
SEARCH FOR CHAPERONE-LIKE ANTICATARACT PREPARATIONS — THE ANTIAGGREGANTS OF EYE LENS CRYSTALLINS

COMMUNICATION 3. Possibilities of A FOLLOW-UP of cataractogenesis processes ON a prolonged rat MODEL of UV-induced cataract

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Abstract

To study the mechanisms of action of new-generation anticataract drugs, it is necessary to have an accessible and adequate model of age-related cataract. A model of UV-induced cataract is pathogenetically closest to that of age-related cataract. A prolonged rat model of UV-induced cataract developing within 10 months is proposed; the clinical features of UV-induced cataract have been established at different stages of its development. A moderate homogeneous cloud-like lenticular opacity was observed at the end of the experiment; a less pronounced homogeneous opacity was seen in the anterior and posterior cortical layers. Cataract development was assessed by the appraisal method using the developed rat lenticular transparency scale, as well as by microdensitometry of biomicroscopic lenticular optical sections. Within the proposed model, the pathomorphological lenticular changes are largely similar to the histological pattern of age-related cataract.

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In order to investigate the mode of action of anticataract preparations, it is necessary to develop an adequate and accessible model of age-related cataract. Based on UV-induced cataract pathogenesis data analysis, it may be concluded that UV-induced cataract is the most similar to age-related cataract. As a matter of fact, UV radiation is the factor that contributes to the initiation and progression of pathological changes in crystalline lens [3, 5, 9, 10].

Photo-oxidation and aggregation processes in water-soluble proteins occur under the influence of UV radiation and visible light, while accumulation of photolysis products serves as a pathogenetic factor of age-related cataract progression [6, 8].

However, currently used UV radiation-induced cataract models ($\lambda=280-400$ nm) involve the effects of high-intensity UV radiation during relatively short damaging exposure periods (up to 10–20 weeks) [11–13]. That fact makes unjustified the correlation of such models with age-related cataract progression which is a fairly long process.

The purpose of the research is to develop an accessible experimental model of UV radiation-induced cataract with the aim of assessing the efficiency of anticataract preparations.

Materials and Methods

The experiment was performed on 15 male Wistar rats (30 eyes) aged 20 to 23 days, weighing 39–41 g. The follow-up period was 10 months (43 weeks). The control group consisted of 7 rats. The experimental group (8 rats) was irradiated by the full light of 2 quartz (ultraviolet) lamps “URLQ-01 «Solnyshko»” ($\lambda=280-400$ nm) for 16 minutes every other day. The mean radiation intensity on the floor of the cage was determined by photochemical reaction (Hatchard and Parker's method involving the formation of phenanthroline ferrous complex) [1]. The mean intensity for 313 and 366 nm wavelength equaled to 23.7 ± 4.4 and 21.5 ± 4.0 joule/sec·m² respectively¹.

Instrumental estimation of lenticular opacity degree included microdensitometry of biomicroscopic lens optical section by means of special software programmed for ophthalmological digital images analysis [2, 4].

Biomicroscopy was performed using slit lamp SL-75 (Opton, Germany) at 45° angle and 0.1 mm diaphragm opening.

Cataract progression was also assessed by expert evaluation method. Three researchers gave independent opinions on the digital images using six-point measurement scale (**Fig. 1**).

0 points — transparent cortex and nucleus of the crystalline lens;

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¹ The data listed as $\bar{X} \pm s$, where \bar{X} — mean value, s — mean square deviation.

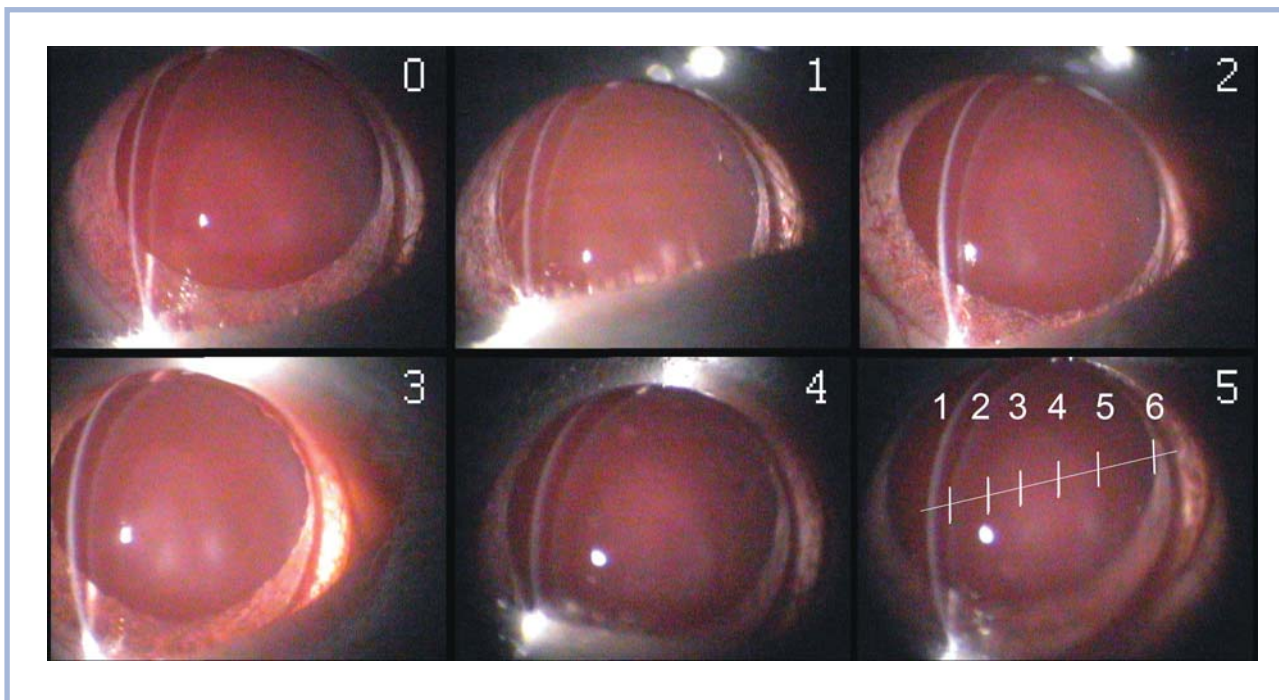


Fig 1. The measurement scale of lens opacifications used for data analysis by expert evaluation method.

Special zones used for graphical analysis are marked on image 5: 1—2— anterior cortex; 2—3— anterior nucleus; 3—4— middle nucleus; 4—5— posterior nucleus; 5—6— posterior cortex.

1 point — insignificant induration under anterior capsule of the lens, optical transparency of the cortex and the nucleus;

2 points — slight diffuse opacification of the nucleus, granularity of the anterior and posterior cortex;

3 points — moderate diffuse opacification of the nucleus, slight homogeneous opacification of the anterior and posterior cortex, slight thickening of the anterior crystalline lens capsule in optical section;

4 points — more pronounced opacification in the nucleus area with, flaky-like opacity development and fusing tendency, slight increase of homogeneous opacification in the anterior and posterior cortex;

5 points — moderate nebulous homogeneous opacification of the nucleus, less pronounced homogeneous opacification of the anterior and posterior cortex.

All the photos were presented for estimating by blind method. When the estimations varied, in case of two opinions versus one, the opinion of majority was preferred. The mean estimation value was chosen in case of full discordance of opinions. The total score of left and right eyes was taken as the final level of the eyes damage. Statistical analysis of the results was performed with the use of distribution-free statistics (the Mann—Whitney U test and Spearman’s rank correlation coefficient).

After 10 months follow-up, animals were sacrificed using chloroform anesthesia according to the Association for *Research in Vision and Ophthalmology* Statement for the Use of Animals in Ophthalmic and Visual Research

[7]. Crystalline lenses were then fixated in order to create semifine sections according to standard practice. Light-optical morphometry of histologic specimens was performed on “Photomicroscope-III” (“Opton”, Germany) with the use of automated morphodensitometry hardware and software complex “DiaMorph Objective” (“ZAO Diamorph”). The photorecording was done using “Diamorph” digital camera which was supplied with the complex.

Results and Discussion

Experimental observations showed that opacifications in crystalline lenses could be detected in both experiment and control groups, as early as 1 month after the experiment started. This initially observed opacification had the form of slight induration of the exterior cortex under the anterior capsule. Optical transparency of the cortex and the nucleus in the experimental group was assessed by expert evaluation method which showed that slight diffuse opacification in the nucleus, and granularity in the anterior and posterior cortex appeared no earlier than after 1.5 months. Then, under the exposure of UV irradiation, opacities progressed faster in nucleus than in cortex. After 10 months, the experimental group was diagnosed with moderate nebulous homogeneous opacification in nucleus, less significant homogeneous opacification in the anterior and posterior cortex. This cataract modeling method didn’t result in mature cataract formation.

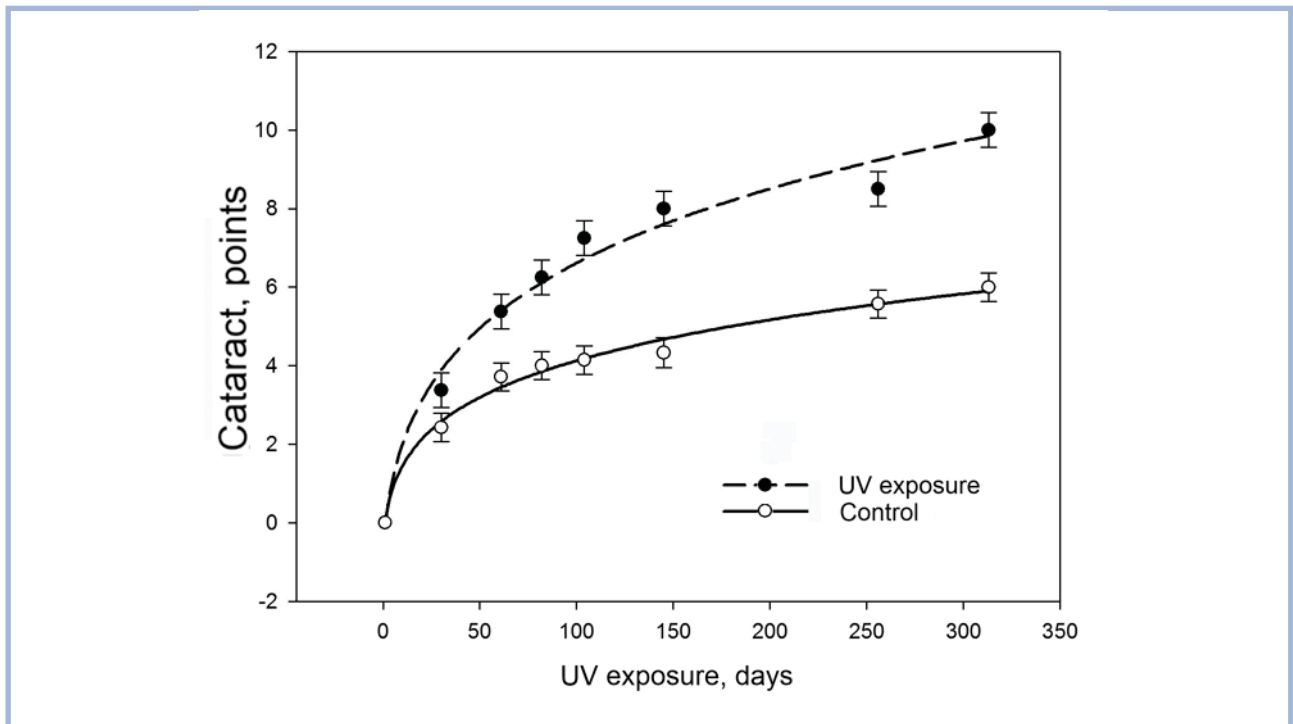


Fig. 2. The dynamics of lens opacification intensity rates in animals of the control and experimental groups (expert evaluation method). The data on fig. 2, 3 and 4 is presented in form of mean values per group with an indication of 95 per cent confidence interval.

The clinical picture of the cataract progression in both groups was the same. However, the opacification in experimental group progressed slower, and only moderate diffuse opacification in nucleus and slight homogeneous opacification in anterior and posterior cortex were observed by the end of the experiment. The evaluation results of lens opacification dynamics are shown in **Fig. 2**.

The microdensitometry data shows that optical density of all lens cortex layers in animals of the experimental group increases significantly during the first 3 months of the radiation exposure, after that the density growth rate slightly decreases. Therefore, the dynamics of opacification development may be described by a logarithmical function (see extrapolation lines drawn through the experimental points in the figures). It should be noted that optical density index (ODI) of the lens anterior capsule had close to no changes throughout the experiment with an increase of 10 u. in both experimental and control groups, which occurred in the form of induration in the capsule. The most significant increase of ODI by the end of the experiment was noticed in nucleus layers. The ODI increase in the middle nucleus was 115 u. (**Fig. 3**). Opacities growth was similar in the posterior and anterior cortex, and the growth rate was less than in nucleus. The ODI increase in the cortex was 95-100 u. (**Fig. 4**).

During the follow-up, the control group exhibited slight steady increase of ODI in all examined layers (from 85-90 u. in the beginning of the experiment to 115-120 u. at the final stage). This may be an indication of natural

age-related processes in crystalline lens in studied animal species (**Fig. 3, 4**).

High correlation level of the results was discovered after comparing the estimations of cataract development dynamics, using expert evaluation method and microdensitometry of biomicroscopic optical sections. Spearman's rank correlation coefficient of the scoring and ODI in various parts of the crystalline lens ranged from 0.65 to 0.74. At that, the highest value $R=0.74$ was observed in pairs of "score — anterior cortex layers" and "score — centre (nucleus) layers".

According to morphological study, the animals of the control group had no abnormalities in the process of new crystalline fibers formation, and the cortex structure remained unchanged. However, some minor changes were observed in the architectonics and in fiber structures under the anterior capsule, wherein the interfiber swelling was attended with local vacuolization of the crystalline lens cells (**Fig. 5**). Fibers induration and homogenization were noticed in crystalline lens nucleus area smoothing over their boundaries.

Crystalline lenses of the animals in irradiated group showed dissection of external cortex, destruction and ejection of anterior lens capsule due to weakening of interfiber bonding, partial fragmentation and divergence of fibers (**Fig. 6**). Destructive changes of crystalline lens fibers in middle cortex occurred in the form of lens fibers homogenization and the absence of nuclei due to karyolysis.

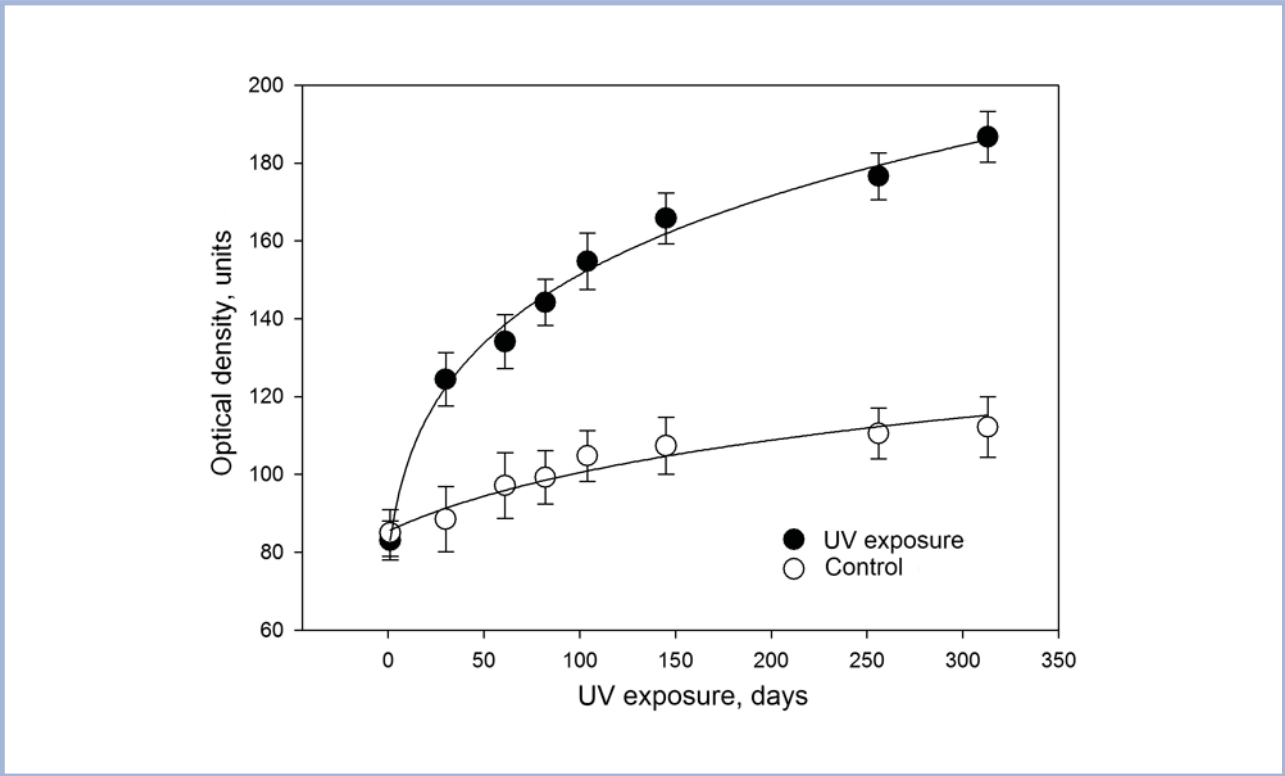


Fig. 3. The dynamics of opacification development in the anterior lens cortex in animals of the control and experimental groups (microdensitometry method).

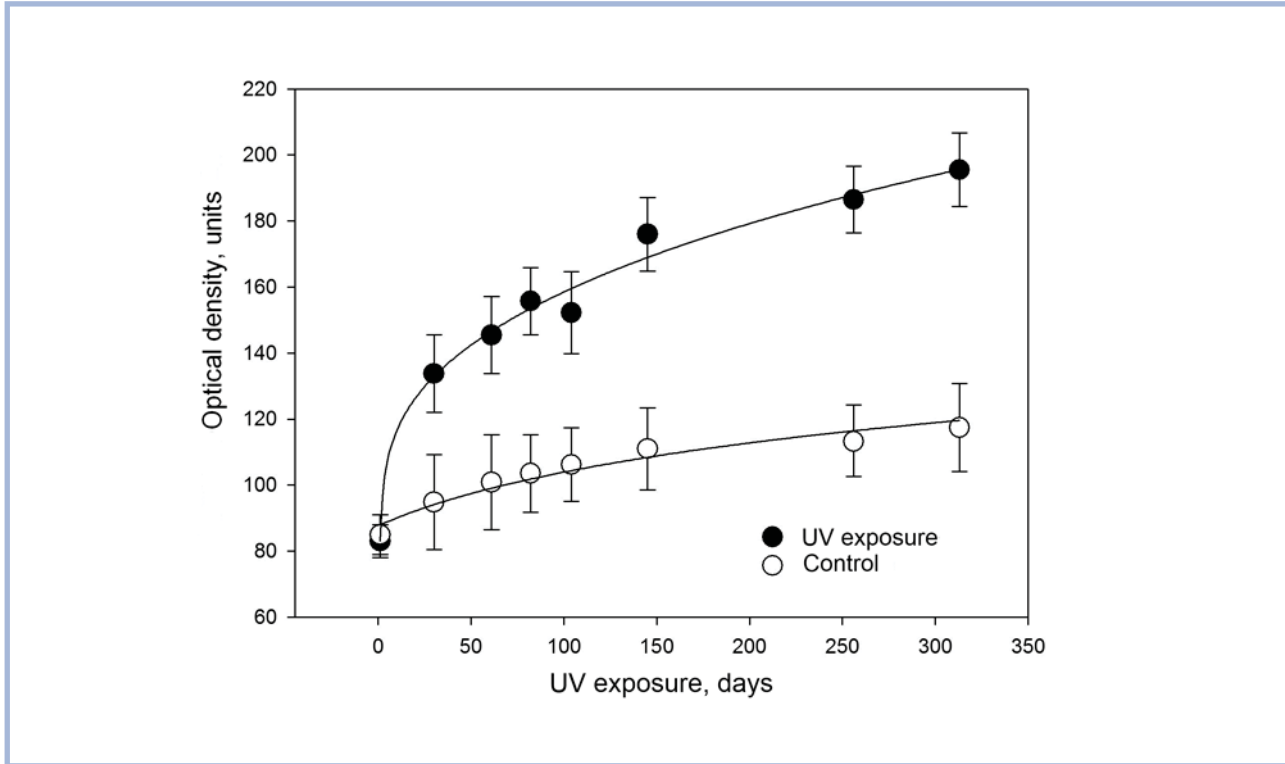


Fig. 4. The dynamics of opacification development in the middle nucleus in animals of the control and experimental groups (microdensitometry method).

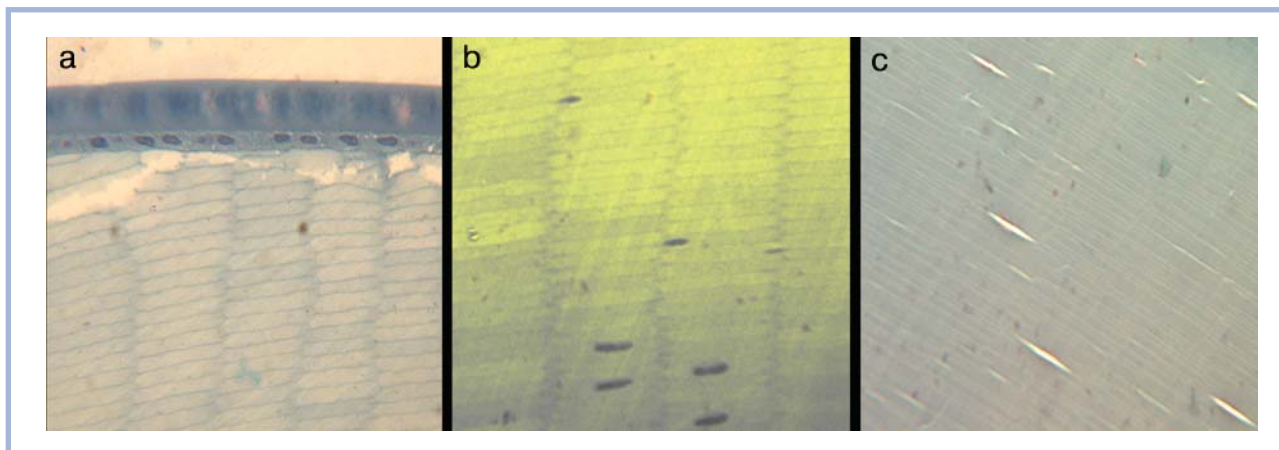


Fig. 5. Histologic pattern of the crystalline lens in a control group animal.

a — capsule and exterior cortex of crystalline lens with local vacuolization and interfiber swelling; b — cortex fibers; c — crystalline lens nucleus, induration and homogenization of lens fibers in the nucleus area.

In fig. 5 and 6: semifine section, polychromatic coloration. Magnification $\times 250$.

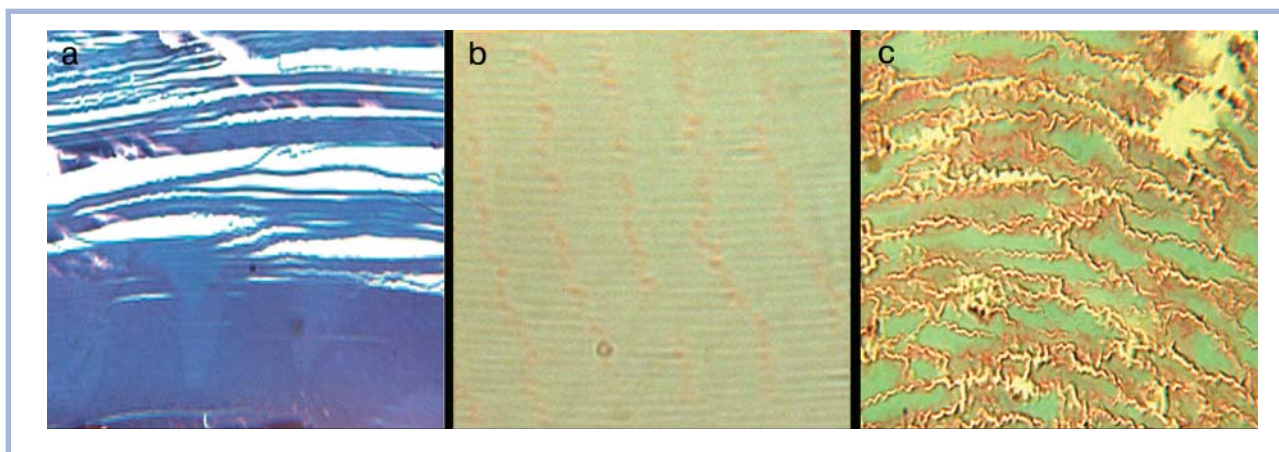


Fig. 6. Histologic pattern of the crystalline lens in a control group animal.

a — dissection of the exterior lens cortex and its partial fragmentation; b — induration of the middle lens cortex; c — crystalline lens nucleus and fragmented lens fibers.

Minor disturbance of lens transparency in animals of the control group is apparently associated with spatial and structural changes of crystalline lens fibers and the appearance of interfiber swelling areas with local vacuolization. Primary changes settled in the nucleus and anterior subcapsular lens cortex. The disturbance of lens transparency due to protracted exposure to UV rays in animals of the control group could be related to the development of apparent structural disturbances that occur in forms of interfiber bond weakening, partial fiber fragmentation and intercellular edema nearly in all parts of the crystalline lens.

As a result, an accessible experimental model of UV-induced cataract has been developed. In future, it could be used to study the effectiveness of anticataractal preparations. Cataract is known to have relatively long forma-

tion period with no biomicroscopic clinical features to detect. After that period, crystalline lens fiber structure gradually becomes nonhomogeneous causing the formation of thicker homogeneous part of the nucleus which later progresses into moderate homogeneous cloud-like opacification. At that, less significant homogeneous opacification occurs in anterior and posterior cortex. During the experiment, anterior lens capsule showed only insignificant induration with no other changes.

Such alterations in anterior capsule are typical for involuntional processes in crystalline lenses in humans and animals. It should be noted, that capsule induration was identical in both control and chronically UV-exposed experimental groups. It is known that short time modeling of UV-induced cataract, which involves harsh UV exposure of the eye that causes formation of opacification of

the anterior capsule. In the conducted research, animals of both groups showed homotypic changes in lens capsule in the tenth month of the experiment, while animals of the experimental group had opacifications in crystalline lenses. These facts indicate that the amounts of UV exposure used in the experimental research were adequate and allowed creating a prolonged model of cataract, which is the most correct one from the physiological point of view.

Conclusions

1. The suggested “prolonged” model of UV-induced cataract in rats is comparable with clinical progression of the age-related cataract and may be utilized to evaluate the efficacy of anticataract preparations in experimental studies.

2. Subjective evaluation of the transparency level in Wistar rats lenses was done using expert evaluation meth-

od, and, consequently, an appropriate measurement scale was developed. The clinical features of UV-induced cataract have been determined for various stages of the disease.

3. Intravital micro-densitometry of biomicroscopic optical sections of rats’ lenses allows objective assessing of the dynamics of lens transparency changes in rats.

4. Pathomorphological changes in crystalline lenses in the context of suggested model were highly similar with histologic pattern of age-related cataract.

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