UDC 617.7-007.681-085.849.19-091.8-092.9
DOI https://doi.org/10.32782/2226-2008-2024-4-2

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HISTOPATHOLOGICAL CHANGES IN RABBIT EYES AFTER TRANSSCLERAL DIODE CYCLOPHOTOCOAGULATION

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Currently, the issue of choosing the energy characteristics of laser radiation to ensure the optimal effect on the structures of the ciliary body during diode (810 nm) transscleral cyclophotocoagulation (TSC CPC) remains debatable.

The research purpose is to determine histopathological changes occurring in the sclera and ciliary body after transscleral diode cyclophotocoagulation with different energy characteristics of laser radiation in the experiment.

Material and methods. The study was conducted on the eyes of 2 rabbits (4 eyes). TSC CPC was carried out using a diode laser with a wavelength of 810 nm and a contact fibre-optic G-probe. Two energy regimes were used: 1 – power 2000 mW, exposure 1.5 sec (3 J energy per pulse) and 2 – power 1000 mW, exposure 1.5 sec (1.5 J energy per pulse). The analysis of the experimental studies' results included light microscopy of histological sections on the 10th day after TSC CPC.

Results. After 2000 mW/1.5 sec TSC CPC (energy 3 J) per pulse, pronounced destruction of ciliary processes and underlying stroma of the ciliary body, as well as pigmented and non-pigmented ciliary epithelium, was observed. The coagulation necrosis of collagen fibres of the sclera was detected. After 1000 mW/1.5 sec TSC CPC (energy 1.5 J) per pulse, the destruction of the pigmented and non-pigmented epithelium of the ciliary body was observed, with less disorganisation of the stroma of the ciliary body. The sclera was not affected when the energy was reduced.

Conclusions. Diode TSC CPC (810 nm) with a laser radiation power of 1000 mW (exposure 1.5 sec) is a more selective form of cyclophotocoagulation, which leads to less destruction of the ciliary body and sclera and at the same time ensures damage to the epithelium of the ciliary body, compared to the use of laser radiation with 2000 mW power.

Key words: diode laser, transcleral cyclophotocoagulation, histopathology, necrosis.

УДК 617.7-007.681-085.849.19-091.8-092.9

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ГІСТОПАТОЛОГІЧНІ ЗМІНИ В ОЧАХ КРОЛИКІВ ПІСЛЯ ТРАНССКЛЕРАЛЬНОЇ ДІОДНОЇ ЦИКЛОФОТОКОАГУЛЯЦІЇ
ДУ «Інститут очних хвороб та тканинної терапії імені В. П. Філатова Національної академії медичних наук України», Одеса, Україна

Стаття присвячена проблемі забезпечення оптимального впливу на структури циліарного тіла діодної (810 нм) трансскліральної циклофотокоагуляції (ТСК ЦФК). Мета роботи – вивчити гістопатологічні зміни у склері та циліарному тілі після ТСК ЦФК в експерименті з використанням двох енергетичних режимів (1 – потужність 2000 мВт, експозиція 1,5 с та 2 – потужність 1000 мВт, експозиція 1,5 с). В результаті 1-го режиму спостерігалося виражене руйнування циліарних відростків, циліарного епітелію і підлеглої строми циліарного тіла, а також коагуляційний некроз колагенових волокон склери. Використання 2-го режиму має більш селективну дію, що супроводжувалось руйнуванням пігментного і безпігментного епітелію циліарного тіла, з меншою дезорганізацією архітектоніки, збереженням строми циліарного тіла та неушкодженої склери.

Ключові слова: діодний лазер, трансклеральна циклофотокоагуляція, гістопатологія, некроз.

Introduction. Neovascular glaucoma (NVG) is a refractory form of secondary glaucoma, which is associated with high morbidity in patients with retinal ischemic diseases. It often manifests itself as a terminal stage of the disease, leading to blindness, constant pain and, ultimately, the loss of an eye. Treatment aims to reduce intraocular pressure (IOP), relieve pain and preserve the eyeball. To achieve this goal, there is no consensus on the most effective and safe procedure. The main treatment methods are trabeculectomy with the use of antimetabolites, implantation of filtration devices, and cyclodestructive procedures. However, when patients refuse surgery or their general health does not allow for surgery, cyclophotocoagulation (CPC) becomes the method of choice.

In the process of transscleral (TSC) CPC, the laser beam passes through the sclera and is absorbed by melanin at the level of the pigment epithelium of the ciliary body and converts into heat with a coagulation effect [1]. The melanin-dependent mechanism of action of CPC was confirmed by Dotson in the ineffective treatment of a patient with classic signs of cutaneous-ocular albinism [2].

Different types of lasers were used for the TSC CPC. Today, the diode laser is considered the most optimal. The radiation of the diode laser (wavelength 810 nm) is best absorbed by the melanin of the pigment epithelium. TSC CPC provides a continuous supply of laser energy. Continuous-wave TSC CPC is effective for reducing IOP but has a risk of serious complications such as decreased visual acuity, hypotony, chronic uveitis, and phthisis bulbi. These complications are probably the result of damage to the surrounding tissues due to the spread of thermal energy [3; 4]. The continuous-wave diode laser can affect the pars plana and pars plicata of the ciliary body, the ciliary muscle and sclera.

According to Barac, a micropulse diode laser can affect the choroidal thickness, as the authors suggest, due to the improvement of uveoscleral outflow [5]. Some authors have reported that when using a micropulse diode laser (2000 mW), sound phenomena of the "popcorn effect" are often observed, and severe ciliary body damage is noted after TSC CPC. At the same time, with the power of laser radiation up to 1000 mW against the background of a significant decrease in IOP, no significant structural changes or tissue damage to the ciliary body, sclera, or ciliary muscles was observed [6].

Therefore, we believe that to avoid some serious complications after TSC CPC, it is advisable to avoid the use of powerful laser radiation, which leads to thermal damage not only to the epithelium of the ciliary body, which directly participates in the production of intraocular fluid but also causes the destruction of other ocular structures. The selection of optimal conditions for TSC CPC requires additional experimental studies of histopathological changes in the ciliary body after exposure to laser radiation with different energy parameters.

Currently, there is no consensus on the optimal choice of laser type and laser radiation parameters to effectively reduce IOP and the risk of complications during TSC CPC in patients with NVG.

We analyzed data from our previous histopathological studies, both of rabbit eyes after TSC CPC of the ciliary body using a neodymium laser [7], and of the structures of the removed eye of a patient with choroidal melanoma after palliative diode TSC CPC of painful neovascular glaucoma with effective reduced IOP [8].

The research purpose is to determine histopathological changes in the sclera and ciliary body after transscleral diode cyclophotocoagulation with a wavelength of 810 nm depending on the laser energy in an experiment on rabbits.

Material and methods. The experimental procedures in this study were carried out following the rules of safety, ethical treatment and work with experimental animals according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty". The study was conducted at the State Institution "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine" and was approved by the ethics committee of the institute for the care and use of animals (research protocol No. 3 of 2024). Rabbits were housed in a standard animal facility with appropriate temperature and humidity control (22°C ± 3°C and 55% ± 10%, respectively) and a 12-h light/dark cycle. The rabbits were given water and food according to the ration.

Experimental studies were carried out on 2 rabbits (4 eyes) of the Chinchilla breed weighing 5–5.5 kg, aged 1 year. Before the TSC CPC, the experimental animals underwent ophthalmoscopy to exclude the pathology of the intraocular structures. The experimental TSC CPC was carried out in an equipped vivarium. Local anaesthesia: epibulbar (oxybuprocaine 0.4% 3 times) and retrobulbar (lidocaine hydrochloride solution 2% 1 ml). Material from intact animals (2 rabbits, 4 eyes) was used as a control.

TSC CPC was performed using a diode laser with a wavelength of 810 nm and a contact fibre-optic G-probe attached to a Vitra 810 semiconductor laser (Quantel Medical, France). The sole of the G-probe was held parallel to the visual axis, with the shorter edge of the sole firmly held between the anterior border and the middle of the limbus, placing the laser fibre over the pars plicata. Pars plicata is defined as the area that includes the ciliary processes with non-pigmented and pigmented epithelium, as well as the stroma of the ciliary body.

TSC CPC was carried out concentrically around a 360º circle, at a distance of 1–1.5 mm from the surgical limbus. Interventions in the 3 and 9 o'clock zones were not performed to avoid damage to the ciliary vascular-nerve bundle. Two energy modes were used: 1 – power of 2000 mW, exposure of 1.5 sec (energy of 3 J per one pulse) and 2 – power of 1000 mW, exposure of 1.5 sec (energy of 1.5 J per pulse). Previously, control transscleral laser coagulation of the ocular tissues was performed using the above-mentioned energies in the area in front of the equator with ophthalmoscopy.

Evaluation of the results of experimental studies included observation in the postoperative period and histopathological examination on the 10th day after TSC CPC. Ophthalmoscopy was performed every day after TSC CPC, and on the 10th day, colour photography of the fundus was performed.

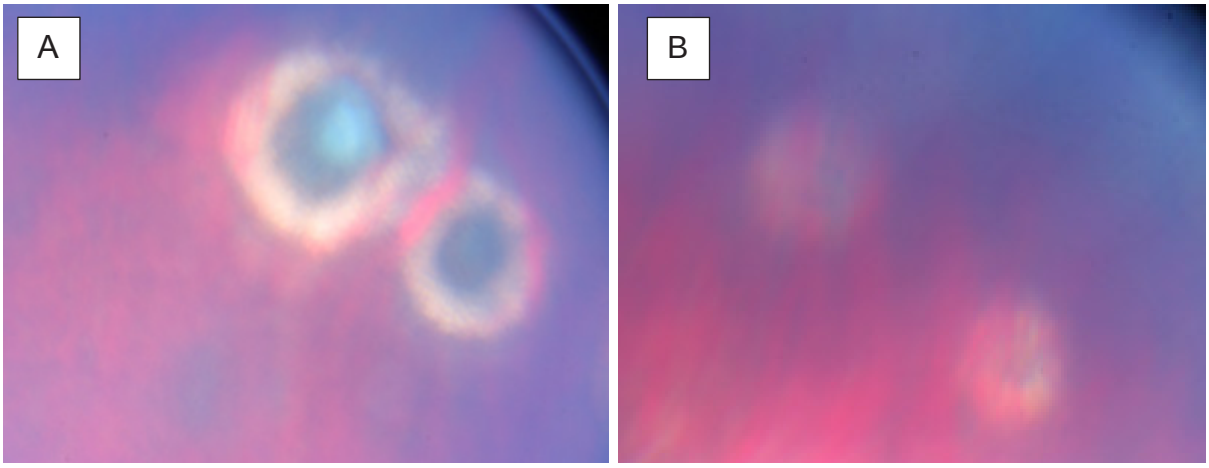

Fig. 1. Colour photo of control laser coagulates of rabbit's retina: A – power 2000 mW and exposure 1.5 sec (3 J); B – power 1000 mW and exposure 1.5 sec (1.5 J)

Histopathological studies were conducted in the laboratory of pathomorphological and electron microscopic studies of the State Institution "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine". After 10 days, the animals were removed from the experiment by the method of air embolism, the eyes were enucleated and fixed in a 10% solution of neutral formalin. After washing in water, the central block of the eye was cut out, dehydrated in a battery of alcohols of increasing concentration, clarified in xylene and embedded in paraffin. Paraffin sections with a thickness of 6–8 μm were stained with hematoxylin-eosin and embedded in Canadian balsam. Histopathological examination of enucleated eyes was carried out on 147 tissue samples stained with hematoxylin-eosin within 10 days after TSC CPC with magnification of 100×, 200× and 400×.

Results.

In the initial stage, control transscleral laser coagulations of rabbit fundus structures were ophthalmoscopically evaluated in the area anterior to the equator. Thus, immediately after transscleral laser intervention, the presence of local foci of oedema and coagulation of the retina and choroid was determined due to the use of both energies of laser exposure. On the 10th day after transscleral laser intervention using energy of 3 J, destruction of all layers of the retina and a larger area of atrophy of the fundus structures were found compared to energy of 1.5 J, the use of which was accompanied by damage mainly to the outer layers of the retina (Fig. 1).

At a laser power of 2000 mW with an exposure of 1.5 s during laser treatment, the sound phenomena "popcorn effect" was observed, and at a power of 1000 mW with an exposure of 1.5 s, the sound phenomena were isolated.

On the 10th day after TSC CPC using 3 J of energy, swelling of the stroma of the ciliary body, foci of the coagulation necrosis, including detritus of pigmented epithelium cells and destroyed non-pigment epithelium of the ciliary body, dilated choroidal vessels with coagulation of blood cells in the lumen, swollen scleral stroma, foci of the destruction and coagulation necrosis of adjacent collagen fibres of the sclera were revealed (Fig. 2–4).

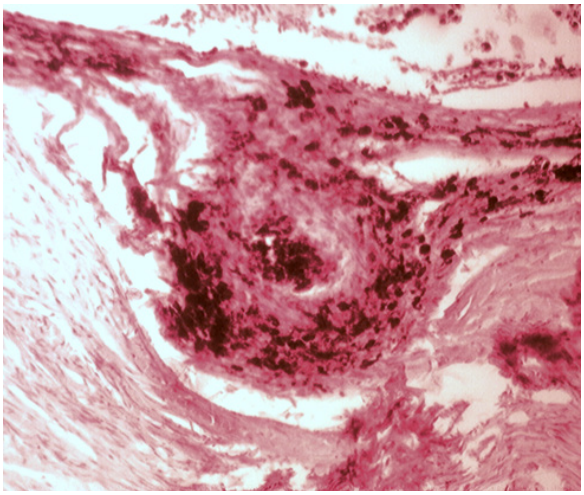

Fig. 2. Microscopic image of ocular tissue section after exposure to a 3 J diode laser in the projection of the ciliary body. Near the ora serrata there is a massive focus of coagulative necrosis, including detritus of pigmented epithelial cells and destroyed non-pigmented cellular elements of the choroid and ciliary body. A sharply swollen scleral stroma is visible on the periphery. Hematoxylin-eosin staining. Magnification 200×

On the 10th day after TSC CPC with the 1.5 J energy in the projection of the ciliary body, the areas of destruction of the non-pigmented and pigmented epithelium of the ciliary body, swelling and pigment deposits in the stroma of the ciliary body prevailed (Fig. 5, 6). However, the destruction of the ciliary body and sclera in the area of laser exposure and surrounding tissues were not as vividly represented as after exposure to laser radiation with higher energy.

Discussion. Neovascular glaucoma is a resistant form of glaucoma secondary to ischemic retinal lesions. It is believed that such patients have a significantly increased risk of developing hypotony after TSC CPC [9]. However, the results of laser exposure are difficult to predict, since direct imaging of ciliary body structures is limited. The level of pigmentation of the ciliary body can additionally

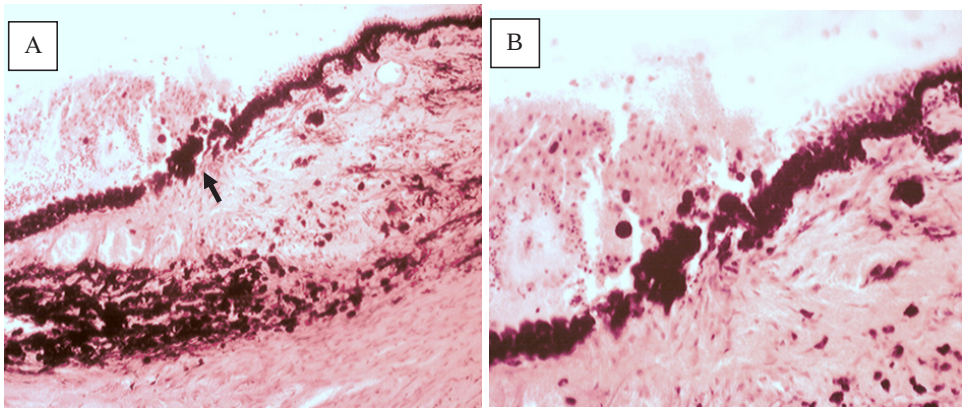

Fig. 3. Microscopic images of ocular tissue section after exposure to a 3 J diode laser in the projection of the ciliary body. A – the focus of destruction of the non-pigmented epithelium (massive cellular detritus), a strip of preserved cells of the non-pigmented epithelium of the ciliary body. A small area of destruction of the pigmented epithelium in the central part (arrow). Hematoxylin-eosin staining. Magnification 100×. B – Fragment of Figure 3A at higher magnification. Details of destructive changes in the ciliary non-pigmented and pigmented epithelium. Hematoxylin-eosin staining. Magnification 200×

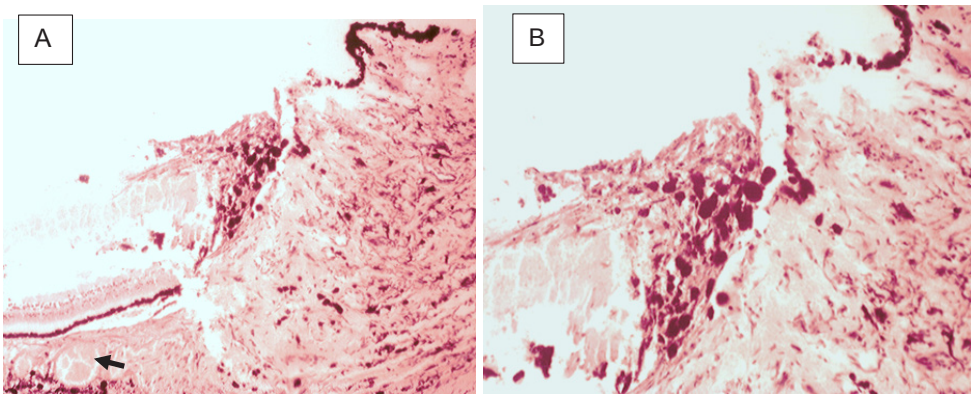

Fig. 4. Microscopic images of ocular tissue section after exposure to 3 J diode laser in the projection of the ciliary body. A – an extended focus of damage near the ora serrata with destruction of the non-pigmented epithelium and the adjacent retina. Dilated vessels with coagulation of blood cells in the lumen (arrow). A zone of destruction of the pigmented epithelium with swelling of the stroma of the ciliary body. Hematoxylin-eosin staining. Magnification 100×. B – a fragment of Fig. 4A at higher magnification. Details of destructive changes in the ciliary non-pigmented and pigmented epithelium. Hematoxylin-eosin staining. Magnification 200×

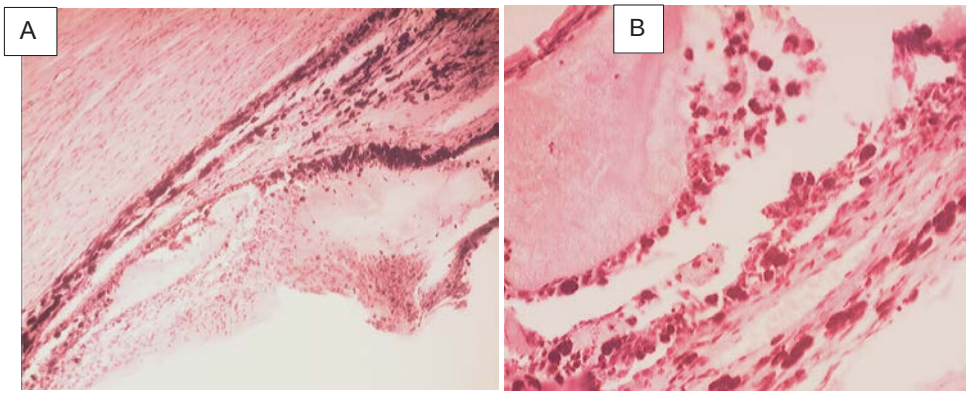

Fig. 5. Microscopic images of ocular tissue after exposure to 1.5 J diode laser in the projection of the ciliary body. A – the focus of the destruction of non-pigmented and partially pigmented epithelium (massive cellular detritus), a strip of the retina with the destruction of cellular layers. Hematoxylin-eosin staining. Magnification 100×. B – the focus of the destruction of the pigmented epithelium of the adjacent part of the ciliary body, numerous deposits of the destroyed pigment. Hematoxylin-eosin staining. Magnification 400×

influence the success of treatment. Another important issue is the optimal setting of the laser radiation power and exposure. Thus, it is essential to carefully select the average energy per session and the frequency of repeated treatment sessions.

It is known that for a successful TSC CPC, the laser radiation pulse energy of 2.5–4.5 J is needed. Even though the power of 2000 mW is often used for human studies, during the CPC at such power, there was a distinct "popcorn effect", which indicated excessive ciliary body exposure. Given the risks of complications when using high laser energy and based on our previous study, which determined the effectiveness of TSC CPC and reduced complication rates when using energy of 1.5 J [8], we decided to conduct an experimental study with the confirmation of sufficient (to achieve a hypotensive effect) destruction of pigmented and non-pigmented epithelium under the condition of avoiding pronounced coagulation necrosis of the ciliary body.

To control the imaging of the coagulation effect when using different diode laser energy, we made several TSC burns on the peripheral ocular fundus. Laser spot formation was observed ophthalmoscopically. Their appearance was noted immediately after application, and formation after a few days. Depending on the power, we detected foci of different diameters and levels of fundus damage, more pronounced at a power of 2000 mW.

Tsujisawa et al. noted that a potential mechanism of IOP reduction after micropulse transscleral cyclophotocoagulation in the experiment is the dysfunction of intraocular fluid transport due to damage to non-pigmented epithelial cells of the pars plicata of the ciliary body and destruction of the basal infoldings [10].

We also relied on the results of a histopathological examination of enucleated eyes of patients with hypotony after TSC CPC using high-energy laser radiation. Data from enucleated eyes after diode laser treatment showed destruction of pigmented and non-pigmented ciliary epithelium with pigment accumulation, coagulation necrosis, and extensive destruction [11]. Williams also identified pronounced coagulation necrosis with complete loss of cell nuclei and a large amount of damaged pigment epithelium of the ciliary body, as well as exudative retinal detachment after CPC (with an energy of 1800–2000 mW and an exposure of 2 sec) [12].

Numerous histological changes in the ciliary body of cadaveric eyes have been described in the available literature following CPC procedures. Moussa noted splitting of the epithelium of the ciliary body, separation of the pigmented epithelium from the stroma, collagen coagulation and damage of the stroma of the ciliary body with full-layer destruction of the epithelium [13]. Similar extensive destruction of ciliary processes was described in rabbits [14].

Our findings align with results from studies on animal eyes, cadaveric eyes, and the enucleated eyes of patients [11; 13–16]. We found clear differences in tissues treated with two different diode laser energies. Histological changes of the rabbit eye after low-energy (1.5 J) TSC CPC showed the destruction of the mainly pigmented and non-pigmented epithelium of the ciliary body, but no ciliary body necrosis and scleral damage were noted (Fig. 5, Fig. 6). And when

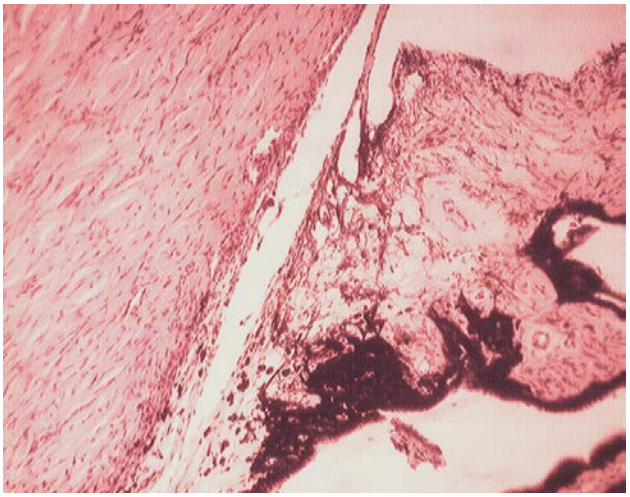

Fig. 6. Microscopic image of ocular tissue after exposure to a 1.5 J diode laser in the projection of the ciliary body. The focus of the destruction of the pigmented and non-pigmented epithelium of the ciliary body, and numerous deposits of the destroyed pigment (pigment knock-out effect) in the underlying stroma of the ciliary body. Exudative detachment of the ciliary body, and destructive changes. Hematoxylin-eosin staining. Magnification 200×

the energy was increased to 3 J, full-thickness destruction of the pigmented ciliary epithelium with swelling of the stroma of the ciliary body with wide, full-layer coagulation necrosis was observed, including the detritus of pigment epithelium cells and destroyed non-pigmented cellular elements of the ciliary body and choroid. Swelling of the scleral stroma and coagulation necrosis of its collagen fibres (Fig. 2–4). These damages can subsequently threaten visual functions and be the basis of a pronounced inflammatory reaction and lead to hypotony and phthisis [17–19].

In the previous study, we determined that two components are associated with the mechanism of hypotensive action of neodymium TSC CPC: coagulation and hydrodynamic cavitation [7]. Different authors emphasize that the mechanism of the hypotensive effect of diode TSC CPC may be related to the thermal destruction of ciliary processes, improvement of uveoscleral outflow of intraocular fluid, formation of biologically active substances or changes in perfusion [12; 19]. Some authors determine the marked thinning of the capillary network in the treated areas of the ciliary body [14], and also that the level of intraocular fluid production depends on the ciliary blood flow [20]. Reitsamer noted that in rabbits' fluid production depends on ciliary blood flow until ciliary blood flow critically drops below 74% of control [21]. Also, the decrease in IOP correlates with the thickening of the choroid [5].

One of the main limitations of our study is the difference in the anatomy of human and rabbit eyes. In the rabbit eye, the size of the ciliary body is smaller compared to the human ciliary body, and the pigmented ciliary processes extend to the back of the peripheral iris. Another anatomical difference is that the rabbit sclera is thinner than the human sclera, potentially leading to a more pronounced

treatment effect in the rabbit compared to the human eye. Despite the small number of eyes included in this study, there was a clear difference in the histological pattern between eyes after exposure to a diode laser with a power of 1000 mW/1.5 sec and 2000 mW/1.5 sec.

Conclusions. Transscleral cyclophotocoagulation using diode laser (810 nm) with a power of 1000 mW and exposure of 1.5 sec caused, according to histopathological findings, mainly selective damage to the ciliary epithelium (pigmented and non-pigmented) with less disorganization of the architecture and preservation of the stroma of the ciliary body, in contrast to laser radiation with power 2000 mW, which revealed gross damage to the ciliary body and sclera.

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Надійшла до редакції 03.06.2024 р.
Прийнята до друку 28.11.2024 р.
Електронна адреса для листування olga.v.guzun@gmail.com