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BASIC SCIENCE

Ameliorative effects of SkQ1 eye drops on cataractogenesis in senescence-accelerated OXYS rats

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Abstract
Background Antioxidant supplements have been suggested as a strategy to decrease the risk of age-related cataract, but there is no evidence that antioxidants can reduce the signs of the disease. Recently, we showed that the mitochondrial antioxidant SkQ1 can partially reverse cataract signs in senescence-accelerated OXYS rats. The aim of the present study was the histomorphological examination of the influence of SkQ1 eye drops on the cataract development in OXYS rats.
Methods OXYS rats received SkQ1 eye drops (250 nM) from 9 to 12 months of age. Ophthalmoscopic examination was carried out before and after treatment. Light and electron microscopy were used for histomorphological examination. Expression of the Cryaa and Cryab genes was determined using real-time PCR. αB-crystallin expression was detected using Western blotting.
Results SkQ1 completely prevented the cataract development in OXYS rats, and in some of the animals diminished the signs of the disease. Light and electron microscopy showed that SkQ1 attenuated the (typical for cataract) alterations in the lens capsule and epithelial cells, ameliorated disturbances of the hexagonal packing geometry of lens fibers, and improved ultrastructure of the epithelial cells. The levels of mRNA of α-crystallins genes which encode small heat shock proteins αA- and αB-crystallin that play a central role in maintaining lens transparency were significantly lower in the OXYS rats' lenses than in Wistar rats (control). SkQ1 normalized the level of mRNA of Cryaa, and significantly increased the level of Cryab mRNA as well as αB-crystallin protein in the lens of OXYS rats to the level of the control Wistar rats.
Conclusion SkQ1 eye drops hold promise as a treatment of cataract.

Keywords Cataract · Mitochondria-targeted antioxidant · SkQ1 · Alpha-crystallin · OXYS rats

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Introduction

Age-related cataract is a major cause of visual impairment in the aging population worldwide. Age is the main risk factor of cataract, and it is sometimes assumed that cataract is simply an extension of the aging process [1]. The prevalence of this age-related disease is increasing dramatically as the proportion of the elderly in the population continues to rise. To date, a surgical procedure remains the only generally accepted treatment, whereas pharmacological prevention has been unsuccessful so far. Surgical cataract removal, however, is associated with some risks. In particular, it can lead to the development of early age-related macular degeneration (AMD), which is the most important cause of blindness among the elderly [2]. Estimates show that a delay of cataract development by 10 years reduces the need for a surgical intervention by 50 %. Therefore, creation of novel therapeutic and prophylactic modalities for cataract is necessary.

Animal research and observational studies suggest that antioxidant supplementation can decelerate aging of the eye and possibly provide some protection from cataract [3]. Indeed, oxidative stress is implicated in the aging process and in the

pathogenesis of a wide range of age-related disorders including cataract formation and progression. Nonetheless, randomized controlled trials showed that supplementation with antioxidants neither prevents nor decelerates progression of age-related cataract [4, 5]. Moreover, a recent study reported an increased risk of cataract in people with increased intake of vitamins C and E [6]. According to the above studies, there is no evidence that antioxidant supplementation can reduce preexisting signs of cataract.

On the other hand, recently, using senescence-accelerated OXYS rats as an animal model of senile cataract, we showed that the antioxidant 10-(6′-plastoquinonyl) decyltriphenylphosphonium (SkQ1) at nanomolar concentrations can not only prevent the development of cataract but also partially reverse clinical signs of incipient stages of the disease in the lens [7–9]. Studies show that SkQ1 has a therapeutic potential against other age-related eye diseases including AMD and glaucoma [7–9]. SkQ1 in the form of eye drops (brand name Visomitin) was approved for the treatment of dry eye syndrome. Currently, clinical evaluation of anticataract effects of this drug is being actively conducted, but the evidence of its effectiveness is still not convincing enough. We believe that persuasive evidence of clinical efficacy of SkQ1 drops can come from a randomized experimental controlled study, such as the present work. Therefore, the purpose of this work was histomorphological examination of the influence of SkQ1 eye drops on the development of cataract in OXYS rats. Furthermore, we evaluated the effect of SkQ1 on mRNA expression of the *Cryaa* and *Cryab* genes, which encode subunits of α-crystallin. α-Crystallin is a major lens protein that acts as a chaperone, and plays a central role in the maintenance of lens transparency and refractive properties [10]. Most of crystallins are downregulated and dysregulated during development of age-related cataract, both in humans [11] and in OXYS rats [12–14]. In OXYS rats at the age of 20 days, clinical manifestations of cataract are absent according to our observations [12, 15]. The first signs of cataract (in 20 % of OXYS rats) appear by age 1.5 months, and at age 3 months, the morbidity reaches 100 % with predominance of a mild form of cataractous changes [12, 15]. A mature cataract is detectable in more than a half of 12-month-old OXYS rats, whereas Wistar rats (control parent strain) of the same age do not show any signs of this pathology. To assess the effectiveness of SkQ1 eye drops, we used OXYS rats from 9 to 12 months of age, when clinical signs of severe cataract are known to develop.

Materials and methods

The ethics statement

All animal procedures were in compliance with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research as well as the European Communities Council Directive No. 86/609/EES. All manipulations with animals were approved by the Institutional Review Board (session No. 9) of the Institute of Cytology and Genetics (ICG) of the Siberian Branch of the Russian Academy of Sciences (SB RAS), according to "The Guidelines for Manipulations with Experimental Animals" (the decree of the Presidium of the Russian Academy of Sciences of April 02, 1980, No. 12000–496).

Animals and the treatment

Male senescence-accelerated OXYS rats and age-matched male Wistar rats (control animals) were obtained from the Breeding Experimental Animal Laboratory of ICG, SB RAS (Novosibirsk, Russia). The OXYS rat strain was derived from the Wistar strain at ICG as described previously [16, 17] and registered in the Rat Genome Database (http://rgd.mcw.edu/). At the age of 4 weeks, the pups were weaned and housed in groups of five animals per cage (57×36×20 cm). They were kept under standard laboratory conditions (22±2 °C, 60 % relative humidity, and natural light), provided with standard rodent feed, PK-120-1, Ltd. (Laboratorsnab, Russia), and given water *ad libitum*.

SkQ1 was synthesized at the Institute of Mitoengineering of Moscow State University (Moscow, Russia). To assess the effects of SkQ1, male OXYS rats were randomly assigned to one of two groups (n=15 each): {1} control or {2} instillation of one drop of a 250 nM SkQ1 solution in 0.9 % NaCl daily from 9 to 12 months of age. In addition, 15 untreated Wistar rats served as another control group. In total, 45 animals were used in the study (OXYS control, OXYS experiment, and untreated Wistar).

Ophthalmoscopic examination

An ophthalmologist examined all animals twice: before and after SkQ1 treatment, at the age of 9 and 12 months respectively. All rats were examined under a Heine BETA 200 TL Direct Ophthalmoscope (Heine, Germany) after dilatation with 1 % tropicamide. Assessment of stages of cataract was carried out according to the Lens Opacities Classification System III (LOCS III) [18]: grades NO1–NO6 and NC1–NC6 (nuclear opalescence and nuclear color, rated using standard samples, 1 through 6), grades C1–C5 (according to standards of cortical cataracts), and grades P1–P5 (according to standards of posterior subcapsular cataracts). Grades NO1, NC1, P1, and C1 correspond to a transparent lens. NO2, C2, and P2 correspond to ratings 1–4 on the standard decimal scale AREDS and are estimated as the first stage of cataract; NO3–NO4, C3–C4, and P3 correspond to ratings 5–8 on the standard decimal scale, and are estimated as the second stage

of cataract. Grades NO5–NO6, C5, and P5 mean intensive cortical or nuclear opacity of the lens (corresponding to ratings 9–10 on the standard decimal scale), and are estimated as the third stage of cataract. The degree of lens opacification was classified using the double-blind method.

Morphological examination using light microscopy and electron microscopy

Histological and morphometric analyses were conducted on lenses of untreated Wistar rats and of SkQ1 eye drop–treated and untreated OXYS rats (eight randomly selected lenses in each of the three groups of animals). The lens of an eye was excised, fixed in 4 % paraformaldehyde, and prepared in the DMEM culture medium, which was used as a buffer in all processing procedures. Then the lenses were cut identically into four pieces, which were washed 3 times in DMEM and postfixed in 1 % OsO_4 (SPI, USA), dehydrated in ethanol and acetone, soaked in a 1:1 mixture of epon–araldite and acetone for 12 h, and embedded in an epon–araldite mixture (SPI, USA) according to the manufacturer's instructions. Semithin sections for light microscopy were made on an ultramicrotome EM UC7 (Leica, Germany), and the regions for electron microscopy were selected there as well; the sections were stained with 1 % azure II. The images for light microscopic examination were acquired using an AxioScope and the AxioVision software (Zeiss, Thornwood, NY). Ultrathin sections were also prepared on an EM UC 7 ultramicrotome from the regions selected on semithin sections, and contrast was enhanced using uranyl acetate and lead citrate. The sections were examined under a JEM 1400 transmission electron microscope (Jeol, Ltd., Japan) supplied with a digital camera Veleta (Olympus SIS, Germany).

Assessment of gene expression of αA- and αB-crystallin

RNA isolation Total cellular RNA was isolated from a rat lens using phenol–chloroform extraction as follows. A tissue sample was placed in water-saturated phenol (pH 7.0; 10 volumes per gram of tissue) with addition of 0.5 % SDS (5 volumes per gram of tissue); then the tissue was homogenized for 15 min. After addition of 0.1 volume of 2 M sodium acetate pH 4.2, the tissue was homogenized again, the mixture was incubated for 10 min, and then chloroform extraction was performed. The supernatant was mixed with 2.5 volumes of 96 % ethanol, and the mixture was kept at −70 °C for 2 h. After that, the mixture was centrifuged (15 min at 13,000 rpm on an Eppendorf 5414 centrifuge; Eppendorf, Germany), and the pellet was dried and dissolved in 25 μl of RNA-grade H_2O. The amount of isolated RNA was assessed by means of electrophoresis of 1 μl of RNA in a 1 % agarose gel. The RNA concentration was quantified in each sample on a spectrophotometer at 260 nm using absorbance ratios A_{260}/A_{280} and A_{260}/A_{320}. The RNA samples were stored at −70 °C. Traces of genomic DNA were removed by means of DNase I (Promega, USA) according to the vendor's instructions. After that, we repeated RNA extraction with the phenol–chloroform mixture and pure chloroform, followed by precipitation with ethanol as described above.

Reverse transcription RNA (10 μg) and random hexamer primers (0.5 μg) were mixed in 11 μl of PCR-grade water. After RNA denaturation (65 °C for 5 min) and primer annealing (37 °C for 5 min), the solution of reverse transcriptase (19 μl) was added. The final reaction mixture contained the buffer for reverse transcriptase (20 mM Tris–HCl pH 8.3, 10 mM DTT, 100 mM KCl, 5 mM $MgCl_2$), 500 μM deoxynucleotide triphosphates, and 40 U of MoMLV reverse transcriptase (Biosan, Novosibirsk, Russia). Synthesis of cDNA was performed at 37 °C for 1 h, 42 °C for 30 min, and 50 °C for 10 min. The polymerase was inactivated by heating this mixture at 75 °C for 5 min. For subsequent PCR, we used 0.25–0.50 μl of the resulting cDNA solution.

Preparation of standard cDNA Aliquots (3 μl) from all cDNA samples were mixed, and the "average" solution was used to construct calibration curves, which were used for quantification of the relative cDNA levels of genes of interest, and of a reference gene in the cDNA samples.

Real-time PCR Expression of the *Cryaa* and *Cryab* genes was determined using real-time PCR in the presence of the SYBR Green I dye (Molecular Probes, USA) on an iCycler iQ4 real-time PCR detection system (Bio-Rad Laboratories, USA). The housekeeping gene *Rpl30* (encoding large ribosomal subunit protein 30) served as a reference gene. The following primer pairs were used: *Rpl30*, 5′-ATG GTG GCT GCA AAG AAG AC-3′ and 5′-CAA AGC TGG ACA GTT GTT GG-3′; *Cryaa*, 5′-AGC CGA CTG TTC GAC CAG TTC-3′ and 5′-AAC TTG TCC CGG TCA GAT CG-3′; and *Cryab*, 5′-CTT CGG AGA GCA CCT GTT GG-3′ and 5′-GAG AGA AGT GCT TCA CGT CCA-3′. The reaction mixture (final volume 20 μl) contained the standard PCR buffer (67 mM Tris–HCl pH 8.9, 16 mM $[NH_4]_2SO_4$, 0.01 % Tween 20, 10 mM β-mercaptoethanol), 3 mM $MgCl_2$, 0.2 mM dNTPs, SYBR Green I (1:20,000 dilution), 150 nM primers, and 0.4 U of Taq polymerase (ICG, Novosibirsk, Russia). The reaction was conducted under the following conditions: initial denaturation at 95 °C for 3 min, then five long cycles: denaturation at 95 °C for 30 s, annealing 30 s, elongation at 72 °C for 60 s; after that, 35 main cycles: denaturation at 95 °C for 20 s, annealing 20 s, elongation at 72 °C for 30 s, data collection based on fluorescence of the *Rpl30* reaction at 84 °C for 30 s, and data collection based on fluorescence of the reactions of interest (*Cryaa* and *Cryab* at 87 °C) for 10 s. After completion of the PCR, the melting curves were recorded for specificity control.

In each experiment, samples of a cDNA under study were mixed with primers for a gene of interest (fouir repeats per cDNA sample) in one multiwell plate, the same samples with primers for the reference gene (also four repeats), and "standard" cDNA diluted from 1:3 to 1:243 and mixed with the same primers (two to three repeats). For each cDNA sample, the PCR assay was repeated at least twice. The initial level of a cDNA in question was determined using standard calibration curves (versus standard cDNA), and the value obtained for each gene of interest was compared with the amount of the reference gene cDNA; thus, we determined differences in the expression of genes among the groups of rats [19]. The number of animals in each group was six or seven.

Western blotting

Protein concentrations were measured using a protein assay reagent (DC; Bio-Rad) with BSA as a standard. Equal amounts of protein (5 μg) were loaded in each lane. The blots were stained with Ponceau S to confirm equal protein loading. Proteins were separated in 12 % Tris-glycine gels (Novex; Invitrogen) and transferred to nitrocellulose membranes using standard methods. Each membrane was blocked with 5 % bovine serum albumin in Tris-buffered saline with 0.1 % Tween 20 (TBS-T buffer) for 1 h, and incubated overnight at 4 °C with a rabbit polyclonal antibody to αB-crystallin (1:1,000; Abcam, USA). The secondary antibody was a horseradish peroxidase–conjugated sheep anti-rabbit IgG antibody (1:10,000; Abcam, USA). After incubation with the secondary antibody, the chemiluminescent signal was measured and scanned, and emission intensity of the bands was quantified using the Image J software (NIH, Bethesda, MD, USA). β-Actin served as an internal loading control in each Western blot. The number of animals in each group varied from five to seven.

Statistical analysis

The design of the above experiments is a randomized experimental controlled study. Sampling of rats and eyes from each group for all types of experiments above was random; simple randomization was also used for distribution of the animals into the treated and untreated groups. All analyses in the *Ophthalmoscopic examination* section were double-blind (double-masked); the other experiments were not masked. The main variable was the cataract stage and the secondary variables were histomorphological parameters and the levels of α-crystallin expression. The data were analyzed using repeated-measures analysis of variance (ANOVA) and non-parametric tests using the statistical package Statistica 6.0 (StatSoft, USA). To assess the therapeutic effectiveness, we performed a dependent pairwise comparison of the cataract stages before and after treatment for every eye (*t*-test for dependent samples). One-way ANOVA was used to evaluate the differences between OXYS and Wistar rats and the effects of treatment with SkQ1 eye drops. The Newman–Keuls post hoc test was applied to significant main effects and interactions in order to assess the differences between some sets of means. The data were presented as mean±SEM. The results were considered statistically significant if the p value was <0.05.

Results

Effects of SkQ1 treatment: ophthalmoscopic examination

Analysis of these data is shown in Fig. 1. Already during the first examination of OXYS rats (before treatment, age 9 months), we did not find a single eye that was free of pathological changes. All three types of cataract were detected in OXYS rats: posterior subcapsular (45 %), nuclear (38 %), and cortical (17 %). Pathological changes that were found during this first preliminary examination in one half of the eyes of untreated OXYS rats corresponded to the first stage of cataract, and in the other half to the second stage. According to the dependent pairwise comparison of the cataract stages at 9 and 12 months of age in the untreated OXYS rats, the cataract progressed ($p<0.00001$), and the second examination of the eyes (age 12 months) revealed that 32 % of the eyes had the first stage of cataract, 62 % the second stage, and 4 % of the eyes reached the third stage.

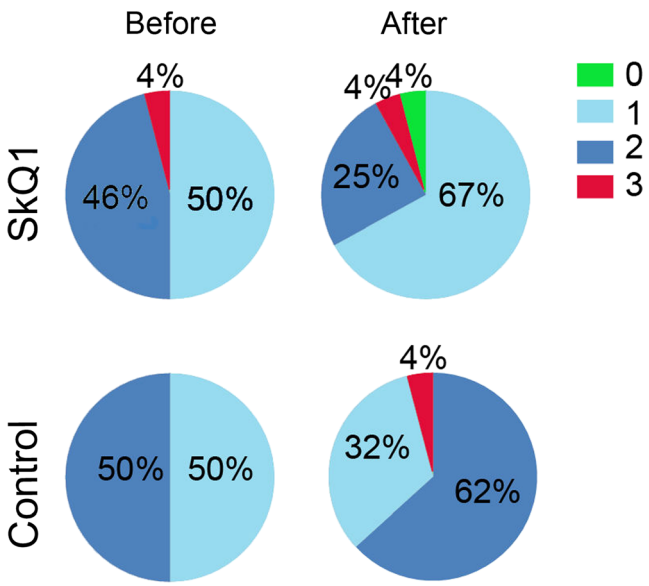

Fig. 1 SkQ1 eye drops inhibit the development of cataract in OXYS rats. The data are presented as a percentage of eyes at the appropriate stage of the disease. *0*: no change of the lens; *1*, *2*, and *3*: stages of cataract (see *Methods*). Age of the animals is 12 months; the SkQ1 treatment (one drop of a 250 nM SkQ1 solution daily) was started at age 9 months

The third stage of cataract was found in 4 % of eyes in OXYS rats treated with SkQ1 eye drops, already during preliminary examination. The number of the eyes with the third-stage disease was the same upon reexamination 3 months after the start of treatment: we did not expect clarification of lenses with such gross disruptions. At the same time, the number of eyes with second-stage cataract was reduced from 50 to 25 %, whereas with the first stage increased from 46 to 67 %. In 4 % of eye lenses, pathological changes were not detectable. Statistical analysis using the method of dependent pairwise comparison of the cataract stages before and after treatment confirmed that SkQ1 eye drops reduced the stage of cataract ($p<0.001$).

ANOVA showed that before treatment, the groups did not differ in the stage of cataract (1.50±0.50 and 1.53±0.57 of AREDS units [mean±SD]; $p=0.812$). Ophthalmoscopic examination after SkQ1 treatment showed that the stage of cataract was reduced compared to the control (1.30±0.59 and 1.68±0.547 of AREDS units [mean±SD] respectively; $p<0.016$). Thus, the SkQ1 eye drops were moderately effective against cataract because Cohen's *d* (effect size based on standard deviation) was 0.68.

Effects of SkQ1 treatment: histological and ultrastructural analysis

In the untreated group of OXYS rats, we identified typical (for age 12 months) structural alterations of the lens capsule under a light microscope (Fig. 2): substantial differences in the thickness of capsules (this change is a sign of cataract), and seals and cracks in the capsule body. We can assume that the cause of such changes is a failure to form a 3-dimensional network of protein capsules; this failure can be caused by aberrant posttranslational modifications of proteins and proteoglycans in the lens capsule. The lens epithelial layer of untreated OXYS rats was different from that in untreated Wistar rats: a more jagged edge of the nucleus, and fewer vesicles. Treatment with SkQ1 eye drops attenuated the interstrain differences. The capsules varied in thickness, and pronounced structural changes were not identified. Only occasional mild age-related alterations were detected in the lenses of both control and SkQ1-treated groups. As a result, the condition of lens epithelial cells of SkQ1-treated OXYS rats was approaching the condition of epithelial cells in (untreated) Wistar rats. Fibers in the lenses of untreated OXYS rats had the characteristic mosaic coloration, and changes of the fiber size were observed (increases and decreases) along with various disturbances of the hexagonal structure and packing of the fibers (Fig. 2). The fibers in the lenses of SkQ1-treated OXYS rats had a more correct hexagonal shape in comparison with the untreated OXYS rats.

Comparative evaluation of ultrastructure of the lens epithelial cells also revealed significant differences between the untreated OXYS and untreated Wistar rats (Fig. 3). The epithelial cells in the germinative region of Wistar rats (Fig. 3a and b) showed granular cytoplasm with short cisterns of the rough endoplasmic reticulum (ER), a small Golgi apparatus, and few lysosomes. Prominent folds of the basolateral plasma membrane and widened intercellular space were frequent in the epithelial layer in untreated Wistar rats, as was endocytosis of the membrane folds. Although nuclear structure was similar in the two rat strains, the cytoplasm in the epithelial cells of untreated OXYS rats looked "empty": cell organelles were scarce (Fig. 3c and d). Although a cross-section of the cytoplasm of epithelial cells in Wistar rats contained 10–15 ER cisterns, a similar cross-section of the cytoplasm in the cells of untreated OXYS rats contained only 2–3 cisterns. Folds of the basolateral plasma membrane were less developed (one to three folds were found along the plasma membrane from apical to basal surface, in contrast to four to five folds in Wistar rats). Widening of the intercellular space was evidently smaller, and the epithelial layer in untreated OXYS rats looked less "vesiculated." The reduced folding of the plasma membrane reflects a slowing of transport processes across the membrane. The plasma membrane in the apical zone of untreated OXYS rats frequently had high electron density suggestive of alterations. Many cells contained small osmiophilic bodies in the cytoplasm (Fig. 3d), which represent so-called residual bodies and are related to alterations in the intracellular structure. The ultrastructure of epithelial cells was indicative of a slowing of the metabolism and degenerative changes in the lens of 1-year-old untreated OXYS rats, while the epithelial layer in the lens of Wistar rats showed normal morphology.

Electron microscopy (lenses of OXYS rats treated with SkQ1 drops) revealed that the cells of the lens germinative region had enlarged intercellular space in the basolateral region ("friable" basal labyrinth); this enlargement became substantial: up to 1.5–2.0 μm in width (Fig. 3e and f). A noticeable increase in endocytosis of plasma membrane folds was not observed. Another striking change in the structure of the epithelial cells of the germinative region was a large amount of rough ER in the cytoplasm (15–20 cisterns could be found in a cross-section of the cytoplasm); the surface of ER was connected with clusters of granular material, as was the case in untreated Wistar rats. The degree of development of rough ER in the epithelial cells of the germinative region was visibly higher compared to the cells of untreated OXYS rats, and approximately the same as in Wistar rats.

In the epithelial cells of the germinative region of SkQ1 eye drop–treated OXYS rats, the number of osmiophilic structures was considerably smaller compared to the

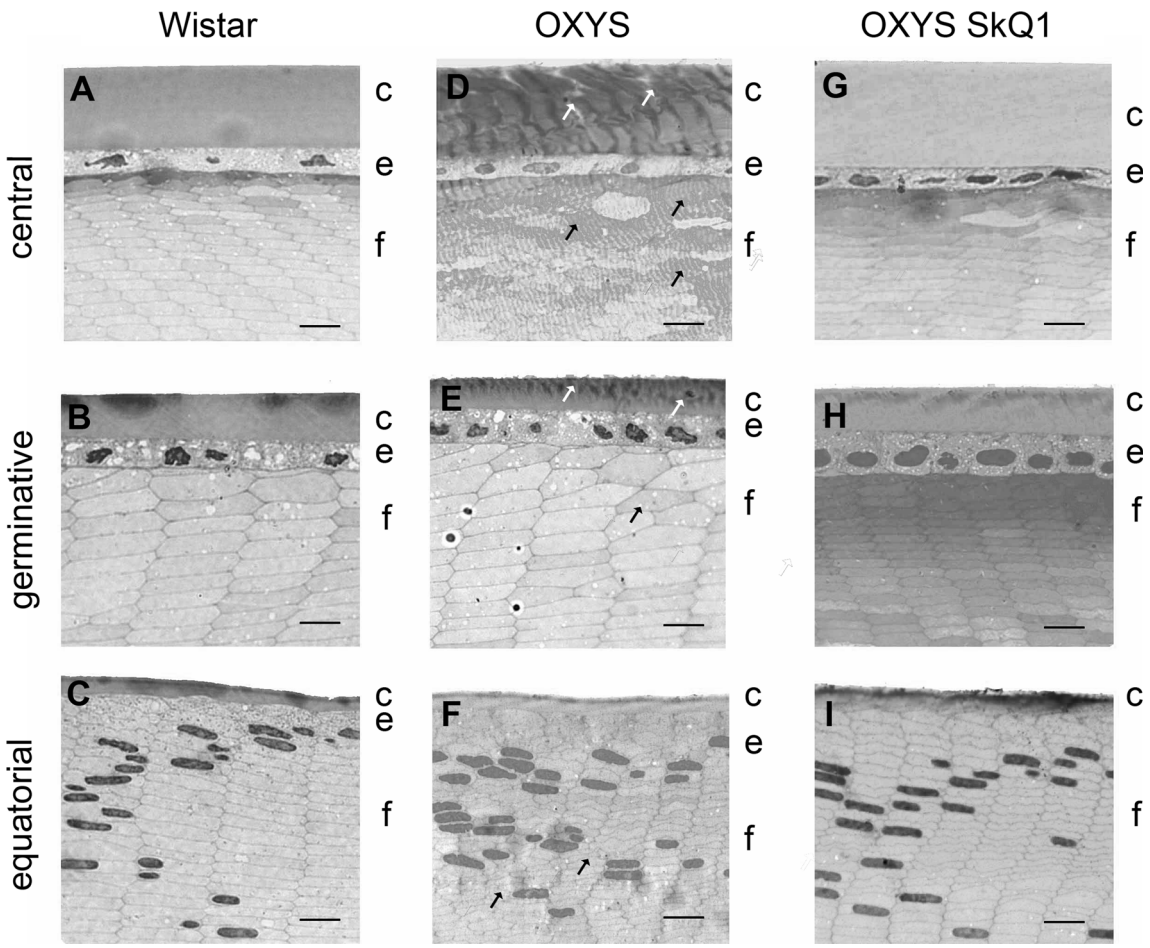

Fig. 2 Comparison of lens morphology of untreated Wistar (**a**, **b**, and **c**) and untreated OXYS rats (**d**, **e**, and **f**) and the lens capsule of OXYS rats treated with SkQ1 eye drops (**g**, **h**, and **i**). The age of all rats is 12 months. *Black arrows* show disruption of the packing of lens fiber cells, and *white arrows* indicate disturbances in the lens capsule. The lens capsule of Wistar rats appears homogeneous, and the lens capsule of OXYS rats starts to show areas of "seals" with age; the capsule of the lens treated with SkQ1 appears homogeneous. The fiber cells of Wistar rats have a regular packing geometry. The fiber cells of intact OXYS have typical (for this age and stage of disease) deviations; fiber cells of OXYS rats treated with SkQ1 are arranged into a regular 3-dimensional hexagonal packing pattern. The Venetian blind artifact in **d** was caused by vibration of a tissue block/knife. Azure II staining; the *scale bar* is 10 μm

untreated OXYS rats. Osmiophilic structures were absent in the sections of the majority of cells, and those that were found had a comparatively small size. Osmiophilic areas of the plasma membrane were absent in the contact zone of the apical plasma membrane of cells of the epithelium and of fiber cells.

Effects of SkQ1 eye drops on α-crystallin gene expression

These effects were evaluated in rats of both strains. Two-way ANOVA showed that αA-crystallin mRNA expression in the lenses was not affected by genotype (strain), but was affected by SkQ1 drops ($F_{1,23}$=8.67, p=0.008), and those two factors were interacting ($F_{1,23}$=5.84, p=0.006). Post-hoc analysis revealed a decreasing mRNA level of the αA-crystallin gene in the lens of untreated OXYS rats ($F_{1,12}$=15.01, p=0.004) compared to the Wistar strain. SkQ1 drops did not change αA-crystallin mRNA expression in Wistar rats, but increased it in OXYS rats ($F_{1,12}$=41.45, p=0.000) to the normal level of Wistar rats (p=0.056).

Expression of the αB-crystallin gene was affected by genotype ($F_{1,23}$=230.81, p<0.0001), and was not influenced by SkQ1 eye drops, but those two factors were interacting ($F_{1,23}$=81.06, p<0.0001). One-way ANOVA revealed a lower level of αB-crystallin mRNA expression in the lenses of untreated OXYS rats compared to untreated Wistar rats ($F_{1,11}$=191.25, p<0.0001). Treatment with SkQ1 drops did not affect the αB-crystallin mRNA expression in Wistar rats, but increased it in OXYS rats ($F_{1,11}$=19.33, p=0.001), albeit not to the normal level of Wistar rats ($F_{1,11}$=165.04, p<0.000). As a result (Fig. 4), in the lens of OXYS rats treated with SkQ1 drops, the transcriptional activity of the αA-crystallin gene was 2.7-fold higher compared to untreated rats, and reached the level of Wistar rats. αB-Crystallin expression

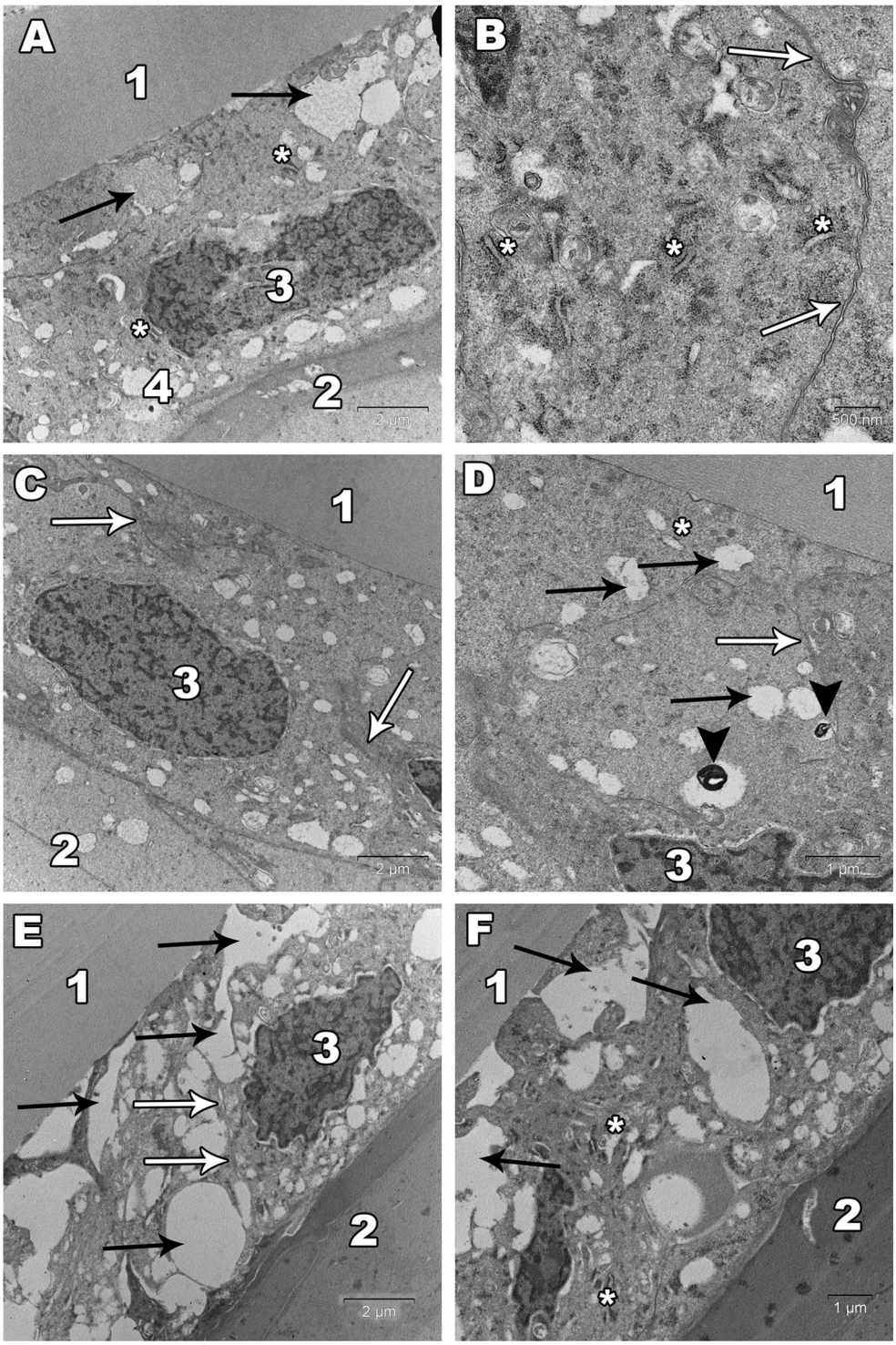

Fig. 3 Epithelial cells of the lens in untreated Wistar (**a**, **b**) and OXYS rats (**c**, **d**) and in OXYS rats (**e**, **f**) treated with SkQ1 eye drops (250 nM). The age of all rats is 12 months. Transmission electron microscopy of ultrathin sections. Epithelial cells in OXYS rats show the absence of cytoplasmic structures and tight contacts with neighboring cells. *1*: a lens capsule adjacent to the basal plasmalemma, *2*: cortical lens fibers adjacent to the apical plasmalemma of an epithelial cell, and *3*: the nucleus. *Asterisks* show cisternae of ER; *black arrows* show the intercellular space among basolateral outgrowths; *white arrows*: the lateral plasmalemma; *arrowheads*: myelinlike structures

increased 3.4-fold compared to the untreated OXYS rats, but was still 5-fold lower than in untreated Wistar rats (Fig. 4). Moreover, a negative correlation was found between the signs of cataract in OXYS rats and αA-crystallin and αB-crystallin gene expression levels (Spearman's $r=-0.53$, $p<0.009$, and $r=-0.67$, $p<0.001$, respectively).

Western blot data on αB-crystallin protein in the lens of OXYS and Wistar rats are shown in Fig. 5. The lens cells of OXYS rats at the age of 12 months showed a significant decrease (by 25.4 %) in the amount of αB-crystallin compared to Wistar rats of the same age ($F_{1,11}=7.03$, $p=0.036$; Fig. 5). After treatment with SkQ1 eye drops, there was a significant

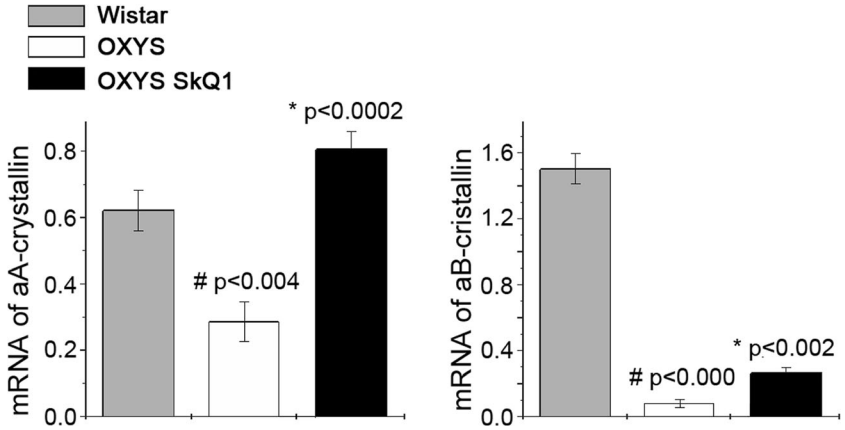

Fig. 4 Relative gene expression levels of αA- and αB-crystallin genes in lens epithelial cells. The figure shows effects of SkQ1 eye drops from 9 to 12 months of age on mRNA expression of αA- and αB-crystallin in the lens OXYS rats (compared to untreated Wistar rats, according to real-time PCR, n=6 to 7; the data are normalized to *Rpl30* gene expression and presented as mean±SEM). The level of expression (a relative fluorescence signal in the PCR reactions) is expressed in arbitrary units. Statistical significance (p<0.05): #interstrain differences, *effects of SkQ1

increase (by 23 %) in the amount of αB-crystallin in the lens cells of OXYS rats ($F_{1,11}$=14.53, p=0.005).

Discussion

The primary purpose of this study was validation of anticataract effects of SkQ1 eye drops on OXYS rats as an animal model of the disease. OXYS rats are already being used to study the mechanisms of cataract formation [13, 20, 21] and to evaluate the efficacy and possible side-effects of new treatments of this disease [7, 22–24] including SkQ1 [8, 25–27]. Previously, to evaluate the therapeutic effects of SkQ1 eye drops and of nutritional supplementation with SkQ1, we used clinical assessment by means of ophthalmoscopy and biomicroscopy only [7–9]. Here, along with the clinical evaluation, histological examination convincingly confirmed that administration of eye drops containing submicromolar concentrations of SkQ1 reverses pathological changes in middle-aged OXYS rats.

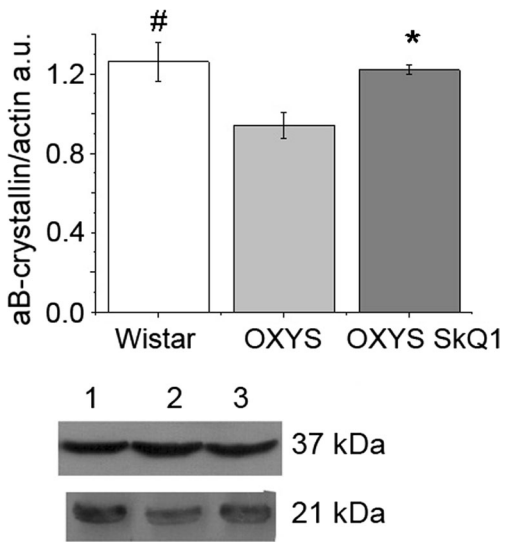

Fig. 5 Alterations in αB-crystallin levels and effects of SkQ1 on OXYS rats. The figure shows Western blot analysis of αB-crystallin in the lens of rats. The relative quantity of the αB-crystallin protein was calculated as intensity of an αB-crystallin band divided by intensity of the corresponding β-actin band. The data are shown as mean±SEM. Statistical significance (p<0.05): #interstrain differences, *effects of SkQ1

Previously, using light and electron microscopy, we demonstrated morphological and pathological changes involving all structural components of the lens in OXYS rats, and we showed that they correspond to senile cataract [28, 29]. In support of our previous reports, we show here that OXYS rats develop cataract resembling human senile cataract with typical disruption or disappearance of normal fiber structure in the opaque region of the lens. All lenses of control OXYS rats exhibit aberrations of varying severity; these problems include large vesicles in cortical fiber cells, swelling and disorganization of fiber cells, and defective migration and elongation of the fiber cells. All of the above leads to worsening of lens transparency. These alterations are thought to be related to dysfunction of transmembrane and cytoskeletal proteins, such as vimentin, nestin, tropomodulin, ephrin, and others [30–32], as well as to disruption of water and ion exchange homeostasis [33, 34]. Furthermore, lens fiber cells in OXYS rats appear rounded and irregular in a cross-section, in contrast to their normal hexagonal appearance in Wistar lenses.

It is known that enhancement of vesicular transport may be associated with disruption of transport systems in the lens, disturbances of the osmotic balance, accumulation of water in the vesicles [35], or with activation of the processes that remove collapsing cells from the cortical layer [36]. The formation of intracellular "vesicles" and expansion of the intercellular space are associated with excessive hydration of

the lens, and are typical for lens fibers in various forms of cortical cataract [37].

In the present work, ophthalmoscopic examination shows that the mitochondria-targeted antioxidant SkQ1 completely prevents the cataract development in OXYS rats, and in some of the animals, it reduces signs of the disease in the lens. As expected, we did not see clarification of lenses in rats with third-stage cataract and markedly impaired vision. Such aberrations are detectable in 4 % of eyes in OXYS rats before and after SkQ1 treatment. At the same time, the number of eyes with the second stage of cataract falls from 46 to 25 %. Some mechanisms of this therapeutic action of SkQ1 eye drops are explored in this study.

Treatment with SkQ1 eye drops was started at the age of 9 months, and it elevates the number of "vesicles" in the lenses of both Wistar and OXYS rats at the age of 12 months. These data may be indicative of enhancement of metabolism of damaged macromolecules and cellular components. As a result, SkQ1 significantly improves the size and packing of cortical fibers in OXYS rats, and thus prevents the development of lens opacification in the cortical areas. It is possible that this effect is due to improvement of the state of cells in the epithelial layer.

The lens capsule is a modified basement membrane that surrounds the ocular lens. The capsule of the avascular lens serves as the sole filter for the transit of solutions and molecules between the ocular environment and the lens cells [38, 39]. Abnormal molecular architecture of the lens capsule may cause spatial variation in its permeability to macromolecules. According to the literature, during aging, there are structural changes in lenticular collagen with a concomitant decrease in solubility; this alteration may play some role in deprivation of the lens of its essential nutrients, thereby causing deficiencies that initiate the development of opacity [40]. Structural changes caused by the damage to proteins are compounded by decreased synthesis of extracellular matrix proteins in the lens epithelium. Accumulation of damaged collagen in the capsule itself can lead to activation of a stress signaling pathway called *unfolded protein response* and to cellular changes underlying cataract formation [41]. At the severe stages of cataract in OXYS rats, we observe characteristic alterations of the lens capsule, which can cause disturbances in the 3-dimensional network formed by a major protein of the lens capsule, collagen IV [41]. As a consequence, a loss of strength, bad permeability, and impaired protective properties of the capsule are expected to ensue. According to the present results, SkQ1 eye drops significantly reduce the anomalies in the lens capsule of OXYS rats. This effect can be a consequence of general improvement of the epithelium functionality and thus of up-regulation of extracellular matrix proteins.

In the germinative region of 1-year-old untreated OXYS rats, electron microscopic analysis of the lens epithelial cells reveals weakening of metabolic and biosynthetic processes compared to age-matched untreated Wistar rats. The structure of the cells clearly shows a lowered level of protein synthesis and signs of destructive processes. The "seal" of the basal labyrinth is suggestive of reduced intensity of transport processes. This downregulation of biosynthetic processes can be interpreted as a consequence of ER stress as a result of accumulation of misfolded proteins. Aberrant posttranslational modifications of lens proteins can cause the unfolded protein response and ER stress, which, according to recent data, perform an important function in cataract pathogenesis [41–44]. Overloading of ER with unfolded or misfolded proteins can cause ER stress and activate the unfolded protein response [44, 45].

In the present work, electron microscopic analysis of the cells shows a low degree of development of the rough ER in the OXYS lens compared to Wistar rats; this deficiency may be due to prolonged exposure to ER stress. Our data show that treatment of lens epithelial cells with SkQ1 normalizes protein synthesis and the development of the rough ER, and thereby may contribute to recovery of lens transparency at early stages of cataract development.

An increase in the number of osmiophilic structures in the cytoplasm of lens epithelial cells takes place in the lens of patients with senile cataract; this change may have something to do with the accumulation of damage to cellular structures and with the degenerative processes [46, 47]. The relevant structures are membrane osmiophilic whorls, i.e., degenerative, often autophagic, bodies belonging to the lysosomal class of cytoplasmic organelles, which may occur in a healthy lens but much less frequently than in a cataract-affected one. In the ultrastructural analysis of OXYS lenses, we uncovered an elevated number of osmiophilic structures compared to the untreated Wistar rats. In OXYS rats that receive the SkQ1 eye drops, the number of cells containing osmiophilic structures is significantly lower than in untreated OXYS rats; this phenomenon may reflect a decrease in oxidative damage to cellular components.

During the development of age-related cataract, in humans, over 1,300 genes are downregulated; among them, 241 genes undergo more than a 5-fold diminution of mRNA expression; α-crystallin genes are also usually downregulated [48]. We showed previously that the development of cataract in OXYS rats is linked to substantial downregulation of αA- and αB-crystallins in the lens [12]. Recently, using high-throughput RNA sequencing (RNA-Seq), we also uncovered extreme downregulation of mRNA of α-crystallin subunits in the OXYS retina during development of AMD-like retinopathy in OXYS rats [49]. Here we confirmed that in the lens of 1-year-old untreated OXYS rats, the levels of mRNA of α-crystallins are significantly lower than in untreated Wistar rats. Treatment with SkQ1 eye drops from the age of 9 months increases the expression of αA-crystallin in the lens of OXYS rats to the level of untreated Wistar rats and significantly

increases the level of αB-crystallin, but expression of the latter gene remains below the healthy level of Wistar rats.

Changes in gene expression during cataract development are associated with damage to the lens epithelial-cell layer or its enzymatic system. This pathology is typical for senile cataract [49]. Using light and electron microscopy, we demonstrated here that cataract development in OXYS rats is also associated with dystrophic and atrophic changes in the lens epithelium, and with insufficiency of its drainage and plasticity function. The SkQ1 eye drops improve the morphological and ultrastructural parameters of the lens epithelium and enhance the expression of α-crystallin subunits, i.e., the heat shock proteins facilitating the functioning of the ER in lens epithelial cells.

α-Crystallin is a major lens protein, constituting up to 40 % of total lens proteins. In the lens, α-crystallin's structural function is to support maintenance of the proper refractive index of the lens. α-Crystallin is the predominant member of the family, and is composed of two types of subunits, A and B, which noncovalently associate to form aggregates with an average molecular weight of 800 kD. α-Crystallin performs a crucial function in preserving lens transparency, not only as a structural protein but also as a lens chaperone, keeping proteins in their native conformation [50]. Factors that suppress the chaperone function of α-crystallin are thought to accelerate aggregation of other crystallins that are undergoing age-related modifications and are losing their native conformation. Consistent with this hypothesis, decreased chaperone activity, increased crystallin aggregation and light scattering, and a loss of lens transparency have indeed all been demonstrated in the aged human lens [51]. One of the proposed mechanisms behind the formation of cataract in humans is aggregation of crystallins that occurs in a highly oxidative environment [52]. Furthermore, cells deficient in αB-crystallin are susceptible to ER stress-induced cell death, whereas αB-crystallin overexpression has protective effects [53]. The pathogenesis of senile cataract is thought to involve accumulation of aberrant posttranslational modifications in proteins, including α-crystallin subunits, with the consequent inhibition of their chaperone activity and formation of insoluble aggregates [54, 55].

There are several reports showing a connection between apoptosis of lens epithelial cells and cataractogenesis [56]. We believe that the effectiveness of SkQ1 in the treatment of cataract is linked to the ability of SkQ1 to reduce mitochondrial reactive oxygen species and, as a consequence, to inhibit mitochondria-mediated apoptosis [26] and to improve expression of α-crystallin subunits, i.e., the heat shock proteins facilitating the workings of ER in lens epithelial cells. The diminished formation of free radicals can hypothetically halt the chain reactions caused by the unfolded protein response, and may reduce ER stress.

In general, our data confirm that treatment with SkQ1 eye drops can not only prevent the development of cataract in senescence-accelerated OXYS rats but also partially reverse signs of early or mild cataract. These data indicate that treatment with SkQ1 may be a promising strategy for cataract prevention in humans.

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References

1. Chang JR, Koo E, Agrón E, Hallak J, Clemons T, Azar D, Sperduto RD, Ferris FL 3rd, Chew EY (2011) Age-related Eye Disease Study Group. Risk factors associated with incident cataracts and cataract surgery in the Age-related Eye Disease Study (AREDS): AREDS report number 32. Ophthalmology 118(11):2113–2119
2. Hooper CY, Lamoureux EL, Lim L, Fraser-Bell S, Yeoh J, Harper CA, Keeffe JE, Guymer RH (2009) Cataract surgery in high-risk age-related macular degeneration: a randomized controlled trial. Clin Exp Ophthalmol 37(6):570–576
3. Gritz DC, Srinivasan M, Smith SD, Kim U, Lietman TM, Wilkins JH, Priyadharshini B, John RK, Aravind S, Prajna NV, Duraisami Thulasiraj R, Whitcher JP (2006) The antioxidants in prevention of cataracts study: effects of antioxidant supplements on cataract progression in South India. Br J Ophthalmol 90(7):847–851
4. Mathew MC, Ervin AM, Tao J, Davis RM (2012) Antioxidant vitamin supplementation for preventing and slowing the progression of age-related cataract. Cochrane Database Syst Rev 6:CD004567. doi: 10.1002/14651858.CD004567.pub2
5. Chew EY, Sangiovanni JP, Ferris FL, Wong WT, Agron E, Clemons TE, Sperduto R, Danis R, Chandra SR, Blodi BA, Domalpally A, Elman MJ, Antoszyk AN, Ruby AJ, Orth D, Bressler SB, Fish GE, Hubbard GB, Klein ML, Friberg TR, Rosenfeld PJ, Toth CA, Bernstein P (2013) Age-related Eye Disease Study 2 (AREDS2) Research Group. Lutein/zeaxanthin for the treatment of age-related cataract: AREDS2 randomized trial report No. 4. JAMA Ophthalmol 131(7):843–850
6. Zheng SJ, Rautiainen S, Lindblad BE, Morgenstern R, Wolk A (2013) High-dose supplements of vitamins C and E, low-dose multivitamins, and the risk of age-related cataract: a population-based prospective cohort study of men. Am J Epidemiol 177(6):548–555
7. Skulachev MV, Antonenko YN, Anisimov VN, Chernyak BV, Cherepanov DA, Chistyakov VA, Egorov MV, Kolosova NG, Korshunova GA, Lyamzaev KG, Plotnikov EY, Roginsky VA, Savchenko AY, Severina II, Severin FF, Shkurat TP, Tashlitsky VN, Shidlovsky KM, Vyssokikh MY, Zamyatnin AA Jr, Zorov DB, Skulachev VP (2011) Mitochondrial-targeted plastoquinone derivatives. Effect on senescence and acute age-related pathologies. Curr Drug Targets 12(6):800–826
8. Neroev VV, Archipova MM, Bakeeva LE, Fursova AZ, Grigorian EN, Grishanova AY, Iomdina EN, Ivashchenko ZN, Katargina LA, Khoroshilova-Maslova IP, Kilina OV, Kolosova NG, Kopenkin EP, Korshunov SS, Kovaleva NA, Novikova YP, Philippov PP, Pilipenko DI, Robustova OV, Saprunova VB, Senin II, Skulachev MV, Sotnikova LF, Stefanova NA, Tikhomirova NK, Tsapenko IV,

Shchipanova AI, Zinovkin RA, Skulachev VP (2008) Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 4. Age-related eye disease. SkQ1 returns vision to blind animals. Biochemistry (Mosc) 73:1317–1328
9. Markovets AM, Fursova AZ, Kolosova NG (2011) Therapeutic action of the mitochondria-targeted antioxidant SkQ1 on retinopathy in OXYS rats linked with improvement of VEGF and PEDF gene expression. PLoS One 6:e21682
10. Andley UP (2009) Effects of alpha-crystallin on lens cell function and cataract pathology. Curr Mol Med 9:887–892
11. Sousounis K, Tsonis PA (2012) Patterns of gene expression in microarrays and expressed sequence tags from normal and cataractous lenses. Hum Genom 6:14
12. Rumyantseva YV, Fursova AZ, Fedoseeva LA, Kolosova NG (2008) Changes in physicochemical parameters and alpha-crystallin expression in the lens during cataract development in OXYS rats. Biochemistry (Mosc) 73(11):1176–1182
13. Kopylova LV, Cherepanov IV, Snytnikova OA, Rumyantseva YV, Kolosova NG, Tsentalovich YP, Sagdeev RZ (2011) Age-related changes in the water-soluble lens protein composition of Wistar and accelerated-senescence OXYS rats. Mol Vis 17:1457–1467
14. Yanshole LV, Cherepanov IV, Snytnikova OA, Yanshole VV, Sagdeev RZ, Tsentalovich YP (2013) Cataract-specific posttranslational modifications and changes in the composition of urea-soluble protein fraction from the rat lens. Mol Vis 19:2196–2208
15. Kolosova NG, Lebedev PA, Dikalova AE (2004) Comparison of antioxidants in the ability to prevent cataract in prematurely aging OXYS rats. Bull Exp Biol Med 137(3):249–251
16. Markovets AM, Saprunova VB, Zhdankina AA, Fursova AZ, Bakeeva LE, Kolosova NG (2011) Alterations of retinal pigment epithelium cause AMD-like retinopathy in senescence-accelerated OXYS rats. Aging (Albany NY) 3:44–54
17. Obukhova LA, Skulachev VP, Kolosova NG (2009) Mitochondria-targeted antioxidant SkQ1 inhibits age-dependent involution of the thymus in normal and senescence-prone rats. Aging (Albany NY) 1:389–401
18. Chylack LT Jr, Wolfe JK, Singer DM, Leske MC, Bullimore MA, Bailey IL, Friend J, McCarthy D, Wu SY (1993) The Lens Opacities Classification System III. The Longitudinal Study of Cataract Study Group. Arch Ophthalmol 111(6):831–836
19. Nolan T, Hands RE, Bustin SA (2006) Quantification of mRNA using real-time RT-PCR. Nat Protoc 1(3):1559–1582
20. Snytnikova OA, Kopylova LV, Chernyak EI, Morozov SV, Kolosova NG, Tsentalovich YP (2009) Tryptophan and kynurenine levels in lenses of Wistar and accelerated-senescence OXYS rats. Mol Vis 15: 2780–2788
21. Dobretsov EA, Snytnikova OA, Koptyug IV, Kaptein R, Tsentalovich YP (2013) Magnetic resonance imaging (MRI) study of the water content and transport in rat lenses. Exp Eye Res 113: 162–171
22. Kolosova NG, Trofimova NA, Shcheglova TV, Sergeeva SV (2005) Increased stress reactivity as a possible factor of early degenerative changes in OXYS rats. Bull Exp Biol Med 139(4):397–399
23. Stefanova NA, Fursova AZ, Sarsenbaev KN, Kolosova NG (2011) Effects of Cistanche deserticola on behavior and signs of cataract and retinopathy in senescence-accelerated OXYS rats. J Ethnopharmacol 138(2):624–632
24. Snytnikova OA, Tsentalovich YP, Stefanova NA, Fursova AZ, Kaptein R, Sagdeev RZ, Kolosova NG (2012) The therapeutic effect of mitochondria-targeted antioxidant SkQ1 and Cistanche deserticola is associated with increased levels of tryptophan and kynurenine in the rat lens. Dokl Biochem Biophys 447:300–303
25. Skulachev VP (2009) New data on biochemical mechanism of programmed senescence of organisms and antioxidant defense of mitochondria. Biochemistry (Mosc) 74(12):1400–1403
26. Skulachev VP, Anisimov VN, Antonenko YN, Bakeeva LE, Chernyak BV, Erichev VP, Filenko OF, Kalinina NI, Kapelko VI, Kolosova NG, Kopnin BP, Korshunova GA, Lichinitser MR, Obukhova LA, Pasyukova EG, Pisarenko OI, Roginsky VA, Ruuge EK, Senin II, Severina II, Skulachev MV, Spivak IM, Tashlitsky VN, Tkachuk VA, Vyssokikh MY, Yaguzhinsky LS, Zorov DB (2009) An attempt to prevent senescence: a mitochondrial approach. Biochim Biophys Acta 1787(5):437–461
27. Stefanova NA, Fursova AZ, Kolosova NG (2010) Behavioral effects induced by mitochondria-targeted antioxidant SkQ1 in Wistar and senescence-accelerated OXYS rats. J Alzheimers Dis 21(2):479–491
28. Kolosova NG, Lebedev PA, Aidagulova SV, Morozkova TS (2003) OXYS rats as a model of senile cataract. Bull Exp Biol Med 136(4): 415–419
29. Kolosova NG, Lebedev PA, Fursova AZ, Morozkova TS, Gusarevich OG (2003) Prematurely aging OXYS rats as an animal model of senile cataract in human. Adv Gerontol 12: 143–148
30. Gold MG, Reichow SL, O'Neill SE, Weisbrod CR, Langeberg LK, Bruce JE, Gonen T, Scott JD (2012) AKAP2 anchors PKA with aquaporin-0 to support ocular lens transparency. EMBO Mol Med 4(1):15–26
31. Nowak RB, Fischer RS, Zoltoski RK, Kuszak JR, Fowler VM (2009) Tropomodulin1 is required for membrane skeleton organization and hexagonal geometry of fiber cells in the mouse lens. J Cell Biol 186(6):915–928
32. Song S, Landsbury A, Dahm R, Liu Y, Zhang Q, Quinlan RA (2009) Functions of the intermediate filament cytoskeleton in the eye lens. J Clin Invest 119(7):1837–1848
33. Donaldson PJ, Chee KS, Lim JC, Webb KF (2009) Regulation of lens volume: implications for lens transparency. Exp Eye Res 88(2):144–150
34. Varadaraj K, Kumari S, Shiels A, Mathias RT (2005) Regulation of aquaporin water permeability in the lens. Invest Ophthalmol Vis Sci 46(4):1393–1402
35. Hara A, Matsumoto M, Uga S (1999) Morphological study on cataractogenesis of the Nakano mouse lens. Graefes Arch Clin Exp Ophthalmol 237(3):249–255
36. Brennan LA, Kantorow WL, Chauss D, McGreal R, He S, Mattucci L, Wei J, Riazuddin SA, Cvekl A, Hejtmancik JF, Kantorow M (2012) Spatial expression patterns of autophagy genes in the eye lens and induction of autophagy in lens cells. Mol Vis 18:1773–1786
37. Feeney-Burns L, Burns RP, Anderson RS (1980) Ultrastructure and acid phosphatase activity in hereditary cataracts of deer mice. Invest Ophthalmol Vis Sci 19(7):777–788
38. Danysh BP, Patel TP, Czymmek KJ, Edwards DA, Wang L, Pande J, Duncan MK (2010) Characterizing molecular diffusion in the lens capsule. Matrix Biol 29(3):228–236
39. Tholozan FM, Quinlan RA (2007) Lens cells: more than meets the eye. Int J Biochem Cell Biol 39(10):1754–1759
40. Ferrell N, Cameron KO, Groszek JJ, Hofmann CL, Li L, Smith RA, Bian A, Shintani A, Zydney AL, Fissell WH (2013) Effects of pressure and electrical charge on macromolecular transport across bovine lens basement membrane. Biophys J 104(7):1476–1484
41. Firtina Z, Danysh BP, Bai X, Gould DB, Kobayashi T, Duncan MK (2009) Abnormal expression of collagen IV in lens activates unfolded protein response resulting in cataract. J Biol Chem 284(51):35872–35884
42. Mulhern ML, Madson CJ, Danford A, Ikesugi K, Kador PF, Shinohara T (2006) The unfolded protein response in lens epithelial cells from galactosemic rat lenses. Invest Ophthalmol Vis Sci 47(9): 3951–3959
43. Ikesugi K, Yamamoto R, Mulhern ML, Shinohara T (2006) Role of the unfolded protein response (UPR) in cataract formation. Exp Eye Res 83(3):508–516

44. Watson GW, Andley UP (2010) Activation of the unfolded protein response by a cataract-associated αA-crystallin mutation. Biochem Biophys Res Commun 401(2):192–196
45. Reneker LW, Chen H, Overbeek PA (2011) Activation of unfolded protein response in transgenic mouse lenses. Invest Ophthalmol Vis Sci 52(5):2100–2108
46. Hayes BP, Fisher RF (1981) The structure of the lens epithelium and its basement membrane in the diabetic state. Curr Eye Res 1(6):317–326
47. Bassnett S (1997) Fiber cell denucleation in the primate lens. Invest Ophthalmol Vis Sci 38(9):1678–1687
48. Hawse JR, Hejtmancik JF, Huang Q, Sheets NL, Hosack DA, Lempicki RA, Horwitz J, Kantorow M (2003) Identification and functional clustering of global gene expression differences between human age-related cataract and clear lenses. Mol Vis 9:515–537
49. Kozhevnikova OS, Korbolina EE, Ershov NI, Kolosova NG (2013) Rat retinal transcriptome: effects of aging and AMD-like retinopathy. Cell Cycle 12(11):1745–1761
50. Nahomi RB, Wang B, Raghavan CT, Voss O, Doseff AI, Santhoshkumar P, Nagaraj RH (2013) Chaperone peptides of α-crystallin inhibit epithelial cell apoptosis, protein insolubilization, and opacification in experimental cataracts. J Biol Chem 288(18): 13022–13035
51. Moreau KL, King JA (2012) Protein misfolding and aggregation in cataract disease and prospects for prevention. Trends Mol Med 18(5): 273–282
52. Benedek GB (1997) Cataract as a protein condensation disease: the Proctor Lecture. Invest Ophthalmol Vis Sci 38(10):1911–1921
53. Dou G, Sreekumar PG, Spee C, He S, Ryan SJ, Kannan R, Hinton DR (2012) Deficiency of αB crystallin augments ER stress-induced apoptosis by enhancing mitochondrial dysfunction. Free Radic Biol Med 53(5):1111–1122
54. Michael R, Bron AJ (2011) The ageing lens and cataract: a model of normal and pathological ageing. Philos Trans R Soc Lond B Biol Sci 366(1568):1278–1292
55. Sharma KK, Santhoshkumar P (2009) Lens aging: effects of crystallins. Biochim Biophys Acta 1790(10):1095–1108
56. Zhang L, Yan Q, Liu JP, Zou LJ, Liu J, Sun S, Deng M, Gong L, Ji WK, Li DW (2010) Apoptosis: its functions and control in the ocular lens. Curr Mol Med 10:864–875