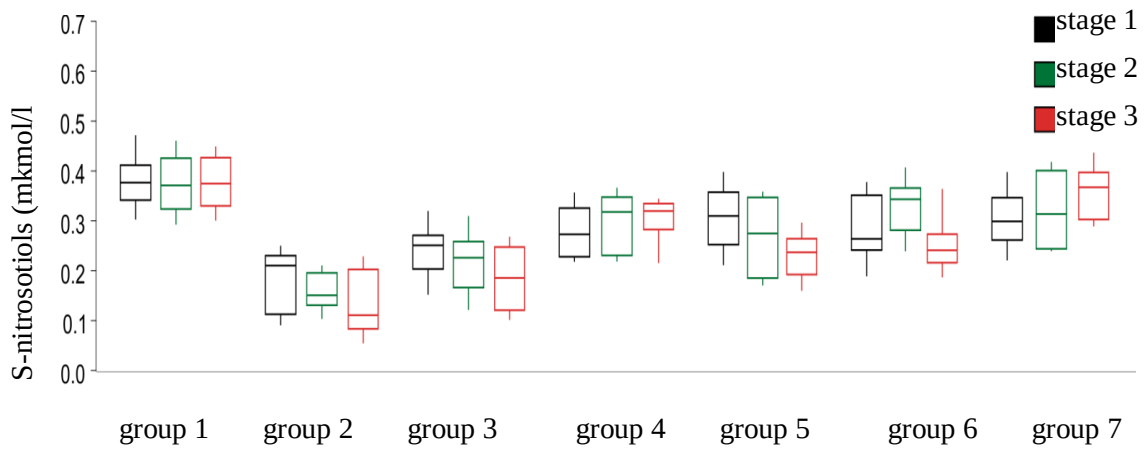

Figure 1. - Content S -nitrosothiols in the blood of experimental animals with simulated diabetic retinopathy and with different methods of its correction on the 30th, 60th and 180th day. Boxing rafts illustrate the distribution of the values of the level of the studied indicator in all groups of the experiment at each stage of the study (n = 20 in each of the groups).

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