Structural Changes in Components of the Blood-Retina Barrier in Rat Retina during Photodamage in Alloxan Diabetes and Their Correction with Ascovertin

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The outer part of the blood-retina barrier was most sensitive to light exposure (6000 lx, 6 h) during photodamage. It was manifested in hemodynamic disturbances, endothelial dysfunction, and focal death of the pigment epithelium. The photo effects increased during alloxan diabetes. The specific area of open vessels decreased, while the number of thrombotic vessels in the choroid increased. Administration of ascovertin improved hemodynamic parameters of the eye and decreased the specific area of focal damage.

Key Words: *light; alloxan diabetes; blood-retina barrier; ascovertin*

The retina has 2 sources of blood supply. The first source is the choroid. Endotheliocytes of the choriocapillaris in combination with the basal complex and pigment epitheliocytes (PE) form the outer part of the blood-retina barrier. The second source is the central retinal artery. Intraretinal capillaries of this artery in combination with the basal membrane and perivascular processes of radial glial cells form the inner part of the blood-retina barrier. Light exposure causes damage to all cells in the retina [6, 11,12]. Diabetes mellitus is accompanied by dysfunction and pathological changes in the microcirculatory bed of the retina [7,12], which results in hemodynamic disturbances and hypoxia. Vascular disorders in diabetes are mainly related to a decrease in viscoelastic properties of the blood and dysfunction of the endothelium and pericytes. Induction of oxidative stress and lipid peroxidation during light exposure increases the severity of damage [5,6].

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Ascovertin is a promising retinoprotective agent. This medical product exhibits pronounced hemorheological properties. Ascovertin is a complex of the bioflavonoid diquertin and ascorbic acid. Ascovertin was synthesized and patented at the Institute of Pharmacology [2,9].

Here we studied morphological changes in vessels and blood-retina barrier of the retina during photodamage in alloxan diabetes. We also evaluated whether ascovertin can be used for the correction of this disorder.

MATERIALS AND METHODS

Experiments were performed on 100 adult male outbred albino rats weighing 200-250 g. The animals were divided into 5 groups (20 rats per group). Groups 1 consisted of intact rats maintained under 12:12-h light/dark conditions (daylight 25 lx). The animals of groups 2-5 were subjected to total light exposure with LB-40 lamps (6000 lx) for 6 h. Group 2 rats were not subjected to additional treatments. Group 3 animals intragastrically received ascovertin in a dose of 70 mg/kg for 5 days. Ascovertin treatment was started 2 days before light exposure. Dia-

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betes mellitus in group 4 animals was induced by intraperitoneal injection of alloxan (single dose 15 mg/100 g) 1 month before light exposure. The severity of disease was estimated from hyperglycemia (more than 15 mmol/liter), body weight loss, and degree of polyuria and polydipsia. Group 5 animals with alloxan diabetes were subjected to light exposure and intragastrically received ascovertin.

Samples of the retina were taken immediately after euthanasia of rats. The animals were killed under ether anesthesia on days 1, 7, 14, and 30 after light exposure. Central regions from the posterior wall of the eye were fixed with a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4). The samples were postfixed with 2% solution of OsO_4 and embedded into Epon. Semithin sections were stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate, examined, and photographed using a JEM-7A electron microscope. For light microscopy, the posterior wall of the eye was fixed with Carnoy's fluid. Sections (5-7 μ) were stained with hematoxylin and eosin. The specific area of focal damage to the retina was measured. The specific area of intact open vessels and thrombotic vessels in the choroid was estimated on semithin sections using an Avtandilov grid. The significance of differences between the means was evaluated by nonparametric Mann—Whitney test.

RESULTS

The choriocapillaris was most sensitive to treatment. Reactive changes (swelling) in capillary endotheliocytes from animals of all groups were revealed 1 day after light exposure. Destruction of mitochondria and widening of cisternae of the granular endoplasmic reticulum were found in the cytoplasm. The size and phagocytic activity of PE increased under these conditions.

Focal damage to the retina and severe focal destruction of neurosensory cells and PE were observed on day 7 after light exposure. The specific area of focuses in group 2 rats reached 26.80±1.72%, which significantly differed from group 4 animals (56.10±1.81%, $p<0.05$). The course of ascovertin treatment reduced the severity of destruction, which was manifested in a significant decrease in the specific area of focuses (16.06±0.62 and 36.1±1.9% in rats of groups 3 and 5, respectively, $p<0.05$).

The size and location of focal damage to the retina coincided with changes in choroidal vessels. The lumen of most vessels in these focuses was

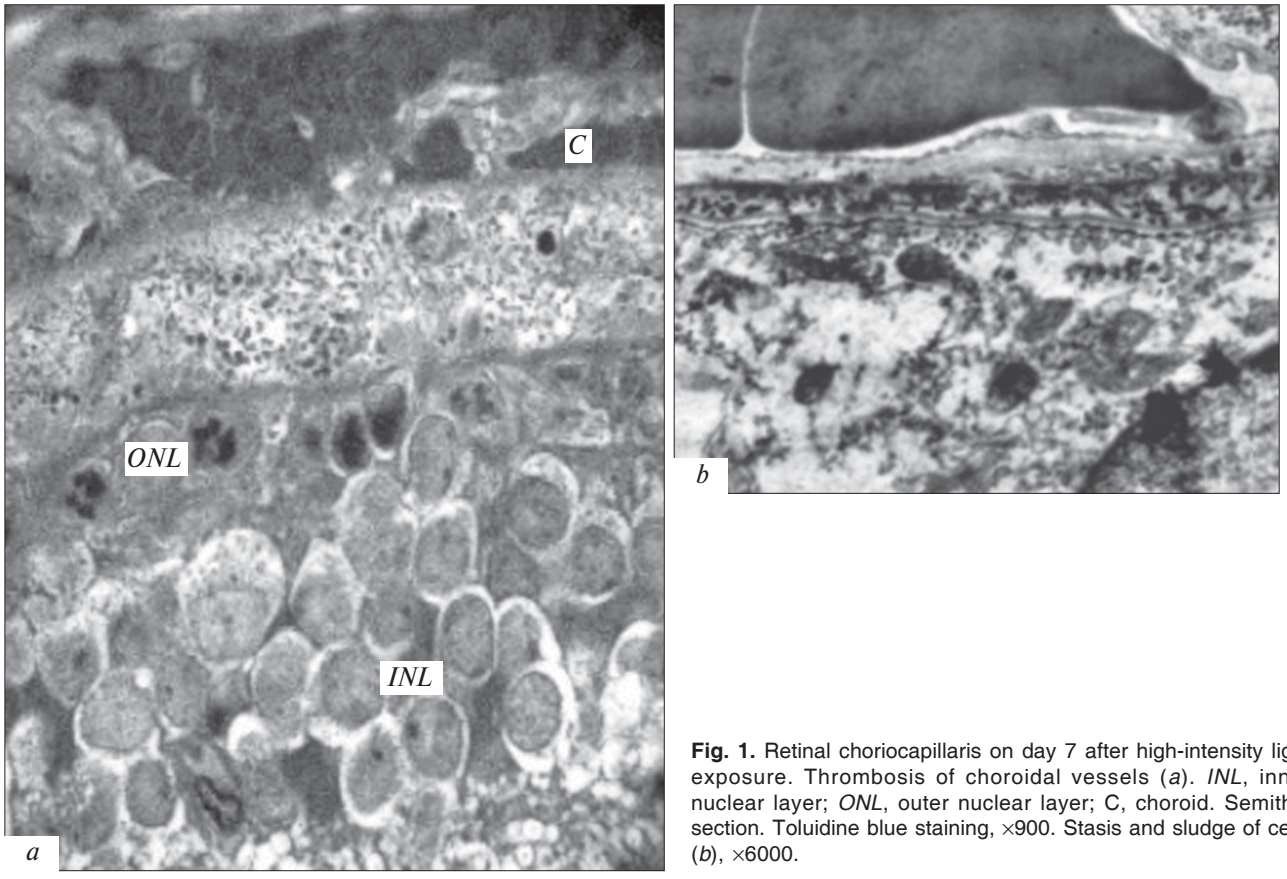

Fig. 1. Retinal choriocapillaris on day 7 after high-intensity light exposure. Thrombosis of choroidal vessels (*a*). *INL*, inner nuclear layer; *ONL*, outer nuclear layer; C, choroid. Semithin section. Toluidine blue staining, ×900. Stasis and sludge of cells (*b*), ×6000.

sharply narrowed in animals of all groups. Some vessels did not contain cells, while others were characterized by thrombosis, stasis, and sludge (Fig. 1). These changes were accompanied by a sharp decrease in the specific area of intact open vessels (Fig. 2, *a*) in group 2 and 4 animals ($p<0.05$). We revealed a significant increase in the specific area of choroidal vessels, sludge of cells, and thrombosis (Fig. 2, *b*). The specific area of intact open vessels in group 3 and 5 rats was much higher than in group 2 animals (by 1.7 and 1.9 times, respectively, $p<0.05$).

Ultrastructural study of the choriocapillaris revealed irregular thickening and stratification of the basal membrane and degenerative changes in several endotheliocytes (destruction of organelles and appearance of myelin-like bodies). The size of most PE decreased in focal damage. These cells had pyknotic nuclei and strongly osmiophilic and vacuolated cytoplasm. Microvilli were not identified. Hypertrophic PE prevailed in ascovertin-treated animals. Some regions did not contain PE and photosensory or outer nuclear layer. Newly formed vessels invaded through defects of the basal complex. They included large scalloped nucleus and had no basal membrane. Intraretinal vessels were relatively resistant to damaging treatments. Changes in pericapillary elements of the radial glia prevailed under these conditions. Processes of glial cells around the capillary were characterized by swelling and low electron density. The cytoplasm of these processes had vacuoles and considerable amounts of lysosomes and phagosomes. The pericapillary space was widened. The basal membrane of capillaries was of high electron density and irregular thickness. The cytoplasm of endotheliocytes had a small number of organelles, but included pinocytotic vesicles and large vacuoles.

On days 14-30 after light exposure, most choriocapillaries in focal damage were replaced by low differentiated fibroblastic cells. Vacuolization of the endotheliocyte cytoplasm and decrease in the number of organelles were revealed in preserved vessels of the choroid. Morphometry of focal damage to the retina in animals with alloxan diabetes showed that the area of thrombotic vessels significantly increases, while the area of intact open vessels decreases on day 30 after light exposure (Fig. 2). Ascovertin significantly decreased the area of thrombotic vessels ($p<0.05$). Mosaic changes were revealed in PE from group 3 and 5 animals (pyknosis and hypertrophy).

Our results show that photodamage in intact animals and rats with alloxan diabetes is accompanied by small changes in intraretinal vessels. Significant changes were found in the choriocapillaris and outer part of the blood-retina barrier. Variations in functional activity of PE (important component in antiradical and antihypoxic protection of the retina) and microcirculatory bed of the choroid due to specific structural characteristics of retinal vessels (dichotomic division of each vascular trunk, terminal pattern of blood supply to retinal zones, and irregular distribution of microvessels in various regions of the retina) contribute to the appearance of ischemic zones in the choroid, which results in focal damage to structural elements of the retina [7].

Dysfunction of the microcirculatory bed, ischemia, and destruction of the pigment epithelium and basal complex during high-intensity light exposure

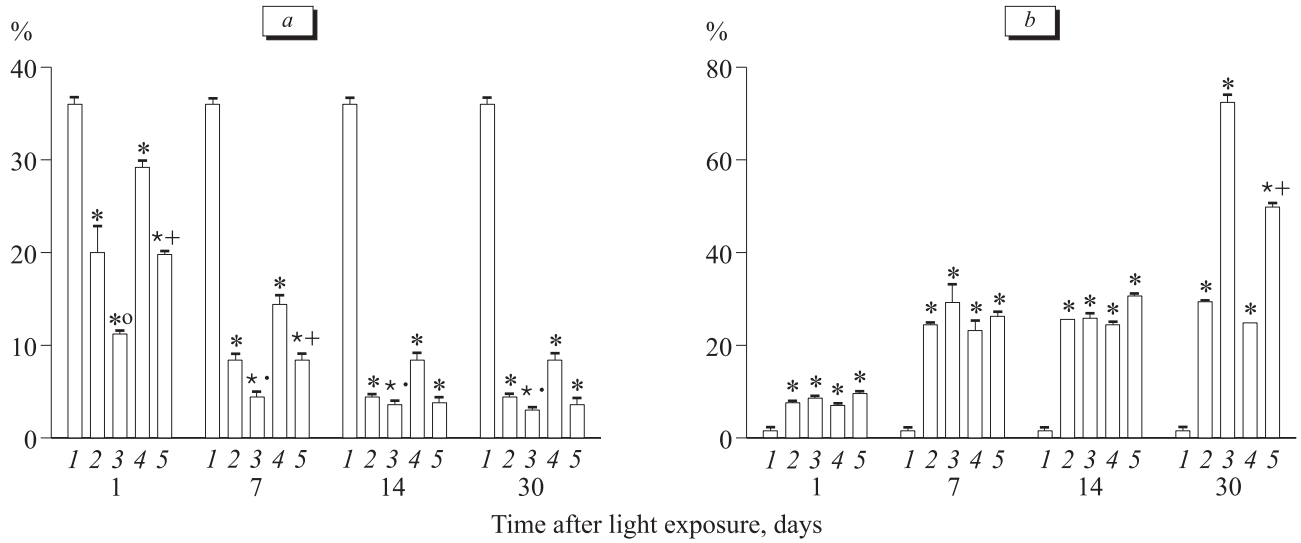

Fig. 2. Specific area of open (*a*) and thrombotic choroidal vessels (*b*) in rat retina. *1-5*: groups of animals. $p<0.05$: *compared to group 1; °compared to group 3; +compared to group 5.

are followed by impairment of the blood-retina barrier and formation of new vessels on day 30 after light exposure. Degenerative changes in vessels and appearance of new vessels in animals with alloxan diabetes were observed on day 7 after light exposure. They were associated with microangiopathy due to diabetic retinopathy. Glucose toxicity is manifested in nonenzymatic glycosylation of proteins, activation of protein kinase C and aldehyde reductase, changes in lipid metabolism, variations in the function of growth factors and renin—angiotensin system, and development of oxidative stress and tissue hypoxia and results in microangiopathy [1,3,10,11]. These changes in combination with high-intensity light exposure have a strong synergistic effect and increase the severity of retinal photodegeneration. Ascovertin has a strong modulatory effect on rheology and microcirculation and exhibits antioxidant properties. This compound improves vascular blood flow in the retina, contributes to a decrease in the specific area of focal damage, and prevents degenerative processes.

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