Effect of acute insulin therapy on the concentration of vascular endothelial growth factor A (VEGF-A) in the intraocular fluid in an experiment

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ABSTRACT

Purpose — to study the effect of insulin therapy on the concentration of vascular endothelial growth factor A (VEGF-A) in the intraocular fluid of rats with alloxan model of diabetes mellitus.

Material and methods. The experiment was conducted on 80 mongrel rats. In 65 rats, the alloxan model of diabetes mellitus was simulated by a single intraperitoneal injection of 100 mg/kg alloxan hydrate saluted in 0.4 ml of citrate buffer. 72 hours after intraperitoneal administration of alloxan monohydrate, these animals were divided into 2 groups. The main group (group 1) consisted of animals with alloxan model of diabetes mellitus, who started daily single intraperitoneal administration of prolonged-acting insulin at a therapeutic dose of 0.9 U/kg body weight. The comparison group (group 2) consisted of animals with alloxan model of diabetes mellitus who did not receive specific therapy. 15 healthy rats constituted the control group (group 3). Experimental animals were removed from the study on day 31 of insulin therapy. The concentration of VEGF-A was determined in 80—90 μl of intraocular fluid collected from both eyes of each animal.

Results. In the main group, the median of VEGF-A concentration [25th; 75th percentiles] in the intraocular fluid was 140 [136; 210] pg/ml, which is 1.94 times higher than in the comparison group (72 [58; 86] pg/ml) and 1.84 times higher than in the control group (76 [62.5; 88] pg/ml). The concentration of VEGF-A in the intraocular fluid in the main group was statistically significantly higher, as compared with the comparison group \(p_{\text{m—u}}<0.0004\), and compared with the control group \(p_{\text{m—u}}=0.0045\). The comparison group had no statistically significant differences when compared with the control group \(p_{\text{m—u}}=0.9979\).

Conclusion. Insulin therapy for 31 days increases the concentration of VEGF-A in the intraocular fluid of rats with alloxan model of diabetes mellitus.

Keywords: diabetes mellitus, insulin, insulin therapy, VEGF-A, HIF-1a, alloxan, diabetic retinopathy, hyperglycemia.

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The incidence of diabetes mellitus (DM) has now become a global epidemic with high mortality, which affects the population of not only highly developed countries, such as the USA, Great Britain, Germany, but also developing countries, including China, India, countries of South America and Africa [1–5]. It is estimated that by 2040, out of 600 million patients with diabetes, 400–500 million will be located in low- and middle-income countries [6].

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes. It develops with a long duration of diabetes and is associated with insufficient control of the level of glycemia, blood pressure and lipid status [7]. A number of studies have shown that strict control of glycemia reduces the frequency and progression of DR [8]. Long-term insulin therapy helps to stabilize DR and leads to a marked reduction in the risk of its progression [9, 10].

Nevertheless, clinical studies show that replacing insulin therapy causes a transient aggravation of DR in patients with type 1 and type 2 diabetes [9, 11–13]. The mechanisms underlying the transient aggravation of DR on the background of insulin therapy remain the topic for discussion.

The aim of the present study was to study the effect of insulin therapy within 1 month from its start on the concentration of vascular endothelial growth factor A (VEGF-A) in the intraocular fluid (IF) of rats with alloxan model of diabetes mellitus.

Materials and methods

The experiment was carried out in compliance with the principles of humanity set forth in the directives of the European Community and the Helsinki Declaration, in
accordance with the «Rules for work using experimental animals.»

To simulate diabetes, 80 outbred rats with an average body weight of 256.07 ± 24.12 g (180–335 g) were used. All animals were kept in vivarium in individual cages on a standard diet with free access to water. In 65 rats, the alloxan model of diabetes was simulated by a single intraperitoneal injection of alloxan hydrate (La Chema, Czech Republic) at a dose of 100 mg / kg in 0.4 ml of citrate buffer solution. The remaining 15 healthy rats formed the control group.

Since the diabetogenic effect of alloxan is more pronounced in animals on a preliminary diet, all animals did not receive food, but had free access to water for 24 hours before the start of experiment.

When planning the experiment design, we took into account the three-phase reaction of organism to injection of alloxan monohydrate to experimental animals, which consists in the initial increase in blood sugar, reaching a maximum 2–4 hours after injection of the drug, which is replaced by the phase of hypoglycemia lasting for 15–20 hours. If the laboratory animal does not die during this phase, then secondary hyperglycemia occurs, indicating the development of diabetes. Therefore, the establishment of diabetic status and the start of insulin therapy was performed 7 days after intraperitoneal injection of alloxan monohydrate.

During first 7 days after injection of alloxan monohydrate, 20 rats died. The study therefore had 45 experimental animals with diabetes and 15 healthy rats. To establish the diabetic status, the concentration of glucose in the blood taken from the tail vein was monitored.

Experimental animals were studied in three groups. The main group (group 1; n = 21) consisted of animals with an alloxan model of diabetes, which started a single daily intraperitoneal injection of prolonged-acting insulin at a therapeutic dose of 0.9 U / kg of body weight. The comparison group (group 2; n = 24) included animals with an alloxan model of diabetes, which did not receive specific therapy. The control group (3rd group; n = 15) consisted of healthy animals that did not receive specific therapy. Instead of insulin, the animals of comparison group and control group were injected intraperitoneally with saline solution.

Experimental animals were withdrawn from the study 31 days after the start of insulin therapy. By the end of experiment, the control group still consisted of 15 rats, the main group — 17, the comparison group — 20.

An 80–90 µl of IF was taken from both eyes of each animal and placed into individual Eppendorf tubes for subsequent analysis. The concentration of VEGF-A was determined in the laboratory of Cheboksary branch of the Federal State Autonomous Institution Scientific Research Center «Eye Microsurgery» named after Acad. S.N. Fedorov” by enzyme immunoassay analysis using the ELISA Kit for Vascular Endothelial Growth Factor A test system (Cloud-Clone Corp., USA).

Since the obtained digital data did not obey the normal distribution, statistical processing and revealing of statistical significance was carried out non-parametrically using the Kruskal — Wallis and Mann — Whitney criteria with the Bonferroni correction in the IBM SPSS Statistics 20. The data are presented as median (Me) [25th; 75th percentile].

Results

In the main group (n = 17), the concentration of VEGF-A in the intraocular fluid was 140 [136; 210] pg / ml, which is 1.94 times higher than in comparison group (n = 20; 72 [58; 86] pg / ml), and 1.84 times higher than in control group (n = 15; 76 [62.5; 88] pg / ml; see figure).

![Concentration of VEGF-A in the intraocular fluid of rats in the studied groups after 31 days of insulin therapy, Me [25th; 75th percentiles]](image-url)
Using the Kruskal – Wallis criterion, statistically significant differences between the studied groups by the concentration of VEGF-A in the IF were established (pk <0.001). Pair-wise comparisons using the Mann – Whitney test with Bonferroni correction showed that VEGF-A concentration in the IF in the main group was statistically significantly higher than in comparison group (pm-u <0.0004) and in control group (pm-u = 0.0045). The comparison group did not have statistically significant differences when comparing the indicators of the control group (pm-u = 0.9979).

Discussion

The alloxan model of diabetes is one of the most common and studied, it is actively used by researchers around the world.

Alloxan is a structural analogue of glucose, due to which it accumulates in the B cells of the pancreas and leads to their death with the subsequent development of diabetes. Defeat of B-cells is accompanied by degenerative changes in the kidneys and liver, which leads to high mortality of laboratory animals on the 1st day after the injection of alloxan [14]. Moreover, the individual sensitivity to alloxan among animals of the same species varies in a very wide range. The reasons for the different sensitivity in response to the injection of diabetogenic doses of alloxan are determined by a large number of factors, including age, gender, state of the nervous and endocrine systems, the nature of metabolism, and the condition of internal organs. An example is the work of N.A. Palchikova et al. [15], in which the hormonal-biochemical features of alloxan and streptozocin models of diabetes in rats were studied. The authors revealed a high individual heterogeneity of animals in response to the injection of alloxan already on the 1st day after injection of the drug. During the first 5 days of the study, having the gradual abandonment of water consumption and urine excretion, 31% of the experimental animals died.

The high mortality of laboratory animals is one of the drawbacks of this model of diabetes that we encountered during the experiment. So, 7 days after intraperitoneal injection of alloxan monohydrate, the mortality of laboratory animals was 30.8% (20 rats), after 10 days, mortality was 41.5% (27 rats).

The pathogenesis of DR contains many pathological processes, of which oxidative stress, structural changes in the microvasculature, hypoxia and expression of VEGF-A are considered as key issues. Y. Chen et al. first showed in vivo for cattle endothelial cell culture that oxidative stress and hypoxia via the Wnt signaling pathway lead to an increase in the concentration of β-catenin in the nuclei of cells. High nuclear concentrations of β-catenin through hypoxia-induced factor 1a (HIF-1α) contribute to the expression of VEGF-A [16].

VEGF-A is the most important and well-studied signal protein. Along with other proteins, it is part of the system responsible for restoring oxygen supply to tissues with insufficient blood circulation by stimulating angiogenesis. VEGF-A plays one of the main roles in the progression of DR, contributing to the violation of hematoretinal barrier and the appearance of newly formed vessels [17, 18]. Moreover, a number of experimental studies on animals have shown that specific inhibition of VEGF prevents the destruction of the hematoretinal barrier and the development of neovascularization [18–23].

V. Poulaki et al. proved that seven-day insulin therapy leads to increased levels of VEGF and HIF-1α in rats with streptozocin model of diabetes, which leads to a clinically significant violation of the hematoretinal barrier. Moreover, it was demonstrated that insulin-induced expression of VEGF is HIF-1α-dependent and is carried out by p38 MAPK and phosphoinositide-3-kinase (PI3Ks), while hyperglycemia-induced VEGF expression is HIF-1α-independent and is carried out by protein kinases C and p42/p44 MAPK [19].

Apparently, insulin therapy increases the concentration of VEGF-A, which can lead to a transient aggravation of DR up to the manifestations of hematoretinal barrier violation. The mechanisms of this phenomenon and its role in clinical practice have yet to be studied more fully.

In this study, we demonstrated that insulin therapy for 31 days leads to a statistically significant increase in the concentration of VEGF-A in IF in vivo in rats with alloxan model of diabetes. Moreover, we determined that in the above terms, the development of the alloxan model of diabetes does not lead to a statistically significant increase in the concentration of VEGF-A in the IF compared with healthy animals.

Conclusion

Insulin therapy during 1 month causes an increase in the concentration of VEGF-A in the IF of rats with alloxan model of diabetes.

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