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Comparing histopathological effects of the neodymium and diode laser transscleral cyclophotocoagulation: an experimental study

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Background: Cyclodestructive procedures with high laser energy settings achieve their IOP reduction effect at the expense of damage to the secretory epithelium of the ciliary processes and adjacent structures, which may result in such complications as hypotony and ocular subatrophy.

Purpose: To experimentally evaluate the histopathological features in the rabbit eye after exposure of the distal ciliary body to transscleral selective laser radiation at the 810 nm wavelength versus the 1064 nm wavelength, and to compare the histopathological effects of the diode and neodymium:yttrium-aluminum-garnet (Nd:YAG) lasers.

Material and Methods: Four Chinchilla rabbits (8 eyes) were included in this experimental study. In four eyes, transscleral cyclophotocoagulation (TSCPC) of the ciliary body was performed with an 1064-nm Nd:YAG laser (energy, 1.0 J/pulse; pulse duration, 3 ms) equipped with a 600- μ m fused-silica fiber optic tip. In another four eyes, an 810-nm diode laser TSCPC of the ciliary body was performed using a Vtra 810 apparatus (Quantel Medical Instruments, France) with a laser power of 1W and exposure duration of 1.5 s (energy, 1.5 J/pulse).

Results: Our experimental histopathological study of rabbit eyes demonstrated no significant difference in the development of ciliary stromal edema ($p = 0.425$) and focal necrosis of the non-pigmented ciliary epithelium ($p = 0.764$) between the eyes that received the transscleral contact cyclodestruction with an 810-nm diode laser at an energy of 1.5 J and the eyes that received transscleral contact-and-compression cyclodestruction with a 1064-nm Nd:YAG laser at an energy of 1.0 J.

Conclusion: The use of 810-nm laser radiation at energy of 1.5 J in the transscleral contact cyclodestruction and the use of 1064-nm laser radiation at energy of 1.0 J in the transscleral contact-and-compression cyclodestruction were similar in enabling selective thermal effects on the ciliary epithelium with limited damage to adjacent structures in rabbits.

Keywords:

ciliary body, diode laser, Nd:YAG laser, transscleral cyclophotocoagulation, histopathology

Introduction

Transscleral cyclophotocoagulation (TSCPC) techniques have been increasingly used for cyclodestruction in the treatment of secondary neovascular glaucoma [1]. The use of this approach in patients with preserved visual function is, however, limited due to insufficiently selective laser effects on the structures of the ciliary body. The risk of excessive damage to the ciliary body and surrounding tissue substantially increases with the application of high laser energy settings for TSCPC [2, 3]. In a study by Alabduljabbar and colleagues [4], TSCPC was used for the treatment of neovascular glaucoma, with the starting power set at 1500 mW for 1500 ms and gradually increased until soft "pops" were heard during treatment. Others [5], however, used a higher power of the diode laser (laser power, 1.5–2 W; exposure time per burn, 2 s), which can be accompanied by additional damage to the ocular tissue.

Most authors prefer performing TSCPC with a 810-nm diode laser, whereas others advocate for 1064-nm Nd:YAG laser [6, 7]. Discussion is still going on the advantages of

different types of laser for TSCPC, efficacy of techniques for the transscleral laser exposure, and laser energy settings for performing transscleral cyclodestructive procedures.

With the TSCPC, radiant energy from a 1064-nm or 810-nm infrared laser is absorbed by melanin granules of ciliary pigment epithelium, but the 810-nm diode wavelength is absorbed better by melanin than the 1064 nm Nd:YAG wavelength [8-10]. Consequently, the 810-nm diode wavelength has an advantage with regard to less energy required for TSCPC when compared to the 1064 nm Nd:YAG wavelength. However, scleral transmission is better and light backscattering is lower at the 1064 nm Nd:YAG wavelength than at the 810-nm diode wavelength. In addition, in TSCPC, dosed scleral compression additionally increases scleral penetration by laser and enables reducing the energy of laser exposure [11, 12].

We have previously demonstrated that experimental transscleral contact-and-compression (TSCC) CPC with a 1064-nm Nd:YAG laser at an energy of 1.2J resulted in subconjunctival tissue and scleral damage and disorganization of stromal collagen fibers along the direction of laser radiation with the formation of a canal, and was accompanied by extensive detachment of the ciliary epithelium [13]. Our subsequent experimental study [3] on TSCPC with an 810-nm diode laser demonstrated that an increase in laser energy to 3 J not only caused coagulative necrotic lesions (detritus of pigment epithelial cells and loss of structure in non-pigmented cells in the ciliary body) but also damage to ciliary and scleral stroma.

Given the findings of our previous studies, we hypothesized that a reduction in laser energy in TSCPC with either of the types of laser (i.e., an 810-nm diode laser or an 1064-nm Nd:YAG laser) will result in selective and controlled damage to the ciliary body at the level of pigmented and non-pigmented epithelium), with a reduced risk of damage to surrounding tissues in the course of laser cyclodestruction and preservation of the therapeutic effect of the latter [14].

Therefore, the **purpose** of this experimental study was to evaluate the histopathological features in the rabbit eye after exposure of the distal ciliary body to transscleral selective laser radiation at the 810 nm wavelength versus the 1064 nm wavelength, and to compare the histopathological effects of the diode and Nd:YAG lasers.

Material and Methods

All animal experiments were performed in compliance with the Law of Ukraine on Protection of Animals from Cruel Treatment No. 3447-IV dated February 21, 2006 and European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes from the European Treaty Series (Strasbourg, 1986), and approved by a local Bioethics Committee of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine" (Meeting Minutes No. 4 dated July 9, 2024).

Four Chinchilla rabbits (8 eyes) were included in this experimental study. In four eyes, transscleral ciliary body coagulation was performed with an 1064-nm Nd:YAG laser (energy, 1.0 J/ pulse; pulse duration, 3 ms) equipped with a 600- μ m fused-silica fiber optic tip for dosed scleral compression. In another four eyes, an 810-nm diode laser TSCPC was applied using a fiber optic G-probe connected to the Vitra 810 (Quantel Medical Instruments, Courmon d'Auvergne Cedex, France), and was performed with a laser power of 1,000 mW and exposure duration of 1.5 s (corresponding to an energy of 1.5 J/pulse). Epibulbar conjunctiva was anesthetized with three drops of 0.4% oxybuprocaine, and one milliliter 2% lidocaine hydrochloride was administered retrobulbarly. The probe was placed 1.5-2 mm from the limbus and held parallel to the visual axis. At average, 20 laser spots were applied over 180 degrees. The TSCC CPC with a 1064-nm Nd:YAG laser included scleral compression by the waveguide face.

Histology was performed at the Pathology and Electronic Microscopy Laboratory of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine". Ten days after TSCPC, 273 histological sections were prepared and stained with hematoxylin-eosin, and their images were taken at magnifications of 70 \times , 100 \times , 180 \times , 200 \times and 400 \times , and included in the analysis. Histological changes, which are not typically seen in normal rabbit globes, were scored as present or absent in each section, and the percentage of total sections involved was calculated. Sections missing the ciliary epithelium were excluded from the analysis. The most common histopathological features were included in the analysis, and the two groups of eyes (subjected to the 810-nm diode laser and the 1064-nm Nd:YAG laser) were compared for these features.

Statistical analyses were conducted using Statistica 10.0 (StatSoft, Tulsa, OK, USA) software. Quantitative data were evaluated for normality using the Shapiro-Wilk test. Data are presented graphically as percentages. Fisher's exact test was used to analyze a 2 x 2 feature contingency table for the significance of difference between the two used lasers. The level of significance $p \leq 0.05$ was assumed. The Cramer's V test was used to assess the strength of associations between the CPC with a 1064-nm Nd:YAG laser and the CPC with an 810-nm diode laser and histological features in the ciliary body.

Results

Ten days after TSCPC, 125 and 148 ocular tissue sections containing ciliary epithelial cells were prepared from the eyes of the rabbits that received TSCPC with a 1064-nm Nd:YAG laser and an 810-nm diode laser, respectively, and included in the analysis.

The most common histological features observed and analyzed in histological sections after TSCPC with either laser type were as follows: (1) ciliary stromal edema; (2) focal necrosis of the non-pigmented ciliary epithelium; (3) destruction and focal necrosis of the pigmented and non-pigmented ciliary epithelium; and (4) separation of the pigmented ciliary epithelium from the stroma.

Detachment and destruction of the non-pigmented ciliary epithelium, homogenized stroma and destruction of a portion of the ciliary processes were seen in most sections prepared from the eyes subjected to TSCPC with an 1064-nm Nd:YAG laser (energy, 1.0 J/ pulse) (Fig. 1). The iris root was swollen, a portion of the epithelial cells of its posterior surface appeared necrotic, and the stromal cells appeared loose. Destruction of the iris pigment epithelium was noted (Fig. 1A). At some locations, not only the ciliary stroma was edematous, but the detachment of the pigmented ciliary epithelium with formation of vacuole-like structures, and accumulation of homogenous protein-containing inclusions at the anterior chamber angle between ciliary processes was observed (Fig. 1B).

In the sections obtained from the eyes subjected to TSCPC with a 810-nm diode laser (energy, 1.5 J/ pulse), there was ciliary stromal edema, but more common findings

included destruction of the pigmented and non-pigmented ciliary epithelium and edema and pigment lumps in the ciliary stroma (Fig. 2A). In addition, we observed the destruction and focal necrosis of the pigmented and non-pigmented ciliary epithelium, and separation of the pigmented ciliary epithelium from the stroma (Fig. 2B).

However, evidence of collagen coagulation and ciliary stromal and scleral destruction at the site of laser exposure and surrounding tissues was common in our previous study [3] after laser exposure with higher pulse energy, but not in the current study.

After exposure to either of the two lasers, histological evaluation found ciliary stromal changes, mostly of a reactive nature (edema, vacuolation and abnormal microcirculation), which were not associated with the use of a laser of any wavelength. In addition, after exposure to either of the two lasers, isolated histological sections exhibited collagen coagulation and ciliary stromal destruction, likely due to repeated exposure of the same site. No full-thickness destruction of the ciliary epithelium was observed, which is in agreement with findings of others [15-17] on diode laser TSCPC performed with conventional energy settings.

Fig. 3 shows the percentages of sections showing histological features, and Table 1 compares four histologic outcomes between two treatment conditions (1064-nm laser and 810-nm laser).

Fisher's exact test showed no significant differences in the development of ciliary stromal edema ($p = 0.425$) and focal necrosis of the non-pigmented ciliary epithelium ($p = 0.764$) between the 810-nm and 1064-nm laser exposure groups. The rate of destruction and focal necrosis of the pigmented and non-pigmented ciliary epithelium and the rate of separation of the pigmented ciliary epithelium from the stroma, were, however, higher in the former group (83.7% versus 72% and 73.5% versus 61.6%, respectively), with the differences between the groups being significant ($p = 0.026$ and $p = 0.038$, respectively).

The Cramer's V test was used to assess the strength of associations of the wavelength of laser used for cyclodestruction with the destruction and focal necrosis of the pigmented and non-pigmented ciliary epithelium and the separation of the pigmented ciliary epithelium from the stroma. There was a weak association between the wavelength of laser used for cyclodestruction and the destruction and focal necrosis of the pigmented and non-pigmented ciliary epithelium (Cramer's V value = 0.141) and between the wavelength of laser used for cyclodestruction and the detachment of the pigmented ciliary epithelium from the stroma (Cramer's V value = 0.127).

Discussion

Not only the selection of laser wavelength but also the specific laser settings (power and duration) are important in transscleral cyclodestruction treatment with laser for refractory glaucoma. Audible "pops" are now believed to indicate overtreatment, and their presence can be used to quantify laser parameters and that the maximum level

of energy used should be slightly below the threshold required to produce said "pops" [18].

A disadvantage of TSCPC is limited selectivity of the target tissue (the ciliary body) which may cause damage to the surrounding structures and complications. TSCPC is associated with a risk of complications like pain, conjunctival burns, scleral thinning, long-germ inflammation and hyphema [14; 19-23], and rarely causes serious complications like chronic hypotony, choroidal detachment, choroiditis, retinal detachment, scleral perforation and phtthis bulbi [24 - 27]. Conjunctival burns have been reported in up to 80% of patients undergoing TSCPC [28]. Pupillary distortion, disturbed accommodation, cystoids macular edema have been also reported in TSCPC, affecting treatment outcomes in eyes with preserved visual function [29].

Factors impacting the selection of laser wavelength for TSCPC include the type of glaucoma, history of prior treatment, visual potential, the surgeon's and patient's preferences, and the potential for success. Unfortunately, the literature lacks studies on the analysis of ocular histology after Nd:YAG laser CPC versus diode laser CPC.

Recently, there has been an increase in studies on the use of a diode laser to perform TSCPC. The wavelength of a diode laser (810 nm) is worse transmitted by the sclera, but better absorbed by melanocytes from the secreting ciliary epithelium compared to the wavelength of the Nd:YAG laser (1064 nm) [11]. In a rabbit study on TSCPC by Brancato and colleagues [30], gross examination revealed threshold lesions at 1 J energy for the Nd:YAG laser and 0.8 J for the diode laser. The histologic and ultrastructural study showed that diode laser radiation produced more remarkable damage to the ciliary pigmented structures, causing deep coagulation necrosis of the pigmented epithelium, wide disorganization of the collagen in the stroma, and intravascular coagulation phenomena in the ciliary vessels [30]. In our previous study [14], at the 12-month follow-up, the Nd:YAG laser TSCPC (1064 nm; power, 1.0 J) was as effective as the diode laser TSCPC (810 nm; power, 1.5 J) in the management of painful neovascular glaucoma (NVG) associated with proliferative diabetic retinopathy. The Nd:YAG laser TSCPC resulted in a reduction in intraocular pressure (IOP) to ≤ 21 mmHg at month 12 in 75%, and diode laser TSCPC, in 77% of patients with diabetic NVG. The rate of side effects was higher for eyes treated with the diode laser TSCPC than for those treated with the Nd:YAG laser TSCPC (71% versus 33%, $p = 0.004$). Inflammation was the most common complication in both groups, but was significantly less common in eyes treated with the Nd:YAG laser TSCPC than in those treated with the diode laser TSCPC (20% versus 46%, $p = 0.03$) [14]. Our findings are in agreement with those by Brancato and colleagues [30].

In the current histopathological study, on day 10 after TSCPC, there was no significant differences in the percentage of sections that showed ciliary stromal edema ($p = 0.425$) and focal necrosis of the non-pigmented

ciliary epithelium ($p = 0.764$) between the group treated with the diode laser TSCPC (power, 1.5 J) and the group treated with the Nd:YAG laser TSCPC (power, 1.0 J). The rate of destruction and focal necrosis of the pigmented and non-pigmented ciliary epithelium and the rate of separation of the pigmented ciliary epithelium from the stroma, were, however, higher in the former group (83.7% versus 72% and 73.5% versus 61.6%, respectively), with the differences between the groups being significant ($p = 0.026$ and $p = 0.038$, respectively). The post-hoc analysis, however, showed a weak association between the wavelength of laser used for cyclodestruction and the destruction and focal necrosis of the pigmented and non-pigmented ciliary epithelium (Cramer's V value = 0.141) and the separation of the pigmented ciliary epithelium from the stroma (Cramer's V value = 0.127); this indicates similarity in the pattern of ciliary lesions at the used laser settings between the groups. Assia and colleagues [31] investigated the cyclodestructive effects of the Nd:YAG laser TSCPC and diode laser TSCPC in cadaver eyes. Histologic changes using both lasers were coagulation necrosis with fragmentation and detachment of the ciliary body epithelium, which is in agreement with our study. Assia and colleagues [31] also noted that laser TSCPC "may have a minimum acute effect on lens structures when done clinically with the usual parameters" (i.e., using 4 J of energy for the Nd:YAG and 1.2 J for the diode laser). In the current study, while using conventional approaches to the dosage of laser energy, we did not observe other gross changes (collagen coagulation, destruction of the ciliary stroma, and full-thickness destruction of ciliary epithelium) noted by others [16]. This also confirms our hypothesis that, in order to reduce the rate of complications of transscleral laser cyclodestruction, it is reasonable to review the current conventional approaches to the selection of diode laser energy settings and favor low laser energy settings that enable selective effects on the ciliary epithelium.

Conclusion

Our experimental histopathological study of rabbit eyes demonstrated no significant difference in the development of ciliary stromal edema ($p = 0.425$) and focal necrosis of the non-pigmented ciliary epithelium (p

$= 0.764$) between the eyes that received the transscleral contact cyclodestruction with an 810-nm diode laser at an energy of 1.5 J and the eyes that received transscleral contact-and-compression cyclodestruction with a 1064-nm Nd:YAG laser at an energy of 1.0 J. The use of 810-nm laser radiation at energy of 1.5 J in the transscleral contact cyclodestruction the use of 1064-nm laser radiation at energy of 1.0 J in the transscleral contact-and-compression cyclodestruction were similar in enabling selective thermal effects on the ciliary epithelium with limited damage to adjacent structures in rabbits.

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Table 1. Histological features in the ciliary body after treatment with the 1064-nm or 810-nm laser

Histological features in the ciliary body after laser exposure	Two-sided Fisher's exact test
Ciliary stromal edema	$p = 0.425$
Focal necrosis of the non-pigmented ciliary epithelium	$p = 0.764$
Destruction and focal necrosis of the pigmented and non-pigmented ciliary epithelium	$p = 0.026^*$
Separation of the pigmented ciliary epithelium from the stroma	$p = 0.038^*$

Note: This table represents a 2 x 2 (fourfold) table that compares four histologic outcomes between two treatment conditions (1064-nm laser and 810-nm laser); *, significant difference

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Disclosures

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Ethics statement. The work with experimental animals and their withdrawal from the experiment was carried out in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelty”. The study was approved by the Bioethics Committee of the State Institution “The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine”, Protocol No. 4 dated July 9, 2024.

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Abbreviations: CPC, cyclophotocoagulation; IOP, intraocular pressure; Nd:YAG, Neodymium:yttrium-aluminum-garnet; NVG, neovascular glaucoma; TSCC, transscleral contact-and-compression; TSCPC, transscleral cyclophotocoagulation

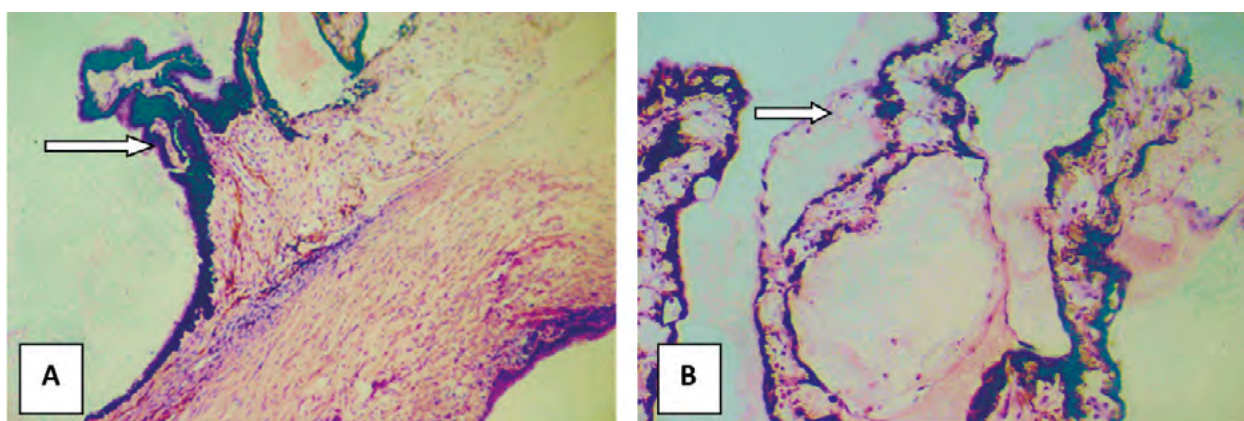


Fig. 1. Microscopic images of rabbit ocular tissue sections showing the effects of ciliary exposure to 1064-nm laser radiation at energy of 1.0 J. A: Ciliary stromal edema and focal necrosis of non-pigmented ciliary epithelium (arrow). Hematoxylin and eosin staining. Magnification, x70. B: Destruction and separation of non-pigmented ciliary epithelium with formation of cyctoid structures (arrow). Hematoxylin and eosin staining. Magnification, x180

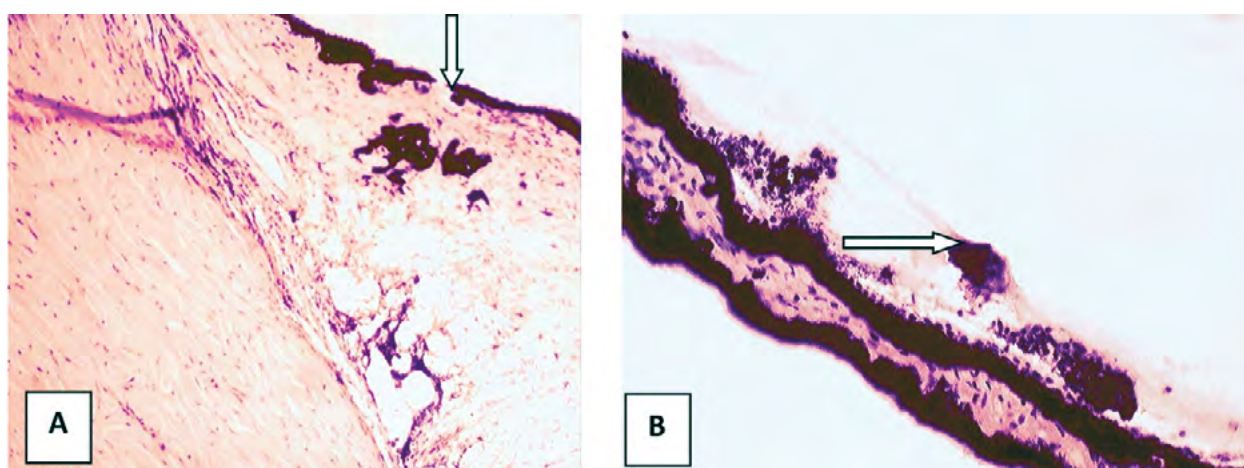


Fig. 2. Microscopic images of rabbit ocular tissue sections showing the effects of 810-nm laser radiation at energy of 1.5 J (1000 mW/1.5 s) in the superior portion of the projection of the ciliary body. A: Destructive lesion of the pigmented and non-pigmented ciliary epithelium (arrow). Numerous destructed pigment lumps, marked dispersion of pigment lumps within ciliary process stroma (with effect of pigment loss indicated by arrow) and ciliary body edema. Hematoxylin and eosin staining. Magnification, x100. B: Moderate destruction of ciliary process epithelium with pigment dispersion on its surface, and non-pigmented epithelium is preserved in some areas. Hematoxylin and eosin staining. Magnification, x100.

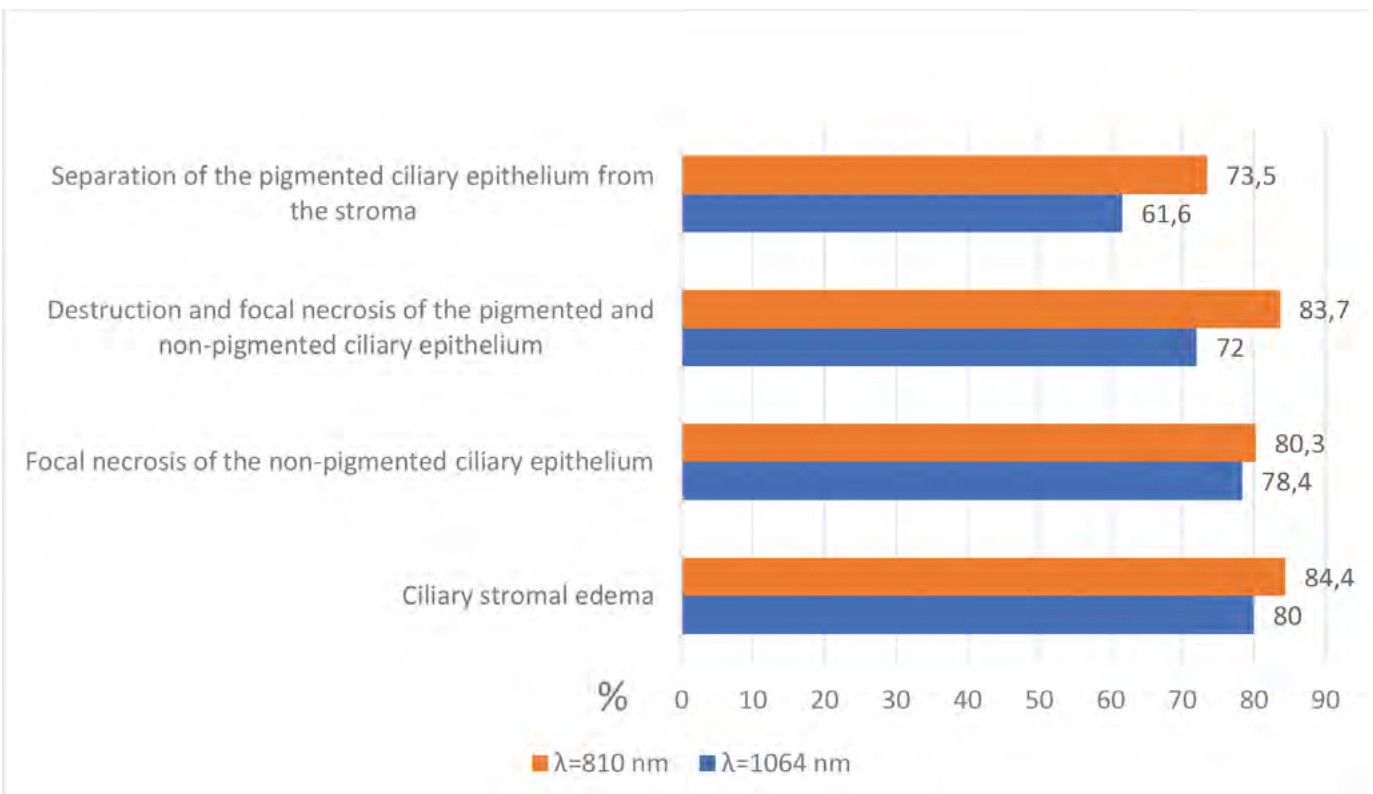


Fig. 3. Percentages of sections exhibiting particular histological features in the ciliary body for the 1064-nm laser exposure group versus the 810-nm laser exposure group