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Neurodegenrative changes in retina of rats with streptozotocin-induced diabetes under conditions of treatment with niacin-oxy-ethylidene-diphosphonate germanate (MIGU-4)

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Background: *Diabetes is accompanied by neurodegenerative changes in the retina. The determination of retinal function parameters under conditions of experimental diabetes may allow for the development of new approaches to pharmacological vision correction.*

Purpose: *To assess changes in the metabolic (lipid peroxidation and antioxidant defence) and functional characteristics (electroretinography (ERG)) of the retina in the rat with streptozotocin (STZ)-induced diabetes, treated with niacin-oxy-ethylidene-diphosphonate germanate (MIGU-4) and a reference drug, diazepam.*

Material and Methods: *Diabetes was induced intraperitoneally (i.p.) in Wistar male rats by streptozotocin (STZ 65 mg/kg). Four weeks thereafter, the rats received a two-week course of a daily dose of MIGU-4 (5.0 mg/kg or 25.0 mg/kg) or diazepam (0.5 mg/kg or 1.5 mg/kg). The electroretinogram (ERG) was obtained, the animals were euthanized and retinal malondialdehyde (MDA) levels and superoxide dismutase (SOD) and catalase (CAT) activity were determined.*

Results: *In rats with untreated STZ-induced diabetes, the retinal MDA level was 3.71 times increased and CAT activity was 35% lower, compared to controls, whereas the SOD activity was half of the activity found in controls ($p < 0.05$). In diabetic animals treated with MIGU-4 i.p. at a 25.0-mg/kg daily dose, the retinal MDA level was 63.2% lower, whereas the CAT activity and SOD activity were 41.9% and 27.6%, respectively, higher, than in untreated diabetic rats ($p < 0.05$). In diabetic animals treated with diazepam i.p. at a 1.5-mg/kg daily dose, the retinal MDA level was 59.2% lower, whereas the CAT activity and SOD activity were 44.4% and 32.1%, respectively, higher, than in untreated diabetic rats ($p < 0.05$). In untreated diabetic rats, the ERG b-wave amplitude was 39.2% lower, ERG a-wave and b-wave latencies, 23.4% and 14.0%, respectively, higher, and the a-wave amplitude recovery rate, 38.8% lower than in controls ($p < 0.05$). The use of a 25.0-mg/kg daily dose of MIGU-4 resulted in a 29.7% and 33.9%, respectively, increase in the ERG b-wave amplitude and the a-wave amplitude recovery rate compared to untreated diabetic rats ($p < 0.05$). The use of a 1.5-mg/kg daily dose of diazepam resulted in a 25.0% and 30.0%, respectively, increase in the above ERG parameters compared to untreated diabetic rats ($p < 0.05$). In untreated diabetic rats, the number of squares crossed (SK) was 29.4% lower ($p < 0.05$); the number of central squares crossed (CSC), 52.1% lower ($p < 0.05$); the number of upright postures (UP), 34.2% lower; and the defecation bolus number, 32.4% higher than in controls ($p < 0.05$). After MIGU-4 (25-mg/kg) withdrawal, there were no more differences in SK, CSC, UP and defecation bolus numbers between treated diabetic rats and controls. In addition, the number of CSC was 4.27 times lower than in controls, and half of that in untreated diabetic animals ($p < 0.05$). In diabetic rats treated with diazepam, the number of UP was 2.56 times lower than in controls ($p < 0.05$), and 40.7% lower than in untreated diabetic rats ($p < 0.05$), whereas the defecation bolus number was 40.5% higher than in controls ($p < 0.05$).*

Conclusion: *STZ-induced diabetes is accompanied by an impairment of the oxidant/ antioxidant balance in the retina with an increase in retinal MDA, decrease in retinal SOD and CAT activity, and ERG abnormalities such as reduced ERG amplitude and increased ERG latency. A course treatment with niacin-oxy-ethylidene-diphosphonate germanate (MIGU-4) i.p. at a daily dose of 25.0 mg/kg provides for a decrease in retinal MDA and an increase in retinal SOD and CAT activity and ERG wave amplitude The withdrawal of a two-week treatment with MIGU-4 i.p. at a daily dose of 25.0 mg/kg decreased open-field anxiety-like behaviors, whereas the withdrawal of a two-week treatment with diazepam i.p. at a daily dose of 1.5 mg/kg increased open-field anxiety-like behaviors. The corrective effect of treatment with MIGU-4 corresponds to the corrective effect of treatment with diazepam.*

Keywords:

streptozotocin-induced diabetes, retinopathy, niacin-oxy-ethylidene-diphosphonate germanate (MIGU-4), oxidative stress, neuroprotection, retina

Introduction

A typical diabetic retinopathy mechanism involves the formation of peroxy compounds followed by retinal alteration with metabolic, neurodegenerative and functional abnormalities of the retina [1, 2]. Correspondingly, antioxidant administration prevents neuroimmune inflammation, promotes the correction of diabetic retinopathy symptoms and prevents progressive impairment [3, 4].

In recent years, the anti-inflammatory activity of germanium containing organic compounds has been demonstrated [5, 6]. In addition, germanium-containing agents have demonstrated anti-oxidative capacity and thus prevent degenerative cellular changes [7]. Compounds of germanium with oxy-ethylidene phosphonic acid have a low toxicity and high biological activity. In addition, derivatives of these compounds have demonstrated a high clinical efficacy for the regulation of mineral metabolism, in anti-tumor and anti-inflammatory therapy, particularly under conditions of the onset of neuroimmune inflammation-based pathological processes [5, 8]. Niacin oxy-ethylidene-diphosphonate germanate (MIGU-4) promptly enters systemic circulation, is readily available to body tissues, have antioxidative and hepathoprotective effects, and decreases the excitability of brain structures [5-8].

Therefore, **the purpose** of the study was to assess changes in the metabolic (lipid peroxidation and antioxidant defence) and functional characteristics (electroretinography (ERG)) of the retina in the rat with streptozotocin (STZ)-induced diabetes, treated with niacin-oxy-ethylidene-diphosphonate germanate (MIGU-4) and a reference drug, diazepam.

Material and Methods

Experimental animals

Sixty-one Wistar male rats (age, 2 months; mass, 180–220 g) were used in this experimental study. They were maintained in an environmentally controlled room (23 ± 2 °C, 60% humidity) on a 12:12-h light:dark cycle, and fed and watered ad libitum. All animal experiments adhered to the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and the Helsinki Declaration. The study was approved by the Bioethics Committee of Odesa National Medical University (Meeting Minutes No.3 dated March 14, 2018).

Model of diabetic retinopathy and groups of animals used in this study

STZ was dissolved in 0.01M sodium citrate (Sigma-Aldrich), pH of 4.5, and diabetes was induced by a single intraperitoneal (i.p.) injection of STZ at 65 mg/kg [9]. One and two weeks after injection, each animal's diabetic status was confirmed by measuring blood glucose levels from tail tip samples taken under nonfasted condition. Only rats with a blood glucose level ≥ 300 mg/dl (16.7 mmol/L) were included in subsequent studies [4]. Blood glucose was tested at 9 am following ad libitum night feeding. Insulin was injected at a dose of 0-2 U subcutaneously 2-5 times a week throughout the experiment [1-4].

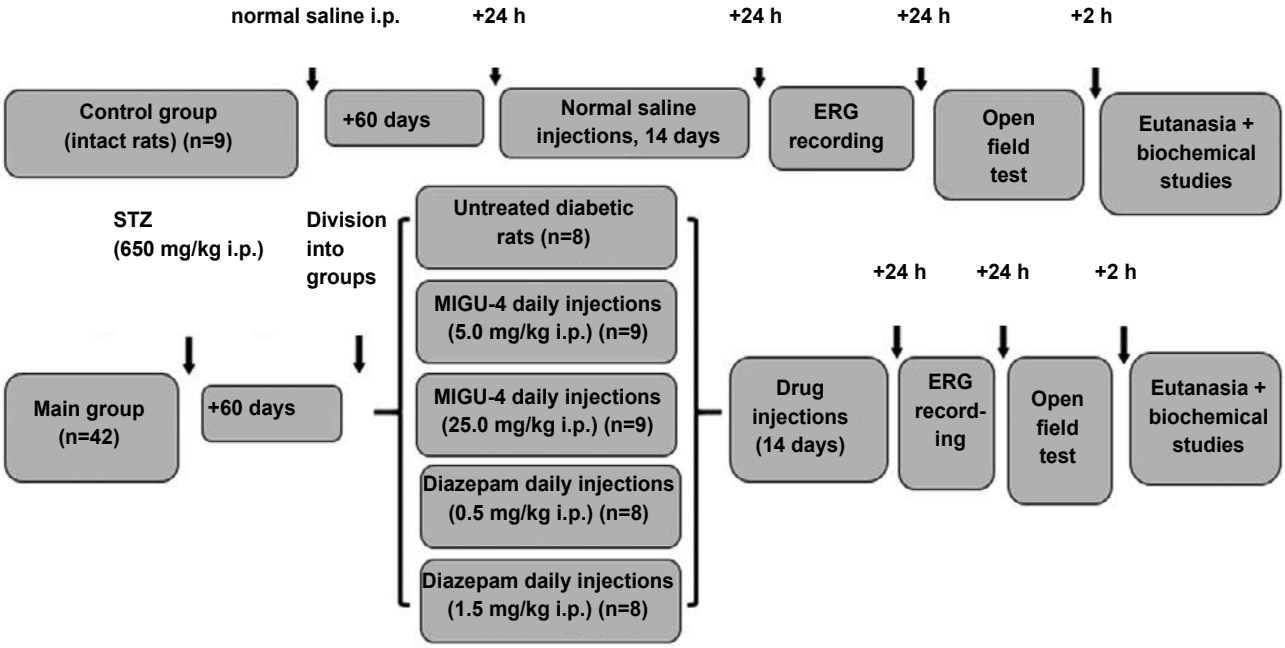

Fig. 1. Experiment design

Sixty days after the use of STZ, experimental animals were divided into observation groups. These groups of animals were treated with MIGU-4, a compound that was synthesized by a group guided by Prof. I.J. Seifullina at Odesa National Medical University. MIGU-4 dissolved in normal saline was administered intraperitoneally (i.p.) at a daily dose of 5.0 mg/kg or 25.0 mg/kg for 14 days. Given a high neurotropic activity of MIGU-4, diazepam was used as a reference drug since its high anti-inflammatory activity is well known [10, 11]. Rats were treated with diazepam (Calmpose, Ranbaxy Diagnostics, India) i.p. at a daily dose of 0.5 mg/kg or 1.5 mg/kg for 14 days (Fig. 1). Nine intact animals (controls) and eight untreated diabetic animals received similar ip doses of normal saline.

Therefore, the following groups of animals were used in this study (Fig. 1):

(i) the control group of 9 intact animals;

(ii) untreated diabetic animals (8 rats);

(iii) diabetic animals treated with MIGU-4 i.p. at a daily dose of 5.0 mg/kg (9 rats);

(iv) diabetic animals treated with MIGU-4 i.p. at a daily dose of 25.0 mg/kg (9 rats);

(v) diabetic animals treated with diazepam i.p. at a daily dose of 0.5 mg/kg (8 rats); and

(vi) diabetic animals treated with diazepam i.p. at a daily dose of 1.5 mg/kg (8 rats).

Methods

The electroretinogram (ERG) was obtained 24 hours after the last use of MIGU-4 or diazepam, as described previously [1]. A day thereafter, the animals were euthanized and decapitated, and the removed tissues were frozen and stored in liquid nitrogen. The removed retinal tissues were washed in phosphate-buffered saline (PBS) to remove blood components and then homogenized in 0.1 M PBS (pH 7.0; 10% w/v). Homogenized samples were subjected to centrifugation at 13,000 rpm for 15 min at 4oC. Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined and expressed in units (U) mg−1 protein [12]. Catalase (CAT, EC 1.11.1.6) activity was also determined and expressed as nmol H2O2 decomposed min-1 mg−1 protein [13]. After a homogenate was incubated at high temperature in an acid medium in the presence of sodium thiobarbiturate, malondialdehyde (MDA) content was measured spectrophotometrically at 532 nm [14]. MDA content was expressed as nmol mg−1 protein. The protein content was determined as described by Lowry et al [15].

Use (particularly, long-term use) of benzodiazepines can cause withdrawal symptoms such as increased anxiety [10, 11]. Motility effects of MIGU-4 were compared to those of diazepam in the open-field test [10], the latter being informative with regard to such symptoms. Withdrawal symptoms were assessed by the open-field test which was performed 24 hours after the last injection of MIGU-4 or diazepam.

The statistical software package Primer Biostatistics was used for statistical analysis. Values were compared using one-way ANOVA and Newman-Keuls test. P values < 0.05 were considered significant. Data are reported as mean plus or minus standard error of mean.

Results

In rats with untreated STZ-induced diabetes, the retinal MDA level was 3.71 times increased compared to controls ($p < 0.05$) (Table 1). In diabetic animals treated with MIGU-4 i.p. at a daily dose of 5.0 mg/kg, the retinal MDA level was 64.3% higher than in controls ($p < 0.05$). The retinal MDA level was 63.2% lower in diabetic animals treated with MIGU-4 i.p. at a high daily dose (25.0 mg/kg) than in untreated diabetic rats ($p < 0.05$). The retinal MDA level was 66.7% higher in diabetic animals treated with diazepam i.p. at a daily dose of 0.5 mg/kg, than in controls ($p < 0.05$), whereas in diabetic animals treated with diazepam i.p. at a daily dose of 1.5 mg/kg, the retinal MDA level was 59.2% lower than in untreated diabetic rats ($p < 0.05$).

In rats with untreated STZ-induced diabetes, the SOD activity was half of the activity found in controls ($p < 0.05$). The SOD activity was significantly lower in diabetic animals treated with MIGU-4 at a daily dose of 5.0 mg/kg and in those treated with diazepam at a daily dose of 0.5 mg/kg, than in controls ($p < 0.05$). However, the SOD

Table 1. Parameters of peroxidation in the retina of rats with streptozotocin-induced diabetes under conditions of treatment with MIGU-4 or diazepam

№	Parameter under study	Control (n=9)	Diabetes (n=8)	Diabetes + MIGU-4 (5.0 mg/kg) (n=9)	Diabetes + MIGU-4 (25.0 mg/kg) (n=9)	Diabetes + diazepam (0.5 mg/kg) (n=8)	Diabetes + diazepam (1.5 mg/kg) (n=8)
1	Malondialdehyde (nmol/mg protein)	1.34±0.15	4.97±0.51#	3.75±0.64#	1.83±0.22*	4.02±0.55#	2.03±0.28*
2	Superoxide dismutase (U/mg protein)	13.65±1.52	6.67±0.75#	8.13±0.90#	11.48±1.60*	7.52±0.69#	12.0±1.71*
3	Catalase (H2O2/min/mg protein, nM)	3.37±0.22	2.31±0.18#	2.50±0.21	3.19±0.30*	2.68±0.15	3.40±0.24*

Note: #, significant difference compared to controls ($P < 0.05$); *, significant difference compared to diabetic rats ($P < 0.05$; ANOVA+ Newman-Keuls test)

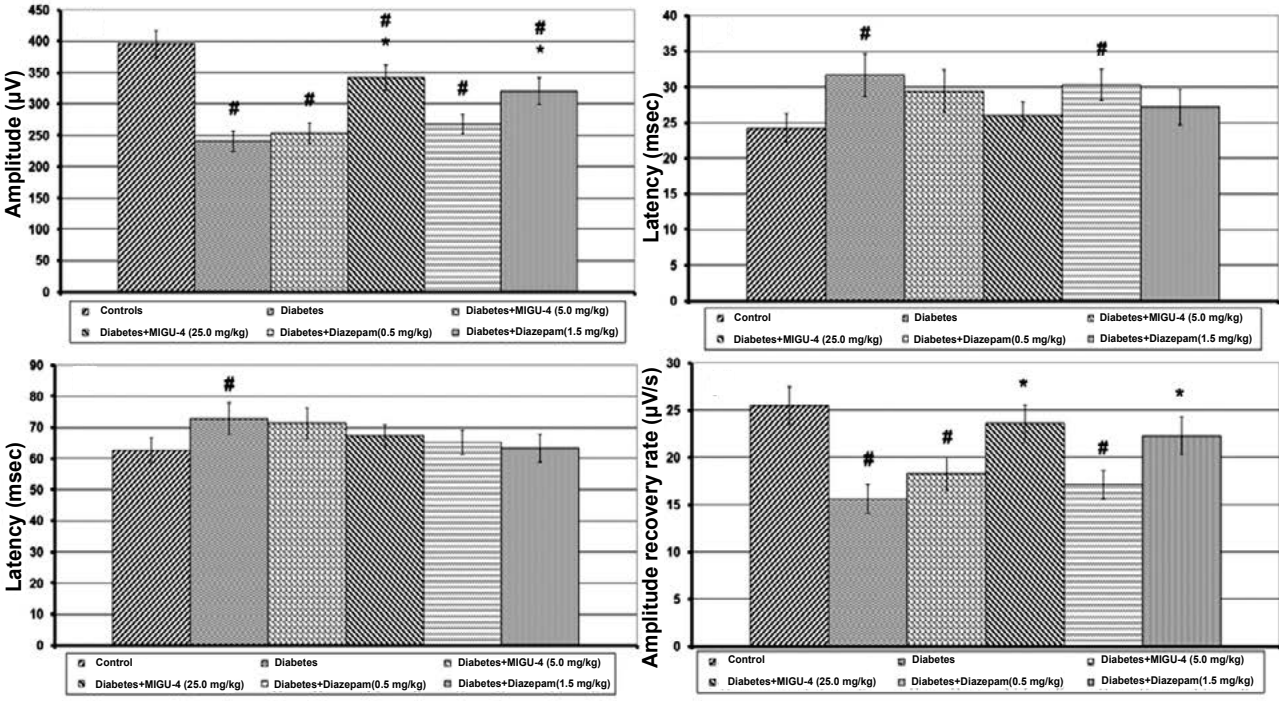

Fig. 2. Parameters of the ERG of rats with streptozotocin-induced diabetes under conditions of course treatment with MIGU-4 or diazepam. Note: A, b-wave amplitude; B, a-wave latency; C, b-wave latency; D, a-wave amplitude recovery rate; #, $p < 0.05$; *, $p < 0.05$, ANOVA+ Newman-Keuls test. The abscissa indicates animal groups used in the study, and the ordinate indicates ERG parameters.

activity was 41.9% and 44.4%, respectively, higher in diabetic animals treated with a high daily dose of MIGU-4 (5.0 mg/kg) and diazepam (1.5 mg/kg), than in untreated diabetic animals ($p < 0.05$).

CAT activity was 35% lower in rats with untreated STZ-induced diabetes than in controls ($p < 0.05$). In addition, CAT activity was 27.6% lower in diabetic rats treated with MIGU-4 at a daily dose of 25.0 mg/kg and 32.1% lower in those treated with diazepam at a daily dose of 1.5 mg/kg, than in controls ($p < 0.05$) (Table 1).

ERG b-wave amplitude was 39.2% lower in untreated diabetic rats than in controls ($p < 0.05$) (Fig. 2A). The use of a 5.0-mg/kg MIGU-4 daily dose or a 0.5 mg/kg diazepam daily dose resulted in an insubstantial (5.1% or 10.1%, respectively) increase in the ERG b-wave amplitude compared to untreated diabetic rats ($p > 0.05$), with the parameter being significantly lower than in controls ($p < 0.05$). The use of a 25.0-mg/kg MIGU-4 daily dose or a 1.5-mg/kg diazepam daily dose resulted in a 29.7% or 25.0%, respectively, increase in the ERG a-wave amplitude compared to untreated diabetic rats ($p < 0.05$).

ERG a-wave and b-wave latencies were 23.4% and 14.0%, respectively, higher in untreated diabetic rats than in controls (both $p < 0.05$) (Fig. 2. B and C). The use of MIGU-4 or diazepam resulted in no significant change ($p > 0.05$) in ERG a-wave and b-wave latencies compared to controls or untreated diabetic rats. The only exception was a 20.1% increase in ERG a-wave latency in diabetic rats treated with a low daily dose of diazepam compared to controls ($p < 0.05$).

A-wave amplitude recovery rate was 38.8% lower in untreated diabetic rats than in controls ($p < 0.05$) (Fig. 2D). Compared to controls, diabetic rats treated with a 5.0- mg/kg daily dose and those treated with 25.0-mg/kg daily dose of MIGU-4 showed 28.2% ($p < 0.05$) and 7.5% ($p > 0.05$), respectively, lower a-wave amplitude recovery rates. The parameter was 33.9% higher in diabetic rats treated with treated with 25.0-mg/kg daily dose of MIGU-4 than in untreated diabetic rats ($p < 0.05$). Compared to controls, diabetic rats treated with a low (0.5-mg/kg) daily dose and those treated with a high (1.5-mg/kg) daily dose of diazepam showed 33.0% ($p < 0.05$) and 12.5% ($p < 0.05$), respectively, lower a-wave amplitude recovery rate, with the latter animals showing a 30.0% higher rate compared to untreated diabetic rats ($p < 0.05$) (Fig. 2D).

In diabetic rats, the number of squares crossed was 29.4% lower ($p < 0.05$), and the number of central squares crossed, 52.1% lower ($p < 0.05$), compared to controls (Fig.3, I and II, respectively). There was no significant difference ($p > 0.05$) in the number of squares crossed between diabetic animals treated with MIGU-4 i.p. at a high daily dose (25.0 mg/kg) and controls. In diabetic rats treated with a high (1.5-mg/kg) daily dose of diazepam, the number of squares crossed was half of that in controls ($p < 0.05$) and 30.5% lower than in untreated diabetic rats ($p < 0.05$) (Fig.3, I). In diabetic rats treated with MIGU-4, the

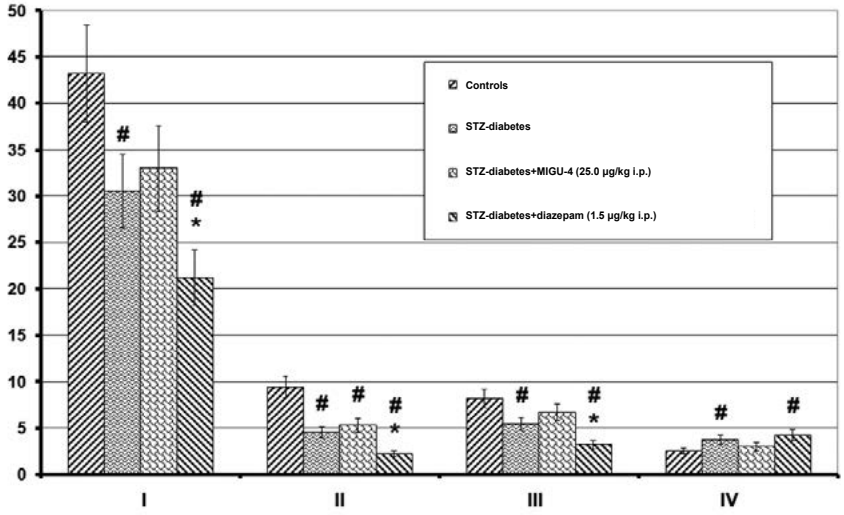

Fig. 3. Parameters of behavior of rats with STZ-induced diabetes under conditions of course treatment with MIGU-4 or diazepam.

Note: The abscissa indicates squares crossed (I), central squares crossed (II); upright postures (III), and boluses (IV). The ordinate indicates the numbers of squares crossed, central squares crossed, upright postures, and defecation boluses. #, significant difference ($P < 0.05$) compared to controls (intact rats injected with normal saline); *, significant difference ($P < 0.05$; ANOVA+ Newman-Keuls test) compared to untreated diabetic rats

number of central squares crossed was 43.6% lower than in controls ($p < 0.05$), and 15.1% higher than in untreated diabetic animals ($p > 0.05$). In diabetic rats treated with diazepam, the number of central squares crossed was 4.27 times lower than in controls, and half of that in untreated diabetic animals ($p < 0.05$) (Fig.3, II).

Compared to controls, the number of upright postures in untreated diabetic animals was 34.2% lower ($p < 0.05$), but there was no significant difference ($p > 0.05$) in this parameter between diabetic rats treated with MIGU-4 and controls (Fig.3, II). In diabetic rats treated with diazepam, the parameter was 2.56 times lower than in controls ($p < 0.05$), and 40.7% lower than in untreated diabetic rats ($p < 0.05$).

In untreated diabetic rats, the defecation bolus number was 32.4% higher than in controls ($p < 0.05$) (Fig.3, IV). There was no significant difference in this parameter between diabetic rats treated with MIGU-4 and controls. However, in diabetic rats treated with diazepam, the defecation bolus number was 40.5% higher than in controls, and this difference was statistically significant ($p < 0.05$).

Discussion

Therefore, we found that untreated STZ-diabetic rats showed typical symptoms of diabetic retinopathy, with reduction in ERG b-wave amplitude, increase in a-wave and b-wave latency and the presence of retinal oxidative stress symptoms like increased retinal content of MDA, a lipid peroxidation product, and decreased antioxidant defence with low retinal SOD and CAT activity. These diabetic retinopathy symptoms were seen within two months after diabetes induction.

In STZ-diabetic rats, ERG wave recovery was observed with the two-week treatment with a germanium-containing drug, MIGU-4, i.p. at a daily dose of 25.0 mg/kg. It is interestingly that correction of diabetic abnormalities was more effective in terms of ERG wave amplitude and less effective in terms of ERG wave latency. Given a potential key value of neurodegenerative retinal changes in the presence of developing STZ-induced diabetes [1, 3], the above effects could be caused by a MIGU-4-induced increase in neuronal excitability. The effects similar to the above were seen also in diabetic rats treated with diazepam, although the action of the latter is associated with the suppression of neuronal excitability. However, a two-week diazepam course used in the current study is associated with the development of benzodiazepine tolerance, with an increased excitability of neuronal ensembles after medication withdrawal [10]. In addition, such an increase in excitability provides for the development of behavioral benzodiazepine withdrawal symptoms like deterioration in open field test performance of rats. A two-week MIGU-4 course, however, caused an improvement in anxiety symptoms, indicating that the drug under study has promising advantages compared to the reference drug.

Therefore, our findings indicate that the mechanisms of the corrective effect of MIGU-4 may be considered in the context of the involvement of gamma-aminobutyric acid inhibitory system as a major component of brain excitability control and a key component in the implementation of neurotropic effects of diazepam. In addition, it should be noted that the anti-inflammatory effects of germanium-containing organic compounds and MIGU-4 may be associated with their inhibitory effects on the release of anti-inflammatory mediators, with the majority of these mediators actively regulating nervous system excitability [5-7]. It is also important to note that the anti-inflammatory effects are also promoted by reduced lipid peroxidation and increased activity of antioxidant defence mechanisms which have been found in MIGU-4 and diazepam.

Conclusion

First, STZ-induced diabetes is accompanied by an impairment of the oxidant/ antioxidant balance in the retina with an increase in retinal MDA, decrease in retinal SOD and CAT activity, and ERG abnormalities such as reduced ERG amplitude and increased ERG latency.

Second, a course treatment with niacin-oxy-ethylidene-diphosphonate germanate (MIGU-4) i.p. at a daily dose of 25.0 mg/kg provides for a decrease in retinal MDA and an increase in retinal SOD and CAT activity and ERG wave amplitude, demonstrating the antioxidative and neuroprotective effects of the compound under study.

Third, the corrective effect of treatment with MIGU-4 i.p. at a daily dose of 25.0 mg/kg on the examined parameters of the retinal oxidant/ antioxidant status and ERG corresponds to the corrective effect of treatment with diazepam i.p. at a daily dose of 1.5 mg/kg.

Finally, the withdrawal of a two-week treatment with MIGU-4 i.p. at a daily dose of 25.0 mg/kg decreased open-field anxiety-like behaviors, whereas the withdrawal of a two-week treatment with diazepam i.p. at a daily dose of 1.5 mg/kg increased open-field anxiety-like behaviors.

Further studies have been planned on (a) metabolic and functional effects in experimental retinopathy modeling, and (b) application of MIGU-4 as monotherapy and as an adjunct to neuroptotective therapy.

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Disclosures

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Author Contribution. *All authors participated in data collection, analysis of results, writing the manuscript and approved the final version of the manuscript.*

Conflict of interest. *The authors declare that they have no conflicts of interest that could influence their opinions on the subject matter or materials described and discussed in this manuscript.*

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Abbreviations: *CAT, catalase; ERG, electroretinogram; MDA, malondialdehyde; MIGU-4, niacin-oxy-ethylidene-diphosphonate germanate; SOD, superoxide dismutase*