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Human retinal photoreceptor cells in glaucoma: destructive changes of mitochondria and mitophagy

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The study was conducted in accordance with the principles of the Helsinki Declaration of the World Medical Association “Ethical Principles for Scientific Medical Research with the Participation of Human”, Federal Law of the Russian Federation of November 21, 2011 № 323 “About the Basics of Protecting the Health of Citizens in the Russian Federation”, and the requirements Federal Law of July 27, 2006 № 152 (as amended on July 21, 2014) "About Personal Data" (as amended and supplemented, entered into force on September 1, 2015). The study was conducted with the permission of the bioethical committee. The written informed consent of the patients to study the biological material was obtained.

Background

Glaucoma is a multifactorial disease characterized by progressive damage and death of retinal ganglion cells, axon atrophy, and, as a result, optic neurodegeneration. It is known that glaucoma-associated cell death occurs by apoptosis. Apoptosis is caused by oxidative stress through mitochondrial damage, inflammation, endothelial dysregulation and dysfunction, and hypoxia. Dysfunction of autophagy at different stages in neurons leads to the emergence of many neurodegenerative diseases, in particular – glaucoma. The pathogenesis of glaucoma is being studied on various animal models (mice, rats, rabbits, cats, monkeys, etc.). However, data obtained in animal models cannot be fully interpreted for the human eye due to anatomical and physiological differences. There are existing data about damage and ultrastructural changes in retinal ganglion cells, inner nuclear layer, inner plexiform layer, but there is no information about ultrastructural changes in the photoreceptors in glaucoma.

Materials and methods

The object of the study were retinal fragments of patients enucleated by medical indications of the eye with terminal stage of glaucoma of the Novosibirsk branch of Academician S.N. Fyodorov Federal State Institution «Intersectoral Research and Technology Complex «Eye Microsurgery», Ministry of Healthcare of Russian Federation.

Ultrathin sections were obtained from the selected material, contrasted with a saturated aqueous solution of uranyl acetate and lead citrate, and studied using a electron microscope JEM 1400 (Japan). Morphometry was performed using Image J software (Wayne Rasband, USA) with a closed test system at x25K magnification. The mean (M) and standard deviation (SD) were calculated using Microsoft Excel software. The significance of differences between the parameters was determined by Statistica 6.0 software (StatSoft, Inc.) with Mann-Whitney U-test. The differences were considered significant at $p < 0.05$.

Results

The volume densities (V_V) of mitochondria and autophagosomes containing mitochondria in ellipsoids of both rod and cone cells inner segment were counted (Fig. 1). In the rod cells outer segments swelling of membrane disks and their disorganization were noted (Fig. 2).

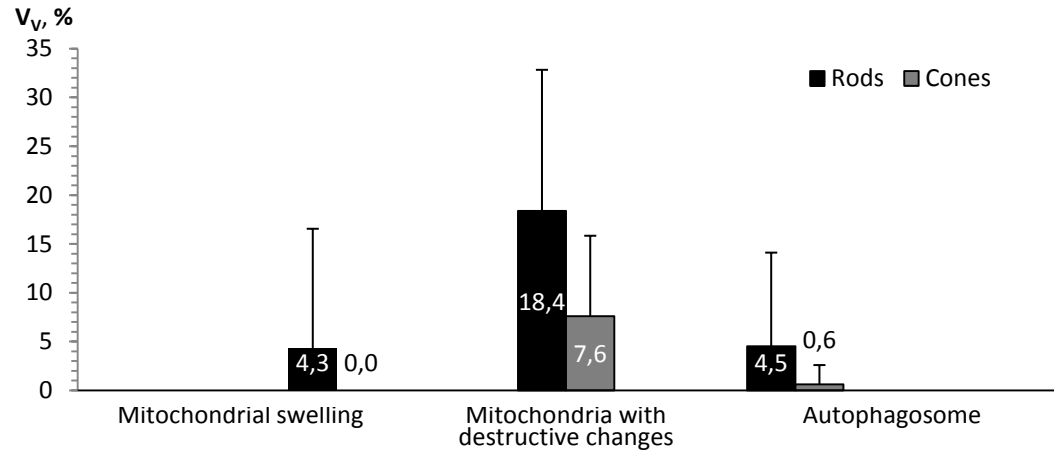


Figure 1. The volume densities (V_V) of damaged mitochondria and autophagosomes.

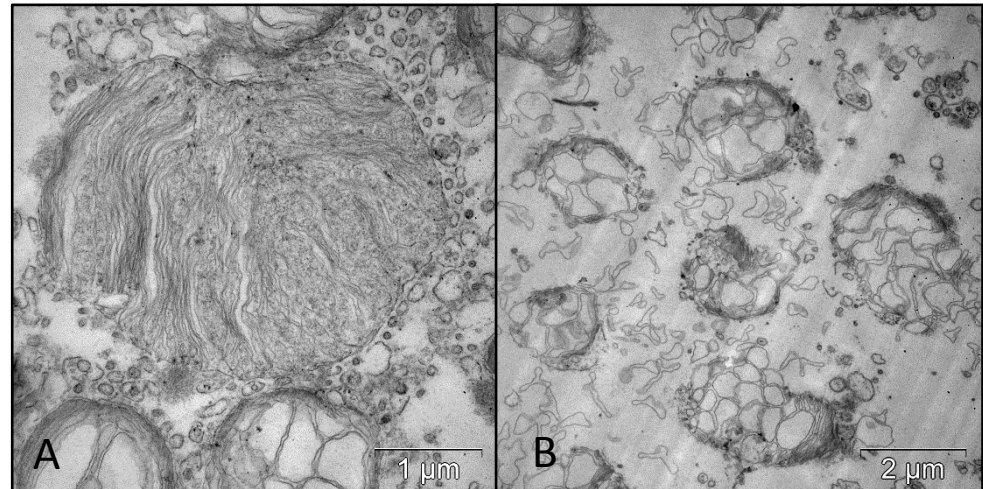


Figure 2. Outer segments of cone (A) and rods (B), TEM.

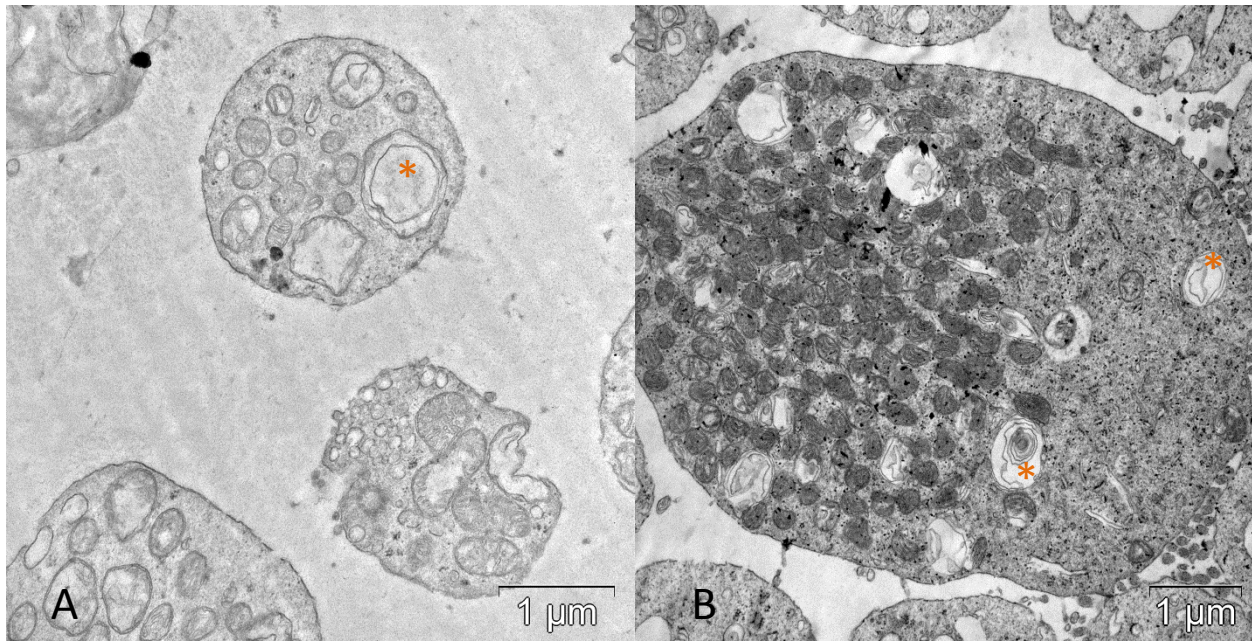


Figure 3. Autophagosomes (*) in ellipsoids of rods (A) and cones (B), TEM.

Cell death associated with glaucoma occurs through apoptosis. It is initiated by many factors: a) relating to mitochondria directly (oxidative stress); b) affecting the development of apoptosis indirectly (Fas-mediated signaling pathway, the effects of neurotoxic proteins: amyloid beta and tau protein). During the study, apoptosis was not detected in the human photoreceptors. However, noted the autophagic activity may lead to the death of photoreceptors.

Conclusion

In our study mitochondria with destructive changes in cristae were detected both in rod and cone cells ellipsoids of all types of photoreceptors. In rods also present mitochondrial swelling and mitophagy. It was revealed that with glaucoma, rods are subject to greater disturbances in contrast to cones. It indicates their more pronounced reaction on an increase of intraocular pressure. Probably, autophagy can play a dual role in retinal cells, on the one hand, contribute to the survival of cells by removing damaged structures, and on the other hand, initiate the apoptosis, that requires further research.