Effects of Carovertin on the Reaction of Retinal Pigmented Epithelium and Radial Glia to Bright Light

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Light exposure (6000 lux, 6 h) caused reactive changes in rat retinal pigmented epithelium and radial glia on day 1. Foci of lesions with virtually complete absence of the layers formed by neurosensory cells appeared on day 7. The number of destructively changed radial gliocytes in these foci was by one order of magnitude higher than in the control. Carovertin reduced destruction of pigmented epithelium and radial glia and reduced the area of lesion foci.

Key Words: *radial glia; pigmented epithelium; light; carovertin*

Activation of free radical LPO accompanies various eye diseases [1-3], including the photoinjuries to the retina. The mechanisms of these injuries includes photooxidation of membrane structures [5,6]. Antioxidants can be pathogenetically justified correctors of lesions developing as a result of LPO activation [4]. Carovertin (CV), a composition of water- and oil-soluble antioxidants, including β-carotene, dihydroquercetin flavonoid, and ascorbic acid, was developed at Institute of Pharmacology; it is an interesting potential corrector of these disorders.

We studied the effects of CV on the type and dynamics of structural changes in the pigmented epithelium (PE) and radial glia of eye retina caused by high-intensity photoexposure.

MATERIALS AND METHODS

Experiments were carried out on 60 outbred adult male albino rats (200-250 g). Group 1 animals (n=20) were exposed to light from LB-40 fluorescent lamps with the maximum radiation in the yellow-green spectrum for 6 h. A special device consisting of rectangular reflector lamps illuminating the cage from 5 sides was used. The illumination intensity was 6000 lux.

Group 2 animals (n=20) were exposed to light according to the same protocol and received intragastric CV (10 mg/kg dihydroquercetin, 50 mg/kg ascorbic acid, and 1 mg/kg β-carotene) in 1% starch gel for 5 days; the treatment was started 2 days before photoexposure. All animals were kept under conditions of artificial day/night regimen (12/12 h), with 25 lux intensity of daytime illumination.

Intact rats (n=20) kept under similar conditions served as the controls. The rats were decapitated under ether narcosis 1, 7, 14, and 30 days after photoexposure. The posterior wall of the eyeball was fixed in Carnoy solution for light microscopy. Vertical sections (5-7 μ) were stained with hematoxylin and eosin. For electron microscopy, the central areas of the posterior wall of the eye were fixed in a solution containing 4% paraformaldehyde and 0.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4). The material was postfixed in 2% OsO_4 and embedded in epon. Semithin sections were stained with toluidine blue, ultrathin sections were contrasted with uranyl acetate and lead citrate, exa-

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mined and photographed under a JEM-7 A electron microscope.

The relative area of foci of retinal lesions was evaluated in paraffin sections using an Avtandilov grid. Pyknomorphic radial gliocytes were counted per 200 cells in semithin sections, and the glioneuronal index in the internal nuclear layer was estimated. Specific thickness of PE section was calculated using Avtandilov's ocular measuring grid.

The significance of differences between the means was evaluated using Mann—Whitney nonparametric test.

RESULTS

Examination of group 1 animals 24 h after exposure to high-intensity light showed that some PE cells were hypertrophic and the content of phagosomes in their cytoplasm increased. Ultramicroscopic study showed hypertrophic microvilli phagocytizing degenerative external segments of neurosensory cells (NSC) (Fig. 1). On day 7 after exposure foci of lesions characterized by destruction of the outer layers appeared in the retina. The area of destruction foci in retinal sections reached 26.8±0.55%, which differed significantly ($p<0.05$) from the corresponding parameter in group 2 (13.2±0.6%).

Morphometric analysis showed that specific thickness of the retinal PE section in foci of destruction significantly decreased in delayed periods after exposure (Fig. 2). This characteristic was significantly higher in group 2 than in group 1 and did not differ from the control level (Fig. 2). Being an antioxidant complex, CV presumably protected PE from the detrimental effects of free radicals, thereby limiting NSC destruction. For example, 1-2 rows of NSC nuclei were detected in lesion foci in group 1 *vs.* 2-4 rows in group 2.

After death of NSC from exposure to high-intensity light the apical processes of radial gliocytes grew and proliferated in the subretinal space (Fig. 3, *a*). Together with macrophages and PE cells they participated in utilization of degenerative NSC displaced into the subretinal space. Pyknotic NSC remaining in the outer nuclear layer was surrounded by multilamellar glial plates isolating them from intact tissues (Fig. 3, *b*). The larger the focus of lesions after exposure to high-intensity light, the more significant glia proliferation was [2]. In addition to proliferation, we observed destructive changes in radial gliocytes manifesting by high osmiophilia of the cytoplasm and nuclear pyknosis. The number of destroyed cells reached 10.25±0.13% as soon as 24 h after exposure, which differed significantly ($p<0.05$) from the control (2.65±0.13%). The maximum values in this parameter were observed in the focus of destruction on day 7 after exposure: 29.50±0.51%; later the count of pyknomorphic radial gliocytes decreased. In group 2 the count of destructively changed gliocytes was significantly lower than in group 1 during all periods of the experiment. Presumably, this was due to antioxidant activity of CV protecting membrane structures of the glia.

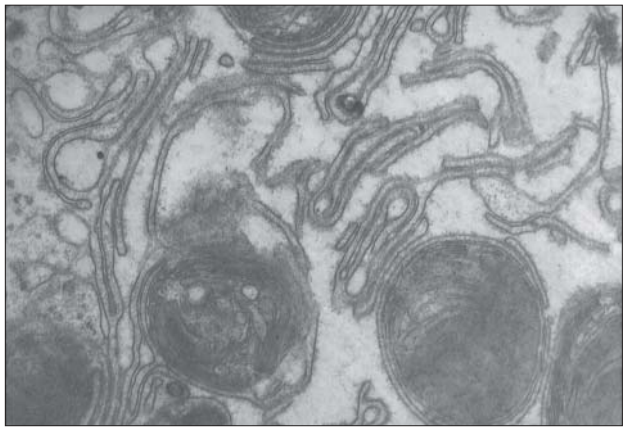

Fig. 1. Hypertrophic microvilli in PE, phagocytosis of membrane disks on day 1 after photoexposure, ×11,000.

The increase of the glioneuronal index in the inner nuclear layer was observed in group 1 animals as early as on day 7 after exposure; the maximum values (0.670±0.007) were recorded on day 30, which was significantly higher than in the control (0.370±0.007; $p<0.05$). Presumably, this was due to death of associative neurons and capacity of the glia to reproduction via mitotic division. The values in group 2 virtually did not differ from those in group 1.

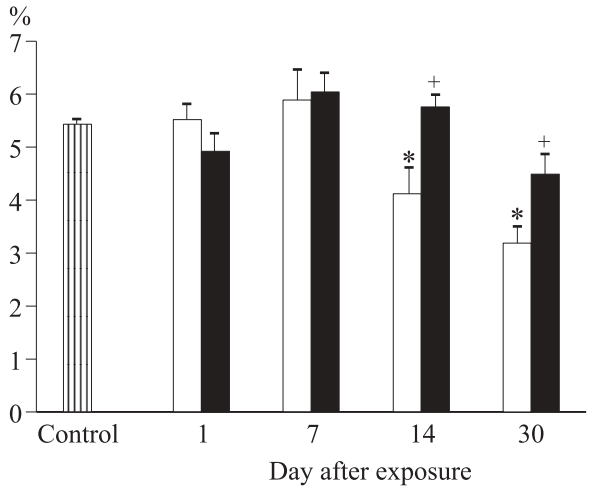

Fig. 2. Specific thickness of rat retinal PE section after photoexposure during CV treatment. Light bars: group 1; dark bars: group 2. $^{*}p<0.05$ compared to the control; $^{+}p<0.05$ compared to group 1.

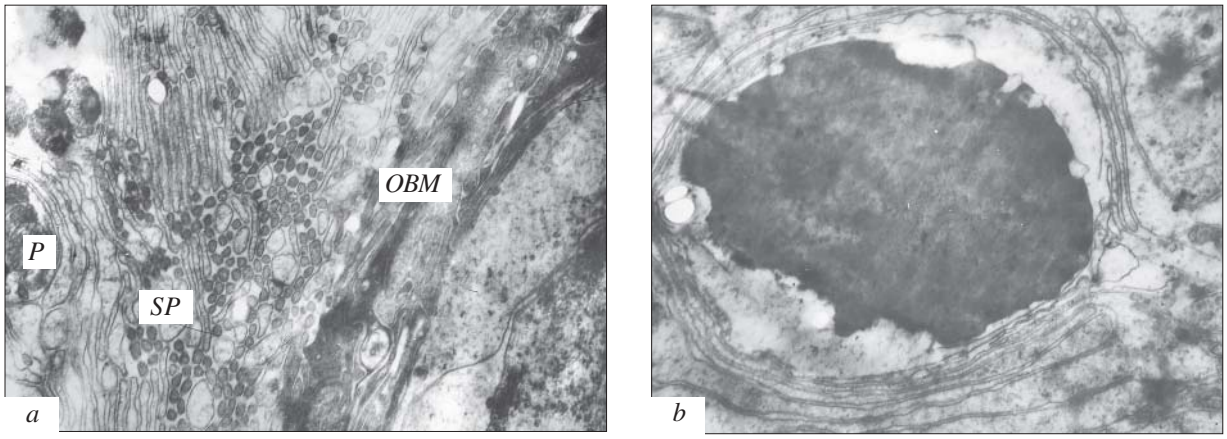

Fig. 3. Glial proliferation on day 30 after exposure against the background of CV treatment. *a*) growth of hypertrophic microvilli of radial glia into the subretinal space, ×9000. *OBM*: outer borderline membrane; *SP*: scleral processes; *P*: phagosomes, *b*) pyknotic neurosensory cell surrounded by glial plates, ×8000.

The role of PE during photoexposure consists in utilization of destructively changed NSC due to capacity to phagocytosis and in the protection of NSC from photodamage due to increased expression of the neuroprotective factors and activity of PE antioxidant enzymes [7,12]. Under conditions of high-intensity illumination, PE cannot utilize increased amounts of destroyed photoreceptor membranes accumulating in it in the form of lipofuchsin granules. Lipofuchsin exposed to visible light generates oxygen and functions as a photosensitizer [8]. Failure of adaptation leads to death of PE and NSC. During the same period macrophages and microglia migrate into the subretinal space; in addition to phagocytosis of the degradation products, they participate in the generation of superoxide and hydroxyl radicals and release factors inducing apoptosis of NSC and PE.

Radial glia permeates all layers of the retina and participates in neuronal damage and reparation processes. After photoexposure it is characterized by high proliferative activity and along with phagocytes participates in elimination of destructive elements and limitation of the destruction focus. This process is important for prevention of secondary necrosis and development of inflammatory reaction [10]. Due to its antiradical activity, CV protects membrane structures of cells and stimulates their proliferation and hypertrophy.

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