Effect of Ascovertin on Morphological Changes in Rat Retina Exposed to High-Intensity Light

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Experiments on rats showed that high-intensity light exposure (6000 lux, 6 h) caused focal injuries in the retina. The most sensitive structures were neurosensory cells, pigmented epithelium, radial gliocytes, and choroid capillaries. Injection of ascovertin led to disappearance of foci of injuries, limited blood supply disorders in the retina, and destruction of neurosensory and glial cells.

Key Words: *retina; photoinjury; ascovertin; neurosensory cells*

Photoinjuries to the eyes and particularly the ocular nervous system remain an important problem for humans: technological progress led to creation and application of high intensity artificial sources of light [2]. One of the mechanisms of photoinjury is photosensitized free-radical oxidation [3,5,9]. Disorders in the retinal hemodynamics also play an important role in photoinjury to the retina [8]. Bioflavonoid compounds attract special interest in this respect [1]. Ascovertin, a drug including a complex of dihydroquercetin (bioflavonoid) and ascorbic acid (1.0:2.5) was developed at Institute of Pharmacology. The drug is characterized by pronounced antioxidant activity, promotes normalization of blood rheology [7], and is effective in ischemia [4].

We studied the effect of ascovertin on the development of morphological changes in the retina during exposure to bright light.

MATERIALS AND METHODS

Experiments were carried out on 30 outbred male albino rats (200-250 g). The animals were divided into 3 groups (10 per group). Groups 1 and 2 were exposed to fluorescent light (LB-40 lamps with maximum radiation in the violet and green spectra) for 6 h. A special device consisting of rectangular reflectors with build-in lamps was used for exposure. The illumination intensity was 6000 lux. Group 2 rats were injected with ascovertin (20 mg/kg dihydroquercetin, 50 mg/kg ascorbic acid) in 1% starch gel for 5 days (the treatment was started 2 days before photoexposure). The animals were decapitated under ether narcosis on days 7 (n=5) and 30 (n=5) after irradiation. Intact rats kept under conditions of artificial day/night regimen (12:12 h) with daylight intensity of 25 lux served as controls.

Central areas of the posterior eyeball wall were fixed in a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.2 M cacodilate buffer (pH 7.4). The material was postfixed in 2% osmium tetroxide and embedded in epon. Semithin sections were stained with toluidine blue, ultrathin sections were contrasted with uranyl acetate and lead citrate, examined and photographed in a JEM-7 A electron microscope. For light microscopy the posterior ocular wall was fixed in Carnoy fluid. The sections (5-7 μ) were stained with hematoxylin and eosin. Neurosensory cells (NC) with signs of nucleus destruction (pyknosis, rhexis, lysis) were counted on semithin sections per 1000 photoreceptors,

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the number of layers and density distribution of nuclei (per mm^2 of a section) in the external nuclear layer (ENL) and the level of hyperchromatic pyknomorphic radial gliocytes per 200 cells from each retina were evaluated. The specific area of open retinal choroid capillaries was counted using Avtandilov measuring grid.

The data were processed using Statistica 6.0 software by methods of variation statistics. The significance of differences between the mean values was evaluated using Mann—Whitney nonparametric test.

RESULTS

Destructive changes in the structural components of the retina were observed in group 1 animals during all stages of observations, with sharply pronounced focal injuries. In group 2 lesions of the ocular retinal membrane were diffuse and less pronounced. On day 7 pigmented epithelium in the foci of lesions was flattened in group 1; sites without pigmented epithelial cells were seen. By day 30 the majority of pigmented epithelial cells were pyknotic. In group 2 during the same period changes in pigmented epithelial cell were reactive, which manifested in activation of their phagocytic function. At later terms proliferation and hypertrophy of pigmented cells was observed. The photosensory layer was absent in the foci of injuries in group 1 rats on day 7 of the experiment. Solitary NC were seen in ENL; new blood capillaries were seen (Fig. 1, *a*). The number of NC with destruction of the nuclei in the foci of lesions increased (49.3±0.47 *vs.* 2.03±0.08% in the control, $p<0.05$). Numerical density of the nuclei in ENL was significantly lower

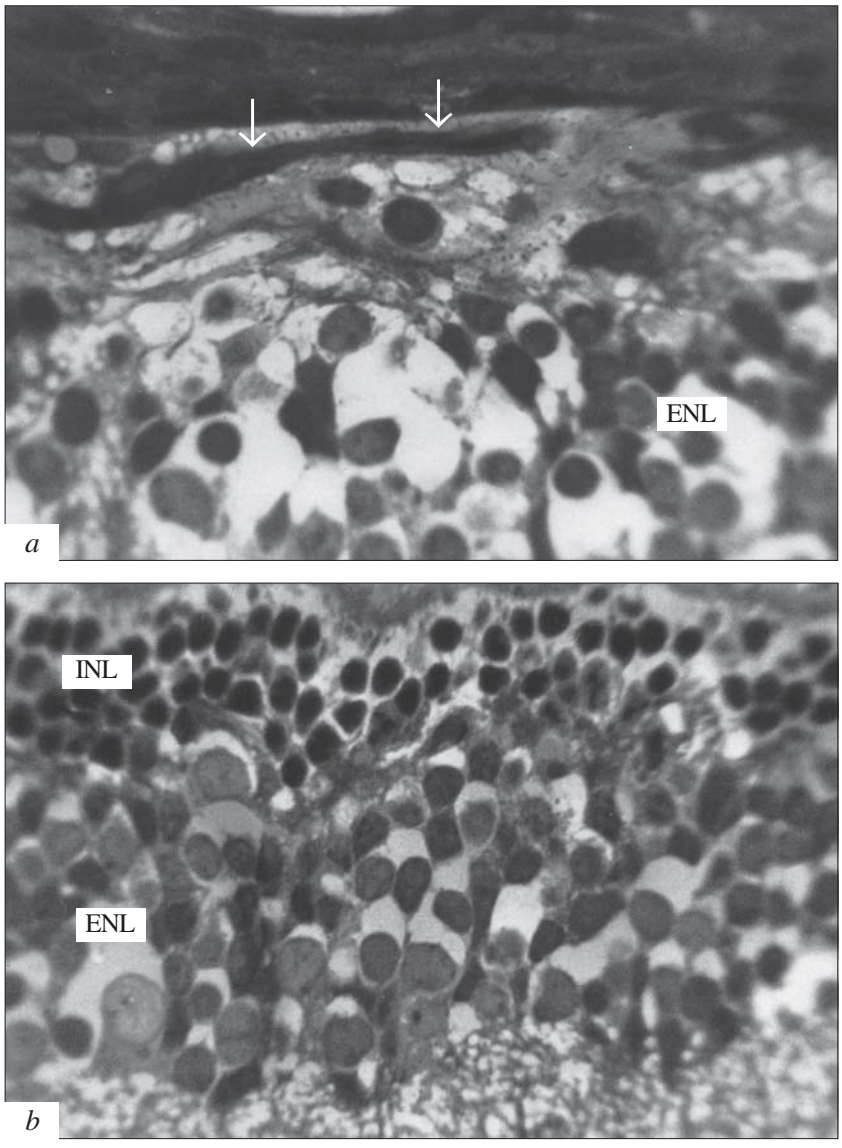

Fig. 1. Structural changes in retina after exposure to high-intensity photoradiation. *a*) complete destruction of photosensory and external nuclear layers, neovasculogenesis (arrows) on day 7 after photoexposure in the focus of lesions; *b*) partial destruction and thinning of external nuclear layer on day 30 after photoexposure after ascovertin treatment; semithin sections, toluidine blue staining, ×900. ENL: external nuclear layer; INL: inner nuclear layer.

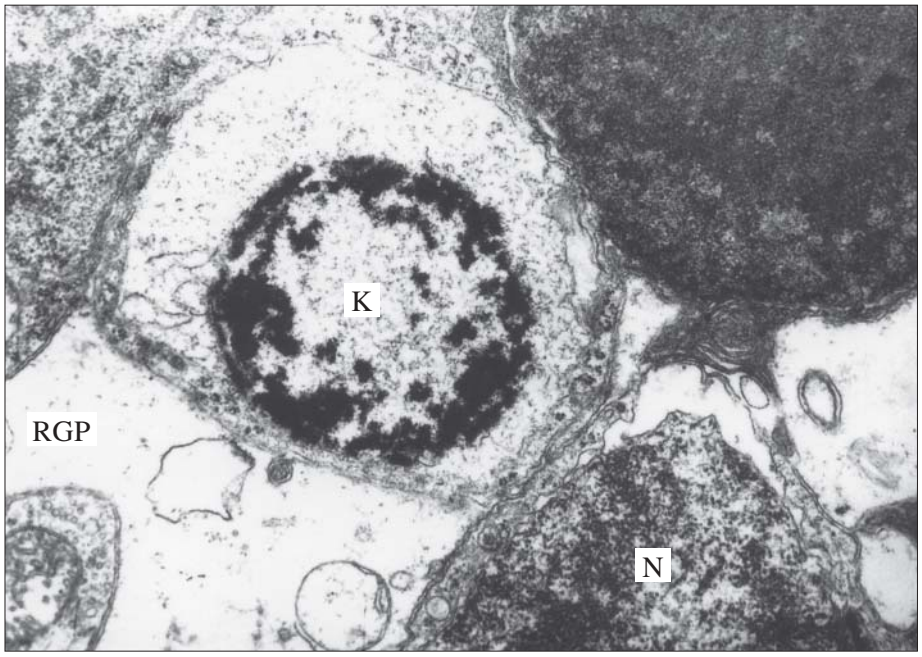

Fig. 2. Karyorrhexis of a neurosensory cell surrounded by hypertrophic and edematous processes of radial glia in the external nuclear layer on day 7 after irradiation after ascovertin treatment (×10,000). K: karyorrhexis; N: neurosensory cell nuclei; RGP: radial gliocyte processes.

in comparison with the control (1532.70±11.49 *vs.* 3491.00±67.12 in the control) because of phagocytosis of dead cells. By day 30 the count of NC with destroyed nuclei decreased (5.37±0.11% in the foci), but their level remained above the control. The cytoplasm volume in the remaining cells was decreased, deformation of the nuclei was observed. Numerical density of the nuclei outside the foci was the same as in the control. Disruption and fragmentation of NC outer segments were observed in ascovertin-treated rats on day 7 of the experiment. The number of destructively modified NC corresponded to the control level; the density of NC nuclei decreased significantly, the number of layers decreased to 4-5 *vs.* 10-12 in the control. Presumably, this was due to activation of the phagocytic function of pigmented epithelial cells, radial gliocytes, and macrophages migrating from blood vessels during the initial period after the exposure and also due to improvement of retinal blood supply after ascovertin injection. On day 30 ENL consisted of 3-4 layers of cells (Fig. 1, *b*). A typical apoptosis picture was observed in some NC: chromatin condensation, fragmentation of nuclear material, phagocytosed apoptotic bodies in radial glial processes surrounding dead cells. Some scientists consider that programmed cell death is one of the key moments in the photoinjury to the retina [6]. On day 7 the count of pyknomorphic radial gliocytes in the foci increased to 29.70± 0.27% in group 1 *vs.* 2.65± 0.15% in the control ($p<0.05$). By day 30 their count appreciably decreased, but remained above the control. In group 2 glial reactions on day 7 after experimental exposure were characterized by elevated phagocytic activity. Gliocytes with cytoplasm of low electron density, containing numerous lysosomes and phagosomes, were detected in the inner nuclear layer. Edematous and hypertrophic scleral processes of gliocytes surrounded destructively modified NC perikaryons (Fig. 2) and, presumably, participated in phagocytosis. The content of hyperchromatic pyknomorphic radial gliocytes was 3.9 times lower than in group 1 ($p<0.05$), but 2.9 times surpassed the control ($p<0.05$). By day 30 the content of hyperchromatic pyknomorphic gliocytes

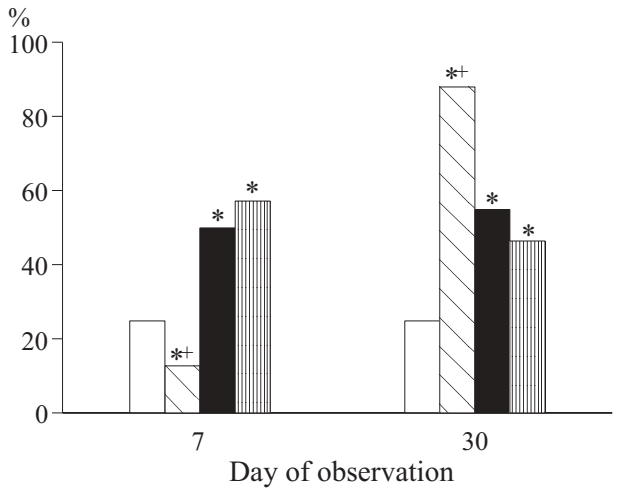

Fig. 3. Specific area of open choroid capillaries of the retina after high-intensity photoexposure in animals treated with ascovertin. Light bars: control; cross-hatched bars: group 1; horizontal hatching: group 2. p<0.05 *compared to the control, +compared to group 2.

increased to 13.3±1.2% ($p<0.05$), but was significantly lower than in group 1.

On day 7 after exposure the specific area of open choroid capillaries decreased significantly in the foci (Fig. 3). On day 30 choroid capillaries were dilated, the basal complex was thinned, nuclear pyknosis and vacuolation of endotheliocyte cytoplasm were observed. Stasis and sludge of blood cells were detected in choroid capillary lumens, which presumably augmented retinal hypoxia and the severity of injuries inflicted to its structures. In group 2 the specific area of open retinal choroid capillaries on day 7 increased 2.3 times in comparison with the control ($p<0.05$) and remained at this level until day 30.

The data of light and electron microscopy confirm the positive effect of ascovertin on the retina of rats exposed to bright. Two major mechanisms the effect of light on the retina are distinguished: the first of them (direct) is realized via photooxidtion of rhodopsin and phospholipids of retinal membrane structures and leads to degeneration of the neurosensory cells, the second (indirect) leads to irreversible changes in the vessels and blood-retinal barrier, which causes retinal hypoxia [3]. Presumably, ascovertin inhibits free radical oxidative processes and improves blood supply to the retina due to its antioxidant and hemorheological characteristics [4,6]. This leads to disappearance of foci of injuries and helps to retain the worst damaged structures, such as NC, and pigmentoepitheliocytes, actively phagocytosing destroyed external segments and inactivating free radicals, thus preventing their penetration into the inner layers of the retina and inhibiting glial destruction. Presumably, that is why the glioneuronal relationships are characterized mainly by progressive proliferative changes in the radial glia.

Hence, the results indicate a protective effect of ascovertin on the eye retina exposed to intense light.

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