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Experimental Studies and the Search for New Innovative Approaches to Treat Dry Eye Disease

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Abstract

Introduction and purpose. The main manifestation of eyelids, tear ducts, and orbits disorders is a dry eye disease considered as a serious disease that affects the quality of life. Nowadays, one in ten people worldwide faces this disease. Increased workload, development of multimedia technologies, hypodynamia, and environmental degradation are the main modern causes of the disease. **Material and methods.** In this work, we used an experimental model of dry eye in rats with the development of inflammation of the anterior surface of the eye. Alkali burns were used to develop inflammation. A fluorescein test performed immediately after alkaline erosion and on days 1, 5, and 10 after burns revealed a gradual increase in corneal epithelialization and vascularization. **Results.** One day after induction of inflammation, there was a dramatic and statistically significant increase in tear production in experimental rats. By the seventh day, tear production declined to the control level with a subsequent tendency to decrease tear production. **Conclusions.** In the group of rats with

inflammation of the anterior surface of the eye that received polarized red-light therapy, there was no significant decrease in tear production on day 7, which may be indicative of a possible anti-inflammatory effect of light therapy under these conditions.

Keywords: dry eye disease; inflammation of the anterior surface of the eye; fluorescein test; Schirmer test; polarized light; Bioptron-Pailer therapy.

Introduction

Dry eye disease (*xerophthalmia*) is a multifactorial disease of the eye surface, characterized by loss of lacrimal homeostasis and accompanied by visual symptoms in which instability of the lacrimal film and hyperosmolarity, inflammation, and damage to the ocular surface and neurosensory abnormalities play an etiological role. The disease can change daily activity and significantly affect the quality of life as a result of decreased visual acuity, obsessive-compulsive disorder [1].

Under physiological conditions, a normally functioning lacrimal apparatus secretes a tear, which is a humectant fluid of a certain qualitative composition and in an amount sufficient to create a stable film, consisting of the outer lipid, middle aqueous, and inner mucin layers. A change in the composition of at least one of them leads to a failure of the whole system. But it is not only instability or qualitative inferiority of tears that leads to xerophthalmia development. The surface of the eye is a functionally important integrative unit, which anatomically consists of seven main interactive and interdependent components, namely: lacrimal film, lacrimal and auxiliary lacrimal apparatus, nasolacrimal drainage system, eyelids, bulbar and tarsal parts of the conjunctiva, and V and VII cranial nerves [2]. A disorder in at least one link leads to unpleasant symptoms: obsessive-compulsive disorder, impaired visual acuity, redness, mucous secretions, irritation, and intense tearing, photophobia.

Dry eye disease or dry keratoconjunctivitis (H.19.3) affects millions of people worldwide and is one of the most common ophthalmic diseases [3]. Among US people over 50 years of age, there are 3.23 million women and 1.68 million men, who suffer from this pathology. Estimates of the disease prevalence range from about 10 to 30 percent of the total population. Data on racial and ethnic predisposition are limited, but the incidence is higher in the Spanish-speaking population and Asian countries [4].

The 2017 DEWS II (The International Dry Eye WorkShop) updated the classification, definition, diagnosis, monitoring, and treatment of dry eye disease. Dry eye is an autoimmune disease characterized by chronic inflammatory infiltration of the lacrimal and salivary glands.

Its pathogenesis is attributed to the defect of Fas-mediated apoptosis, which makes possible penetration of CD4+ T-lymphocytes into the exocrine tissue with its subsequent damage [5]. However, it is not only the inflammatory mechanism that underlies the development of xerophthalmia. Defects in the transmembrane and secretory expression of mucin, disturbance of nervous stimulation of the tear production process, dysfunction of meibomian glands also lead to the development of various xerophthalmia forms. Undoubtedly, there are several adverse factors, such as environmental influences and anatomical features, the presence of endocrine, inflammatory, autoimmune diseases, and the use of contact lenses, the use of drugs, or ophthalmic surgery [6].

Anterior surface inflammation (ASI) and the development of dry eye syndrome can have irreversible and serious consequences. The chronic defect in the corneal epithelium results in corneal pannus and squamous metaplasia, leading to blindness. Unfortunately, there is only a symptomatic treatment that can only reduce the suffering of patients but not cure the problem. Today, the vital task for ophthalmology is the search for new methods of treatment, and one of them is the application of Piler therapy.

Bioptron-Pailer light therapy is a local and/or systemic effect of polarized electromagnetic waves of the biologically necessary (solar) range using receptor or sensory gates, a transporting connective tissue framework, and a photochemical reaction cascade to deliver electromagnetic energy to regulatory systems or areas experiencing its deficiency or imbalance.

Piler light is a linear polarized incoherent polychromatic light with a wavelength of 400–2000 nm (visible and light infrared spectrum of light except for UVI). When polarized, light waves pass only in parallel planes. The degree of polarization is about 95%. The acceptor of light, along with other substances, is the oxygen of the cell, which selectively absorbs light – a chain of biological reactions is triggered, lipid peroxidation is activated, which induces a stress response, *i.e.* a nonspecific adaptive response is observed. Under the influence of polarized light, the energy activity of the cell membrane increases. Regenerative processes are activated, oxygen uptake by tissue increases with the formation of adenosine triphosphate (ATP) in mitochondria, the bioenergetic potential of cells, and the blood-groove speed increase in tissue, transport through a vascular wall is activated, vessels are intensively formed. Piler light affects tissue regeneration, as well as immune protection [7].

Purpose

The work aims to study the pathophysiological mechanisms of action and clinical efficacy of poly- and monochromatic Piler light with an experimental model of dry eye disease.

Material and methods

The work was carried out within the study *Clinical and experimental substantiation of diagnosis, treatment, and prevention of refractive, dystrophic, traumatic, and inflammatory diseases of the eye* conducted by the Ophthalmology Department of the Shupyk National Healthcare University of Ukraine.

Adult Wistar rats, males weighing 250-300 grams, were used for the experiments. Xylazine 10 mg/kg body weight (Biovet Pulawy, Poland) and ketamine 60 mg/kg body weight (Farmak, Ukraine) were used for anesthesia. All experimental procedures were performed in compliance with the regulations of the Committee on Animal Bioethics of the Bogomolets Institute of Physiology (Kyiv, Ukraine) and following the directives of the European Commission (86/609 / EEC). Every effort has been made to reduce the suffering of the animals and minimize their number. All manipulations were performed under antiseptic and aseptic conditions.

A combined experimental model of dry eye disease (DED) on rats. For our study, the rats were split by 10 animals into the control and experimental groups. The combined representation of DED was achieved with alkali burns. Under anesthesia with xylazine 10 mg/kg body weight and ketamine 60 mg/kg body weight and additional irrigation of each eye with 2% lidocaine, 25 ul 0.2% NaOH [8] was instilled into each eye to cover the entire corneal surface with the solution. After 30 seconds, the eyes were washed with isotonic NaCl solution. To confirm corneal erosion, labeling was performed using fluorescein test strips. Confirmation of this was the complete staining of both eyes, which indicated the complete defeat of the entire surface of the eye. After the operation, the animals were transferred to a dry, heated cage and observed until their functions were restored, after which they were transported to the vivarium. Subsequently, rats were monitored and clinically evaluated for corneal transparency and vascularization.

Corneal transparency was assessed on a scale, where 0 points are the completely transparent cornea; 1 point is a slightly blurred cornea (iris and pupil are easily visible); 2 points is the slightly cloudy cornea (iris and pupil are still detected); 3 points is the cloudy cornea (the pupil is difficult to detect); 4 points is the completely cloudy cornea (the pupil is not visualized).

Corneal vascularization was assessed on the 4-point scale, where 0 points designate the absence of the limb vessels; 1 point is the vessels 2 mm from the limbus of the cornea; 2 points is the vessels 4 mm from the limbus of the cornea; 3 points are the vessels in the center of the cornea.

On the 1st day after the DED induction, Piler-light therapy was performed using a red filter of the Bioptron device (Bioptron AG, Zepter Group, Switzerland). The animals were fixed with the left eye shielded. Pieler light therapy only for the right eye was performed from a 30 cm distance for 3 minutes. Each animal underwent a daily course of treatment for 10 days. The experiments were performed in a darkened room in the absence of direct natural and artificial lighting.

Fluorescein test. The test was performed on the anesthetized animals after burning the cornea with alkali on the 5th and 10th days to assess epithelialization. Standard fluorescein strips were placed in the lower conjunctival sac, after which the eyes were closed and artificially blinked several times to fully distribute the dye over the entire surface of the cornea. Fluorescein was excited using an ophthalmoscope with the 470 nm wavelength lamp, then the eyes were photographed and epithelialization assessed.

Schirmer test. The test was performed without anesthesia on the 1st, 3rd, 5th, 7th, and 9th days after induction of the DED model. 2 mm wide strips were cut from standard Schirmer test strips to adapt to the size of the rat's eye. The strips were inserted into the lower arch of the conjunctiva and held for 5 minutes. The length of the wet part was measured in mm.

Statistical data processing was performed in Windows MS Exel 2010 using SPSS statistical software. The results were processed by methods of variance statistics using Student's t-criterion.

Results

The results of the study are presented in Figs. 1-3.

A combined experimental model of DED in rats. There are many models to study DED. They all differ according to the etiopathogenetic factor [9-12]. During the study, we adapted and used a model of combined lesions of the lacrimal duct, meibomian glands, and damage to the cornea and conjunctiva with the development of inflammation. The model was implemented as follows: after irrigation of the corneal surface with alkaline solution and washing with isotonic NaOH solution, the erosions were evaluated optically using a binocular microscope in a fluorescein sample, which confirmed the effectiveness and specificity of this model. According to the results of the fluorescein test, which was performed immediately

after alkali erosion and on the 1st, 5th, and 10th days, the stages of development of corneal epithelization were recorded.

Using a score scale, the clinical evaluation of corneal transparency and neovascularization was performed for 10 days.

Study of the effect of red polarized light on the clinical course of DED in rats. The course of Piler-light therapy using the red filter of the Biopton device did not show a statistically significant difference between the control in the assessment of transparency (Fig. 1) and vascularization of the cornea (Fig. 2). During the study, there was a tendency for slowing down the processes of epithelialization and vascularization.

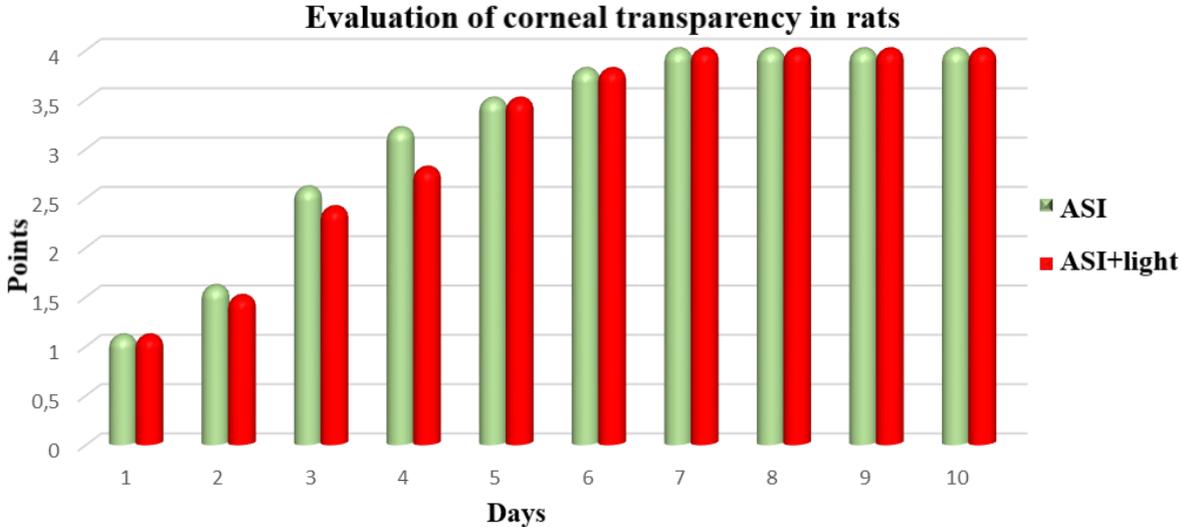


Figure 1. Evaluation of corneal transparency on a scale from 0 to 4

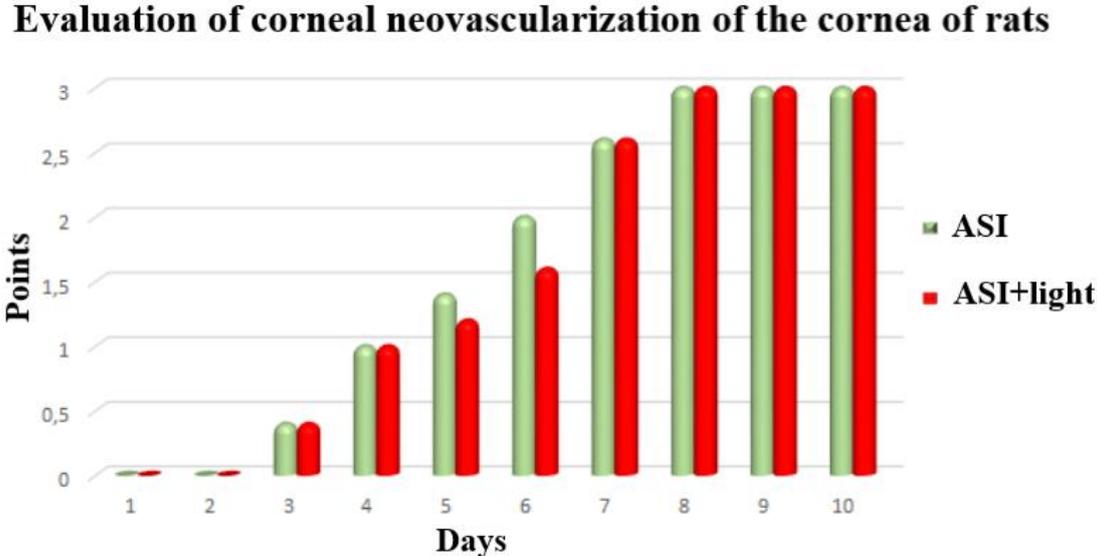


Figure 2. Evaluation of corneal neovascularization on a 4-point scale

Owing to several studies [11, 12] it is known that stimulation of cytochrome oxidase by light can lead to increased energy metabolism in mitochondria, increased metabolism within the cell, activate cell proliferation and migration. Thus, during our studies, there was a slowing of the processes of epithelialization and vascularization of the cornea with the use of Pailer-light red light therapy, which has a positive anti-inflammatory effect.

Influence of red polarized light on tear production. In the course of our study, we divided all animals in both groups into two subgroups. There was the control intact group that did not receive treatment with Pailer-light therapy (n=5); the control group (n=5), in which each animal received therapy with polarized light with a red filter using the Biopton device. The experimental group of animals, which was induced a combined model of ASI with the development of DED (n=5); who did not receive daily treatment and the group with the development of ASI, receiving a course of treatment with polarized red light (n=5). The left eye of the animals was shielded. The polarized red light was exposed to the right eye for 3 min from a distance of 30 cm. After 10 sessions of Piler-light therapy, the animals were excluded from the experiment.

Tear production was assessed using the Schirmer test (Fig. 3). The mean values of tear production were 11.2 ± 0.49 mm in the control group and 11.08 ± 0.38 mm in the group derived from the course of Pailer-light red light therapy. Analysis of the results showed no statistical difference between the groups, which allowed us to conclude that this exposure and treatment protocol does not cause toxic reactions from tear production in rats, and is safe for further use and study.

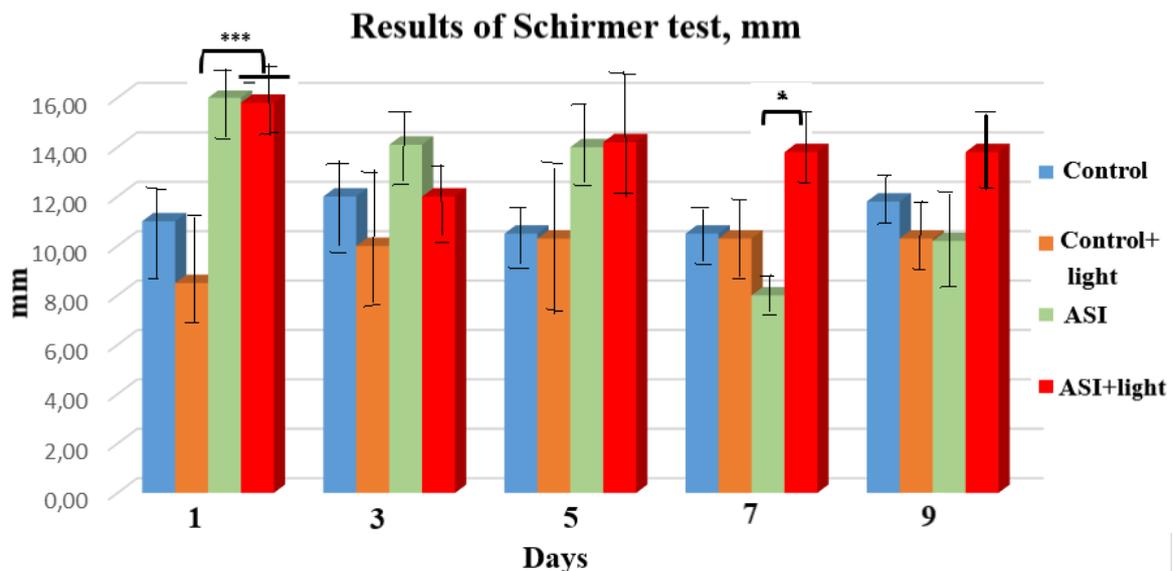


Figure 3. Results of Schirmer test tear production measurements in control animals, animals with ASI, and animals of both groups with polarized red light therapy

The results of the study demonstrated a significant and statistically reliable increase in tear production of 15.75 ± 1.01 mm ($p = 0.003$) on the first day after induction of burns as compared to the control. By comparing the results in the group treated with red polarized light we obtained a similar statistically reliable relationship in the range of 16.25 ± 0.6 mm ($p = 0.011$).

Assessment of the dynamics of tear production over the next days and on the 7th day after the burn showed a dramatic decrease in tear production to 8 ± 0.63 mm ($p = 0.02$) in the group with induced ASI, excluding the group of animals treated with polarized red light. The trend continued on day 9.

The decrease in tear production can be explained by the development of DED in rats after the chemical induction of ASI. In the group of rats with ASI, which received a course of polarized red light, changes in tear production throughout the course were not significant, and on day 7 only in this group, no significant decrease in tear production was observed, which may indicate a possible anti-inflammatory and protective effect of Piler-light therapy in these conditions.

Conclusions

Using the experimental model of dry eye disease allowed establishing a positive anti-inflammatory effect from red polarized light therapy. The biological effect of light energy was utilized by the photochemical transformation in mitochondria through cytochrome oxidase interaction and influenced energy processes within the cell, cell proliferation, cell migration, neutralizing reactive oxygen compounds. The results obtained are evidence of the possibility for further study of the properties of the effect of polarized light on the biological processes inside cells and may be considered as a promising direction in the development of alternative therapy in the treatment of patients with diseases of the anterior surface of the eye.

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