Research Results in Pharmacology

Research Article

Neuroprotective properties of Na+/H+-exchanger isoform-1 inhibitor in experimental POAG

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Abstract

Introduction: Worldwide glaucoma is the leading cause of irreversible vision loss. The processes associated with the loss of retinal ganglion cells are multifactorial and have much in common with neurodegenerative diseases. Therefore the search for means to prevent the death of retinal neurons is an important task of modern pharmacology.

Materials and Methods: The study was conducted on male Wistar rats. Glaucoma was modeled by injecting a 1% solution of hyaluronic acid into the anterior chamber of the eye. The IOP level was recorded on the 0th, 63rd and 73rd days of the experiment. The effectiveness of the drugs was evaluated based on the results of ophthalmoscopy, electroretinography, followed by the determination of gene expression.

Results and Discussion: In the group with RU-1355 correction, the fundus picture improved; the index in the group was 18.0% lower compared to the model. The introduction of the RU-1355 compound provided an increase in the a-wave amplitude by 18.1%, and b-wave amplitude by 39.0% relative to the group with pathology. The most pronounced effect was observed on the expression level of BDNF, Bcl-2, Caspase 3 and NF-κB p65, which indicates that the compound has the capacity to influence the slowdown of the apoptosis process through an increase in the neurotrophic factor and the anti-apoptotic factor Bcl-2.

Conclusion: RU-1355 has neuroprotective properties, which was expressed by a decrease in ophthalmoscopic manifestations, preservation of the b-wave amplitude of the electroretinogram and the influence on gene expression of factors involved in apoptosis and neuroprotection. Based on the pharmacological activity of the RU-1355 compound in relation to POAG, further study of its action against other retinal diseases is promising.

Graphical Abstract

Neuroprotective properties of a type 1 Na+/H+ exchanger inhibitor in experimental POAG in rats

Group

1) Intact group (n=10)   2) POAG (n=10)   3) Negative control (n=10)   4) POAG + RU-1335 (1,12mg/kg)(n=10)   5) POAG + Zoniporide (1mg/kg) (n=10)   6)POAG + Timolol (0,009ml/kg)(n=10)

Study design

25 mcl Hyaluronic acid injection into the anterior chamber of the eye	Instillation of group 6 once for 10 days	Itraperitoneal injection of the drug in group 4 and 5 once for 10 days	IOP level	IOP level,ERG, ophthalmoscopy	CO 2 euthanasia
1	63	63	63	73	73

Day

Result

Created in BioRender.com

Keywords

Intraocular pressure, retinal ganglion cells, glaucoma, neurodegeneration, ophthalmoscopy, retina, electroretinography

Introduction

Worldwide glaucoma is the leading cause of irreversible vision loss. The processes associated with the loss of retinal ganglion cells are multifactorial and have much in common with neurodegenerative diseases (Beykin et al. 2021). Worldwide there are more than 60 million people with glaucomatous optic neuropathy, of which 8.4 million are blind. The global incidence of glaucoma is expected to rise to 111.8 million by 2040 (Downs 2015).

The most common clinical sign of glaucoma is increased intraocular pressure (IOP). Under conditions of increased IOP, there is a decrease in eye perfusion, which causes oxygen and nutritional starvation, which leads to disruption of cell energy metabolism and the accumulation of free radicals. In addition, under conditions of increased intraocular pressure, the retrograde transport of neurotrophins is disrupted and the internal pathway of apoptosis is activated (Qu et al. 2010). Also a prolonged increase in IOP in combination with oxidative stress and neurotrophin deprivation leads to a malfunction in the regulation of the local immune response, which causes a neuroinflammatory process that contributes to the progression of the disease (Baudouin et al. 2020).

Current drug therapy aimed at reducing IOP does not always stop the process of vision loss. Moreover the disease can progress despite significant IOP control (Pitts et al. 2022).

Therefore, there is a need to search for drugs that can prevent the progression of the disease and protect ganglion cells in the context of ongoing pathological processes (Boia et al. 2020).

Given the critical importance of neuronal death in the pathogenesis of many disorders and neurodegeneration being mediated by multiple mechanisms that may overlap (Fan et al. 2017), a better understanding of the types, mechanisms, and roles of neuroprotection is fundamental to the development of strategies to combat neurodegeneration in glaucoma (Monteiro et al. 2017).

The role of Na^+/H^+-exchanger isoform-1 (NHE-1) in cerebral ischemia, disorders of the cardiovascular system, and the effect on the atioxidant system of the body and the blood system have been extensively studied (Perfilova et al. 2019; Ovsyankina 2022; Spasov et al. 2022).

Considering that NHE-1 is widely expressed in various tissues including the retina and has a wide range of actions, the study of compounds of this type in neuroprotection in glaucoma is very promising.

The aim of the study was to study the pharmacological activity of the NHE-1 inhibitor, compound RU-1355, in an experimental model of primary open-angle glaucoma (POAG).

Materials and Methods

Animals

Ethical principles for experiments on laboratory rats were observed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, ETS No. 123. Animals were obtained from the nursery of the Research Institute of Pharmacology of Living Systems, Russia. Experimental studies were carried out on male rats (6 groups, 10 animals in each group); for the experiment, there were selected animals weighing 180-220 g. All manipulations on rats were carried out under general anesthesia with intraperitoneal injection of chloral hydrate solution. The experiments were approved by the Local Ethics Committee of Belgorod State National Research University, Belgorod, Russia (Minutes No. 08/19 of March 12, 2019).

Design of the Experiment

The following groups were included in the experiment: 1) intact group; 2) group with a model of primary open-angle glaucoma (POAG); 3) negative control group; 4) group with POAG correction with NHE-1 inhibitor, RU-1355, compound at a dose of 1.12 mg/kg; 5) group with POAG correction with zoniporide at a dose of 1.0 mg/kg (Sigma Aldrich, USA); 6) group with POAG correction with timolol, 0.5% eye drops (Pharmaceutical Manufacturer "Renewal", Russia) at a dose of 0.009 mL/kg instillation.

The study compounds were administered daily at the same time in the morning for 10 days. In the correction groups of RU-1355 at a dose of 1.12 mg/kg and zoniporide at a dose of 1.0 mg/kg, the compounds were administered intraperitoneally. In the group with timolol, eye drops 0.5%, instillation (inst.) of 0.002 mL was carried out, which corresponds to the conversion of doses from humans to rats. The test compounds were administered from the 63rd day of the study once a day of the study once a day for 10 days daily.

Experimental glaucoma was modeled in rats for 9 weeks by introducing a 1% solution of hyaluronic acid (Sigma Aldrich, USA) in a volume of 25 μL into the anterior chamber of the eye (Moreno et al. 2005; Kalatanova et al. 2021). General anesthesia (chloral hydrate at a dose of 300 mg/kg) with continuous monitoring of the depth of anesthesia was used to carry out all painful and animal-immobilizing manipulations.

The IOP level was determined on the 0th, 63rd and 73rd days of the experiment. On the 73rd day of the experiment after recording control parameters (IOP, ophthalmoscopy, electroretinography (ERG)), the animals were euthanized in a CO_2 chamber, followed by retinal sampling to determine gene expression.

Intraocular pressure

The IOP level in laboratory animals was determined using an Icare® TONOVET tonometer (Finland) (Dolzhikov et al. 2020).

Ophthalmoscopy

Ophthalmoscopy to study the fundus of laboratory rats was performed using a V78C lens, Volk Optical (USA). A detailed description was provided earlier (Pobeda et al. 2021). For further statistical processing, the degree of changes in the fundus was assessed in points (Table 1), the points were summed for each criterion for the animal and the data in the group were calculated as the average of all animals in the group.

Electroretinography

The electrophysiological study was performed after a 30-minute dark adaptation on the software and hardware complex of Biopac System, Inc. (USA) according to the previously described method (Shchurovskaya et al. 2021).

Table 1. The scale of evaluation of the ocular fundus during ophthalmoscopy on the POAG model

Parameter	Assigned point		
	0	1	2
General view of the retina	Pink, uniform in all parts	The retina is pale; there are almost white areas of ischemia	The retina is pale, with white areas of ischemia, thinned; there are areas of retinal hemorrhages
ONH	Pale pink, clear borders	Pale, pale gray, clear borders	Gray, clear borders
ONH excavation	There is no excavation or it takes no more than 10% of the diameter of ONH	Excavation is extended, but does not reach the edge of the disc	The excavation reaches the edge of the disk or total
Neuroretinal belt	Uniform throughout	Local or general slight thinning	Marked thinning
Course of vessels through excavation	Smooth course	Slight bending of the vessel at the site of ONH excavation	Sharp bending of a vessel at the site of excavation or slight bending of 2 or more vessels
Arteries	Smooth course, not convoluted, uniform caliber, in a 2:3 ratio with the veins	Narrowed, smooth course, uniform caliber	Filamentous, convoluted, uneven caliber
Veins	Smooth course, not convoluted, uniform caliber, in a 2:3 ratio with the arteries	Convoluted, expanded, uniform caliber	Sharply convoluted, dilated, microaneurysms, small hemorrhages along the veins, uneven caliber

Quantitative PCR

Quantitative polymerase chain reaction was carried out according to the standard procedure described earlier (Pobeda et al. 2022). Primers were selected using the Primer-BLAST resource (NCBI, USA) (Table 2).

Table 2. List of primers

Gene	Primer sequence (5'→3')	Annealing temperature (°C)
ACTB F	GACATCCGTAAAGACCTCTATGCC	59
ACTB R	ATAGAGCCACCAATCCACACAGAG	
Opa1 F	ACACCAGGTGACCACGAGAA	61
Opa1 R	AGGCGGGGGTAGTTGTTCTT	
BDNF F	TTGATGAGACCGGGTTCCCT	60
BDNF R	ACTCAGGGTCCACACAAAGC	
NF-κB p65 F	TTCCCTGAAGTGGAGCTAGGA	61.3
NF-κB p65 R	CATGTCGAGGAAGACACTGGA	
Bax F	TCACGTGACCGGGGCG	61
Bax R	TGATCTGTTCAGAGCTGGTGG	
Bcl-2 F	GGTGAACTGGGGGAGGATTG	61
Bcl-2 R	AGAGCGATGTTGTCCACCAG	
Caspase 3 F	GAGCTTGGAACGCGAAGAAA	60.4
Caspase 3 R	GAGTCCATCGACTTGCTTCCA	

Statistical data processing

The obtained results were subjected to statistical processing using Microsoft Excel 2016 and Statistica 10 (StatSoft, USA). For quantitative indicators, standard descriptive statistics were calculated: the average and the standard error of the average. For intergroup comparisons of average quantitative indicators of independent groups, Student's t-test or U-test (Mann-Whitney test) was used depending on the type of distribution of indicators (normal/abnormal). Using statistical procedures, the differences were considered statistically significant at values of $p \leq 0.05$ (Pobeda et al. 2023).

Results and Discussion

Results of IOP level examination

The IOP level was calculated in each group, the values of which are presented in Fig. 1.

Modeling glaucoma on the 63rd day of the study, an increase in IOP by 2.3-2.5 times was observed in groups with pathology modeling in comparison with the group of intact animals ($p < 0.05$).

On day 73 of the study, a slight decrease in IOP was observed in the group with the introduction of Na+/H+-exchanger isoform-1 inhibitors, compared with the group with the model. This is probably due to the effect of Na+/H+ exchanger inhibitors on the production of aqueous humor (Naumenko et al. 2021; Spasov et al. 2021).

The most significant decrease was achieved in the group with the comparison drug timolol; with its use IOP decreased by 35.6% of the values in the group with the model, which is explained by its pharmacodynamic effect.
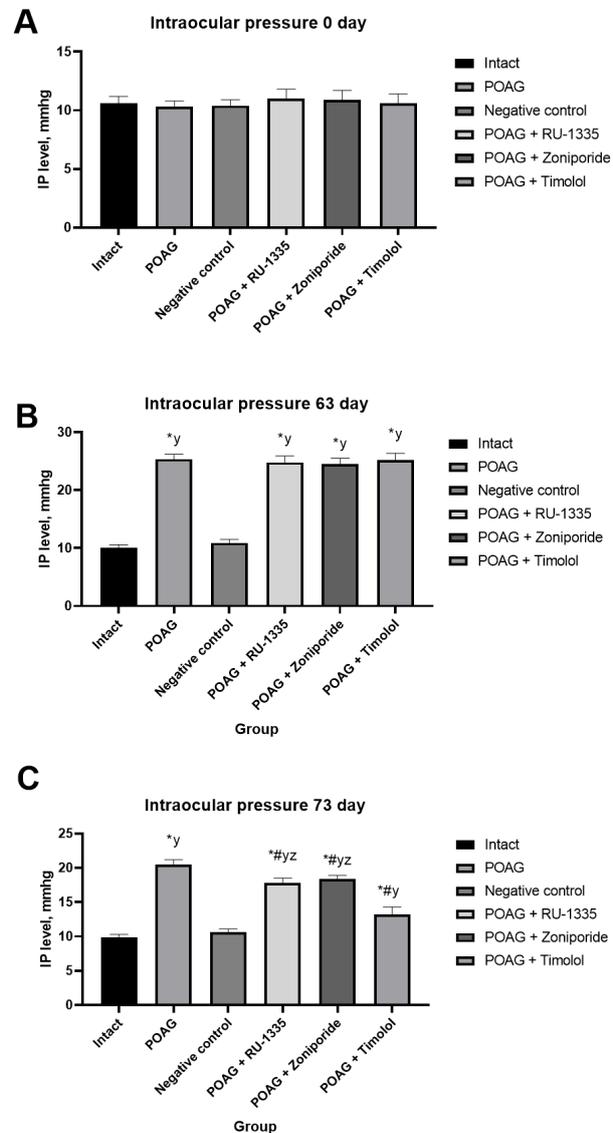
Figure 1. The results of determining the IOP level on the POAG model. (**A**) 0 day of the experiment, (**B**) 63 day of the experiment, (**C**) 73 day of the experiment. ***Note:*** * – p<0.05 in comparison with intact group; # – p<0.05 in comparison with POAG group; y – p<0.05 in comparison with negative control group; x – p<0.05 in comparison with zoniporide group; z – p<0.05 in comparison with timolol group.

Results of ophthalmoscopic examination

An example of an ophthalmoscopic picture of the fundus of an intact animal is shown in Figure 2a. The ophthalmoscopic picture of the fundus of the animals was characterized by uniform pink-yellow coloring of the retina in all areas. ONH is clearly visible, pink, lies in the plane of the retina. Veins and arteries are of uniform caliber, not convoluted; their ratio is 3:2.

Pallor of the retina with areas of retinal hemorrhages and thinning were observed in the group with glaucoma modeling (Fig. 2b). The optic disc was pale and often turned gray. There was an increase in the excavation of
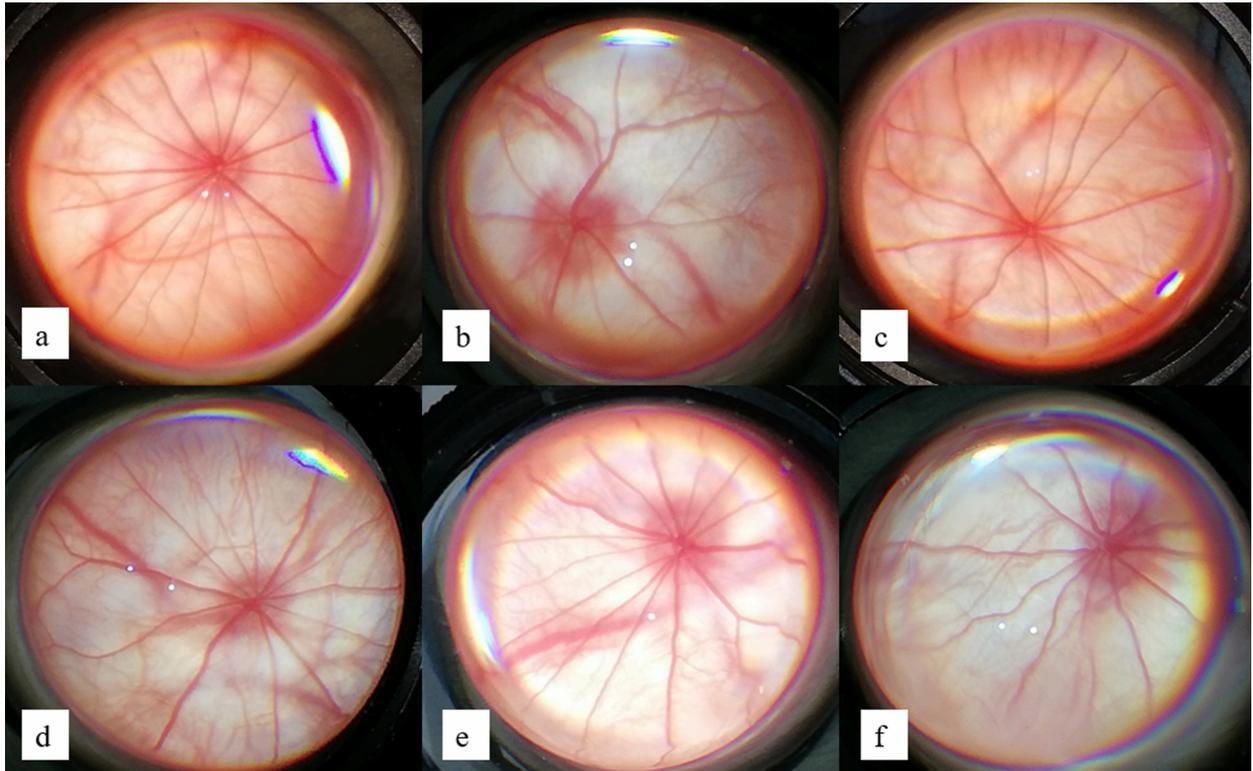
Figure 2. Ophthalmoscopic picture of the fundus in rats on the 73rd day of the experiment: (**a**) an animal of the intact group; (**b**) an animal of the POAG group; (**c**) animal of the negative control group; (**d**) animal with correction of RU-1355 at a dose of 1.12 mg/kg; (**e**) animal with correction of zoniporide at a dose of 1 mg/kg; (**f**) animal with correction of timolol at a dose of 0.009 mL/kg.

the optic disc and thinning of the neuroretinal belt. The caliber and course of veins and arteries changed as well as and their bending through excavation increased. Hemorrhages were observed along the course of the veins.

The ophthalmoscopic picture of the fundus in the negative control group did not differ from the intact animals group (Fig. 2c).

An improvement in the ophthalmoscopic picture of the fundus in the group with the introduction of RU-1355 (Fig. 2d) was observed due to the general appearance of the retina, a decrease in the pallor of the fundus, the degree of grayness of ONH and a significant improvement in the vascular component; the caliber of the vessels was uniform throughout; the ratio of veins and arteries was approaching normal, and vascular tortuosity remained.

In the group with the introduction of zoniporide, the pallor of the retina remained, the optic nerve disk retained a grayish color, and changes in the caliber and course of the veins and arteries remained (Fig. 2e).

In the group with the treatment regimen of timolol administration, the ophthalmoscopic picture of the fundus did not improve significantly, the pallor of the retina remained, the optic nerve disc acquired a grayish color, and changes in the caliber and course of the veins and arteries persisted (Fig. 2f).

The results of ophthalmoscopic fundus evaluation in each group are presented in Fig. 3.

In the group with pathology modeling, the index of the ophthalmoscopic picture of the fundus was 10.0±0.4, which significantly differed from those in the intact animals group and the negative control group (p<0.05). Thus, in animals with increased IOP, the changes

observed on the fundus are close to the same changes as in people suffering from POAG.

The introduction of RU-1355 improved the fundus picture. The index in the group was 18.0% lower than in the group with the pathology model and was significantly different from those in the group with the pathology model, the intact animals group and the negative control group (p<0.05).

In the group with zoniporide introduction, the index was comparable to the values in the timolol group, 9.1±0.4 and 9.3±0.4 points, respectively, which significantly differed from the indices in the intact animals group and the negative control group (p<0.05).
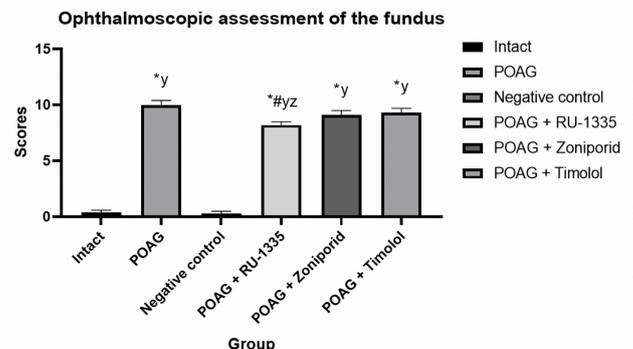
Figure 3. Results of ophthalmoscopic evaluation of the ocular fundus during correction of retinal damage with Na+/H+-exchanger isoform-1 inhibitor on the POAG model. ***Note:*** * – p<0.05 in comparison with intact group; # – p<0.05 in comparison with POAG group; y – p<0.05 in comparison with negative control group; x – p<0.05 in comparison with zoniporide group; z – p<0.05 in comparison with timolol group.

Results of ERG examination

The a-wave and b-wave amplitudes were determined in each group (Fig. 4).

In the intact animals group, the amplitude of the a-wave of ERG was 116.0 ± 6.7 µV and the b-wave was 205.4 ± 8.8 µV.

On the 73rd day of the study, the a-wave amplitude in the group with the model decreased by 29.9% compared to that in the intact group; this index was significantly different ($p<0.05$). The b-wave amplitude decreased by 35.8% compared to that in the intact group and the index was significantly different ($p<0.05$).
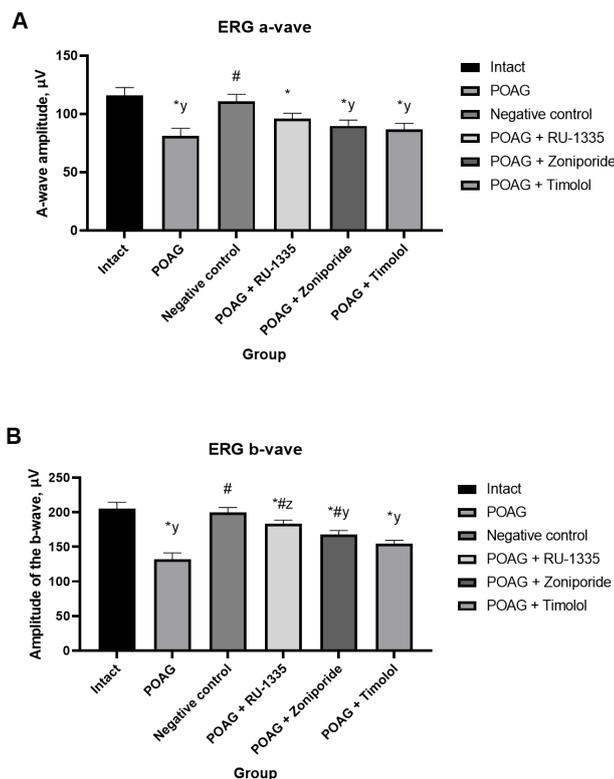
Figure 4. Results of an electrophysiological study for the correction of retinal damage with Na$^+$/H$^+$-exchanger isoform-1 inhibitor on a POAG model (M±m; n=10), µV. ***Note:*** * – $p<0.05$ in comparison with intact group; # – $p<0.05$ in comparison with POAG group; y – $p<0.05$ in comparison with negative control group; x – $p<0.05$ in comparison with zoniporide group; z – $p<0.05$ in comparison with timolol group.

The introduction of the RU-1355 compound provided an increase in the a-wave amplitude relative to the group with the pathology model by 18.1%, while this index was significantly different from that in the intact animals group ($p<0.05$) and not statistically significantly different from those in the group with pathology model and the negative control group. The b-wave amplitude increased by 39.0% relative to the group with the model and was statistically significantly different from those of the intact group, the group with the pathology model and timolol group ($p<0.05$).

In the group of animals administered with zoniporide, the a-wave amplitude increased by 10.5% and the b-wave amplitude increased by 27.2% relative

to the group with the pathology model; these indexes were significantly different from those in the intact animals group and the negative control group ($p<0.05$); the a-wave amplitude was also significantly different from that in the group with the pathology model ($p<0.05$).

In the group of animals administered with timolol, the a-wave amplitude increased by 6.6% and the b-wave amplitude by 17.1% relative to the group with the pathology model; these indexes were significantly different from those in the intact animals group and the negative control group ($p<0.05$), but not significantly different from that in the group with the pathology model ($p>0.05$).

Results of evaluation changes in gene expression

The choice in determining the expression of a particular gene was determined by its participation in the implementation of apoptosis or a possible role in neuroprotection.

The results of the determination of gene expression are presented in Fig. 5.

The administration of the substance RU-1355 relative to the group with pathology modeling resulted in: a decrease in the expression level of the Caspase 3 gene by 42.2% ($p<0.05$); a decrease in the expression of the NF-κB p65 gene by 43.9%, which was significantly different from those in both the group with the pathology model and the intact animals group ($p<0.05$); an increase in the expression of the Opa1 gene by 28.6%, which was significantly different from those in both the group with the pathology model and the intact animals group ($p<0.05$); the level of expression of the BDNF gene increased 2.8 times, which was significantly different from that in the group with the pathology model ($p<0.05$); the expression level of the Bax gene was 24.6% lower, which was significantly different from that in the group with the pathology model ($p<0.05$); the expression level of the Bcl-2 gene increased by 83.7%, which was significantly different from those in the group with the pathology model, the intact animals group and the negative control ($p<0.05$).

Conclusion

The above results show that the presented model of POAG is accompanied by a high level of IOP, which leads to characteristic ophthalmoscopic, electrophysiological changes in the retina with subsequent changes in gene expression.

The most pronounced neuroprotective results were observed in the group with the correction by using RU-1355. This was expressed by a decrease in ophthalmoscopic manifestations, the greatest preservation of b-wave amplitude and changes in gene expression. Compared to the group with the pathology model, there was a decrease in the expression of the Caspase 3, NF-κB p65, Bax genes and an increase in the expression of the Opa1, BDNF, Bcl-2 genes. The most pronounced effect was observed on the expression level of BDNF, Bcl-2, Caspase 3 and NF-κB p65, which indicates that the compound has the possibility to influence the slowdown of the apoptosis process through an increase in the neurotrophic factor and the anti-apoptotic factor Bcl-2.
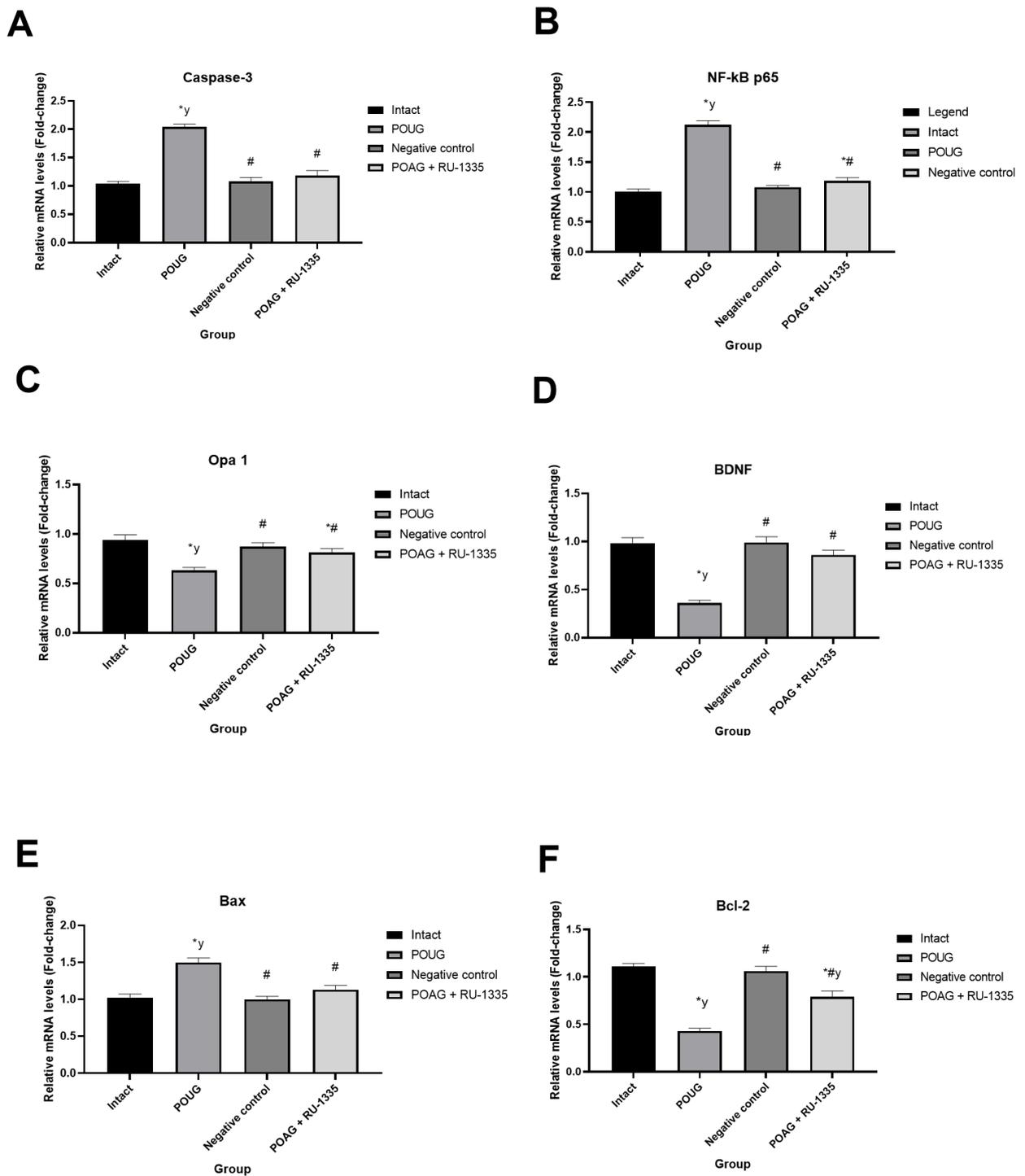
Figure 5. Effect of Na+/H+-exchanger isoform-1 inhibitor, RU-1355 on gene expression in the retina during POAG modeling. *Note:* * – p<0.05 in comparison with intact group; # – p<0.05 in comparison with POAG group; y – p<0.05 in comparison with negative control group.

Conflict of interests

The authors declare no conflict of interests.

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