April 30, 2021

PharmacologyOnLine

Archives • 2021 • vol.1 • 90-95

DYNAMICS OF VASOCONSTRUCTOR-VASODILATION POTENTIAL ON THE BACKGROUND OF THE DEVELOPMENT OF EXPERIMENTAL DIABETIC RETINOPATHY

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Abstract

Proved development of endothelial dysfunction in rats that was modeled experimental diabetic retinopathy, as evidenced by increased endothelin-1 levels. It was found that at each stage of the study, pathological vasoconstriction increases ($p < 0.001$ compared with the intact group). It was revealed that simulated diabetic retinopathy negatively affects vasodilation potential, as evidenced by the analysis of the content of S-nitrosothiols in the blood of experimental animals ($p < 0.001$ compared with the data of intact animals). In the analysis of vasoconstrictor-vasodilyatatsiynogo potential at each stage of the experiment proved the prevalence of pathological vasoconstriction ($p < 0.001$).

The weakening of the vasodilatory potential was step by step more gradual in comparison with the dynamics of the increase in the vasodilation potential. In a comparative experiment characteristic pathological stages most pronounced shifts mounted on stage III of diabetic retinopathy study simulated (180-day).

Key words: *experimental diabetic retinopathy, endothelial dysfunction, endothelin-1, S-nitrosothiols, vasoconstrictronovasodilation potential*

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] and is diagnosed in 40-85% of patients with diabetes.

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. Other important reasons include the role of hypertension and the development of macular edema [5-9]. To date, the key role of endothelial dysfunction in the occurrence and progression of DR has been proven [10, 11]. It was determined that 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 disruption of vascular capillary diameter and hemodynamics [4, 12, 13].

It has been proven that 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 purpose of the study: analysis of the dynamics of markers of vasoconstriction and vasodilation at different stages of development of experimental diabetic retinopathy.

Methods

The study was performed on white Wistar rats weighing 180-200 g. According to the tasks, the animals were divided into 2 groups:

1st group - 60 intact animals;

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

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 [16].

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).

The content of S-nitrosothiols, which are known to be stable metabolites of NO, was determined by spectrofluorimetric method [17, 18, 19]. The principle of the method is as follows: There is a conversion of the group of S-nitrosothiols into nitrites (using mercury chloride) and subsequent acid-catalyzing nitrosotation of 2,4-diaminonaphthalene. Before that, NO_2 is completely removed with ammonium sulfate (reduces NO_2 to N_2 in acidic conditions). The method is as follows: a solution of ammonium sulfate with a concentration of 0.1 mm / l precipitated nitrites with diluted saline 7 times serum. Centrifuged. To 50 µl of the supernatant was added 200 µl of the reaction mixture (1 part 1.1 mm $HgCl_2$ and 4 parts 0.05 g / l diaminonaphthalene in 0.62 g HCl), incubated for 10 minutes in the dark at room temperature. The reaction was stopped by adding 20 µl of 2.7 M NaOH. Fluorescence was measured at 360/450 nm. The calculation was performed on a calibration curve constructed using different concentrations of reduced glutathione (from 0.15 to 10.0 µmol / l of reduced glutathione in 1M HCl). Incubation of reduced glutathione with sodium nitrite (10.0 µmol / l) at room temperature produces nitroso-glutathione [18].

The content of endothelin-1 in blood serum was performed by enzyme-linked immunosorbent assay using reagent kits from DRG (Germany) [18].

Statistical processing of the obtained 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 the 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 EI Pustylnyk.

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. This is a comparison between different groups of animals or a comparison between the same group of animals (but since there is no correspondence between animals in the samples, they will also be independent). The value of p <0.05 was chosen as a criterion of reliability. An analysis was made as to whether the mean values differed. The results of determining 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. This difference was calculated as a percentage. Thus, we demonstrated a comparison of the mean values between different groups of animals.

Results

1. Study of the level of endothelin-1 (E1) - an indicator of vasoconstriction and a marker of endothelial dysfunction in the dynamics of experimental diabetic retinopathy

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. The level of the marker of endothelial dysfunction is increased at this stage by 56.7% (p <0,001). In the second stage, the level of E1 in group №2 increased by 62.29%, 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 12.91% 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 180th day, differences in the level of significance p <0.001 (endothelin 1 level in the blood of experimental animals was higher by 63.55%). Analyzing the dynamics of E1 during all stages of the experiment, we found that compared with the 30th day, the value of this indicator increased by 15.83% (p <0.001), when comparing the data of the 2nd and 3rd stages, the differences were no longer statistically significant and amounted to 3.36%, indicating insignificant stat., but there is an increase in endothelial dysfunction, ie 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.

2. Study of the content of S-nitrosothiols - an indicator of vasodilation and an indirect marker of nitric oxide synthesis in the dynamics of experimental diabetic retinopathy

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 2 -th group by 111.12% compared with the data of the 1st group (p <0.001)). On the 60th day (stage II) an even more pronounced decrease in vasodilatory potential was detected: the S-NO content was lower by 137.5% compared to intact animals (p <0.001). Comparing the data of the 2nd group of the 1st and 2nd stages, a weakening of vasodilation by 12.5% was revealed, but no statistically significant differences were found. 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 192.31% 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 step-by-step 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 38.47% (p <0.05), differences between the second and third stages - 23,08% (statistically significant differences were not detected).

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

Conclusions

1. The development of endothelial dysfunction in rats in which experimental diabetic retinopathy was simulated has been proved, as evidenced by the increase in endothelin-1 levels.

2. It was found that at each stage of the study increases pathological vasoconstriction ($p < 0.001$ compared with the intact group).

3. It was found that the simulated diabetic retinopathy has a negative effect on vasodilatory potential, as evidenced by the analysis of the content of S-nitrosothiols in the blood of experimental animals ($p < 0.001$ compared with intact animals).

4. In the analysis of vasoconstrictor-vasodilatory potential at each of the stages of the experiment proved the pathological predominance of vasoconstriction ($p < 0,001$).

5. The weakening of the vasodilating potential was gradually smoother than the dynamics of increasing the vasodilating potential.

6. At the comparative characteristic of stages of experiment the most expressed pathological shifts are established at the III stage of research of the modeled diabetic retinopathy (on the 180th day).

Acknowledgments

The authors declare that there are no conflicts of interest.

Relationship of the publication with the planned research works. The work presented is a fragment of the research project "Diabetic nephropathy pathogenesis and substantiation of chronic kidney disease diagnostics, № state registration 0120U102210.

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Table 1. Dynamics of endothelin-1 in the blood of laboratory animals (pkg / l)

Stages Groups	I stage	II stage	III stage
1 group	3.04 ± 0.19	3.04 ± 0.16	3.04 ± 0.17
2 group	7.02 ± 0.23	8.06 ± 0.21	8.34 ± 0.2

Table 2. Dynamics of the level of S-nitrosothiols in the blood of laboratory animals against the background of the development of experimental diabetic retinopathy (M ± m) (μmol / l)

Stages of Group	I stage	II stage	III stage
1 group	0.38 ± 0.02	0.38 ± 0.02	0.38 ± 0, 02
2 group	0.18 ± 0.02	0.16 ± 0.01	0.13 ± 0.02