Photo-induced Damage to Neurosensory Retinal Cells after Preliminary X-Ray Irradiation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 8, pp. 237-240, August, 2000
Original article submitted March 2, 2000

Preliminary X-ray irradiation (5 Gr) enhances damages to neurosensory retinal cells by visible light (3500 lx, 48 h) in rats. This combined treatment caused early destruction of the external and preterminal processes, karyopyknosis and karyolysis of about 30% neurosensory cells followed by focal destruction of the corresponding retinal layers.

Key words: *neurosensory cells; light; X-ray radiation*

Neurosensory cells (NSC) of the retina are extremely vulnerable structures during exposure to both light [9,11] and ionizing radiation [8,10]. Sometimes these two factors can act synchronously [2,6]. However, combined effect of these factors on the structure of photoreceptors has not been studied yet.

The aim of the present study was to evaluate the effect of preliminary X-ray irradiation on changes in retinal NSC induced by bright light.

MATERIALS AND METHODS

The experiments were conducted with 150 outbred adult rats of both sexes. Group 1 rats (n=30) were subjected to uniform 48-h illumination under LB-40 luminescent lamps (peaks in violet and green bands of the spectrum). Rectangular reflectors illuminating the cage from 4 sides were used [3]. The illumination intensity was 3500 lx. Group 2 rats (n=30) were subjected to single whole-body X-ray irradiation (5 Gr) on a RUM-17 device. In group 3 rats (n=45) X-ray irradiation was followed after 1 h by illumination with the same parameters. Intact rats kept under the conditions of artificial illumination with 12-h light-dark cycle and illumination intensity of 200 lx (n=25, control I) and 25 lx (n=20, control II) served as the control. The animals were decapitated 30 min, 1, 10, 30, and 180 days after the experiment. Central segment of the posterior eyeball wall was fixed in 2.5% glutaraldehyde on cacodylate buffer (pH 7.4), postfixed in 2% OsO_4 and embedded in araldite. Semithin sections were stained with toluidine blue, ultrathin sections were contrasted with uranyl acetate and lead citrate and examined and photographed under a JEM-100 CX electron microscope. On semithin sections NSC with nucleus destruction (pyknosis, rhexis, lysis) were counted per 1000 photoreceptors and the density of these nuclei in the external nuclear layer (ENL) was determined. The significance of differences was estimated by Student's t test.

RESULTS

At early terms (30 min, 1 day), disorientation of membrane disks, focal enlargement or shrinkage of intermembrane spaces, rhexis and lysis of external membranes in most NSC were observed in group 1 (Fig. 1, *a*). Mitochondrial swelling and enlargement of endoplasmic reticulum were observed in the internal segments. Karyopyknosis was noted in some cells. Agglutination of synaptic vesicles and shortening of synaptic contacts were found in the preterminal processes. After 10 days most external segments were restored due to renewal of membrane disks. However, some areas of photosensory layer (PL) contained fragments of destroyed external segments; ENL was presented by solitary NSC nuclei with hypertrophied radial glial processes between them. In some preterminal proces-

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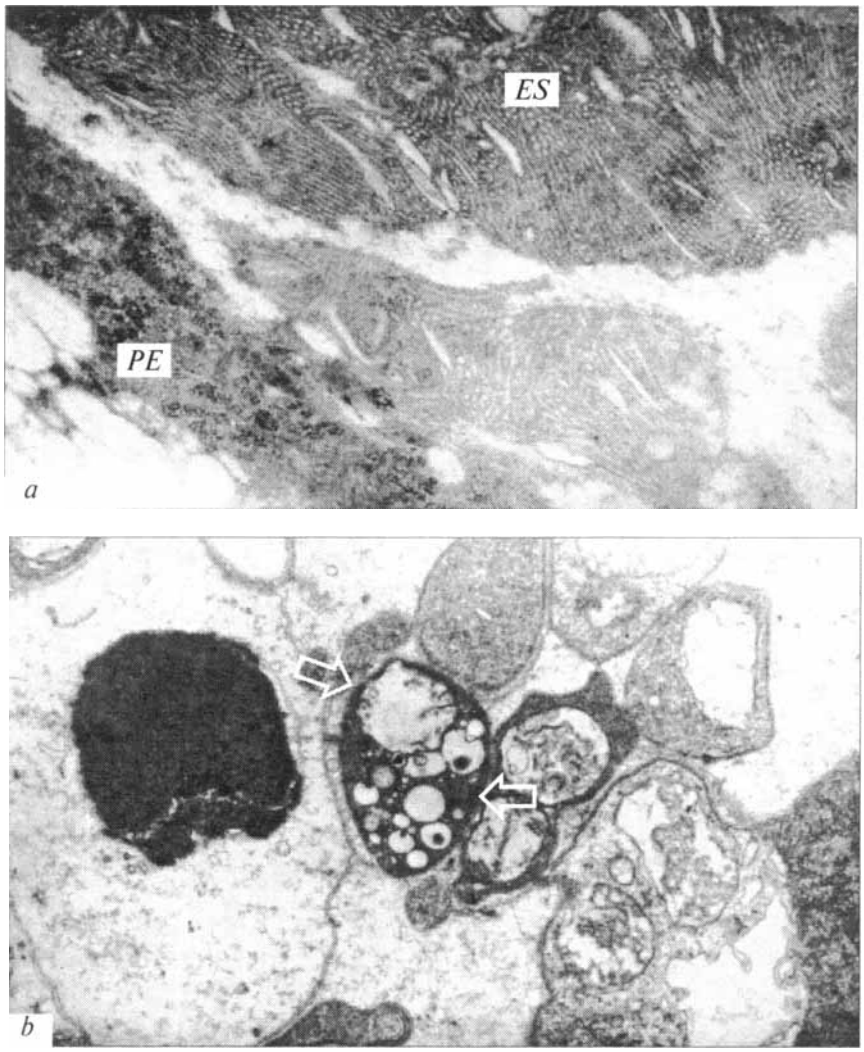

Fig. 1. Ultrastructural changes in neurosensory cells after light illumination. *a*) vesicular degeneration, disorientation of membrane disks in external segments of retinal neurosensory cells 30 min after illumination, ×14,000; *b*) agglutination of synaptic vesicles, vacuolization and destruction of preterminal processes (arrowheads) 10 days after illumination, ×48,000. *PE*: pigment epitheliocyte, *ES*: external segments.

ses increased osmiophilia, edema, destruction of mitochondria with the formation of large electron dense vesicles, and agglutination of synaptic vesicles were noted (Fig. 1, *b*).

Discomplexation of membrane disks in the distal parts of external segments one day after X-ray irradiation were less pronounced than after light illumination. Some NSC showed chromatin condensation and increased electron density of the nuclei. On day 10 post-irradiation, multivesicular bodies and myelin-like structures were found in some NSC, cisterns of the endoplasmic reticulum and perinuclear spaces were enlarged. At later stages (6 months), ultrastructure of NSC in group 2 did not differ from the control.

At the early stages of the experiment with combined treatment, PL were completely necrotic in some retinal zones, while other parts contained sharply decreased number of external segments and pyknotic and lysed NCS nuclei (Fig. 2). After 10 days, ENL nuclei were not numerous and pyknomorphic, while subretinal space contained solitary external segments, phagosome-rich macrophages, and hypertrophied glial processes.

At later terms (1 and 6 months) in groups 1 and 3, the content of external segments in PL significantly decreased, but their structure was somewhere completely restored. In some retinal zones, the majority of NSC and pigment epitheliocytes died and focal adhesions between the internal nuclear layer and basal complex and intensive neovasculogenesis were observed.

The integrity of nuclei is the main criterion of reversible NSC changes. The number of degenerated NSC nuclei (karyopyknosis, karyolysis) 30 min after exposure and up to 30 days was significantly higher than in control ($p<0.05$, Table 1). Thirty minutes after X-ray irradiation the number of nuclei per 1 mm^2 ENL decreased to 22,590±1777 compared to 34,576±

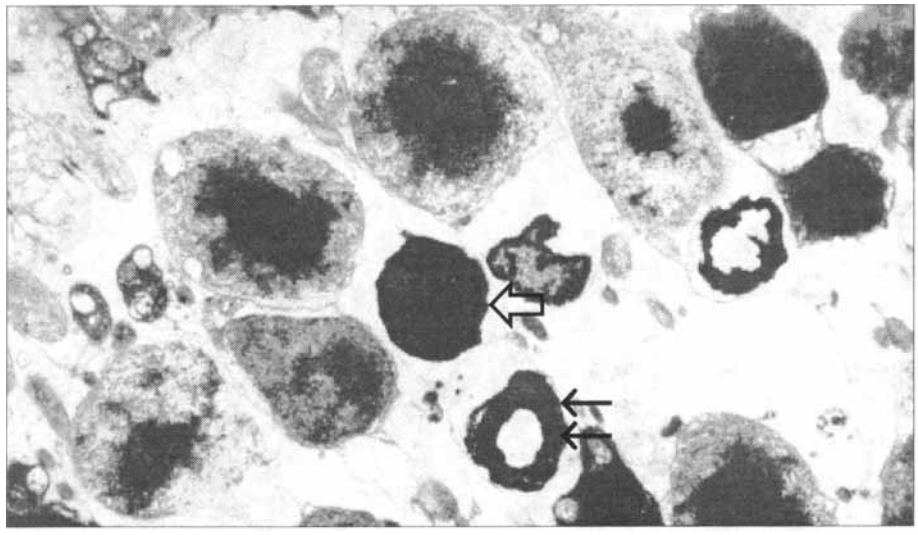

Fig. 2. Karyopyknosis (arrow) and karyolysis (double arrow) in neurosensory cells 1 day after combined irradiation, ×4,800.

TABLE 1. Content (%) of Destructively Changed NSC Nuclei after Irradiation of the Retina ($M \pm m$)

Time after exposure	Control	Exposure		
		light	x-ray	combined
30 min	1.9±0.2	7.50±0.43*	3.80±0.31*	32.5±4.3*+
1 day	1.9±0.2	8.00±0.51*	4.20±0.49*	33.2±5.0*+
10 days	1.9±0.2	7.40±0.82*	3.0±0.4	24.2±8.1*+
30 days	1.6±0.1	7.6±1.1*	2.6±1.1	3.8±1.1
180 days	2.0±0.4	4.40±0.96*	2.5±0.6	3.3±0.8

Note: $p<0.05$: *compared to the control or +combined treatment.

793 and 41,373±200 in controls I and II, respectively ($p<0.05$). This decrease was due to destruction and phagocytosis of nuclei and because of hypertrophy and swelling of radial glia processes between NSC. Thirty min and 1 day after combined exposure, the content of destructively changed NSC nuclei 8-8.5-fold surpassed that after light illumination ($p<0.05$, Table 1). A marked decrease in this parameter was observed on day 30 of the experiment due to utilization of dead NSC by glial cells and macrophages. Thirty minutes after combined treatment, the density of nuclei in ENL decreased to 11,304±3193 ($p<0.05$ compared to group 1), but later this parameter increased due to alleviation of glial edema and after 10 days it did not differ from that group 1.

Thus, preliminary X-ray irradiation promoted photodegeneration of retinal NSC, which was most pronounced at the early stages after combined treatment. This synergistic effect attests to a key role of lipid peroxidation in light- and radiation-induced damage to the retina [4,5,7]. NSC are most vulnerable to free radical damage because of extremely high content of polyenoic fatty acids in membrane phospholipids vulnerable to lipid radicals and reactive oxygen species. Intensive vascularization and aeration of the retina are also important [7]. Combined effect of X-rays and light can be most pronounced in albino animals, because melanin of the retinal pigment epithelium protects eye structures from ionizing radiation [5]. Moreover, thrombosis and obliteration of some choriocapillaries, pyknosis in their endothelium due to its high radiosensitivity [1], and increased permeability of the blood-retina barrier can also contribute to the effect of these two factors on NSC.

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