BIOCHEMISTRY AND BIOPHYSICS

Experimental Models for Culturing of Eye Tissues of *Pleurodeles Waltl* for Evaluation of Specific Effects of Sclera Bioregulator in Ultra-Low Doses

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Experiments on stationary culture of posterior eye and roll-bottle culture of the whole eye from adult water lizards *Pleurodeles waltl* showed that sclera bioregulator produces a stabilizing effects on adhesion interactions between the sclera, choroid, and pigment epithelium and on the maintenance of viability of sclera fibroblasts and pigment epithelium cells.

Key Words: *sclera bioregulator; ultra-low doses; culturing*

Myopia is a prevalent eye disease associated with disturbances in the organization of the extracellular matrix of the sclera and changes in these cells. Here we developed models for culturing of sclera tissues from eye of *Pleurodeles waltl* water lizards and studied specific effects of sclera bioregulator acting in ultra-low doses (ULD) using these models.

MATERIALS AND METHODS

Experiments were carried out on *Pleurodeles waltl* adult water lizards, obtained from aquarium of N. K. Kol'tsov Institute of Developmental Biology. In each experiment, at least 10 animals were used (20 eyes). The fraction of sclera bioregulator in a concentration of 10^{-9} mg/ml was added to vials of experimental series before culturing. Sclera bioregulator was isolated from cattle sclera tissue using a previously developed approach [2]. Previously developed method of biotesting [4] was used at each stage of purification to identify the bioregulator in fractions. Cattle sclera tissue was incubated in Ca^{2+}-containing Ringer solution under 4°C for 2 h, the extract was fractionated using the method including sedimentation with ammonium sulfate and isoelectric focusing. The bioactive fraction of acidic sclera proteins collected at pH<3 was then analyzed. The corresponding amount of physiological solution was added to culture medium in control series. Culturing of posterior eye tissues was performed in darkness at 20-22°C for 7 days. Posterior sectors of the eyes including the retina, pigment epithelium (PE), choroid, and sclera were placed in penicillin vials with serum-free medium [1], onto cellulose A/E filters (Gelman). Roll-bottle culturing of the whole eyes was carried out in culture medium [1] containing 10% ETS (BioLot) using roller (Assistant RM5) with rotation velocity of 35 rpm for 28 days at 20-22°C. After culturing, the state of the explants was evaluated on serial paraffin sections. For qualitative evaluation of the state of sclera tissue, fibroblasts were counted on histological sections of

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cultures. Eyepiece grid (quadrant size 0.00015625 mm^2) was used for quantification. The number of fibrioblasts per 0.0225 mm^2 (12×12 quadrants) was calculated on more than 300 sections in both control and experimental series.

Since control and experimental samples were independent and corresponded to normal distribution, two-sample Student's *t* test was used for evaluation of significance and errors in estimation of cell number per area unit.

RESULTS

Bioregulators active in ULD were found in various mammalian tissues [1-9]. They constitute a new group of protein compounds characterized by the capacity to modulate vital biological processes (migration, adhesion, proliferation, and cell differentiation) in ULD. We previously found a bioregulator active in ULD in bovine sclera [2]. By its physicochemical properties it corresponded to this group of bioregulators. To investigate specific effects of sclera bioregulator, the model of stationary culture of posterior eye [1] and a new model of roll-bottle culturing of the whole water lizard eye were developed.

Destructive processes in all tissues of the posterior segment of the eye from adult vertebrates are observed during *in vitro* culturing. In control cultures, impairment of adhesive interactions between the sclera and choroid and disturbances in compact structure of fibers in sclera were observed. In the retina, complete cell degradation and destruction of the choroid and PE were seen. In the sclera, fibroblasts with atypical irregular roundish nuclei were present; they were surrounded by empty spaces formed due to destruction of collagen fibers (Fig. 1, *a*). Addition of sclera bioregulator to the medium in a concentration of 10^{-9} mg/ml preserved spatial organization of the posterior eye wall. This was seen from improved the interactions between the choroid and sclera and preserved compact adhesive interactions in the choroid. PE layer was also in a better state compared to control cultures: the cells were viable with even distribution of the pigment and the adhesive interactions between cells were preserved. In the sclera we observed more compact and dense arrangement of fibers with less pronounced stratification compared to control cultures. Sclera fibroblasts also looked viable judging from morphology of their nuclei; in addition, their number was approximately twofold higher (51.00±0.79 *vs.* 27.42±0.73 in the control; $p<0.05$; Fig. 1, *b*).

Roll-bottle culturing of the whole eye in the control was associated with impairment of adhesion interactions between tissues in the posterior eye

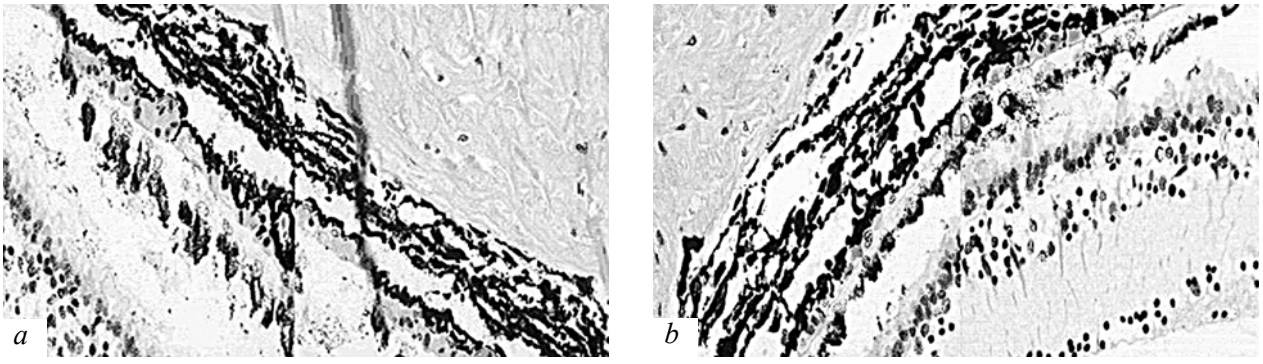

Fig. 1. Posterior eye tissues of *Pleurodeles waltl* water lizard after 7 days of stationary culturing. Here and on Fig. 2: *a*) culturing without sclera bioregulator (control); *b*) culturing in the presence of sclera bioregulator in a concentration 10^{-9} mg/ml (experiment). Hematoxylin and eosin staining, ×200.

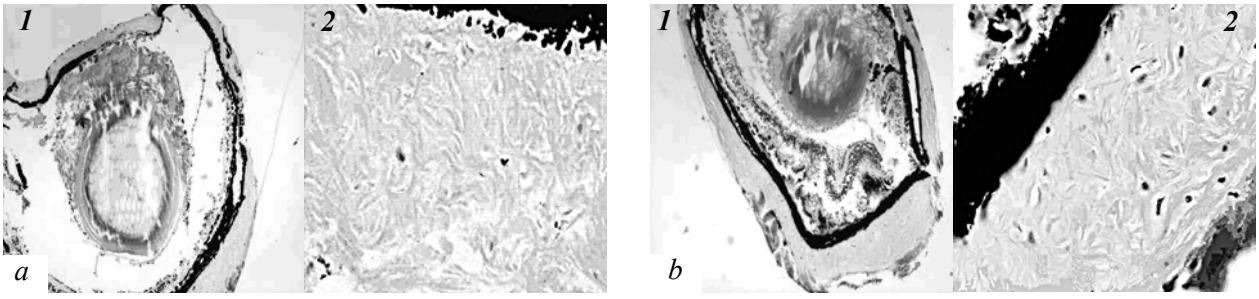

Fig. 2. Tissues of *Pleurodeles waltl* water lizard eye after 28-day roll-bottle culturing. Magnification: ×40 (*1*) and ×200 (*2*).

segment despite their preserved spatial organization (Fig. 2, *a*). In the retina, cells of main types died, the integrity of PE layer was impaired: the cells formed individual melanophages. In the growth zone of the eye, numerous newly formed dedifferentiated cells were found; they migrated to the dying retina and replaced photoreceptor cells and higher-order neurons. Almost complete loss of fibroblasts was observed in the sclera. In control and experimental cultures, death of epithelial layer cell was observed in the cornea. Adhesion interactions between tissues of the posterior eye sector were preserved in experimental cultures (Fig. 2, *b*). Viable fibroblasts were present in the sclera (3.75±0.21 in control and 18.94±0.61 in experiment; $p<0.05$), collagen fibers were densely arranged.

Thus, protective effects of the studied bioregulator were demonstrated on the models of organotypic cultures of the sclera of the posterior eye segment and roll-bottle culture of the whole eye from *Pleurodeles waltl* water lizards; these effects manifested in better viability of sclera fibroblast and preserved spatial organization of collagen fibers. Moreover, bioregulator extracted from the sclera was shown to be able to maintain adhesion between posterior eye tissues and cell differentiation status in PE.

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