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Retinoprotective Effect of SkQ1, Visomitin Eye Drops, Is Associated with Suppression of P38 MAPK and ERK1/2 Signaling Pathways Activity

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Abstract—Visomitin eye drops are the first and, so far, the only drug based on SkQ1 – the mitochondria-targeted antioxidant 10-(6′-plastoquinonyl) decyltriphenylphosphonium, developed in the laboratories of Moscow State University under the leadership of Academician V. P. Skulachev. SkQ1 is considered as a potential tool to combat the aging program. We have previously shown that it is able to prevent and/or suppress development of all manifestations of accelerated senescence in OXYS rats, including retinopathy, similar to the age-related macular degeneration (AMD). Here, we assessed the effect of Visomitin instillations on progression of the AMD-like pathology and p38 MAPK and ERK1/2 activity in the OXYS rat retina (from the age of 9 to 12 months). Wistar and OXYS rats treated with placebo (composition identical to Visomitin with the exception of SkQ1) were used as controls. Ophthalmological examination showed that in the OXYS rats receiving placebo, retinopathy progressed and severity of clinical manifestations did not differ from the intact OXYS rats. Visomitin suppressed progression of the AMD-like pathology in the OXYS rats and significantly improved structural and functional parameters of the retinal pigment epithelium cells and state of microcirculation in the choroid, which, presumably, contributed to preservation of photoreceptors, associative and ganglion neurons. It was found that the activity of p38 MAPK and ERK1/2 in the retina of 12-month-old OXYS rats is higher than that of the Wistar rats of the same age, as indicated by the increased content of phosphorylated forms of p38 MAPK and ERK1/2 and their target protein tau (at position T181 and S396). Visomitin decreased phosphorylation of p38 MAPK, ERK1/2, and tau indicating suppression of activity of these MAPK signaling cascades. Thus, Visomitin eye drops are able to suppress progression of the AMD-like pathology in the OXYS rats and their effect is associated with the decrease in activity of the MAPK signaling cascades.

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Keywords: age-related macular degeneration, mitochondrial antioxidant SkQ1, Visomitin, p38MAPK, ERK1/2, phosphorylation, OXYS rats

INTRODUCTION

Eye drops Visomitin is the first and, so-far, the only one pharmaceutical preparation based on SkQ1 – the mitochondria-targeted antioxidant 10-(6′-plastoquinonyl) decyltriphenylphosphonium, developed in the laboratories of Moscow State University under the leadership of Academician V. P. Skulachev [1]. Its development was initiated by investigation in the framework of the project “Skulachev’s ions” using OXYS rats, model of accelerated senescence, created in the Institute of Cytology and Genetics, Siberian Branch of

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Russian Academy of Sciences. Accelerated senescence of the OXYS rats is manifested by the early development of a complex of geriatric diseases including cataract and retinopathy according to the clinical, morphological, and molecular signs of the similar age-related macular degeneration (AMD) in humans [2, 3]. Several months of research was sufficient to demonstrate that administration of SkQ1 with feed is capable of preventing development of the whole complex of phenotypic manifestation of accelerated aging in OXYS rat, which later was confirmed in multiple studies. During the first discussion of the results of pilot studies at the V. P. Skulachev seminar, it was concluded that the most remarkable effect of SkQ1 was its ability to suppress development of cataract and retinopathy. A. M. Olovnikov suggested that it would be very promising to develop eye drops based on the mitochondria-targeted antioxidant. Already in 2005 we started investigation of the ability of SkQ1 to affect development of cataract and signs of AMD in the OXYS rats not only during administration with feed, but also administering the drug in a form of eye drops; results of this study were published in 2008 [4]. At the same in the framework of the "Shulachev's ions" project the work began on the development of the therapeutic preparation on its basis followed by clinical trials. As a result, in 2012 the Ministry of Health of the Russian Federation approved the use of Visomitin preparation as a keratoprotector for prophylactics of dry eye syndrome. Later it was also approved for prophylactics of early cataract [5]. The studies with OXYS rats provide hope that in future Visomitin could be also used for treating AMD, a multifactorial neurodegenerative disease of retina, which is the main cause of vision deterioration and loss in individuals of 60 years and older in the developed countries [6]. As has been shown in our studies, retinopathy developing in OXYS rats is similar to the dry or atrophic type of AMD in humans [3, 7]. Clinical manifestations of retinopathy develop in OXYS rats by the age of ~3-4 months at the background of structural and functional changes in the retinal pigment epithelium (RPE) cells and disruption of choroidal microcirculation, which progress with age and lead to decrease of the thickness of photoreceptor layer and of the outer nuclear layer – events at the basis of vision loss in AMD [8, 9].

Considering the mitochondria-targeted antioxidant SkQ1 as a tool in the fight against aging, V. P. Skulachev explained its effects primarily by its anti-radical activity, ability to suppress generation of reactive oxygen species directly in mitochondria [10]. It was shown in our studies that the accelerated ageing in OXYS rats is associated with dysfunctions of mitochondria, but we did not observe any direct associations with oxidative stress. Nevertheless, SkQ1 is capable to prevent and/or suppress development of all manifestations of aging in OXYS rats including signs of AMD [11]. In particular, it was demonstrated that administration of SkQ1 with feed starting at the age of 1.5 months prevents development of clinical signs of retinopathy in OXYS rats up to the age of 2 years and is capable to significantly suppress progression of the disease in the animals with already developed signs of the disease [4, 10]. Retinoprotective effects of this preparation are associated with normalization of microcirculation in the choroid vessels, of ultrastructure of RPE and photoreceptors [12, 13], with its effect on expression of the genes of angiogenesis regulation – vascular endothelium growth factor (VEGF) and its antagonist – pigment epithelium-derived factor (PEDF) [8], as well as with normalization of autophagy processes in the retina of OXYS rats [14]. Accumulation of toxic amyloid beta (Aβ) in the retina of OXYS rats is a sign of proteostasis disruption typical for AMD, which is significantly suppressed on the background of SkQ1 administration [15]. It seems logical that administration of SkQ1 with feed provided a systemic effect closely associated with prevention of structural-functional disruptions of mitochondrial parameters and/or with their restoration [16, 17]. Remarkably, the long-term administration of SkQ1 suppressed the mTOR (mammalian target of rapamycin) signaling pathway [15], activation of which is considered as one of the main mechanisms of aging and development of age-related neurodegenerative diseases [18].

We have demonstrated previously that manifestation and progression of the signs of AMD and Alzheimer's disease in the OXYS rats occurred on the background of activation in the retina and brain of the mitogen-activated protein kinase (MAPK) signalling pathway [19-23], and the ability of SkQ1 to suppress their development and progression, including accumulation of toxic Aβ aggregates and hyperphosphorylated tau-protein in the OXYS rat brain was found to be associated with the decrease of activity of the p38 MAPK and ERK1/2 (extracellular signal-regulated kinase 1 and 2) signaling pathways [20, 21]. Changes in activity of MAPK have been recognized lately as important players in pathogenesis of neurodegenerative diseases, and they are considered as potential targets for therapeutic interventions [24]. In this study we evaluated association between the ability of SkQ1 (Visomitin drops) to suppress progression of AMD manifestations in the OXYS rats and its effect of activity of p38 MAPK and ERK1/2 in the retina.

Abbreviations: Aβ, amyloid beta; AMD, age-related macular degeneration; ERK1/2, extracellular signal-regulated kinase 1 and 2; MAPK, mitogen-activated protein kinases; mTOR, mammalian target of rapamycin; RPE, retinal pigment epithelium; SkQ1, 10-(6′-plastoquinonyl)decyltriphenylphosphonium; VEGF, vascular endothelial growth factor.

MATERIALS AND METHODS

Animals. Male Wistar and OXYS rats were used in the study, which were housed under standard laboratory conditions (22 ± 2°C and 12-h light/dark cycle) in cages (57×36×20 cm) five animal in each with access to standard feed for rodents (PK-120-1; Laboratorsnab, Russia) and water *ad libitum*.

Exposure. OXYS rats from the experimental group (n = 15) starting from the age of 9 months (age of active development of retinopathy manifestations) received daily instillations of one drop of SkQ1-based eye drops Visomitin (NII Mitoinzhenerii MGU, Russia). OXYS rats from the control group (n = 15) and Wistar rats (n = 15) received placebo – preparation with composition identical to Visomitin, but without SkQ1.

Ophthalmological examinations. At the age of 9 and 12 months (before and after instillations) the state of fundus of animal eyes was examined with the help direct ophthalmoscope Beta (HEINE, Germany) using pupil dilation with 1% tropicamide. Degree of pathological changes in retina was evaluated using the commonly accepted clinical classification according to the Age-Related Eye Disease Study (AREDS) protocol (https://eyephoto.ophth.wisc.edu): score 0 – no changes; score 1 – 1st nonexudative state of disease, with dot hemorrhages, edemas, drusen in the posterior pole of the eye, defects in RPE, redistribution of pigment, atrophy of choriocapillaris layer and RPE; score 2 – 2nd exudative stage – exudative detachment of RPE, neuroepithelia; score 3 – 3rd stage – exudative-hemorrhagic detachment of the retinal pigment and/or neuroepithelia, neovascularization, scarring.

Histological examination. Posterior part of an eye was fixed in a 12% solution of neutral buffered formalin for 1 day as described previously [2]. Next, a sample was washed in a running water for two hours, dehydrated, flatten out, and immersed in paraffin according to the standard technique. Slices of the posterior part of an eye with thickness 4-5 μm were prepared with the help of a rotary microtome and stained with hematoxylin and eosin. Samples were analyzed using a Carl Zeiss Axiostar plus microscope (Carl Zeiss, Germany) with the help of Carl Zeiss AxioVision 8.0 program under magnification 10×100. Calculations of the specific surface area of choroidal vessels (open, with stasis, with sludge of formed blood elements, or thrombosis), specific surface area of RPE, of photoreceptor and outer nuclear layers, open intraretinal vessels in the retina slice, as well as average surface of cytoplasm and nuclei in the RPE were conducted for cells of each retina (50 samples in each group). Number of retinal slices and density of cells in the outer nuclear layer were determined with the help of Avtandilov ocular grid over the surface of 900 μm^2. Five slices of each retina imaged with magnification 10×100 were used for analysis. Enumeration of photoreceptors with nuclear pyknosis were carried out for 1000 photoreceptors, pyknomorphic radial gliocytes, neurons of inner nuclear and ganglion layers (200 cells from each retina).

Western blotting analysis. Retina of Wistar and OXYS rats exposed to placebo instillations and retina of the OXYS rats (n = 5) subjected to Visomitin instillations were homogenized with the help of a RIPA buffer supplemented with protease and phosphatase inhibitors (Sigma-Aldrich, USA) as described previously [22, 23]. Total protein concentration was determined with the help of bicinchoninic acid (BCA) (Thermo Fisher Scientific, USA) assay. Proteins were separated using electrophoresis in a 12% polyacrylamide gel, transferred onto a nitrocellulose membrane (Bio-Rad, USA), and blocked with a 5% bovine serum albumin solution in a phosphate buffered saline (10 mM; pH 7.4) for 1 h. Next, the membrane was incubated overnight at 4°C with primary antibodies against p38 MAPK, phospho-p38 MAPK (Thr180, Tyr182), ERK1/ERK2, phospho-ERK1/ERK2 (Thr202, Tyr204) (# 33-1300, # 36-8500, # 36-8800, # 13-6200, respectively; dilution 1 : 1000; Invitrogen, USA), Tau, phospho-Tau (S396), phospho-Tau (T181) or GAPDH (ab80579, ab75679, ab109390, ab8245, respectively; dilution 1 : 1000; Abcam, USA) followed by 1-h incubation with secondary anti-rabbit and anti-mouse antibodies (ab6721, ab6808, Abcam; dilution 1 : 5000). Intensity of luminescence was recorded with the help of a ChemiDoc MP visualization system (Bio-Rad) and evaluated with the help of ImageJ software (NIH, USA).

Statistical analysis. Statistical analysis was carried out with the help of Statistica 10.0 software package (Statsoft, USA). One-way analysis of variance (ANOVA) with *post hoc* comparison of differences in means (Newman–Keul test) were used. Data are presented as a median (q1-q3). To estimate significance of the differences during comparison of mean values Mann–Whitney test was used. Differences were considered significant at the value $p < 0.05$.

RESULTS

Visomitin slowed down progression of clinical signs of retinopathy. Preliminary ophthalmological examination of 9 months old rats from experimental and control groups of OXYS rats revealed that there were no differences in manifestations of pathological signs in retina (p = 0.74), which were presented by the first stage of retinopathy. At the age of 9-12 months the OXYS rats receiving placebo demonstrated significantly progressing pathological changes, which in 87% of the cases corresponded to the second stage of the disease, and in 13% of the cases – to the third most severe stage of AMD. Manifestations of pathological changes were

pronounced significantly less in the group of animals subjected to instillation of Visomitin: in 90% of the cases pathological changes corresponded to the first stage of AMD, and only 10% – to the second stage. Average levels of manifestation of pathological changes were 2.14 ± 0.07 and 1.33 ± 0.09, respectively, and were significant (according to one-way ANOVA, $F_{1.59} = 53.8$, $p < 0.0000$ and Mann–Whitney test – $p = 0.000003$). In the Wistar rats pathological changes were not revealed in both initial and second examinations – placebo instillation did not affect the state of retina.

Visomitin suppressed pathological changes in the retina of OXYS rats. Histological examination of all retina layers of 12 months old OXYS rats receiving placebo revealed changes typical of AMD contrary to the retina of Wistar rats (Fig. 1, table). Specific surface area of open choroidal vessels in OXYS rats was twice less ($p < 0.01$), and fraction of the vessels with signs of occlusion (with stasis, sludge, or thrombosis) – twice higher ($p < 0.01$) than in the Wistar rats (table). With regards to the intraretinal vessels in retina of OXYS rats, microcirculatory changes have been also noted such as appearance of swelled blood vessels with stasis and sludge of formed elements, but no statistically significance of the differences has been revealed. Average surface area of the RPE cells in the slice of OXYS rat retina was twice less than in the retina of Wistar rats ($p < 0.01$) mainly due to flattening of nuclei and decrease of the cytoplasm volume, however, nuclei with signs of pyknosis were also revealed (Fig. 1, table).

Part of photoreceptors in the retina of OXYS rats was also subjected to destruction: percent of nuclei with pyknosis was twice as high in comparison with the Wistar rats ($p < 0.01$). Cell death was, respectively, accompanied with the 1.7-fold decrease in the number of receptors rows ($p < 0.01$). Fraction of the associative neurons with cytoplasm edema and shrinkage of nucleus as well as of pyknomorphic hyperchromic radial gliocytes in the inner nuclear layer of retina of the OXYS was increased in comparison with the Wistar rats (1.4- and 1.84-fold, respectively, $p < 0.05$; Fig. 2, a and b; table). Percent of neurons with focal and total chromatolysis and of neurons with signs of pyknosis increased 1.4- ($p < 0.05$), 6.4-, and 11.2-fold ($p < 0.01$; table), respectively, in the ganglion layer in the retina of OXYS rats.

Daily instillations of Visomitin slowed down progression of retinopathy in the 9-12 months old OXYS rats. Visomitin administration led to significant improvement in the state of microcirculation in the choroidal vessels: specific surface area of the vessels with signs of occlusion decreased 2-fold in the OXYS rats ($p < 0.01$), and surface area of the open vessel increased 1.6-fold ($p < 0.05$) in comparison with the animals receiving placebo. Analysis of the RPE state revealed activation of the compensatory-adaptive processes, which was manifested in OXYS rat as the increase of average surface area of nuclei in the RPE cells by 7.5% ($p < 0.01$), thus offsetting the differences in this parameter in comparison with the Wistar rats (table).

Visomitin revealed clearly pronounced neuroprotective and glia-protective effects protecting neurons and radial gliocytes in the retina of OXYS rats from damage. As a result, the percent of pyknotic nuclei in photoreceptors of the retina of OXYS rats treated with Visomitin was not higher than the level of

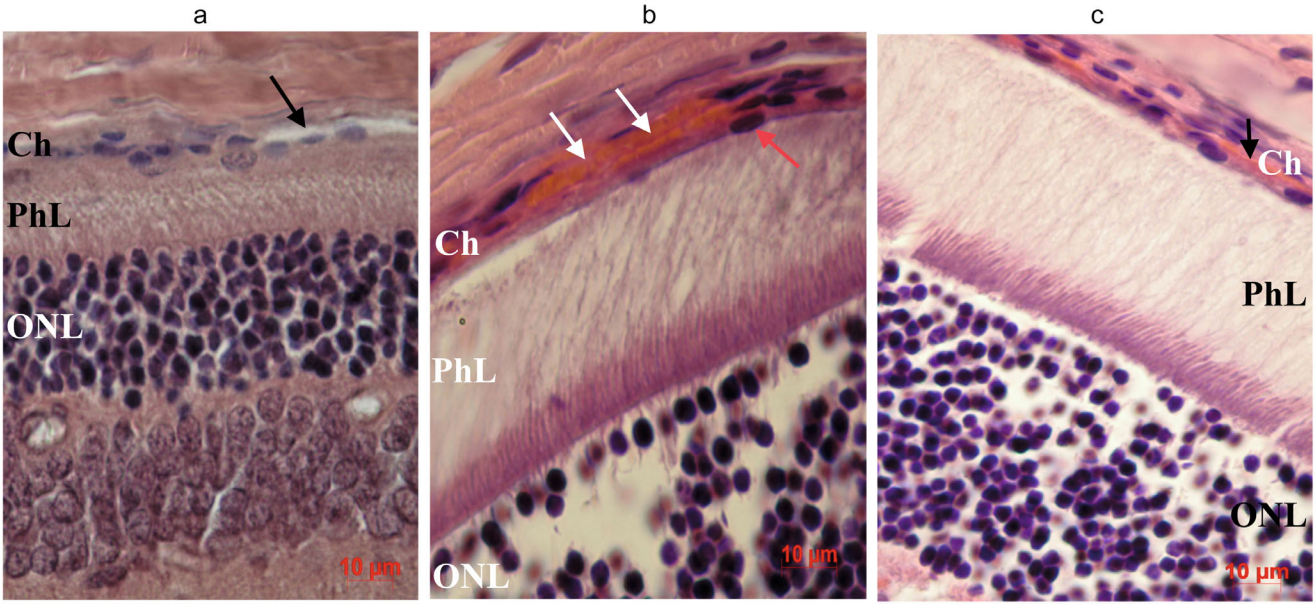

Fig. 1. Choroid complex in retina of Wistar (a) and OXYS (b) rats receiving placebo and of OXYS rats receiving Visomitin (c) at the age 9-12 months. Designations: Ch, choroid; PL, photoreceptor layer; ONL, outer nuclear layer. Black arrows, choriocapillaris with open lumen; white arrows, choriocapillaris blocked due to thrombosis; red arrows, pyknosis of RPE nucleus.

Effect of Visomitin on morphometric parameters of retina in rats

Parameters	Wistar	OXYS	OXYS + Visomitin
Specific surface area of open vessels, %	19.50 ± 1.93	9.67 ± 1.06* $p < 0.01$	15.04 ± 1.43# $p < 0.05$
Specific surface area of vessels with stasis or thrombosis, %	6.10 ± 0.44	11.36 ± 0.90* $p < 0.01$	5.71 ± 0.83# $p < 0.01$
Specific surface area of pigment epithelium in the retinal slice (%)	5.77 ± 0.41	2.64 ± 0.19* $p < 0.01$	2.47 ± 0.19* $p < 0.01$
Average surface area of RPE cell, μm^2	247.49 ± 3.96	183.65 ± 2.82* $p < 0.01$	207.98 ± 3.52*# $p < 0.01$
Average surface area of the nucleus of RPE cell, μm^2	53.71 ± 0.82	48.11 ± 0.59* $p < 0.01$	51.72 ± 0.90# $p < 0.01$
Number of photoreceptor rows	11.0 ± 0.42	6.62 ± 0.35* $p < 0.01$	7.57 ± 0.29* $p < 0.01$
Pyknosis of photoreceptor nuclei, %	0.37 ± 0.09	0.72 ± 0.06* $p < 0.01$	0.57 ± 0.07
Pyknomorphic associative neurons, %	0.54 ± 0.06	0.75 ± 0.06* $p < 0.05$	0.48 ± 0.05# $p < 0.01$
Pyknomorphic radial gliocytes, (%)	3.61 ± 0.78	6.67 ± 0.65* $p < 0.01$	3.40 ± 0.59# $p < 0.01$
Ganglion neurons with focal chromatolysis, %	3.25 ± 0.31	4.41 ± 0.30* $p < 0.05$	4.54 ± 0.16* $p < 0.01$
Ganglion neurons with total chromatolysis, %	0.71 ± 0.19	4.53 ± 0.59* $p < 0.01$	0.85 ± 0.08#
Ganglion pyknomorphic neurons, (%)	0.28 ± 0.05	3.13 ± 0.38* $p < 0.01$	0.44 ± 0.05#
Specific surface area of intraretinal vessels, %	0.97 ± 0.10	1.13 ± 0.23	0.76 ± 0.13

Note. Data are presented as a mean ± SEM.

* Statistically significant differences between the OXYS and Wistar rats receiving placebo.

Statistically significant effect of Visomitin instillations. Differences were considered statistically significant at the value $p < 0.05$.

RPE, retinal pigment epithelium.

this parameter in the Wistar rats, fraction of the pyknomorphic associative neurons decreased by 36% ($p < 0.01$) and of radial gliocytes – by 51% ($p < 0.01$) in comparison with the OXYS rats receiving placebo, thus reaching the levels typical for the Wistar rats (Fig. 2; table). Fractions of the cells with signs of destruction in the population ganglion neurons were comparable in the OXYS rats receiving Visomitin and Wistar rats receiving placebo (table).

Visomitin decreased the level of phosphorylation of p38 MAPK and ERK1/2 in the retina of OXYS rats. At the age of 12 months content of the p38 MAPK protein and its phosphorylated form (p-p38MAPK) in the retina of OXYS rats receiving placebo was higher than in the Wistar rats ($p < 0.005$ and $p < 0.001$, respectively; Fig. 3, a-c). The level of phosphorylated p38 MAPK (evaluated based on the ratio of p-p38MAPK to p38MAPK) in the OXYS rats was higher than in the Wistar rats ($p < 0.017$; Fig. 3d). Instillations of Visomitin did not affect the content of p38 MAPK, but there was a decreasing trend observed in the content of p-p38 MAPK ($p = 0.054$; Fig. 3, b and c). The p-p38 MAPK to p38 MAPK ratio in this group was significantly lower than in the OXYS rats from the placebo group ($p < 0.050$), but was still higher than in the Wistar rats (Fig. 3d).

Content of ERK1/2 in the retina of 12 months old OXYS rats receiving placebo did not differ from the content in the Wistar rat retina (Fig. 3e). As expected,

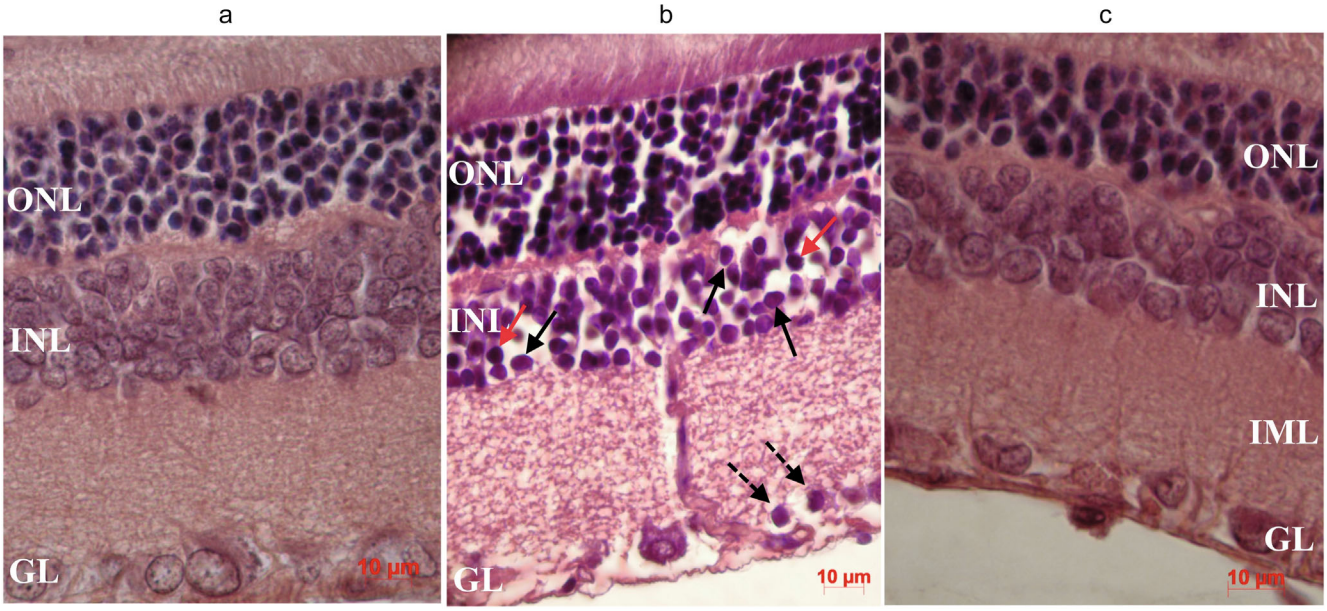

Fig. 2. Retina of Wistar (a) and OXYS (b) rats receiving placebo, and of OXYS rats receiving Visomitin (c). Black arrows – associative neurons with edema; red arrows – hyperchromic pyknomorphic associative neurons; dashed arrows associative neurons – pyknomorphic ganglion neurons. Designations: ONL, outer nuclear layer; INL, inner nuclear layer; ORL, inner retinal layer; GL, ganglion layer. Staining with hematoxylin and eosin.

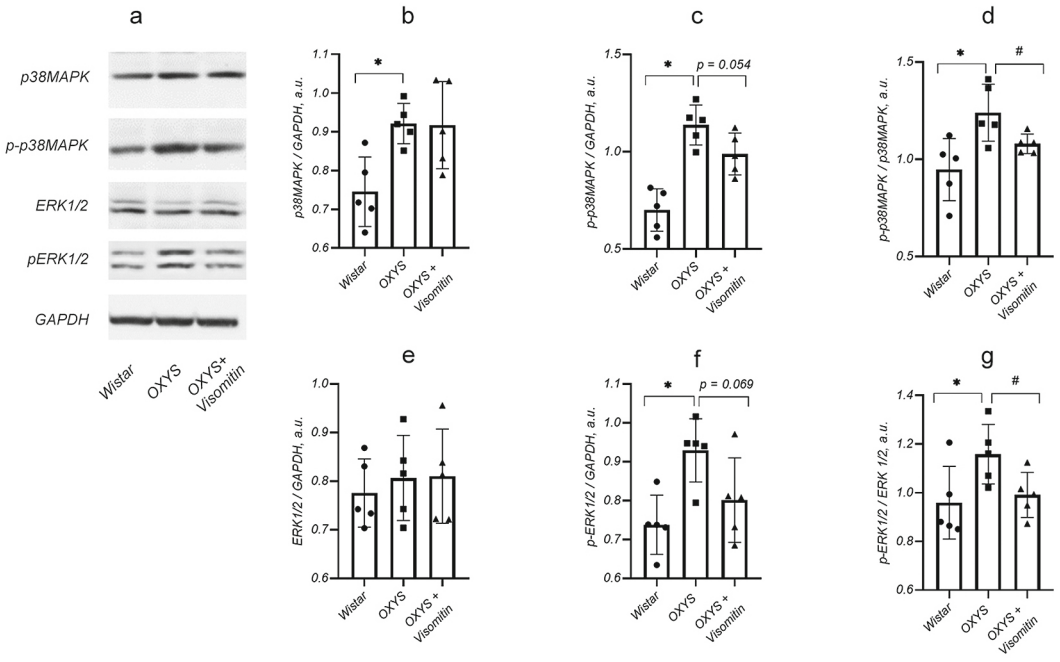

Fig. 3. Effects of Visomitin instillations on the content of p38 MAPK, p-p38 MAPK, ERK1/2, and p-ERK1/2 in the retina of OXYS rats. Representative images of blots (a), content of p-38 MAPK (b), p-p38 MAPK (c), ratio p-p38 MAPK to p38 MAPK (d), ERK1/2 (e), p-ERK1/2 (f), and ratio p-ERK1/2 to ERK1/2 (g) in the retina of Wistar and OXYS rats receiving placebo, and of OXYS rats receiving Visomitin. GAPDH was used as a control. Data are presented as a median (q1-q3). * Statistically significant differences between the OXYS and Wistar rats receiving placebo; # Statistically significant effect of Visomitin instillations. Differences were considered significant at the level of $p < 0.05$.

content of the phosphorylated form (p-ERK1/2) in the OXYS rats was higher than in the Wistar rats ($p < 0.005$; Fig. 3f). In agreement with these data, the level of phosphorylation of ERK1/2 in the OXYS rats receiving placebo was higher than in the Wistar rats ($p < 0.005$; Fig. 3g), which indicates activation of the ERK1/2 signaling pathway in the retina of these animals. Despite the fact that Visomitin instillations did not affect the

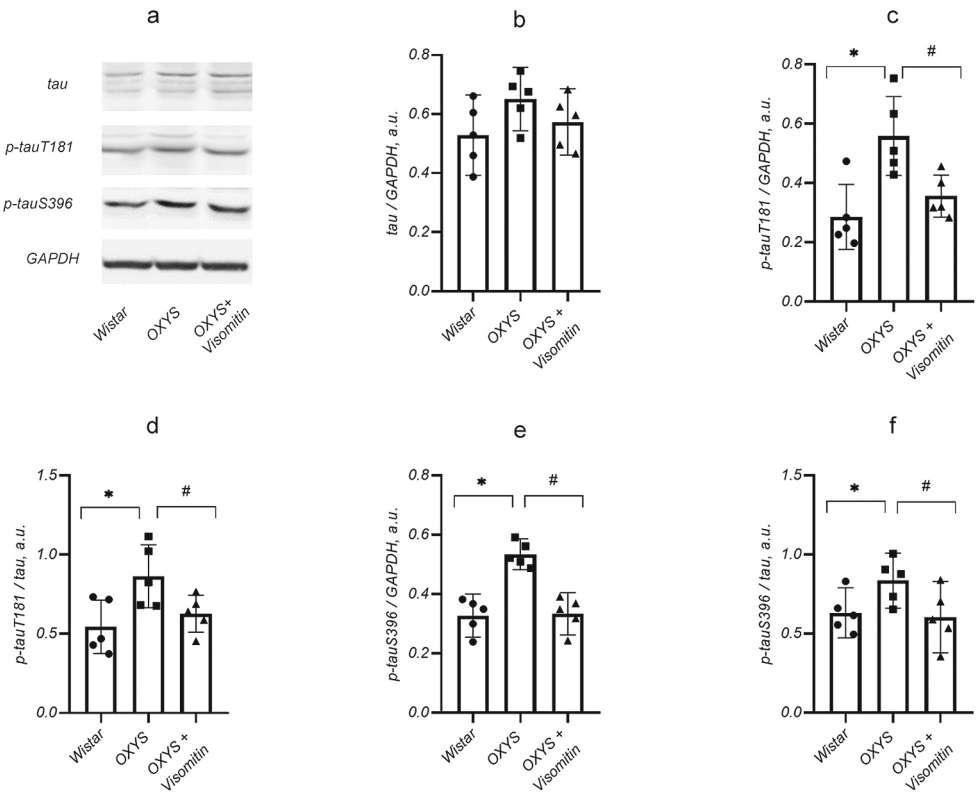

Fig. 4. Effect of Visomitin of content of tau, p-tauT181, and p-tauS396 in the retina of OXYS rats. Representative images of blots (a), content of tau (b), p-tauT181 (c), ratio p-tauT181/tau (d), p-tauS396 (e), ratio p-tauS396/tau (f) in the retina of Wistar and OXYS rats receiving placebo, and of OXYS rats receiving Visomitin. GAPDH content was used as a control. Data are presented as a median (q1-q3). * Significant differences between the OXYS and Wistar rats receiving placebo. # Statistically significant effect of the preparation. Differences were considered significant at the level of $p < 0.05$.

content of ERK1/2 in the retina of OXYS rats, there was a decreasing trend observed in the content of p-ERK1/2 ($p = 0.069$; Fig. 3, e and f). It is important to note that Visomitin administration significantly decreased the ratio of p-ERK1/2 to ERK1/2 in the OXYS ($p < 0.040$; Fig. 3g) to the level observed in the Wistar rats.

Effect of Visomitin on the content of tau, p-tauT181, and p-tauS396 in the retina of OXYS rats. In order to confirm inhibitory effect of Visomitin instillations of activity of p38 MAPK and ERK1/2 in the retina, the level of site-specific phosphorylation of their target, tau protein, was evaluated. Total content of tau protein in the retinas of 12 months old Wistar and OXYS rats receiving placebo did not differ, and Visomitin did not affect its content in the retina of OXYS rats (Fig. 4, a and b). Increased content of the p-tauT181 and p-tauS396 in the retina of OXYS rats in comparison with the Wistar rats was observed ($p < 0.025$ and $p < 0.001$, respectively). The content of p-tauT181 and p-tauS396 was lower in the retina of OXYS rats receiving Visomitin in comparison with the control OXYS rats ($p < 0.017$ and $p < 0.001$, respectively), as well as the ratios of p-tauT181/tau and p-tauS396/tau ($p < 0.05$ and $p < 0.05$, respectively; Fig. 4, c and d).

DISCUSSION

It has been shown previously in our study devoted to investigation of the efficiency of SkQ1 instillation (250 nM aqueous solution) that this form of preparation is capable of not only preventing development and progression of AMD, but, similar to the case of administration with feed, also of decreasing to a certain degree manifestation of the changes in the retina of OXYS rats [4, 10, 25]. In this study we for the first time present the results of evaluation of the effects of the SkQ1-based pharmaceutical preparation, Visomitin eye drops, on the development of retinopathy and activity of MAPK in the retina of OXYS rats.

As can be seen from the results of ophthalmologic examination, Visomitin instillations that started at the age of 9 months suppressed progression of AMD signs in the OXYS rats: at the age of 12 months manifestations of pathological changes in the animals receiving preparation decreased 1.6-fold in comparison with the control animals. The preparation significantly improved structural and functional parameters of RPE, photoreceptors, neurons, and radial glia. It could be suggested that neuroprotective effect of

the preparation is associated with its ability to restore microcirculation in the choroidal vessels – administration of this preparation led to significant decrease of the number of vessels with disrupted blood flow.

AMD pathogenesis is closely associated with inflammation, oxidative stress, and progressive disruptions in proteostasis, which are the processes regulated via the MAPK signaling cascades mediating signal transduction from the cell membrane to the nucleus [26]. Sequential phosphorylation of downstream kinases eventually causes phosphorylation and activation of the target proteins and nuclear transcription factors. This event serves as an indicator of both efficiency of signal transduction, and activity of the signal pathway as a whole. Suppression of activity of the MAPK signaling pathway is considered as a promising approach for treatment of AMD, despite the fact that information on their state during the development of AMD is very limited and is based on the data obtained using the RPE cell cultures [24].

We have shown previously that manifestation and progression of AMD signs in OXYS rats occur on the background of activation of the p38 MAPK and ERK1/2 signaling cascades in the retina [22, 23]. This was confirmed in the current study: activity of the p38 MAPK and ERK1/2 signaling cascades in the retina of 12 months old OXYS rats was higher than in the Wistar rats. Visomitin instillations affected activity of the p38 MAPK and ERK1/2 signaling cascades in the retina of OXYS rats. In the process, the preparation decreased the content of p-p38MAPK and p-ERK1/2 only at the level of trend, but the ratio of phosphorylated forms of the kinases to their total content decreased significantly.

Retina cells have unique morphology, lifespan, and functional organization, which to a large degree depend on structural and functional preservation of proteins. Dysfunction of mitochondria during AMD development triggers disruption of proteostasis – accumulation of pathological aggregates, which are detected in the retina of the patients with AMD before their vision is affected [27, 28]. Such aggregates formed from lipofuscin, toxic Aβ, and hyperphosphorylated tau-protein are capable of damaging neurons. The MAPK signaling pathways participate in regulation of proteostasis via phosphorylation of target proteins, including tau. We have shown previously that at the age of 18 months, when the changes in the retina of OXYS rats are clearly pronounced, the content of Aβ and of tau protein phosphorylated at the MAPK-specific sites is significantly increased [3, 23]. In the current study we demonstrated that phosphorylation of the tau protein in the retina of OXYS rat is increased already at the age of 12 months, and Visomitin instillations decreased it due to suppression of the MAPK-dependent phosphorylation. Similar results were obtained during investigation of the effects of administration of SkQ1 with feed on the dynamics of accumulation of the phosphorylated tau and Aβ in the brain and retina of OXYS rats [15, 21].

Accumulation of pathological aggregates is facilitated by the decrease of activity of autophagy processes (cellular system controlling protein quality) occurring with aging. Dysfunction of autophagy results in disruption of homeostasis, accumulation of damaged organelles and toxic proteins, which, in turn, initiate disruption of mitochondrial functions and oxidative stress [29]. Enhanced accumulation of lipofuscin and Aβ in the retina on the background of disruption of the autophagy process is typical for both the OXYS rats with pronounced signs of AMD [30, 31], as well as for the patients at the late stage of the disease [32]. Administration of SkQ1 with feed facilitated activation of autophagy in the retina of OXYS rats [14]. The data available in the literature allow suggesting existence of direct association between the cellular systems of quality control, changes in mitochondria dynamics, and MAPK signaling pathways. We have shown previously that the mechanisms of neuroprotective effects of SkQ1 both in the course of prophylactic administration with feed starting at early age, and in the case of its administration to the OXYS rats with pronounced neurodegenerative changes are associated with restoration of structural and functional parameters of mitochondria in the brain [33, 34].

Decrease in the activity of MAPK signaling cascades by antioxidants has been demonstrated previously in different models of the human RPE damage. In particular, protective effect of resveratrol has been associated with inhibition of ERK 1/2 activity in the RPE cells [35]. Blueberry extract increased the level of endogenous antioxidant enzymes with simultaneous decrease of reactive oxygen species and MAPK inhibition in the model of oxidative damage *in vitro* [36]. It must be mentioned that the abovementioned effects of antioxidants were associated not only with partial inhibition of MAPK, but also with inhibition of other signaling pathways. This occurs due to existence of relationships between the MARK signal transmission and such signaling pathways such as VEGF, mTOR, autophagy, and others. It is known that p38 MAPK and ERK1/2 facilitate increase of expression of VEGF [37, 38], which is the main proangiogenic factor involved in AMD pathogenesis [39]. The eye drops with SkQ1 (its aqueous solution) decreased expression of VEGF in the retina of OXYS rats both at the level of mRNA and at the protein level [25].

CONCLUSIONS

In this work we demonstrated that the eye drops Visomitin are capable of suppressing progression of retinopathy in OXYS rat and could, potentially,

be recommended for treating AMD in humans. The results obtained in our previous studies and results of the current study indicate that the mechanisms of therapeutic effects of SkQ1 – active compound in Visomitin eye drops – are multifaceted and are associated with its effect on redox-dependent signaling pathway, including decrease of activity of the MAPK-dependent signaling pathways.

Contributions. N.A.M. concept of the study, conducting experiments, discussion of the results of the study; A.Zh.F. ophthalmological examination of animals; A.A.Zh. histomorphometric analysis of retina samples; A.Zh.F., A.A.Zh., N.A.M., and N.G.K. preparation and editing of the paper text.

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