Experimental Model of Acute Ischemia of the Retina in Rats

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We studied the development of retinal ischemia in rat eye after laser coagulation of blood vessels. Typical signs of ischemia manifested in the retina after 24 h: development of stable retinal edema, decrease in the b/a index (ratio of the electroretinogram b and a-wave amplitudes) to 1-2 units, pronounced disorders in the retinal microcirculation system, leading to ischemia of the inner layers of the retina. The proposed model is convenient for studies of the development of acute retinal ischemia, is easily realized, and reproduces some acute ischemic diseases of the retina.

Key Words: *ischemia; retina; experimental model; rats*

Experimental study of retinal ischemia is essential for understanding of the pathogenesis of such diseases as retinal artery occlusion, glaucoma, macular degeneration, *etc.* [4]. The most adequate are rat models of retinal ischemia, because the ratio of retinal and choroidal blood supply in these animals is close to that in humans. Several models of experimental ischemia are known, the most prevalent of them are artificial elevation of the intraocular pressure [2] or ligation of blood vessels in the optic nerve region [5]. None of these approaches is without faults [4,6]. Elevation of intraocular pressure requires rigid fixation of the needle, through which high pressure is created for a long time (up to 1 h). This method is highly sensitive to all mechanical factors, which makes its results unstable. Application of a ligature inevitably damages not only blood vessels, but also the optic nerve, which leads to pathological changes in the retina not related to ischemia [3].

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These faults can be escaped by direct local laser exposure of the main retinal vessels, which is fairly well reproducible, because the vessels are easily seen and available for laser coagulation. We studied the possibility of inducing the characteristic signs of acute retinal ischemia by a direct short-term laser coagulation of retinal vessels.

MATERIALS AND METHODS

The study was carried out on 12 male Wistar rats (24 eyes) weighing 200-230 g. Before the study, the animals were intraperitoneally narcotized with chloralhydrate (50 mg/100 g).

Laser coagulation was carried out by the photocoagulation Visuals Kombi II laser (argon laser) creating vascular obturation. The parameters of exposure were as follows: mean radiation power 200-300 mW, spot diameter 100-200 μ, pulse duration 0.1-0.2 sec. First-order vessels were coagulated in the zone with a diameter of $^1/_2$ optic disk.

Electroretinogram (ERG) was recorded using EYE Handheld ERG Unit Mjolner device (Ephios) after preliminary 24-h darkness adaptation, directly before, and 24 h after laser coagulation of the reti-

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nal vessels. Hanzfeld ERG was recorded in response to a solitary (1 Hz) and rhythmic (RERG; 30 Hz) stimulation by standard flashes (0 log=1.7 cd×sec/m^2).

The rats were decapitated 24 h after laser exposure. After enucleation, the eyes were fixed in 2.5% glutaraldehyde solution for 2 h. The eye was then dissected along the limbus and fragments (1.5×1.5 mm) were cut out through all layers of the eyeball in the central, equatorial, and peripheral zones (along the coagulated retinal vessel). The samples were postfixed for 2 h in 1% osmic acid and after dehydration were embedded in epoxy resin mixture (epon-araldite). Semithin sections (0.5-1.5 μ) prepared on an Ultratome-IV (LKB) were stained with toluidine blue or methylene blue and fuchsine (polychromatic staining).

RESULTS

Ophthalmoscopy during laser coagulation showed sharp constriction of the main vessels with complete or almost complete blood flow arrest directly after coagulation; it was retained during several minutes, after which the blood flow was partially resumed. The vessels remained significantly constricted (Fig. 1).

On the next day after coagulation, the optic disc interface in the fundus oculi was blurred in some rats because of adjacent retinal edema; the arteries were constricted, sometimes completely obstructed; the blood flow in these arteries was intermittent. Dilatation of the veins was seen, presumably as the compensatory effect (Fig. 1, *c*). Hence, laser exposure caused stable occlusion of the selected retinal vessels, which after 24 h led to

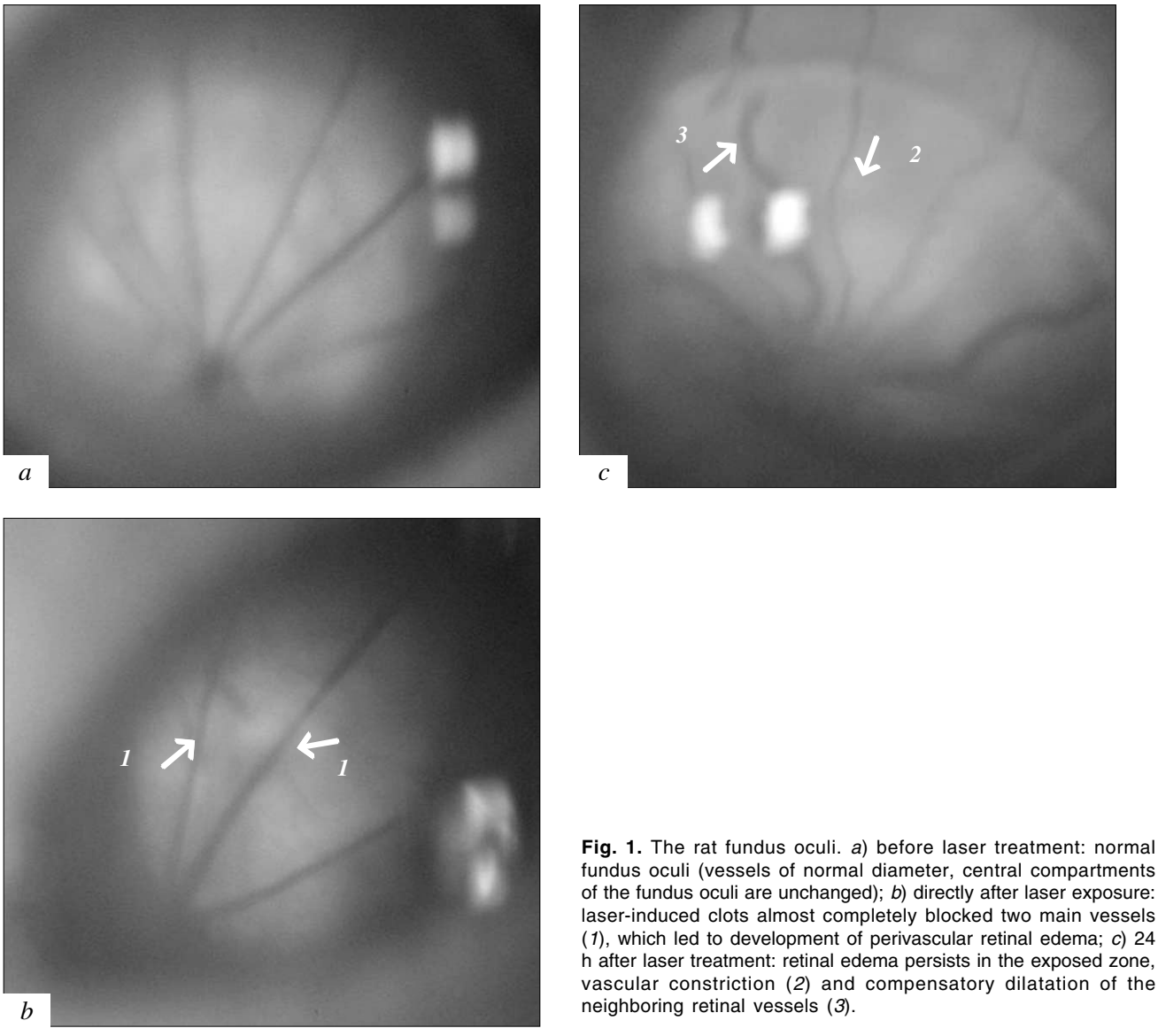

Fig. 1. The rat fundus oculi. *a*) before laser treatment: normal fundus oculi (vessels of normal diameter, central compartments of the fundus oculi are unchanged); *b*) directly after laser exposure: laser-induced clots almost completely blocked two main vessels (*1*), which led to development of perivascular retinal edema; *c*) 24 h after laser treatment: retinal edema persists in the exposed zone, vascular constriction (*2*) and compensatory dilatation of the neighboring retinal vessels (*3*).

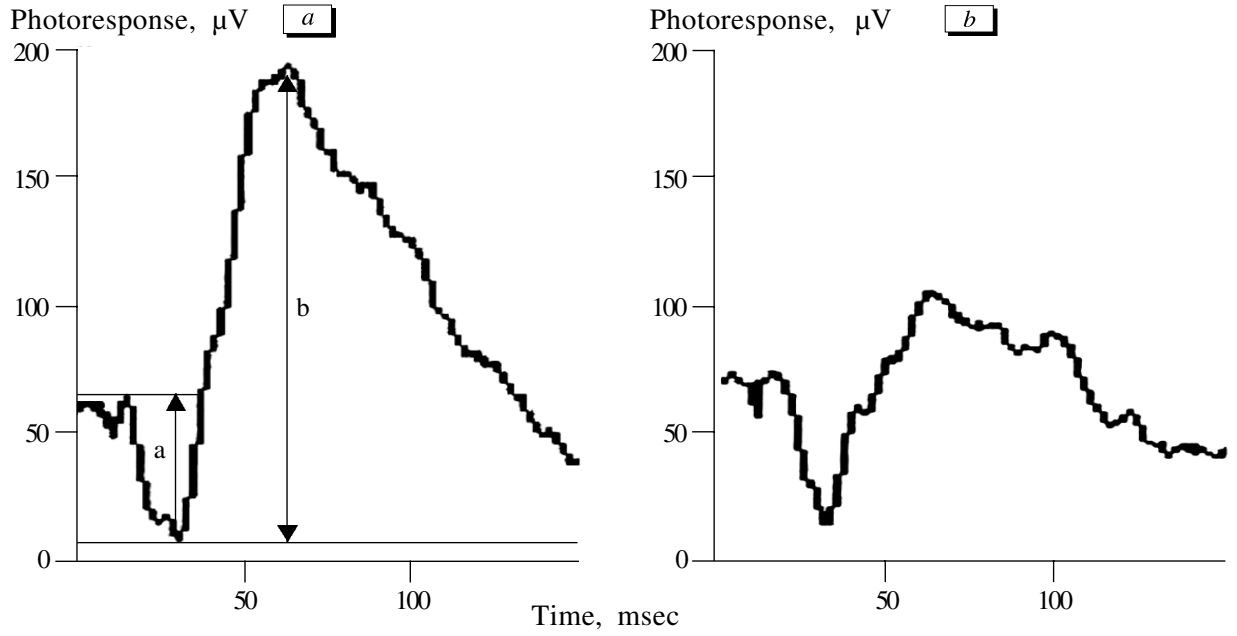

Fig. 2. Rat ERG. *a*) eye of control animal; *b*) the same eye 1 day after laser coagulation of retinal vessels.

anatomical changes in the retina, characteristic of the developing ischemia.

Electrophysiological studies also showed characteristic changes. The ratio of ERG b- and a-wave amplitudes, or the b/a index [1], is widely used for evaluation of retinal ischemia. ERG a-wave characterizes functional activity of photoreceptor cells, while b-wave characterizes activity of the retinal inner layers, primarily the bipolar cells. One day after laser coagulation of retinal vessels, the amplitude of ERG b-wave decreased by 40-70% of its initial value (Fig. 2). Suppression of ERG a-wave was observed not in all animals after 24 h. In cases when a-wave amplitude decreased, the inhibition of b-wave was more pronounced. Changes in the b-wave developed usually before a-wave inhibition and were more pronounced because of poor trophics of the neurons in the retinal inner nuclear layer under conditions of retinal circulation disorders. In our study, the development of acute retinal ischemia was associated with a drop of the b/a coefficient (1.0-2.0 arb. units *vs.* 3.3-4.8 arb. units in intact rats).

Histological findings confirmed electroretinography data. Pronounced hemodynamic disorders

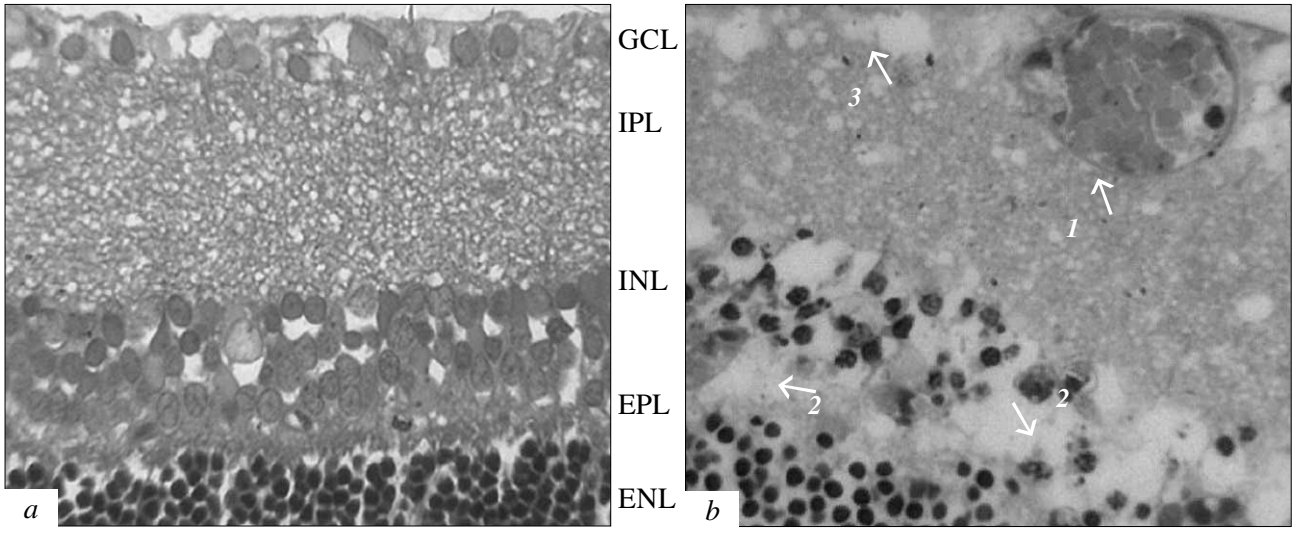

Fig. 3. Histology of rat retina. Toluidine blue staining (1 μ; ×400). *a*) normally; *b*) 1 day after laser coagulation of vessels. ENL: external nuclear layer; INL: internal nuclear layer; IPL: internal plexiform layer; EPL: external plexiform layer; GCL: ganglionar cell layer. *1*) retinal arteriolar thrombosis; *2*) decreased density of neurons in bipolar cell layer; *3*) ganglionar cell edema.

in the retinal microcirculation, leading to ischemia of the retinal inner layers, were observed 24 h after laser coagulation and thrombosis of the main retinal vessels (Fig. 3). Periapedetic hemorrhages, microcyst-like changes in the layer of nerve fibers, ganglionar cell edema, local reduction of the neuron density in the bipolar cell layer were the typical manifestations of ischemic injury. Perivascular edema resulted in thinning and microruptures of the inner borderline membrane, through which mononuclear cells migrated from vascular lumen to the cortical layers of the adjacent vitreous body.

Importantly that these changes involved mainly the inner compartments of the retina (up to the inner nuclear layer), to which the blood was delivered through retinal vessels. No apparent morphological changes were seen in the outer compartments fed from the choriocapillary layer (a layer of external segments of photoreceptors, external nuclear and external plexiform layers).

Hence, our results indicate that laser coagulation of retinal vessels leads to the development of characteristic ischemic changes in the retina. Selective occlusion of vessels created by laser coagulation permits creation of local and extensive retinal ischemia by choosing various retinal vessels and modifying the exposure dose, which can be used in simulation of ischemia.

This model has certain advantages in comparison with the known methods. First, the bloodflow velocity is changed not after a long (1-2 h) treatment, but immediately after laser exposure. This makes possible studies of ischemia during the acute period. Second, the model is realized rapidly and easily if laser is available; and third, laser coagulation induces such an ophthalmological disease as retinal vascular thrombosis.

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