MORPHOLOGY AND PATHOMORPHOLOGY

Ultrastructure of the Endothelium of the Drainage System of the Eye

O. N. Kuleshova, G. I. Nepomnyashchikh, S. V. Aidagulova, and E. V. Shvedova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 5, pp. 574-577, May, 2008
Original article submitted March 11, 2008

The endothelium of the ocular drainage system (Schlemm's canal, collector tubules, and aqueous veins) in primary juvenile glaucoma undergoes degenerative dystrophic changes with compensatory hypertrophy and proliferation at the initial stages of the glaucomatous process and atrophy and desquamation at advanced and terminal stages. Progressive decrease in the pinocytous function of endotheliocytes, reduction of the protein-synthesizing and mitochondrial compartments of the cytoplasm, and formation of autophagosomes reflect the process of endotheliocyte degeneration in general.

Key Words: *primary juvenile glaucoma; ocular drainage system; endothelium; electron microscopy*

Glaucoma is a group of neurodegenerative disorders characterized by the death of retinal ganglion cells and degeneration of the optic nerve axons as a result of lasting ophthalmic hypertension caused by, among other things, disorders in the intraocular hemodynamics and dysfunction of the drainage system of the eye [1,6,9]. Primary open-angle glaucoma (POAG) is the most prevalent and best studied form of the glaucomatous process; primary juvenile glaucoma (PJG) is a less incident form, usually manifesting during the second-third decades of life [2].

Mechanical injury to the endothelium in ophthalmic hypertension impairs the homeostasis of ocular drainage system components, triggering cascade development of the pathological process [12]. The search for indicators of structural and functional changes in the endothelium, which can serve as diagnostic and prognostic markers in various forms of glaucoma and in the course of its correction, is in progress [4,5,7,8,10,11].

We studied ultrastructure of the endothelium of the drainage system of the eye in PJG.

MATERIALS AND METHODS

Fifty-five patients with glaucoma were divided into 2 groups: 1) 25 patients with PJG of different stages (13 with initial and advanced stages, 8 with far advanced, and 4 with the terminal stage) and 2) 30 patients with POAG, 10 with each stage of glaucoma. Group 1 consisted of 13 men and 12 women aged 11-35 years (24.8±4.6 years), group 2 of 21 men and 9 women aged 50-78 years (63.8±14.2 years). The operation material (juxtacanalicular tissue and Schlemm's canal (SC) outer wall) collected during nonpenetrating deep sclerectomy was analyzed.

The specimens were fixed in 4% paraformaldehyde. Paraffin sections (stained with hematoxylin and eosin in combination with Perls reaction,

Institute of Regional Pathology and Pathomorphology, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** pathol@soramn.ru. O. N. Kuleshova

0007-4888/08/1455-0634

after Van-Gieson with post-staining of elastic fibers by Weigert's resorcin-fuchsin, and PAS reaction), semithin sections (stained with azur II), and ultrathin sections (contrasted with uranyl acetate and lead citrate) were examined. Light microscopy was performed using a universal Leica DM 4000B microscope with digital photocamera (Leica DFC 320). For ultrastructural studies, a JEM 1010 electron microscope (accelerating voltage 80 kV) was used.

RESULTS

The endothelial lining of the outer SC wall at the initial and advanced stages of the glaucomatous process was thinned in the majority of cases, in 25% cases the endothelium was partially desquamated; the nuclei in retained cells were pyknotic. Foci of compensatory proliferation of endotheliocytes were detected in 3 preparations: pseudomultilamellar accumulations of large basophilic cells with euchromatic nuclei. In one case we observed pigmented imbibition of the endothelial lining of SC and the juxtacanalicular tissue with supra- and intercellular, rarely intracellular localization of large and finely dispersed melanin granules. The endothelium of intrascleral draining vessels was characterized by intracellular edema, degeneration, and compensatory proliferation, which led to splitting and thickening of the endothelial basal membrane.

Advanced stage of PJG was characterized by more pronounced structural changes in elements of the draining system of the eye. Dystrophic degenerative processes usually predominated during this stage in the absence of compensatory proliferative cellular reactions. The endothelial lining of the outer wall of SC exhibited signs of degenerative dystrophic changes: endotheliocyte desquamation (Fig. 1, *a*), karyorrhexis and coagulation of the cytoplasm in remaining cells (Fig. 1, *b*). In 2 cases, pigmented imbibition of the borderline connective tissue with deposition of large amounts of melanin in the interfibrous zone was noted in the juxtacanalicular tissue exposed after endothelial desquamation. The collector tubules and intrascleral veins in the majority of cases looked "immured" in the fields of fibrous tissue.

The terminal stage of PJG is associated with pronounced destruction of the juxtacanalicular tissue. The SC wall consists of solitary necrobiotically transformed cells adjacent to reduced and sharply changed juxtacanalicular tissue with rare fibroblasts, fragmented elastic and collagen fibers, optically empty spaces forming as a result of high pressure of intraocular humor and due to incompetence of contacts between structural components of the connective tissue.

Electron microscopy of endothelial associations detected ultrastructural changes, corresponding to developing degenerative process. At the initial stages of PJG, the endothelium of the outer SC wall with signs of cytoplasmatic edema was integrated into continuous layer due to tight cell-cell contacts, some of these contacts were destroyed (Fig. 1, *c*). Floccular substrate was adsorbed on the luminal cytolemma; some cells had polymorphic cytoplasmatic processes oriented along the SC wall.

The pinocytotic function of endotheliocytes, evaluated by the number of pinocytous vesicles, varied; the protein-synthesizing compartment of the cytoplasm was appreciably reduced, the mitochondria were vacuolated. Numerous microfilaments and lysosome-like incorporations forming autophagosomes were seen, some of them included pinocytous vesicles reflecting endotheliocyte degeneration processes (Fig. 1, *d*).

With the disease progress and at the stage of photo-optically detected atrophy and desquamation of SC endothelium, the ultrastructural organization of remaining cells was characterized by high electron density of the matrix, destruction and reduction of cytoplasmatic organelles (particularly membranous), which was paralleled by reduced functional activity. Endotheliocyte disintegration, degeneration, and desquamation resulted in denudation of the subendothelial matrix (Fig. 1, *e*).

The most significant ultrastructural changes in the collector tubules and aqueous veins also involved endothelial lining: vacuolation and destruction of mitochondria, formation of autophagosomes, extracellular edema, and violation of the luminal cytolemma integrity, which was paralleled by disorganization of the basal membrane: thickening, uneven condensation, loss of fibers, multiplication, and interweaving of collagen fibrils (Fig. 1, *f*).

Ultrastructural changes in the endothelium of the ocular drainage system in POAG reflected stereotypical heterogeneous dystrophic/atrophic restructuring of cells at different stages of the glaucomatous process, the intensity and dissemination of these processes augmenting at later stages of the disease. Approximation of the inner and outer walls of SC and filling of the lumen by amorphous substance were observed at the far-advanced stage in the majority of cases.

On the whole, PJG was characterized by more pronounced degenerative dystrophic changes in the endothelial lining of the ocular drainage system in comparison with POAG, while pigmented imbibition of the outer SC wall was less pronounced and

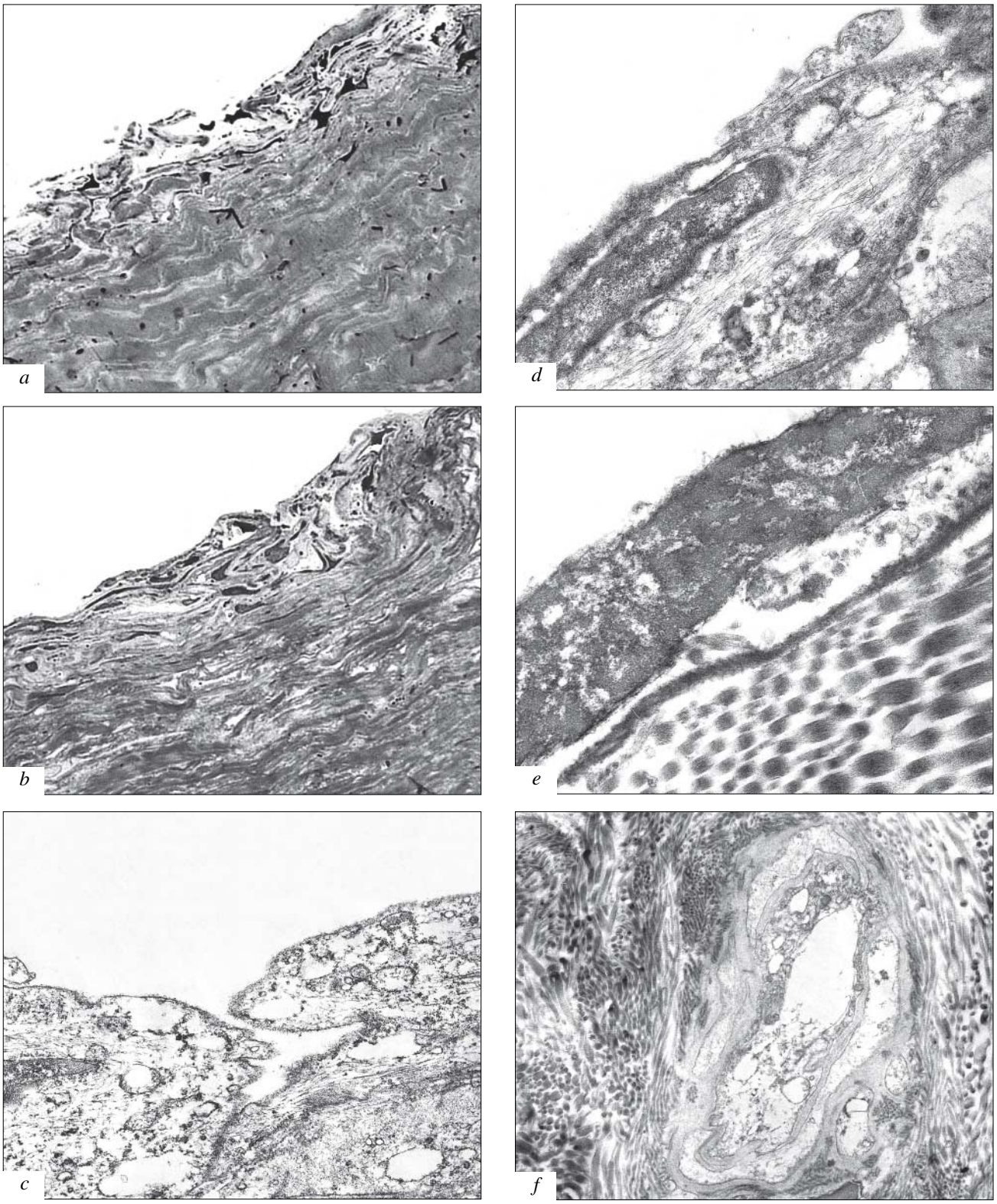

Fig. 1. Structural characteristics of the endothelium of the drainage system of the eye (*a*, *e*: SC; *f*: collector tubule) in PJG. *a*, *b*) semithin sections, azur II staining; *c-f*) electronograms. *a*) desquamation of endothelium, ×250; *b*) endothelial degeneration, ×250; *c*) vacuolation of endotheliocyte cytoplasm, ×10,000; *d*) karyopyknosis, autophagosomes, multiple tonofilaments, ×6000; *e*) osmiohilia and disorganization of endotheliocyte cytoplasm, ×15,000; *f*) disorganization of endothelial lining, multiplication of basal membrane, ×4000.

cellular infiltration of the juxtacanalicular tissue was atypical.

Hence, the findings of photo-optic and electron-microscopic analysis of components of the ocular drainage system indicate stereotypical and systemic structural changes of different severity in PJG. The endothelium of the SC outer wall, collector tubules, and scleral veins undergoes gradual degenerative dystrophic changes (reduction of the pinocytous function, destruction and reduction of cytoplasmatic organelles) with compensatory hypertrophy and proliferation at the initial stages of the glaucomatous process and atrophy and desquamation during advanced and terminal stages.

In general, the prognosis of PJG largely depends on the balance between the two basic cell reactions of the endothelium of the ocular drainage system: degeneration and regeneration. This balance is one of the components of PJG pathogenesis, including also genetically determined qualitative and quantitative changes in the connective tissue glycosaminoglycans [3].

REFERENCES

1. N. I. Koshits, O. V. Svetlova, K. E. Kotlyar, *et al.*, *Glaukoma*, No. 1, 41-62 (2005)
2. M. M. Krasnov, *Microsurgery for Glaucoma* [in Russian], Moscow (1980).
3. O. N. Kuleshova, A. M. Zaidman, and A. V. Korel', *Byull. Eksp. Biol. Med.*, **143**, No. 3, 354-357 (2007).
4. P. T. Atalar, E. Atalar, H. Kilic, *et al.*, *Int. Heart J.*, **47**, No. 1, 77-84 (2006).
5. K. H. Baratz, C. B. Nau, E. J. Winter, *et al.*, *Cornea*, **25**, No. 9, 1046-1052 (2006).
6. B. T. Gabelt and P. L. Kaufman, *Prog. Retin. Eye Res.*, **24**, No. 5, 612-637 (2005).
7. M. A. Kass, D. K. Heuer, E. J. Higginbotham, *et al.*, *Arch. Ophthalmol.*, **120**, 701-713 (2002).
8. F. W. Price Jr. and M. O. Price, *J. Cataract Refract. Surg.*, **32**, No. 3, 411-418 (2006).
9. V. Senatorov, I. Malyukova, R. Fariss, *et al.*, *J. Neurosci.*, **26**, No. 46, 11,903-11,914 (2006).
10. C. C. Tham, Y. Y. Kwong, J. S. Lai, and D. S. Lam, *J. Glaucoma*, **15**, No. 6, 482-485 (2006).
11. N. Yepes, F. Segev, M. Hyams, *et al.*, *Cornea*, **26**, No. 5, 530-533 (2007).
12. K. Wnuczko and M. Szczepanski, *Pol. Mercur. Lekarski*, **23**, No. 133, 60-65 (2007).