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Relationship between changes in retinal brain-derived neurotrophic factor (BDNF) concentration and morphological changes in retinal neurons in rats with induced diabetes and axial myopia

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Background: *Myopia significantly decreases the frequency and severity of diabetic retinopathy (DR). The proliferative form of this diabetic complication in the retina is known to be very uncommon in diabetic myopes. The mechanisms of this phenomena are, however, still unclear. Studies on these mechanisms and the clarification of the structural changes in retinal neurons in animal models of both diabetes and myopia are critical to the research of the pathogenesis and further identification of therapeutic and preventive targets for these pathological conditions.*

Purpose: *To examine changes in the retinal brain-derived neurotrophic factor (BDNF) concentration and the relationship of the latter with the structure of retinal neuronal cells in rats with induced both diabetes and axial myopia.*

Material and Methods: *Rats (age, 2 to 10 weeks) were assigned to four groups: group 1 (myopia only, n = 15), group 2 (diabetes only, n = 15), group 3 (myopia plus diabetes, n = 15), and group 4 (healthy controls, n = 10). Axial myopia was produced in two-month-old animals by surgically fusing the eyelids of both eyes. Streptozotocin (STZ) (15 mg/kg body weight, intraperitoneally consequently for 5 days) was used to induce diabetes. Diabetes was induced in group 3 at 3 weeks after the initiation of the experiment. At 2 months, all rats were euthanized under anesthesia, and their eyes were enucleated. To perform a histomorphological study, serial retinal sections were made and stained with hematoxylin and eosin, and microscopy was performed and images were collected and evaluated on a light microscope Jenamed 2. Rat BDNF enzyme-linked immunosorbent kits (Elabscience, Houston, TX) were used to determine BDNF concentrations in retinal supernatant and plasma.*

Results: *Rats with both diabetes and myopia exhibited smaller reductions in plasma and, especially, retinal BDNF concentrations compared to rats with diabetes only. Retinal BDNF concentrations in rats with both diabetes and myopia were 36.1% higher than in rats with diabetes only. Unlike rats with STZ-induced diabetes only, those with STZ-induced diabetes in the presence of experimental myopia exhibited a rather high neuronal cell density in the retinal ganglion cell layer. No noticeable change in the cell density in the inner nuclear layer and photoreceptor layer was observed in the latter animals.*

Conclusion: *Axial length elongation secondary to experimental myopia in animals facilitates the protection against diabetic changes in the retina, which was confirmed at the molecular and morphological levels, with BDNF being a possible component of this protective mechanism.*

Keywords:

diabetic retinopathy, myopia, rats, retina, BDNF, retinal neuronal cells, structural changes, ganglion cells

Introduction

Although numerous studies have been conducted around the globe on the pathogenesis and methods of treatment of such a severe disease as diabetic retinopathy (DR), there are still open questions in this area.

The onset and development of DR under conditions of axial elongation or myopization of the globe have specific clinical features. It is known from clinical observations that, in myopia (particularly, high myopia), the frequency of the development of diabetic changes in the retina and the rate of retinopathy progression may be low. The number of publications on a reduced risk of DR in patients with myopia has been growing steadily [1-5].

At present, the mechanism of the protective effect of myopia in DR is not understood, and the relationship with the structural changes in the eye and ocular components is still to be elucidated. Although different metabolic factors of the pathogenesis of DR and myopia have been investigated in recent decades, the features of pathogenetic mechanisms of diabetic changes in the retina and choroid in the presence of high myopia have not been studied sufficiently yet.

In a study with a large population of diabetics with available axial length measurements [3], a shorter axial length was the major factor associated with a higher prevalence of DR. This was also found in our previous experimental animal studies [6].

Brain-derived neurotrophic factor (BDNF) interacts with tropomyosin-related kinase B (TrkB) to promote neuronal growth, survival, differentiation, neurotransmitter release, and synaptic plasticity. It plays an important role in neuronal survival and growth. It has been demonstrated that BDNF is neuroprotective against ischemic injury [7]. This neurotrophin is also released from retinal ganglion cells. Because retinal ganglion cells are damaged in DR, it has been suggested that BDNF is a potential biomarker for DR [8].

Akamatsu and colleagues (2001) conducted a study on chicks with experimentally induced myopia [9] and found that neurotrophin (NT)-3, nerve growth factor (NGF) and BDNF were not differentially expressed in experimentally induced myopic retinal samples compared with controls, which might indicate the absence of apoptosis of retinal ganglion cells in myopia.

The purpose of this study was to examine changes in the retinal BDNF concentration and the relationship of the latter with the structure of retinal neuronal cells in rats with induced both diabetes and axial myopia.

Material and Methods

All animal experiments were performed in compliance with the provisions of the European Convention on the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and Guidelines for Works Involving Experimental Animals approved by the Law of Ukraine on the Protection of Animals from Cruelty (No. 1759-VI dated December 15, 2009) and decision of the local bioethics committee (dated September, 2021). Rats (age, 2 to 10 weeks) were assigned to four groups: group 1 (myopia only, n = 15), group 2 (diabetes only, n = 15), group 3 (myopia plus diabetes, n = 15), and group 4 (healthy controls, n = 10).

High axial myopia was produced in animals by surgically fusing the eyelids of both eyes and exposure to reduced lighting conditions for 2 weeks [10]. Streptozotocin (STZ) (a subdiabetic dose of 15 mg/kg body weight, intraperitoneally consequently for 5 days) was used to induce diabetes, given the age of animals. Control animals were housed in normal lighting conditions and fed conventionally. At 3 weeks after the initiation of the experiment, diabetes was induced in group 3. In addition, diabetes was induced in intact rats. At 2 months, all rats were euthanized under anesthesia, and their eyes were enucleated.

Histological studies of rat ocular tissues were conducted. The globes were fixed with 10% neutral buffered formalin, processed and paraffinized. Paraffin blocks were sectioned serially and stained with hematoxylin and eosin. Thereafter, microscopy was performed, and images were collected and evaluated on a light microscope Jenamed 2 (Carl Zeiss Jena, Jena, Germany) at 100x, 200x and 400x magnifications.

ELISA kits (Rat BDNF ELISA kit, Elabscience, Houston, TX) were used to determine BDNF concentrations in retinal supernatant and plasma as per the manufacturer's instructions, and total protein concentrations were determined by the Lowry method.

Retina was homogenized ultrasonically in 50 mM Tris-HCl buffer, pH 7.5, with protease inhibitors. Thereafter, the retinal homogenates were centrifuged for 15 min at 12,000 g and 4 °C, and retinal supernatants were collected. BDNF concentration was expressed in pg/mg protein.

Table 1. Retinal and plasma brain-derived neurotrophic factor (BDNF) levels (expressed in pg/mg protein) in control rats, rats with streptozotocin-induced diabetes only and rats with streptozotocin-induced diabetes in the presence of form-deprivation myopia

Statistical characteristics	Controls (n = 10)	Diabetes only (n = 15)	Myopia plus diabetes (n = 15)
Plasma BDNF levels			
M±m	5.24±0.35	3.76±0.24	4.29±0.32
%	100.0	71.8	81.9
p	-	<0.05	<0.05
%1	-	100	114.0
p1	-	-	>0.05
Retinal BDNF levels			
M±m	23.78±1.96	14.83±1.24	20.19±1.73
%	100.0	62.4	84.9
p	-	<0.01	>0.05
%1	-	100.0	136.1
p1	-	-	<0.01

Note: n, number of animals; p, significance of difference compared to control; p1, significance of difference compared to diabetes only

Statistical analyses were conducted using Statistica software (StatSoft, Tulsa, OK, USA). Data are presented as mean and standard error. The level of significance $p < 0.05$ was assumed.

Results

There were no significant differences between myopic rats and controls in plasma BDNF concentration or retinal BDNF concentration, which is in agreement with the findings of a study by Akamatsu and colleagues [9].

In addition, we determined plasma BDNF concentration and retinal BDNF concentration in rats with diabetes only and in those with diabetes plus myopia (Table 1).

We found changes in BDNF concentrations in experimental groups, but there was a difference in the magnitude of changes between them. Retinal and plasma BDNF concentrations in rats with diabetes only were 37.6% and 28.2% lower, respectively, than in controls ($p < 0.05$).

The reductions in blood and retinal BDNF concentrations were, however, less pronounced in rats with both diabetes and myopia than in those with diabetes only. Thus, plasma and retinal BDNF concentrations in rats with both diabetes and myopia were 18.1% ($p < 0.05$) and 15.1% ($p > 0.05$) lower, respectively, than in controls. In addition, plasma and retinal BDNF concentrations in rats with both diabetes and myopia were 14.0% ($p > 0.05$) and 36.1% ($p < 0.01$) higher, respectively, than in rats with diabetes only.

Therefore, in the current experimental study, reductions in the plasma BDNF concentration and especially retinal BDNF concentration were significantly lower in rats with retinal diabetic changes accompanied by myopization of the globe than in rats without myopia. We believe that in the group of diabetic rats with myopia, BDNF could act largely as a neuroprotective factor and exert retinal protection.

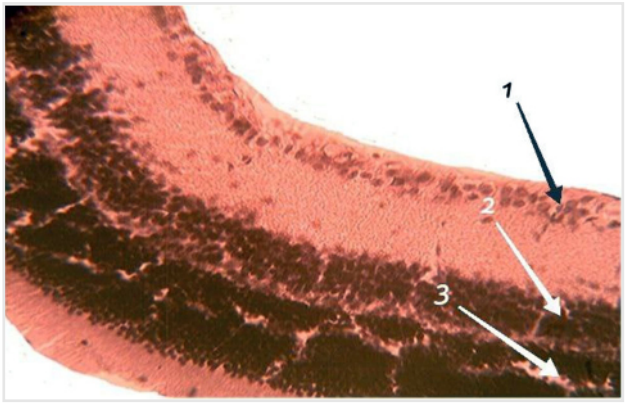

Fig. 1. Histological slide of the retina from a control rat. Magnification, 200x. Note: 1, ganglion cell layer; 2, inner nuclear later; 3, retinal photoreceptor cell layer

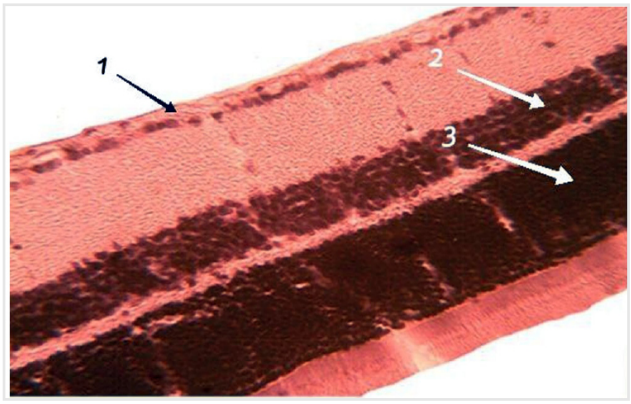

Fig. 2. Histological slide of the retina from a rat with axial myopia. Magnification, 200x. Note: 1, ganglion cell layer; 2, inner nuclear later; 3, photoreceptor cell layer

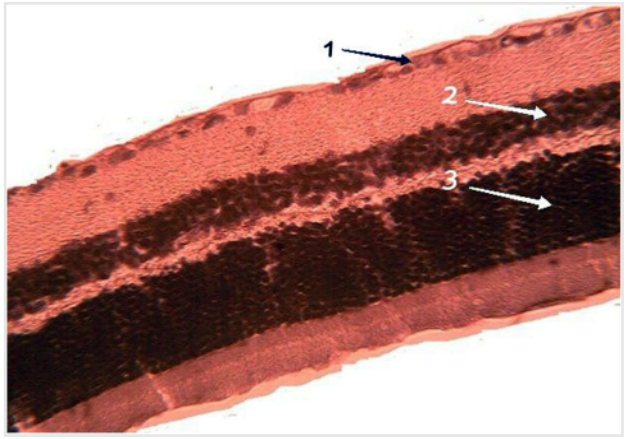

Fig. 3. Histological slide of the retina from a rat with streptozotocin-induced diabetes only. Magnification, 200x. Note: 1, ganglion cell layer; 2, inner nuclear later; 3, photoreceptor cell layer

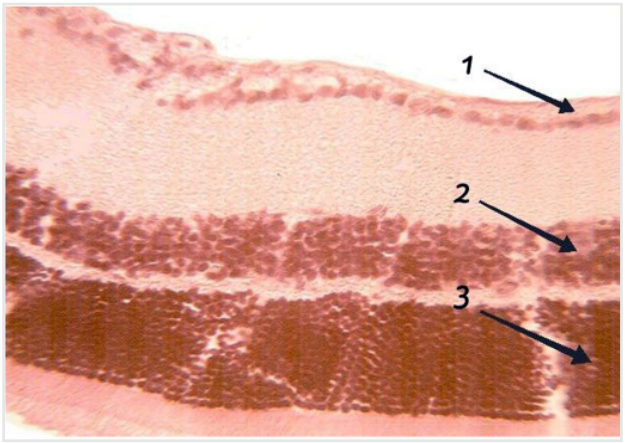

Fig. 4. Histological slide of the retina from a rat with streptozotocin-induced diabetes in the presence of myopia. Magnification, 200x. Note: 1, ganglion cell layer; 2, inner nuclear later; 3, photoreceptor cell layer

Morphological state of retinal neuronal cells

The next phase of the study was the examination of the retinal structure in rats with induced diabetes only, rats with induced axial myopia only, and rats with induced diabetes in the presence of induced myopia. The results of histomorphological studies indicated that these groups exhibited substantial differences in the state of retinal neuronal cells (Figs. 1-4).

Histological examination at high power of magnification (200x) demonstrated a high density of ganglion cells (20 to 25 cells in the field of vision) in the retina of control rats (Fig. 1).

The retinal inner nuclear layer and photoreceptor cell layer showed high densities of cells. There was a difference in the severity of morphological abnormalities in retinal neuronal cells between histological preparations from the three experimental groups. The density of neurons in the ganglion cell layer in rats with axial myopia (15 to 20 cells in the field of vision; Fig. 2) was close to but somewhat lower than in controls. No change in the neuronal density in the retinal inner nuclear layer and photoreceptor cell layer was observed in the slides from rats with axial myopia.

The histological picture of the retina in rats with STZ-induced diabetes only (Fig. 3) was characterized by the non-uniform density of neurons in the ganglion cell layer, with some sites showing some loss of ganglion cells and less than 10 ganglion cells in the field of vision at a large magnification. No noticeable change in the neuronal density in the inner nuclear layer and photoreceptor layer was observed in the slides from rats with STZ-induced diabetes only.

Unlike those from rats with STZ-induced diabetes only, slides from rats with STZ-induced diabetes in the presence of experimental myopia (Fig. 4) exhibited a rather high neuronal cell density (sometimes as high as 20 cells in the field of vision) in the retinal ganglion cells layer. No noticeable change in the neuronal density in the inner nuclear layer and photoreceptor layer was observed in the slides from rats with STZ-induced diabetes in the presence of experimental myopia.

Discussion

Rats with STZ-induced diabetes only were found to have the lowest retinal and plasma BDNF concentrations and also to show the greatest changes in the neuronal retinal structure (particularly, the lowest ganglion cell density) of the four groups. Of note, it is abnormalities in the ganglion cell layer that are the major pathological sign of both diabetes-induced retinal neuronal degeneration and the development of DR [11, 12].

The histomorphological and biochemical changes in the retina in rats with both experimental myopia and STZ-induced diabetes seem to corroborate the concept that myopic eyes with elongated axial length have capacity to buffer the development of signs of severe DR.

The model of STZ-induced diabetes has been proposed by Rakieten and colleagues in 1963 [13], and since that time has been widely used in research, with typical DM symptoms (persistent hyperglycemia, polyuria and polydipsia) reproduced in animals (rats, dogs and, less commonly, rabbits). STZ causes abnormalities in the islets of Langerhans and loss of beta cells [13]. The mechanism of action of STZ is inducing cell death via DNA fragmentation. The onset of retinal lesion formation in a rat model of STZ-induced diabetes is observed due to blood-retinal barrier breakdown about two weeks after diabetes induction. ONL thinning begins in the fourth week following induction, which forms the signs of DR [14, 15]. It is at these time points (4-5 weeks after diabetes induction) that we assessed both structural changes and the state of BDNF in the retina in the current study.

In a study by Guo and colleagues [16], decreased serum levels of BDNF were associated with DR and vision-threatening DR in Chinese type 2 diabetic patients, suggesting a possible role of BDNF in the pathogenesis of DR complications. Multivariate logistic regression analysis adjusted for common risk factors showed that serum BDNF levels were independent risk factors for DR and vision-threatening DR.

Ola and colleagues [17] observed reduced levels of BDNF both in the serum of patients with DR and in the retina of rats with STZ-induced diabetes. They concluded that the results indicated that reduced levels of BDNF in diabetes may cause apoptosis and neurodegeneration early in diabetic retina, which may lead to neurovascular damage later in DR.

Conclusion

We found a reduction in retinal and plasma BDNF concentrations in a rat model of DM. These metabolic changes were accompanied by the structural changes in retinal neurons with the features (e.g., a reduced number of ganglion cells) typical of DR. Diabetes modeling in rats with primarily induced axial myopia was characterized by significantly smaller changes in retinal and plasma BDNF concentrations, and was accompanied by substantially better retinal neuronal morphology. Histological studies indicated some preservation of cell density in the retinal ganglion layer in experimental diabetes in the presence of myopia, which could serve as a structural equivalent of better preservation of retinal function in this experimental condition compared to experimental diabetes only or experimental myopia only. No notable structural changes in inner nuclear later or retinal photoreceptor layer were observed. Therefore, axial length elongation secondary to experimental myopia in animals facilitates the protection against diabetic changes in the retina, which was confirmed at the molecular and morphological levels, with BDNF being a possible component of this protective mechanism.

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Disclosures

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