

BENZODIAZEPINE RECEPTOR AGONIST CARBACETAM MODULATES THE LEVEL OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE RETINA OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

S. V. ZIABLITSEV¹✉, D. B. ZHUPAN¹, A. O. TYKHOMYROV², O. O. DYADYK³

¹Bogomolets National Medical University, Kyiv, Ukraine;

²Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine;

³Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine;

✉ e-mail: zsv1965@gmail.com

Received: 07 September 2023; **Revised:** 19 October 2023; **Accepted:** 01 December 2023

One of the primary mechanisms of retinal neurodegeneration in diabetes mellitus is gamma-aminobutyric acid (GABA) deficiency that makes the use of GABA-benzodiazepine receptor modulators a promising option for the correction of this diabetic complication. The aim of this study was to determine the effect of the benzodiazepine receptor agonist carbacetam on the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1 α (HIF-1 α) in retina of rats with hyperglycemia. Experimental diabetes was modeled by a single administration of streptozotocin (50 mg/kg) to three-month-old male Wistar rats. Immunoblotting and immunohistochemical studies were performed using monoclonal antibodies against VEGF and HIF-1 α . It was shown that the development of diabetic retinopathy (DR) at the early stages was accompanied by a progressive multifold increase in the retina content of VEGF on 7-28 days and HIF-1 α on 28th day. Insulin and insulin+carbacetam treatment significantly alleviated diabetes-induced overexpression of both HIF-1 α and VEGF. Carbacetam was shown to block the diabetogenic increase in VEGF content in retina. The introduction of insulin with carbacetam significantly reduced the expression of VEGF and the development of specific morphological manifestations of DR. Thus, restoration of GABA-ergic signaling can be used as a promising therapeutic option for the correction of DR disorders.

Key words: VEGF, HIF-1 α hyperglycemia, streptozotocin-induced diabetes, retinopathy, GABA-benzodiazepine receptors, carbacetam.

One of the main mechanisms of retinal damage in diabetes mellitus (DM) is the dysfunction of the retinal neurovascular unit, which includes retinal neurons, glia, and vascular endothelium [1]. The first manifestations of diabetic retinopathy (DR) are intraretinal microvascular abnormalities, accompanied by edema due to increased vascular permeability and neovascularization due to hypoxia and activation of a powerful transcription factor – hypoxia-inducible factor (HIF-1 α) and its dependent vasculoendothelial growth factor (VEGF) [2, 3].

Microvascular abnormalities include capillary basement membrane thickening, endothelial cell tight junction failure, pericyte loss, and acellular capillary formation [4]. As a result, capillary

insufficiency leads to hypoxia/ischemia and triggers pathological angiogenesis, which in turn exacerbates retinal hypoxia, inflammation, immune dysfunction and completes the pathological cycle of DR progression [5]. The vessels that form are immature, highly permeable, and rupture easily, leading to intraocular hemorrhages [6, 7].

Neurodegeneration of the retina develops interdependently with the development of circulatory disorders, which may even precede microvascular manifestations [8]. Scanning laser polarimetry and optical coherence tomography identify retinal ganglion cell loss concomitant with a decrease in nerve fiber layer thickness [9] even in the absence of any microvascular deficit [8]. Neurodegeneration of the retina is manifested by apoptosis of neurons and re-

active gliosis, which complements the dysfunction of the retinal neurovascular unit and, in turn, increases the development of vasoregression [10].

Most current therapeutic strategies, including anti-VEGF antibodies (anti-VEGF therapy), target the late stages (diabetic macular edema and proliferative DR) and are unable to prevent neuronal damage [10, 11]. Therefore, the research of early factors leading to diabetic retinal lesions and the justification of new therapeutic approaches aimed at eliminating not only vascular, but also neuronal disorders is relevant.

Primary mechanisms underlying neurodegeneration in DM include accumulation of extracellular glutamate (glutamate excitotoxicity), oxidative stress, imbalance between neuroprotective and neurotrophic factors, and chronic inflammation [12]. Gamma-aminobutyric acid (GABA), dopamine deficiency and excitatory glutamate toxicity are the neurochemical mechanisms specific to DR that trigger metabolic disturbances, the caspase cascade, and apoptosis [13].

The first drug to be approved by the US Food and Drug Administration (FDA) for the treatment of diabetic neuropathy is the GABA receptor agonist pregabalin [14]. In diabetic rats, pregabalin blocked retinal-specific neuroinflammation, apoptosis, and oxidative stress through enhanced GABA-ergic regulation. Therefore, the impact on GABA-ergic processes is a promising direction for the pathogenetic treatment of retinal neuronal dysfunction in DR.

In this regard, a promising drug is carbacetam, which has a number of positive effects in various pathological processes [15, 16], including diabetes [17]. The drug was developed at the Institute of Physical and Organic Chemistry and Carbon Chemistry of the National Academy of Sciences of Ukraine, belongs to endogenous modulators of the GABA-benzodiazepine receptor complex, β -carboline derivatives and is a carboline isostere (1-oxo-3,3,6-trimethyl-1,2,3,4-tetrahydroindolo [2,3c]quinoline). It has anti-amnestic, anxiolytic, anti-hypoxic, anti-edema and anti-shock effects, thanks to which it can become a promising means of neuroprotection [15-17].

The aim of this study was to determine the effect of the benzodiazepine receptor agonist carbacetam on the retinal expression of VEGF and HIF-1 α in DR.

Materials and Methods

Experiments. The work was carried out in accordance with the norms and principles of the EU Directive 2010/63 on the protection of animals, the Declaration of Helsinki (2008) and the requirements of the Law of Ukraine “On the Protection of Animals from Cruelty” (No. 1759-VI dated 15.12.2009). The animals were kept in vivarium conditions on a standard diet.

DM was modeled in 35 three-month-old male Wistar rats weighing 140-160 g by a single intraperitoneal injection of streptozotocin (50 mg/kg; Sigma-Aldrich, USA). Immediately after injection 3 rats were removed from the experiment for immunoblotting studies (pre-DM control). After 7 days, 5 rats were removed from the experiment (pre-treatment control). Other animals ($n = 27$) were divided into three groups – in the 1st group, insulin (Actrapid HM Penfill, Novo Nordisk A/S, Denmark) was administered at a dose of 30 Units ($n = 9$), in the 2nd group – insulin was combined with carbacetam (5 g/kg in 0.5 ml of saline solution; $n = 9$). In the control group ($n = 9$), only drug solvents were administered (untreated control). The drugs were injected intraperitoneally every other day, starting from the 7th day after the administration of streptozotocin, for 28 days.

Carbacetam was synthesized in the Department of Chemistry of Biologically Active Compounds of the Institute of Physical-Organic Chemistry and Carbon Chemistry named after L. M. Lytvynenko of the National Academy of Sciences of Ukraine (Kyiv, Ukraine) under the supervision of Doctor of Chemical Sciences, senior researcher S. L. Bogza.

Glucose content was monitored using a glucometer and disposable test strips (ACCU-Chek Instant, Roche, Germany) in fasting blood taken from the tail vein. Blood glucose content was stably high during the observation and on the 7th day (pre-treatment control) was 27.93 ± 1.36 mmol/l, in 14 days – 29.80 ± 1.38 vs 17.6 ± 1.21 vs 15.58 ± 0.81 mmol/l in untreated controls, 1st and 2nd groups, respectively. On the 28th day, blood glucose content in the untreated controls was 29.32 ± 1.25 mmol/l, in the 1st and 2nd groups 17.02 ± 1.03 and 14.38 ± 1.25 mmol/l, respectively ($P < 0.05$ when compared with pre-treatment control). During the experiment, the animals significantly lost weight: before DM simulating, the average weight was 145.1 ± 2.5 g, and on the 28th day – 115.3 ± 1.6 g ($P < 0.05$). The given data made

it possible to consider the applied model of DM in rats as adequate.

Immunohistochemical study. Animals were euthanized after 7 ($n = 3$), 14 ($n = 9$; 3 in each group), and 28 days ($n = 9$; 3 in each group) by lethal injection of thiopental (75 mg/kg) and decapitation. The eyes were immersed in a 10% solution of neutral formalin and embedded in paraffin. Serial histological sections with a thickness of 2–3 μm were made from paraffin blocks on a rotary microtome NM 325 (Thermo Shandon, England). Immunohistochemical study was performed using monoclonal mouse antibodies against VEGF (no. MA5-12184, Invitrogen, USA). Sections were additionally stained with hematoxylin. Microscopic research and photo archiving were carried out using a light-optical microscope ZEISS (Germany) with the results processing system “Axio Imager. A2”. The evaluation of expression severity was carried out according to D. J. Dabbs (2014) based on the visual-analog scale: 0 points – no coloring; 1 point (+) – weak color intensity; 2 points (++) – average color intensity; 3 points (+++) – high color intensity [18]. The percentage of VEGF-positive cells with different intensities of staining was calculated in the inner nuclear layer per 100 cells, the results were presented in %.

Immunoblotting. Animals were euthanized after 7 ($n = 2$), 14 ($n = 9$; 3 in each group), and 28 days ($n = 9$; 3 in each group) by lethal injection of thiopental. The content of VEGF and HIF-1 α in retinal tissue lysates was determined by immunoblotting. Tissue samples were preserved in liquid nitrogen, crushed and homogenized in 50 mM Tris-HCl buffer (pH 7.4) with the addition of phosphatase and protease inhibitors (Pierce Protease and Phosphatase inhibitor, ThermoScientific, USA). Electrophoresis was performed in an 8% polyacrylamide gel with sodium dodecyl sulfate in a chamber for vertical gel electrophoresis (BioRad, USA). Proteins from the gel were transferred onto the nitrocellulose membrane using an electroblot. Membranes were incubated with monoclonal antibodies to VEGF (no. MA5-12184, Invitrogen, USA) and HIF-1 α (no. HPA001275, rabbit, Sigma Aldrich, USA). Antibodies to actin (β -actin, no. MA5-15739, mouse, Invitrogen, USA) were used for its detection as a control of protein application. After primary incubation, membranes were washed and treated with anti-species secondary antibodies conjugated with horseradish peroxidase (Invitrogen, USA). Semiquantitative analysis was performed densitometrically using TotalLab soft-

ware (TL120, Nonlinear Inc, USA). The results of the immunoblot analysis were expressed in arbitrary units from the control value of the optical density of the corresponding polypeptide zone on the blotogram normalized to the actin content in each sample.

Statistical procedures. Statistica 10 software (StatSoft, Inc., USA) was used for statistical analysis. Means and their standard errors were calculated. Sample means were compared using analysis of variance (ANOVA), for posterior pairwise comparisons – Scheffe’s test; Fisher’s exact test was used to compare semiquantitative characteristics. P values < 0.05 were considered significant.

Results and Discussion

The study of the VEGF content by immunoblotting revealed its multiple increase in the retina tissue of rats with experimental diabetes (Fig. 1).

On the 7th day, the VEGF content exceeded the initial level (pre-DM control) by 10.3 times ($P < 0.05$). After 28 days, it was increased by 8.6 times compared to the initial level ($P < 0.05$). On the blotograms of the control samples after 7 and 28 days, bands at the level of about 28 kDa were detected, which corresponded to the content of the monomeric form of VEGF and indicated a high level of its synthesis.

Against the background of insulin administration, the content of VEGF in the retinal tissue (Fig. 1) remained high and, after 28 days, exceeded the initial level by 3.7 times ($P < 0.05$), but it was significantly lower than in the untreated control at day 28 (2.3 times; $P < 0.05$).

Administration of insulin with carbacetam blocked the hyperglycemia-induced increase in retina VEGF content (Fig. 1), which was practically no different from the initial level on the 28th day ($P > 0.05$). In the samples of this group, no band of the monomeric form of VEGF (28 kDa) was detected on the blotograms (Fig. 1, A), which indicated a low level of its synthesis.

Given the close connection between VEGF expression and the activity of the transcription factor HIF-1 α [3], the content of the latter in retinal tissue was analyzed by immunoblotting (Fig. 1). The initial level of HIF-1 α (pre-DM control) was almost indistinguishable from the basal values, reflecting the actual absence of its expression. The content of HIF-1 α rose to maximal values on the 28th day, reaching a maximum in the untreated control. When insulin was administered, it was the smallest (27.2 times

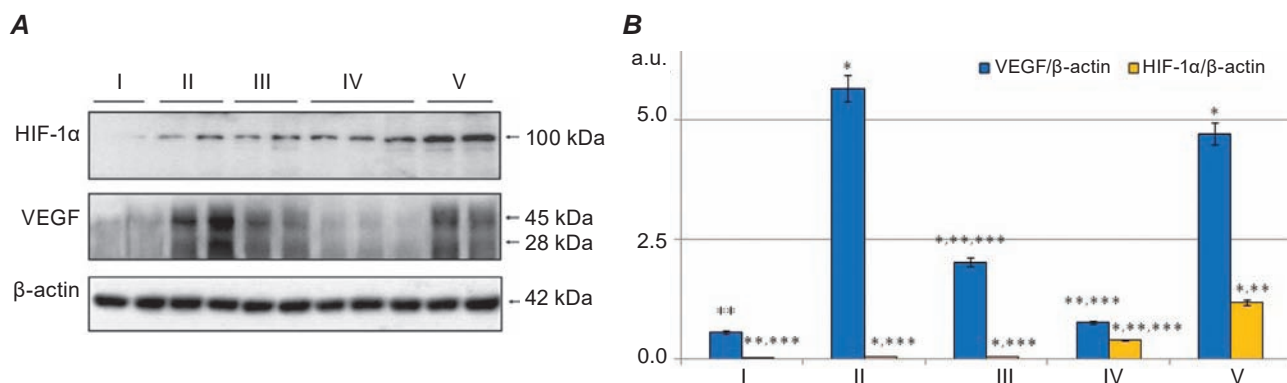


Fig. 1. Western blot analysis of hypoxia-inducible factor-1 α (HIF-1 α) and vascular endothelial growth factor (VEGF) levels in the retinal tissue: pre-DM control (I); 7 days – pre-treatment control (II); 28 days after insulin administration (III) and insulin and carbacetam co-administration (IV); 28 days – untreated control (V). **A** – representative blotograms of HIF-1 α , VEGF, and β -actin (loading control); **B** – results of densitometric analysis of protein levels normalized to β -actin content; vertical axis shows arbitrary units (a.u.); * $P < 0.05$ compared to the pre-DM control; ** $P < 0.05$ compared to the pre-treatment control; *** $P < 0.05$ compared to the untreated control

compared to untreated control; $P < 0.05$), and when insulin was administered with carbacetam, it was intermediate (3.0 times lower than untreated control; $P < 0.05$).

Thus, in this study, the development of DR already in the early stages (7-28 days) was accompanied by a progressive multiple increase in the content of VEGF and HIF-1 α in the retina, which was inhibited by the introduction of both insulin and insulin with carbacetam. At the same time, the effect of carbacetam actually blocked the increase in the VEGF content and significantly reduced the increase in the HIF-1 α content.

The obtained results also made it possible to establish an interesting feature of the VEGF/HIF-1 α ratio. If in the late period (28 days) the increase of VEGF could be associated with the increase of HIF-1 α , then in the early period there was no such connection. Therefore, it was possible to assume the presence of another, different from HIF-1 α , mechanism of VEGF overexpression in the early stages of DR development, for example, reactive gliosis, when activated glia become a source of inflammatory and growth factors [12]. Accordingly, the blocking of the increase in VEGF with the use of carbacetam indicated that the action of the drug is aimed at these pathways.

Morphologically, the development of DR was confirmed, which could be observed in the dynamics of observation in the untreated control (Fig. 2, a, d, g). A decrease in the density of cells in the nuclear

layers of the retina, swelling of all layers, especially the inner plexiform layer, vascular anomalies in the form of microaneurysms, dilatation of vessels, microthrombus formation, areas of ischemia were noted. Cytoplasmic vacuolization, cell swelling, nuclear pyknosis were noted in nerve cells, which was especially noticeable in ganglion cells. This reflected the development of microcirculation disorders, metabolism and degeneration of nerve cells.

In the dynamics, such changes increased according to the observation period (7th, 14th, and 28th days), while if signs of non-proliferative DR were detected at first, specific signs of proliferative DR were added on the 28th day (Fig. 2, g). Thus, microaneurysms were formed on the inner surface of the retina, in which foci of angiogenesis were formed (white arrows in Fig. 2, g) with proliferation of the endothelium and the formation of several microvascular lumens, closely located to each other and surrounded by a thick perivascular membrane. A large number of VEGF-positive cells were noted around such vessels. The accumulation of such cells in the inner layers of the retina preceded the formation of foci of angiogenesis (black arrows in Fig. 2, a, d), which corresponded to the initial phenomena of neovascularization in DR [19].

Another manifestation of proliferative DR was the formation on the 28th day in the outer retina layers of untreated control rats of cell proliferates, formed from intensely basophilic rounded cells (yellow arrows in Fig. 2, g). This was likely a reflection

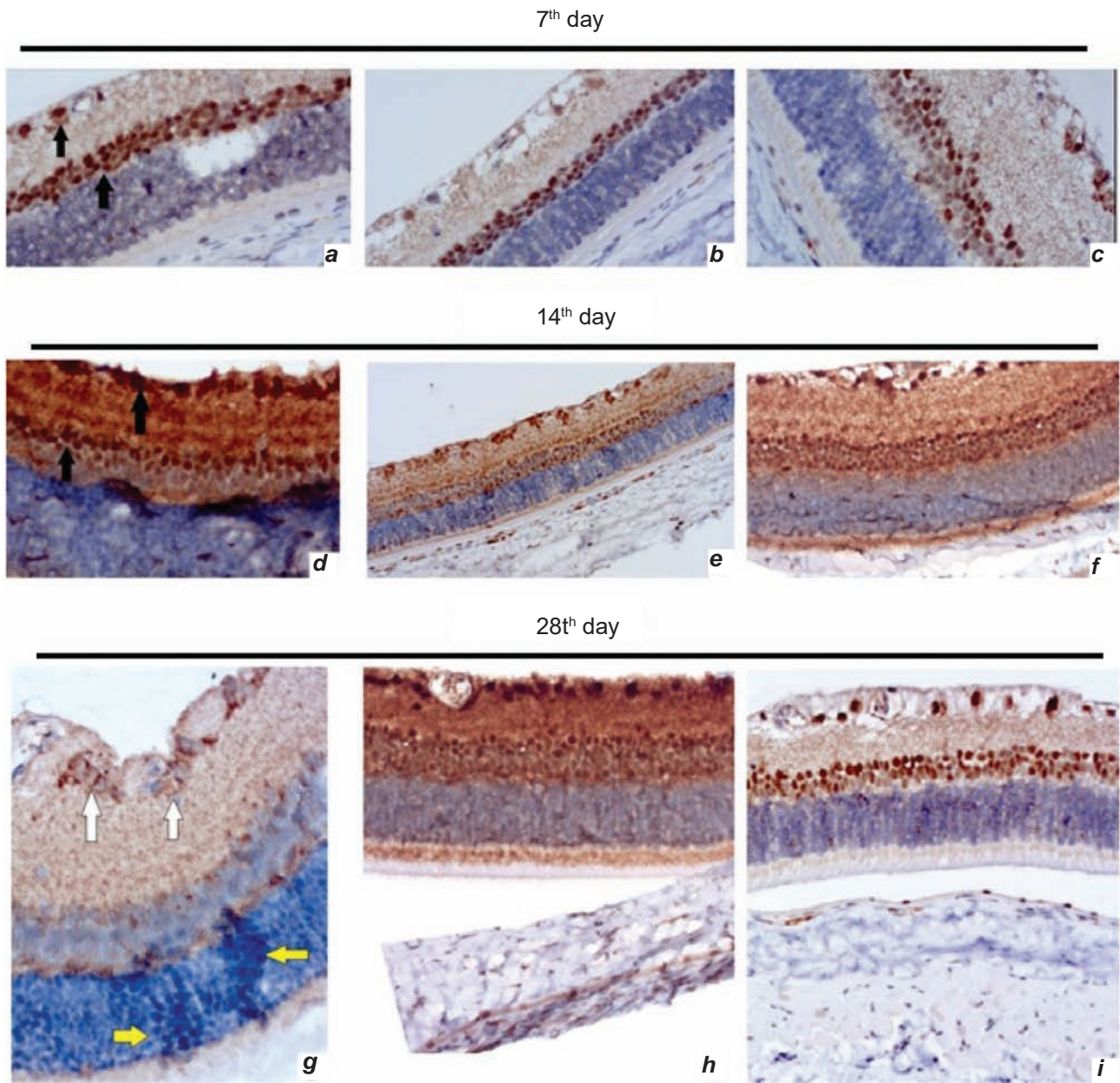


Fig. 2. Vascular endothelial growth factor (VEGF) immunostaining in the rat retina (representative microphotographs, counterstaining with hematoxylin): a-f×200; g-i×400; a, d, g – untreated control; b, e, h – insulin administration; c, f, i – administration of insulin and carbacetam. a, d – black arrows: VEGF-positive cells in the inner retina layers; g – white arrows: microaneurysms with foci of angiogenesis and numerous VEGF-positive cells; yellow arrows: cellular proliferations in the outer retina layers

of the glial-mesenchymal transition of Müller cells, which is the main fibrogenic mechanism in proliferative DR [20]. Transdifferentiation of Müller glial cells into myofibroblasts occurs under the influence of trophoblastic growth factor (TGF- β) against the background of VEGF overexpression [20]. As shown above, overexpression of VEGF according to immunoblotting data was noted at earlier terms.

Against the background of insulin administration, a decrease in the intensity of VEGF expression was noted (Fig. 2, b, e, h). On the 7th day (Fig. 2, b), immunospecific staining remained in the rounded cells of the inner nuclear layer (probably Müller cells). Morphologically, insignificant signs of non-proliferative DR were identified at this time, mainly in the form of microaneurysms of the retina. On the

14th day (Fig. 2, *e*), immunospecific staining was also detected in the inner layers of the retina, surrounding microaneurysms, and in addition, in the vessels of the choroid plexus. On the 28th day (Fig. 2, *h*), the intensity of VEGF-positive staining was the greatest, but no signs of proliferative DR were observed at this time, unlike the control group.

The combined administration of insulin and carbacetam made it possible to significantly prevent the development of DR (Fig. 2, *c, f, i*). Weak VEGF-positive staining was noted in individual cells surrounding slightly dilated vessels in the inner layers of the retina, individual rounded cells of the inner nuclear layer. At the same time, expanded vessels did not form foci of angiogenesis, and the formation of cellular proliferates was not detected in the outer layers. Morphologically, the layers of the retina were in a preserved state at all observation periods.

The highest intensity of VEGF-specific staining was noted in cells of the inner nuclear layer, probably Müller cells [3, 10]. To assess the degree of expression of VEGF in these cells, their number was calculated depending on the intensity of staining in animal groups on the 28th day (Fig. 3).

When insulin and both insulin with carbacetam were administered, the proportion of non-stained and weakly stained cells with an intensity of 0-1 points increased against the background of a decrease in the number of intensively stained cells (2-3 points). Such results also confirmed the inhibitory effect of insulin and carbacetam on the activation of VEGF expression.

Thus, the high intensity of VEGF-positive staining was characteristic of foci of specific morphological manifestations of DR: on the inner retina surface – the formation of microaneurysms with proliferation of the endothelium and foci of angiogenesis, in the outer retina layers – the formation of cell proliferations. Administration of insulin and, to an even greater extent, insulin with carbacetam significantly reduced the expression of VEGF and the development of specific morphological manifestations of DR.

As it has been established today, the dysfunction of the retinal neurovascular unit contributes to such microvascular changes as microaneurysms, microhemorrhages, infarcts of nerve fibers (cotton wool spots), deposition of exudate (hard exudates), the formation of acellular and non-perfused capillaries, a decrease in the integrity and an increase in the permeability of retinal vessels [21]. We observed the beginning of the development of such changes on the 28th day of the experiment in the control group.

It is believed that hypoxia, through protection of HIF-1 α from degradation and formation of HIF-1 that activates VEGF expression, leading to vascular hyperpermeability and retinal neovascularization [3]. However, in our study, against the background of VEGF overexpression already from the 7th day, the increase in the content of HIF-1 α occurred only on the 28th day and, accordingly, did not explain the activation of VEGF in the early stages. The ability of carbacetam to reduce the early expression of VEGF indicated the involvement of GABA-ergic

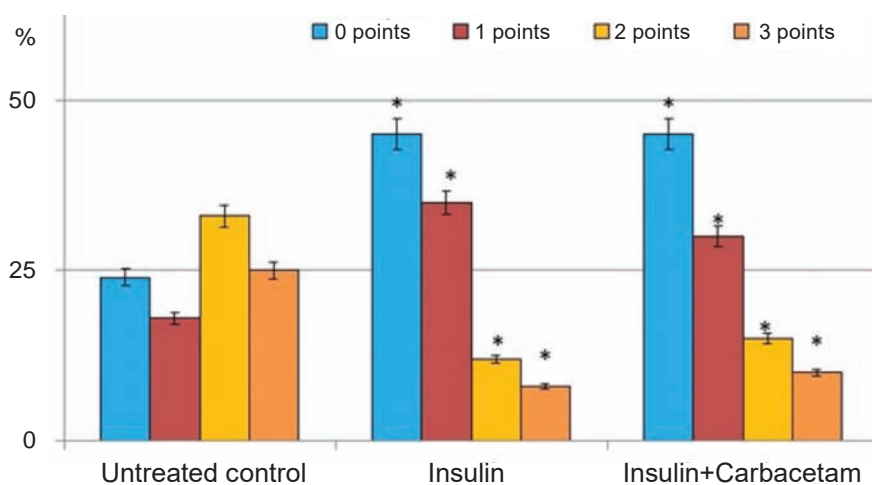


Fig. 3. Quantification of vascular endothelial growth factor (VEGF)-positive cells (%) in the rat retinal tissue at the 28th day of onset of diabetes (immunohistochemical data), according to the scale of D. J. Dabbs [18]; * $P < 0.05$ compared to the untreated control (Fisher's exactly test)

mechanisms in this process. It is significant that in our studies VEGF activation explosively covered all layers of the retina, starting with the layer of ganglion cells, then nuclear layers, and later all layers of the retina. Logically, this can be connected with the insufficiency of the inhibitory mechanisms of nervous regulation, namely, the insufficiency of GABA-ergic signaling [13], the deficiency of which under the conditions of DR was restored by carbacetam.

The principle possibility of VEGF expression activation through HIF-1-independent mechanisms was shown for Hep3B hepatocellular carcinoma cells [22], where VEGF regulation is mainly controlled by the Akt/PI3K and SP1 pathways and is independent of HIF-1 under hypoxic conditions.

In addition, during hyperglycemic stress, microglia are activated and can contribute to neuronal damage due to the secretion of neurotoxic and proinflammatory factors [23]. Our studies do not exclude the possibility of VEGF expression by activated microglia. Involvement of Müller cells and astrocytes leads to the development of reactive gliosis, which is also associated with overexpression of VEGF and proinflammatory cytokines [24]. In DM, Müller cells have a number of abnormal functions, they become gliotic, impair potassium deposition, glutamate and GABA uptake, and express angiogenesis modulators [25]. It is the excessive activation of glia that is the cause of specific diabetic neurodegeneration of the retina [26]. Therefore, the effect of carbacetam could also be explained by the inhibitory effect on micro- and macroglia under DR conditions.

In our study, it was clearly shown that the main pathological processes in the retina, namely reactive gliosis, angiopathy, and cellular proliferations, occur almost simultaneously already in the early stages of DR. The analysis of literary data proves that the cause of their development is a universal mechanism of activation of pathological cellular cascades with the formation of an excessive amount of proinflammatory mediators and growth factors [3, 5, 6, 12]. However, the positive effects of the GABA-ergic drug highlight the role of neurochemical disorders, the correction of which can significantly restore the dysfunction of the retinal neurovascular unit and inhibit the overexpression of cellular regulators. *In vitro* studies have shown that changes in the activity of retinal neurons in DR are associated with a decrease in inhibitory processes due to GABA deficiency, an increase in the release of glutamate, and an increase in the excitation of ganglion cells [27].

The study of neuroretinal dysfunction in the early stages of DM [28] sheds light on possible cellular mechanisms of the obtained results. Normally, amacrine cells of type A17 provide inhibition of GABA-ergic feedback on bipolar cells. In DM, the calcium permeability of synaptic receptors of A17 cells is reduced, which leads to inhibition of GABA release with subsequent disinhibition and increase of glutamate release from bipolar cells [28].

Our study shows the perspectives of a trial of carbacetam for the prevention of diabetic retina damage. Its main effect is modulating, which prevents excessive activation of regulatory systems under the influence of pathogenic factors [15-17]. Such an effect is generally characteristic of benzodiazepines, which, for example, in Alzheimer's disease improve clinical outcomes due to allosteric modulation of the GABA effect [29]. The results of our study are in agreement with very recent report, in which N-GABA treatment of rats with streptozotocin-induced diabetes has been shown to improve angiogenic signaling, inhibit apoptosis, and preserve the integrity of blood-brain barrier in brain tissue [30]. Thus, restoration of GABA-ergic signaling can be used as a promising therapeutic option for the correction of neuronal dysfunction in DR.

Conclusions. The conducted study showed that during the development of experimental DM, morphological signs of DR developed in the animal eyes after 7 days and within 28 days, which was accompanied by a multiple increase in the retinal VEGF content. Insulin administration decreased VEGF content, while administration of insulin with carbacetam blocked the increase in retina VEGF content. The content of HIF-1 α in the retina increased only on the 28th day, the introduction of insulin, as well as insulin with carbacetam, reduced this increase. Immunohistochemical study revealed a significant activation of VEGF expression both in the inner (in foci of angiogenesis) and in the outer (in newly formed cell proliferates) retina layers. Administration of insulin and, to a greater extent, insulin with carbacetam allowed to significantly prevent the DR development and reduce the intensity of VEGF-positive staining in all retina layers.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. The authors declare that this study was conducted at the initiative of the Department of Pathophysiology of the Bogomolets National Medical University (Kyiv, Ukraine) and was funded by the budget program of the Ministry of Health of Ukraine, state registration No 0122U001308.

АГОНІСТ БЕНЗОДІАЗЕПІНОВИХ РЕЦЕПТОРІВ КАРБАЦЕТАМ МОДУЮЄ ВМІСТ ФАКТОРА РОСТУ ЕНДОТЕЛІЮ СУДИН У СІТКІВЦІ ЩУРІВ ЗІ СТРЕПТОЗОТОЦИН-ІНДУКОВАНИМ ДІАБЕТОМ

С. В. Зяблицев¹✉, Д. Б. Жупан¹,
А. О. Тихомиров², О. О. Дядик³

¹Національний медичний університет імені О. О. Богомольця, Київ, Україна;

²Інститут біохімії ім. О. В. Палладіна НАН України, Київ, Україна;

³Національний університет охорони здоров'я України імені П. Л. Шупика, Київ, Україна;
✉e-mail: zsv1965@gmail.com

Одним із основних механізмів нейродегенерації сітківки при цукровому діабеті є дефіцит гамма-аміномасляної кислоти (ГАМК), що робить використання модуляторів ГАМК-бензодіазепінових рецепторів перспективним варіантом корекції цього діабетичного ускладнення. Метою цього дослідження було визначити вплив агоніста бензодіазепінових рецепторів карбацетама на експресію васкулярного ендотеліального фактора росту (VEGF) і гіпоксія-індуцибельного фактора-1 α (HIF-1 α) у сітківці ока щурів із гіперглікемією. Експериментальний діабет моделювали одноразовим введенням стрептозотоцину (50 мг/кг) тримісячним щурам-самцям лінії Вістар. Імуноблотинг та імуногістохімічні дослідження проводили з використанням моноклональних антитіл проти VEGF і HIF-1 α . Показано, що розвиток діабетичної ретинопатії (ДР) на ранніх стадіях супроводжувався прогресуючим багаторазовим збільшенням у сітківці вмісту VEGF на 7-28 добу та HIF-1 α на 28 добу. Лікування інсуліном та інсуліном+карбацетамом значно зменшувало спричинену діабетом гіперекспресію як HIF-1 α , так і VEGF. Показано, що карбацетам блокує діабетогенне збільшення вмісту VEGF у сітківці. Введення інсуліну з карбацетамом

значно знижувало експресію VEGF і розвиток специфічних морфологічних проявів ДР. Таким чином, відновлення ГАМК-ергічної сигналізації може бути використано як перспективний терапевтичний варіант для корекції розладів ДР.

Ключові слова: VEGF, гіперглікемія HIF-1 α , стрептозотоцин-індукований діабет, ретинопатія, ГАМК-бензодіазепінові рецептори, карбацетам.

References

1. Antonetti DA, Silva PS, Stitt AW. Current understanding of the molecular and cellular pathology of diabetic retinopathy. *Nat Rev Endocrinol.* 2021; 17(4): 195-206.
2. Simó R, Simó-Servat O, Bogdanov P, Hernández C. Neurovascular Unit: A New Target for Treating Early Stages of Diabetic Retinopathy. *Pharmaceutics.* 2021; 13(8): 1320.
3. Wang W, Lo ACY. Diabetic Retinopathy: Pathophysiology and Treatments. *Int J Mol Sci.* 2018; 19(6): 1816.
4. Potente M, Carmeliet P. The Link Between Angiogenesis and Endothelial Metabolism. *Annu Rev Physiol.* 2017; 79: 43-66.
5. Wong TY, Cheung CM, Larsen M, Sharma S, Simó R. Diabetic retinopathy. *Nat Rev Dis Primers.* 2016; 2: 16012.
6. Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. *JCI Insight.* 2017; 2(14): e93751.
7. Usui Y, Westenskow PD, Murinello S, Dorrell MI, Schepke L, Bucher F, Sakimoto S, Paris LP, Aguilar E, Friedlander M. Angiogenesis and Eye Disease. *Annu Rev Vis Sci.* 2015; 1: 155-184.
8. Sohn EH, van Dijk HW, Jiao C, Kok PH, Jeong W, Demirkaya N, Garmager A, Wit F, Kucukevcilioglu M, van Velthoven MEJ, DeVries JH, Mullins RF, Kuehn MH, Schlingemann RO, Sonka M, Verbraak FD, Abramoff MD. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. *Proc Natl Acad Sci USA.* 2016; 113(19): E2655-E2664.
9. Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, Lois N. The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res.* 2016; 51: 156-186.

10. Simó R, Hernández C, Porta M, Bandello F, Grauslund, J, Harding SP, Aldington SJ, Egan C, Frydkjaer-Olsen U, García-Arumí J, Gibson J, Lang GE, Lattanzio R, Massin P, Midea E, Ponsati B, Ribeiro L, Scanlon P, Lobo C, Costa MÁ, Cunha-Vaz J. Effects of Topically Administered Neuroprotective Drugs in Early Stages of Diabetic Retinopathy: Results of the EUROCONDOR Clinical Trial. *Diabetes*. 2019; 68(2): 457-463.
11. Maturi RK, Glassman AR, Josic K, Antoszyk AN, Blodi BA, Jampol LM, Marcus DM, Martin DF, Melia M, Salehi-Had H, Stockdale CR, Punjabi OS, Sun JK. Effect of Intravitreal Anti-Vascular Endothelial Growth Factor vs Sham Treatment for Prevention of Vision-Threatening Complications of Diabetic Retinopathy: The Protocol W Randomized Clinical Trial. *JAMA Ophthalmol*. 2021; 139(7): 701-712.
12. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia*. 2018; 61(9): 1902-1912.
13. Eggers ED. Visual Dysfunction in Diabetes. *Annu Rev Vis Sci*. 2023; 9: 91-109.
14. Ali SA, Zaitone SA, Dessouki AA, Ali AA. Pregabalin affords retinal neuroprotection in diabetic rats: Suppression of retinal glutamate, microglia cell expression and apoptotic cell death. *Exp Eye Res*. 2019; 184: 78-90.
15. Ziablitsev SV, Starodubskaya OO, Dyadyk OO. Influence of carbacetam on neurologic destruction processes under the experimental traumatic brain injury. *J Educ Health Sport*. 2017; 7(2): 601-611.
16. Kmet OG, Ziablitsev SV, Filipets ND, Kmet TI, Slobodian XV. Carbacetam effect on behavioral reactions in experimental Alzheimer's disease. *Arch Balk Med Union*. 2019; 54(1): 124-129.
17. Kmet OG, Ziablitsev SV, Filipets ND. Peculiarities of the antioxidant protection and nitrogen oxide systems of the brain in rats with experimental type 2 diabetes mellitus after carbacetam administration. *Int J Endocrinol*. 2019; 15(5): 376-380. (In Ukrainian).
18. Dabbs D. Diagnostic Immunohistochemistry, 4th Edition Theranostic and genomic applications. 2014. 960 p.
19. Proia AD, Caldwell MC. Intraretinal neovascularization in diabetic retinopathy. *Arch Ophthalmol*. 2010; 128(1): 142-144.
20. Wu D, Kanda A, Liu Y, Noda K, Murata M, Ishida S. Involvement of Müller Glial Autoinduction of TGF- β in Diabetic Fibrovascular Proliferation Via Glial-Mesenchymal Transition. *Invest Ophthalmol Vis Sci*. 2020; 61(14): 29.
21. Arboleda-Velasquez JF, Valdez CN, Marko CK, D'Amore PA. From pathobiology to the targeting of pericytes for the treatment of diabetic retinopathy. *Curr Diab Rep*. 2015; 15(2): 573.
22. Choi SB, Park JB, Song TJ, Choi SY. Molecular mechanism of HIF-1-independent VEGF expression in a hepatocellular carcinoma cell line. *Int J Mol Med*. 2011; 28(3): 449-454.
23. Chang KC, Shieh B, Petrash JM. Role of aldose reductase in diabetes-induced retinal microglia activation. *Chem Biol Interact*. 2019; 302: 46-52.
24. Sundstrom JM, Hernández C, Weber SR, Zhao Y, Dunkleberger M, Tiberti N, Laremore T, Simó-Servat O, Garcia-Ramirez M, Barber AJ, Gardner TW, Simó R. Proteomic Analysis of Early Diabetic Retinopathy Reveals Mediators of Neurodegenerative Brain Diseases. *Invest Ophthalmol Vis Sci*. 2018; 59(6): 2264-2274.
25. Fletcher EL, Phipps JA, Ward MM, Puthussery T, Wilkinson-Berka JL. Neuronal and glial cell abnormality as predictors of progression of diabetic retinopathy. *Curr Pharm Des*. 2007; 13(26): 2699-2712.
26. Lynch SK, Abramoff MD. Diabetic retinopathy is a neurodegenerative disorder. *Vision Res*. 2017; 139: 101-107.
27. Eggers ED, Carreon TA. The effects of early diabetes on inner retinal neurons. *Vis Neurosci*. 2020; 37: E006.
28. Castilho Á, Ambrósio AF, Hartveit E, Veruki ML. Disruption of a neural microcircuit in the rod pathway of the mammalian retina by diabetes mellitus. *J Neurosci*. 2015; 35(13): 5422-5433.
29. Al-Kuraishy HM, Al-Gareeb AI, Saad HM, Batiha GE. Benzodiazepines in Alzheimer's disease: beneficial or detrimental effects. *Inflammopharmacology*. 2023; 31(1): 221-230.
30. Tykhonenko T, Guzyk M, Tykhomyrov A, Korska V, Yanitska L, Kuchmerovska T. Modulatory effects of vitamin B3 and its derivative on the levels of apoptotic and vascular regulators and cytoskeletal proteins in diabetic rat brain as signs of neuroprotection. *Biochim Biophys Acta Gen Subj*. 2022; 1866(11): 130207.